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(54) Title: ELECTROCHEMICAL BLOOD MIMICKING FLUID

(57) Abstract: A fluid mimicking electrochemical properties of blood includes a base fluid and particles dispersed in the base fluid. The fluid has rheological properties that mimic blood, and the dispersion of the particles may be maintained for a range of hematocrit equivalent levels. In addition, a method for preparing a blood mimicking fluid for use with an electrochemical biosensor includes dispersing particles into a base fluid, and introducing an analyte that is compatible with the electrochemical biosensor into the base fluid. Further, a method of testing an electrochemical biosensor includes the steps of obtaining a blood mimicking fluid having a predetermined concentration of the analyte and having rheological and electrochemical properties in combination mimicking blood, assaying the blood mimicking fluid to measure an analyte concentration, and comparing the measured analyte concentration and the predetermined analyte concentration.

ELECTROCHEMICAL BLOOD MIMICKING FLUID

Cross-Reference to Related Application

[0001] This application is related to U.S. Patent Application Serial No. 13/810,639, which issued as U.S. Patent No. 9,052,278 B2 on June 9, 2015, which is hereby incorporated herein by reference in its entirety.

Technical Field

[0002] This application generally relates to the field of biosensors and more specifically to electrochemical blood mimicking fluids which may be compatible with biosensors and methods of preparing and using such fluids.

Background

[0003] Electrochemical biosensors, such as glucose sensors, may be used to detect or measure analytes, such as glucose, in a biological fluid, such as blood. For example, an electrochemical biosensor, may be in the form of a test strip which is used in conjunction with a test meter having electrodes which are applied to the test strip. In such a case, the test strip may include a reagent mixture that includes an electron transfer agent, or electron mediator, and an analyte specific enzyme, for example specific to glucose. In operation, the biosensor senses electron transfer between the mediator and electrode surfaces and function by measuring electrochemical redox reactions when a biological fluid is applied to a test strip. In other examples, a biosensor may continuously monitor a biological fluid, and may be used to monitor fluids such as tears, saliva, urine, etc. A patient, for example, may self-manage testing of blood glucose levels as part of an ongoing treatment regimen for diseases such as diabetes mellitus.

[0004] Such electrochemical biosensors must be fabricated in a manner that ensures performance variability is bounded within acceptable tolerances. As such, it is necessary during the fabrication process to have reliable techniques with which to test the fabrication variability and accuracy of biosensors. Typically, variability of the

biosensors, such as test strips, arise due to lot-to-lot and strip-to-strip differences attributable to process and materials variations during fabrication.

[0005] In order to ensure that fabrication variability falls within the acceptable tolerances, conventionally whole human blood has been used to measure the performance of sample biosensors during the fabrication process. However, because of blood-related variability between different batches of blood from different donors, testing sample biosensors using whole human blood introduces an extra set of variables in addition to factors that are attributable to the fabrication process and materials variability. The presence of this blood-related variability complicates the interpretation of performance evaluations and tests, because, for example, it may not be possible to separate out variability arising from strip-to-strip and lot-to-lot differences in test strip properties from differences in the human blood used in testing.

[0006] Indeed, differences in the human blood used for testing may arise due to multiple different factors, including donor to donor differences, differences in hematocrit levels, levels of interference compounds, and changes that occur in the human blood over the course of time and due to handling, such as changes in levels of analyte and interferent compounds, lysis of cells, etc. Therefore a need exists for enhanced techniques for testing biosensors that avoid the use of inherently variable whole human blood.

Summary of the Disclosure

[0007] Therefore and according to a first aspect, there is provided a fluid for mimicking electrochemical properties of blood. The fluid may include a base fluid and a plurality of particles dispersed in the base fluid. The base fluid and the plurality of particles may be selected to have rheological properties in combination mimicking blood. The dispersion of the plurality of particles in the base fluid may be maintained for a range of hematocrit equivalent levels.

[0008] According to another aspect, a method of preparing a blood mimicking fluid for use with an electrochemical biosensor is provided. The method includes dispersing a plurality of particles in a base fluid to form the blood mimicking fluid. The base fluid and the plurality of particles may be selected to have rheological properties in combination mimicking blood. The method further includes introducing an analyte into the blood mimicking fluid, the analyte being compatible with the electrochemical biosensor.

[0009] In accordance with yet another aspect, a method for testing an electrochemical biosensor for measuring an analyte is presented. The method includes assaying a blood mimicking fluid to measure an analyte concentration; and comparing the measured analyte concentration and a predetermined analyte concentration.

[0010] These and other embodiments, features and advantages will become apparent to those skilled in the art when taken with reference to the following more detailed modes of carrying out the invention in conjunction with the accompanying drawings that are first briefly described.

Brief Description Of The Drawings

[0011] The accompanying drawings, which are incorporated herein and constitute part of this specification, illustrate presently preferred embodiments of the invention, and, together with the general description given above and the detailed description given below, serve to explain features of the invention (wherein like numerals represent like elements).

[0012] FIG. 1 illustrates an electrochemical blood mimicking fluid, in accordance with aspects set forth herein;

[0013] FIG. 2 is a flowchart of a method for preparing an electrochemical blood mimicking fluid, in accordance with aspects set forth herein;

[0014] FIG. 3 depicts flow curves of a base fluid of an electrochemical blood mimicking fluid as compared to human plasma, in accordance with aspects set forth herein;

[0015] FIG. 4 depicts a dispersed particle distribution of an electrochemical blood mimicking fluid, in accordance with aspects set forth herein;

[0016] FIG. 5 depicts voltammetry of different embodiments of a base fluid as compared with human plasma, in accordance with aspects set forth herein;

[0017] FIG. 6 is a Levich plot of an electrochemical blood mimicking fluid, in accordance with aspects set forth herein;

[0018] FIG. 7 depicts diffusion coefficients calculated from the Levich plot of FIG. 6, in accordance with aspects set forth herein; and

[0019] FIG. 8 depicts measured current transients for an electrochemical blood mimicking fluid as compared to human blood, in accordance with aspects set forth herein.

Detailed Description

[0020] The following detailed description should be read with reference to the drawings, in which like elements in different drawings are identically numbered. The drawings, which are not necessarily to scale, depict selected embodiments and are not intended to limit the scope of the invention. The detailed description illustrates by way of example, not by way of limitation, the principles of the invention. This description will clearly enable one skilled in the art to make and use the invention, and describes several embodiments, adaptations, variations, alternatives and uses of the invention, including what is presently believed to be the best mode of carrying out the invention.

[0021] The present disclosure provides, in part, electrochemical blood mimicking fluids, for example, for use in testing electrochemical biosensors during fabrication processes. The use of whole human blood in the testing ofthese biosensors is fraught with difficulties. For example, process improvement engineering may be hindered because when conducting tests, the inherent variability of human blood may not be

separable from the variability of the fabrication process. In addition, false indications of batch failure, due to problems with the human blood used to test and monitor the fabrication processing, may decrease yield during the manufacturing process, as biosensors that are actually within required tolerances are discarded.

[0022] Described herein is a fluid for mimicking electrochemical properties of human blood. Different types of synthetic fluids may be contemplated to mimic various aspects of whole human blood, which has numerous properties. For instance, a synthetic fluid that matches the oxygen content of human blood may be include perfluorocarbon emulsions or modified bovine hemoglobin, and may be used to test the performance of blood oxygen equipment. In addition, a synthetic fluid that matches acoustic properties of human blood may be used test the performance of Doppler ultrasound equipment. In such a case, the fluid may match properties such as ultrasound backscatter and acoustic velocity to whole human blood.

[0023] Advantageously, an electrochemical blood mimicking fluid as described herein overcomes the limitations inherent in the use of whole human blood for the testing of electrochemical biosensors. For example, an electrochemical blood mimicking fluid may be prepared using precisely controlled preparation steps so that any variability in the electrochemical response of the fluid is small enough so that the fluid may be used to accurately test or calibrated processes for fabricating biosensors. Therefore, the present disclosure describes the necessary properties of a synthetic blood mimicking fluid, provides various suitable embodiments of such a fluid, methods of preparing the fluids and test results verifying that the fluid performs electrochemical blood mimicking functions.

[0024] In addition, reproducible, synthetic test fluids, such as electrochemical blood mimicking fluids may be used for a wide range of purposes beyond testing and quality control during fabrication of sensors. For example, the fluids may be used to calibrate existing test equipment, test human blood to determine its properties, etc. In addition, such fluids may be used to characterize a measurement system where the physical

process underlying the measurement principle involves mass transport by diffusion through heterogeneous media.

[0025] By way of explanation, in whole human blood, the majority of the cell portion consists of red blood cells, which are biconcave discs with a diameter of about 7 μm. In addition, the packed cell volume of human blood is approximately 40 %. Turning to the electrochemical properties of blood, the blood cells act as barriers to diffusion, as redox species must travel around them rather than through them. In such a case, the time taken to diffuse from the solution to an electrode surface of a biosensor is increased by the blood cells, thus lowering the effective diffusion coefficient of whole human blood. In addition, diffusion theory suggests that the diffusion coefficient will vary with the volume fraction and shape, but not the size, of the suspended particles.

[0026] FIG. 1 illustrates an electrochemical blood mimicking fluid 100, in accordance with aspects set forth herein. In the embodiment of FIG. 1, the mimicking fluid 100 includes a base fluid 110 and a plurality of particles 120 that are dispersed in the base fluid 110. Therefore, the base fluid 110 may be analogous to plasma, and the plurality of particles 120 may be analogous to blood cells.

[0027] In one or more embodiments, base fluid 110 and the plurality of particles 120 are selected to have specific rheological properties, such as density, viscosity, diffusion constants, etc. For example, the rheological properties may be chosen to mimic the properties of whole human blood. In addition, the rheological properties may be selectable in a range to mimic properties of whole human blood, such as hematocrit level.

[0028] In one example, the density of the particles 120 may be matched to that of the base fluid 110 so that they form a stable dispersion. For example, matching of the densities may prevent the particles 120 from either floating or sinking within the base fluid 110.

[0029] In a specific working example, base fluid 110 may be prepared as a solution of 5 % bovine serum albumin (BSA) in pH 7.4 phosphate buffered saline (PBS). For example, the PBS may include 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride. In another example, base fluid 110 may contain a sufficient supporting electrolyte (*e.g.*, > 0.1 M) to ensure that internal resistance (IR) losses will be minimized. For example, IR losses may be limited to less than 200 ohms / cm. Suitable electrolytes may include simple buffers for example citrate, phosphate, tri(hydroxymethyl)methylamine (TRIS) , 2-(N-morpholino)ethanesulfonic acid (MES), piperazine-N,N′-bis(2-ethanesulfonic acid) (PIPES), 4-2-hydroxyethyl-1-piperazineethanesulfonic acid (HEPES), or any electrolyte that is compatible with an enzyme used in the biosensor. In another example, a suitable range of pH of an electrochemical blood mimicking fluid may be from pH 5 to pH 9.

[0030] In addition, in another specific example, particles 120 may be polyamide particles having a density close to that of water (*e.g.*, 1.03 g cm^{-3}). For instance, particles 120 may have nominal mean particle diameters of 10 μm, with a distribution with a distribution such that the majority of the particle diameters are distributed between 5 and 30 microns. By way of example, polyamide particles with such properties may be obtained from Arkema of France. In another example, ultrafine polyamide powders may be used, and may include range of polymers and copolymers of lauryllactam (PA 12) and/or caprolactam (PA 6). In a further example, particle diameters between 5 and 60 microns may be selected.

[0031] Because the diffusion rate within a fluid may decrease with increasing viscosity, the viscosity of the base fluid 110 may be matched to that of human plasma. In addition, the base fluid 110 may have an appropriate voltammetric window that matches that of human blood. Further, base fluid components may be selected so that they are not electrochemically active in the same potential range as the mediator in a biosensor under evaluation.

[0032] Advantageously, in conjunction with the electrochemical properties, the herein described fluid is capable of forming a stable dispersion over a wide range of volume percentages of particles 120. In such a case, the fluid 100 may mimic rheological properties of human blood with hematocrit levels in a range from 20% to 60% depending on the amount of particles in the dispersion.

[0033] FIG. 2 is a flowchart of a method 200 for preparing an electrochemical blood mimicking fluid, in accordance with aspects set forth herein. In the example of FIG. 2, method 200 at block 210 may include preparing a base fluid, *e.g.*, having rheological properties of blood plasma. In addition, method 200 at block 220 may include dispersing a plurality of particles in a base fluid to form the blood mimicking fluid, where the base fluid and the plurality of particles are selected to have rheological properties in combination mimicking blood. Further, method 200 at block 230 may include introducing an analyte into the blood mimicking fluid, the analyte being compatible with the electrochemical biosensor.

[0034] In one working example, dispersing the particles at block 220 may be achieved by adding a surfactant. For example, Tergitol NP-7 (available from Sigma Aldrich of the United Kingdom), a nonylphenyl ethoxylate, may be added, with a HLB value of 12. In another example, a suspension of 40 vol % 10 μm particles may be prepared using various surfactant loadings (*i.e.,* weight percentage of surfactant in the suspension). In one example, partial dispersion may be achieved at a surfactant loading of 0.6 wt % (≤ 0.004 g m^{-2}). In another example, a milky dispersion may be obtained at a surfactant loading of 1.0 wt % (≤ 0.006 g m^{-2}). In a further example, at surfactant loadings of $\geq$ wt 5.0 % (≤ 0.03 g m^{-2}) the particles may disperse and formed a thick gel.

[0035] In one specific example, a surfactant loading of 1 wt % Tergitol NP-7 may be used to adequately for disperse up to 40 vol % of 10 μm particles. One having skill in the art will understand that for higher volume percentages, or for smaller particle diameters, the surfactant loading may be adjusted such that the loading per m^2 of particle surface area is maintained. An alternate surfactant that may be used Brij C10 (Polyethylene

glycol hexadecyl ether, Polyoxyethylene (10) cetyl ether) available from Sigma Aldrich of the United Kingdom.

[0036] In one specific working example, a base fluid is prepared as follows. First, pH 7.4 phosphate buffered saline (PBS, 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride) may be prepared by first adding one PBS tablet (available from Sigma Aldrich of the United Kingdom) to 200 ml of deionized water. Next, to 60 ml of PBS so prepared, 3 g of bovine serum albumin (BSA) (BioReagent >= 96 %, available from Sigma Aldrich of the United Kingdom may be added. Next, the solution may be stirred using a magnetic stirrer until completely dissolved to give a solution of 5 % BSA in PBS.

[0037] Continuing with the specific example, to the solution of 5 % BSA in PBS 1 g of non-ionic surfactant Tergitol NP-7 (available from Sigma Aldrich of the United Kingdom) may be added. Next, the solution may be stirred using a magnetic stirrer while adding 41.2 g of 10 μm mean particle diameter polyamide spheres (*e.g.*, Orgasol, available from Arkema of France) over the course of 5 minutes, such that the electrochemical blood mimicking fluid is formed, resembling a milky dispersion. Next, the fluid may be left on oscillating rollers for a further 2 hours to promote formation of the suspension.

[0038] FIGS. 3–8 depict test results obtained on various formulations of electrochemical blood mimicking fluids prepared in accordance with aspects set forth herein. As described below, the test results confirm that the fluids have various properties that mimic those of whole human blood.

[0039] FIG. 3 depicts flow curves of a base fluid of an electrochemical blood mimicking fluid and human plasma, in accordance with aspects set forth herein. For example, a measurement may be conducted using a rheometer with 60 mm 2 degree cone and plate, *e.g.*, an AR 550 Rheometer available from TA Instruments of New Castle, Delaware. As depicted, for a base fluid including 5% BSA in PBS, flow curves closely match flow curves of human plasma, indicating that the fluids have closely matched

viscosities across a range of shear rates. From this example, a suitable range of viscosity of an electrochemical blood mimicking fluid may be between 0.001 to 0.1 Pa s at a shear rate of 100 s^{-1}.

[0040] FIG. 4 depicts a dispersed particle distribution of an example electrochemical blood mimicking fluid, in accordance with aspects set forth herein. In the example of FIG. 4, a fluid with 40 vol% of 10 μm particles was dispersed in 5% BSA in PBS, using 1 wt % surfactant loading of Tergitol NP-7. In this example, effective dispersion was verified using a Malvern Mastersizer 2000.

[0041] Effective dispersion may be verified by a particle size distribution as depicted, and inadequate dispersion would be evident as poorly dispersed particles with diameter greater than 30 μm.

[0042] FIG. 5 depicts voltammetry of different embodiments of a base fluid as compared with human plasma, in accordance with aspects set forth herein. For example, voltammetry may be tested using a 1.6 mm gold (Au) disc with a platinum (Pt) coil counter electrode, at a potential scan rate of 50 mV s^{-1}. FIG. 5 In FIG. 5, surrogate human plasma and a base fluid as described in FIG. 4 are compared to a formulation of the base fluid of FIG. 4 with 10 mM added ferrocyanide.

[0043] As illustrated, the flat baseline of the base fluid without the ferrocyanide demonstrates that the base fluid has minimal IR losses and that no components of the base fluid are electrochemically active over the potential range -0.1 to 0.7 V vs Ag|AgCl.

[0044] The base fluid with added ferrocyanide demonstrates that this potential range is adequate for the assessment of the ferricyanide / ferrocyanide redox couple. For example, the peak currents are less than predicted by theory (20 cf 32 μA) and that the peak separation is much wider than predicted by theory (500 mV cf ~60 mV). These differences are interpreted as variability introduced at the electrode surface by BSA.

[0045] In another example, an appropriate surface treatment (*e.g.*, 2-mercaptoethanesulphonate) may applied to the Au surface to reduce the variability at the electrode surface.

[0046] FIG. 6 is a Levich plot of rotating disk voltammetry measurements for an electrochemical blood mimicking fluid, in accordance with aspects set forth herein. A Levich plot shows the limiting current as a function of electrode rotation rates, and may be used to determine the diffusion coefficients from the slope of the plot. In the example of FIG. 6, a rotating disc electrode was used to measure the diffusion coefficient of potassium ferrocyanide in dispersions of particles at volume fractions of 17 and 51 % in the base fluid of FIG. 3. As illustrated, the Levich plot verifies that the dispersed particles have the desired effect of acting as an impediment to diffusion within the sample.

[0047] In another example, for particle dispersions, plots of limiting current versus the square root of the rotation rate are nonlinear at high rotation rates because the presence of the particles constrains the height of the diffusion layer, increasing effective mass transport.

[0048] FIG. 7 depicts diffusion coefficients calculated from the Levich plot of FIG. 6, in accordance with aspects set forth herein. Using the Levich equation, diffusion coefficients may be determined from the plot of FIG. 6, and are presented in FIG. 5.

[0049] For example, the Levich equation may be used to predict currents at a rotating disk electrode and may show that the current is proportional to the square root of rotation speed. This is because, in a rotating disk electrode, electrolytes may flow past the electrode by convection. In one non-limiting example, the Levich equation may have the form:

[0050] $I_L = (0.620)n\,F\,A\,D^{\frac{2}{3}}\omega^{\frac{1}{2}}v^{\frac{-1}{6}}C$, where:

[0051] I_L is the Levich current (A)

[0052] n is the number of electrons transferred (mol^{-1})

[0053] F is the Faraday constant (C/mol)

[0054] A is the electrode area (cm^2)

[0055] *D* is the diffusion coefficient (cm^2/s)

[0056] *w* is the angular rotation rate of the electrode (rad/s)

[0057] *v* is the kinematic viscosity (cm^2/s)

[0058] *C* is the analyte concentration (mol/cm^3)

[0059] As depicted, the presence of dispersed particles of the blood mimicking fluid effectively reduces the diffusion coefficients in the same manner as do red blood cells in whole human blood.

[0060] By way of example, the fluid may be prepared to mimic the effect of hematocrit on diffusion in a range available in human samples, including neonates. For example, the effective hematocrit level to be mimicked can be > 65 %, and may be in a range of 0 - 80 %. In one example, the effective diffusion coefficient may be reduced by 1 % per % hematocrit. In one specific example, for potassium ferricyanide, which has a diffusion coefficient of 6.7 E-06 cm^2 s^{-1} , the equivalent diffusion constant value at 20 % hematocrit would be 1.35 E-06 cm^2 s^{-1}.

[0061] FIG. 8 depicts measured current transients for an electrochemical blood mimicking fluid and human blood, in accordance with aspects set forth herein.

[0062] For example, the measurements depicted may be taken by using a test strip with each of the fluids. In the embodiment of FIG. 8, the blood mimicking fluid of FIG. 7 has been spiked with an analyte, namely glucose at 100 mg / dL, followed by measurements of current transients. In addition, the performance of the blood mimicking fluid has been compared to 20% hematocrit human blood spiked 100 mg / dL glucose, followed by similar measurements. In the measurement process for each fluid, a specific test current may be applied from t=0 s to t=1 s, another current may be applied from t=1 s to t=4 s, and another current may be applied from t=4 s onward. The specific currents applied may vary depending on the specific parameters of the biosensor and a test system

being used. In the specific example of FIG. 8, current transients of the fluid were measured using a OneTouch® Verio glucose sensor.

[0063] As depicted, subtle differences may be present because human blood includes oxidizable interferent species such as ascorbate and uric acid. For example, for human blood, the current in the first second, where a potential of + 0.02 V is typically applied, may be higher than for the blood mimicking fluid, due to the presence of species such as ascorbate that may be oxidized at low potentials. In addition, the current between 1 and 4 s, where a potential of + 0.3 V, is typically applied, may be higher for human blood than the blood mimicking fluid, for example due to the presence of species that undergo oxidation at higher potentials.

[0064] Advantageously, the blood mimicking fluid presented herein demonstrates excellent agreement between measured current transients as compared with whole human blood with an equivalent analyte level, as shown in FIG. 8. By correctly mimicking rheological and/or diffusional properties of blood, such a fluid is suitable for the evaluation of strip-to-strip and lot-to-lot differences in dimensional properties (*e.g.*, electrode area or chamber height) and compositional properties (*e.g.*, enzyme loading). As noted above, the electrochemical properties of such a fluid will not be susceptible to variability arising through differences in the properties of blood.

[0065] By way of summary, the electrochemical blood mimicking fluid described herein may be used for testing an electrochemical biosensor for measuring an analyte. In one embodiment, the method uses a blood mimicking fluid with a predetermined concentration of an analyte. In such a case, the blood mimicking fluid is assayed by the electrochemical biosensor to measure the analyte concentration and compare it with the predetermined concentration.

[0066] While the invention has been described in terms of particular variations and illustrative figures, those of ordinary skill in the art will recognize that the invention is not limited to the variations or figures described. In addition, where methods and steps described above indicate certain events occurring in certain order, those of ordinary skill in the art will recognize that the ordering of certain steps may be modified and that such

modifications are in accordance with the variations of the invention. Additionally, certain of the steps may be performed concurrently in a parallel process when possible, as well as performed sequentially as described above. Therefore, to the extent there are variations of the invention, which are within the spirit of the disclosure or equivalent to the inventions found in the claims, it is the intent that this patent will cover those variations as well.

WHAT IS CLAIMED IS:

1. A fluid for mimicking electrochemical properties of blood, the fluid comprising:

a base fluid; and

a plurality of particles dispersed in the base fluid, wherein the base fluid and the plurality of particles are selected to have rheological properties in combination mimicking blood, and wherein the dispersion of the plurality of particles in the base fluid is maintained for a range of hematocrit equivalent levels.

2. The fluid of claim 1, wherein a blood mimicking hematocrit level of the fluid corresponds to a predetermined quantity of the plurality of particles dispersed in the base fluid.

3. The fluid of claim 1, further comprising a concentration of an analyte, wherein the fluid mimics electrochemical properties of blood having the analyte concentration.

4. The fluid of claim 3, wherein the analyte comprises glucose.

5. The fluid of claim 1, wherein the dispersion of the fluid is stable for hematocrit equivalent levels of approximately 20% to 80%.

6. The fluid of claim 1, wherein densities of the particles and the base fluid are selected to facilitate the stable dispersion of the fluid.

7. The fluid of claim 1, wherein a quantity of the plurality of particles dispersed in the base fluid facilitates the fluid having a blood-mimicking viscosity.

8. The fluid of claim 1, wherein the particles comprise polyamide particles.

9. The fluid of claim 1, wherein the particles are approximately 7 to 10 microns in size.

10. The fluid of claim 1, wherein the fluid demonstrates an electrochemical current output similar to an electrochemical current output of blood.

11. The fluid of claim 1, further comprising an electrolyte, the electrolyte providing the fluid with a blood-mimicking electrical resistance.

12. The fluid of claim 1, further comprising a buffer, the buffer facilitating the fluid having a blood-mimicking pH level.

13. A method for preparing a blood mimicking fluid for use with an electrochemical biosensor, the method comprising:

dispersing a plurality of particles in a base fluid to form the blood mimicking fluid, wherein the base fluid and the plurality of particles are selected to have rheological properties in combination mimicking blood; and

introducing an analyte into the blood mimicking fluid, the analyte being compatible with the electrochemical biosensor.

14. The method of claim 13, wherein the dispersing comprises using a surfactant to achieve a stable dispersion of the plurality of particles in the base fluid.

15. The method of claim 13, wherein the dispersing comprises selecting a quantity of the plurality of particles to select a hematocrit equivalent level of the blood mimicking fluid.

16. The method of claim 13, further comprising providing the fluid with a selected concentration of an analyte, wherein the fluid mimics electrochemical properties of blood having the selected analyte concentration.

17. A method for testing an electrochemical biosensor for measuring an analyte, the method comprising:

obtaining a blood mimicking fluid, the blood mimicking fluid comprising a predetermined concentration of the analyte and having rheological and electrochemical properties in combination mimicking blood;

assaying the blood mimicking fluid to measure an analyte concentration; and

comparing the measured analyte concentration and the predetermined analyte concentration.

18. The method of claim 17, wherein the obtaining comprises providing the blood mimicking fluid with a predetermined quantity of the plurality of particles dispersed in the base fluid, and the assaying comprises determining a hematocrit equivalent level of the blood mimicking fluid, wherein the hematocrit equivalent level corresponds to the predetermined quantity of the dispersed particles.

19. The method of claim 18, wherein the assaying comprises performing an electrochemical test on the blood mimicking fluid, wherein the fluid mimics electrochemical properties of blood having the predetermined analyte concentration.

20. The method of claim 19, wherein the assaying comprises measuring an electrochemical current output of the blood mimicking fluid.

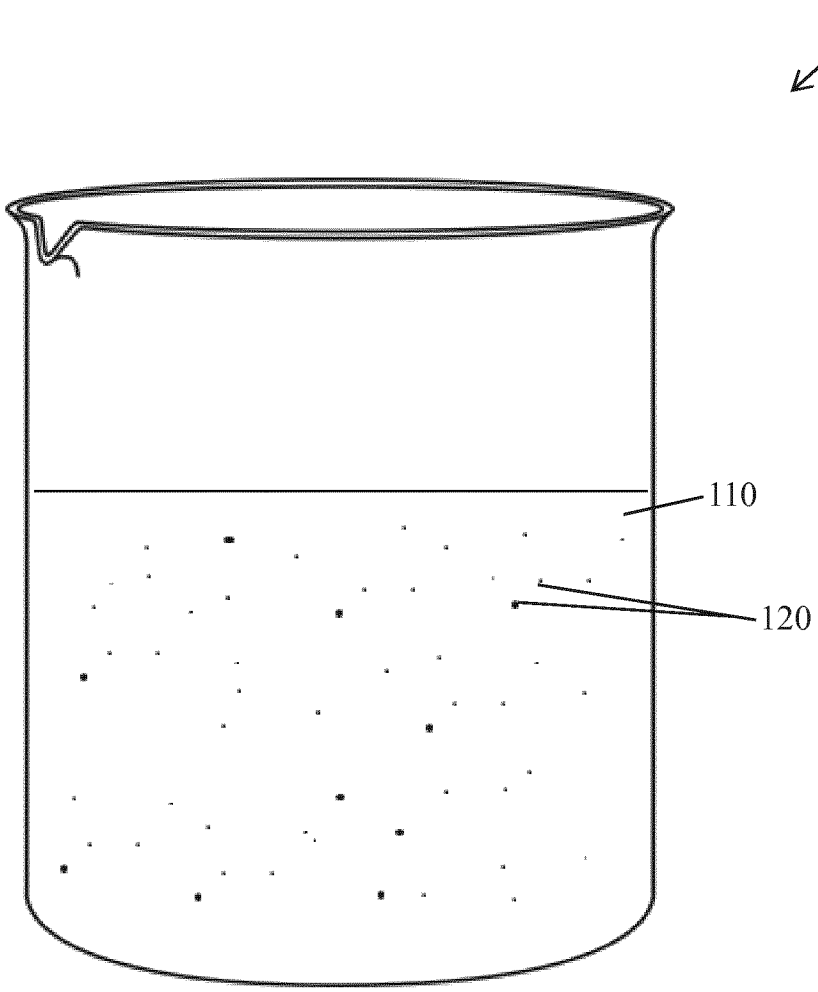


FIG. 1

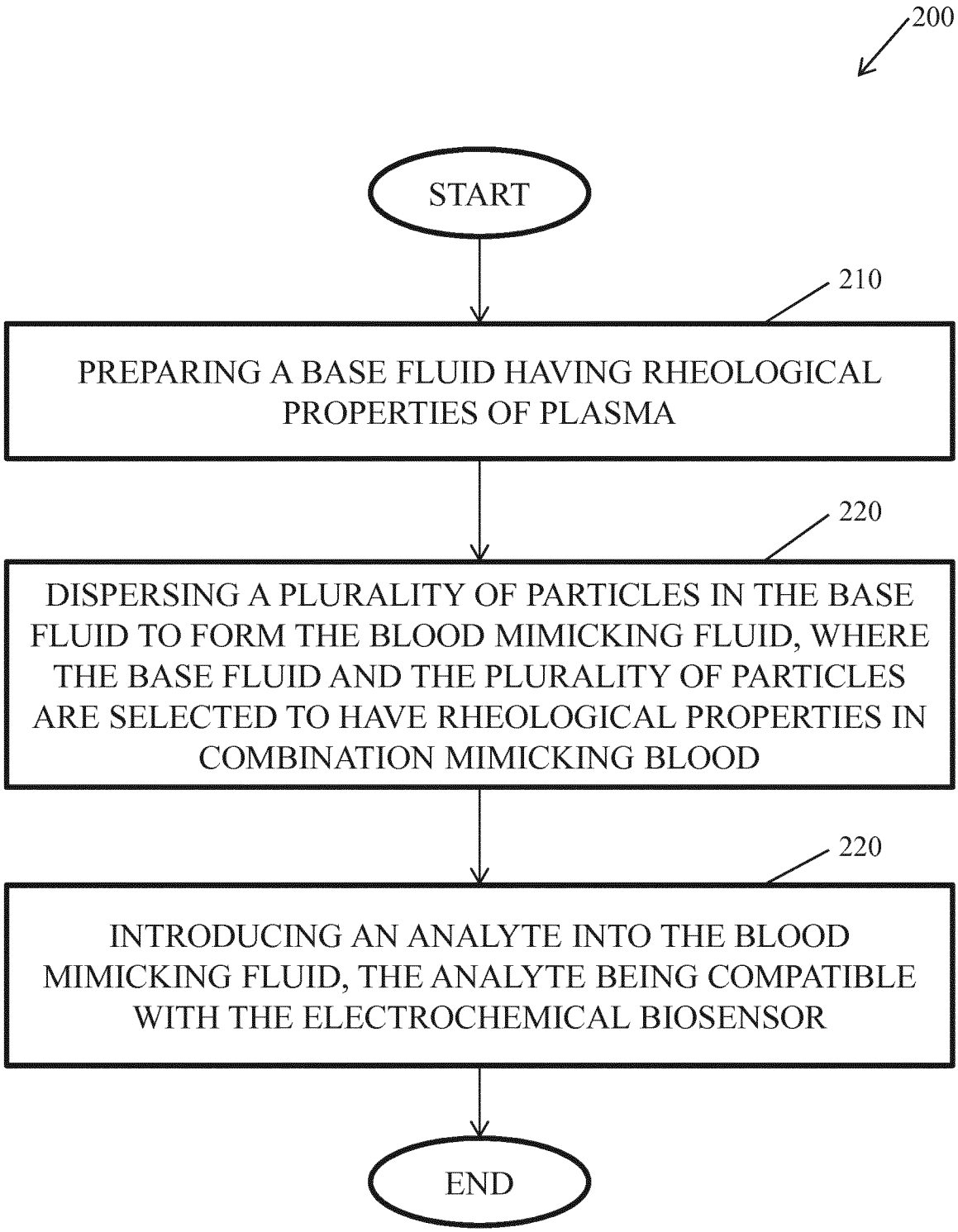

200
START
210
PREPARING A BASE FLUID HAVING RHEOLOGICAL PROPERTIES OF PLASMA
220
DISPERSING A PLURALITY OF PARTICLES IN THE BASE FLUID TO FORM THE BLOOD MIMICKING FLUID, WHERE THE BASE FLUID AND THE PLURALITY OF PARTICLES ARE SELECTED TO HAVE RHEOLOGICAL PROPERTIES IN COMBINATION MIMICKING BLOOD
220
INTRODUCING AN ANALYTE INTO THE BLOOD MIMICKING FLUID, THE ANALYTE BEING COMPATIBLE WITH THE ELECTROCHEMICAL BIOSENSOR
END

FIG. 2

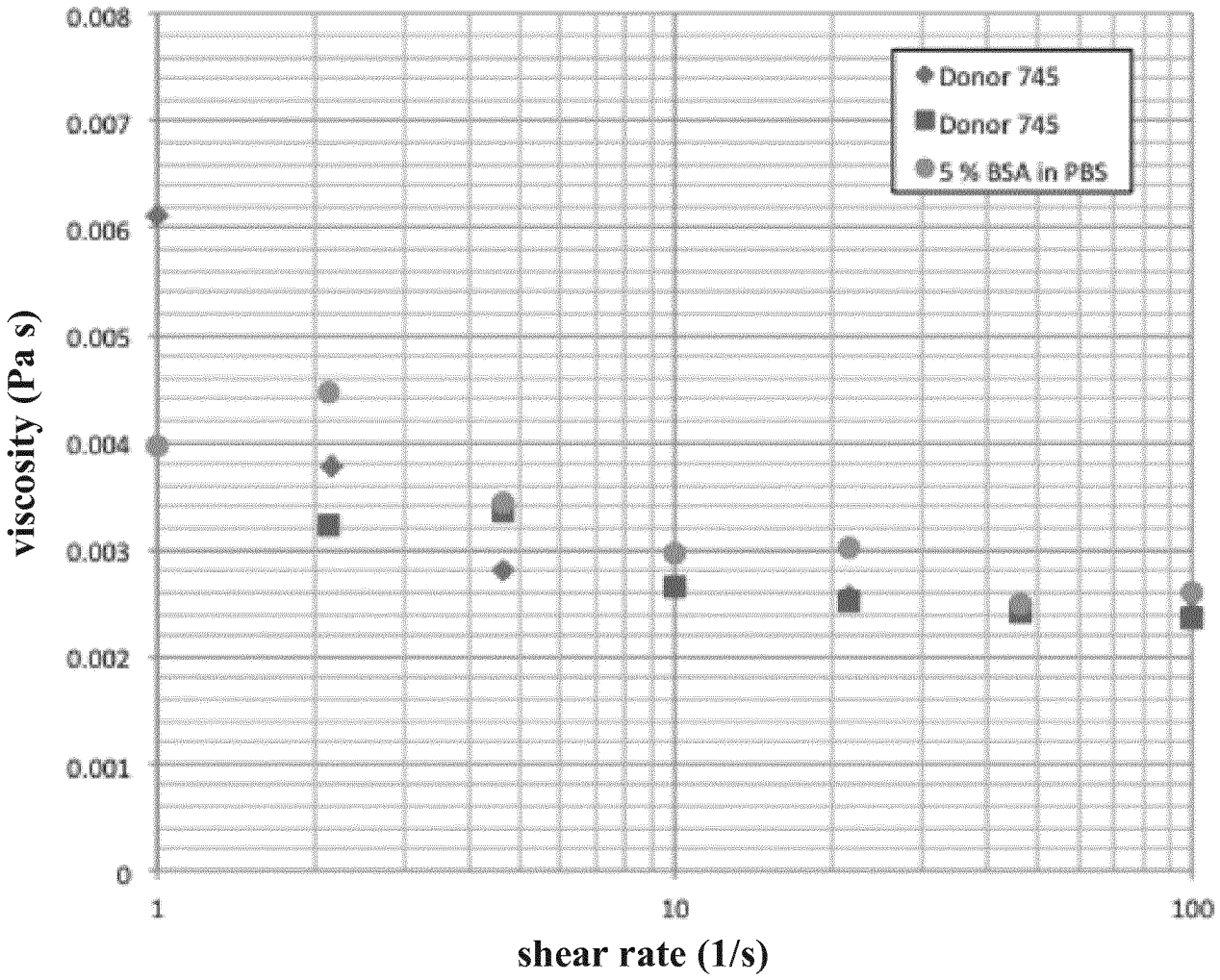

0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
viscosity (Pa s)
1
10
100
shear rate (1/s)
Donor 745
Donor 745
5 % BSA in PBS

FIG. 3

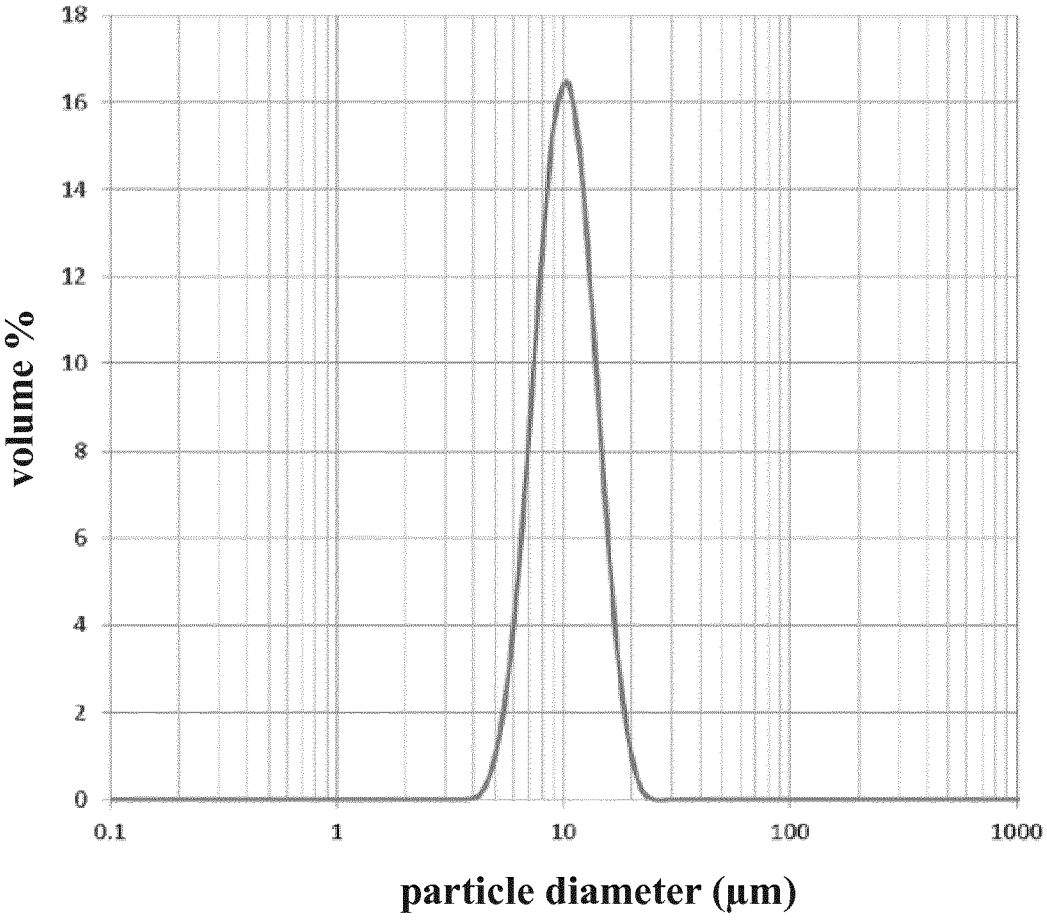

18
16
14
12
10
8
6
4
2
0
volume %
0.1
1
10
100
1000
particle diameter (µm)

FIG. 4

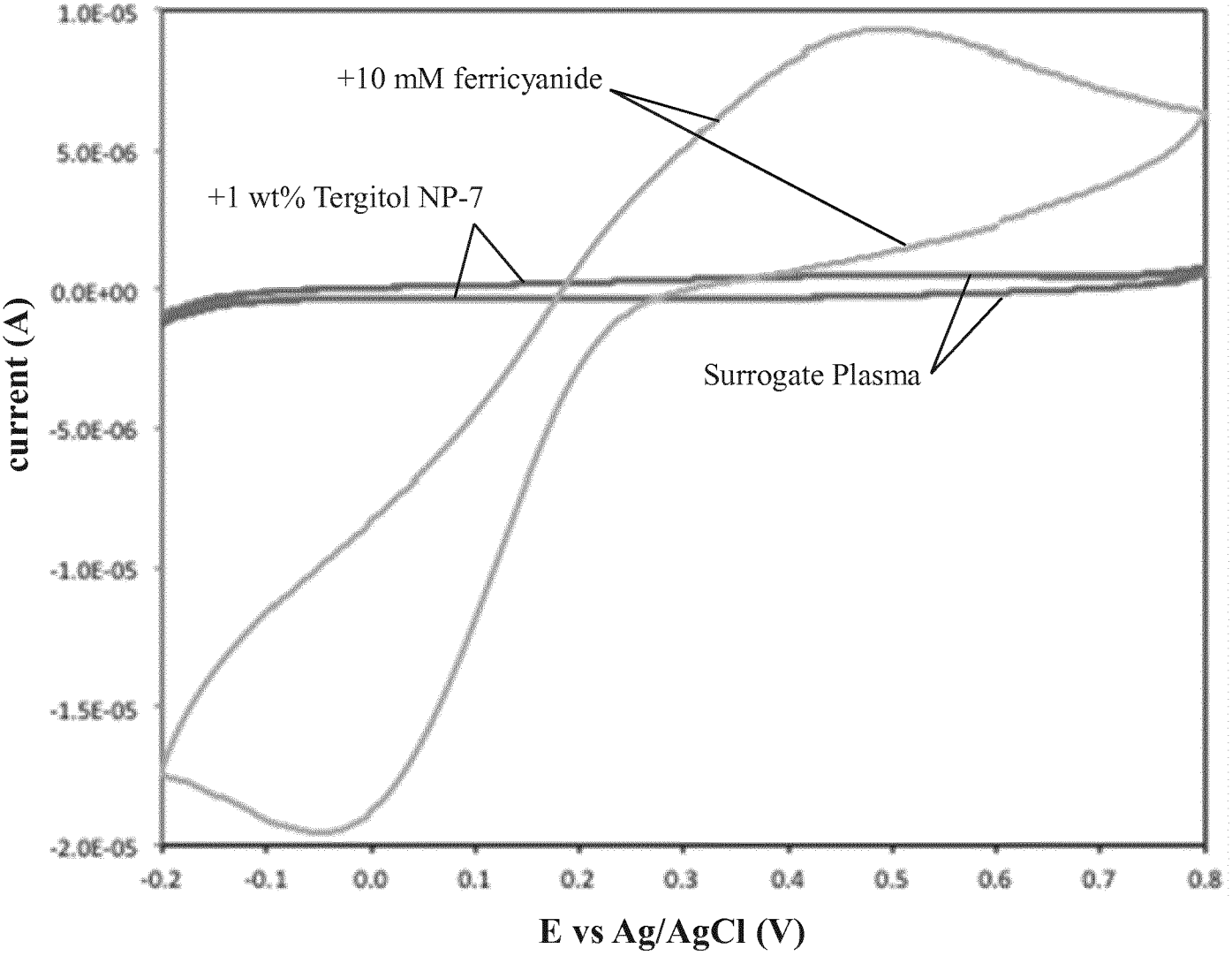

1.0E-05
5.0E-06
0.0E+00
-5.0E-06
-1.0E-05
-1.5E-05
-2.0E-05
current (A)
+10 mM ferricyanide
+1 wt% Tergitol NP-7
Surrogate Plasma
-0.2
-0.1
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
E vs Ag/AgCl (V)

FIG. 5

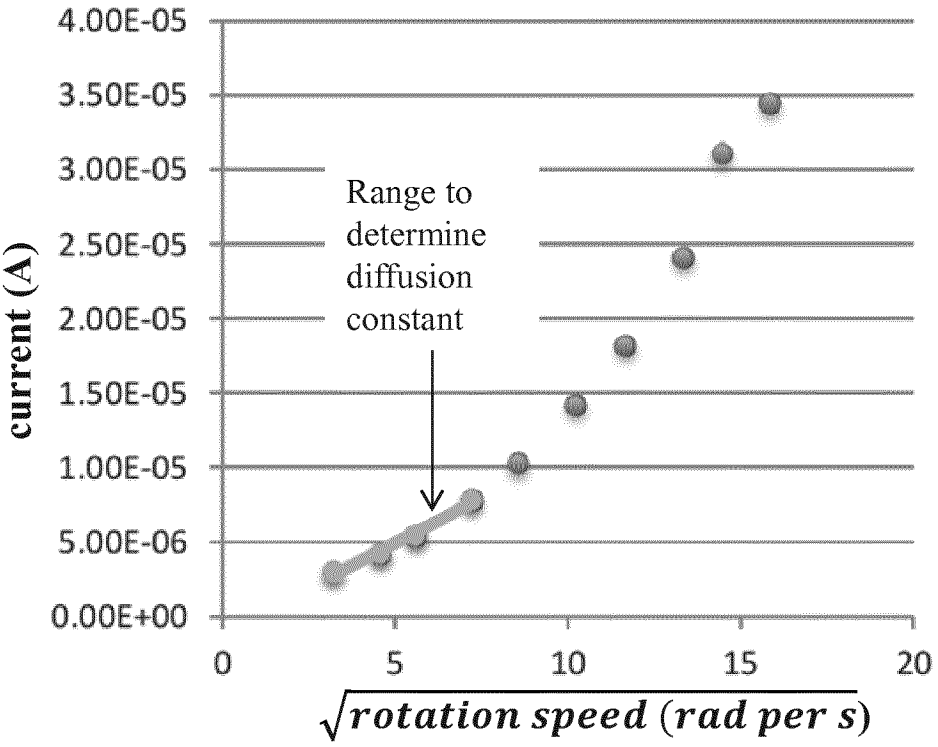

4.00E-05
3.50E-05
3.00E-05
2.50E-05
2.00E-05
1.50E-05
1.00E-05
5.00E-06
0.00E+00
current (A)
Range to determine diffusion constant
0
5
10
15
20
√rotation speed (rad per s)

FIG. 6

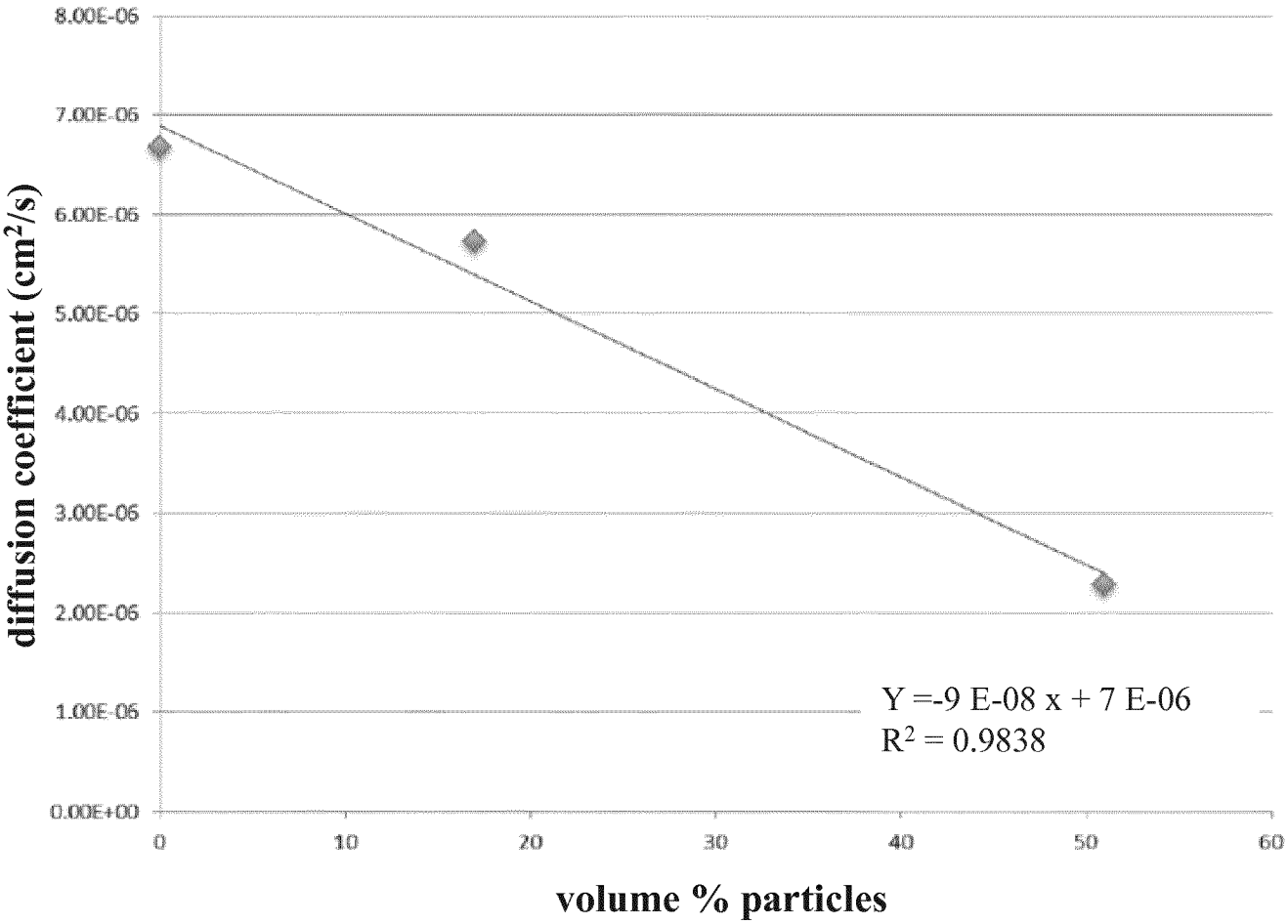

8.00E-06
7.00E-06
6.00E-06
5.00E-06
4.00E-06
3.00E-06
2.00E-06
1.00E-06
0.00E+00
diffusion coefficient (cm²/s)
0
10
20
30
40
50
60
volume % particles
Y =-9 E-08 x + 7 E-06
R² = 0.9838

FIG. 7

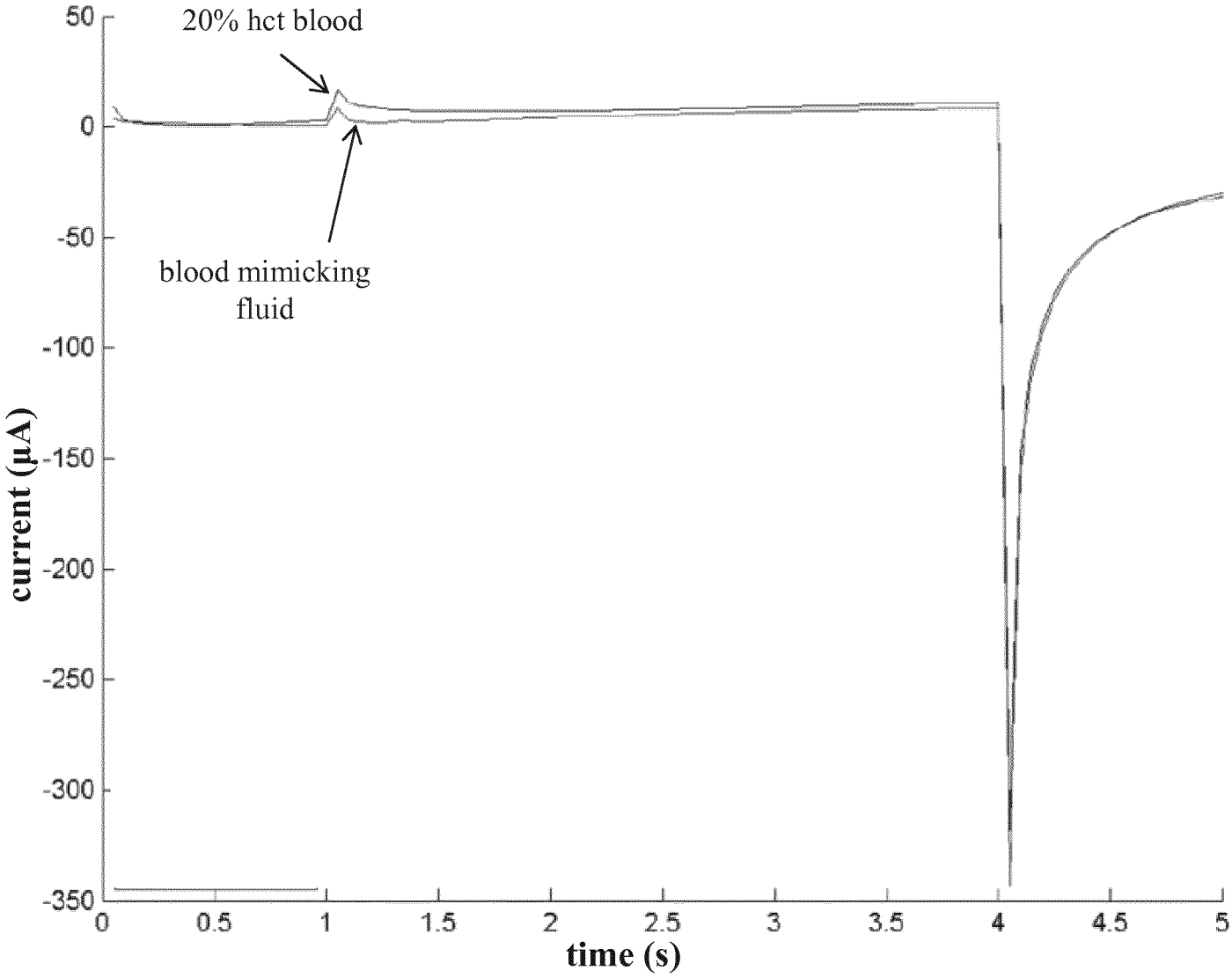

20% hct blood
blood mimicking fluid
current (µA)
time (s)
50
0
-50
-100
-150
-200
-250
-300
-350
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5

FIG. 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/081890

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/00 G01N27/327
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)
C12Q 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, BIOSIS, COMPENDEX, EMBASE, FSTA, INSPEC, 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	WO 95/13536 A1 (BOEHRINGER MANNHEIM CORP [US]) 18 May 1995 (1995-05-18) abstract page 1, line 4 - line 9 page 4, line 5 - line 23 page 7, line 1 - page 10, line 3 examples 3, 4 ----- -/--	1-7, 9-14,16, 17,19,20

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

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
23 March 2017	31/03/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Adida, Anne

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/081890

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 7 390 663 B2 (RYAN WAYNE L [US] ET AL) 24 June 2008 (2008-06-24)	1-7, 9-11,13, 15-17, 19,20
Y	abstract column 1, line 10 - line 24 column 1, line 40 - line 50 column 5, line 30 - column 6, line 11 figure 2 claims 1,21; example 2	18
X	KUMAR V RAMNARINE ET AL: "VALIDATION OF A NEW BLOOD-MIMICKING FLUID FOR USE IN DOPPLER FLOW TEST OBJECTS", ULTRASOUND IN MED. & BIOL., vol. 24, 1 January 1998 (1998-01-01), pages 451-459, XP055356454, abstract page 453, right-hand column, paragraph 3 - page 454, left-hand column, paragraph 1 page 459, left-hand column, paragraph 2	1-9
X	FUKADA E ET AL: "Blood modeling using polystyrene microspheres.", BIORHEOLOGY 1989, vol. 26, no. 2, 1989, pages 401-413, XP008183802, ISSN: 0006-355X abstract page 402, paragraph 1 - paragraph 2 page 405, last paragraph page 408, paragraph 2 - paragraph 3 page 410, paragraph 1 - paragraph 3 page 411, paragraph 2 figures 6,8	1-7,9,11
Y	SHIN J-Y ET AL: "Estimation of glucose concentration using adaptive calibration curve in different hematocrit levels", ELECTRONICS LETTERS, IEE STEVENAGE, GB, vol. 49, no. 9, 25 April 2013 (2013-04-25) , pages 584-585, XP006044664, ISSN: 0013-5194, DOI: 10.1049/EL.2012.3715 abstract	18

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2016/081890

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		EP 0728306 A1	28-08-1996
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