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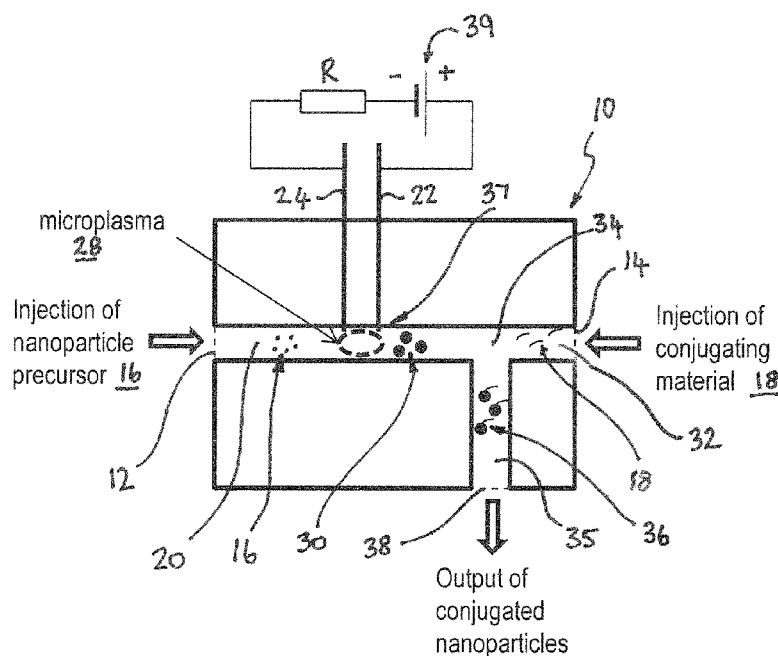
(54) **Title:** METHOD AND SYSTEM FOR THE PRODUCTION OF NANOPARTICLES

Fig. 1

(57) **Abstract:** A method for the production of conjugated nanoparticles in a microfluidic device. The method comprises: adding a nanoparticle precursor to the microfluidic device through one or more flow channels; generating microplasma in the microfluidic device; causing the microplasma to interact with the nanoparticle precursor to generate nanoparticles; adding a conjugate material into the microfluidic device through one or more flow channels; and causing the nanoparticles to mix with the conjugate material in a continuous flow to form conjugated nanoparticles.



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METHOD AND SYSTEM FOR
PRODUCTION OF CONJUGATED NANOPARTICLES

5 **Field of the Invention**

The present invention relates to a method and system for the production of conjugated nanoparticles, and more particularly conjugated nanoparticles for pharmaceutical and biomedical applications.

Background of the Invention

10 Recent advances in nanotechnology provide materials known as nanoparticles in the nanometer range with many potential industrial applications. Due to their unique size-dependent properties, nanoparticles offer the possibility to develop both new therapeutic and diagnostic tools.

15 Existing techniques for the synthesis and conjugation of nanoparticles generally use chemical synthesis which is a multifaceted and complicated procedure. In chemical synthesis, liquid solutions are generally used as precursors and the desired nanoparticles are formed using appropriate temperature conditions and reducing agents such as sodium borohydrate or sodium citrate. The surface characteristics of the nanoparticles are critical for application purposes. However, use of such reducing
20 agents can be detrimental to nanoparticle functionality. In addition, use of such chemical synthesis methods and temperature requirements result in prolonged multi-step processes which can range from hours to days, require skilled staff and are not cost effective. Additionally, the use of harsh chemicals can be environmentally harmful.

25 Microplasmas are a special class of electrical discharges where at least one dimension is reduced to sub-millimetre length scales. Recently microplasma technology has attracted interest in the area of nanoparticle synthesis due to their stable operation at atmospheric pressure and non-thermal characteristics. These properties make microplasmas suitable for a wide range of materials applications which have been described in a recent review by an inventor of the present invention (see "Microplasmas
30 for nanomaterials synthesis", J. Phys. D: Appl. Phys. 43 (2010) 323001 (21pp)).

Summary of Invention

35 Using a combination of microplasma technology and microfluidic systems, preferred embodiments of the present invention attempt to overcome the complex, environmentally adverse and time consuming problems of chemical synthesis of nanoparticles.

Thus, in a first aspect of the present invention there is provided a method for the production of conjugated nanoparticles in a microfluidic device at atmospheric pressure and low temperature in a continuous flow comprising the steps of:

- 5 1) addition of a nanoparticle precursor, or more than one nanoparticle precursors, to the microfluidic device through one or more flow channels;
- 2) generation of microplasma using one or more electrodes wherein the electrodes are powered by a power supply;
- 10 3) interaction of microplasma with said nanoparticle precursor to generate nanoparticles;
- 4) addition of a conjugate material into the microfluidic device through one or more flow channels;
- 15 5) mixing of said nanoparticles with said conjugate material in a continuous flow to form conjugated nanoparticles;
- 20 6) collection of desired conjugated nanoparticles.

In alternative embodiments, the fluidic device need not necessarily be microfluidic and the plasma need not necessarily comprise microplasma.

25 In a second aspect of the present invention there is provided a system for the production of conjugated nanoparticles wherein the system comprises;

- 1) a microfluidic device comprising one or more flow channels for entry of a nanoparticle precursor and a conjugate material;
- 2) flow control means;
- 30 3) a microplasma reactor for the generation of microplasma, the reactor having one or more electrodes;
- 4) a power supply to provide a voltage to at least one or more of the electrodes;
- 5) an outlet for exit flow of the desired conjugated nanoparticles.

35 Preferably the nanoparticle precursor is in solution. The nanoparticle precursor may be a metal salt, for example, HAuCl_4 , or other such soluble material that is capable of chemical reduction by electrons.

The conjugation of the nanoparticle precursor with the conjugate material may occur by any covalent or non-covalent/electrostatic means known in standard chemistry.

5 Embodiments of the invention may use any one or more of a range of conjugate materials for production of conjugated nanoparticles, more particularly for pharmaceutical and biomedical applications. Such conjugate material may include but are not limited to diagnostic agents, bio-sensing agents, drug delivery carriers, therapeutic drugs and/or prophylactic agents. Such drugs or agents may further include, but are not limited to small molecules, peptides, proteins, lipids, organometallic
10 compounds, nucleic acids, carbohydrates, hormones, silicon and polymer-based conjugates (for example PLGA (Poly(Lactide-co-Glycolide), poly(lactic acid) (PLA) and poly(glycolic acid(PGA)) or any target ligand or drug, metals such as gold, silver, palladium, ruthenium, radioactive compounds, vaccines, immunological agents or combinations thereof.

15 Preferably each flow channel is further provided with an inlet.

Optionally each flow channel is provided with an outlet

In one embodiment, the conjugated nanoparticles can be collected through an outlet of the flow channel and stored for later use. In another embodiment, the conjugated nanoparticles can be used directly for injection in other applications for example as
20 described herein.

The temperature is preferably low. In one embodiment, the temperature is between 15 to 80°C. In another embodiment, the temperature is the ambient temperature surrounding the system.

25 Most plasma generation methodologies require low-pressure which requires pumps and other equipment to work optimally. Systems embodying the present invention preferably operate at or around (e.g. substantially at) atmospheric pressure such that they are operable at ambient conditions with no specific need of varying the pressure with pumps or a vacuum system. This has the benefit of reducing the cost of the system, lowering maintenance costs and simplifying the system to reduce the risk of malfunctioning. The
30 further advantage of using an atmospheric pressure microplasma system is that it provides a more stable environment for plasma formation compared with large scale plasma systems which often lead to instabilities and gas heating, conditions that are unsuitable for materials applications.

35 Preferably, the microplasma reactor is located within the flow channel which carries the nanoparticle precursor such that a microplasma is in contact with the nanoparticle precursor.

In one embodiment, said electrodes comprise an anode electrode and a cathode electrode. The anode electrode may comprise any suitable type of anode such as a metal rod, for example, a stainless steel rod. The cathode electrode may comprise any
5 suitable type of cathode, for example, a microcapillary hollow tube. In one embodiment, the microcapillary tube is made of metal e.g. stainless steel. In a further embodiment, the microcapillary tube is approximately 250µm in diameter.

Each electrode may be in direct contact with the nanoparticle precursor, for example, the electrode may be immersed in the nanoparticle precursor solution or the electrode
10 may be located near or on an interface with the nanoparticle precursor solution.

In operation, the microplasma may be generated by electrodes driven by a power supply. By applying a sufficient voltage to one or more electrodes, a region of plasma can be generated. Non-thermal, atmospheric-pressure operation makes it possible to couple microplasmas with liquid as well. When a microplasma is formed at the surface
15 or with a liquid, the ions, electrons and other excited species in the gas phase interact with and initiate reactions in the solution. This approach can be useful to nucleate and grow colloidal nanoparticles or modify the surface chemistry of nanoparticles.

The power supply may be any suitable power supply capable of generating microplasma, and may supply ac or dc voltages. In one embodiment the power supply
20 has an operating voltage of 1.2kV and a resistor at 100 kΩ. Other examples of operational power ranges include but are not limited to; 1) frequency in the range dc to microwave; 2) voltage in the range 500 V to 5 kV; 3) current in the range from µA to mA. In a further embodiment, the power supply is in a miniature form.

Optionally, the microplasma generation may be coupled with supplied gas flow wherein
25 the gas is introduced into the microplasma reactor through a gas flow inlet. In one embodiment, the gas is carried through a hollow electrode such as in the form of a microcapillary hollow tube. The gas may include but is not limited to any one of following: air, helium or argon.

In some embodiments, the flow channels are of uniform shape. In some embodiments, the width and/or height of each flow channel ranges from approximately 1 µm to approximately 1000 µm. In some embodiments, the length of each flow channel ranges from approximately 100 µm to approximately 10 cm. Flow channels may be composed of any material suitable for the flow of fluid through the channels, such as Perspex. Typically, the material is one that is resistant to solvents and non-solvents that may be
35 used in the preparation of nanoparticles and conjugates thereof.

Preferably the flow channels join at a convergence point. In one embodiment, the nanoparticle precursor flow channel and the conjugate material flow channel converge

to form a T-Shape channel or the like to provide optimum interaction of the nanoparticle precursor and conjugate material.

5 In some embodiments, the system may comprise a mixing means for aiding the interaction between the microplasma and the conjugate material. The mixing means may comprise an additional enlarged channel section which forms part of one or more flow channels to help mixing and control flow speed of the nanoparticle formation. Preferably the mixing means is located proximate to or at the convergence point of the flow channels for supplying the nanoparticle precursor and the conjugating material
10 respectively.

In use of preferred embodiments, the application of pressure to the nanoparticle precursor and/or conjugate material causes optimal flow of such fluid through each channel which is controlled by a flow control means. In one embodiment, the flow control means is a pressure means for aiding the flow of fluid. Pressure means may be
15 any suitable means such as a syringe, a microcapillary force, a pump, and/or by gravity. In one embodiment, the pressure means is regulatable i.e. the applied pressure may be increased, decreased, or held constant. In other embodiments, the flow rate of the nanoparticle precursor or conjugate material is adjustable and/or regulatable by adjusting the size e.g. length, width, and/or height of the flow channel. The flow rate
20 may be regulated either manually or by automation.

In one embodiment the dimensions of the system can be approximately 10 cm x 5 cm x 5 cm. Optionally, the system is portable.

An advantage of continuous flow in preferred embodiments of the present invention is to allow the manufacturing of large quantities of nanoparticle precursors with a fast rate
25 and high yield so as to provide a high throughput. Continuous flow is a non-stop process analogous to roll-to-roll type manufacturing. In addition, once the parameters have been set initially, the process provides continuous production of conjugated nanoparticles with no need of supervision or intervention beyond the actual collection of nanoparticles.

30 Preferred embodiments of the invention provide rapid generation of conjugated nanoparticles, for example within a range from 30 seconds up to a several minutes. In one embodiment, the processing time is approximately 2 minutes.

In use, in one embodiment, the nanoparticle precursor and conjugate material are injected into the microfluidic device through two separate flow channel inlets.
35 Microplasma is generated at the electrode(s) and interacts with the nanoparticle precursor. The microplasma generation then produces nanoparticle nucleation and growth. The synthesized nanoparticles are mixed with the conjugate material. This

process allows nanoparticles functionalization and drug attachment. These can then be collected at the outlet or directly used as required.

5 In preferred embodiments, a combined microfluidic-microplasma method and system produce bespoke functionalized nanoparticles at atmospheric-pressure and ambient temperature with no need for specific temperature conditions and pressure regulations which are required for chemical synthesis or other nanoparticle synthesis in gas phase.

10 A disadvantage of the generation of gas-phase plasma-generated nanoparticles is that the nanoparticles are collected on a substrate initially and conjugated later making the process multi-stepped and prolonged. The use of liquid-phase plasma-generated nanoparticles in preferred embodiments of the present invention overcomes this time consuming step as the synthesized nanoparticles remain dispersed in solution. This is also desirable for biological applications such as bio-sensing and drug delivery as they are generally performed in liquid environments and manipulation of conjugated
15 nanoparticles can be done with ease and with no risk of contamination with air.

20 In preferred embodiments, the combined microplasma-microfluidic system produces nanoparticles with a controlled size and shape and optimized functionality without the use of damaging reducing agents. This also facilitates the functionalization of the nanoparticles, reduces the steps and enhances the application effectiveness of the nanoparticles. This is particularly critical in the area of therapeutics and drug delivery systems which are dependent on particle size and surface characteristics.

Preferred embodiments of the present invention are capable of synthesizing conjugated nanoparticles with bespoke characteristics which can be used for many applications such as in therapeutic use, drug delivery, or biosensing applications.

25 It is further envisaged that the shape and size of each conjugated nanoparticle can be synthesized to any bespoke specification for use in such applications by regulating the process parameters; for instance precursor concentration and flow channel size can be manipulated to determine nanoparticle size and shape whilst electrical parameters can control production rate and the degrees of interaction with conjugating materials.

30 In one specific embodiment and as exemplified in the examples, the method and system of the present invention produce gold nanoparticles. Gold nanoparticles exhibit unique Surface Plasmon Resonance (SPR) enhanced properties such as Mie scattering, two-photon luminescence, Surface Enhanced Raman Scattering (SERS). These unique properties are useful for optical diagnostics and detection of cancer.
35 Furthermore, by changing the morphology of the gold nanoparticles, the SPR can be shifted to the NIR region of the 'biological window' which enables the use of gold nanoparticles in imaging and therapy techniques.

A further advantage of preferred embodiments is that synthesis and conjugation of nanoparticles is achieved at very low cost with no need for complex chemical procedures and processes. Embodiments of the invention typically also provide a very
5 clean process with improved performance and reduced costs that can be operated in any environment such as laboratories, hospitals and clinics by a lay person who is not skilled in the art.

The invention advantageously may also extend to a modular system and thus viewed from a third aspect, the invention provides a system of the second aspect of the present
10 invention for use with other like modular systems of the second aspect of the invention for the production of conjugated nanoparticles.

In a fourth aspect of the present invention, the invention provides conjugated nanoparticle products of the method and systems of the present invention as described herein.

15 In a further aspect of the present invention, there is provided use of conjugated nanoparticles produced according to the method and systems of the present invention wherein the conjugated nanoparticles are used for pharmaceutical and biomedical applications.

The features of each aspect of the invention are as for each of the other aspects mutatis
20 mutandis.

Brief description of the Drawings

Embodiments of the present invention will now be described, by way of example only, and with reference to the drawings, in which like numerals are used to denote like parts and in which:

25 Figure 1 shows a schematic diagram of a system for the production of conjugated nanoparticles embodying one aspect of the present invention;

Figure 2 shows a schematic diagram of an alternative embodiment of a system for the production of conjugated nanoparticles embodying one aspect of the present invention;

30 Figure 3 shows TEM (transmission electron microscopy) images of gold nanoparticles produced using embodiments of the method and system of the present invention;

Figures 4A to 4C show respective size distributions with the radius in nm for three different gold precursor concentrations, namely 4A: 0.2 mM, 4B 1 mM, and 4C 5 mM, of HAuCl₄ precursor.

Detailed Description of Specific Embodiment

Referring to Figure 1, there is shown a system for the production of conjugated nanoparticles embodying one aspect of the present invention, the system comprising a fluidic device, preferably a microfluidic device 10, an inlet 12 for receiving a nanoparticle precursor 16 (or more than one) and an inlet 14 for receiving a conjugate material 18, for example a conjugate drug. Either or both of the nanoparticle precursor 16 and conjugate material 18 may be introduced to the system and in particular to the respective inlet 12, 14 by and/or under the control of flow control means, for example a syringe, pump or other pressure device (or injection means) capable of injecting or moving fluid, especially liquid, by pressure. Typically, any one or both of the nanoparticle precursor 16 and conjugate material 18 are in liquid form, e.g. in solution, but may take other forms e.g. fluid (gas), solid or suspension.

The device 10 includes a flow channel 20 for the precursor 16 and a flow channel 32 for the conjugate material 18. The channels 20, 32 meet at an intersection 34. An outlet channel 35 is typically provided, leading to an outlet 38. Advantageously the outlet channel runs between the intersection 34 and the outlet 38. The illustrated flow channels 20, 32 are substantially co-linear. The outlet flow channel 35 may be substantially perpendicularly disposed to the inlet flow channels 20, 32. Hence, the channels 20, 32, 35 may form a substantially T-shaped composite channel.

In preferred embodiments, at least one and preferably all of the channels 20, 32, 35 are shaped and dimensioned to serve as microfluidic channels. A typical width and/or height of each flow channel 20, 32, 35 ranges from approximately 1 μm to approximately 1000 μm . In some embodiments, the length of each flow channel 20, 32, 35 ranges from approximately 100 μm to approximately 10 cm. The channels 20, 32, 35 may be composed of any material suitable for the flow of fluid through the channels, such as Perspex, and are typically formed in a body of the device 10. The channels 20, 32, 35, or any one or more of them, conveniently has a substantially uniform cross-section, except where a mixing chamber is provided. In alternative embodiments, any one or all of the channels do not have microfluidic dimensions.

The device 10 includes a plasma generator 37, preferably a microplasma generator, for example a microplasma reactor. The plasma generator 37 typically comprises electrodes, in particular an anode 22 and a cathode 24, connected to an electrical power supply 39, a DC supply in this example. The generator 37 typically also includes means for supplying gas to the generator 37 for ionization by the action of the electrodes 22, 24 to produce plasma, preferably microplasma. The plasma generator 37 is conveniently located in the device 10 to generate plasma in the nanoparticle precursor flow channel 20. For example this may be achieved by locating the generator 37, or at least the electrodes 22, 24, in the channel 20.

In use, the nanoparticle precursor 16 is injected into the device 10 via inlet 12 and flows through the precursor flow channel 20. The electrode pair, i.e. anode 22 and cathode 24 are in this example placed through the flow channel 20. The preferred cathode 24
5 comprises a stainless steel microcapillary tube having an inner diameter of approximately 250µm and the preferred anode 22 comprises a stainless steel rod. Other types and sizes of anode and/or cathode may alternatively be used. The DC power supply 39 is switched on at an operating voltage of 1.2Kv for an operating period, for example 2 minutes. The electrode circuit includes a current limiting resistor which in this
10 example has a resistance of 100 kΩ. The power supply drives the electrodes 22, 24 to generate microplasma 28. The microplasma 28 is generated on the interface with the nanoparticle precursor 16 and interacts with the precursor resulting in nucleation and growth of nanoparticles 30. Optionally, gas for creating the microplasma may be supplied via a hollow cathode 24, or by any other convenient means.

15 The nanoparticle precursor 16 may comprise a metal salt, for example, HAuCl₄, or other such soluble material that is capable of chemical reduction by electrons. It is typically in liquid form but may take other forms e.g. fluid (gas), solid or suspension.

The conjugating drug or other conjugating material 18 is injected into the device 10 via inlet 14 and flows through the conjugate material flow channel 32. At the channel
20 intersection 34 the nanoparticles 30 mix with the conjugated drug/material 18 to cause nanoparticle functionalization and/or drug attachment and to form conjugated nanoparticles 36. The conjugated nanoparticles 36 can be collected at outlet 38 or directly used, as required.

The conjugating material 18 may comprise a diagnostic agent, bio-sensing agent, drug
25 delivery carrier, therapeutic drug and/or prophylactic agent. Such drugs or agents may further include small molecules, peptides, proteins, lipids, organometallic compounds, nucleic acids, carbohydrates, hormones, silicon and polymer-based conjugates (for example PLGA (Poly(Lactide-co-Glycolide), poly(lactic acid) (PLA) and poly(glycolic acid(PGA)) or any target ligand or drug, metals such as gold, silver, palladium,
30 ruthenium, radioactive compounds, vaccines, immunological agents or combinations thereof. It is typically in liquid form but may take other forms e.g. fluid (gas), solid or suspension.

The operating temperature of the system and in particular within device 10 is
35 advantageously low, for example ambient temperature or between approximately 15 to 80°C. The system advantageously operates substantially at atmospheric pressure and as such is operable at ambient conditions.

The device 10 may include mixing means (not illustrated) for aiding interaction between the microplasma and the conjugate material. The mixing means may comprise an

enlarged channel section forming part of one or more flow channels 20, 30, 35 to help mixing and control flow speed of the nanoparticle formation. Preferably the mixing means is located proximate to or at the intersection 34.

5 Figure 2 illustrates a system for the production of conjugated nanoparticles embodying one aspect of the present invention, the system being an experimental example showing the synthesis of gold (Au) nanoparticles using AMP (Atmospheric-pressure Microplasma) in the presence of helium gas prior to conjugation with a conjugate material.

10 The nanoparticle precursor comprises a gold precursor solution 44, e.g. Gold (III) chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) having a concentration (%) of 0.2 mM in distilled water, held in a tank 45.

Figure 2 is a schematic configuration of part of the apparatus for the generation of the gold nanoparticles prior to conjugation with a conjugate material. In this configuration,
15 the anode 42 is immersed in the gold precursor solution 44. Helium 46 is passed through a microcapillary cathode 48 at a rate of 25 SCCM (standard cubic centimetres per minute). Upon switching on the power supply 50 at an operating voltage of 1.2Kv and with a 100 k Ω resistor for 2 minutes, a current flows through the gold precursor solution 44. A microplasma 52 is generated at the interface with the gold precursor
20 solution 44. The microplasma 52 interacts with the gold precursor 44 resulting in the formation of gold nanoparticles.

Figure 3 shows Transmission Electron Microscopy (TEM) images of gold nanoparticles using a method embodying the present invention which show a typical five-fold crystal structure and in this case the particle size is about 80 nm in diameter.

25 Figures 4A to 4C show respective size distributions of the gold nanoparticles with the radius in nm for three different gold precursor concentrations, i.e. 4A) 0.2 mM of precursor, 4B) 1 mM of precursor, 4B) 5 mM of precursor.

It will be understood that the above examples are of just preferred embodiments and that it will be apparent to one of ordinary skill in the art that many changes and
30 modifications are possible without departing from the scope of the invention.

CLAIMS:

- 5 1. A method for the production of conjugated nanoparticles in a fluidic device, the method comprising:
- adding a nanoparticle precursor to the fluidic device through one or more flow channels;
- generating plasma in said fluidic device;
- 10 causing said plasma to interact with said nanoparticle precursor to generate nanoparticles;
- adding a conjugate material into the fluidic device through one or more flow channels; and
- causing said nanoparticles to mix with said conjugate material in a continuous flow to form conjugated nanoparticles.
- 15 2. A method as claimed in claim 1, wherein said adding of said nanoparticle precursor involves causing said nanoparticle precursor to flow through said one or more flow channels.
3. A method as claimed in claim 1 or 2, wherein said adding of said conjugate material involves causing said conjugate material to flow through said one or more flow channels.
- 20 4. A method as claimed in any preceding claim, wherein the or each of said one or more flow channels for said nanoparticle precursor comprises a microfluidic flow channel.
5. A method as claimed in any preceding claim, wherein the or each of said one or more flow channels for said conjugate material comprises a microfluidic flow channel.
- 25 6. A method as claimed in any preceding claim, wherein said nanoparticle precursor is in liquid form, preferably in solution.
7. A method as claimed in any preceding claim, wherein said conjugate material is in liquid form, preferably in solution.
- 30 8. A method as claimed in any preceding claim, further including collecting said conjugated nanoparticles.

9. A method as claimed in any preceding claim, further including maintaining said fluidic device at substantially atmospheric pressure.
- 5 10. A method as claimed in any preceding claim, further including maintaining either or both of said nanoparticle precursor and said plasma at substantially atmospheric pressure.
11. A method as claimed in any preceding claim, further including maintaining said conjugate material at substantially atmospheric pressure.
- 10 12. A method as claimed in any preceding claim, further including maintaining said fluidic device at ambient temperature or between approximately 15°C and approximately 80°C.
13. A method as claimed in any preceding claim, further including maintaining either or both of said nanoparticle precursor and said plasma at ambient temperature or between approximately 15°C and approximately 80°C.
- 15 14. A method as claimed in any preceding claim, further including maintaining said conjugate material at ambient temperature or between approximately 15°C and approximately 80°C.
15. A method as claimed in any preceding claim, further including producing said conjugated nanoparticles in a continuous flow.
- 20 16. A method as claimed in any preceding claim, wherein said generation of said plasma involves application of an electrical power supply to one or more electrodes.
17. A method as claimed in any preceding claim, including causing said conjugated nanoparticles to be output from said device in a continuous flow.
- 25 18. A method as claimed in any preceding claims, including causing said plasma to form in said one or more flow channels for receiving said nanoparticle precursor.
19. A method as claimed in claim 16, including providing said electrodes in said one or more flow channels for receiving said nanoparticle precursor.
- 30 20. A method as claimed in any preceding claim, wherein said nanoparticle precursor comprises a soluble material that is capable of chemical reduction by electrons.
- 35 21. A method as claimed in claim 20, wherein said nanoparticle precursor comprises a metal salt.

22. A method as claimed in any preceding claim, wherein said conjugate material comprises any one or more of a diagnostic agent, bio-sensing agent, drug delivery carrier, therapeutic drug or prophylactic agent.

23. A method as claimed in any preceding claim, wherein said conjugate material comprises any one or more of a peptide, a protein, a lipid, an organometallic compound, a nucleic acid, a carbohydrate, a hormone, a silicon-based conjugate, a polymer-based conjugate, a target ligand, a target drug, a metal, a radioactive compound, a vaccine, an immunological agent, or any combination of any two or more thereof.

24. A method as claimed in any preceding claim, including introducing said nanoparticle precursor into said one or more flow channel under pressure, and optionally regulating said pressure.

25. A method as claimed in any preceding claim including controlling the flow, for example the flow rate, of said nanoparticle precursor in said one or more flow channel.

26. A method as claimed in any preceding claim, including introducing said conjugate material into said one or more flow channel under pressure, and optionally regulating said pressure.

27. A method as claimed in any preceding claim including controlling the flow, for example the flow rate, of said conjugate material in said one or more flow channel.

28. A method as claimed in any one of claims 24 to 27, including selecting one or more dimensions, for example length width and/or height, of said one or more respective flow channels to control the pressure and/or flow of said nanoparticle precursor and/or said conjugate material.

29. A method as claimed in claim 16, including providing a gas around at least one of said electrodes, said gas being capable of forming said plasma upon application of electrical power to said electrodes.

30. A system for the production of conjugated nanoparticles, the system comprising
a fluidic device comprising one or more flow channels for receiving a nanoparticle precursor and one or more flow channels for receiving a conjugate material,
a plasma generator for the generation of plasma,
wherein said plasma generator is located such that said plasma contacts, in use, said nanoparticle precursor in said one or more flow channels to generate nanoparticles,

and wherein said respective one or more flow channels for receiving said nanoparticle precursor and said conjugate material are configured to cause, in use, said conjugate material and said nanoparticles to mix to form conjugated nanoparticles.

- 5 31. A system as claimed in claim 1, wherein said plasma generator comprises a plasma reactor.
32. A system as claimed in claim 31 or 32, wherein said plasma generator comprises one or more electrodes.
- 10 33. A system as claimed in claim 32, wherein at least one of said one or more electrodes is located in said one or more flow channels for receiving said nanoparticle precursor.
34. A system as claimed in claim 33, including an electrical power supply for providing electrical power to said one or more of the electrodes.
- 15 35. A system as claimed in any one of claims 30 to 34, wherein said fluidic device includes an outlet for said conjugated nanoparticles.
36. A system as claimed in any one of claims 30 to 35, wherein the or each of said one or more flow channels for said nanoparticle precursor comprises a microfluidic flow channel.
- 20 37. A system as claimed in any one of claims 30 to 36, wherein the or each of said one or more flow channels for said conjugate material comprises a microfluidic flow channel.
38. A system as claimed in any one of claims 35 to 37, wherein said outlet opens from an outlet flow channel formed in said device.
39. A system as claimed in any one of claims 30 to 38, wherein said respective one or more flow channels for receiving said nanoparticle precursor and said conjugate material meet at an intersection.
- 25 40. A system as claimed in any one of claims 35 to 39, wherein said fluidic device includes an outlet channel leading to said outlet.
41. A system as claimed in claim 40 when dependent on claim 39, wherein said outlet channel runs between said intersection and said outlet.
- 30 42. A system as claimed in any one of claims 39 to 41, wherein said respective one or more flow channels for receiving said nanoparticle precursor and said conjugate material are substantially co-linear.

43. A system as claimed in any one of claims 39 to 42, wherein said outlet flow channel is substantially perpendicularly disposed to said respective one or more flow channels for receiving said nanoparticle precursor and said conjugate material.
- 5 44. A system as claimed in any one of claims 30 to 43, further including gas supply means for supplying gas to said fluidic device for the creation of said plasma.
45. A system as claimed in claim 42 when dependent on any one of claims 32 to 41, wherein said gas supply means is configured provide gas around at least one of said electrodes, said gas being capable of forming said plasma upon application of electrical
10 power to said electrodes.
46. A system as claimed in any one of claims 30 to 44, further including flow control means for controlling the flow of said nanoparticle precursor and/or said conjugate material through said fluidic device.
47. A system as claimed in claim 46, wherein said flow control means is operable to
15 cause said conjugated nanoparticles to form in flow, and preferably to be output from said device in flow.
48. A system as claimed in claim 46, wherein said flow control means comprises one or more pump or syringe.
49. A system as claimed in any one of claims 30 to 48, further including mixing means
20 for mixing said conjugate material and said nanoparticles to form conjugated nanoparticles.
50. A system as claimed in any one of claims 32 to 49, wherein the or each electrode is located to be, in use, in contact with the nanoparticle precursor, for example immersed in the nanoparticle precursor, or located near or on an interface with the nanoparticle
25 precursor.
51. A system as claimed in claim 44 when dependent on any one of claims 32 to 50, wherein said gas is delivered, in use, through a hollow one of said electrodes, which electrode may comprise a microcapillary hollow tube.
52. A system as claimed in any one of claims 30 to 51, comprising a plurality of
30 interconnected modules, each module comprising a respective one of said fluidic devices and said plasma generators.
53. A system as claimed in any one of claims 30 to 52, wherein said fluidic device comprises a microfluidic device.
54. A conjugated nanoparticle product formed by the method of any one of claims 1 to
35 29.

55. Use of a conjugated nanoparticle product formed by the method of any one of claims 1 to 29 in a pharmaceutical or biomedical application.

5 56. A method for the production of nanoparticles in a fluidic device, the method comprising:

adding a nanoparticle precursor to the fluidic device through one or more flow channels;

generating plasma in said fluidic device; and

10 causing said plasma to interact with said nanoparticle precursor to generate nanoparticles.

57. A system for the production of conjugated nanoparticles, the system comprising

a fluidic device comprising one or more flow channels for receiving a nanoparticle precursor and one or more flow channels for receiving a conjugate material,

a plasma generator for the generation of plasma,

15 wherein said plasma generator is located such that said plasma contacts, in use, said nanoparticle precursor in said one or more flow channels to generate nanoparticles.

58. A method as claimed in any one of claims 1 to 29 or 56, wherein said plasma comprises microplasma.

20 59. A system as claimed in any one of claims 30 to 53 or 57, wherein said plasma generator comprises a microplasma generator for generating microplasma.

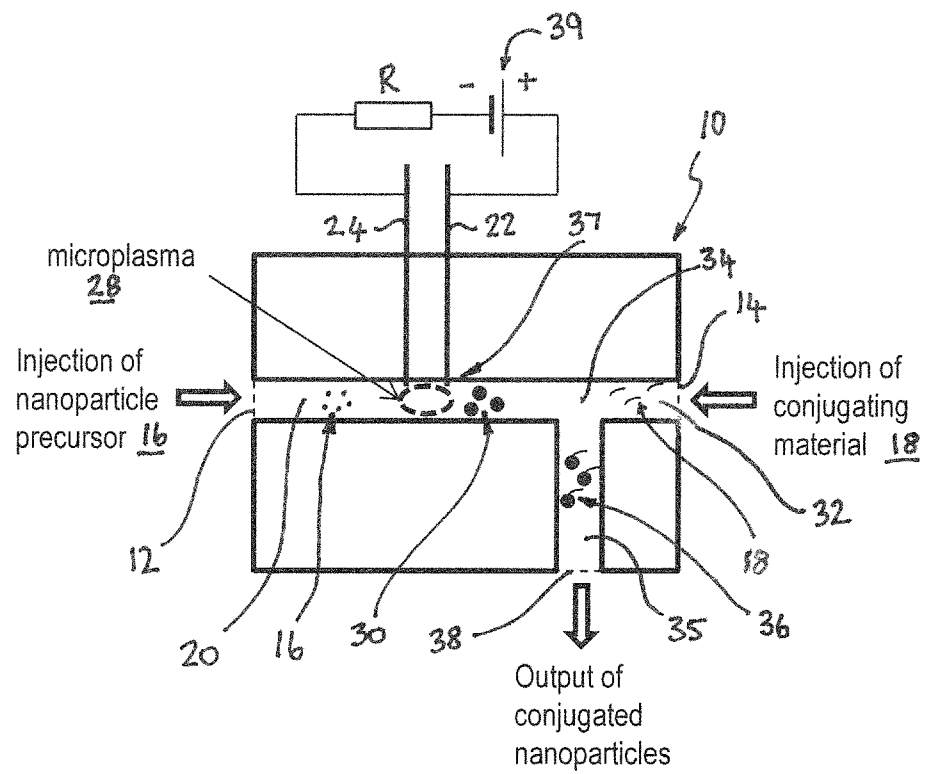


Fig. 1

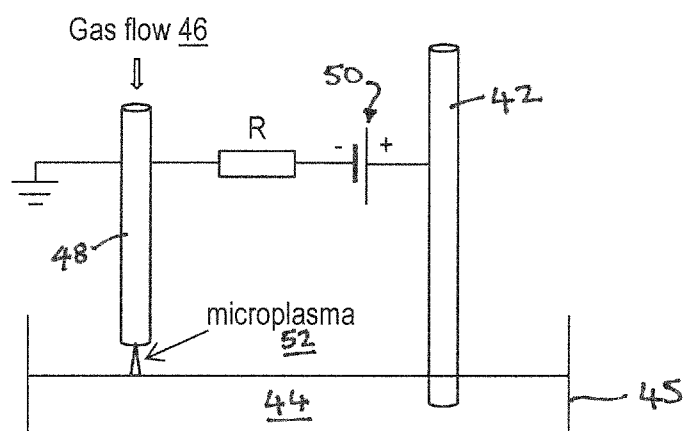
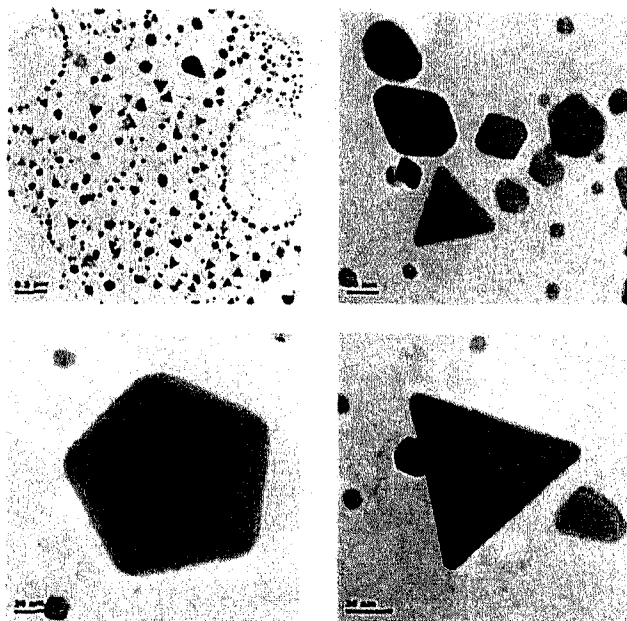


Fig. 2



TEM Images of Au NPs

FIG. 3

Size Distribution by Intensity

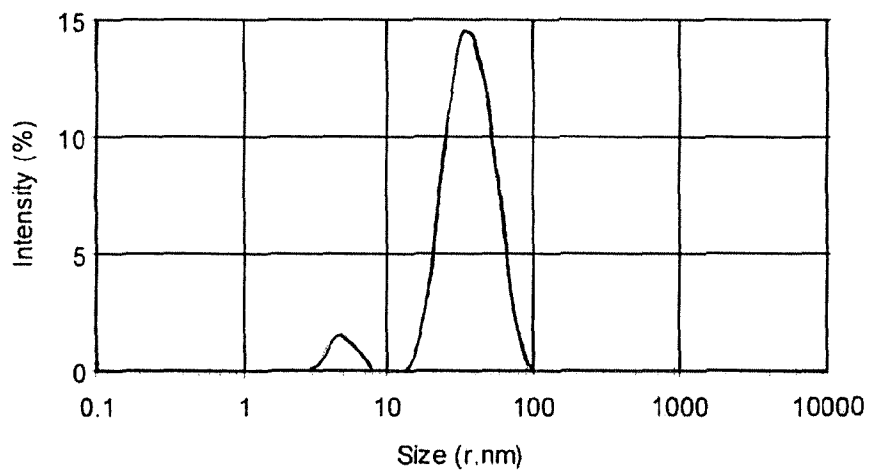
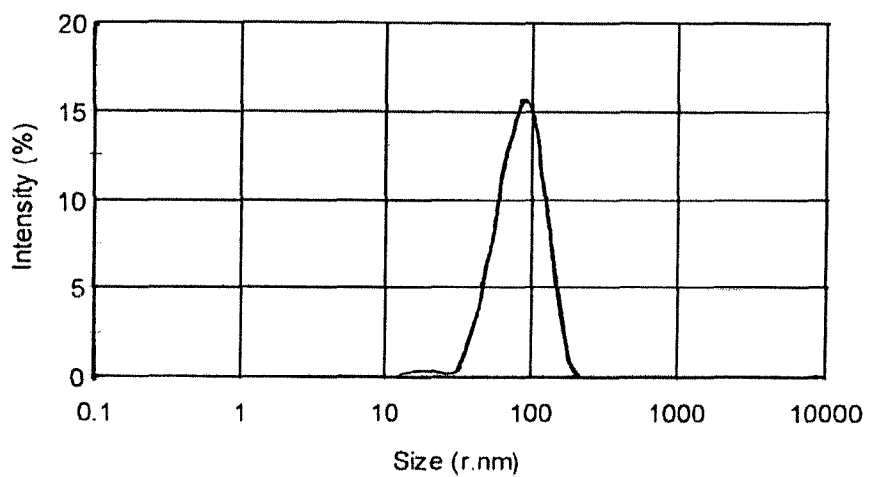
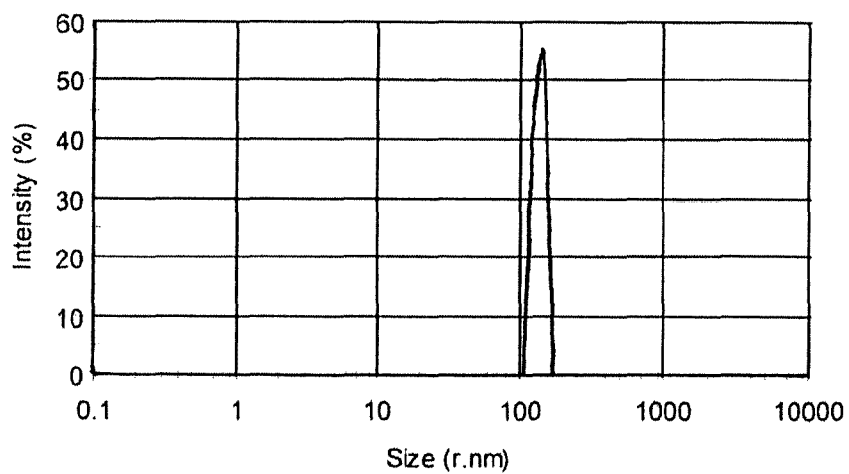


FIG. 4A

Size Distribution by Intensity

FIG. 4B

Size Distribution by Intensity

FIG. 4C

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/065937

A. CLASSIFICATION OF SUBJECT MATTER
INV. B82Y30/00
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)
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, BIOSIS, EMBASE, 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	YANG P ET AL: "Synthesis of intrinsic fluorescent polypyrrole nanoparticles by atmospheric pressure plasma polymerization", APPLIED SURFACE SCIENCE, ELSEVIER, AMSTERDAM, NL, vol. 255, no. 15, 15 May 2009 (2009-05-15) , pages 6924-6929, XP026093114, ISSN: 0169-4332, DOI: 10.1016/J.APSUSC.2009.03.016 [retrieved on 2009-03-17] page 6924 - page 6925 ----- -/-	1-59



Further documents are listed in the continuation of Box C.



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Date of the actual completion of the international search

21 November 2013

Date of mailing of the international search report

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Vázquez Lantes, M

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2013/065937

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CAO QIN ET AL: "Organic layer-coated metal nanoparticles prepared by a combined arc evaporation/condensation and plasma polymerization process; Synthesis organic layer-coated nanoparticles", PLASMA SOURCES SCIENCE AND TECHNOLOGY, INSTITUTE OF PHYSICS PUBLISHING, BRISTOL, GB, vol. 16, no. 2, 1 May 2007 (2007-05-01), pages 240-249, XP020120296, ISSN: 0963-0252, DOI: 10.1088/0963-0252/16/2/005 page 240 - page 242 -----	1-59
X	SRIKANTH H ET AL: "Magnetic studies of polymer-coated Fe nanoparticles synthesized by microwave plasma polymerization", APPLIED PHYSICS LETTERS, AIP, AMERICAN INSTITUTE OF PHYSICS, MELVILLE, NY, US, vol. 79, no. 21, 19 November 2001 (2001-11-19), pages 3503-3505, XP012029487, ISSN: 0003-6951, DOI: 10.1063/1.1419237 the whole document -----	1-59
X	WO 2007/087708 A1 (UNIV MCGILL [CA]; COULOMBE SYLVAIN [CA]; TAVARES JASON [CA]) 9 August 2007 (2007-08-09) paragraphs [0005], [0026], [0045] - [0061] claims 1,11,23,24,28 -----	1-59
X	"Microplasmas for nanomaterials synthesis", J. PHYS. D: APPL. PHYS., vol. 43, 2010, page 21, XP020195471, cited in the application the whole document -----	1-59

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2013/065937

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007087708	A1	09-08-2007	NONE
