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(54) Title: SENSOR APPARATUS AND USE THEREOF

(57) Abstract: A sensor apparatus for analysing a sample by a stripping voltammetric technique to determine the presence and/or amount of an analyte species in solution in the sample. The apparatus comprises: a sensor unit (1) having a liquid flow path therethrough between an inlet (3) and an outlet (4) of the unit, said unit comprising (a) an electrode assembly comprising at least one working electrode comprised of a conductive carrier substrate having a surface coated with mercury immobilised on the surface of the substrate, (b) a counter electrode, and (c) a reference electrode (12), a liquid supply arrangement (10) for supplying a flow of electrolyte to the inlet of the sensor unit, a sample supply arrangement (9) for introducing the sample to be analysed into electrolyte flow to the inlet (3) of the sensor unit, and (iv) stripping voltammetric means (13) for stripping a species immobilised on the mercury surface of the working electrode and for determining the presence and/or amount of said analyte species The sample supply arrangement is adapted to introduce a metered amount of said sample into the electrolyte flow.

SENSOR APPARATUS AND USE THEREOF

The present invention relates to a sensor apparatus, and also to a method, for determining the presence and/or amount of an analyte in solution in a sample by means of a stripping voltammetric technique using a mercury film electrode. The preferred stripping voltrammetric technique employed in the invention is anodic stripping voltammetry.

Anodic stripping voltrammetry is a technique well known to persons skilled in the art for the determination of specific ionic species, and in particular such species that may be converted to metals that will amalgamate with mercury (e.g. copper, lead, cadmium and zinc). The analyte metal (which, if present) will be in ionic form is caused to be reduced and electroplated on to the mercury surface to form an amalgam. On application of an appropriate potential, the metal is oxidised resulting in a sharp peak which is representative of the presence and amount of the metal in the analyte.

There are generally four steps in an anodic stripping voltammetric technique using a mercury film electrode. The first step is a cleaning step (e.g. effected in "pure" buffer) in which the potential of the mercury film electrode is held at a value that will oxidise any analyte metal amalgamated on the surface of the mercury in order fully to remove analyte metal from the electrode which is therefore cleaned. Subsequently, in the second step, the sample to be analysed is brought into contact with the mercury and the potential is held at a lower value which causes reduction of the analyte metal ion and deposition thereof on the mercury film. A third step is equilibration to allow the deposited metal to become more evenly distributed in the mercury. In the final step, the potential of the working electrode is raised to a higher, anodic potential which causes the deposited analyte metal to be oxidised into an ionic form. It is this latter step that is referred to as "stripping". The oxidation of the metal in this "stripping" step gives off electrons and these give rise to a current that may be measured.

Other stripping techniques are known, e.g. adsorptive stripping voltammetry.

The present invention relates to a development of the biosensor described more fully in our earlier PCT application published as WO 2009/016366. Briefly, the aforementioned prior PCT specification discloses an electrode assembly comprising at

least one working electrode comprised of a conductive carrier substrate (e.g. platinum) having a surface coated with mercury immobilised on the surface of the substrate. The biosensor apparatus further includes a counter electrode and a reference electrode. The biosensor apparatus of the WO specification is intended to determine whether a species has biomembrane activity and for this purpose the surface of the mercury remote from the substrate is coated with a phospholipid layer.

Fig 10 of the WO 2009/016366 discloses a flow cell arrangement for use in effecting measurement of the biomembrane activity. In the arrangement of Fig 10 of the earlier PCT application, there is a single port (referenced as 406) through which (i) phospholipid to be deposited on the mercury electrode, and (ii) sample to be monitored are separately introduced at various stages of the measurement procedure.

The development of the present invention is based on modifications of the biosensor disclosed in WO 2009/016366 so it is eminately suitable for use in determining the amount of analyte present or potentially present in solution in a sample by means of a stripping voltammetric technique. The present invention arises from a development of the biosensor disclosed in WO 2009/016366 but with the modification that the phosopholipid layer is not required for a stripping voltammetric technique.

According to a first aspect of the present invention there is provided a sensor apparatus for analysing a sample by a stripping voltammetric technique to determine the presence and/or amount of an analyte species in solution in the sample, the apparatus comprising:

(i) a sensor unit having a liquid flow path therethrough between an inlet and an outlet of the unit, said unit comprising

(a) an electrode assembly comprising at least one working electrode comprised of a conductive carrier substrate having a surface coated with mercury immobilised on the surface of the substrate,

(b) a counter electrode, and

(c) a reference electrode,

(ii) a liquid supply arrangement for supplying a flow of electrolyte to the inlet of the sensor unit,

(iii) a sample supply arrangement for introducing the sample to be analysed into electrolyte flow to the inlet of the sensor unit, and

(iv) stripping voltammetric means for stripping a species immobilised on the mercury surface of the working electrode and for determining the presence and/or amount of said analyte species

wherein the sample arrangement is adapted to introduce a metered amount of said sample into the electrolyte flow.

According to a second aspect of the present invention there is provided a method of analysing a sample to determine the presence and/or amount therein of an analyte using the apparatus in accordance with the first aspect of the invention, said analyte being one that, if present in the sample, is capable of being deposited on the exposed mercury surface of the working electrode, the method comprising the steps of

(i) supplying to the inlet of the sensor unit a flow of electrolyte,

(ii) introducing into said flow of electrolyte upstream of the sensor a metered amount of the sample to be analysed,

(iii) effecting a technique that will cause the analyte if present in the sample to be deposited on the mercury surface of the working electrode, and

(iv) effecting a stripping voltammetric technique to cause analyte deposited on the mercury surface to be stripped therefrom and determining the presence and/or amount of said analyte.

Step (iv) of the method may be effected by anodic stripping voltammetry. The method may comprise the steps of:

(a) cleaning the mercury electrode,

(b) depositing the analyte (if present) on the mercury surface of the working electrode,

(c) equilibrating the electrode, and

(d) effecting anodic stripping.

The analyte may be a heavy metal, e.g. selected from the group consisting of copper, lead, cadmium and zinc. The sample to be analysed may be a water sample (e.g. drinking water).

In particularly preferred embodiments of the invention, the liquid supply arrangement is adapted selectively to supply either control electrolyte or electrolyte containing sample to the inlet of the sensor unit. The liquid supply arrangement may be adapted to provide a first flow path for supplying a control electrolyte of the sensor unit and a second flow path for supplying electrolyte containing sample to the inlet of the sensor unit. In such a case, the first flow path may include a first reservoir for holding control electrolyte. Alternatively or additionally the second flow path may be adapted to operate as a continuous loop. The second flow path may incorporate a second reservoir for holding electrolyte (preferably in a predetermined volume). The sample supply arrangement may introduce the sample into the second reservoir.

Conductive substrates for use in the invention (i.e. the substrate on which the mercury is deposited) preferably have a resistivity of less than 1 ohm metre, more preferably less than 1×10^{-2} ohm metre and ideally less than 1×10^{-3} ohm metre.

The carrier substrate may be a metal selected from the group consisting of iridium, platinum, palladium and tantalum which are selected as "carriers" because of their refractory inert properties and their low solubility in mercury and because the mercury can be deposited on such metals (e.g. by electrodeposition) to give a uniform

film. The smooth mercury surface allows for defect-free formations of the phospholipid layer. A further conductive carrier substrate that may be employed in the invention is carbon, e.g. in the form of graphite or glassy carbon. Platinum is the preferred carrier substrate.

Without wishing to be bound by theory) we believe platinum has a particularly appropriate solubility with respect to mercury to allow production of an "amalgam-like" joint which holds the mercury relatively strongly on the platinum whilst providing a good surface for the mercury to allow phospholipid deposition and provide good membrane activity.

The working electrode may for example be circular and/or have a maximum surface dimension in any direction of 2µm to 1mm, although dimensions outside this range are not precluded. Alternatively, the working electrode may be a microelectrode and preferably sized such that the mercury has a maximum surface dimensions of 2µm to 10µm in any direction. The or each working electrode may, for example, be circular with a diameter of 2µm to 10µm. Such dimensions serve to maximise the edge-to area and thereby enhance stability of the phospholipid layer.

The mercury layer may be formed by eletrodeposition. The amount of mercury deposited should be sufficient to give a continuous film of the mercury on the conductive carrier substrate. Increasing the thickness of the mercury layer will enhance the stability of that layer and allow repeated the mercury layer to allow repeated cycles of phospholipid layer deposition, sample measurement and removal of the phospholipid layer so that one electrode may be used many times without disruption of the mercury. Additionally thickness of mercury layer will be a consideration for use of the electrode assembly in a flow cell where the mercury layer is required to withstand forces associated with liquid flows through the cell. The amount of charge required for the electrodeposition process will depend on the required thickness of the mercury layer and this will in turn depend on factors such as the surface area of the conductive substrate (on to which the mercury is to be electrodeposited) and the concentration of mercury ions in the mercury deposition electrolyte.

Controlled electrodeposition of mercury from a solution containing Hg^{2+} ions (e.g. provided by $Hg(NO_3)_2$) may be effected within an electrochemical cell. The mercury deposition electrolyte may contain $HClO_4$ which prevents oxidation of the Hg species. However the lipid interrogation will not take place in this acidic solution and will instead be in a more benign electrolyte such as 0.1M KCl. Alternatively electrodeposition of mercury can be effected by pipetting a drop of the base electrolyte (e.g. $HClO_4$ + Hg(II)) onto the surface of the first insulator. The electrodeposition may be facilitated by applying a potential of ~-0.4V vs. 3.5 M KCl Ag/AgCl to the working electrode surface in the deposition solution and recording the charge passed which is directly proportional to the mass of mercury deposited the deposition can be stopped by breaking the circuit. This allows precise control over the amount of Hg deposited.

Once the mercury layer has been deposited, the assembly can be transferred to a neutral electrolyte such as 0.1M KCl and the electrode surface electrochemically cleaned by applying an extreme negative potential <-2V evolving hydrogen gas to 'scrub' the surface free from organics.

The invention is illustrated by way of example only with reference to the accompanying drawings, in which:

Figs 1(a)-(h) illustrate one embodiment of sensor apparatus in accordance with the invention in successive stages of operation; and

Figs 2 to 4 show the results of the Example detailed below.

The illustrated apparatus comprises a sensor unit 1 (in the form of a flow cell) incorporating a sensor 2 of the type described more fully in WO 2009/016366 and comprising mercury immobilised on a conductive substrate platinum although other conductive substrate as disclosed in WO 2009/016366 may be employed. Sensor unit 1 has a liquid inlet 3 and liquid outlet 4 associated with a single channel peristaltic pump 5 (available from Cole Palmer Instrument) located upstream of inlet 3 and control valves 6 and 7 which regulate supply and discharge of liquid to and from sensor unit 1 along various flow paths in the manner described more fully below. Valves 6 and 7 are universal valve switching modules with six channels.

A syringe 9 is provided holding a sample to be analysed and is arranged to discharge a known (metered) amount of this sample into a reservoir 10 containing a known volume of buffered electrolyte.

Control electrolyte (buffered) is provided in a further reservoir 11.

Argon is fed to reservoirs 10 and 11 as depicted by the labelled arrows.

Sensor unit 1 may be of a similar device to the flow cell illustrated in Fig 10 of WO 2009/016366 save that inlet port 406 of that flow cell is not required. In the present case, sensor unit 1 comprises the working electrode (i.e. mercury deposited on conductive substrate) and a counter electrode. A separate Ag/AgCl reference electrode 12 is provided for the sensor unit 1, all three electrodes (working, counter and reference) being connected to a potentiostat 13 controlled by AUTOLAB software and associated with a monitor 14 for displaying results.

Fig 1(a) shows the apparatus in its "non-operational" condition in which there is no liquid circulation through the sensor unit 1. In this "non-operational" condition reservoirs 10 and 11 are both charged with electrolyte, and syringe 9 is charged with sample to be analysed.

In the first stage of operation (Fig 1(b)), pump 5 is operated and valves 6 and 7 are set so that control electrolyte from reservoir 11 passes in sequence through valve 7, pump 5, sensor unit 1, valve 7, valve 6 and then to discharge, all as represented by the arrows shown in Fig 1(b). Cleaning of the electrode may be effected at this stage by appropriate adjustment of the potential of the working electrode, in the manner well known to persons skilled in the art. At this stage, a trace may be displayed on monitor 14 showing the signal obtained from the bare mercury electrode (see Fig 1(c)).

In the next stage of operation (Fig 1(d)), valves 6 and 7 are set so that electrolyte from reservoir 10 runs round a continuous flow path whereby this electrolyte from reservoir 10 is passed in sequence through valve 7, pump 5, sensor unit 1, back to valve 7, to valve 6 and then returned to reservoir 10 (see arrows in Fig 1(d)). Once the system has equilibrated, the plunger of syringe 9 is depressed (Fig 1(e)) to

discharge a known volume of sample into reservoir 10, the sample then circulating around the continuous flow path as described for Fig 1(d).

A voltammetric technique (e.g. stripping anodic voltammetry) may now be effected to deposit analyte on to the mercury electrode, equilibrating of the electrode (if desired), and stripping the analyte from the electrode so that a current is generated to demonstrate the presence of the analyte, the current being representative of the amount of the analyte.

Once the measurement has been made, valve 6 is set (Fig 1(f)) so that the electrolyte (containing sample) previously circulating back to reservoir 10 is now discharged to waste as depicted by the arrows in Fig 1(f)

Once electrolyte (containing sample) has been discharged, valves 6 and 7 are set (Fig 1(g)) so that electrolyte from reservoir 11 passes through the apparatus as depicted by the arrows and is then discharged.

Finally, valve 6 is set (Fig 1(h)) in readiness for a further cycle of the apparatus.

The invention is further illustrated by the following non-limiting Example.

Example

Apparatus as illustrated in Fig 1 was used for the determination of Zn^{2+} in zinc oxide (ZnO) centrifugate and the results compared with those obtained using inductively coupled plasma-mass spectrometry (ICP-MS).

The buffer used – A 0.2 μmol dm^{-3} acetate buffer (CH_3COOK) was used, which gives smooth clear peaks, and does not combine with Zn^{2+} in solution – meaning the Zn concentration measured is a representation of that leached into solution from ZnO nanoparticles. However, the pH of this buffer is 4.5 – and therefore it was decided to remove nanoparticles from solution by centrifugation, before adding 0.5 ml of supernatant to a total of 20 ml of buffer in the system. Thus, the dilution was 40x.

Deposition potential and time – It was found that a deposition potential of -1.5

V was sufficient to deposit Zn^{2+} from solution as Zn(Hg) on the fabricated Pt/Hg film electrode in the preconcentration step. A deposition time of 150 s was used to allow enough time for sufficient Zn to accumulate at the working electrode.

Equilibration time – 30 s was found to be enough time to allow the system to reach equilibrium after the deposition step.

Initial and end potential – It was advised that the zinc peak would appear around -1 V. Therefore, the anodic sweep was performed between -1.2 V and -0.5 V to include the zinc peak. The actual zinc peaks appeared at -0.85 V.

Pump speed – The pump was manually turned on for the deposition step at a speed of 2/10 (to allow mixing and full accumulation of Zn at the electrode) and then turned off for equilibration and for the anodic sweep (to minimise disruption to the system and give smoother measurements). Pump speed was sufficiently fast for mixing purposes, without causing increased pressure in the valve switches.

Time between repeated measurements – 5 min (with the pump on) was given between successive repeats of measurements to allow the system to fully restore back to its original conditions. This gave tight repeatability of oxidation curves.

System cleaning – The system was cleaned by circulating with buffer for several minutes between samples.

Using these experimental conditions: Cu^{2+}, Pb^{2+} and Cd^{2+} can also be determined *on-line* using the nanosensor in waters.

The following is a direct application of the nanosensor to determine Zn^{2+} in the centrifugate of ZnO nanoparticles and proof of concept results are illustrated.

Calibration: A calibration curve in Figure 2, showing the relationship between peak area and concentration, was first plotted by injecting samples of 20 mmol dm^{-3} zinc chloride ($ZnCl_2$) into the system using a 5 µl syringe. It was important here to ensure that the tip of the syringe was submerged in the buffer solution as the $ZnCl_2$ was being injected. This ensured that all $ZnCl_2$ was injected into the system. $ZnCl_2$

completely dissociates in solution – therefore the concentration of $ZnCl_2$ in solution is equal to the concentration of zinc in solution. To obtain an idea of the range to plot the calibration curve for,the supernatant from a few zinc oxide samples were injected into the system and the peak areas compared with those of the $ZnCl_2$ oxidation peaks. It was found that concentrations of Zn in the system were between 2 and 5 μmol dm^{-3} – so a calibration curve was plotted for concentrations of $ZnCl_2$ up to 10 μmol dm^{-3}. Then, after centrifugation for 20 min at 14500 rpm, the supernatant was immediately removed from each ZnO sample to replicate treatment given to ICP-MS samples. 0.5 ml of this was added to the system for measurements, and the resulting peak area (see Figure 3) was measured using a peak area measurement function software. This involves selecting two points at either side of the base of the peak. For continuity, these points were chosen at the lowest tangent of the peak corners, as illustrated in Fig 3.

Figure 4 is proof that the ASV detection method provides reliable answers since they report similar concentrations irrespective of the ionic strength of KCl in which the ZnO particles are initially suspended. At low ionic strength of KCl (0.1 mol dm^{-3}) both ASV and inductively coupled plasma-mass spectrometry (ICP-MS) give identical results for Zn in the centrifugate. At higher ionic strength of KCl (0.1 mol dm^{-3}), IPC-MS gives a significantly higher result than ASV for Zn in the centrifugate. This can be explained by the fact that a higher concentration of KCl solubilises the ZnO to give Zn complex species which are not detected by ASV. ASV only detects free Zn^{2+}.

CLAIMS

1. A sensor apparatus for analysing a sample by a stripping voltammetric technique to determine the presence and/or amount of an analyte species in solution in the sample, the apparatus comprising:

(i) a sensor unit having a liquid flow path therethrough between an inlet and an outlet of the unit, said unit comprising

(a) an electrode assembly comprising at least one working electrode comprised of a conductive carrier substrate having a surface coated with mercury immobilised on the surface of the substrate,

(b) a counter electrode, and

(c) a reference electrode,

(ii) a liquid supply arrangement for supplying a flow of electrolyte to the inlet of the sensor unit,

(iii) a sample supply arrangement for introducing the sample to be analysed into electrolyte flow to the inlet of the sensor unit, and

(iv) stripping voltammetric means for stripping a species immobilised on the mercury surface of the working electrode and for determining the presence and/or amount of said analyte species

wherein the sample supply arrangement is adapted to introduce a metered amount of said sample into the electrolyte flow.

2. Apparatus as claimed in claim 1 wherein the liquid supply arrangement is adapted selectively to supply either control electrolyte or electrolyte containing sample to the inlet of the sensor unit.

3. Apparatus as claimed in claim 1 or 2 wherein the liquid supply arrangement is adapted to provide a first flow path for supplying control electrolyte to the inlet of the sensor unit and a second flow path for supplying electrolyte containing sample to the inlet of the sensor unit.

4. Apparatus as claimed in claim 3 wherein the first flow path includes a first reservoir for holding control electrolyte.

5. Apparatus as claimed in claim 3 or 4 wherein the second flow path is adapted to operate as a continuous loop.

6. Apparatus as claimed in claim 5 wherein the second flow path incorporates a second reservoir for holding electrolyte and said arrangement for introducing the sample to be analysed into electric flow to the inlet of the sensor unit introduces the sample into said second reservoir.

7. Apparatus as claimed in any one of claims 1 to 6 wherein the stripping voltammetric means is adapted to perform stripping anodic voltammetry.

8. A method of analysing a sample to determine the presence and/or amount therein of an analyte using the apparatus of any one of claims 1 to 7, said analyte being one that, if present in the sample, is capable of being deposited on the exposed mercury surface of the working electrode, the method comprising the steps of

(i) supplying to the inlet of the sensor unit a flow of electrolyte,

(ii) introducing into said flow of electrolyte upstream of the sensor a metered amount of the sample to be analysed,

(iii) effecting a technique that will cause the analyte if present in the sample to be deposited on the mercury surface of the working electrode, and

(iv) effecting a stripping voltammetric technique to cause analyte deposited on the mercury surface to be stripped therefrom and determining the presence and/or amount of said analyte

9. A method as claimed in claim 8 wherein in step (ii) the flow of electrolyte containing the sample to be analysed circulates continuously through the sensor unit.

10. A method as claimed in claim 8 or 9 wherein step (iv) is effected by anodic stripping voltammetry.

11. A method as claimed in claim 13 which comprises the steps of:

(a) cleaning the mercury electrode,

(b) depositing the analyte (if present) on the mercury surface of the working electrode,

(c) equilibrating the electrode, and

(d) effecting anodic stripping.

12. A method as claimed in any one of claims 8 to 11 wherein the analyte is a heavy metal.

13. A method as claimed in claim 12 wherein the heavy metal is selected from the group consisting of copper, lead, cadmium and zinc.

14. A method as claimed in any one of claims 8 to 13 wherein said sample is a water sample.

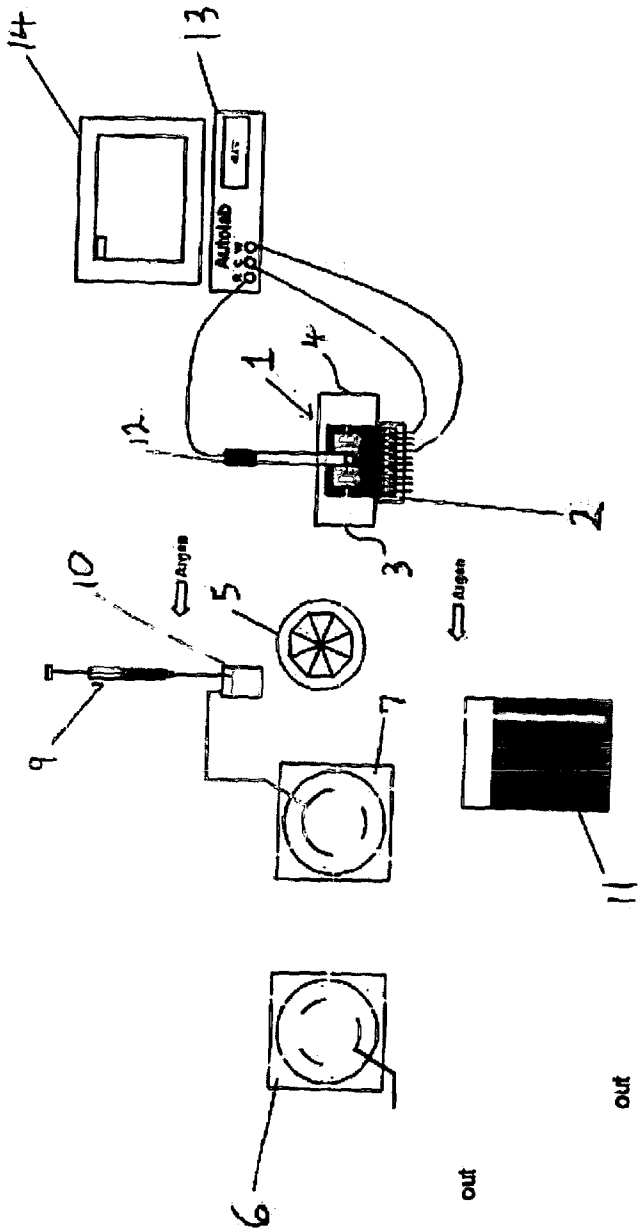


Fig 1a

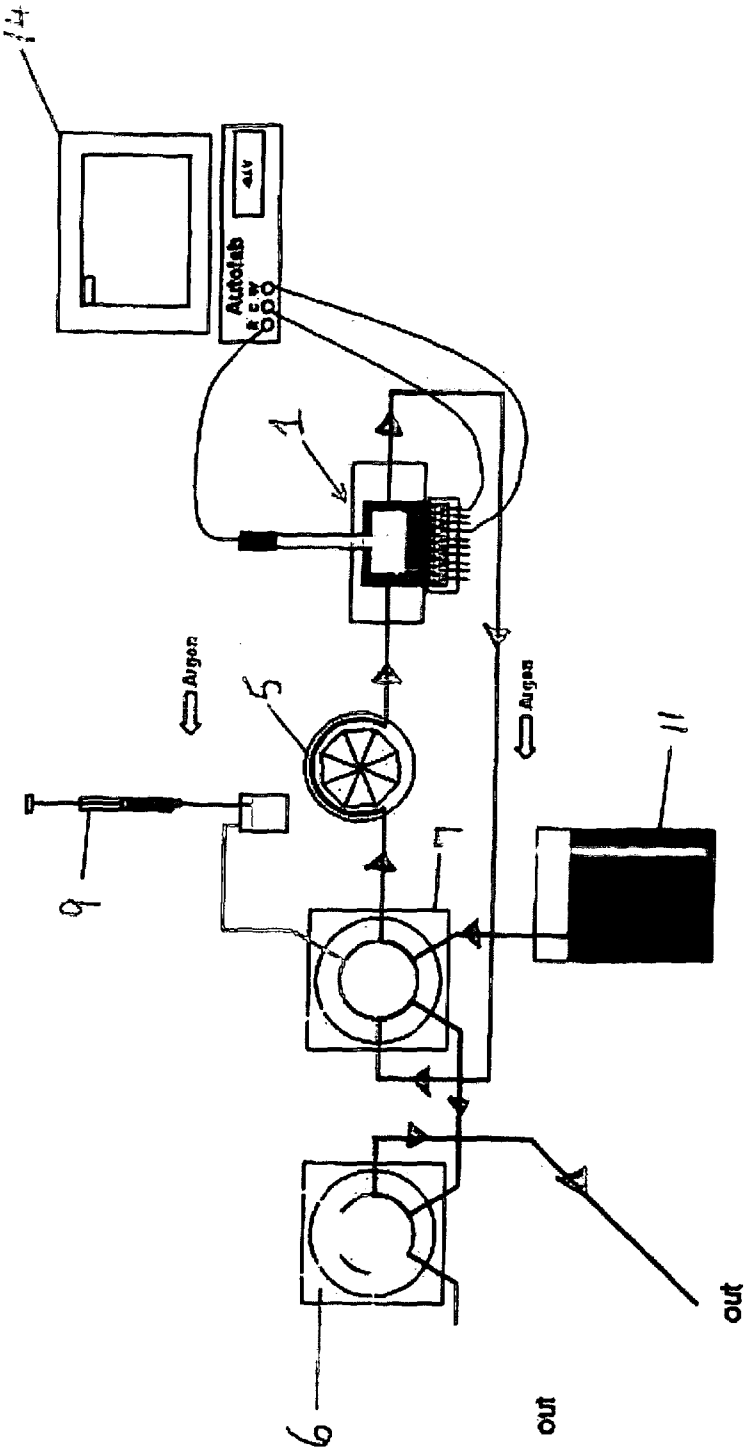


Fig 1b

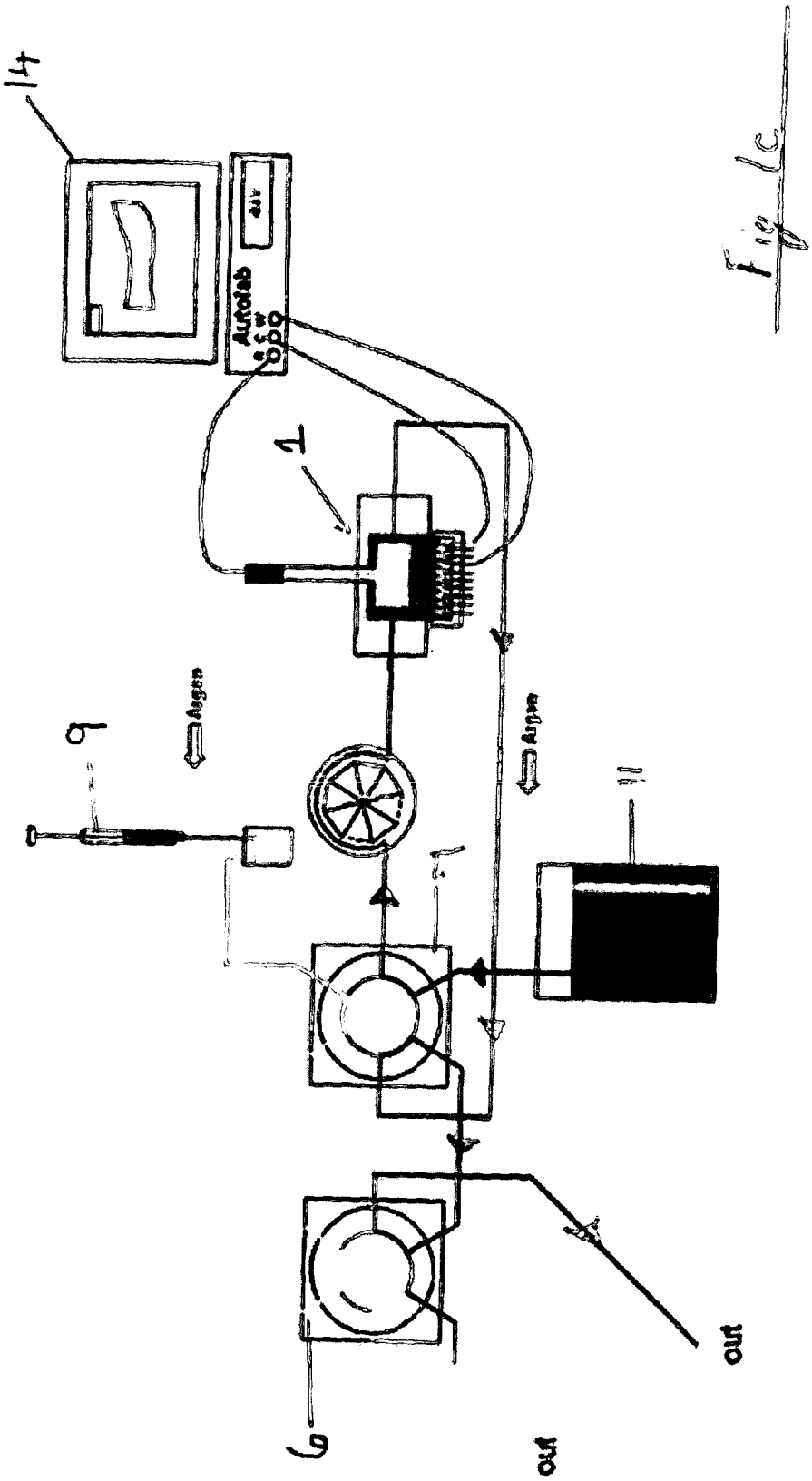


Fig 1c

Fig 1d

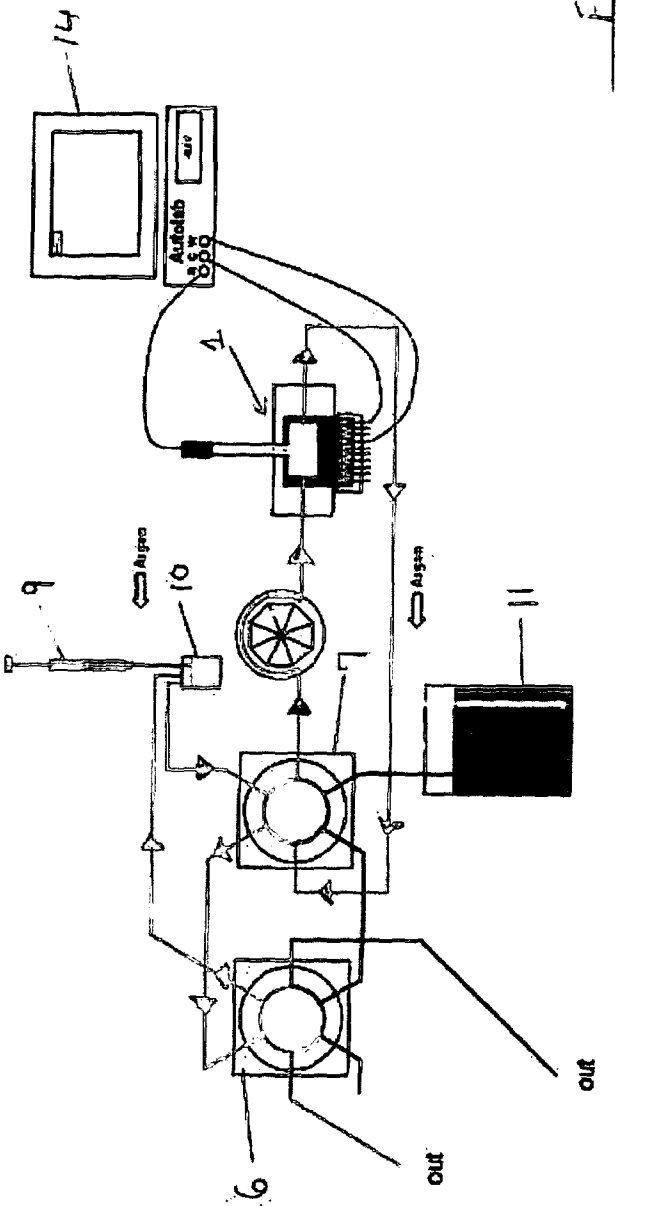


Fig 1e

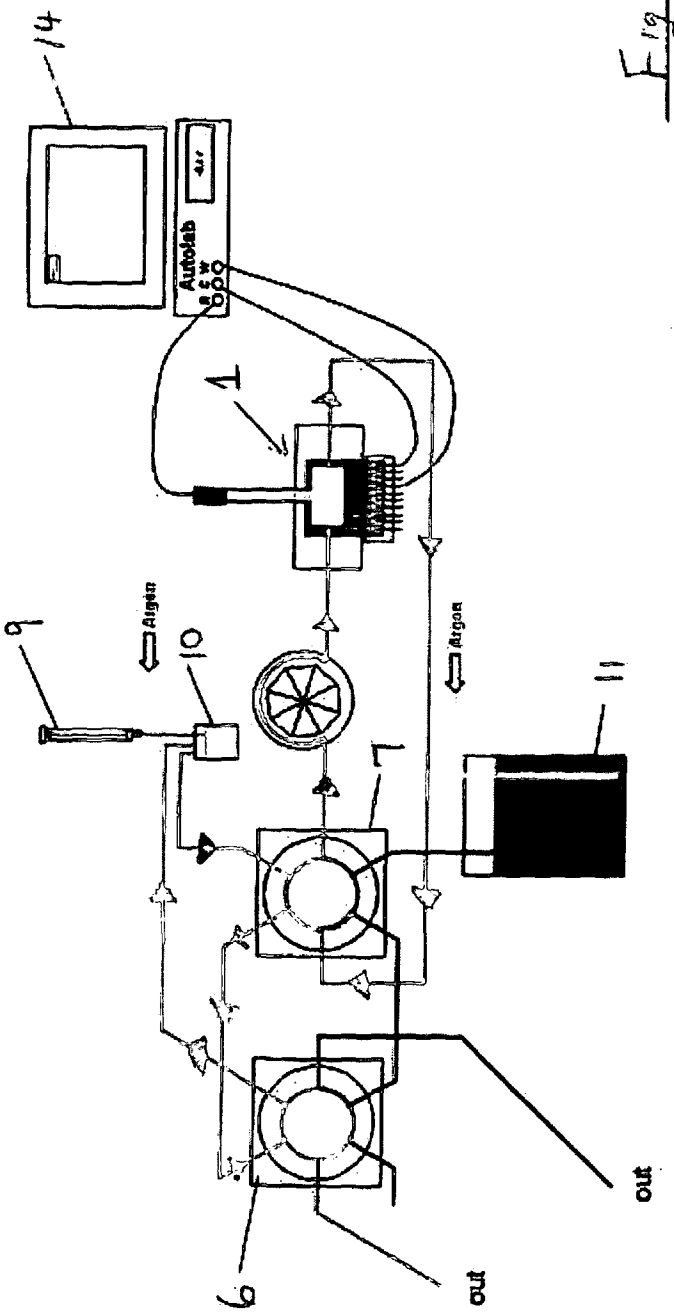


Fig 1F

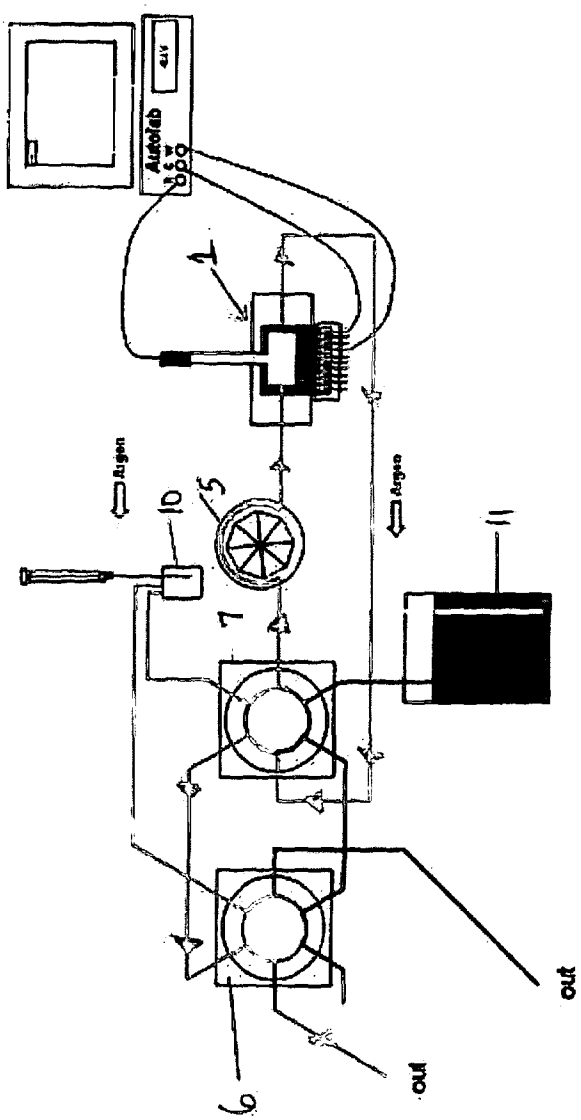


Fig 1g

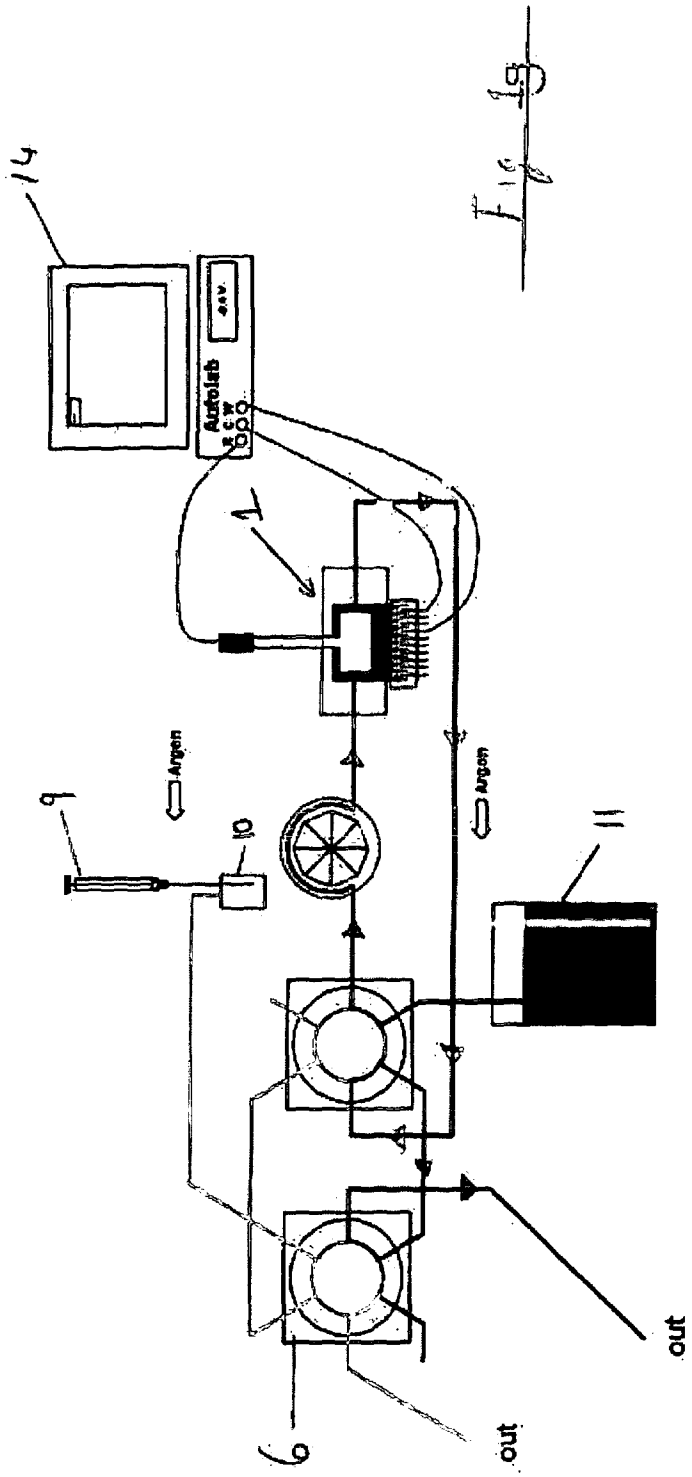


Fig 1h

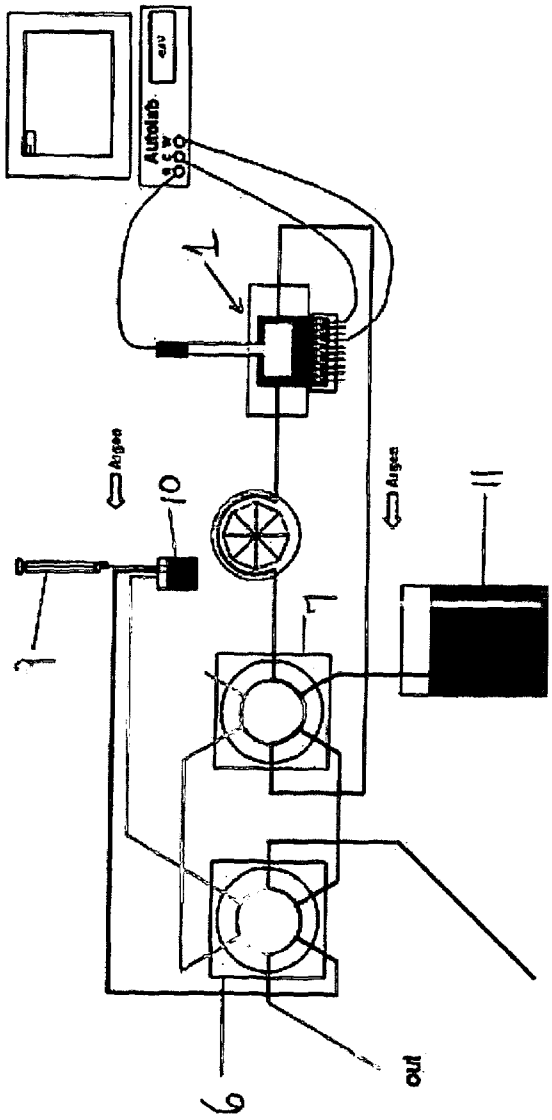


Figure 2. Calibration curve for oxidation peak area vs Zn concentration measurements.

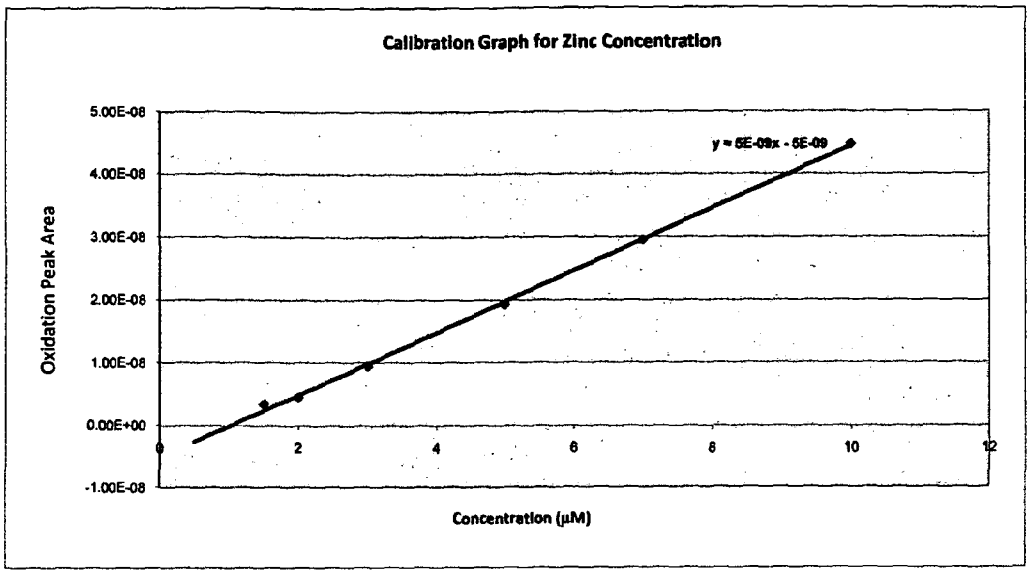


Figure 3. Illustration of peak area measurements on ASV oxidation peak.

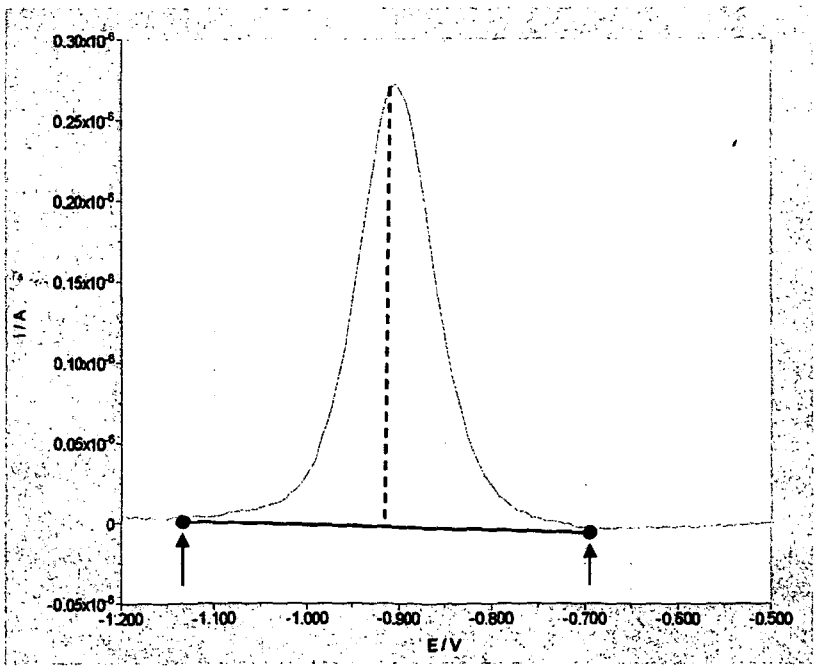
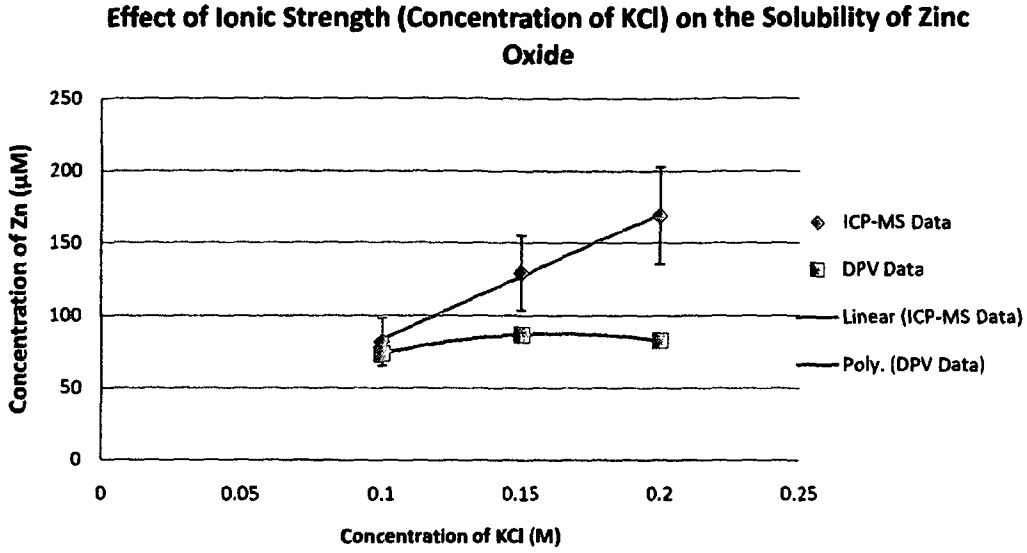


Figure 4. Graph to show the relationship between ionic strength of solution and solubility of ZnO nanoparticles.



INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2012/000235

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N27/42 G01N27/48 G01N33/18
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, COMPENDEX, INSPEC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/016366 A1 (UNIV LEEDS [GB]; NELSON LAWRENCE ANDREW [GB]; COLDRICK ZACHARY [GB]) 5 February 2009 (2009-02-05)	1-4,7,8, 10-14
Y	figure 10	5,6,9
Y	US 2008/318203 A1 (TRAN PHUONG-LAN [FR] ET AL) 25 December 2008 (2008-12-25) paragraph [0120]; figure 2	5,6,9
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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14 June 2012	28/06/2012

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INTERNATIONAL SEARCH REPORT

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