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Tuning stable noble metal nanoparticles dispersions to moderate their interaction with model membranes

Nicola William[a], Faith Bamidoro[b], Paul A. Beales[a,c,d], Rik Drummond-Brydson[b,c], Nicole Hondow[b,c], Sarah Key[a], Alexander Kulak[a], Aidan Charles Walsh[a], Sophia Winter[a], Laurence Andrew Nelson[a]*

[a] *School of Chemistry, University of Leeds, Leeds LS2 9JT, UK*
[b] *School of Chemical and Process Engineering, University of Leeds, Leeds LS2 9JT, UK*
[c] *Bragg Centre for Materials Research, University of Leeds, Leeds LS2 9JT, UK*
[d] *Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds LS2 9JT, UK*

**Corresponding author: email: a.l.nelson@leeds.ac.uk*

ABSTRACT

Hypothesis: The properties of stable gold (Au) nanoparticle dispersions can be tuned to alter their activity towards biomembrane models.
Experiments: Au nanoparticle coating techniques together with rapid electrochemical screens of a phospholipid layer on fabricated mercury (Hg) on platinum (Pt) electrode have been used to moderate the phospholipid layer activity of Au nanoparticle dispersions. Screening results for Au nanoparticle dispersions were intercalibrated with phospholipid large unilamellar vesicle (LUV) interactions using a carboxyfluorescein (CF) leakage assay. All nanoparticle dispersions were characterised for size, by dynamic light scattering (DLS) and transmission electron microscopy (TEM).
Findings: Commercial and high quality home synthesised Au nanoparticle dispersions are phospholipid monolayer active whereas Ag nanoparticle dispersions are not. If Au nanoparticles are coated with a thin layer of Ag then the particle/lipid interaction is suppressed. The electrochemical assays of the lipid layer activity of Au nanoparticle dispersions align with LUV leakage assays of the same. Au nanoparticles of decreasing size and increasing dispersion concentration showed a stronger phospholipid monolayer/bilayer interaction. Treating Au nanoparticles with cell culture medium and incubation of Au nanoparticle dispersions in phosphate buffered saline (PBS) solutions removes their phospholipid layer interaction.

Keywords: Au nanoparticle dispersions; Ag nanoparticle dispersions; Ag coated Au nanoparticles; Nanoparticle screening; Electrochemical membrane sensor; Vesicle leakage assay.

1. Introduction

Currently Au and Ag nanoparticles are being increasingly used in health-care applications [1] and sanitation [2]. Au nanoparticles are employed in nanomedicine for photothermal therapy [3] drug delivery [4] and tissue imaging [5]. Ag nanoparticles, which have antimicrobial properties, have been utilised for disinfection of water [6] and conveying antimicrobial properties to materials [7]. Accordingly there is an understandable concern about their biological activity to living organisms and potential release into environmental systems. Au nanoparticles have a low water solubility as well as showing a strong tendency to agglomerate [8] and their biological activity remains uncertain with conflicting reports as to their hazard to environmental and human health [9]. In contrast Ag nanoparticle dispersions are soluble in water [10] releasing Ag^+ ions in the presence of oxygen [10]. Dispersions of Ag microsized particles are well known to be toxic, indeed Ag^+ has been documented for many years to be toxic to living organisms. However it is not certain whether the particulate species is the more biologically active form [11]. Due to these concerns about the characteristics and possible toxicity of Au and the nature of the biological activities of Ag nanoparticle dispersions, a considerable amount of work has been carried out investigating the problem. The empirical toxicities

of Au and Ag nanoparticle dispersions have been fairly well characterised using both *in-vitro* [12,13] and *in-vivo* [13] screens over the past decade. In spite of this, considerable uncertainty still relates to their possible mechanisms of interaction with biological tissues and organisms. Physicochemical model systems are particularly valuable in underpinning the detailed mechanisms of interaction that arise in highly complex living matter [14], with a considerable amount of work looking at the interaction of Au and Ag nanoparticles with biological membranes (biomembranes) [15,16] and biomembrane models [17-31]. The reason for the latter approach is that the biomembrane is the first point of entry of a nanoparticle in media to an organism and in addition it is the most vulnerable entity open to attack from a xenobiotic species. Biomembrane activity is defined as the tendency of any species, chemical or nanoparticle to structurally modify and/or permeate in biomembranes and/or biomembrane-like layers.

In general the interactions of Au nanoparticle dispersions on model biomembranes has been quite extensive over the last decade [18-30]. Many different Au particle dispersion types were used with variously reported results [18-29]. Au particles interacted to various degrees with model membranes. Specific investigations have shown that thiol [22], organic [23,24] and organo-thiol [25] and multiple functionality [29] capped Au particles interact with model membranes of lipid bilayers [23-29] and monolayers [22]. Although most studies worked with capped home-synthesised Au particles, two studies showed interactions between commercial citrate coated Au nanoparticles and model membranes [27,28]. A series of simulations have predicted an interaction between thiol coated [30] and citrate coated [31] particles and model lipid membranes. The experimental studies have used several different end-points to diagnose an interaction including lipid flip-flop [27], liposome leakage [28], ion transfer across membrane [24], cell-membrane integrity [25], lipid packing and pore formation [20], membrane fluidity [21] and electrochemical parameters of charge transfer and capacitance [22]. In most Au particle/lipid membrane studies to date, the Au nanoparticles are stabilised not only with citrate but also with additional capping agents. These capping agents are sometimes only weakly bonded to the particle and readily displaced by membrane binding [26]. Capping agents are utilised to enhance colloidal stability and performance reproducibility [32] which is necessary when the Au nanomaterials are used in an extensive series of testing experiments. It is significant that in most of the experimental studies, surprisingly little information has been gained on the effect of the capping agent or surface coating on the interaction except in one case where exchange of the capping ligand affects the interaction to different extents depending on the nature of the ligand [26] and pinpoints the influence of the coating on the interaction [29]. In contrast to the large number of Au nanoparticle/model membrane investigations, fewer studies have investigated Ag nanoparticle dispersion interactions with model membranes. One particular study focused on the interaction of citrate coated Ag nanoparticles [33] with tethered lipid bilayers and another on the effect on fluidity of bilayer liposomes from sterylamine coated Ag particles [34]. A study carried out in this University showed an interaction between weakly agglomerating Ag nanoparticles and the lipid membrane of GUVs [17].

Previous studies in this laboratory have looked at SiO_2 [35], ZnO [36, 37], CdTe [38] and TiO_2 [39] nanoparticles using a high throughput electrochemical phospholipid monolayer activity screen and correlating the particles' chemical and physical characteristics with their activity towards the phospholipid monolayers. Although these previously examined nanoparticles differ considerably from each other in terms of their structural properties and functionality, it has been shown that for all these nanoparticle classes their monolayer activity is dependent on their particle size. Apart from coated CdTe, all nanoparticles studied so far have been metal oxides which makes this present study interesting since Au and Ag were studied in their apparent native elemental state. In spite of this, the commercial Au nanoparticle dispersions used contained a "proprietary" stabiliser in addition to the citrate ion and it has been reported that Ag nanoparticles have a layer of amorphous Ag_2O on their surface [40,41]. The rapid screen employed enabled a large number of monolayer activity assays to be carried out and the results were related to the dispersions' physical and chemical properties.

Monolayer activity refers to the ability of dispersions to modify and/or disrupt the monolayer or sensor element structure and is taken to be some guide to their biomembrane activity.

This study advances the theme of relating the physicochemical properties of nanoparticles to their activity towards phospholipid model membranes working with representative Au and Ag nanoparticles and the above described [35-39] original experimental approach. The objectives of this work are twofold: (a) to validate the electrochemical membrane model as a rapid nanoparticle/lipid membrane interaction sensor platform and (b) to study the moderation of Au particle phospholipid membrane activity by combining protocols of particle surface treatment with rapid screening. In view of the increasing use of Au nanoparticle dispersions in health care and diagnostics, the main application of the results in this study are within the Safety by Design paradigm. In this, nanoparticle production technology can be developed to tune particle dispersions to have a controlled interaction with phospholipid layers and by inference cell membranes without compromising their stability and application. This study used mainly commercial high quality Au and Ag dispersions. These dispersions were characterised by their stability and reproducibility which was essential for the large number of manipulations involved. Home-synthesised citrate stabilised Au dispersions depend on the citrate concentration in the dispersion for their properties [42] and these properties would be affected by the extensive dilutions and manipulations required for this study. In such a dispersion an alteration of the solution concentration of citrate will alter the citrate cap on the particle and thus the particle morphology. Because of this, although the phospholipid monolayer activity of these dispersions was related to that of the less stable home-synthesised dispersions, the commercial dispersions were employed for the bulk of the experiments in this work. All dispersions were extensively characterised for their physical and chemical properties.

2. Experimental

2.1. Materials

1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) was purchased from Avanti Polar Lipids and had a purity of >99%. Gold (III) chloride trihydrate ($HAuCl_4$), used for Au nanoparticle synthesis, silver nitrate ($AgNO_3$), used for Ag thin film synthesis, and $Na_3C_6H_5O_7$ were all purchased from Sigma Aldrich and had a purity of >99.9%. The electrolyte used in the electrochemical and nanoparticle incubation experiments was 0.138 mol dm^{-3} NaCl and 0.0027 mol dm^{-3} KCl buffered at pH 7.4 with 0.0119 mol dm^{-3} phosphate (hereinafter in the text referred to as PBS). The PBS was of analytical grade and purchased from Sigma-Aldrich. The microfabricated platinum electrodes (MPE) [43] were supplied by the Tyndall National Institute, Ireland. Hg was electrodeposited from $AgNO_3$ solution on a Pt disc of radius 0.480 mm to give a Pt/Hg electrode as described previously [44].

Au nanoparticles dispersions of nominal primary particle size of 5, 20 and 50 nm were purchased from Alfa Aeser. Au concentrations were measured by inductively coupled plasma mass spectrometry (IPC-MS) to be between 0.2 and 0.3 mmol dm^{-3} in 0.39 mmol dm^{-3} sodium citrate ($Na_3C_6H_5O_7$). Ag nanoparticle dispersions of nominal particle size 10 and 20 nm were also purchased from Alfa Aesar and ICP-MS analysis indicated a Ag concentration of 0.19 mmol dm^{-3} in 2 mmol dm^{-3} $Na_3C_6H_5O_7$. The Au commercial dispersions were essentially monodisperse with a polydispersity index (PDI) of below 0.2 but the Ag commercial dispersion had a PDI of 0.42 as measured by dynamic light scattering (DLS). Dispersions were diluted with 18.2 MΩ MilliQ water to give a concentration of Au of 0.033 mmol dm^{-3} prior to screening. *In-house* synthesised Au nanoparticle dispersions were also used for some of the experiments. Au nanoparticle dispersions prepared by the general Turkevich [45] procedure possessed a PDI > 0.2, greater than that of the commercial Au dispersions which meant that their screening results could not be cross correlated. Because of this, the following modified procedure for Au nanoparticle synthesis was used. A stock solution of 10 mmol dm^{-3} $HAuCl_4$ solution was prepared in MilliQ water. 1 cm^3 of the $HAuCl_4$ stock was diluted to 20 cm^3 in water. 0.5 cm^3 of 0.1 mmol dm^{-3} $Na_3C_6H_5O_7$ stock was diluted to 5 cm^3 in

water and then added to the Au solution. The initial solution pH was 3.76, and then 0.5 mmol dm^{-3} NaOH solution was added dropwise until the pH reached 5. The reaction was heated to 25 °C whilst stirring. At this stage, one further drop of 0.5 mmol dm^{-3} NaOH was added which resulted in the dispersion changing to a dark blue/purple colour. The dispersion was stored in a fridge at approximately 3 °C until use and had a particle size of ~20 nm and PDI <0.2. The addition of NaOH made available OH^- ions which react with $AuCl_4^-$ ions to produce the less reactive $AuCl_3(OH)^-$ species. This $AuCl_3(OH)^-$ species slows down the reaction, restricting the nucleation and growth process, resulting in a more monodisperse product. Note also that, unlike the Turkevich procedure, this reaction is kept at 25° (Method 1). Dispersions produced by this procedure were also heated to 100 ° to produce a more polydisperse sample (Method 2) with a particle size of 64 nm and an average PDI of 0.51. Au dispersions synthesised by Methods 1 and 2 were diluted with MilliQ water by 2-3 times prior to characterisation and electrochemical screening.

To treat commercial Au dispersions with PBS, specific volumes of the Au dispersions were diluted with appropriate volumes of PBS to give a final Au concentration of 0.033 mmol dm^{-3}. Coating commercial Au nanoparticles with protein and Ag respectively was carried out as follows. For the protein coating, the Au nanoparticle dispersion and a cell culture medium (CCM) (1:10 respectively by volume) were mixed and placed in an incubator at 37 °C for 24 hours. The CCM used was Dulbecco's modified eagle medium (DMEM) [46] with 25, 44, 1 and 0.04 mmol dm^{-3} glucose, sodium bicarbonate, sodium pyruvate, and phenol red respectively, as well as 10% by weight of foetal bovine serum (FBS). Following incubation, samples were centrifuged at between 2000 and 35000 g for 10–20 minutes (the larger the nanoparticle size, the lower the speed). This method has been well proven to be effective for coating Au nanoparticles with protein [47,48]. Following removal of the supernatant the centrifuged particles were resuspended in MilliQ water. For the Ag coating, 50 nm Au nanoparticles were used. Ag coated Au nanoparticles (Ag/Au) were prepared using a method adapted from Shankar *et al* [41]. 2 cm^3 of the Au nanoparticle dispersion (0.2 mmol dm^{-3}) were added to 9 cm^3 Milli-Q water and brought to the boil. $AgNO_3$ (0.2 cm^3 of between 2 to 50 mmol dm^{-3} as appropriate) and 1 cm^3 of 1.35 mmol dm^{-3} $Na_3C_6H_5O_7$ were added simultaneously dropwise to the boiling solution. More than 10 times excess of Ag over the Au concentration in the dispersion was necessary to coat the Au nanoparticles with Ag [41]. The reaction was stopped after 24 minutes reaction time yielding a light orange solution. A longer reaction time gave rise to the production of individual Ag nanoparticles in the dispersion.

2.2. Nanoparticle characterisation: Dynamic light scattering (DLS) was employed to determine the hydrodynamic diameter, zeta potential and PDI of the nanoparticle dispersions. The results are displayed in Table 1. When measuring particle size, samples were placed in disposable semi-micro 1.5 cm^3 cuvettes and results are displayed as intensity versus hydrodynamic diameter. Measurements were performed using a Malvern Zetasizer Nano ZSP. The equilibration time for samples was 120 seconds and 12 measurements were performed without delay. Measurements were carried out in triplicate. Scanning electron microscopy (SEM) was initially used to observe particle morphologies and sizes. Sample preparation was carried out by dropping the wet sample on to a SEM grid on a silicon chip attached to a sample holder by double-sided copper tape. The sample was then dried on the grid in an oven, prior to measurement on the field emission scanning electron microscope (FE-SEM), JEOL 7100F. Accurate measurements of particle size were undertaken using transmission electron microscopy (TEM) and are also displayed in Table 1. For each sample, the mean particle diameter and standard deviation from 150 particles were analysed from TEM bright field images. TEM samples were prepared by placing a drop of the nanoparticle dispersion onto a continuous carbon-coated copper grid and allowing them to dry. The measurements were conducted on a FEI Titan^3 Themis G2 operated at 300 kV and fitted with 4 energy dispersive X-ray (EDX) silicon drift detectors, multiple scanning transmission electron microscope (STEM) detectors, and a Gatan One-View CMOS charge-coupled device (CCD). EDX spectroscopy and mapping was undertaken using Bruker Esprit v1.9 software. High angle annular dark field (HAADF) STEM was utilised alongside

EDX measurements to identify possible bimetallic nanoparticles and to confirm the presence of a coating. HAADF STEM images exhibit atomic number contrast, with heavier atoms appearing brighter than lighter atoms, which allow Au and Ag to be distinguished. Electron microscopic imaging of the protein modified Au nanoparticles can be equivocal due to the low atomic number and density of the proteins (as compared to a Au nanoparticle) and the electron beam sensitivity of these species which can lead to considerable artifacts [49]. Because of this and in view of the fact that an established method was used to coat the particles with protein, a detailed imaging investigation of the adsorbed protein was considered outside the scope of this paper.

2.3. Inductively coupled mass spectrometry (ICP-MS)

The concentration in mmol dm^{-3} of Au and Ag in the nanoparticle dispersions was determined via ICP-MS using a Perkin Elmer SCIEX ELAN DRC-e. For the analysis, 3 replicates were prepared for each of the Au and Ag nanoparticle dispersions. Each replicate was analysed 10 separate times by the ICP-MS equipment and the concentration value reported by the machine is an average of those 10 individual measurements with a % relative standard deviation (RSD). Furthermore, the instrument performed 10 separate data collection events per replicate, reporting an average of these 10 measurements in μg cm^{-3} which was accordingly converted to mmol dm^{-3}. This was used throughout this study to characterise the concentration of Au and Ag respectively in the nanoparticle dispersions. ICP-MS results for the nanoparticle dispersions are included in Table 1. In the sample preparation and analysis, 1 cm^3 of the stock Au and Ag nanoparticle dispersion respectively was dissolved in 9 cm^3 of a 2 % v/v nitric acid to obtain a 10 times dilution sample, while 100 μL was dissolved in 9.9 cm^3 of 2 % v/v nitric acid to prepare 100 times dilution samples. The 10 times and 100 times dilutions were repeated 3 times. The samples were then sonicated for 30 minutes to ensure complete dissolution of particulate matter prior to analysis of the solutions by ICP-MS.

2.4. Preparation of 5(6)-carboxyfluorescein (CF) encapsulated large unilamellar vesicles (LUV) and leakage assay Lipid thin films were deposited by evaporating 100 μL of 34.1 mmol dm^{-3} DOPC solutions in chloroform in glass vials and then dried overnight under vacuum. The lipid film was rehydrated with CF buffer (120 mmol dm^{-3} CF, 130 mmol dm^{-3} NaCl and 20 mmol dm^{-3} HEPES at pH 7.4) and vortexed to form a lipid suspension. The sample was frozen in liquid nitrogen before thawing in a water bath. Vortex-freeze-thaw cycling was carried out 5 times in total. The dispersion was extruded by passing through a polycarbonate membrane with 400 nm pores 11 times using an Avanti mini-extruder (Avanti Polar Lipids). Unencapsulated CF was removed via separation using a Sephadex G50 size exclusion column equilibrated with HEPES buffer (130 mmol dm^{-3} NaCl and 20 mmol dm^{-3} HEPES, pH 7.4). The LUVs were stored in a plastic vial. Assays were performed on a Spex Fluoromax 3 Spectrofluorometer. Samples were excited at 493 nm and monitored using 2 nm excitation and emission bandwidths with emission recorded from 502-600 nm. Au nanoparticle dispersions at concentrations ranging from 10^{-5} mmol dm^{-3} to 0.1 mmol dm^{-3} were incubated with the LUVs for one minute in fluorimeter cuvettes prior to screening for fluorescence (*I(Au)*). The incubation time was chosen to match as closely as possible the time period that the Au dispersion was in contact with the sensor element in the RCV experiments described in Section 2.5 below. As shown in later experiments, the short contact time of the buffer with the nanoparticle dispersion had an insignificant effect on the nanoparticle agglomeration. The incubation and fluorimetry was carried out in the following way. 1.5 cm^3 of the HEPES buffer with 30 μL of the LUV dispersion was added to the cuvettes, giving a final lipid concentration of ~0.05 μmol dm^{-3} measured accurately by phosphorus assay [50,51]. 1.5 cm^3 of prepared Au nanoparticle working dispersions in MilliQ water was then added to the cuvette and the stoppered cuvette was inverted. Concentrations of Au in the working dispersions were previously measured by IPC-MS and the additions were such that the Au concentration in the cuvette exactly matched that exposed to the sensor element in the RCV experiments. Maximum leakage signal (*I(max)*) was obtained by addition of 50 μL of 10% Triton X-100 causing complete lysis of vesicles. A control signal was obtained by measuring the fluorescence

of a diluted LUV sample (*I(0)*). Percentage leakage in the nanoparticle/LUV dispersions was calculated using Equation (1):

$$\%\ Leakage = \frac{I(Au)-I(0)}{I(max)-I(0)} \times 100 \quad (1)$$

A similar technique using calcein as the fluorophore has been used previously to characterise SiO_2 nanoparticle-phospholipid interactions [52].

2.5. Electrochemical measurements

For the electrochemical assay, the fabricated Pt/Hg electrode was contained in a flow cell. A constant flow of PBS was passed over the electrode with a flow rate of 5 cm^3 min^{-1} maintained by a peristaltic pump [53]. A valve and injection system allowed: (a) PBS solution; (b) DOPC in PBS dispersion; and (c) the appropriate nanoparticle dispersion either diluted or not with MilliQ water, to be introduced into the flow cell. The exposed Pt/Hg electrode in the flow cell was connected to the PGSTAT 30 Autolab potentiostat (Ecochemie, Utrecht, Netherlands) interfaced to PowerLab 4/25 signal generator (AD Instruments Ltd.) where the input voltage functions were applied through ScopeTM software (AD Instruments Ltd.) controlled by Scope software. Scope software was also used to record rapid cyclic voltammetry (RCV) scans at a scan rate of 40 Vs^{-1}. In the RCV experiments the fabricated rectangular Pt electrode on the wafer was employed as a counter electrode and an Ag/AgCl/3.5 mol dm^{-3} KCl reference electrode was inserted into the flow cell. All potentials in this paper are quoted versus this reference electrode. Starting twenty minutes prior to each experiment, a blanket of argon gas (Air Products) was maintained above the control and test electrolytes and DOPC dispersion to exclude O_2 which interferes with the assay.

Phospholipid deposition on the Pt/Hg electrode was carried out as described previously [43,44,53]. Phospholipid behaviour in response to potential changes has been studied extensively on Hg electrodes [43,44,53]. Monolayers of DOPC on Hg and Pt/Hg electrodes undergo two potential induced phase transitions characterised by two sharp capacitance current peaks, respectively. These peaks correspond to the ingress of electrolyte into the layer and the re-organisation of the layer to form bilayer patches [43,44,53,54]. Through observing the capacitance current peaks in the voltammograms, alterations in the peak configuration can be detected when nanoparticles and/or chemical compounds interact with the layers. A nanoparticle and/or compound's monolayer activity is manifested by characteristic changes in the capacitance current–voltage plots which represent modifications to the phospholipid layer conformation. The DOPC dispersion for electrode coating was prepared by gently shaking DOPC with PBS to give 0.25 mol cm^{-3} dispersion. Prior to DOPC deposition a valve was switched to allow control PBS to flow through the flow cell. Subsequently, phospholipid deposition on the Pt/Hg electrode was carried out as described in the following. A potential excursion was applied from −0.4 V to −3.00 V at a scan rate of 100 V s^{-1} at which point100–200 μL DOPC dispersion was introduced into the flow cell. After 1 to 2 s, the characteristic voltammetric peaks appear corresponding to the potential enabled phase transitions of the phospholipid [43,44,53]. At this point, the valve was switched back to allow the control PBS entry to flow cell and the potential excursion was altered to −0.4 to −1.2 V. Subsequently, by repetitive cycling, the characteristic RCV profile of DOPC on Hg with two voltammetric peaks is maintained which confirms the coverage and stability of the monolayer. Once the DOPC monolayer was formed and tested, 2 cm^3 of the nanoparticle dispersion was injected into the line entering the flow cell using a syringe. This initially showed up as the effect of water on the current response which causes a separation and depression of the capacitance current peaks due to a transient increase in the solution resistance. Once this had passed, the effect of the nanoparticle dispersion on the phospholipid sensor element was observed. Taking account of the flow rate, the flow dynamics and the volume of

dispersion injected, the maximum contact time of the Au nanoparticle dispersion with the DOPC sensor element was of the order of one minute. RCV plots were generated from the electrochemical experiments scanning from -0.4 to -1.4 V and all measurements were carried out in triplicate. Any change in the two voltammetric peaks heights and baseline current indicated an interaction. Subsequently the electrode was cleaned *in-situ* by repetitive cycling in control PBS from −0.4 to −3.0 V. Owing to the dynamic, induced mobility of the DOPC sensor element, the results from the RCV assay related to the way in which the nanoparticle/DOPC association influenced the DOPC assembly. Using this electrochemical method in flow is advantageous for nanoparticle dispersion characterisation. Most nanoparticle dispersions agglomerate over time, so screening immediately after their preparation is vital. Full details of this screening procedure have been described previously [53].

3. Results and discussion

The physical and chemical properties of the commercial Au and Ag dispersions are shown in Table 1. The nanoparticle zeta potential is accordingly negative reflecting the citrate anion coating and this increases with particle size on the Au dispersions. In principle the zeta potential should not change. On the other hand, the nanoparticle size can affect the isoelectric point of the particle surface and this would in turn alter the zeta potential at a given solution pH [55]. In contrast, the zeta potential of the Ag dispersions becomes more negative with decrease in particle size which might be associated with the higher citrate concentration and ionic strength in these dispersions and the greater tendency of smaller Ag particles to dissolve [10]. Further, the commercial Ag dispersions have a higher PDI (0.42 and 0.57) than the commercial Au dispersions (< 0.2). Figure 1 summarises the DLS results and TEM images for the commercial Au (three) and Ag (one) nanoparticle dispersions. The sizes measured by DLS were larger than those observed using TEM which reflects the fact that TEM measures the physical size of the core nanoparticle and does not include any "soft" capping agent, whereas DLS measures a hydrodynamic size of the whole nanoparticle assembly. In addition, DLS biases to larger particles in the sample due to the dependence of the scattering cross-section of particles on the square of the volume. Nanoparticle sizes derived by TEM generally reflect the sizes quoted by the supplier. At the time of testing, the samples were not agglomerated and were well dispersed as shown Figure 1. Figure 2 (a) displays representative voltammograms of the DOPC-coated Pt/Hg electrode in both the absence and presence of 20 nm Au nanoparticle dispersions at a concentration of 0.033 mol dm^{-3} of Au. In the absence of the Au dispersions, the voltammograms of the DOPC on Pt/Hg electrode exhibited a flat baseline and the two well-defined voltammetric current peaks [43,44,53]. An indication of the interaction of the Au nanoparticle dispersion is shown by the depression of the two capacitance current peaks labelled 1 and 2 respectively which represent nanoparticles adsorbing on the DOPC surface [35]. This was previously evidenced by TEM images of the SiO_2 in a close-packed configuration on the lipid monolayer surface. The capacitance current peak depression was shown to be proportional to the quantity of particles adsorbed [35]. Penetration of the DOPC layer by the nanoparticles is shown by an increase in the capacitance current baseline. The baseline capacitance current corresponds to the monolayer adsorbed on the Hg through its alkyl chains of low relative dielectric constant (~2). In the case of a higher dielectric such as a particle and/or water penetrating the layer coupled with a disorganisation of the layer, the average relative dielectric constant will increase as indicated by an increase in the capacitance current [56]. The height of the capacitance current peak 1 is generally ~-20 ± 4 μA in the control specific to the electrode age and condition and its depression is utilised as an indicator of interaction in all plots. Interestingly Figure 2(b) shows that the 20 nm Ag dispersion, unlike the 20 nm Au nanoparticle dispersion, has an insignificant interaction with the DOPC sensor element. This insignificant association might be associated partly with the less negative zeta potential (-10.8 mV) on the particle and also with the higher PDI (0.42) although other factors discussed later may also be responsible for the lack of interaction. Figure 2(c) displays a voltammogram of the effect on the DOPC of 1 mmol dm^{-3} $Na_3C_6H_5O_7$ which is present in both the Au and Ag dispersions and which has an insignificant interaction. Figure 3 (a) shows that Au nanoparticle dispersions synthesised with the

modified Turkevich procedure (Method 1) with a ~17 nm nanoparticle size (shown in TEM image) and PDI < 2 (Table 1) had a significant interaction with the DOPC. On the other hand Figure 3(b) indicates that Au nanoparticle dispersions, synthesised by Method 1 and heated to 100° (Method 2), with ~40 nm nanoparticle size (shown in TEM image) and a PDI > 0.2 (Table 1) had little effect on the DOPC. We note that the Method 1 home-synthesised Au nanoparticle dispersions did not effect an increase in the capacitance current baseline or broaden the capacitance peak current in the same way as the commercial Au dispersions (cf Figure 2(a)). In addition the Method 1 home-synthesised dispersions of 0.12 mmole dm^{-3} effected a similar or even slightly less depression of the capacitance peaks on monolayer interaction as did the commercial Au dispersions of 0.033 mmole dm^{-3}. Thus the commercial Au dispersions show a greater activity towards the DOPC layer. Figure 3 (c) displays and confirms the contrast between the particle properties prepared by Methods 1 and 2 respectively whereby the particles prepared by Method 2 show a larger hydrodynamic particle size and PDI than those prepared by Method 1.

Figure 4(a) displays the capacitance peak 1 current plotted against concentration of between 10^{-5} and 0.1 mmol dm^{-3} Au nanoparticle dispersions as an exposure-response curve. For each of the dispersions, the capacitance peak current decreased with increasing concentration of the Au. A significantly stronger interaction was noted in the presence of the 5 nm Au nanoparticle dispersion. The electrochemical results are supported by the CF leakage assay results which are plotted as a function of Au concentration in the dispersion and shown in Figure 4(b). CF leakage assays were performed using 400 nm DOPC LUVs incubated with commercial Au nanoparticle dispersions of between 10^{-5} and 0.1 mmol dm^{-3}. Release of the CF dye from a vesicle is due to bilayer perturbation or damage by interaction with the nanoparticles. Significant increases in fluorescence intensity were observed upon addition of all three Au particle sizes indicating dye release linked to bilayer integrity being compromised. Increasing the concentration of the Au in the nanoparticle dispersion increased the extent of dye release and the 5 nm Au nanoparticles induced the highest leakage at all concentrations. In both the RCV and leakage assay calibrations three replicate experiments were carried out and results were expressed as mean and standard error (SE) in Figure 4(b). The extent of voltammetric peak suppression aligned with the CF leakage assay indicating that the CF assay and electrochemical results are apparently consistent with each other and relate to the extent of interaction of the Au dispersions with the DOPC layer. A plot of the LUV leakage % versus the RCV capacitance peak current when exposed to a given concentration and size of Au nanoparticles shows a positive correlation. The correlation is good for the 5 nm particle size (R^2=0.96) but for 20 and 50 nm particle sizes it is not so tight (R^2 = 0.84 and 0.82 respectively). Most interestingly the leakage assay shows that the LUV are *more sensitive* to the smaller sized Au dispersions than the larger particle sizes (Figure 4(b) and (c) whereas the DOPC monolayer on Hg has a similar sensitivity to all particle sizes (Figure 4(a)). The RCV results in Figure 4(a) show however that the DOPC monolayer on Hg shows a lower *detection limit* [53] to the 5 nm dispersions than to the larger particle dispersions. This discrepancy between the two assays is observed since the LUV assay monitors DOPC free-standing bilayer breakdown whereas the DOPC supported monolayer assay reflects the initial Langmuirean adsorption of the particle on the layer which is the event preceding monolayer disruption [35].

Figure 5 summarises the DLS results of the 50 nm Au nanoparticles dispersed in different media. The DLS results indicated that in the presence of DMEM, the Au nanoparticles attain a larger hydrodynamic diameter due to adsorption of protein but remain relatively stable (Figure 5 (a)). DLS results for Au nanoparticles dispersed in PBS showed insignificant agglomeration initially but after 5 hours displayed some increase in particle size (Figure 5(b)). Figure 5 also displays representative voltammograms of DOPC on a Pt/Hg electrode exposed to 50 nm Au nanoparticles which were (c) treated with DMEM supplemented with 10% FBS for 24h, centrifuged and then resuspended in MilliQ water; and (d) incubated in PBS for 5 hours. In both cases, activities of the Au nanoparticle dispersions to the DOPC sensor element were suppressed. Clearly the adsorption of protein on the Au particles inhibited the interaction of the nanoparticles with the DOPC and in the same way the

PBS which facilitated increase of Au particle size also impeded nanoparticle/DOPC interaction.

To extend the investigation on the effects of coatings on Au nanoparticle properties, the decreased lipid monolayer activity of Ag particles was exploited as a potential moderator of Au nanoparticle lipid membrane activity. A Ag shell/Au core nanoparticle structure was synthesised. DLS results of Ag-coated Au nanoparticles are displayed in Figure 6(a). The dispersion was most stable for a synthesis time of under 20 minutes. The observed increase in hydrodynamic diameter could be unsystematically variable. Towards 40 minutes synthesis time, a second DLS peak appeared at smaller diameters, indicating the production of discrete Ag nanoparticles. The effect of Ag coating of Au nanoparticles on the interaction of the Au nanoparticle dispersion with DOPC layers was subsequently investigated. Figure 6(b) shows that the Ag coating of the 50 nm Au nanoparticle dispersion alleviated the interaction of the Au nanoparticle dispersion with the DOPC sensor element. This indicates that the Ag coating plays a part in the minimisation of Au nanoparticle interaction with the DOPC monolayer. As mentioned above, a small number of Ag nanoparticles were produced as a by-product of the Ag coating procedure. This effect was minimised in subsequent syntheses by keeping the reaction time to ~20 minutes. However there was a concern that these Ag nanoparticles would compete with the Au for adsorption on the surface of DOPC providing an additional mechanism for impeding the Au nanoparticle interaction. This was checked by investigating the interaction of Au nanoparticles in the presence of an equal concentration of Ag nanoparticles with the DOPC surface. The results displayed in Figure 6(c) show that commercial 10 nm Ag nanoparticles at a molar ratio Ag:Au of 1:1 have an insignificant effect on the commercial Au nanoparticle induced depression of the capacitance current peaks in the RCV plot (cf. Figure 6(b)).However an effect of the Ag nanoparticle dispersion on lowering the voltammogram baseline increase is observed.

To provide a more definitive characterisation of the core-shell nature of the Ag coated Au nanoparticles, HAADF STEM imaging and EDX elemental mapping was carried out. Figure 7 shows a HAADF STEM image of a Ag-coated Au nanoparticle along with the corresponding EDX maps for Ag and Au (Figure 7(a-c). In the HAADF STEM image, the Au core was observed as a bright central region while the Ag appeared as a thin shaded uniform coating around the brighter core. Correspondingly, STEM-EDX mapping confirmed that the Au is spatially resolved to the core while the Ag is present in the shell. In addition SEM images of the Ag coated Au showed no close aggregation of the particles (Figure 7(d)). The Ag coated Au particles were studied further by varying the concentration of $AgNO_3$ used in the coating treatment procedure. The subsequent alleviation of the Au nanoparticle interaction with the DOPC layer was then plotted against the $AgNO_3$ concentration used in the treatment. The results are displayed in Figure 8(a) and showed that the threshold for alleviation of the Au nanoparticle dispersion interaction with DOPC was at a concentration of ~0.45 mmol dm^{-3} $AgNO_3$ which represents a ratio of 13.6:1 to the Au concentration of 0.033 mmol dm^{-3} in the dispersion. The hydrodynamic size of the Au particles as displayed in Figure 8(b) did not vary consistently with the concentration of $AgNO_3$ used in the coating procedure and no significant overall increase in the mean hydrodynamic particle size was indicated.

The commercial Au nanoparticle dispersions in contrast to the commercial Ag dispersions were quoted by the manufacturer to contain a “proprietary” stabiliser in addition to $Na_3C_6H_5O_7$. The rationale in this study for the use of the commercial dispersions was the greater stability and quality as evidenced by the lower polydispersity index (PDI < 0.2) than that of the dispersions synthesised by the citrate reduction procedure. The “proprietary” stabiliser possessed no apparent interaction with the phospholipid in the solution phase, since when the sourced Au nanoparticle dispersions were incubated in PBS or coated with Ag, the phospholipid monolayer interaction was alleviated (see Figures 5(d) and 6(b)). A soluble surfactant if used to cap the particles has to be maintained in the solution phase to preserve an equilibrium with the particle surface. If the particle monolayer-activity is deactivated by a change in particle properties, the soluble surfactant properties will not be affected.

The lipid monolayer sensor is highly sensitive to the presence of surfactant in the aqueous phase above the μmol dm^{-3} level [53] and therefore the sensor would detect any soluble surfactant if present in the deactivated particle dispersion. Fourier transform infrared spectroscopy (FTIR) spectroscopy showed the presence of only water in the dried commercial Au dispersions. Low magnification SEM-EDX elemental mapping indicated Au and a homogenous dispersion of carbon presumably as a result of the presence of citrate in the samples with no detectable sulphur. We thus assume that there is no significant concentration of soluble lipid monolayer-active surfactant in, or thiol capping agent on, the commercial Au dispersions. This is interesting since soluble highly lipid layer-active surfactants [57] such as PVP/CTAB/SDS and thiol [22,58] capping agents respectively are commonly used to stabilise Au nanoparticle dispersions [32,59]. Similarly the presence of a positively charged amine [60] capping agent is not evidenced due the negative zeta potential on the particle. Significantly the coating of these particles by a thin film of Ag (Figure 6) would not be possible if the Au particle was coated with an *irreversibly bound* organic moiety since the Ag actually adheres to the Au presumably through a local solid solution formation [61]. An intimate contact between the two metals is necessary to maintain a stable core-shell structure [41]. It should be noted however that "soft" soluble surfactants have been used to facilitate the formation of Ag/Au alloyed nanoparticles [61] and more recently Ag coated Au nanoparticles [62]. In spite of this, a clean Au surface is required for plasma Ag deposition on Au nanorods [63]. It is probable that the "proprietary" stabiliser is a relatively lipid layer-inactive organic anion such as ascorbate [64] which can be readily displaced from the Au surface. A variation of the Turkevich method for Au nanoparticle synthesis was developed to synthesise high quality monodispersed (PDI < 0.2) Au nanoparticle dispersions and a similar, but not identical, interaction of the nanoparticles with the phospholipid monolayer was seen compared to that of the commercial Au dispersion as displayed in Figure 3. This indicated that the monodispersity contributed to the Au nanoparticle interaction with the DOPC monolayer. The difference in the voltammogram resulting from home-synthesised, compared to, commercial Au nanoparticle/monolayer interaction can be related to an increase in the voltammogram baseline current reflecting a penetration of the commercial particle into the lipid monolayer. Some capping agent on the commercial particle surface will influence this and contribute to the observed increased activity of the particle on the monolayer. Since the main aim of this study was to develop ways to tune the lipid layer activity of lipid layer active particles, the commercial (as opposed to home-synthesised) dispersions were used throughout the work due to their enhanced stability and low PDI.

This study also showed that Ag in contrast to Au nanoparticle dispersions exhibited no significant interaction with the phospholipid sensor element. This is correlated with a parallel study on the interaction of Ag nanoparticle dispersions with giant unilamellar vesicles (GUV) which found that only when they were weakly agglomerating did they exhibit a significant interaction with a phospholipid bilayer [17]. Significantly when the commercial monodispersed Au nanoparticles were coated with a thin film of Ag, no interaction with the phospholipid was observed. In addition to factors previously mentioned, the insignificant Ag/phospholipid interaction in the electrochemical membrane model could be related to the formation of a water insoluble Ag(I)-citrate complex [65] on the Ag nanoparticle surface which is formed from solubilised Ag [10] oxidised to Ag^+. This complex would not be readily displaced at the phospholipid/water interface. Further, in the presence of trace or higher concentrations of Cl^- ions, insoluble AgCl will also form [10] on the available Ag surface inhibiting interaction with phospholipid. Indeed low magnification SEM-EDX mapping of the commercial Ag nanoparticle dispersion showed the presence of Cl^- at ~14-22 weight % in the dried Ag dispersion. In contrast to Ag, Au nanoparticles do not solubilise in water and the citrate coating is readily displaced allowing the Au nanoparticle to associate with the phospholipid [65]. Interaction of Au surfaces with lipid films is not surprising in view of the high adsorption energy of lipids on Au predicted by modelling procedures [68] and estimated from experiment [31,66,67]. Indeed citrate-coated Au nanoparticles have been shown to be hydrophobic [68] and would have an affinity for phospholipid layers. Previously it was considered that van der Waals forces were responsible for the interaction between nanoparticles, in particular SiO_2, and lipid membranes [69]. However, when considering the

interaction of Au and Ag dispersions with lipid layers, we note that the Hamaker constant for these two metals in nanoparticulate form is similar for 20 nm nanoparticles: 170 and 150 x 10^{-21} J for Au and Ag respectively [70]. The present paper shows that Au nanoparticles can interact strongly depending on their agglomeration state and coating whereas Ag nanoparticles interact insignificantly with lipid layers. So it appears it is the surface and size of these nanoparticles, rather than their bulk Hamaker constant, which determines the nature of their interaction with lipid layers.

5. Conclusions

Commercial and high quality home synthesised Au nanoparticle dispersions are phospholipid monolayer active whereas Ag nanoparticle dispersions are not. If Au nanoparticles are coated with Ag then the particle/lipid interaction is suppressed. The rapid electrochemical screen of the monolayer activity of Au nanoparticle dispersions was found to align with the Au nanoparticle activity to bilayer vesicles. Au nanoparticles of decreasing size and increasing dispersion concentration showed a stronger phospholipid monolayer/bilayer interaction leading to increasing layer penetration/disruption. Incubation of Au nanoparticle dispersions in PBS media caused them to increase in particle size decreasing their phospholipid layer interaction. Coating of Au nanoparticles with protein increased their size and alleviated their phospholipid layer interaction. These results imply that the biomembrane activity of nanoparticle dispersions can be delicately tuned through a combination of surface treatments and rapid screening leading to the development of direct Safety by Design protocols for nanoparticle manufacture.

Generally the findings in this study are commensurate with those of other studies for Au nanoparticles. Thus Au nanoparticles interact with model lipid membranes [18-29] and this is consistent with predictive and experimental modelling [31,66,67] which shows a significant interaction energy between the Au surface and phospholipid. Interestingly the toxicity of Au nanoparticles to living organisms remains uncertain [9]. In addition, there is little data on the interaction of Ag nanoparticles with lipid membranes. Ag nanoparticles are characteristically toxic to living organisms [15] presumably due to their solubility in water to give toxic Ag^+ in the presence of oxygen [10]. Future work should correlate the evidenced Au nanoparticle/phospholipid layer interaction with their activity to well defined *in-vitro* cellular systems. An understanding of the physical chemistry of their interaction with phospholipid layers should be widened and deepened with investigations on the rate and energetics of the interaction.

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Figure legends

Figure 1.
DLS nanoparticle size distribution versus nanoparticle hydrodynamic diameter (where figures on plot refer to peak diameter) and TEM images of 0.2 mmol dm^{-3} Au commercial nanoparticle dispersion of nominal particle sizes: (a) 5 nm, (b) 20 nm and (c) 50 nm; and of (d) 0.16 mmol dm^{-3} Ag commercial nanoparticle dispersion of nominal particle size 20 nm.

Figure 2.
RCV (-I vs –E) at scan rate 40 Vs^{-1} of DOPC coated MPE in PBS at pH 7.4 before (black line) and after (red line) exposure to: (a) 0.033 mmol dm^{-3} Au commercial 20 nm nanoparticle dispersion in < 0.06 mmol dm^{-3} $Na_3C_6H_5O_7$ (figures 1 and 2 on plot refer to capacitance current peaks in text) (b) 0.16 mmol dm^{-3} Ag commercial 20 nm Ag nanoparticle dispersion in 1 mmol dm^{-3} $Na_3C_6H_5O_7$ (red line) and (c) 1 mmol dm^{-3} $Na_3C_6H_5O_7$ in the absence of nanoparticle dispersion.

Figure 3.
RCV (-I vs –E) at scan rate 40 s^{-1} of DOPC coated MPE in PBS at pH 7.4 before (black line) and after (red line) exposure to: (a) 0.12 and (b) 0.27 mmol dm^{-3} Au nanoparticle dispersions (in < 0.01 mmol dm^{-3} $Na_3C_6H_5O_7$) synthesised as described in text by (a) Method 1 and (b) Method 2 respectively with accompanying TEM images of respective dispersions and (c) DLS plots of Method 1 (black line) and Method 2 (red line) nanoparticle dispersions (figures on plot refer to PDI values).

Figure 4.
(a) RCV peak 1 capacitance current ($-I_p$) of DOPC coated Hg electrode and (b) % leakage of CF release from DOPC LUVs versus Au concentration in commercial nanoparticle dispersions with nominal particle size of 5nm (filled blue circle), 20 nm (open black circle) and 50 nm (red triangle). Mean and error bars (summarising SE) from three replicate experiments are displayed together with best-fit trendline as sigmoid drawn through mean values; and (c) % leakage of CF release from DOPC LUVs versus RCV peak 1 capacitance current of DOPC coated Hg electrode for each Au concentration in commercial nanoparticle dispersions with nominal particle size symbols as above and R^2 values for the correlations as follows: 5 nm: 0.96, 20 nm: 0.84 and 50 nm: 0.82.

Figure 5.
DLS nanoparticle size distribution versus nanoparticle hydrodynamic diameter (where figures on plot refer to peak diameter) of 0.033 mmol dm^{-3} Au 50 nm commercial nanoparticle dispersions in MilliQ water (black line) and (a) sterile-filtered DMEM after supplementing with 10% FBS (red line) and, (b) incubated in PBS for 5 hours (red line).
RCV (-I vs –E) at scan rate 40 Vs^{-1} of DOPC coated MPE in PBS at pH 7.4 before (black line) and after exposure (red line) to 0.033 mmol dm^{-3} Au 50 nm commercial nanoparticle dispersions in < 0.06 mmol dm^{-3} $Na_3C_6H_5O_7$ (c) incubated in DMEM supplemented with 10% FBS for 24h and

resuspended in MilliQ water and (d) incubated in PBS for 5 hours.

Figure 6.
(a) DLS nanoparticle size distributions of 0.033 mmol dm^{-3} Au 50 nm commercial nanoparticle dispersions in MilliQ water with Ag coating treatment after synthesis times of 0 (black line), 10 (red line), 20 (purple dash line) and 40 mins (blue line) respectively (figures on plot refer to peak diameter); (b) RCV (-I vs –E) at a scan rate 40 Vs^{-1} of DOPC coated MPE in PBS at pH 7.4 before (black line) and after exposure to 0.033 mmol dm^{-3} Au commercial nanoparticle dispersion in < 0.06 mmol dm^{-3} $Na_3C_6H_5O_7$ (red line) and in Ag functionalised 50 nm commercial 0.033 mmol dm^{-3} Au nanoparticle dispersion with 0.17 mmol dm^{-3} sodium citrate (dashed blue line); (c) RCV (-I vs –E) at a scan rate 40 Vs^{-1} of DOPC coated MPE in PBS at pH 7.4 before (black line) and after exposure to 0.08 mmol dm^{-3} Ag 10 nm nanoparticle dispersion in 0.84 mmol dm^{-3} $Na_3C_6H_5O_7$ (dashed blue line) and 1:1 mixture of 0.017 mmol dm^{-3} 50 nm commercial Au and 0.017 mmol dm^{-3} 10 nm commercial Ag nanoparticle dispersion in 0.2 mmol dm^{-3} $Na_3C_6H_5O_7$ (red line).

Figure 7.
STEM-HAADF image and EDX elemental maps of a single Ag-coated (shown as grey halo) commercial Au nanoparticle (shown as white core) which used the following EDX lines for Ag and Au respectively: Ag L-α (2.983 keV) and Au L-α (9.713 keV); and (a) Au EDX map; (b) Ag EDX map; (c) an overlay of the Au and Ag EDX maps for a single particle and (d) SEM of Ag coated 50 nm Au nanoparticles.

Figure 8.
(a) hydrodynamic diameter of Ag coated 50 nm Au dispersion versus $AgNO_3$ concentration used to coat particles; and (b) RCV peak 1 capacitance current ($-I_p$) of DOPC coated Hg electrode in presence of 0.033 mol dm^{-3} Au commercial 50 nm nanoparticle dispersion versus $AgNO_3$ concentration used to coat particles.

Table 1

Physical and chemical properties of nanoparticle dispersions used in this study

Nanomaterial	5 nm Au	20 nm Au	50 nm Au	10 nm Ag	20 nm Ag	Au	Au
Source	Alfa Aesar	Alfa Aesar	Alfa Aesar	Alfa Aesar	Alfa Aesar	*in-house (Method 1)*	*in-house (Method 2)*
Particle diameter (nm) (TEM)[a]	4.2 ± 0.54	16.8 ± 1.4	52.2 ± 3.5	10.9± 4.14	22.3 ± 8.7	16.9 ± 0.37	41.1 ± 3.6
Particle diameter (nm) (DLS)	10.1 ± 2.4	33.8 ± 1.1	68.1 ± 1	14.13 ± 0.05	27.5 ± 2	19.6 ±1.5	64.1 ± 4.6
Zeta potential (mV)	-14.3 ± 3.3	-22.6 ± 4.0	-29.4 ± 1.4	-45.9 ± 1.2	-10.8 ± 0.9	-20.8 ± 1.5	-28.6 ± 1.6
PDI (DLS)	0.14 ± 0.01	0.12 ± 0.01	0.19 ± 0.02	0.57 ± 0.01	0.42 ± 0.06	0.13 ± 0.02	0.51 ± 0.11
Dispersion concentration (mmol dm^{-3}) ICP-MS	0.3 ± 0.005	0.25 ± 0.01	0.2 ± 0.002	0.18 ± 0.004	0.19 ± 0.01	0.12 ± 0.004	0.27 ± 0.059

[a]Primary particle size measured from 150 particles in TEM images.