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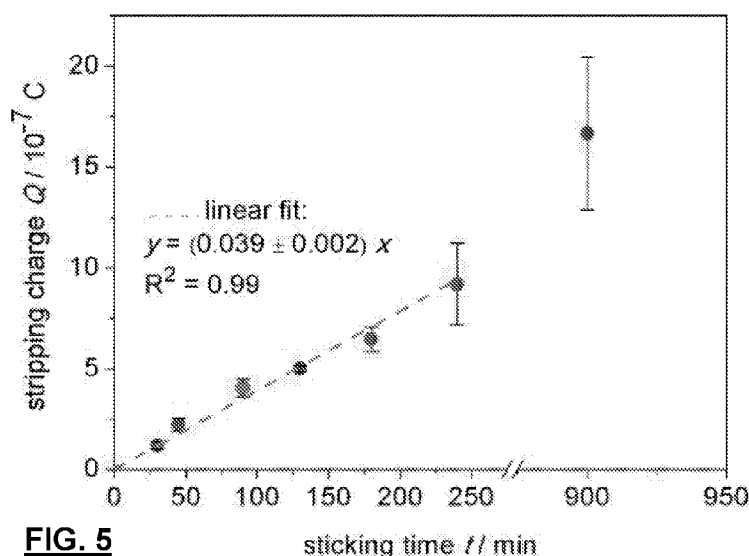
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**FIG. 5**

(57) Abstract: The present application provides a method of detecting nanoparticles in a sample comprising: a. immersion of a substrate in the sample, wherein, if the nanoparticles are present in the sample, at least some of the nanoparticles are immobilized on the substrate; b. analysing the substrate to determine the presence of, identity of the material of and/or quantity of the nanoparticles on the substrate and/or in the sample. A device for carrying out the method is also disclosed. In a preferred embodiment, silver nanoparticles (AgNPs) are detected in situ in the environment, eg. sea water, by immersing a cysteine modified glassy carbon (GC) electrode in a sample and detecting adsorbed silver using anodic stripping voltammetry (ASV).

Detection and quantification of nanoparticles

Field of the Invention

- 5 The present invention relates to the detection and/or quantification of nanoparticles, including, but not limited to, metal nanoparticles (e.g. silver nanoparticles), metal oxide nanoparticles, and organic nanoparticles.

Background

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Many nanoparticles are now in widespread use. For example, the antibacterial and antiseptic effect of silver nanoparticles (Ag NPs), in combination with their cost-efficient mass production, started the precedence of Ag NPs in a wide variety of consumer and medical products. Today's omnipresence of nanoparticles and the corresponding
15 massive release of nanoparticles into the environment in combination with their unknown effect on environmental systems, however, raise the demand for reliable and affordable techniques for their detection.

A number of nanoparticle impact-based methods has been successfully used to
20 determine the composition, concentration, size, surface charge density, adsorption and agglomeration of nanoparticles in various systems, including real environmental samples. A limitation of these methods, however, is that liquid samples have to be taken, transported and analysed, which bear the risk of causing changes to the sample, e.g. by altering the concentration or aggregation state. For example, if a nanoparticle
25 suspension was stored in a sample container, the nanoparticles may, over time, adsorb on the interior walls of the container, altering the concentration of the nanoparticles in suspension. It would be desirable to develop techniques to detect nanoparticles at a site of interest, in an accurate and efficient manner.

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Summary of the Invention

In a first aspect, there is provided a method of detecting nanoparticles in a sample comprising:

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- a. immersion of a substrate in the sample, wherein, if the nanoparticles are present in the sample, at least some of the nanoparticles are immobilized on the substrate;
 - b. analysing the substrate to determine the presence of, identity of the material of and/or quantity of the nanoparticles on the substrate and/or in the sample.

In a second aspect, there is provided a device for detecting nanoparticles in a sample, the device configured to:

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- a. immerse a substrate in the sample, wherein, if the nanoparticles are present in the sample, at least some of the nanoparticles would be immobilized on the substrate;
 - b. analyse the substrate to determine the presence of, identity of the material of and/or quantity of the nanoparticles on the substrate and/or
- 15 in the sample.

The present inventors consider that the method and device described herein can be used for nanoparticle detection, with potential application for long-term field studies. The main advantage thereby is that the sample can be taken on site. In one

20 embodiment, one can immerse the substrate, which may be termed a "sticky electrode", into the sample, wait for NPs from the sample to stick to the surface within a predetermined amount of time, which may be termed the "sticking time", and subsequently (either in field or laboratory) analyse the amount of nanoparticles immobilised on the electrode surface e.g. by anodic stripping voltammetry. Because

25 the nanoparticles can adhere or be immobilised on the electrode without a potential being applied to it, this enables a long sticking time and thus detection of NPs even from media with low concentrations of NPs. In the Examples below, the present inventors demonstrate the use of cysteine-modified glassy carbon electrodes for this purpose as an environmentally friendly modified electrode for silver nanoparticle (Ag

30 NP) detection and quantification. In an embodiment, the electrodes can be screen printed, disposable electrodes.

Brief Description of the Figures

Figure 1 shows two linear sweep voltammograms of a Ag NP modified GC (glassy carbon) electrode; the peak in the 1st sweep (black) suggests quantitative stripping of the Ag NPs from the electrode, which is in accordance with the lack of a stripping peak in the 2nd sweep (grey).

Figure 2 shows five linear sweep voltammograms showing the oxidative stripping of Ag NPs from GC electrodes (without any surface modification) after immersion in Ag NP containing 0.1 M NaClO₄ at open circuit potential (OCP) for x min sticking time: x = 60 (red), 125 (green), 900 (blue) and 1260 min (black). The grey line shows a blank scan of an unmodified GC electrode in 0.1 M NaClO₄; Inset: corresponding stripping charge as function of the sticking time. Mean value and standard deviation of each data point were obtained from at least three repeats.

Figure 3 shows three cyclic voltammograms recorded during the electrochemical modification of GC using a solution of 1 mM L-cysteine and 0.1 M NBu₄BF₄ in acetonitrile.

Figure 4 shows seven linear sweep voltammograms showing the oxidative stripping of Ag NPs from *cysteine-modified* GC electrode after immersion in Ag NP containing 0.1 M NaClO₄ at OCP for x min sticking time: x = 30 (red), 45 (green), 90 (dark cyan), 180 (blue), 240 (purple) and 900 min (black). The grey line shows a blank scan of the cysteine-modified GC electrode in 0.1 M NaClO₄.

Figure 5 shows stripping charge as function of the sticking time (immersion in Ag NP containing 0.1 M NaClO₄ at OCP) for a cysteine-modified GC electrode. Mean value and standard deviation of each data point were obtained from at least two repeats.

Detailed Description

The present invention relates to the first and second aspects described above. Optional and preferable features of the method and device are now described. Unless otherwise stated, any optional or preferred feature may be combined with any other

optional or preferred feature. All features are equally applicable to the method and the device.

In an embodiment, the substrate is immersed in the sample for a predetermined amount of time. The predetermined amount of time can be selected to allow sufficient nanoparticles to immobilise on the substrate to allow their detection and/or quantification in the analysis, for examples in an electrochemical analysis or other analytical technique, as described below. The predetermined amount of time may be at least 30 seconds, optionally at least 1 minute, optionally at least 5 minutes, optionally at least 15 minutes, optionally at least 30 minutes. The predetermined amount of time may be from 30 seconds to 10 hours, optionally from 30 seconds to 5 hours, optionally from 30 seconds to 4 hours, optionally from 15 minutes to 4 hours. The predetermined amount of time may be from 30 seconds to 10 minutes, optionally from 15 minutes to 4 hours, optionally from 15 minutes to 3 hours, optionally from 15 minutes to 1 hour. By immersing the substrate for a predetermined amount of time and then analysing the quantity of the nanoparticle material on the substrate, and using a predetermined relationship between the quantity of the nanoparticle material on the substrate and the concentration of nanoparticles in a reference sample, the concentration of nanoparticles in the sample can be determined.

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After immersion of the substrate in the sample, e.g. for the predetermined amount of time, the substrate may be removed from the sample, and then analysed. In an alternative embodiment, the substrate may remain in the sample while the analysis of the substrate is carried out. The analysis may involve an analytical technique that is useful in determining the identity of and/or quantity of the nanoparticle material on substrate. The analytical technique may involve electrochemical analysis of the nanoparticles immobilised on the substrate, and/or another analytical technique, including, but not limited to a spectroscopic analysis, such as a technique selected from x-ray photoelectron spectroscopy, ultraviolet-visible spectroscopy and IR spectroscopy; microscopic analysis, such as a technique selected from transmission electron microscopy or scanning electron microscopy; and mass spectrometry.

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In an embodiment, the method of detecting nanoparticles in the sample comprises:

- a. immersion of the substrate in the sample for a predetermined amount of time, wherein, if the nanoparticles are present in the sample, at least

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some of the nanoparticles are immobilized on the substrate; and optional removal of the substrate from the sample; and

- 5 b. electrochemically analysing the substrate, wherein the substrate acts as a working electrode, to determine the presence of, identity of the material of and/or quantity of the nanoparticles on the substrate and/or in the sample. The electrochemical analysis may involve using the substrate as a working electrode connected to a potentiostat. The substrate may be connected to a potentiostat in step (a), or step (b) may involve connecting the substrate to a potentiostat. In an embodiment, a potential is applied to the substrate while it is immersed in the sample in step a; this may assist in the immobilisation of the nanoparticles on the substrate. Preferably, during step (a), no potential is applied to the substrate, i.e. it is contacted with the sample under open circuit conditions.

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If an electrochemical analysis is used, the substrate will be suitable for use as an electrode. The electrochemical analysis may involve using the substrate, which was previously immersed in the sample, as a working electrode in an electrochemical analytical device, optionally with the device further comprising a counter electrode, and a reference electrode, applying a potential between the reference electrode and the working electrode, and monitoring the electrochemical response of the working electrode. The electrochemical analytical device may comprise a potentiostat.

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The counter electrode may be made of any suitable material, for example a metal or carbon. The counter electrode may be selected such that it is not itself oxidised or reduced under the conditions at which the electrochemical analysis is carried out. The counter electrode may comprise a metal selected from gold, silver, copper and platinum. The counter electrode may comprise a carbon-containing material, which may be selected from edge plane pyrolytic graphite, basal plane pyrolytic graphite, a glassy carbon, boron doped diamond, highly ordered pyrolytic graphite, carbon powder and carbon nanotubes.

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A reference electrode, for example a Ag/AgCl reference electrode or a saturated calomel reference electrode, may be connected to the working and/or counter electrodes as is known in the art. The working electrode, the counter electrode and the

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reference electrode may be controlled by a potentiostat, as is known by the skilled person.

5 A means for applying a potential is electrically connected to the working, counter and reference electrodes. The means for applying a potential can be any suitable means, for example a potentiostat, as mentioned above.

10 The sample may comprise a liquid, e.g. water, in which the nanoparticles are suspended. The sample may, for example, be selected from sea water, river water, drinking water and effluent.

15 The electrochemical analysis may be carried out in a suitable medium, such as a liquid carrier medium. The liquid carrier medium may comprise, consists essentially of, or consist of (excluding any dissolved solutes and/or suspended nanoparticles), water. If the carrier medium consists essentially of water, preferably the liquid carrier medium (excluding any dissolved solutes and suspended nanoparticles) comprises at least 98% by weight water, preferably at least 99% by weight water, preferably at least 99.5% by weight water. The liquid medium may comprise an electrolyte for use in the electrochemical analysis, including, but not limited to NaClO_4 . Suitable electrolytes
20 should be soluble in the medium and not be oxidised or reduced during the electrochemical analysis. The skilled person could select an appropriate electrolyte.

25 The electrochemical analysis may involve a voltammetry technique, i.e. monitoring of the current at the working electrode as the potential between the working electrode and a reference electrode is changed, or a chronoamperometry technique, or a chronopotentiometric technique. The electrochemical analysis may involve a voltammetry technique selected from cyclic voltammetry, square wave voltammetry, linear sweep voltammetry, pulse voltammetry, such as normal pulse or differential pulse voltammetry; or an amperometry technique such as chronoamperometry. The
30 electrochemical analysis may be used to obtain electrochemical information, such as the potential at which the oxidation or reduction of the immobilized nanoparticles occurs. This may be used, for example, to determine the identity of the immobilized nanoparticles. For example, in the conditions used in the Examples, silver nanoparticles typically are oxidised at a minimum potential of 0.244 V (vs a standard calomel electrode). For example, in voltammetry, e.g. linear sweep voltammetry, silver
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nanoparticles, in the conditions shown in the Examples, typically show a peak in the region of 0.244 V to 0.4 V (vs a standard calomel electrode (SCE)). The location of the peak is dependent on electrolytes, size of the nanoparticles, the liquid medium in which the electrochemical analysis is carried out, temperature, and other factors, as the skilled person will appreciate. The location of a peak in a voltammetry or amperometry technique under chosen conditions for a nanoparticle material of interest may be determined in a reference experiment.

In an embodiment, the nanoparticles are detected by electrochemical stripping of the nanoparticles immobilised on the substrate. The electrochemical stripping may involve varying the potential at the working electrode, i.e. the substrate, (e.g. relative to a reference electrode) until a peak in current is observed at a certain potential, and continued until the current has dropped from this peak and then started to rise again. The varying of the potential may involve raising it or lowering it, for example at a constant rate, for example at a rate of 0.01 to 0.1 Vs⁻¹, optionally at a rate of from 0.01 Vs⁻¹ to 0.05 Vs⁻¹, optionally at a rate of 0.01 to 0.03 Vs⁻¹, optionally about 0.02 Vs⁻¹. The potential may be varied over a range suitable for detecting the type(s) of nanoparticle of interest. For example, the detection of silver nanoparticles may involve varying of the potential from a first value to a second value. The first value is chosen/determined by the skilled person depending on electrochemical parameters such as e.g. the used electrolyte. For example to oxidise silver nanoparticles in an aqueous solution of 0.1 M NaClO₄ the first potential value would be chosen as less than 0.244 V (vs. SCE), while e.g. in 0.10 M KCl the first potential would be chosen as less than 0.1 V (vs. SCE) The a second value, e.g. a value at which all oxidative stripping has ceased, for example a value of 0.5 V (vs. SCE) or more, for example 0.6 V (vs. SCE) or more. The stripping may be oxidative or reductive stripping, depending on the nanoparticle. In an embodiment, the nanoparticles are metallic nanoparticles, e.g. silver nanoparticles, and the metallic nanoparticles are detected during the electrochemical analysis by oxidative stripping of the metallic nanoparticles.

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In an embodiment, the potential at the working electrode (e.g. relative to the reference electrode) is varied over a period of time and the current required to oxidise or reduce the nanoparticles during the stripping is measured during the stripping and then used to calculate the amount of nanoparticles immobilised on the substrate during the immersion of the substrate in the sample. In this instance, the “amount of

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nanoparticles” may indicate the amount of nanoparticle material (e.g. in moles or grams) that is oxidised or reduced. For example, if a peak in current is observed as described above, this peak may be integrated over time, to provide the total amount of current (q , which may be measured in Coulombs) passed during the stripping of the nanoparticles. From knowing the number (n) of electrons required to either oxidise or reduce the nanoparticles (per atom or molecule) during the stripping, the number of moles of the nanoparticle material being either reduced or oxidised can be calculated from q/nF , where F is Faraday’s constant.

10 The electrochemical analysis of the substrate may be used to determine the concentration of the nanoparticles immobilised on the substrate and/or in the sample. In an embodiment, the concentration of nanoparticles in the sample is determined by determining the amount of nanoparticles immobilised on the substrate in the predetermined amount of time and using a predetermined relationship between the concentration of the nanoparticles in a reference sample and the expected amount of nanoparticles that would immobilise on the substrate during the predetermined amount of time. The predetermined relationship may have been determined in a reference experiment in which is measured the variation of the amount of nanoparticles immobilized on the substrate in the predetermined amount of time over a range of concentrations of the nanoparticles in the reference sample. The reference sample and the sample used in step (a) of the method may be similar to one another, for example in that they contain the same liquid medium, e.g. water, in approximately the same amount, and the conditions in the reference experiment and in step (b) of the method, e.g. the temperature, and parameters used in the electrochemical analysis are substantially similar to one another.

The nanoparticles, in step (a) of the method, may be or comprise particles having a diameter of from 1 to 500 nm, optionally from 1 to 200 nm, optionally from 10 to 100 nm. The diameter of a nanoparticle, and/or particle size distributions of nanoparticles in a sample, can be determined by a technique selected from scanning electron microscope (SEM) imaging, atomic force microscopy, dynamic light scattering techniques, UV-visible spectroscopy, and other suitable techniques, as would be appreciated by the skilled person. The nanoparticle can be any suitable shape, including spherical, and elongated, for example a rod-shaped nanoparticle. If a

nanoparticle is non-spherical, the diameter of the nanoparticle as measured herein will be the smallest diameter across the particle.

5 The nanoparticles may comprise, consist essentially of, or consist of, a material, which may be selected, for example, from a metal, a metal compound and an organic material. If a particle consists essentially of a material, this indicates that preferably the particle comprises at least 95 % by weight of this material, preferably 98 % by weight, preferably 99 % by weight, of this material.

10 The nanoparticles may comprise an inorganic metal compound, e.g. a compound selected from, but not limited to, a metal oxide and a metal chalcogenide, such as a metal sulphide.

15 The nanoparticles may comprise an electrically-conducting material. The nanoparticles may comprise a material selected from a metal, a semi-metal, and a semi-conductor. The nanoparticles may comprise a metal, which may be in elemental form. The nanoparticles may comprise, consist essentially of or consist of an element, in elemental or compound form, selected from any of groups 3 to 15 of the periodic table. The nanoparticles, may comprise, consist essentially of or consist of a metal or semi-
20 metal selected from groups 3 to 15 of the periodic table. The nanoparticles may comprise a transition metal of any of groups 3 to 12 of the periodic table. Optionally, the nanoparticles comprise a metal selected from group 11 of the periodic table. Optionally, the nanoparticles comprise, consist essentially of or consist of a metal in elemental form selected from copper, silver, gold, iron, nickel, ruthenium, osmium,
25 rhodium, iridium, palladium and platinum. Preferably, the nanoparticles comprise, consist essentially of or consist of a metal in elemental form selected from silver and gold.

30 In an embodiment, the nanoparticles are silver nanoparticles, i.e. nanoparticles that comprise, consist essentially of or consist of silver in elemental form.

The nanoparticles may comprise carbon-containing materials, such as fullerenes and carbon nanotubes. The nanoparticles may comprise a polymeric material.

As mentioned, the substrate may be suitable for use as an electrode. The substrate used (in step (a) of the method) may comprise an electrically conductive or semi-conductive substance having on a surface thereof a species, e.g. an organic species, capable of binding to the nanoparticle, preferably in the absence of a potential being applied to the substrate. The electrically conductive substance may comprise a metal or carbon. The electrically conductive substance may comprise a metal selected from gold, silver and platinum. In an embodiment, the electrically conductive substance comprises indium tin oxide. In an embodiment, the electrically conductive substance comprises a conductive polymer. The electrically conductive substance may comprise a carbon-containing material, which may be selected from glassy carbon, edge plane pyrolytic graphite, basal plane pyrolytic graphite, boron doped diamond, highly ordered pyrolytic graphite, carbon powder and carbon nanotubes. In a preferred embodiment, the substrate (used in step (a) of the method) comprises a glassy carbon material. In an embodiment, the substrate may be or comprise a screen printed electrode, which may be a screen printed carbon electrode having on a surface thereof a sulphur-containing species, a carboxy-containing species, or an amino-containing species for binding to a nanoparticle, e.g. a silver nanoparticle. In an embodiment, the substrate may be or comprise a screen printed electrode, which may be a screen printed carbon electrode having on a surface thereof cysteine. In an embodiment, the substrate may be a screen printed electrode comprising a screen printed carbon working electrode, on which the nanoparticles can be immobilised when the substrate is immersed in the sample, and a reference electrode that may comprise, for example, silver. The screen printed carbon electrode may comprise a sulphur-containing species, a carboxy-containing species or an amino-containing species on a surface thereof, including, but not limited to cysteine. The screen printed electrode may be reversibly connected to a suitable electrochemical analytical device, such that, after immersion of the screen printed electrode in the sample (and optional subsequent removal), it can then be used in the electrochemical analytical device to carry out the electrochemical analysis in step b. of the method.

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In an embodiment, the substrate has a sulphur-containing species, a carboxy-containing species and/or an amino-containing species on a surface thereof. The sulphur-containing species, carboxy-containing species or an amino-containing species can preferably bind to the nanoparticles, e.g. silver or gold nanoparticles. In an embodiment, the substrate comprises an electrically conducting material having a

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5 sulphur-containing species, a carboxy-containing species and/or an amino-containing species on a surface thereof. The sulphur of the sulphur-containing species may be in a thio group, i.e. with the sulphur covalently bonded to a carbon. The sulphur in the sulphur-containing species may be in a form selected from -SH or -S⁻. In an embodiment, the sulphur in the sulphur-containing species may be in a disulphide group or contain sulphur in S_n form, wherein n is 3 or more. Carboxy may indicate -CO₂⁻ or -CO₂H. The amino group of the amino-containing species may be a primary, secondary or tertiary amino group. The sulphur of the sulphur-containing species, the carboxy of the carboxy-containing species, or the amino of the amino-containing group is preferably a terminal group. In embodiment, the substrate has species on a surface thereof comprising an -alkylenethiol, an -alkylenecarboxy or an -alkyleneamino group, for example as a terminal group. In an embodiment, the substrate has a species of the formula (Y)_m-L-X on a surface thereof, wherein each Y is independently a carboxy or an amino group, m is 1 or 2, L is an organic linker group and X is -SH, -S⁻, -CO₂H, -CO₂⁻ or -N(R₁)(R₂), wherein R₁ and R₂ are each independently selected from H and an alkyl group. The organic linker group may be an alkylene group. Y is preferably bound to the surface of the substrate, and X is free to bond to the nanoparticle.

20 Alkylene, when mentioned herein, includes, but is not limited to, linear, cyclic or branched optionally substituted alkylene, optionally containing from 1 to 20 carbon atoms, optionally from 2 to 10 carbon atoms, not including any substituents that may be present. Alkylene includes, but is not limited to, a species of formula -(CH₂)_n-, where n is 1 to 20, optionally from 1 to 10, optionally from 1 to 5.

25 Alkyl, when mentioned herein, includes, but is not limited to, linear, cyclic or branched optionally substituted alkyl, optionally containing from 1 to 20 carbon atoms, optionally from 2 to 10 carbon atoms, not including any substituents that may be present. Alkyl includes, but is not limited to, a species of formula -(CH₂)_n-, where n is 1 to 20, optionally from 1 to 10, optionally from 1 to 5.

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In an embodiment, the substrate may have a self-assembling monolayer comprising organic molecules that are attached at one end to the substrate and having a free part of the molecule comprising a sulphur-containing group, e.g. a thio group; a carboxy group; and/or an amino group for bonding to the nanoparticles.

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In an embodiment, the substrate has cysteine on a surface thereof. In an embodiment, the substrate is suitable for use as an electrode, and may comprise a carbon-containing material, e.g. a glassy carbon material, having cysteine on a surface thereof. Cysteine, wherever mentioned herein, may be L-cysteine or D-cysteine, or a mixture thereof, e.g. a racemic mixture. The cysteine may be deposited on the surface of the electrode by any suitable technique. Optionally, the cysteine is deposited on the surface of the electrode by an electrochemical technique, e.g. a cyclic voltammetry technique, which may be carried out in a liquid medium, e.g. acetonitrile, with a suitable electrolyte, e.g. tetra-N-butylammonium tetrafluoroborate. After deposition of the cysteine on the electrode, the electrode may be rinsed and sonicated.

As indicated, the present invention also provides a device for detecting nanoparticles in a sample, the device configured to:

- a. immerse a substrate in the sample, wherein, if the nanoparticles are present in the sample, at least some of the nanoparticles would be immobilized on the substrate;
- b. analyse the substrate to determine the presence of, identity of the material of and/or quantity of the nanoparticles on the substrate and/or in the sample.

The present invention also provides a device for detecting nanoparticles in a sample, the device configured to:

- a. immerse an electrode in the sample for a predetermined amount of time and optionally without applying a potential to the electrode, wherein, if the nanoparticles are present in the sample, at least some of the nanoparticles would be immobilized on the electrode;
- b. electrochemically analyse the electrode to determine the presence of, identity of the material of and/or quantity of the nanoparticles in the sample.

The device may be adapted to carry out the process as described herein, for example in an automated way. All features as described above in relation to the method are equally applicable to the device. The device may be programmed to carry out the method.

In an embodiment, the device is configured, in step a., to immerse the substrate in the sample for a predetermined amount of time; and, in step b., electrochemically analyse the substrate to determine the presence of, identity of the material of and/or quantity of the nanoparticles on the substrate and/or in the sample. In an embodiment, after the
5 predetermined amount of time, the substrate is removed from the sample, and the electrochemical analysis of the substrate then carried out. However, in an alternative embodiment, the substrate will remain in the sample, and the electrochemical analysis, e.g. the electrochemical stripping, carried out in the sample, preferably immediately after the predetermined amount of time has elapsed.

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Optionally, the quantity of the nanoparticles on the substrate and/or in the sample is determined by electrochemical stripping of the nanoparticles immobilised on the substrate.

15 Optionally, the device has been configured to use a predetermined relationship between the concentration of the nanoparticle in a reference sample and the amount of nanoparticle that would be expected to be immobilised on the electrode in the predetermined amount of time to calculate the concentration of the nanoparticle in the sample. Where “the sample” is mentioned herein, this indicates the sample in which
20 the substrate is or has been immersed in step (a), unless otherwise stated.

The device may be suitable for remote sensing.

In an embodiment, the device may be a portable device.

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Embodiments of the present invention will now be described with reference to the following non-limiting Examples and the accompanying drawings.

Examples

Cysteine-modified “sticky” glassy carbon electrodes are demonstrated in these Examples to enable immobilisation of Ag nanoparticles suspended in 0.1 M NaClO₄ under open circuit conditions. Subsequent oxidative stripping yields the amount of Ag nanoparticles sticking on the electrode surface, which increases monotonically with the immersion time.

The chemicals used in these Examples were all of analytical grade and, unless stated otherwise, purchased from Sigma Aldrich and aqueous solutions were prepared using ultrapure water (Millipore, resistivity $\sim 18.2 \text{ M}\Omega\cdot\text{cm}$ at 25°C). Detailed information on the synthesis and characterisation of the citrate-capped Ag NPs used can be found in Toh *et al.*¹⁰. Their average size was determined to be ca. 27 nm in diameter and their concentration in the stock suspension was about $1.73 \times 10^{-9} \text{ mol Ag NPs L}^{-1}$ (resulting from quantitative reduction of 1 mM Ag⁺, during the nanoparticle synthesis).

All electrochemical experiments were performed at room temperature in a three electrode setup using a μ Autolab II potentiostat (Metrohm-Autolab BV, Netherlands). For the electrochemical sticking and stripping experiments, a glassy carbon (GC; diameter = 3 mm) electrode was used as the working electrode (WE) and a graphite rod electrode (diameter = 3 mm) served as the counter electrode. Potentials were applied against a saturated calomel reference electrode (SCE, potential $E = 0.244 \text{ V}$ vs. standard hydrogen electrode) and are referenced to SCE throughout this work unless stated otherwise.

Example 1

To characterise the silver nanoparticle (Ag NP) voltammetric response of a Ag NP modified GC electrode in 0.1 M NaClO₄ (sodium perchlorate) aqueous solution, linear sweep voltammetry was performed by shifting the potential from 0.1 V to 0.6 V vs. SCE at a scan rate of 0.020 Vs^{-1} . To prepare Ag NP modified GC electrodes, the Ag NP stock solution was first diluted by a factor of 160 using H₂O and then 3 μL of this diluted suspension was drop cast on the GC surface and dried under N₂ atmosphere. In Fig. 1 an anodic peak is visible in the first sweep, while none is present in the second sweep. This, and the fact that the measured peak charge was equal to that expected for a

quantitative stripping of the drop cast Ag NPs (1.8 μC), indicate an exhaustive oxidation of Ag from the GC electrode under the chosen conditions.

5 This in turn enables the direct quantification of Ag NPs adsorbed on the electrode in a certain period of time (sticking experiment), by subsequent oxidative stripping (stripping experiment).

10 Prior to each sticking experiment, the following pre-treatment was performed. The GC working electrode was polished thoroughly with alumina powder of sequentially decreasing size (1.0 μm , 0.3 μm and 0.01 μm), using polishing paper (micro cloth PSA, Buehler). Then, the WE was rinsed with water and sonicated in water for 5 min to remove remaining Al_2O_3 particles from the GC surface. The unmodified GC electrode prepared this way was either directly used for Ag NP sticking experiments or modified with cysteine to increase the sticking of Ag NPs on the electrode surface. The latter
15 was achieved by cyclic voltammetric deposition from a solution of 1 mM L-cysteine ($\text{C}_3\text{H}_7\text{NO}_2\text{S}$, Lancaster) and 0.1 M Tetra-n-butylammonium tetrafluoroborate (NBu_4BF_4 , Fluka) in acetonitrile (CH_3CN), according to Zhang¹¹. A Pt counter electrode and a Ag wire quasi-reference electrode were employed for this purpose and three scans from 0 V to 1.55 V vs Ag wire (scan rate = 0.02 Vs^{-1}) were performed. The resulting modified
20 GC electrode was first rinsed and then sonicated in water for 5 min to remove physically adsorbed L-cysteine from the surface.

Following this pre-treatment, each unmodified or modified GC electrode was subjected to a cyclic voltammetric scan in an aqueous solution of 0.1 M NaClO_4 . Afterwards, the
25 WE was transferred to a freshly prepared suspension of 0.5 mL Ag NP stock suspension in 25 mL 0.1 M NaClO_4 for the sticking experiment. After holding the electrode at open circuit potential (OCP) throughout the desired sticking time (0 to 900 min), linear sweep voltammetry was used to anodically strip the Ag NPs from the electrode. The potential region from 0.15 V to 0.45 V vs. SCE was used for the
30 stripping since this region has been found to be suitable for quantitative oxidation of Ag NPs from a GC electrode in 0.1 M NaClO_4 , as stated above. The detected oxidative charge is determined by the amount of Ag stripped and therefore allows the detection of Ag NPs originally present in the electrolyte.

Using unmodified GC electrodes, oxidative stripping peaks were observed for sticking times of several hours (see Fig. 2), indicating the low affinity of Ag NPs for GC under open circuit conditions. The inset in Fig. 2 additionally reveals the stripping charge to suffer from irreproducibility. This result is in agreement with studies using micro carbon electrodes, where systematic Ag NP sticking was only reported when a negative bias potential was applied to the electrode¹².

Thus, to increase the sticking rate of Ag NPs on the electrode surface, the latter was chemically modified with cysteine, which is known for its high affinity to Ag¹³. Figure 3 shows cyclic voltammograms recorded during the electrochemical surface modification, which is marked by two oxidative peaks on the forward scan and an oxidative and a reductive peak during the reverse scan. The observed anodic double peak has been reported for solutions with a cysteine concentration of at least 1 mM and both peaks were attributed to oxidation of cysteine to cystine, which adsorbs on the electrode surface^{13,14}. The oxidation of cysteine to cystine continues throughout the reverse scan and can cause a third anodic peak. As the electrochemical oxidation of cysteine to cystine is irreversible, only a small reductive peak is observed at more negative potentials^{13,14}. However, since GC electrodes provide functional groups, among them -OH and -COOH, also an alternative reaction mechanism has been proposed in the literature, assuming binding of cysteine to these functional groups via the amino group of cysteine¹¹.

Example 3

X-ray photoelectron spectroscopy (XPS) analysis of a modified GC surface was performed to prove that the electrochemically produced layer remains on the surface after rinsing and sonicating in water. XPS was carried out using a Thermo KAlpha spectrometer with Al K α radiation operated in constant analyser energy mode. High resolution S 2p, N 1s C 1s and O 1s regions from pristine and cysteine modified GC electrodes were measured. In the treated samples an S environment was observed with S2p_{3/2} at 166.3 eV. A small amount of N was present in the pristine sample, likely due to adsorption of N containing species. After cysteine treatment, the N level increased. This analysis revealed the presence of sulphur and nitrogen species on the surface not present on the untreated electrode, confirming a successful modification.

Further studies may give precise information on how the cysteine (or cystine) is bound to the GC surface. Using these cysteine-modified “sticky” GC electrodes, Ag NP stripping has been observed for sticking times of several minutes and resulting oxidation curves for various sticking times are shown in Fig. 4.

5

Fig. 5 shows that for sticking times of up to 240 min a linear increase of the stripping charge with sticking time was observed. The corresponding linear fit suggests an increase of the sticking charge of about 3.9×10^{-9} C per minute of sticking time, corresponding to about 4×10^4 NP stick to the surface per minute in a suspension of a
10 Ag NP concentration of about 34 pM.

The break in Fig. 5 indicates that for long sticking times (900 min) the sticking charge seems to level off, as a further doubling of the stripping charge is only observed after 900 min of sticking time.

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References

The following documents are referred to in the text of the application or otherwise mentioned as they are useful as background. They are each incorporated herein by reference in their entirety.

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5

CLAIMS

1. A method of detecting nanoparticles in a sample comprising:
 - a. immersion of a substrate in the sample, wherein, if the nanoparticles are present in the sample, at least some of the nanoparticles are immobilized on
10 the substrate;
 - b. analysing the substrate to determine the presence of, identity of the material of and/or quantity of the nanoparticles on the substrate and/or in the sample.
2. A method according to claim 1, wherein step a. involves immersion of the substrate in
15 the sample for a predetermined amount of time, and then optionally removing the substrate from the sample, and step b. involves electrochemically analysing the substrate to determine the presence, identity of the material of and/or quantity of the nanoparticles on the substrate and/or in the sample.
- 20 3. A method according to claim 2, wherein determining the quantity of the nanoparticles on the substrate and/or in the sample involves electrochemical stripping of the nanoparticles immobilised on the substrate.
4. A method according to claim 3, wherein the nanoparticles are metallic nanoparticles
25 and the electrochemical stripping is oxidative stripping.
5. A method according to claim 3 or claim 4, wherein the current required to oxidise or reduce the nanoparticles is measured during the stripping and then used to calculate the amount of nanoparticles immobilised on the substrate during the immersion of the substrate
30 in the sample.
6. A method according to any one of claims 2 to 5, wherein the electrochemical analysis of the substrate is used to determine the concentration of the nanoparticles in the sample.
- 35 7. A method according to claim 6, wherein the concentration of nanoparticles in the sample is determined by determining the amount of nanoparticles immobilised on the substrate in the predetermined amount of time and using a predetermined relationship between the concentration of the nanoparticles in a reference sample and the expected

5 amount of nanoparticles that would immobilise on the substrate during the predetermined amount of time.

8. A method according to any one of the preceding claims, wherein analysing the substrate involves one or more techniques selected from a spectroscopic analysis;
10 microscopic analysis; and mass spectrometry.

9. A method according to claim 8, wherein the spectroscopic analysis involves a technique selected from x-ray photoelectron spectroscopy, ultraviolet-visible spectroscopy and IR spectroscopy.

15

10. A method according to claim 8, wherein the microscopic analysis involves a technique selected from transmission electron microscopy or scanning electron microscopy.

11. A method according to any one of the preceding claims, wherein the nanoparticles are
20 silver nanoparticles.

12. A method according to any one of the preceding claims, wherein the substrate comprises an electrically conductive substance having on a surface thereof a species capable of binding to the nanoparticles.

25

13. A method according to claim 12, wherein the species on a surface of the substrate is capable of binding to the nanoparticles without a potential being applied to the substrate.

14. A method according to any one of the preceding claims, wherein the substrate has a
30 sulphur-containing species, a carboxy-containing species and/or an amino-containing on a surface thereof.

15. A method according to any one of the preceding claims, wherein the substrate has cysteine on a surface thereof.

35

16. A method according to any one of the preceding claims, wherein the substrate comprises a carbon-containing material having cysteine on a surface thereof.

17. A device for detecting nanoparticles in a sample, the device configured to:

- 5 a. immerse a substrate in the sample, wherein, if the nanoparticles are present
 in the sample, at least some of the nanoparticles would be immobilized on the
 substrate;
- b. analyse the substrate to determine the presence of, identity of the material of
 and/or quantity of the nanoparticles on the substrate and/or in the sample.

10

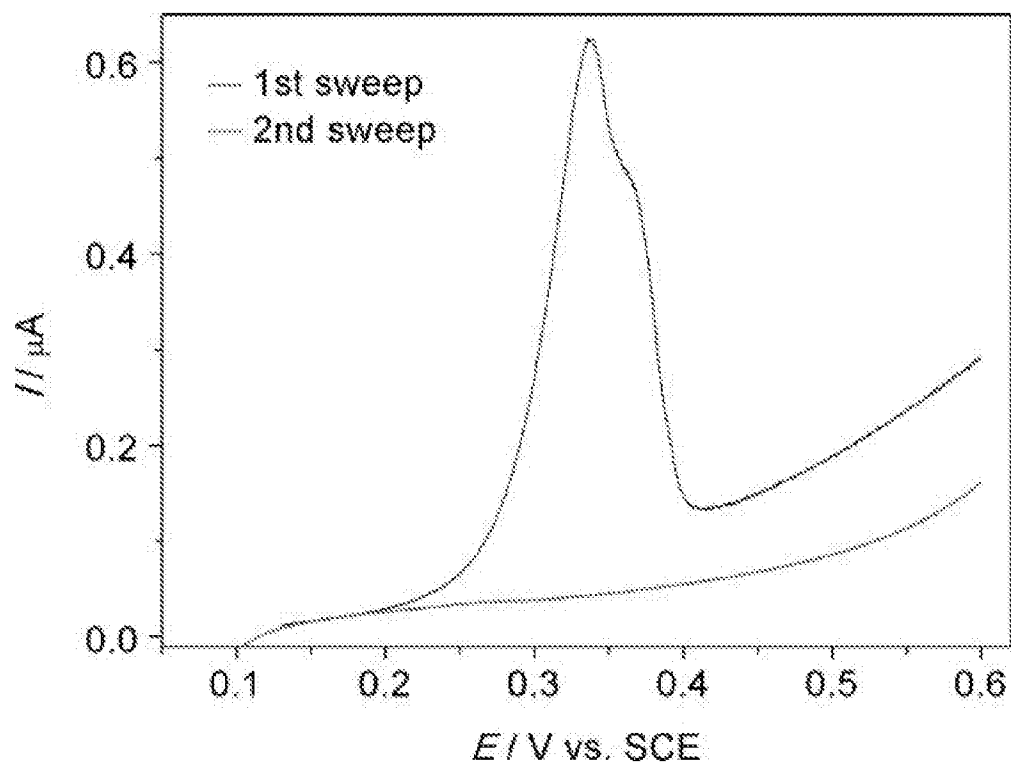
18. A device according to claim 17, wherein the device is configured, in step a., to immerse
the substrate in the sample for a predetermined amount of time, and then optionally remove
the substrate from the sample; and, in step b., electrochemically analyse the substrate to
determine the presence of, identity of the material of and/or quantity of the nanoparticles on
15 the substrate and/or in the sample.

19. A device according to claim 17 or claim 18, wherein the quantity of the nanoparticles on
the substrate and/or in the sample involves electrochemical stripping of the nanoparticles
immobilised on the substrate.

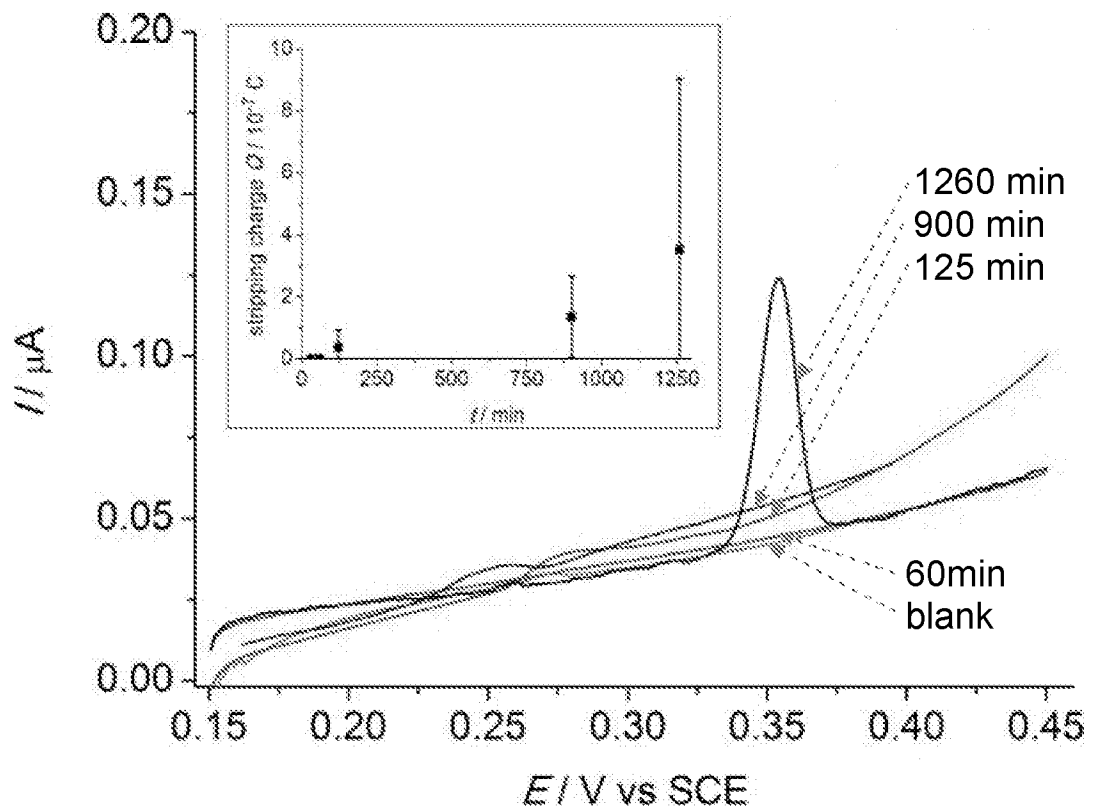
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20. A device according to claim 19, wherein the device has been configured to use a
predetermined relationship between the concentration of the nanoparticle in a reference
sample and the amount of nanoparticle that would be expected to be immobilised on the
electrode in the predetermined amount of time to calculate the concentration of the
25 nanoparticle in the sample.

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FIG. 1

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FIG. 2

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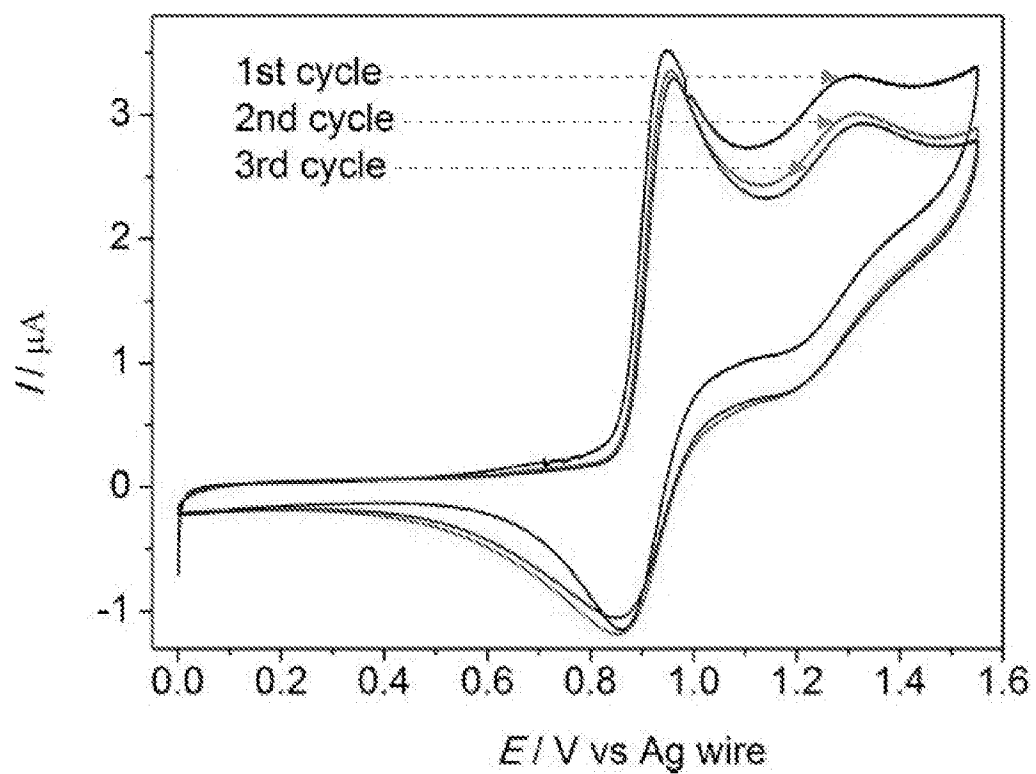
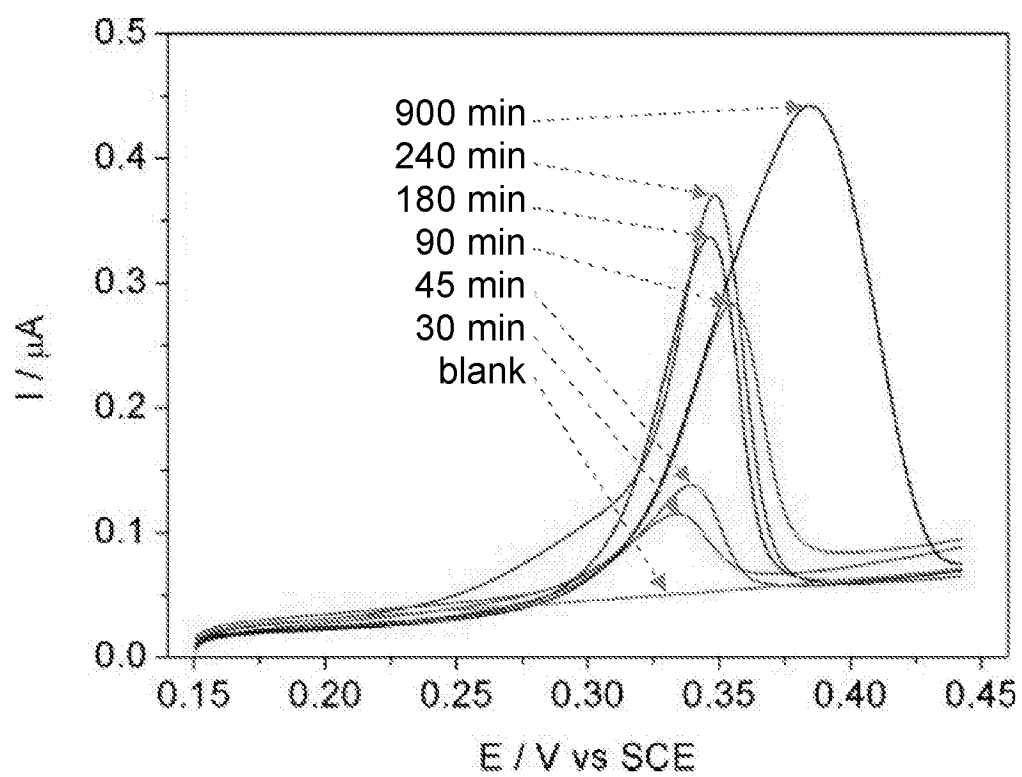
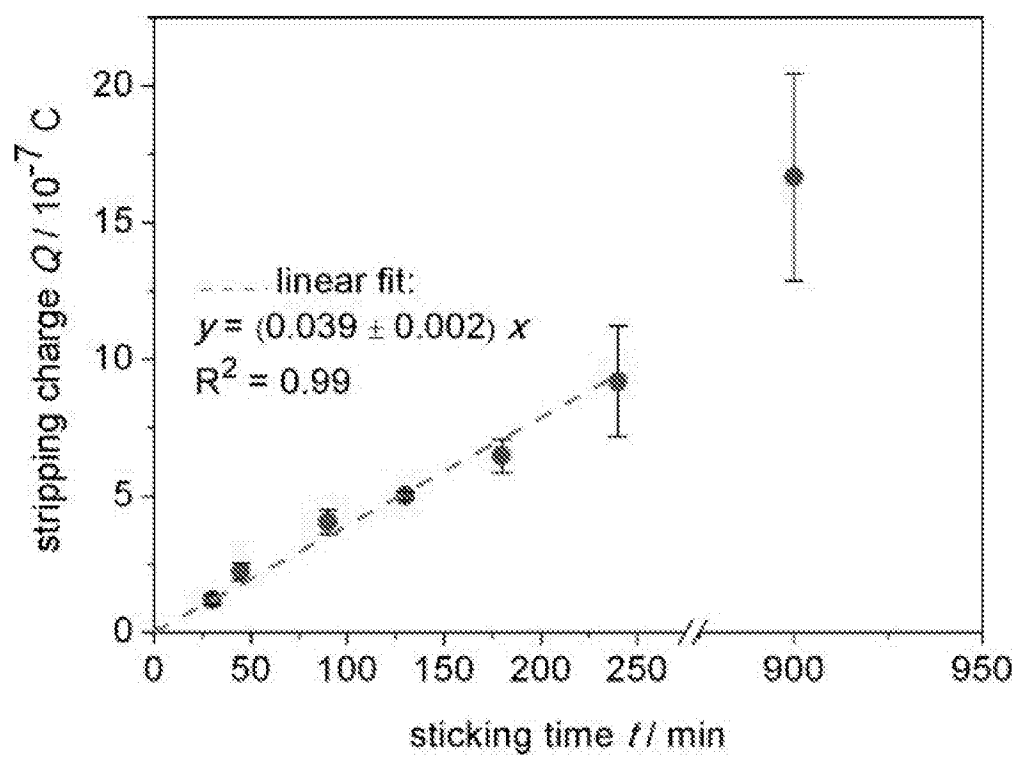
FIG. 3

FIG. 4

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FIG. 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2014/051284

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N27/42 G01N27/48 B82Y35/00
ADD. G01N33/18

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 B82Y

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	"Chapter 4. Formation and Reactions of Sticky Surface with Nanoparticles and Aniline" In: Lee L: "MSc Thesis: Modification of Glassy Carbon Electrodes with Diazonium Cation Terminated Films", 2011, University of Canterbury, Christchurch, NZ, XP055127561, pages 59-83, page 69 - page 79 figures 4.8-4.12	1-8,10, 12-14, 17-20
Y		9,11
A		15,16
Y	----- WO 2012/114087 A1 (ISIS INNOVATION [GB]; COMPTON RICHARD GUY [GB]; REES NEIL VAUGHAN [GB]) 30 August 2012 (2012-08-30) paragraph [0117] - paragraph [0126] figure 1	9,11
A	----- -/--	15,16

☒ Further documents are listed in the continuation of Box C.

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Date of the actual completion of the international search

8 July 2014

Date of mailing of the international search report

17/07/2014

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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2014/051284

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Information on patent family members

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