**Synthesis of Au clusters-redox centre hybrids by diazonium chemistry employing double layer charged gold nanoparticles.**

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**Abstract**

The experimental conditions in the two-phase synthesis of hexanethiolate gold monolayer-protected clusters (C6-Au) have been investigated by varying the thiolation and reduction times to give particles with an average core diameter of 1.66 nm and a capacitance of 0.54-0.59 aF. These clusters were further functionalised with anthraquinone by reaction between the diazonium derivative of 1-amino anthraquinone and electrochemically or chemically charged nanoparticles. Cyclic voltammetry and differential pulse voltammetry measurements demonstrate functionalisation by the redox couple and the presence of quantised double layer (QDL) charging events at the gold core. The feasibility of using a dispersion of charged nanoparticles as reagents for driving a diazotisation reaction to attach redox ligands is demonstrated.

KEYWORDS: gold nanoparticles; quantised double layer charging; Fast Fourier Transform nanoparticle charging analysis; diazonium functionalisation, anthrahaquinone electrochemistry; monolayer protected nanoparticle synthesis

**1. Introduction**

Monolayer protected clusters (MPC) have attracted a great deal of interest1 due to their many possible applications, for example, in plasmonic photothermal cancer therapy [2], for allowing high conductance across molecular wires in long range electron transfer reactions to access redox centres [3,4] and in biological and chemical sensing.5 These require, however, achieving great stability of the hybrid metal-chemically attached ligand structures. Thiols have been extensively employed for this purpose but it is only recently that we are beginning to understand the structure of thiolated nanoclusters, in particular the nature of bonding between thiols and gold, and the importance, in this respect, of Au-Au bonds [6]. Other functionalisation approaches have been extensively explored and one of the most promising employs the reductive attachment of an aryldiazonium cation leading to the formation of a metal-carbon bond [7,8]. In the case of gold, the greater stability of the Au-C over that of the Au-S bond has been experimentally demonstrated [9].

A significant result from the work of McDermott et al [9] was the observation that octadecanethiol, in contrast to its behaviour for replacing other attached thiols, was unable to fully displace a monolayer of organic moieties bound to the Au surface by diazonium chemistry, thus demonstrating the greater strength of the Au-C bond. More recent work with nanoporous gold confirms this preferential stability [10]. These results are in agreement with quantum chemical calculations that indicate a higher bond energy for C-Au than for S-Au bonds, of the order of 0.4 eV [11], a result that has been recently confirmed [12]. Diazonium chemistry has been successfully employed for the functionalisation of nanoparticles by spontaneous reduction reaction with metal particles [13], by two phase reduction of Au(III) [14] and by direct reaction in solution of citrate stabilised Au nanoparticles with a diazonium compound [15]. In addition to good stability, functionalisation through the formation of Au-C bonds offers a wide availability of the amine precursors employed for the synthesis of the required diazonium compounds.

The attachment of functional groups based on diazonium chemistry offers additional synthetic possibilities for surface functionalisation, in particular, as described in the present work, for the incorporation of strongly bound redox group within a thiol functionalised nanoparticle surface. In a series of important original reports, Murray et al [1] recognised that monolayer protected clusters could be regarded as dispersed nanoelectrodes. Importantly, due to their small core size and hence, small electrical capacitance, these particles display quantised double layer (QDL) charging behaviour in electrochemical experiments related to discrete charging events [16]. Using this property, the present work attempted to control, in a rational way, the number of organic residues attached per nanoparticles by reacting a pre-charged dispersion of particles with a diazonium compound.

The paper is divided in two sections. The dependence of average size of hexanethiol protected nanoparticles precursor on reaction conditions is described first and then a redox marker, in this case an anthraquinone molecule, was incorporated by reaction of their charged dispersion with the corresponding diazonium compound.

**2. Experimental**

Full experimental details are given in the Supplementary Information section.

*2.1 Preparation of hexanethiol monolayer protected clusters (C6-Au).*

The synthesis followed a literature protocol [17]. Briefly, a solution of HAuCl4·3H2O in Milli-Q® water (5.18×10-3 mol, 160 ml) was added to a toluene solution of TOABr (0.02 mol, 160 ml; TOA=tetraoctyl ammonium) with vigorous stirring. After 40 minutes, the water phase was discarded and 1-hexanethiol (0.016 mol, 1:3 Au(III):RSH molar ratio) was added to the organic phase. The reaction between Au(III) and the thiol was allowed to proceed for different times, from 30 min to 6 hours, to determine the influence of the formation of the Au(I)-thiol polymer on particle size and monodispersity. A freshly prepared aqueous solution of NaBH4 (6.74×10-2 mol, 160 ml, 1:13 molar ratio of Au:NaBH4) was then rapidly added to the mixture at 0°C with vigorous stirring. The solution turned black immediately and the reaction mixture was left with stirring for 15 to 30 min for the different preparative conditions tested. The phases were separated, the organic phase extensively cleaned with water and then toluene removed by rotatory-evaporation (*T* ≤ 30 °C). The black residue was re-suspended in absolute ethanol (160 ml), sonicated and left to settle overnight. The solution was then filtered through a sintered disc filter funnel and the filtrate transferred to a round-bottomed flask to remove the ethanol under vacuum. The nanoparticles were then suspended in approximately 160 ml of acetonitrile, sonicated for 15 minutes and left standing overnight. Finally, the capped particles were filtered and washed with acetonitrile. The reason for only using the ethanol soluble fraction was to restrict the subsequent analysis to the low molecular weight fraction of the preparation. Precipitation of the reaction mixture with solvents such as acetone leads to a range of sizes that do not display well defined QDL behaviour, a central point of the present work, requiring further fractionation.

*2.2 Attachment of anthraquinone to nanoparticles.*

To simplify notation, we have indicated throughout the text AQ to mean the 1-anthraquinone bonded moiety. Anthraquinone functionalised nanoparticles (AQ-C6-Au) were prepared by reaction of electrochemically or chemically charged nanoparticles with the diazonium derivative of anthraquinone (AQ-N2+). C6-Au particles were used as a stable starting material for the attachment of AQ by reducing AQ-N2+ at the nanoparticle surface according to [7]:

RN2+ + e- → R• + N2 (1)

followed by the reductive coupling of the radical (AQ• in this case) at the Au nanoparticle surface:

R• + e- + Aun →R-Aun (2)

The reduction of the N2+ group must be carried out adjacent to the metal surface to be functionalised. This reaction was achieved by first injecting electrons into a nanoparticle dispersion followed, in a separate step, by reaction in solution with the diazonium compound.

*2.2.1 AQ functionalisation by electrochemical charging*

C6-Au particles were further functionalised with anthraquinone employing its diazonium derivative. Two methods for charge injection were employed, direct electrochemical charging and a two phase redox reaction with borohydride as reducing agent in the aqueous phase. A well-defined number of electrons could therefore, be injected in the nanoparticles and used to carry out the surface functionalisation shown in reactions (1)-(2) without the need of applying external potential for reducing the N2+ group.

A solution of C6-Au nanoparticles (59 μM in a 2:1 toluene-acetonitrile solvent mixture containing 0.1 M TBAPF6 as base electrolyte) was placed in contact under stirring with an electrode held at a potential of -1 V for approximately 6 hours. AQ-N2+ (0.2 mM in a 2:1 a toluene-acetonitrile solvent mixture) was then added to the electrolysed nanoparticles in a 3:1 molar ratio (AQ-N2+:C6-Au) and the reaction mixture kept under stirring for 12 hours. The nanoparticles were then precipitated, filtered and washed with acetonitrile to eliminate the supporting electrolyte and any unreacted AQ-N2+. The solid was dried, redispersed in toluene and precipitated with addition of acetonitrile.

*2.2.2 AQ functionalisation by two-phase redox charging*

A C6-Au nanoparticle dispersion in toluene (59 μM i.e., 5.9 mg in 3 mL) was reduced in a two-phase reaction by mixing it with a 1 mM aqueous solution of NaBH4 for approximately 6 hours under continuous stirring. The organic phase was then washed with Milli-Q® water to remove excess of NaBH4. After phase separation, a AQ-N2+ solution (0.2 mM in the toluene-acetonitrile solvent mixture) was added to the organic phase in a 3:1 molar ratio (AQ-N2+: C6-Au) and the reaction allowed to proceed for 12 hours. The nanoparticles were then separated as described above.

*2.3 Electrochemical measurements*

Electrochemical measurements were performed with an Autolab potentiostat (PGSTAT 10, Eco Chemie B.V., The Netherlands) using the General Purpose Electrochemical System (GPES) software. The gold clusters were dissolved in 0.1 M TBAPF6 in a toluene: acetonitrile (2:1) mixture. A gold electrode embedded in glass (Area = 0.0314 cm2) and a platinum mesh were used as working and counter electrodes, respectively. The potentials are referred to Ag/AgCl/3M NaCl. The reference electrode was separated from the main solution by a salt bridge filled with an aqueous solution of 0.1 M TBACl.

**3. Results and Discussion**

*3.1. Spectroscopy and TEM results*

The UV-vis spectra of the as-synthesised C6-Au and AQ-C6-Au particles are shown in Fig. 1. The 520 nm characteristic plasmon band of Au nanoparticles is not observed for either preparation indicating a size smaller than 2 nm and that no aggregation or significant change in the core size takes place on functionalisation. Two additional absorption bands at 314 and 348 nm are observed for the AQ functionalised particles. These spectral features, albeit slightly shifted in frequency, have been previously observed for free AQ in different solvents and assigned to π→π\* transitions associated with the AQ benzene rings [18-20].

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**Fig. 1.** Comparison of UV-Vis spectra in toluene of the as-synthesised C6-Au particles (1) and after functionalisation with AQ employing the two-phase charging method (2). The concentrations were 0.28 μM and for comparison purposes, the absorbance values of the two datasets were normalised at 800 nm. The C6-Au material corresponds to entry 2 in Table 1 (See later). For clarity, the results are offset in the figure.

Free AQ also shows a weak absorbance at 405-410 nm, with a molar absorption coefficient of 60-90 M-1 cm-1 that has been assigned to the quinoid chromophore [18-21]. This band, however, would be too weak to be observed due to the strong absorbance of the Au cores. Fig. SI-1 shows the measured UV-vis spectrum of the diazonium compound employed, for which no information could be found in the literature. AQ-N2+ has main absorption bands at 460 and 325 nm. No absorbance features are observed in the 460 nm region (Fig. 1) for AQ-C6-Au indicating the absence of a diazonium compound impurity in the nanoparticle preparation after purification. These results indicate the incorporation of AQ as a ligand in the functionalised C6-Au particles.

The average core diameter determined from TEM measurements was 1.66±0.2 nm (Figure 2). Elemental analysis (Table SI-1, Supplementary Information) and the UV-vis spectrum (Fig. 1) are consistent with the values expected for Au140 core clusters [22].



Z

**Fig. 2**. TEM image for C6-Au nanoparticles employed.

The infrared spectra of the hexanethiol capped gold C6-Au showed (data not shown) vibrations at 2871 and 2956 cm-1 (CH3 str), 2852 and 2920 cm-1 (CH2 str) and 1461 cm-1 (CH2 scissors) as already well-known for alkane thiols attached to gold surfaces.[23,24].

*3.2. - Dependence of C6-Au particles size on preparation conditions*

The influence of synthesis conditions on average particle size was investigated by varying the reaction times of the thiol with Au(III) and that of the NaBH4 reduction step. The different preparations were characterised by Differential Pulse Voltammetry (DPV) using the individual nanoparticle Quantised Double-Layer charging (QDL) events to determine sample homogeneity, which provides a sensitive measure of monodispersity [25]. A typical example of the electrochemical responses observed is shown in Figure 3 where the individual one-electron Coulomb staircase charging steps can be clearly observed.



**Fig. 3.** DPV for as-synthesised C6-Au MPC (sample 2 in Table 1). Step amplitude: (1) 5; (2) 10; (3) 20 and (4) 50 mV.

The linear dependence of the peak potential on the quantised charge state (Fig. 4) demonstrates that these correspond to particle charging phenomena and that the particles capacitance is fairly independent of the charge state.



**Figure 4.** Dependence of *E*P on *z* for C6-Au nanoparticles in 0.1 M TBAPF6/Tol:ACN (2:1) (R2 = 0.96). Potential steps: 5 mV; () 20 mV

These results also indicate a reasonable monodispersity for the as-synthesised material. A full set of results for all the conditions investigated is given in Table SI-2 in the Supplementary Information.

Consecutive one-electron charging events for nanoparticle solutions have been analysed as traditional redox reactions for which the different redox states reflect the electrostatics of small metal spheres surrounded by a concentric dielectric consisting of the capping ligand and immersed in an electrolyte solution, usually in an organic solvent. Since the QDLs involve one-electron transfers per nanoparticle, the capacitance of the monolayer protected clusters determines the electrostatic energy difference between successive electron transfers. The electron transfer potential for a given charge state is given by [26]:

(3)

where is the redox potential corresponding to a z/(z-1) electron transfer event referred to *E*PZC, the potential of zero charge for the nanoparticle surface. *z* is the charge state, *e* is the electron charge and *C*MPC is the particle capacitance. The latter can be conveniently obtained from:

(4)

Equation (4) was used to obtain the particle capacitance considering cathodic and anodic scans and for pulse potentials of 5 and 20 mV (Fig. 4). Results at other pulse potentials were very similar.

Girault modelled *C*MPC as a concentric capacitor for the functionalised nanoparticles but importantly, also taking into account the dielectric medium where the particle is present [27,28]. This model leads to a linear dependence on z of the potential for the charge events given by:

(5)

where ԑ0 is the permittivity of free space, ԑd and ԑs are the relative permittivities of the alkanethiol protecting layer of thickness *d* and of the solvent, respectively, and *r*0 is the radius of the metallic core. A value of 4.373 was used for ԑd.[28,29]. Results from calculations using eqn (5) are strongly dependent on the value of the relative permittivity of the Tol:AN mixture employed, which has been reported in a range between 10.6 [30] and 25.2 [31]. In order to avoid any arbitrary choice, ԑs, was calculated from the relative permittivity of the pure toluene and acetonitrile solvents at 21 oC by fitting the data by Ritzoulis at al [31]. Values of 2.39 and 36.58 were obtained. The dielectric permittivity of the solvent mixture was hen calculated from the Bruggeman effective medium approximation, which has been successfully applied to model the dielectric properties of mixtures. This is given by [32]:

(6)

ԑ is the dielectric permittivity of the mixture, ԑTol and ԑAN are the corresponding values for toluene and acetonitrile and φV,AN is the *volume fraction* of acetonitrile in the mixture. A value of ԑ = 10.37 was obtained from equation (6), close to 10.6 calculated from the data in Reference 30.

As an example of the analysis of the DPV results, *C*MPC was obtained from the results in Fig. 4 and the effective thickness of the thiol layer was calculated from equation (5) using the measured average Au core radius of 0.83 nm. A thickness *d* = 0.83 nm was obtained for these results giving a total hydrodynamic radius of 1.66 nm. A collection of DPV results for the different conditions investigated is shown in the Supplementary Information (Fig. SI-2) and the measured nanoparticle capacitance and effective thiol thickness calculated from equation (5) are collected in Table 1.

The analysis of the thickness of the thiol layer obtained from this model presents some difficulties since the organisation of the hydrocarbon chains is not a regular arrangement as is the case for thiols on a flat gold single crystal surface. The extended length of the thiol residue attached to the gold core estimated from a molecular mechanics model [33] is approximately 1.2 nm, considerably larger than the thickness of the thiol layer calculated above. As discussed by Wuelfing *et al* [26], the most likely reason for this apparent discrepancy is the hydrodynamic conditions that determine mass transfer of functionalised nanoparticles. Since mass transfer rates for nanoparticles in solution such as those described in the present work are dependent on an effective average hydrodynamic radius, the slip boundary conditions prevailing at the chain-solution boundary must be taken into account. Due to the high radius of curvature of the core to which the hydrocarbon residues are attached, there is no sharp thiol layer-solvent boundary determining the viscous drag experienced by a moving particle and in consequence, solvent penetration within the thiol chains results in an apparent smaller overall diameter of the nanoparticle than would be experienced if such a sharp boundary existed [26]. The trend in the thiol hydrodynamic thickness calculated (Table 1) supports the above consideration indicating a more compact capping layer for longer reaction times, in particular when comparing the results for samples 1 to 3 with 4**,** 5 and 6, although unexpectedly, the value of *d* obtained for sample 6 was lower than that of sample 5.

**Table 1 Data from different preparation conditions**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample No** | **Thiol reaction time/min** | **NaBH4 reduction time/min** | **Capacitance/**  **aF** | **Thiol hydrodynamic thickness, *d*/nm** |
| **1** | 30 | 15 | 0.591 ± 0.013 | 0.69 ± 0.08 |
| **2** | 45 | 15 | 0.568 ± 0.014 | 0.83 ± 0.10 |
| **3** | 60 | 15 | 0.560 ± 0.014 | 0.92 ± 0.12 |
| **4** | 30 | 30 | 0.581 ± 0.013 | 0.74 ± 0.08 |
| **5** | 180 | 25 | 0.543 ± 0.015 | 1.04 ± 0.12 |
| **6** | 360 | 30 | 0.562 ± 0.014 | 0.88 ± 0.11 |

Although care was taken to ensure that the way these experiments were carried out was the same for all the samples, the details of the potential dependence of the DPV response have poor reproducibility indicating that uncontrollable experimental conditions such as rate of reactant addition and stirring rate, influence the properties of the nanoparticles prepared. However, these results show a clear decrease in nanoparticle capacitance for an increase in the thiol reaction time indicating the formation of larger nanoparticles (Samples 1, 2, 3). A similar general trend is observed for samples 4, 5 and 6. This trend must be related to the type of precursors resulting from the reaction between Au(III) and C6-SH. For RSH:AuCl4 molar ratios larger than 2:1, the formation of a Au(I) thiolate polymer, [Au-S(R)-]n, has been proposed as the precursor [22,34], the reduction of which leads to the formation of the capped nanoparticles. The above results might indicate not only the formation of larger polymeric precursor but also increasing chains ordering for longer reaction times with thiol. More recent work has demonstrated the complexity of the reduction chemistry of the Au-thiol system [35-37] and that these reactions occur in a homogenous organic phase and not in inverse micelles [37-39].

Lennox [35] observed the initial formation of a TOA+Au(I)X2- ion pair (X anion= Cl-, Br-) rather than that of a Au(I)-thiolate polymer after the addition of 2 equivalents of thiol to a Au(III)-TOA toluene solution. More recent work [36,37] has confirmed Lennox ion-pair mechanism and concluded that this species reacts with additional thiol to produce polymeric species since no NMR signal for the additional added thiol is observed, for example:

2 TOA+Au(I)X2- +2 RSH → 2 TOA+X- + (Au(I)-SR)2 + 2 HX (7)

It is concluded that the change with reaction time in nanoparticle size observed for the results shown in Table 1 is mainly due to the slow kinetics of reaction (7) since this involves large bond rearrangement. These results are in agreement with current understanding of the first steps in the two-phase mechanism of nanoparticle synthesis [37].

*3.3. FFT analysis*

Linear plots such as those illustrated in Fig. 4 cannot disentangle minor contributions to the DPV results from different particle sizes present in the preparation. The possibility of using Fast Fourier Transform (FFT) for the analysis of these results was explored [40]. The interest of this technique is to transform the potential dependence of the current responses of quantised charge events to a problem in the time domain, thus allowing the application of well-established signal processing techniques to extract different frequency contributions to the electrochemical response for estimating the main components of the nanoparticle size distribution. In this case, the applied potential is taken as the time variable and the frequencies calculated correspond to the inverse of the potential difference between quantised double layer charging events for particular nanoparticle sizes.

Briefly, the method employed recognises that the frequencies of interest are determined by the potential differences between charging events, which are of the order of less than 0.5 V, in the present example, between 0.27 and 0.29 V (see, e.g., Fig. 3. The equivalent frequency domain is of the order of 2 Hz, i.e., specific features with a separation between charging events much greater than 0.5 V will make a negligible contribution to the Fourier transform of the *potential dependence of the DPV response*. For this reason, frequency components of the electrochemical signal of less than 2.0 Hz do not relate to the quantised charging events. In addition, in order to avoid time domain truncation errors, a Hanning weighting function was employed [40]. Other methods to avoid truncation errors can be employed for the DPV responses discussed here, such as filtering the signal with cut off frequencies at approximately 2Hz. For the purpose of testing the suitability of the FFT method, no arbitrary additional data filtering was applied.

An example of the frequency components for DPV data obtained for sample 4 in Table 1 is shown in Fig. 5, where the main individual QDL contributions have been fitted to Gaussian functions. Transforming the FFT response back to the potential domain, two main components of charging events can be distinguished due to particles having Coulomb staircases with steps of 0.29 and 0.20 V. The characteristic frequencies are 3.4 and 5.0 Hz, respectively (Fig. 5), with an approximate population of 90 % for the smaller particles calculated from the areas under the distribution functions. The average potential separation between the main charging events estimated from the data in Fig. 4 was 0.28 V, in reasonable agreement with the value obtained from the FFT analysis. These results indicate that this sample contained a small concentration of particles with a capacitance of 0.84 aF. From equation (5) this fraction would have a core radius of ~1.1 nm, corresponding to the 225/314 Au atoms particles estimated by Murray *et al* [22] and to the 36 kD fraction measured by Whetten *et al.* by mass spectrometry [41]. This contribution cannot be easily detected from the analysis shown in Fig. 4 or from TEM images. The approach described above appears to provide a rapid semi-quantitative assessment of the main size distribution *of the whole* nanoparticle sample.



**Fig. 5.** FFT response of the cathodic scan of the DPV results for C6-Au, Sample 4, Table 1. Pulse amplitude = 5 mV. The two main components of the DPV response are indicated in the figure.

*3.4. Anthraquinone functionalised particles*

*3.4.1 Electrochemical charging*

Charged particles were prepared from sample 3, Table 1, as described in section ~~2.3~~ **2.2.1**. Fig. 6a shows the cyclic voltammograms (CVs) for these particles functionalised with 1-anthraquinone. The first electron transfer step for AQ reduction is observed between -0.81 to -0.88 V. This is an irreversible process as indicated by the absence of an anodic peak in the return sweep and by a significant dependence of peak potential on sweep rate. The peak current shows the expected linear dependence on ν1/2 (Fig. 6b) from which the number of AQ moieties attached per nanoparticle can be estimated. The peak current (*I*p) for an irreversible reaction at 21 oC is given by [42]:

(8)

where *A* = electrode area (0.0314 cm2, = concentration of AQ in the bulk i.e., of AQ molecules bound to the nanoparticles (mole cm-3), α = transfer coefficient taken as 0.5, *D* = diffusion coefficient of the species responsible for transport of AQ-C6 Au to the electrode surface (cm2 s-1) and ν = sweep rate (V s-1).



Fig. 6a. Cyclic voltammetry AQ-C6-Au MPC (3 mg/mL or 88.8 µM) prepared by reaction of AQ-N2+ with electrochemically reduced C6-Au nanoparticles. Sweep rates: (1) 0.02, (2) 0.05. (3) 0.10 and (4) 0.20 V s-1. Sample No 3, table SI-2, was employed.



**Fig. 6b.** Dependence of the peak current on the sweep rate for the first electron transfer step in the reduction of AQ attached to C6-Au nanoparticles.

The diffusion coefficient was estimated from the Stokes-Einstein equation [43]:

(9)

η = solvent viscosity and *a* = hydrodynamic radius of the diffusing species (nanoparticle carrying the attached AQ groups). The viscosity for Tol:AN 2:1 (η = 4.734x10‑4 Pa s) was calculated from Ref. [44] as shown in the Supplementary Information. The effective hydrodynamic radius of the diffusing species were taken form Table 1 and for the data in Fig. 6aa value of *D* = 2.6x10-6 cm2  s-1 was obtained, in reasonable agreement with other estimates from the literature [28]. From equation (8), the concentration of AQ attached to the functionalised nanoparticles was 63 μM. Since the concentration of C6-Au particles in solution was 88.8 μM, this corresponds on average approximately to 3.4 AQ groups attached per four nanoparticles.

*3.4.2. Two-phase redox nanoparticle charging*

The AQ grafting reaction requires two electrons and therefore, the potential of zero charge of the C6-Au particles is probably slightly more positive than the DPV peak at -0.9 V. Electron injection relying on nanoimpacts has recently attracted considerable attention [45,46]. In the case of monolayer protected clusters, electron transfer rates during collision events can be hindered by the presence of an insulating layer, which could be reflected in a decrease of the actual charge accumulated in the nanoparticle dispersion. For this reason, particle charging was investigated by a two-phase reaction with aqueous BH4**-** as described in Section 2.2.2, employing sample 5 in Table 1.

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**Fig. 7a.** Electrochemistry of AQ-C6-Au prepared by reduction of AQ-on C6-Au particles reduced with sodium borohydride in a two-phase reaction. Sweep rates: (1) 0.02, (2) 0.05. (3) 0.10 and (4) 0.20 V s-1. Sample No 5, table SI-2, was employed. The resolution of the QDL features, although present, cannot be clearly seen in the scale of this figure

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**Fig. 7b.** Dependence of peak current on sweep rate for the first AQ reduction reaction from the results shown in Fig 7a.

Figs. 7a and 7b show the cyclic voltammetry of AQ-C6-Au particles prepared by this method and the dependence of the peak current on sweep rate, respectively. The AQ reduction currents for this material are significantly larger than those for que results shown in Fig. 6a, indicating a larger coverage by AQ for nanoparticles functionalised by the two-phase method. In this case, the second reduction wave for AQ, at approximately -1.5 V for the lower sweep rates, can be clearly seen. The corresponding currents for the data in Fig.6a cannot be distinguished from those of the QDL charging events. Using the same arguments as above, the concentration of AQ calculated was 313 μM and therefore, this preparation led to an average of ~3.5 AQ molecules attached per nanoparticle.



Fig. 8 DPV measurements for a 3 mg/mL solution of AQ-C6 MPCs. Modulation amplitudes: (1) 5, (2) 10, (3) 20 and (4) 50 mV. The 2nd AQ reduction process is chemically irreversible (Fig 7a) and therefore a DPV peak is not observed.

Fig. 8 shows the DPV response for AQ-C6-Au prepared by the two-phase charging method. The AQ redox reaction is clearly visible, with a peak potential at -0.8 V, close to the half-wave potential of the results shown in Fig. 7a. The second reduction peak (Fig. 7a) is a chemically irreversible process [23] that will not result in a sharp DPV response, as observed in Fig. 8 [47].

Although it is difficult to obtain quantitative information on the number of molecules of AQ per nanoparticle by subtracting the charge under the AQ peak from the charge involved in the quantised charging events within this potential range, a qualitative estimate employing the charge for the QDL steps as internal calibration indicated that this number should be greater than 6, nearly twice that obtained from the cyclic voltammetry results (Fig. 7b). This assumes that the 1-electron individual quantised charging events and the current for the faradaic reaction are independent of each other, thus allowing the use of the QDL charges as a calibration of the area under a DPV peak. The very large difference between the estimates from these techniques is probably a consequence of the intrinsic difference between the DPV pulse and cyclic voltammetry methods, where for an irreversible reaction, the time scales of the electrolysis sequence are very different.

The results presented here are an example of the original observation by Murray *et al*. that charged monolayer protected clusters can be viewed as soluble diffusing nanoelectrodes [23]. In the present work, this provides a route for taking advantage of the large stability of the gold-carbon bond produced by reductive diazonium chemistry [48] for a very wide variety of synthetic applications since arylamine precursors are readily available.

**4. Conclusions**

All the synthesis reaction conditions investigated yielded nanoparticles displaying well defined quantised double layer charging effects. An increase in the capping thiol layer thickness was observed for longer reaction times of the thiol with Au(III) before reduction. The absence of the characteristic surface plasmon band confirms that the Au clusters prepared are smaller than 2 nm in diameter.It is also shown that FFT analysis can provide a simple method to assess size distribution of the synthesised material. Cyclic voltammetry and DPV measurements demonstrated the attachment of anthraquinone groups by reaction of the electrochemically or chemically charged nanoparticles with the diazonium derivative of 1-amino anthraquinone The importance of this work is to have demonstrated the possibility of adjusting the number of groups attached by controlling the number of electrons injected into the nanoparticles prior to reaction with a diazonium compound and demonstrating that diazonium chemistry can be used for the direct attachment of organic groups directly to the metal core by the formation of Au-C bonds by charging the particles before reaction.

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