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(54) Title: PARTICLE DETECTION THROUGH CHRONOAMPEROMETRIC PROFILING

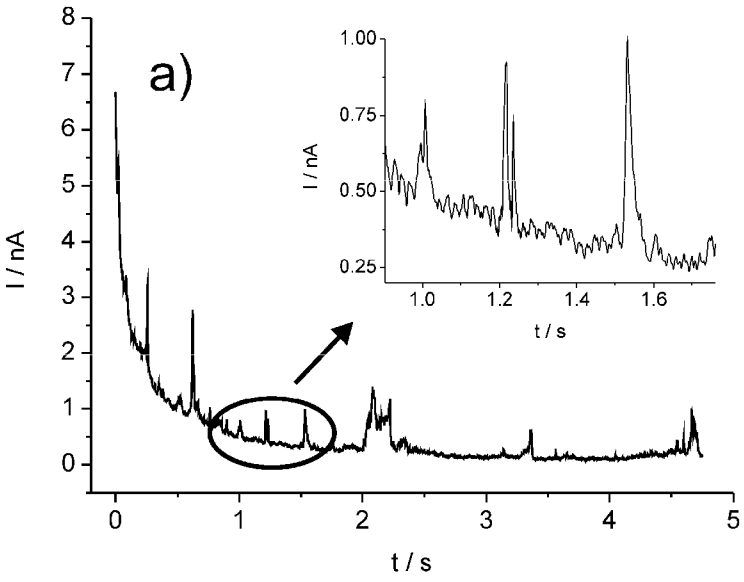

Figure 1

(57) Abstract: The present invention relates to a method of detecting (or identifying) particle(s) in a sample, particularly nanoparticles which, on entering the natural environment, may pose a public health risk. The method generally involves assessing the presence and/or properties of the particles in a sample from electrochemical responses yielded by the oxidative (or reductive) consumption/destruction of particles as they collide with a working electrode. In particular, the present invention allows identifying, quantifying, and characterising of particles in a sample via the electro-oxidation of particles which collide with working electrodes operating at a suitable potential difference.

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PARTICLE DETECTION THROUGH CHRONOAMPEROMETRIC PROFILING

INTRODUCTION

[0001] The present invention relates to an electrochemical method of detecting (or identifying) and quantifying particle(s), particularly nanoparticles, in a sample. The method generally involves assessing the presence and/or properties of the particles from electrochemical responses resulting from the oxidative or reductive consumption/destruction of particles as they collide with an electrode. The present invention also relates to a method of measuring a property of, a method of measuring the size of, a method of measuring concentration of, or a method of modifying (or destroying) particles in a sample, or a method of cleaning a sample. In addition the present invention relates to a cleaned sample, an apparatus for carrying out the above methods, a computer programmed to implement the above methods, and a computer-readable medium comprising computer software for carrying out the above methods.

BACKGROUND OF THE INVENTION

[0002] With the increasing production of engineered nanoparticles (ENPs) for a variety of commercial applications, including water-treatment, and the inevitable ENP contamination of recreational and drinking water supplies that this brings, there is an urgent need to detect and quantify the ENPs in water supplies to assess public health risks, as noted in a recent and thorough review of the area (Weinberg *et al*, *Trends in Analytical Chemistry*, Vol 30, No 1, 2011).

[0003] ENPs are essentially anthropogenic materials of less than 100nm in size in more than one dimension. ENPs come in a variety of forms, including spherical, tubular, and irregular. Nanoparticles (NPs) in general have become ubiquitous with an estimated 1600 commercial products available (Project on Emerging Nanotechnologies, www.nanotechproject.org/news/archive/8277/.April 29, 2009). In particular, silver nanoparticles (AgNPs) are used in clothing for their antibacterial properties, but approximately 50% of AgNPs leach out per washing cycle (T.M. Benn, P. Westerhoff, *Environ. Sci. Tech.* 2008, 42, 4133). Inevitably such leaching leads to contamination of water supplies since an estimated 65 tonnes per annum of AgNPs are released into global river systems alone (Blaser *et al*, *Sci. Total Environ.* 2008, 390, 396). Since AgNPs cause endocrine disruption in amphibians (Hinther *et al*, *Environ. Sci. Technol.*

2010, 44, 8314), and are toxic to many mammalian organs (Ahamed *et al*, *Clin. Chim. Acta*, 2010, 411, 1841), such leaching could pose a serious public health risk.

[0004] As stated in the recent Weinberg review article (above), currently there is no singly effective protocol for examining ENPs in a complex environment, where there is a need to examine properties of ENPs, such as NP size, size distribution, redox potential, and purity, amongst others. The review proposes the extraction of NPs from the water supply by chromatographic techniques such as size-exclusion chromatography (SEC), high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), hydrodynamic chromatography (HDC) or field-flow fractionation (FFF), before subsequent characterization and analysis is carried out upon the extracted NPs. This approach is, however, extremely laborious, and analysis and characterization is still problematic after isolation of the NPs. The same review article considers a number of post-extraction characterization techniques, but again these are either laborious, complex, inefficient, and in many cases lack the accuracy required to obtain meaningful information regarding the properties of the NPs. In particular such techniques cannot accurately determine the difference between the targeted ENPs and naturally-occurring NPs, especially given that the ENPs are typically present at concentrations well below that of naturally-occurring NPs. Finally, the review considers the "formidable challenges" in the development of quantitative sensors for analyzing the ENPs, particularly *"in-situ*, real time, portable devices" that can readily be applied to environmental samples. The review acknowledges that this is "a largely unexplored area of research", and merely reports relatively undeveloped indirect detection methods, which involve "peroxide-driven" metallic silver decomposition (oxidation). However, such techniques are susceptible to interference from Ag^+ ions already in solution.

[0005] It is therefore an object of the present invention to solve at least one of the problems inherent with the prior art by an electrochemical method of detecting and quantifying particles.

SUMMARY OF THE INVENTION

[0006] The present invention essentially employs electrochemical apparatus to directly and quantitatively oxidise (or reduce) particles within a sample to thereby oxidatively (or reductively) consume/destroy said particles, typically upon a single collision of each particle with a working electrode. Electrochemical signals generated during this process allow said particles to be detected and/or quantitatively examined.

[0007] In a first aspect of the present invention there is provided a method of detecting (and/or identifying) a particle in a sample, the method comprising:

i) applying an electric potential difference across the sample between a working electrode and a counter electrode, in the presence of an electrolyte, to allow an electric current to flow through the sample;

ii) monitoring the electric current over time for an electrochemical response caused by the particle colliding with the working electrode and being itself oxidised or reduced; and

iii) determining the presence of the particle(s) in the sample by the observance of an electrochemical response.

[0008] In a second aspect of the present invention there is provided a method of measuring a property of a particle in a sample, the method comprising:

i) applying an electric potential difference across the sample between a working electrode and a counter electrode, in the presence of an electrolyte, to allow an electric current to flow through the sample;

ii) monitoring the electric current over time for an electrochemical response caused by the particle colliding with the working electrode and being itself oxidised or reduced; and

iii) determining a property of the particle in the sample from the electrochemical response.

[0009] In an embodiment, the method of measuring a property of a particle is a method of measuring the size (or mass) of a particle in a sample, wherein step (iii) comprises determining the size (or mass) of the particle in the sample from the size of the electrochemical response.

[0010] In another embodiment, the method of measuring a property of a particle is a method of measuring the concentration of particles in a sample, wherein step (iii) comprises determining the concentration of particles in the sample from the frequency of the electrochemical response.

[0011] In a third aspect of the present invention there is provided a method of modifying (or destroying) a particle or particles in a sample, and/or a method of cleaning a sample, the method comprising:

i) applying an electric potential difference across the sample between a working electrode and a counter electrode, in the presence of an

electrolyte, to allow an electric current to flow through the sample and cause the particle or particles themselves to be oxidised or reduced on colliding with the working electrode; and

ii) optionally:

a. monitoring over time the electric current for an electrochemical response caused by particles colliding with the working electrode and being themselves oxidised or reduced; and

b. determining whether the particle or particles have been modified (or destroyed) from a change in the frequency of the electrochemical response.

[0012] In a fourth aspect there is provided a cleaned sample obtained by, obtainable by, or directly obtained by, the method of cleaning a sample according to the third aspect.

[0013] In a fifth aspect of the present invention there is provided an apparatus configured to carry out any one of the methods of the first to third aspects. The apparatus may suitably be a particle analyser. The particle analyser may suitably be operable to detect (or identify), measure a property of, measure the size(s) of, measure the concentration of, or modify (or destroy) particles in a sample, or to clean a sample.

[0014] In an sixth aspect of the present invention there is provided a computer programmed with computer software to implement one or more steps of any of the methods of the first to fifth aspects.

[0015] In a seventh aspect of the present invention there is provided a computer-readable medium comprising the computer software described in the eighth aspect.

[0016] Reference to "a/the particle in a sample" may suitably include a single particle or a plurality of particles. In most embodiments, the methods of the present invention relate to a plurality of particles.

[0017] All methods of the present invention generally comprise detecting (and/or identifying) particle(s) in a sample. In most embodiments, the method(s) of the present invention involve destroying or modifying the particles, preferably completely destroying the particles, preferably completely oxidising the particles.

[0018] Suitable and preferred features in relation to any aspect of the present invention may also be suitable features in relation to any other aspect.

[0019] The present invention provides a direct, simple, effective and inexpensive means of detecting particles *in situ* in a sample, especially very small (e.g. nanoparticles) and

uncharged particles. Moreover, the present invention provides a simple, effective, and inexpensive means of characterising and quantifying said particles in a sample. Advantageously the present invention allows for detection, characterisation, quantification, and sizing even with highly dilute samples. As such, the present invention addresses the long felt need for accurate and reliable techniques for screening samples, such as environmental samples (e.g. river and seawater samples) for toxic nanoparticles of silver and the like. Moreover, methods of the present invention are not susceptible to interference from the by-products of the methods, especially where such by-products are soluble or otherwise electrochemically inert at a particular selected electric potential difference.

[0020] Recently, Bard et al (Bard et al, J. Phys. Chem. C, 2009, 113, 14978; Bard et al, J. Am. Chem. *Soc*. 2007, *129*, 9610; Bard *et al*, *Isr. J. Chem*. 2010, *50*, 267; Bard *et al*, *J. Am. Chem. Soc*. 2010, *132*, 13165; Bard *et al*, *J. Am. Chem. Soc.* 2008, 130,16669) has used electrochemical techniques to observe electrochemical reactions on the surface of impacting NPs, but did not employ methods which involve destroying or modifying particles via oxidation or reduction of the particles themselves to detect, identify, and measure properties (e.g. size, concentration, etc.) of the particles.

[0021] An advantageous feature of all method(s) of the present invention is that no prior isolation of the particles is carried out prior to carrying out the method(s). Instead, methods of the invention capitalise on the inevitable collisions particles will make with a working electrode (e.g. through Brownian motion) and as such said particles are inevitably registered without any pre-isolation or pre-gathering of particles. Another advantageous feature is that all methods are direct methods (i.e. provide all required information from a single sensory technique). A further advantageous feature is that the particles do not stick to the working electrode.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] For a better understanding of the present invention, and to show how embodiments of the same may be carried into effect, reference is made, by way of example, to the following diagrammatic drawings, in which:

[0023] Fig. 1a shows chronoamperometric profiles showing oxidative collisions of AgNPs in citrate solution (enlarged inset showing detailed impact spikes), and relates to an embodiment of the present invention;

[0024] Fig. 1b shows an overlay plot of a stripping voltammogram for an AgNP-modified GC electrode (left axis) and the impact frequency (right axis) showing the onset of potential of spikes, and relates to an embodiment of the present invention; and

[0025] Fig. 1c shows the distribution of NP radii inferred from Q *via* equation (1) with deconvolution, and relates to an embodiment of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0026] Unless otherwise stated, the following terms shall in the context of this specification have the following definitions:

[0027] "Particle" is used herein to refer to any particulate material present in the sample which generates an electrochemical response upon colliding with the working electrode.

[0028] "Base-line current" refers to the current flowing through the sample between the working and counter electrodes in response to the applied voltage, and is typically observed as the substantially continuous smooth curve or straight line obtained when a measured electric current is graphically plotted against time for standard electrolysis upon a homogeneous electrolyte (i.e. in the absence of any particles). The base-line current need not remain constant over time since it is expected to decay, for instance, as the electrolyte is depleted during electrolysis. However, the base-line current is continuous and smooth, notwithstanding any background noise.

[0029] A "deviation" relative to the base-line current is a discontinuity over and above any background noise in an otherwise continuous function defined by a graphical plot of "base-line current" against time. Typically a deviation is characterised by a peak or spike, albeit overlapping peaks or spikes may occur.

[0030] Herein, reference to "particles" may include a single particle where, for example, only a single particle is being detected or the size or other property of a single particle is being measured.

[0031] Herein, unless otherwise stated a redox potential (i.e. standard electrode potential) or an electric potential difference is given in volts (V) relative to a standard hydrogen electrode (SHE), at 25°C, at an effective concentration of 1 mol/L for each aqueous species or species in a mercury amalgam. Where reference to another standard reference electrode is made (e.g. Ag/AgCl or calomel reference electrode), the

voltage relationship to the SHE can be easily deduced by one skilled in the art but, by way of example, Table 1 shows certain voltage relationships that apply at 25°C:

Table 1

Reference electrode	Electrode potential with respect to SHE (mV)
Standard hydrogen electrode (SHE)	0
Saturated calomel electrode (SCE)	241
Ag/AgCl, 1 M KCl	236
Ag/AgCl, 4 M KCl	200
Ag/AgCl, sat. KCl	197

General Description

[0032] The present invention provides methods to examine particles using the electrochemical response resulting from collisions of the particles with a working electrode. Typically such an electrochemical response is measured as a current deviation (usually a spike) relative to a base-line current. When particles strike the working electrode, the working electrode reduces or oxidises the particles themselves, thereby leading to transient current surges which register an electrochemical response. Although the electrochemical response may be detectable at voltages not affecting the redox state of the particles in question (e.g. spikes at the working electrode caused by electrolysis of the electrolyte on the surface of the particles as they strike the electrode), electrochemical responses are generally larger and hence more detectable when using voltages which do affect the redox state of the particles in question. For instance, at suitable voltages, sudden current surges may be observed following sudden oxidation of a particle (e.g. metallic particle) as it collides with the working electrode. By way of example, silver particles may be oxidised in accordance with the following half-reaction:

$$Ag(np) - e^- = Ag^+(aq)$$

[0033] In the present invention, electrode-particle collisions are detected when the electric potential difference is set to be at or beyond the redox potential of the particles in question. A potential difference "beyond" the redox potential of a particle is defined as a potential difference at which the particle's redox state still changes as it would at the particle's redox potential. For instance, for oxidative collisions, as per metallic particles, an electric potential difference of -0.5 V or +0.5 V is beyond the redox potential of zinc, which is -0.76 V. However, an electric potential difference of -0.5 or +0.5 V is not beyond the redox potential of silver, which is +0.8 V. As such, analysis at a potential difference of -0.5V or +0.5V of a sample containing both zinc and silver particles will register zinc

particle collisions, but fail to register (or only register as a minor signal fluctuation) silver particles given that the potential difference is insufficient to cause oxidation of the silver particles. As such, the electric potential difference applied to the sample may be suitably tuned to examine a particular particle of interest, potentially at the exclusion of other electrochemically reactive particles which may co-exist within the same sample. When the potential difference is appropriately tuned for a given particle of interest, various data can be gathered relating to such particles, for example, their very existence (detection/identification by the existence of current spikes), their size (by measuring the size of the current spikes), their number concentration (measuring collision frequency through the average number of spikes per unit time), or their weight concentration (by combining measurements of particle size with number concentration).

The Sample

[0034] The sample is a liquid sample in which the particles are dispersed or suspended. Any suitably liquid medium may be used (e.g. water). Suitably, the liquid medium is an electrolyte solution.

[0035] In an embodiment, the sample may be formed by mixing a precursor material with a liquid medium. The precursor material may be a solid (e.g. a solid sample taken from the environment), or alternatively a liquid already comprising particles. The sample may be formed following an additional treatment step upon the liquid precursor, for instance further dilution, concentration (e.g. *in vacuo*), or filtration to remove macroparticulates.

[0036] In particular embodiments, the sample is of high dilution relative to the particles in question. The concentration of the particles may suitably be below 10μM, suitably below 1μM, suitably below 1nM, suitably below 100pM, suitably below 50pM. In some cases it may be desirable to dilute a sample or precursor material to produce a sample having a concentration more conducive to providing accurate measurements.

[0037] Suitably the sample does not contain particles which have been especially pre-isolated, pre-concentrated, or pre-gathered prior to use in the methods of the present invention.

The Particles

[0038] In the context of the present invention, a particle or particles refers to the particle(s) of interest in relation to the methods of the present invention – e.g. the

particles to be detected (or identified), quantified, modified (or destroyed), or particles upon which measurements are performed.

[0039] The particles are preferably insoluble in the liquid medium – i.e. the sample preferably comprises undissolved particles in a liquid medium. The particles are preferably dispersed or suspended in a liquid sample. In a particular embodiment the particles are dispersed particles, such as colloidally dispersed particles.

[0040] The dispersed particles may suitably have a particle size (maximum dimension) less than or equal to 100μm, suitably less than or equal to 2.5μm, suitably less than or equal to 1μm (1000 nm), suitably less than or equal to 500 nm, suitably less than or equal to 100nm, suitably less than or equal to 50nm. In a particular embodiment, the dispersed particles suitably have a particle size of at least 0.1 nm, suitably at least 1 nm, suitably at least 10 nm, suitably at least 20 nm. The dispersed particles are suitably nanoparticles (NPs). In certain embodiments, nanoparticles are engineered nanoparticles (ENPs) as opposed to natural NPs. The dispersed particles may suitably exhibit Brownian motion, for instance, in an unagitated dispersion.

[0041] In certain embodiments, the dispersed particles suitably have a particle size (maximum dimension) greater than 100μm, and possible greater than 1mm. Such larger dispersed particles may not exhibit Brownian motion. Where the dispersed particles do not exhibit Brownian motion, the methods of the present invention may include agitating (e.g. through stirring) the dispersion, as this may provide greater accuracy of measurements.

[0042] The particles may be positively or negatively charged - i.e they can have varying zeta potentials. Alternatively, the particles may be uncharged. Uncharged particles may suitably comprise a neutral species, for example, a species in a zero oxidation state. Such uncharged particles may suitably be (substantially) free of ions and/or counter-ions. In a particular embodiment, the uncharged particles essentially consist of a neutral species. The solvent used may or may not be water.

[0043] In a particular embodiment, the particles may suitably comprise a species which is electrochemically oxidisable, suitably electrochemically oxidisable at an electric potential difference at which the solvent is electrochemically stable, that is to say oxidisable at a potential at which the solvent is neither oxidised nor reduced.

[0044] In certain embodiments, the particles may comprise two or more species with different redox potentials. Alternatively the sample may comprise particles comprising one species, and additional auxiliary particulates comprise a species with a different

redox potential to the particles. In either case, examination of the individual species requires performance of the method(s) of the present invention at two or more electric potential difference thresholds (see below).

[0045] In preferred embodiments, the particles are inorganic. In preferred embodiments, the particles comprise a metal, suitably a metal in a zero oxidation state. In preferred embodiments, the particles are metallic particles. "Metallic particles" suitably comprise a metal in a zero oxidation state, and suitably essentially consist of a metal in a zero oxidation state. In preferred embodiments, individual metallic particles comprise or essentially consist of a single metal. In a particular embodiment, the metallic particles comprise or essentially consist of a single metal. In alternative embodiments, the metallic particles comprise two or more metals, suitably where individual metallic particles comprise or essentially consist of a single metal. Where multiple metal varieties comprise the metallic particles, each variety may be examined by the method(s) of the present invention, for example, by multiple runs of the method(s) using different electric potential differences (see below). Alternatively, the metallic particles may be considered to comprise only one of the multiple metal varieties, with the other metal varieties being considered to be comprised of additional auxiliary particulates.

[0046] In a particular embodiment, the metallic particles may suitably be particles that have been released into the environment either by intention, manufacture, or disposal, or through subsequent treatment of commercial products (e.g. the washing of textiles). The metallic particles may suitably have been previously used to combat bacteria or other microorganisms. In a particular embodiment, the metal of the metallic particles is toxic to human and/or animal life.

[0047] In a particular embodiment, the metal of the metallic particles may suitably be a metal which is electrochemically oxidisable, suitably electrochemically oxidisable at an electric potential difference at which the solvent is electrochemically stable, that is to say oxidisable at a potential at which the solvent is neither oxidised nor reduced.

[0048] The metal may suitably be selected from the group including silver, titanium, zinc, and gold. In a particular embodiment, the metal is silver (i.e. Ag^0).

[0049] The methods of the present invention suitably oxidatively (or reductively) consume/destroy each particle, directly from/within the sample (i.e. without any pre-isolation/pre-gathering of the particles from the sample), as each particle undergoes a single collision with the working electrode. The particles are suitably consumed/destroyed through a change in redox state, which then suitably leads to said particles becoming solubilised within the sample.

Electrolyte

[0050] "Electrolyte" is a term well understood in the art and refers to a liquid medium containing free ions so as to render the medium electrically conductive. Although this does not exclude molten electrolytes, an electrolyte of the present invention is preferably an ionic solution, otherwise called an "electrolyte solution". Preferably the electrolyte comprises water as the liquid medium.

[0051] In a particular embodiment the sample itself may comprise the electrolyte. For instance, where the sample is seawater, the sample is already in the presence of an electrolyte. In other embodiments, the sample is mixed with a separate electrolyte.

Potential Difference and Redox Potentials

[0052] "Electric potential difference" is a term well understood in the art and refers to the voltage established between a working and counter electrode. In accordance with the present invention, the electric potential difference (i.e. voltage) applied across the sample suitably affects the redox state of the particles in question.

[0053] In a particular embodiment, the electric potential difference across the sample is provided by a suitable power source *via* an anode and a cathode, or a working electrode and a counter electrode. The working electrode may suitably be the anode. A reference electrode may also be present. In particular embodiments, the method(s) of the present invention are conducted within a faraday cage.

[0054] Although any suitable electrode may be used, in a particular embodiment the working electrode is suitably a glassy carbon (GC) electrode (preferably bare). In a particular embodiment, the counter electrode is a graphite electrode (preferably a graphite rod). The reference electrode, when present, may suitably be either an Ag/AgCl or calomel reference electrode.

[0055] In a particular embodiment, the electric potential difference across the sample is suitably provided by a potentiostat.

[0056] Redox potentials are quoted for a species in terms of the cathodic reduction half-reaction – i.e. a highly negative redox potential demonstrates that the oxidised form of the species is thermodynamically favoured (i.e. the species is readily oxidised). For instance, silver ($Ag^+ + e^- = Ag(s)$) has a redox potential of +0.80 whereas zinc ($Zn^{2+} + 2e^- = Zn(s)$) has a redox potential of -0.76, relative to a SHE. As such, zinc is more readily

oxidised. Unless otherwise stated, references herein to the redox potential of a metal refer to the redox potential as defined by the half-reaction between the metal in its least oxidised form and the unoxidised metal.

[0057] In a particular embodiment, the electrical potential difference across the sample is suitably sufficient for an electrochemical response (i.e. to provide detectable current deviations relative to the base-line current) when the particle(s) collide with one of the electrodes. The electric potential difference may be suitably sufficient to provide an electrochemical response when the particle(s) collide with the working electrode. The electric potential difference may be such that the solvent is electrochemically stable, that is to say oxidisable at a potential at which the solvent is neither oxidised or reduced.

[0058] Presence of certain ions can shift the standard redox potential of a metal species. For instance, the presence of chloride ions renders silver more easily oxidisable since the redox potential of silver chloride ($AgCl + e^- = Ag(s) + Cl^-$) is +0.22 (cf. Redox potential of silver ions *per se* mentioned above). Therefore, the electric potential difference across the sample may be tuned appropriately when performing the method(s) of the present invention. Moreover, the electrolyte and the ions contained therein may be chosen to optimised the method(s) for particular particles of interest. For example, inclusion of the chloride ion within the electrolyte shifts the redox potential of silver, which may allow for greater distinction between deviation signals arising from silver and those arising from other metallic particles that may be present, such as mercury.

[0059] The electric potential difference may be suitably tuned for particular particles of interest (e.g. particles of a particular metal species). In particular embodiments, the electric potential difference is selected to be (substantially) at the redox potential of the particles of interest. Tuning the electric potential difference to be at or beyond the redox potential of the particles of interest suitably increases the signal of a current surge (i.e. increases the size of deviations relative to the base-line current) following a collision event. Therefore this improves the signal to noise ratio, and also increases discrimination between signals provided by the particles of interest and signals provided by other particulates or events.

[0060] Where the particles comprise a mixture of two or more species with different redox potentials, examination of the individual species requires performance of the method(s) of the present invention at two or more electric potential difference thresholds. Performing the method(s) of the present invention at different thresholds requires multiple runs at different electric potential differences. For instance, in a particular embodiment a first run at a first threshold suitably examines and/or provides data on a

first species of particles, and a second run at a second threshold suitably examines and/or provides data on a second species of particles. Typically, in such embodiments, the second run provides deviations relative to a base-line current relating to both the first and second species of particles. In such cases, data on only the second species can be suitably obtained by effectively "subtracting" the data obtained at the first threshold from the data obtained at the second threshold. Second and subsequent runs may be performed at second and subsequent thresholds to examine and/or provide data on second and subsequent species of particle. In preferred embodiments, a first run is performed on a first sample, and second and subsequent runs are suitably performed on a second and subsequent sample, whereby the second and subsequent sample is a refreshed sample identical to the first sample. Such refreshed samples ensures that depletion of the first species in the first sample after the first run does not distort the data obtained in second and subsequent runs.

[0061] By way of example, the method(s) of the present invention may be performed upon a sample containing both metallic silver (E^0 = +0.80V) and metallic zinc particles (E^0 = -0.76V). A first run may suitably include a first threshold set at an electric potential difference of -0.76V, at which voltage all relevant data regarding the zinc particles may be obtained. As this potential difference is insufficient to oxidise the silver particles at the anode, no deviations (e.g. spikes) relative to the base-line current will register when silver particles collide with the anode. Thus any data obtained relates to the zinc particles only. Refreshing the sample so as to provide a second sample identical to the first before it was subject to the method(s) of the present invention, a second run may suitably be performed to include a second threshold set at an electric potential difference of +0.80V. At this voltage, collisions of both zinc and silver particles with the anode will register as deviations from the base-line current, and thus provide a data set on both species of particles. To obtain information regarding silver particles only, it is necessary to "subtract" the data from the first run to leave only data pertaining to silver. For instance, the data from the second run will include collisions from both zinc and silver particles, and as such the collision frequency will be higher than that for the first run. However, subtracting the collision frequency of the first run from the second run will leave the collision frequency of the silver particles only. This data can then be used to deduce the number concentration of the silver particles only.

[0062] Method(s) of the present invention are applicable to any species, particularly to any species whose redox potential is known, since the electric potential difference can then be appropriately tuned for a particular species of particle. Redox potential values

are readily available from standard reference sources (e.g. Standard Potentials in Aqueous Solution, A.J. Bard, R. Parsons, J. Jordan, CRC Press, 1985).

Electrochemical Response

[0063] As previously stated, the detectable electrochemical response is a deviation in the current from a typical base-line value (i.e. a detectable current peak or spike). Monitoring the current flow over time when applying a specific electric potential difference across a homogenous electrolyte solution gives rise to a base-line current. The base-line current can be recorded as a graphical trace (or plot) against time, which is typically characterised as a (substantially) smooth continuous curve, notwithstanding any detectable noise. Typically the smooth curve is a decaying curve as the electrolyte is depleted (assuming new species do not arise which provide a further electrochemical response at the specific electric potential difference applied).

[0064] A deviation relative to this base-line current is characterised as a discontinuity, over and above any noise, in the graphical trace of current against time. A deviation in the form of a current peak or spike is indicative of a collision event whereby, in colliding with one of the electrodes (usually the anode), a particle in a sample induces an electrochemical response at said electrode. Typically the electrochemical response is a current surge resulting from sudden oxidation or reduction of the particle at the electrode. In a particular embodiment, the electrochemical response is a current surge resulting from sudden oxidation of the particle at the working electrode. Usually, collision events fail to register a deviation relative to the base-line current if the electric potential difference is insufficient to reduce or oxidise the particle when it collides with the electrode.

[0065] Interfering electrical noise is readily identifiable and calculable by a skilled practitioner, who would recognise deviations due to particle collisions.

[0066] Typically, the electrochemical response is characterised by a peak or spike in the trace, said peaks or spikes being clearly identifiable above any detectable noise. Peaks or spikes may on occasion overlap, in which case the skilled practitioner can readily extract the data pertaining to the individual overlapping spikes by standard mathematic techniques known in the art.

[0067] Signal interference may be caused by particulates colliding with an electrode (particularly the working electrode) without any change in oxidation state. Current deviations will be suitably distinguished from signal interference by those skilled in the

art. However, by way of example, such distinction may include only considering deviations greater than a certain threshold value relative to the mean current deviations (e.g. >1%, >5%, or >10% of the mean value of all current deviations). Particulates causing such interference at a particular potential difference may be considered electrochemically inert at that particular potential difference.

[0068] Computer software may suitably be employed to analyse the data and reduce the noise in the graphical trace, thus allowing for detection of small particles, which typically produce smaller deviations/spikes when detectably colliding with an electrode.

[0069] Over time, collision events (when particles detectably collide with one of the electrodes) may suitably become less frequent as the particles of interest are destroyed or changed into species which are no longer detectable by method(s) of the present invention.

[0070] Analysis of deviation(s) relative to the base-line current may suitably provide data and information about the particle(s), especially regarding properties of the particles such as size, mass, number concentration, and concentration. Such analysis can advantageously be performed under highly dilute conditions.

[0071] In accordance with the method(s) of the present invention, the size of the deviation(s) correspond to the size (and mass) of the detected particles. In particular, the area under the deviation(s) is suitably proportional to particle size and particle mass. The area under the deviation(s) is straight forward for a skilled practitioner to measure, and involves comparisons between the deviation(s) and an extrapolation of the base-line current. Such comparisons may suitably be performed by a computer running pursuant of computer software.

Particle Detection

[0072] The present invention provides a method of detecting (and/or identifying) a particle in a sample, the method comprising:

i) applying an electric potential difference across the sample between a working electrode and a counter electrode, in the presence of an electrolyte, to allow an electric current to flow through the sample;

ii) monitoring the electric current over time for an electrochemical response caused by the particle colliding with the working electrode and being itself oxidised or reduced; and

iii) determining the presence of the particle(s) in the sample by the observance of an electrochemical response.

[0073] Detection of particles may suitably include recognising a collision event (from current deviation(s)), involving collision of a particle with one of the electrodes, preferably the working electrode. Detection may suitably involve recognising multiple collision events. Identification of particles may suitably comprise identifying the redox potential (in some cases the approximate redox potential) of the particles. The (approximate) redox potential of the particles may be obtained by varying the electric potential difference to find the potential difference at which current deviations start or are at their maximum (e.g. where the largest spikes are obtained). The method may suitably comprise both detecting and identifying particles.

[0074] In a particular embodiment, the particles are pre-determined particles of interest (i.e. a particular pre-selected species of particles is under observation, and any other particles are to be considered auxiliary particulates excluded from observation). As such, the electric potential difference may be set to be near, at, or beyond the redox potential of the particles.

[0075] Detecting (or identifying) particles may suitably include selecting or tuning the electric potential difference for the particles of interest. In particular embodiments, selecting or tuning involves selecting or tuning the electric potential difference to be sufficient to allow for a detectable electrochemical response (i.e. to provide deviations relative to the base-line current) when the particle(s) collide with one of the electrodes. Selecting or tuning may suitably involve selecting or tuning the electric potential difference to the redox potential of the particle(s) of interest.

[0076] Applying an electric potential difference across the sample may suitably involve contacting the sample with working and counter electrodes in the presence of an electrolyte, and causing a current to flow between the electrodes (e.g. using a electrical power source connected to the electrodes, such as a potentiostat). Selecting/tuning the electric potential difference may suitably involve adjusting a potentiostat to supply the desired voltage, which may suitably include contacting the sample with an additional reference electrode and selecting a suitable electric potential difference relative to the reference electrode.

[0077] Monitoring the electric current for deviation(s) relative to a base-line current may occur every millisecond or faster.Monitoring may involve merely taking a current reading in real time. Monitoring may also suitably involve plotting a graphical trace of current

against time to enable deviations to be distinguished from the base-line current. Alternatively, distinguishing of deviations from the base-line current may be performed by a computer programmed by computer software and suitably configured to sample the current over time.

[0078] Determining the presence of the particle(s) in the sample may suitably involve observing a sudden deviation in the current reading taken in real time. For instance, a current reader may either display the current such that a sudden deviation can be physically recognised by an observer, or alternatively the current reader may provide an indication that the particle(s) have been detected. In certain embodiments, determining the presence of the particle(s) comprises physically observing a graphical trace and recognising current deviation(s) relative to the base-line current. Alternatively, determining the presence of particle(s) may comprise a computer programmed with computer software recognising a sudden fluctuation(s) in current and reporting the presence of the particle(s) in response to the fluctuation(s).

[0079] Detecting (or identifying) two or more species of particles may suitably comprise a first run followed by a second and/or subsequent run(s), wherein the first run comprises detecting (or identifying) a first species of particle at a first electric potential difference threshold, and the second and/or subsequent run(s) comprise detecting (or identifying) a second and/or subsequent species of particle at a second and/or subsequent electric potential threshold. Determining the presence of the second and/or subsequent species of particle may suitably comprise subtracting data (e.g. collision frequency data) obtained from the first run from data obtained from the second and/or subsequent run(s). The subtracting of data may suitably be carried out by a computer programmed with computer software.

[0080] In certain embodiments, for instance, where identification the particles present is desired, the method may comprise performing multiple runs at different electric potential differences. For instance, the method may comprise adjusting the electric potential difference whilst screening for the onset of current deviations, and identifying the particles from either the voltage at which the onset of such current deviations occur or another suitable voltage at which current deviations are detectable (e.g. where the size of the current deviations is at a maximum). In a particular embodiment, the electric potential difference is changed incrementally (e.g. changed gradually from a low potential difference to a high potential difference or visa versa) for each subsequent run, in so doing performing a sweep of electric potential differences in search of detectable particles. If particles are present, the method may suitably comprise recognising the particles from deviations relative to the base-line current (i.e. from collision events)

occurring at a particular electric potential difference appropriate for that particular species of particle. The method may further comprise identifying the species of particle by reference to the electric potential difference at which said species of particle are detected. Suitably such identification involves recognising a correspondence between the electric potential difference at the redox potential of the particular species of particle. As such, the method may allow for characterisation of a plurality of species of particle in a sample. Once the species of particles present have been identified, the electric potential difference can then be appropriately tuned for analysis of the particles in accordance with any of the other method(s) of the present invention.

Particle Property Measurements

[0081] The present invention provides a method of measuring a property of a particle in a sample, the method comprising:

i) applying an electric potential difference across the sample between a working electrode and a counter electrode, in the presence of an electrolyte, to allow an electric current to flow through the sample;

ii) monitoring the electric current over time for an electrochemical response caused by the particle colliding with the working electrode and being itself oxidised or reduced; and

iii) determining a property of the particle in the sample from the electrochemical response.

[0082] Measuring a property of particle(s) in a sample may suitably include selecting or tuning the electric potential difference for the particles of interest as described above in relation to detecting particles.

[0083] The property to be measured may suitably be one or more properties selected from the group including particle size, particle mass, particle size distribution, particle mass distribution, particle shape, surface area, concentration, number concentration, weight concentration, redox potential, purity, identity of contaminants, catalytic activity, dissolution potential, aggregation state and agglomeration state.

[0084] In preferred embodiments, the property to be measure is one or more properties selected from the group including particle size, particle mass, particle size distribution, particle mass distribution, particle concentration, particle number concentration, particle weight concentration, and redox potential.

[0085] In an embodiment, the method of measuring a property of a particle is a method of measuring the size (or mass) of a particle in a sample, wherein step (iii) comprises determining the size (or mass) of the particle in the sample from the size of the electrochemical response. Determining particle size(s) and/or particle mass(es) may include correlating the size(s) of the electrochemical response (current deviations) to particle size(s) and/or particle mass(es). This may involve translating the size(s) of the deviation(s) to particle size(s), for instance, through applying a mathematical equation or algorithm. The size(s) of the deviation(s) may involve calculating the area(s) between the deviation(s) and the base-line current (e.g. area(s) under peak(s) or spike(s)). Such a calculation may suitably involve extrapolating the base-line current based on a projected continuous function. Calculating the area(s) may involve integration. In a particular embodiment, the mathematical equation used to convert deviation(s) sizes(s) to particle size(s) is shown as Equation (1):

$$Q_{max} = \frac{4F\pi\rho r_{np}^{3}}{3A_r u} \quad (1)$$

wherein Q_{max} is the charge (in coulombs, C) typically characterised as the area under a peak or spike, r_{np} is the spherical radius of the particle(s) (i.e. calculations yield particle size results based on an assumed spherical form), ρ is the bulk density (kg/m^3) of the particles, A_r is relative atomic mass of the species constituting the particles, u is the unified atomic mass unit and is constant at $1.660\,538\,782(83)\times10^{-27}$ kg, F is the faraday constant - 96,485.3399(24) C mol^{-1}.

[0086] Using equation (1), particle size(s) can be derived from the charge surge(s) measured as deviation(s) or spikes from the base-line current. The size of the deviation(s) is therefore proportional to the particle size(s) (in this case the particle volume). Repeated particle size measurements over time can show particle size distribution, which may be suitably plotted graphically, for instance, as counts at a particular particle size, to produce a particle size distribution curve. The more measurements that are taken, the more accurate the distribution curve will be. An average particle size can be readily determined by the skilled practitioner from the particle size distribution.

[0087] Determining particle mass(es) may involve correlating the size(s) of the deviation(s) relative to the base-line current to particle mass(es). This may involve converting the size(s) of the deviation(s) to particle mass(es), for instance, through

applying a mathematical equation or algorithm. The sample mathematical equation may be used as for particle size measurement, since particle volume ($4\pi r_{np}^3/3$) can be converted to mass by multiplying particle size by bulk density (ρ). As per measuring particle size(s), particle mass measurements over time can show particle mass distribution, which again may be suitably plotted graphically, for instance, as counts at a particular particle mass, to produce a particle mass distribution curve. An average particle mass can be readily determined by the skilled practitioner from the particle mass distribution.

[0088] In particular embodiments where larger particle sizes are evident, the method may suitably comprise agitating the sample, suitably during performance of any of the methods of the present invention. This may compensate for any break down in Brownian motion particle dynamics, for instance, where gravity becomes a factor motion, particularly for larger particles.

[0089] In another embodiment, the method of measuring a property of a particle is a method of measuring the concentration of particles in a sample, wherein step (iii) comprises determining the concentration of particles in the sample from the frequency of the electrochemical response. Determining concentration may involve correlating the frequency of the electrochemical responses to particle concentration. Determining concentration may involve correlating the frequency of the deviations or spikes to particle concentration, including particle number concentration, particle weight concentration, particle molar concentration. Particle number concentration refers to the number of particles as such per unit volume. Particle weight concentration refers to the weight of particles per unit volume, whilst particle molar concentration refers to the number of moles of the species comprising the particles per unit volume.

[0090] As such, measuring concentration may suitably involve counting the number of deviations (or spikes) occurring in a given time, and calculating collision frequency therefrom (collisions per second) – collision frequency is proportional to the deviation/spike count. Preferably a further calculation is conducted to provide collision frequency per surface area of the relevant electrode (the electrode at which collisions are detected - the working electrode), since an electrode having a larger surface area will inevitably register more frequent collisions at a given concentration. Herein, the surface area of the electrode refers to the surface area which is submerged within the sample Collision frequency per electrode surface area can be directly converted into a particle number concentration. This can be achieve where the linear responsiveness of number concentration to collision frequency per electrode surface area (i.e. linear gradient) is already known – this may be established from previous calibration tests using known

concentrations of the particles which are the same as the particles subject to the method(s) of the present invention.

[0091] In particular embodiments, a linear relationship exists between collision frequency and particle number concentration at particle molar concentrations between 0 and 100μM, suitably between 0 and 1 μM, suitably between 0 and 1nM, suitably between 0 and 100pM, suitably between 0 and 50pM, suitably between 0 and 40pM. The number and molar concentrations are related by Avogadro's number. If desired, the sample may be diluted by a pre-determined dilution factor to yield a particle concentration within the abovementioned ranges where the linear relationship is strongest. After performing the method of measuring particle number concentration upon the diluted sample, the number concentration of the original undiluted sample may then be calculated using the dilution factor.

[0092] In particular embodiments, a smaller working electrode is employed to reduce the number of overlapping deviations/spikes registered. This may suitably assist in the counting of the deviations so as to provide more accurate particle concentration measurements. The skilled practitioner would be readily able to adjust the concentration and/or size of the electrode to obtain the most accurate measurements in this regard.

[0093] Particle number concentrations may be readily converted to a weight concentration (and thereafter a molar concentration) by multiplying the particle number concentration by the average particle mass.

[0094] In an embodiment, the method of measuring a property of a particle is a method of measuring the redox potential of a particle, wherein step (iii) includes determining the redox potential of the particle by reference to the electric potential difference beyond which an electrochemical response is observed. Determining the redox potential of the particles may suitably involve first detecting (or identifying) the particles, as hereinbefore described, and determining the redox potential by reference to the electric potential difference at which the particles are detected (or identified). Determining redox potential may additionally involve tuning the electric potential difference, once the particles have been detected, to an electric potential difference at which registered deviations from the base-line current are at a maximum (i.e. the voltage at which collision events produce the largest possible signal response) or have their onset.

[0095] In certain embodiments, the method may comprise measuring a property of two or more species of particles, comprising a first run followed by a second and/or subsequent run(s), wherein the first run comprises measuring a property of a first species of particle at a first electric potential difference threshold, and the second and/or

subsequent run(s) comprise measuring a property of a second and/or subsequent species of particle at a second and/or subsequent electric potential threshold. Determining the property of the second and/or subsequent species of particle may suitably comprise subtracting data (e.g. collision frequency data) obtained from the first run from data obtained from the second and/or subsequent run(s). The subtracting of data may suitably be carried out by a computer programmed with computer software.

[0096] The method may comprise performing multiple runs at different electric potential differences. In a particular embodiment, the electric potential difference is changed incrementally (e.g. changed gradually from a low potential difference to a high potential difference or visa versa) for each subsequent run, in so doing performing a sweep of electric potential differences. If particles are present, the method may suitably comprise recognising the particles from deviations relative to the base-line current (i.e. from collision events) occurring at a particular electric potential difference appropriate for that particular species of particle, and measuring a property of said particles. The method may further comprise identifying the species of particle by reference to the electric potential difference at which said species of particle was detected. Suitably such identification involves recognising a correspondence between the electric potential difference and the redox potential of the particular species of particle. As such, the method may allow for characterisation of a plurality of species of particle in a sample. Once the species of particles present have been identified, the electric potential difference can then be appropriately tuned to for analysis of the particles in accordance with the methods of the present invention.

[0097] As methods of the present invention destroy or modify the particles, data obtained earlier in the time cycle tends to more accurately reflect the real state of the sample than data obtained after a significant amount of time has elapsed. Therefore, it may be desirable to refresh the sample after a certain time has elapsed in order to obtain a statistically representative sample of data. This may be achieved by running the methods for a pre-determined time before refreshing the sample (wherein the refreshed sample is identical to the original sample before it was subjected to the methods). Alternatively, the sample may be provided as a continuous flow so that any distortions in the data arising from the depletion of particles is minimised.

Particle Modification (or Destruction) and Cleaning of Sample

[0098] The present invention provides a method of modifying (or destroying) a particle or particles in a sample, and/or a method of cleaning a sample, the method comprising:

i) applying an electric potential difference across the sample, in the presence of an electrolyte, to allow an electric current to flow across the sample and cause the particle or particles themselves to be oxidised or reduced on colliding with the working electrode;

ii) optionally:

a. monitoring over time the electric current for deviations relative to a base-line current caused by particles colliding with the working electrode and being themselves oxidised or reduced; and

b. determining whether the particle or particles have been modified (or destroyed) from changes in the frequency of the deviations relative to the base-line current.

[0099] Modifying (or destroying) particles in sample may suitably include selecting or tuning the electric potential difference for the particles of interest as described above in relation to detecting particles.

[00100] In a particular embodiment, the method additional comprises measuring the concentration of particles in a sample as hereinbefore described. The method may additionally include monitoring the modification or destruction of the particles using real time concentration measurements; and then terminating the current flow when a predetermined concentration of the particles is reached. In an embodiment, the predetermined concentration is substantially zero.

[00101] In a particular embodiment, the method comprises processing the modified particles or processing a by-product of the destroyed particles. In some embodiments, such processing suitably comprises any one or combination of steps including:

a. collecting the modified particles or by-product of the destroyed particles;

b. removing the modified particles or by-product of the destroyed particles from the sample (e.g. decanting or filtering);

c. disposing of the modified particles or by-product of the destroyed particles;

d. restoring the modified particles or by-product of the destroyed particles to the original redox state;

e. allowing the modified particles or by-product of the destroyed particles to settle by gravity.

In a particular embodiment, the method additionally comprises collecting the sample remaining after the particles have been destroyed. The cleaning of the sample may comprise recovering the sample following performance of the method. In particular embodiments, the sample may suitably have a volume of at least 0.1μL, suitably at least 1mL, suitably at 1L. The sample may comprise at least part of the contents of a reservoir, lake, river, sea, or ocean.

Cleaned Sample

[00102] The present invention provides a cleaned sample obtained by, obtainable by, or directly obtained by the above described method of cleaning a sample. The cleaned sample may suitably be recreational water or drinking water. The cleaned sample may suitably be mineral water, such as bottled mineral water. The clean sample may suitably be water for use in preparing beverages.

[00103] The present invention also provides a beverage comprising the cleaned sample.

Particle Analyser

[00104] The present invention provides an apparatus configured to carry out any one of the methods of the first to fifth aspects. The apparatus may suitably be a particle analyser. The particle analyser may suitably be operable to detect (or identify), measure a property of, measure the size(s) of, measure the concentration of, or modify (or destroy) particles in a sample.

[00105] In a particular embodiment the particle analyser comprises a working electrode and a counter electrode, each connected or connectable to an electrical power source. The working electrode may suitably be the anode. The particle analyser may also comprise a reference electrode may also be present. The particle analyser may be suitably be arranged so that it creates a faraday cage in use.

[00106] In a particular embodiment, the working electrode is suitably a glassy carbon (GC) electrode (preferably bare). The counter electrode is suitably a graphite electrode (preferably a graphite rod). The reference electrode, when present, is suitably either an Ag/AgCl or calomel reference electrode.

[00107] In a particular embodiment, the power source includes a potentiostat. In use, the potentiostat is preferably arranged to supply an electric potential difference across the sample. In preferred embodiments, the potentiostat is adjustable to vary the electric potential difference.

[00108] The particle analyser is preferably arranged to detect and record collision events of particles when the electric potential difference is sufficient to oxidise particles colliding with the electrode in the sample. The particle analyser is preferably arranged not to register collision events of particles when the electric potential difference is not sufficient to oxidise the particles of interest. The particle analyser preferably comprises a current monitor, which is arranged to record current over time. The particle analyser is preferably configured to recognise and/or extrapolate a base-line current. The particle analyser is preferably arranged to record and/or quantify deviations relative to a base-line current.

[00109] The particle analyser is preferably configured to respond to a collision event (i.e. between a particle and the working electrode), for example, by reporting a deviation from the base-line current. The particle analyser may thus comprise a reporting means. Reporting may merely involve activation or operation of the reporting means, which may suitably comprise an alarm, a flashing light, a light pulse (e.g. which pulse upon each collision, optionally the brightness may be indicative of the registered current surge), a graphical trace (where deviation a base-line current indicates a collision), or any other suitable means of reporting. The particle analyser is preferably configured to deduce and/or report a one or more properties of the particles, as described hereinbefore.

[00110] In a particular embodiment, the particle analyser is arranged to perform one or more of the following steps:

a) Receive input *via* a user interface regarding the number of method runs to carry out;

b) Receive input *via* a user interface regarding a selected electric potential difference threshold for the or each method run;

c) Receive input *via* a user interface regarding calibration data for the calculation of one or more properties of the particles;

d) Operate to perform one or more method run, preferably based on the input from step a);

e) Operate at an electric potential difference threshold, preferably based on the input from step b), optionally using a pre-programmed potential difference sweep (e.g. when identifying what unknown particles are present);

f) Receive a current signal in real time during the performance of the one or more method runs of step d);

g) Record the current signal over time;

h) Recognise and/or extrapolate a base-line current;

i) Recognise deviation(s) from the base-line current;

j) Reduce noise in the current signal, preferably using an FFT filter, preferably by selectively blocking the frequency of the electricity mains supply (e.g. 50 Hz) and suitably associated multiples up to 200 Hz.;

k) Operate to report a collision event in response to deviation(s) in the base-line current;

l) Deduce and/or calculate one or more properties of the particles based on the recorded current signal over time;

m) Integrate the deviation(s) or peak(s) to deduce the charge passed in each peak;

n) Plot the charges ascertained from step m) as a histogram and/or plot a distribution of charges;

o) Deconvolute the distribution of step n) into Gaussian distributions;

p) Convert the distribution of step n) or o) into a particle size or particle mass distribution;

q) Report one or more properties of the particles.

Computer-Implementation and Computer Software

[00111] The present invention provides a computer programmed with computer software to implement one or more steps of any of the methods of the first to fifth aspects.

[00112] In a particular embodiment the particle analyser comprises the computer programmed with computer software to perform one or more of the following steps:

a) Receive input *via* a user interface regarding the number of method runs to carry out;

b) Receive input *via* a user interface regarding a selected electric potential difference threshold for the or each method run;

c) Receive input *via* a user interface regarding calibration data for the calculation of one or more properties of the particles;

d) Operate the particle analyser to perform one or more method run, preferably based on the input from step a);

e) Operate the particle analyser at an electric potential difference threshold, preferably based on the input from step b), optionally using a pre-programmed potential difference sweep (e.g. when identifying what unknown particles are present);

f) Receive a current signal in real time during the performance of the one or more method runs of step d);

g) Record the current signal over time;

h) Recognise and/or extrapolate a base-line current;

i) Recognise deviation(s) from the base-line current;

j) Reduce noise in the current signal, preferably using an FFT filter, preferably by selectively blocking the frequency of the electricity mains supply (e.g. 50 Hz) and suitably associated multiples up to 200 Hz.;

k) Operate the particle analyser to report a collision event in response to deviation(s) in the base-line current;

l) Deduce and/or calculate one or more properties of the particles based on the recorded current signal over time;

m) Integrate the deviation(s) or peak(s) to deduce the charge passed in each peak;

n) Plot the charges ascertained from step m) as a histogram and/or plot a distribution of charges;

o) Deconvolute the distribution of step n) into Gaussian distributions;

p) Convert the distribution of step n) or o) into a particle size or particle mass distribution;

q) Report or operate the particle analyser to report one or more properties of the particles.

[00113] Alternatively the particle analyser is connected or connectable to a computer programmed as described above.

[00114] The present invention also provides a computer-readable medium comprising the computer software described above.

EXPERIMENTAL

General Materials and General Methodology

[00115] AgNPs of diameter ranges 20-50nm and 80-120nm were synthesized according to the procedure below. Sodium dihydrogen citrate ($NaC_6O_7H_7$, Aldrich, >99.5%) and KCl (Riedel-de-Haan, >99.5%) were used as received. All solutions (10mM $NaC_6O_7H_7$ and 90mM KCl unless stated) were made using ultrapure water of resistivity ≥18.2 MΩ.cm (Millipore) and degassed thoroughly with N_2 (oxygen-free, BOC Gases plc) and an atmosphere of N_2 maintained during the experiment. Unless otherwise stated, all electrochemical experiments were conducted within a faraday cage using glassy carbon working electrodes (BASI), Ag/AgCl or calomel reference electrode (Radiometer, Copenhagen), and a graphite rod counter electrode. The potentiostat was a μAutolab III (Metrohm-Autolab BV, Utrecht, Netherlands). Impact spikes were analysed using Origin v.8.1 (www.OriginLab.com) for spike identification and integration, and gaussian deconvolution of radius distribution.

Example 1 – Synthesis of Nanoparticles

[00116] The procedure for the preparation of spheroid AgNPs was taken from the work of Pyatenko et al and adapted to synthesize batches of nanoparticles of varied size (F.W. Campbell, S.R. Belding, R. Baron, L. Xiao, R.G. Compton, *J. Phys. Chem. C* 2009, *113*, 9053; A. Pyatenko, M. Yamaguchi, M. Suzuki, *J. Phys. Chem. C* 2007, *111*, 7910; A. Pyatenko, M. Yamaguchi, M. Suzuki, *J. Phys. Chem. B* 2005, *109*, 21608). The nanoparticles are synthesized via citric reduction of a silver salt, where a citrate molecule acts as a reducing and capping agent at high temperatures. 25 mL of a 0.01 M silver nitrate ($AgNO_3$) solution was added to 225 mL of distilled water in a clean 250 mL flask. The flask was then heated in a bath at 100 °C with vigorous magnetic stirring. A solution of trisodium citrate ($C_6H_5Na_3O_7$, Aldrich, 99%) was prepared by dissolving 1 g of the salt in 100 mL of distilled water. When the solution reached boiling point, 5 mL of the citrate solution was then added to the flask. Boiling was then allowed to continue for 1 h, and the reaction mixture was then allowed to cool in a cold water bath. This first procedure generated the initial colloid of AgNPs with the smallest diameter (25-40 nm). The initial colloid was used as a seed particle in subsequent syntheses, allowing growth of larger nanoparticles. The synthesis of nanoparticles of 80-120 nm was achieved by adding a small volume (100 μL) of the initial colloidal solution and 5 mL of citrate solution to 25 mL of (0.01 M) $AgNO_3$ in 225 mL of distilled water as it began to boil and continued for 1 h before cooling the flask. It was necessary for each synthesis to separate, wash, and redisperse the nanoparticles to remove any excess chemicals. One milliliter portions of the reaction solution were pipette into 1.5 mL centrifuge tubes. These tubes were centrifuged at 14 000 rpm for 20 min. The supernatant liquid was then pipette off, and the remaining solid at the bottom of the centrifuge tube was redispersed in 1 mL of water. This was repeated three times to thoroughly wash the nanoparticles. After they were washed, the nanoparticles were redispersed in 1.5 mL of water, giving a yellow colloidal solution, and stored away from direct light.

Example 2 – Characterisation of Nanoparticles

[00117] Colloidal AgNPs in solution were first characterized by UV-vis spectroscopy. As each of the samples was of a different particle size, it was possible to characterize their relative size in terms of their optical absorbance. Smaller particles exhibit an absorbance at shorter wavelengths, and an increase in size causes the

absorbance to shift to longer wavelengths. This is due to the surface Plasmon resonance of conduction band electrons associated with colloidal silver nanoparticles.

[00118] Further characterization was carried out using scanning electron microscopy. Images of the particles showed them to be roughly spherical in shape as well as provided the actual sizes of the nanoparticles in each sample as follows: sample 1, 20-40 nm, and sample 2 80-120 nm. The analysis also provided estimates of the number of NPs per unit area. SEM was performed using a copper grid and a carbon film substrate.

Example 3 – Characterisation of AgNPs *via* instantaneous oxidation during AgNP-electrode collisions

[00119] Fig. 1a shows chronoamperometric profiles showing oxidative collisions of AgNPs in citrate solution (enlarged inset showing detailed impact spikes).

[00120] Fig. 1b shows an overlay plot of a stripping voltammogram for an AgNP-modified GC electrode (left axis) and the impact frequency (right axis) showing the onset of potential of spikes.

[00121] Fig. 1c shows the distribution of NP radii inferred from Q *via* equation (1) with deconvolution.

[00122] In accordance with embodiments of the present invention, the characterisation of AgNPs was conducted using direct methods involving the *in situ* instantaneous oxidation of the AgNPs during collisions according to the half equation:

$$Ag(np) - e^- = Ag^+ (aq); \text{ or}$$

$$Ag\ (np) - e^- + KCl\ (aq) = AgCl\ (s) + K^+ (aq)$$

[00123] First, an AgNP-modified GC electrode (diameter 3mm) was scanned anodically across a range of potentials in in a solution of 10 mM sodium citrate and 90 mM KCl, as per the general methodology above. Fig. 1b presents the stripping voltamogram and shows the electrode potential of the AgNPs (as per those oxidised on the electrode) to approximately +0.05 V (oxidation onset) relative to a saturated Ag/AgCl electrode.

[00124] Next, this experiment was repeated with a bare GC microelectrode (11μm radius) in the presence of dispersed AgNPs (20-50nm diameter). Under potentiostatted conditions, oxidative current spikes were observed (Fig. 1a) showing for the first time that direct oxidation of metal NPs during collision events is both observable and quantitative. The onset of spikes was found to vary with potential, since Fig. 1b shows by the solid squares that spikes are observable at the same onset potential as recorded in relation to the AgNP-modified electrode stripping experiment.

[00125] Assuming that the NPs are spherical (radius r_{np}), the maximum charge passed due to completion oxidation of the AgNP is given by equation (1) described above. Using this equation, Fig. 1c shows the distribution of radii obtained from the analysis of over 1500 impacts, which can be deconvoluted into sub-distributions of radii 13, 26, and 39 nm, corresponding to single NPs and agglomerates.

Summary

[00126] The results presented in Example 3 and Fig. 1(a)-(c) demonstrate the simplicity and effectiveness of the present invention in detecting and identifying AgNPs (*via* comparison of the onset voltammetry of AgNPs) as well as simultaneously determining their size range (by analysis of the charge passed per current spike). The results also show the significant advantages provided by the present invention over electrochemical methods which do not involve oxidation or reduction of particles during particle-electrode collisions. In particular, methods of the present invention provide higher signal strengths than prior art methods (where direct particle oxidation does not occur), as demonstrated by the favourable signal to noise ratios for Fig. 1a (oxidative collisions). Moreover, higher signal strengths improve detectability, especially at high dilution and also allow for greater accuracy when making quantitative measurements, such as particle size measurements. The principles naturally apply to other metal NPs as well as mixed NP systems for direct application in public health and environmental monitoring.

CLAIMS

1. A method of detecting (and/or identifying) a particle in a sample, the method comprising:

i) applying an electric potential difference across the sample between a working electrode and a counter electrode, in the presence of an electrolyte, to allow an electric current to flow through the sample;

ii) monitoring the electric current over time for an electrochemical response caused by the particle colliding with the working electrode and being itself oxidised or reduced; and

iii) determining the presence of the particle(s) in the sample by the observance of an electrochemical response.

2. A method of measuring a property of a particle in a sample, the method comprising:

i) applying an electric potential difference across the sample between a working electrode and a counter electrode, in the presence of an electrolyte, to allow an electric current to flow through the sample;

ii) monitoring the electric current over time for an electrochemical response caused by the particle colliding with the working electrode and being itself oxidised or reduced; and

iii) determining a property of the particle in the sample from the electrochemical response.

3. The method as claimed in claim 2, wherein the property to be measured is one or more properties selected from the group including particle size, particle mass, particle size distribution, particle mass distribution, particle shape, surface area, concentration, number concentration, weight concentration, redox potential, purity, identity of contaminants, catalytic activity, dissolution potential, aggregation state and agglomeration state.

4. A method of modifying (or destroying) a particle or particles in a sample, and/or a method of cleaning a sample, the method comprising:

i) applying an electric potential difference across the sample between a working electrode and a counter electrode, in the presence of an electrolyte, to allow an electric current to flow through the sample and

cause the particle or particles themselves to be oxidised or reduced on colliding with the working electrode; and

ii) optionally:

a. monitoring over time the electric current for an electrochemical response caused by particles colliding with the working electrode and being themselves oxidised or reduced; and

b. determining whether the particle or particles have been modified (or destroyed) from a change in the frequency of the electrochemical response.

5. The method as claimed in any preceding claim, wherein the electrochemical response arises from a change in the redox state of the particle.

6. The method as claimed in any preceding claim, comprising causing the particle to be oxidised to provide the electrochemical response.

7. The method as claimed in any preceding claim, wherein the concentration of the particles in the sample is below 10μM.

8. The method as claimed in any preceding claim, wherein the sample comprises particles which are dispersed in a liquid medium.

9. The method as claimed in claim 8, wherein the particles are dispersed particles with a particles size of at most 100nm.

10. The method as claimed in any of claims 8 or 9, wherein the particles are metallic particles.

11. The method as claimed in claim 10, wherein the metal of the metallic particles is selected from the group including silver, titanium, zinc, and gold.

12. The method as claimed in claim 11, wherein the metal of the metallic particles is silver.

13. The method as claimed in any preceding claim, comprising selecting or tuning the electric potential difference for the particles of interest.

14. The method as claimed in any preceding claim, comprising identifying the species of particles by reference to the electric potential difference beyond which said particles are detected, and recognising the correspondence between the electric potential difference and the redox potential of the particular species of particle.

15. The method as claimed in any preceding claim, comprising performing multiple runs at different electric potential differences.

16. The method of any preceding claim, comprising modifying (or destroying) particles in sample as claimed in claim 5; and further processing the modified particles or a by-product of the destroyed particles by any one or combination of the following steps including:

a. collecting the modified particles or by-product of the destroyed particles;

b. removing the modified particles or by-product of the destroyed particles from the sample (e.g. decanting or filtering);

c. disposing of the modified particles or by-product of the destroyed particles;

d. restoring the modified particles or by-product of the destroyed particles to the original redox state;

e. allowing the modified particles or by-product of the destroyed particles to settle by gravity.

17. A particle analyser operable to carry out any of the method(s) of any preceding claim.

18. The particle analyser as claimed in claim 17, wherein the particle analyser is arranged to perform one or more of the following steps:

a) Receive input *via* a user interface regarding the number of method runs to carry out;

b) Receive input *via* a user interface regarding a selected electric potential difference threshold for the or each method run;

c) Receive input *via* a user interface regarding calibration data for the calculation of one or more properties of the particles;

d) Operate to perform one or more method run, preferably based on the input from step a);

e) Operate at an electric potential difference threshold, preferably based on the input from step b), optionally using a pre-programmed potential difference sweep (e.g. when identifying what unknown particles are present);

f) Receive a current signal in real time during the performance of the one or more method runs of step d);

g) Record the current signal over time;

h) Recognise and/or extrapolate a base-line current;

i) Recognise deviation(s) from the base-line current;

j) Reduce noise in the current signal, preferably using an FFT filter, preferably by selectively blocking the frequency of the electricity mains supply (e.g. 50 Hz) and suitably associated multiples up to 200 Hz.;

k) Operate to report a collision event in response to deviation(s) in the base-line current;

l) Deduce and/or calculate one or more properties of the particles based on the recorded current signal over time;

m) Integrate the deviation(s) or peak(s) to deduce the charge passed in each peak;

n) Plot the charges ascertained from step m) as a histogram and/or plot a distribution of charges;

o) Deconvolute the distribution of step n) into Gaussian distributions;

p) Convert the distribution of step n) or o) into a particle size or particle mass distribution;

q) Report one or more properties of the particles.

19. A cleaned sample obtainable by the method of cleaning a sample according to claim 5.

20. A computer programmed with computer software to implement one or more steps of any of the methods of claims.

21. A computer-readable medium comprising the computer software described in claim 20.

22. A method, particle analyser, cleaned solution, computer, or computer-readable medium as substantially hereinbefore described with reference to the Examples and Figures.

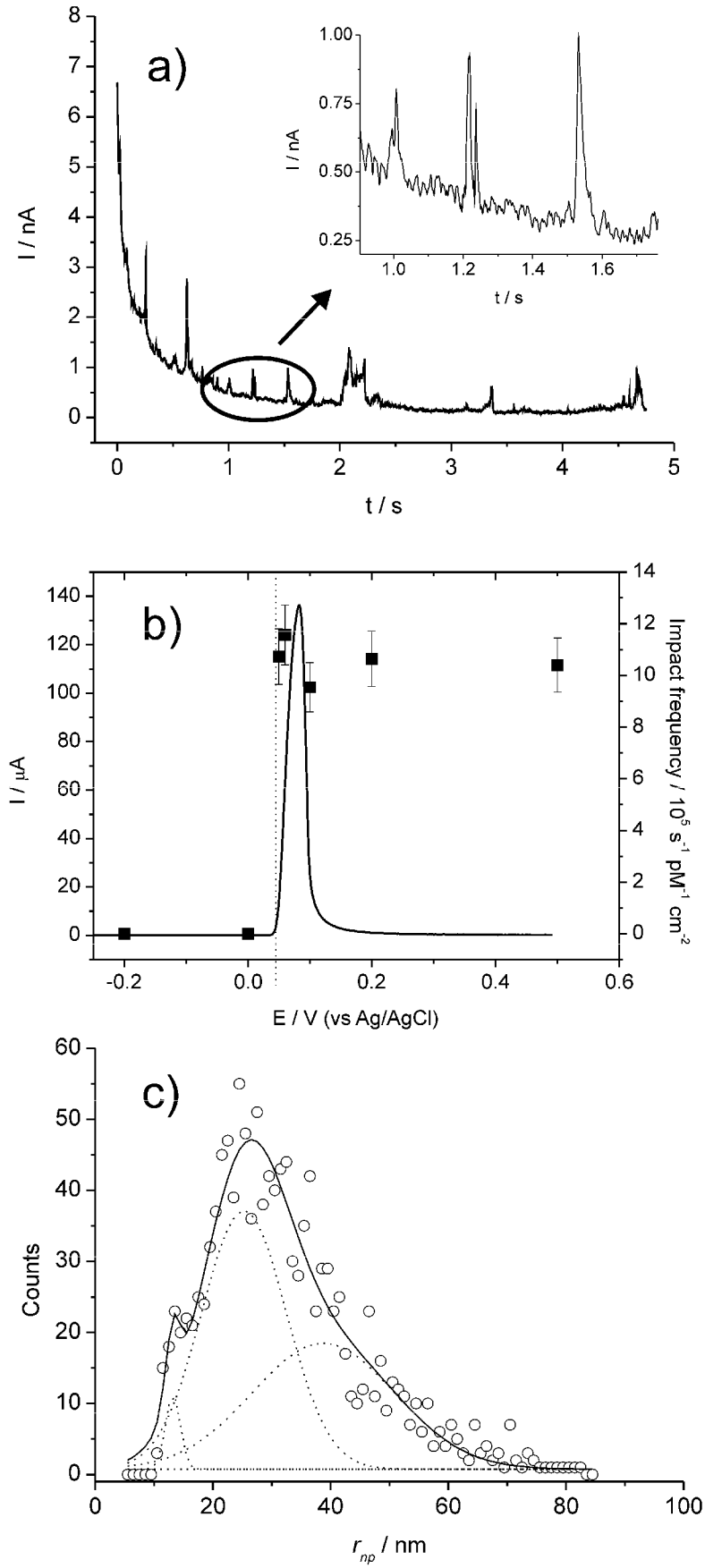
a)
I / nA
t / s
1.00
0.75
0.50
0.25
1.0
1.2
1.4
1.6
b)
I / μA
Impact frequency / 10^5 s^-1 pM^-1 cm^-2
E / V (vs Ag/AgCl)
c)
Counts
r_np / nm

Figure 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2012/050371

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N27/42 G01N15/10
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/065371 A1 (XIAO XIAOYIN [US] ET AL) 12 March 2009 (2009-03-12) paragraph [0054] - paragraph [0058]; figures 4A, 4B -----	1-21
X	US 2009/095643 A1 (SVETLICIC VESNA [HR] ET AL) 16 April 2009 (2009-04-16) paragraph [0073] - paragraph [0074]; figures 8,9 -----	1,2,4, 17-21
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 May 2012	05/06/2012

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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2012/050371

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCHOLZ F ET AL: "Detection of the adhesion events of dispersed single montmorillonite particles at a static mercury drop electrode", ELECTROCHEMISTRY COMMUNICATIONS, ELSEVIER, AMSTERDAM, NL, vol. 6, no. 9, 27 July 2004 (2004-07-27), pages 929-933, XP002396467, ISSN: 1388-2481, DOI: 10.1016/J.ELECOM.2004.07.005 the whole document -----	1,2,17, 18,20,21
X	M HEYROVSKY, J JIRKOVSKY AND B R MÜLLER: "Polarography and voltammetry of aqueous colloidal SnO2 solutions", LANGMUIR, vol. 11, 30 November 1995 (1995-11-30), pages 4293-4299, XP002675897, DOI: 10.1021/la00011a021 page 4297, column 2 - page 4298, column 1; figure 3 -----	1,2,4

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2012/050371

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