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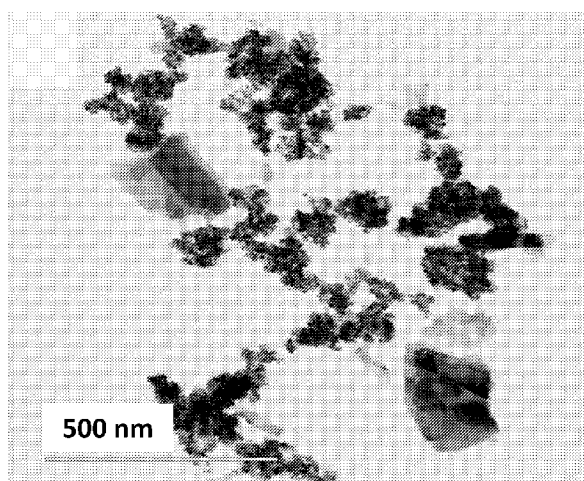


FIG. 1A

(57) Abstract: Methods for making nanocomposites are provided. In an embodiment, such a method comprises combining a first type of nanostructure with a bulk material in water or an aqueous solution, the first type of nanostructure functionalized with a functional group capable of undergoing van der Waals interactions with the bulk material, whereby the first type of nanostructure induces exfoliation of the bulk material to provide a second, different type of nanostructure while inducing association between the first and second types of nanostructures to form the nanocomposite.



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NANOCOMPOSITES, NANOCOMPOSITE SENSORS AND RELATED METHODS**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] The present application claims priority to U.S. Provisional Patent Application No. 62/545,686 that was filed August 15, 2017, the entire contents of which are hereby incorporated by reference.

REFERENCE TO GOVERNMENT RIGHTS

[0002] This invention was made with government support under DMR1507810 awarded by the National Science Foundation. The government has certain rights in the invention.

BACKGROUND

[0003] Two-dimensional (2D) nanomaterials have drawn tremendous interest due to their unique structural and electronic properties.¹⁻⁴ Nanocomposites with 2D nanomaterials as a host matrix have demonstrated enhanced mechanical, thermal, and catalytic properties for a range of applications including optoelectronics, catalysis, energy, and biomedicine.⁵⁻⁹ Regarding biomedicine however, the existing technology for glucose detection is based on an electrochemical method. It requires a glucose test strip and a device called glucometer that is powered by a battery. The patient has to prick blood from his/her finger and transfer onto test strip which is inserted in glucometer which gives a reading of blood glucose level. The test is invasive and blood pricking is inconvenient for many patients, especially elderly people and kids.

SUMMARY

[0004] Nanocomposites, methods for making the nanocomposites, and methods for using the nanocomposites, e.g., for H₂O₂ sensing and non-invasive glucose sensing, are provided.

[0005] In one aspect, methods for making nanocomposites are provided. In an embodiment, such a method comprises combining a first type of nanostructure with a bulk material in water or an aqueous solution, the first type of nanostructure functionalized with a functional group capable of undergoing van der Waals interactions with the bulk material, whereby the first type of nanostructure induces exfoliation of the bulk material to provide a

second, different type of nanostructure while inducing association between the first and second types of nanostructures to form the nanocomposite.

[0006] In another aspect, sensors for a target analyte are provided. In an embodiment, such a sensor comprises a nanocomposite comprising a plurality of a first type of nanostructures and a plurality of a second type of nanostructures, the first type of nanostructures in association with the second type of nanostructure, wherein the nanocomposite exhibits intrinsic catalytic activity in a reaction involving a target analyte; and a chromogenic material capable of exhibiting a color change in the presence of the target analyte.

[0007] Other principal features and advantages of the disclosure will become apparent to those skilled in the art upon review of the following drawings, the detailed description, and the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] Illustrative embodiments of the invention will hereafter be described with reference to the accompanying drawings.

[0009] FIG. 1A depicts a TEM image of MoS₂/Fe₃O₄ nanocomposite. FIG. 1B depicts elemental mapping of the nanocomposite. FIG. 1C depicts the EDX of the nanocomposite.

[0010] FIG. 2A shows an XPS full line scan of Fe₃O₄ nanoparticles, MoS₂ nanosheets, and MoS₂/Fe₃O₄ nanocomposite. FIG. 2B depicts a detailed scan of MoS₂/Fe₃O₄ nanocomposite with characteristic peaks of Fe. FIG. 2C depicts a detailed scan of MoS₂/Fe₃O₄ nanocomposite with characteristic peaks of Mo. FIG. 2D shows a detailed scan of MoS₂/Fe₃O₄ nanocomposite with characteristic peaks of S.

[0011] FIGs. 3A and 3B depict Raman spectroscopy of Fe₃O₄ nanoparticles, MoS₂ nanosheets, and MoS₂/Fe₃O₄ nanocomposite.

[0012] FIG. 4A depicts a demonstration of peroxidase-like activity of MoS₂/Fe₃O₄ nanocomposite in pH 5.0 citrate phosphate buffer at room temperature. Color change was only observed in solution of H₂O₂ + TMB + MoS₂/Fe₃O₄ while solutions of H₂O₂ + TMB and H₂O₂ + MoS₂/Fe₃O₄ remained colorless. [TMB] was 1 mM, and [Mo] and [Fe] were 8 and 22 µg/mL in MoS₂/Fe₃O₄. FIG. 4B shows that, similar to TMB, the other chromogenic substrates such as ABTS and OPD were also oxidized and resulted in green and orange colors,

respectively. Absorbance plots and a picture (inset) of oxidized substrates (ABTS, OPD, and TMB) in the presence of H_2O_2 are shown.

[0013] FIG. 5A shows the time-dependent absorbance changes at 652 nm in the presence of 100 mM H_2O_2 and 1 mM TMB at different concentrations of $\text{MoS}_2/\text{Fe}_3\text{O}_4$. FIG. 5B shows the time-dependent absorbance changes at 652 nm in presence of 100 mM H_2O_2 and 1 mM TMB at different concentrations of $\text{MoS}_2/\text{Fe}_3\text{O}_4$, MoS_2 , and in the presence of Fe_3O_4 at room temperature.

[0014] FIGs. 6A and 6B depict time-dependent absorbance changes at 652 nm in presence of at different concentrations of H_2O_2 in pH 5.0 citrate phosphate buffer at room temperature. [TMB] was 1 mM, and [Mo] and [Fe] were 8 and 22 $\mu\text{g/mL}$ in $\text{MoS}_2/\text{Fe}_3\text{O}_4$.

[0015] FIG. 7A illustrates the mechanism of glucose detection by the $\text{MoS}_2/\text{Fe}_3\text{O}_4$ nanocomposite. FIG. 7B depicts dose dependent behavior for glucose detection in the presence of GOx, TMB, and $\text{MoS}_2/\text{Fe}_3\text{O}_4$, as well as absorbance values at different concentrations of glucose in the presence of GOx (20 mg/ml), TMB (1 mM) and $\text{MoS}_2/\text{Fe}_3\text{O}_4$ ([Mo]=8 $\mu\text{g/mL}$, [Fe]= 22 $\mu\text{g/mL}$).

[0016] FIG. 8A depicts absorbance plots in the presence of 1 mM glucose solution with different concentrations of $\text{MoS}_2/\text{Fe}_3\text{O}_4$, MoS_2 and Fe_3O_4 in presence of 50 μL GOx (20 mg/ml), 50 μL TMB (1 mM). FIG. 8B depicts the selectivity analysis of this detection method by monitoring the absorbance of glucose analogues, as well as fructose, lactose, and maltose in the presence of GOx, TMB, and $\text{MoS}_2/\text{Fe}_3\text{O}_4$. The sharp color change in only the glucose solution demonstrates the highly selective nature of the method.

[0017] FIG. 9 depicts the development of glucose test strips by inkjet printing solutions of GOx (20 mg/ml), ABTS (1 mM) and $\text{MoS}_2/\text{Fe}_3\text{O}_4$ ([Mo]=8 $\mu\text{g/mL}$, [Fe]= 22 $\mu\text{g/mL}$). The strip turns from colorless to green and then to dark green in the presence of different concentrations of glucose.

[0018] FIG. 10 depicts the synthesis of $\text{MoS}_2/\text{Fe}_3\text{O}_4$ nanocomposite via thiol functionalized Fe_3O_4 nanoparticles assisted by exfoliation of MoS_2 .

[0019] FIG. 11 depicts implementation of the test strip diagnostic platform of FIG. 9 using a smartphone.

DETAILED DESCRIPTION

[0020] Nanocomposites, methods for making the nanocomposites, and methods for using the nanocomposites, e.g., for H₂O₂ sensing and non-invasive glucose sensing, are provided.

[0021] In one aspect, a nanocomposite comprising at least two different types of nanostructured materials is provided. By “different type” it is meant that the two nanostructured materials have different chemical compositions, different morphologies, or both. In embodiments, one of the nanostructured materials is composed of a plurality of zero-dimensional (0D) nanostructures. By “zero-dimensional” it is meant a nanostructure having each of its three dimensions on the order of about 100 nm or less. The three dimensions may also be of similar magnitude, e.g., the height, width and thickness of the nanostructure, are similar, e.g., within $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, etc. of each other. A nanoparticle is an illustrative example of a 0D nanostructure. The nanoparticles may be spherical, but this term encompasses irregularly shaped particles which are still reasonably well defined by a sphere. (See FIG. 1A.) The nanoparticles may be characterized by an average diameter. The average diameter may be about 100 nm or less, about 50 nm or less, about 25 nm or less, about 10 nm or less, or in the range of from about 1 nm to about 100 nm.

[0022] In embodiments, one of the nanostructured materials is composed of a plurality of one-dimensional (1D) nanostructures. By “one-dimensional” it is meant a nanostructure having at least two dimensions on the order of about 100 nm or less. The two dimensions may also be of similar magnitude to each other as described above. The other dimension may be substantially greater, e.g., about 25 times greater, about 50 times greater, about 100 times greater, about 200 times greater, etc. A nanowire is an illustrative example of a 1D nanostructure. Other illustrative examples include nanorods, nanofibers, nanowhiskers. The 1D nanostructures may be characterized by an average diameter. The average diameter may be about 25 nm or less, about 10 nm or less, about 5 nm or less, or about 1 nm or less. The other dimension of the 1D nanostructure, e.g., the length, is not particularly limited, but as described above, is substantially greater than the diameter. The term “diameter” is not meant to imply that the 1D nanostructures are limited to circular cross-sections. Other cross-sections, e.g., square, rectangle, ellipse, etc. may be used, in which case “diameter” may refer to a width, height, major diameter, etc.

[0023] In embodiments, one of the nanostructured materials is composed of a plurality of two-dimensional (2D) nanostructures. By “two-dimensional” it is meant a nanostructure

having at least one dimension on the order of about 100 nm or less. The other two dimensions may be substantially greater, e.g., about 25 times greater, about 50 times greater, about 100 times greater, about 200 times greater, etc. These other two dimensions may also be of similar magnitude to each other as described above. A nanosheet is an illustrative example of a 2D nanostructure. The nanosheets may be characterized by an average thickness. The average thickness may be about 25 nm or less, about 10 nm or less, about 5 nm or less, or about 1 nm or less. The other dimensions of the nanosheets, e.g., the length and width, are not particularly limited, but as described above, are substantially greater than the thickness. The terms “length” and “width” are not meant to imply that the nanosheets are limited to square or rectangular shapes. Other shapes, including irregular shapes may be used. (See FIG. 1A.) The nanosheet may be composed of one to a few (e.g., 3) atomic or molecular layers of the element/molecule from which the nanosheet is composed. Thus, the thickness of the nanosheet may be reported as the number of atomic/molecular layers in the nanosheet.

[0024] The term “average” refers to an average value over a representative number of nanostructures. Transmission electron microscopy (TEM) may be used to determine the size of the nanostructures. Raman spectroscopy may be used to determine the number of atomic/molecular layers in a nanosheet.

[0025] In the nanocomposite, the at least two different types of nanostructures are associated with each other, the association which is in the form of noncovalent, van der Waals interactions. The nature of the association (i.e., to confirm the van der Waals interactions) may be determined using Raman spectroscopy as described in the Example, below. To facilitate the association between the two different types of nanostructures in the nanocomposite, at least one of the nanostructures may be functionalized with functional groups. A variety of functional groups may be used, provided the functional group has an affinity (based on the capability of forming van der Waals interactions) for the material from which the other type of nanostructure is composed. Thus, the particular functional group selected will depend upon the chemical composition of the nanostructures. Illustrative functional groups include thiol, sulfate, carboxylate, cholate, sulfonate, and trimethyl ammonium. By way of illustration, thiol groups have a strong affinity towards chalcogenides (e.g., sulfides, selenides, tellurides). Thus, nanostructures functionalized with thiol groups may be used in order to facilitate association between the thiol-functionalized nanostructures and other nanostructures composed of a transition metal chalcogenide. Similarly sulfate, carboxylate, cholate, and sulfonate show strong affinity towards transition metals, thus

nanostructures functionalized with these groups may be used to facilitate association with transition metal chalcogenides.

[0026] The functional groups may be provided by a variety of different types of molecules, e.g., 11-mercaptoundecanoic acid (MUA), oleic acid (OAc), sodium dodecyl sulfate (SDS), sodium cholate, sodium dodecyl benzene-sulfonate, and hexadecyltrimethylammonium bromide (CTAB).

[0027] A variety of materials may be used for the nanostructures of the nanocomposite, depending upon the desired application. Illustrative materials include noble metals (e.g., Au, Pt, Ag), quantum dots (CdS, CdSe, ZnSe, PbS, PbSe, PbTe, CdTe, InP, InAs, Ag₂S), graphene, transition metal chalcogenides (e.g., MoS₂, MoTe₂, MoSe₂, WSe₂, WS₂), transition metal oxides (e.g., Fe₃O₄, Fe₂O₃, MnO, ZnO, MnFe₂O₄, ZnFe₂O₄), and nitrides (e.g., BN).

[0028] The ratio (e.g., concentration ratio) of the at least two different types of nanostructures in the nanocomposite may be adjusted depending upon the desired application. By way of illustration, if the nanocomposite is used as a catalyst for a particular reaction, the ratio may be adjusted to maximize the rate of the reaction.

[0029] In embodiments, the nanocomposite comprises 0D nanoparticles and 2D nanosheets, wherein the 0D nanoparticles are distributed, e.g., uniformly, on the exposed surfaces of the 2D nanosheets. An illustrative nanocomposite comprising thiol-functionalized Fe₃O₄ 0D nanoparticles and MoS₂ 2D nanosheets is described in the Example below. (See also FIGs. 1A and 10.)

[0030] In another aspect, methods of making the nanocomposites are provided. In embodiments, the methods include combining a first type of nanostructure with a material from which a second type of nanostructure is composed, in water or an aqueous solution, whereby the first type of nanostructure induces exfoliation of the material to provide the second type of nanostructure and association between the first and second types of nanostructures to form the nanocomposite. The method may be carried out in the absence of any organic solvents. The use of functional groups on the first type of nanostructure facilitates the exfoliation of the material to provide the second type of nanostructure as well as association between the first and second types of nanostructures as described above. Thus, the methods achieve nanocomposites in a single step in which combining the first type of nanostructure with the material from which the second type of nanostructure is to be formed results in exfoliation to provide the second type of nanostructure as well as association

between the first and second types of nanostructures. In other words, the exfoliation and association happen simultaneously, induced by the first type of nanostructure. However, sonication for a period of time may be used during the combining of the first and second types of nanostructures to further facilitate exfoliation and thus, association. The method may comprise additional steps, e.g., isolating the nanocomposite from unassociated nanostructures (e.g., via centrifuging and collecting a supernatant and dialyzing the supernatant).

[0031] In the paragraph immediately above and throughout the present disclosure, it is to be understood that the phrase “the material from which the second type of nanostructure is composed” (and the like) is meant to differentiate the “material” from the “second type of nanostructure.” That is, the “material” and the “second type of nanostructure” are different physical entities. By way of example, a material may be bulk MoS₂, which is a solid layered material, and the second type of nanostructure may be individual nanosheets of MoS₂.

[0032] Similarly, it is to be understood that the material from which the second type of nanostructure is composed is not an intercalated material, e.g., is not an ion intercalated transition metal chalcogenide such as lithium ion intercalated MoS₂. Similarly, the material from which the second type of nanostructure is composed is not an exfoliated material, i.e., is not a material which has been subjected to an exfoliation technique such as exfoliated MoS₂.

[0033] The material from which the second type of nanostructure is composed is also generally unfunctionalized, i.e., free of other atoms or molecules (aside from the chemical compound making up the material).

[0034] Finally, in view of the description of “nanostructure” above, the first type of nanostructure is not a small molecule, a polymer molecule, a solvent molecule, or a surfactant molecule.

[0035] As an initial step, the method may include functionalizing the first type of nanostructure, e.g., by combining the first type of nanostructure with the functional group selected to have a strong affinity for the second type of nanostructure (or a molecule having the functional group). By “strong affinity” it is meant an affinity sufficiently high to achieve van der Waal interactions between the first and second types of nanostructures. Thus, also provided by the present disclosure is the functionalization method itself, e.g., combining a first type of nanostructure with a functional group having a strong affinity for a second type of nanostructure (or a molecule having the functional group). In the functionalization, the functional group may be covalently bound to the first type of nanostructure. The functional

group is not necessarily directly covalently bound to the first type of nanostructure, i.e., when the functional group is provided by a molecule, the molecule may be covalently bound to the first type of nanostructure via another chemically appropriate functional group. This is illustrated by functionalizing Fe_3O_4 with 11-mercaptoundecanoic acid. The carboxylic acid group of 11-mercaptoundecanoic acid covalently bonds to Fe_3O_4 while the thiol group of the molecule is the functional group having a strong affinity for the second type of nanostructure.

[0036] An illustrative method for forming a 2D- MoS_2 /0D- Fe_3O_4 nanocomposite is shown in FIG. 10 and is further described in the Example, below. As the Example shows, no surfactants or stabilizers are required to achieve the exfoliation of 2D- MoS_2 . Moreover, the nanocomposite is formed in a single step in which the functionalized 0D- Fe_3O_4 nanoparticles (Fe_3O_4 nanoparticles functionalized with 11-mercaptoundecanoic acid) induce simultaneous exfoliation of bulk MoS_2 into 2D- MoS_2 nanosheets and association between the 0D- Fe_3O_4 nanoparticles and 2D- MoS_2 nanosheets to provide the nanocomposite.

[0037] By contrast, conventional methods of making 2D/0D nanocomposites include two steps. First, a bulk material is exfoliated (mechanically or chemically) to provide 2D nanostructures or the 2D nanostructures are grown (chemical vapor deposition). Then 0D nanoparticles are conjugated/grown onto the 2D nanostructures. As described above, the present methods can achieve the formation of 2D/0D nanocomposites in a single step. The present methods are also more flexible and may be applied to any combination of 0D and 2D nanostructures provided that the 0D nanostructures are functionalized with functional groups having a high affinity for the selected material for the 2D nanostructures. Since the method is based on surface functionality, instead of 0D nanostructures, 1D and 2D nanostructures with appropriate functional groups can also be used to exfoliate materials to provide 2D nanostructures and thus, 2D/1D and 2D/2D nanocomposites, respectively.

[0038] Depending upon the materials used for the nanostructures of the nanocomposites, the nanocomposites may exhibit intrinsic catalytic activity which may be exploited to provide a method for sensing a target analyte. Alternatively or in addition, the nanostructures of the nanocomposites may be further functionalized with receptors sensitive to a target analyte for use in a method for sensing the target analyte. Due to their high surface area and multiple functionalities, the nanocomposites may provide a higher limit of detection (LOD) for the target analyte. The type of intrinsic catalytic activity, receptors, and the target analytes are not particularly limited. Illustrative target analytes include biomarkers and toxic elements in fluids or air.

[0039] In embodiments, the nanocomposite exhibits intrinsic peroxidase activity, i.e., the ability to catalyze the oxidation of a substrate by hydrogen peroxide, H_2O_2 . A chromogenic material capable of being oxidized by H_2O_2 may be used as the substrate. The color change induced by the oxidation may be detected by monitoring the absorbance of the chromogenic material at a particular wavelength of light. This wavelength may be one which overlaps with the absorbance spectrum of the oxidized chromogenic material, e.g., at or near an absorbance maximum. The absorbance is proportional to the concentration of H_2O_2 . Illustrative chromogenic materials include 3,3',5,5'-Tetramethylbenzidine (TMB), o-Phenylenediamine (OPD), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), p-nitrophenyl phosphate, 5 aminosalicylic acid, and 3,3' diaminobenzidine. The chromogenic material TMB turns blue when oxidized by H_2O_2 ; OPD turns orange when oxidized by H_2O_2 ; and ABTS turns green when oxidized by H_2O_2 . (See FIGs. 4A and 4B.) The chromogenic material 5 aminosalicylic acid turns yellow when oxidized by H_2O_2 ; p-nitrophenyl phosphate turns yellow when oxidized by H_2O_2 ; and 3,3' diaminobenzidine turns brown when oxidized by H_2O_2 .

[0040] The Example, below, demonstrates that an illustrative 2D-MoS₂/0D-Fe₃O₄ nanocomposite exhibits peroxidase activity and catalyzes the oxidation of TMB, OPD and ABTS by H_2O_2 . Thus, such a nanocomposite may be used as a H_2O_2 sensor, by exposing the nanocomposite to a sample in the presence of a chromogenic material and measuring the absorbance of the chromogenic material. The sample is one which is suspected of containing the target analyte, H_2O_2 . Quantitative determination of the concentration of H_2O_2 may be accomplished by comparing the measured absorbance to a calibration curve. (See FIG. 6B.) Rapid and accurate detection of H_2O_2 is useful in the fields of bioanalysis as well as food security and environmental protection.

[0041] Peroxidase activity may further be exploited to sense other target analytes besides H_2O_2 . By way of illustration, since the oxidation of glucose by glucose oxidase (GOx) produces H_2O_2 , nanocomposites exhibiting peroxidase activity may also be used to sense glucose. The mechanism is illustrated in FIG. 7A, for the illustrative 2D-MoS₂/0D-Fe₃O₄ nanocomposite. In this case, the nanocomposite is exposed to a sample suspected of containing glucose in the presence of a chromogenic material and GOx and the absorbance of the chromogenic material is measured. The sample may be a fluid (e.g., a bodily fluid such as saliva, blood, sweat, urine) from a subject (e.g., a mammalian subject). Since concentration of H_2O_2 is proportional to the concentration of glucose, the measured absorbance of the

chromogenic material is proportional to the glucose concentration. Thus, quantitative determination of the concentration of glucose may be achieved. (See FIG. 7B.)

[0042] Other oxidoreductases besides GOx may be used, similar to the description above.

[0043] In embodiments, the nanocomposite, the chromogenic material, and the GOx (if present) may be in the form of an aqueous solution. However, in other embodiments, these materials may be deposited on a substrate. A variety of substrates may be used, including flexible substrates such as paper or plastics. Ink jet deposition may be used to deposit aqueous solutions of each of the materials on the substrate, e.g., in a layer-by-layer fashion. Next, the substrate may be exposed to the sample for a period of time. The exposed substrate may be heated for a period of time to facilitate drying. As described above, oxidation of the chromogenic material via H_2O_2 either present in the sample or produced due to glucose present in the sample, will induce a color change. The intensity of the resulting color (which may be visually detected) is proportional to the concentration of the H_2O_2 /glucose in the sample. Qualitative and/or quantitative determination of the concentration may be achieved by comparing the intensity of the resulting color to a calibration color chart (i.e., a chart which associates a particular color/intensity with a particular analyte concentration). This embodiment is illustrated in FIG. 9 in the form of a non-invasive glucose diagnostic platform.

[0044] As shown in FIG. 11, the test strip diagnostic platform of FIG. 9 can be implemented using a smartphone-based attachment/module for more automated recognition that can monitor the glucose readings. The strip can be inserted into a cartridge-based device where the colorimetric in presence of glucose can be digitally detected. Then, the device can be attached to a smartphone and the data can be collected, sent to physician/clinic and/or stored in the cloud. This can allow the instant feedback from the physician/clinic. The same device can be extended to detect other biomarkers (target analytes) such as nitric oxide and cortisol. For each biomarker, the device can use a different cartridge which has the sensing elements for that biomarker. For example, for nitric oxide and cortisol, the cartridge can be an electrochemical cell. The presence of nitric oxide and cortisol can be detected based on change in current values.

EXAMPLE

[0045] **Introduction**

[0046] A novel, one-step method where 2D materials can be exfoliated and stabilized in water using functionalized nanoparticles (NPs), resulting in 2D/0D nanocomposites, is reported here. Liquid exfoliation of 2D materials via organic solvents and ion intercalation has been previously reported.¹⁰⁻¹² Ion intercalation is an environmentally sensitive and time consuming process, and in some cases results in the structural deformation of 2D materials.¹³ Exfoliation of 2D materials in organic solvents is promising.^{10, 14} However, for large scale production, environmentally friendly solvents such as water are preferred.¹⁵ Most of the 2D materials cannot be exfoliated in water without any surfactants or stabilizers.¹⁵ This method uses aqueous dispersion of nanoparticles to exfoliate 2D materials, and is a facile and scalable. Three different 2D materials (MoS_2 , WS_2 , BN) have been exfoliated using different NPs (Fe_3O_4 , Au, QDs) to show the versatile nature of this method. Hence, a library of 2D/0D nanocomposites was developed.

[0047] To demonstrate the application of 2D/0D nanocomposites, $\text{MoS}_2/\text{Fe}_3\text{O}_4$ nanocomposites were prepared and their peroxidase-like catalytic property was studied. $\text{MoS}_2/\text{Fe}_3\text{O}_4$ nanocomposite demonstrated significantly higher peroxidase activity than both MoS_2 nanosheets and Fe_3O_4 nanoparticles. The enhanced peroxidase activity resulted in lower limit of detection for H_2O_2 and glucose.¹⁶⁻¹⁷ Finally, a paper based sensor has been developed by inkjet printing $\text{MoS}_2/\text{Fe}_3\text{O}_4$ test strips and showing their application towards point-of-care diagnostics.

Experimental

Materials

[0048] MoS_2 powder, SDS, GOx, TMB, OPD, ABTS, fructose, lactose and maltose were purchased from Sigma Aldrich.

Preparation of $\text{MoS}_2/\text{Fe}_3\text{O}_4$ nanocomposites

[0049] 50 mg of MoS_2 powder, 0.2 ml of thiol functionalized Fe_3O_4 nanoparticles ($[\text{Fe}]=1$ mg/ml) and 10 mL of milli-q water were probe sonicated for 10 mins. The solution was then centrifuged at 4500 rpm for 15 mins, and the green-brown supernatant was collected and dialyzed using a dialysis bag (MWCO = 10 000) for 6 hours in water. Any aggregated particles were removed by centrifugation. Finally, dispersion of $\text{MoS}_2/\text{Fe}_3\text{O}_4$ nanocomposite was stored at room temperature and used as is for further experiments. The concentration of Mo and Fe was calculated via ICP. The preparation is illustrated in Scheme 1, FIG. 10.

Preparation of MoS_2 nanosheets

[0050] 50 mg of MoS₂ powder, 15 mg of SDS and 10 mL of milli-q water were probe sonicated for 10 mins. The solution was then centrifuged at 4500 rpm for 15 mins, and the green supernatant was collected and dialyzed (MWCO = 10 000) for 6 hours. Any aggregated particles were removed by centrifugation. Finally, dispersion of MoS₂ nanosheets was stored at room temperature and used as is for further experiments.

Synthesis of Fe₃O₄ nanoparticles

[0051] The Fe₃O₄ nanoparticles were synthesized using a previously reported thermal decomposition method that resulted in monodispersity and single crystallinity.¹⁸⁻¹⁹ In a typical Fe₃O₄ nanoparticles synthesis, Fe(acac)₃ (2 mmol), 1,2-hexadecanediol (10 mmol), oleic acid (6 mmol), oleylamine (6 mmol), and benzyl ether (20 mL) were charged in a 100 mL three-neck round-bottom flask and magnetically stirred under a flow of nitrogen. The mixture was first heated to 110 °C for 1 h to remove moisture. The temperature was then increased to 210 °C for 1 h, and was finally refluxed for 1 h before cooling down to room temperature. The black-brown mixture was precipitated, washed three times using ethanol, and dispersed in hexane.

Preparation of thiol functionalized Fe₃O₄ nanoparticles

[0052] The as-synthesized oleic acid coated hydrophobic Fe₃O₄ nanoparticles were functionalized with MUA and citric acid (CA) via ligand exchange process.¹⁸ Chloroform dispersion (2 ml) of Fe₃O₄ nanoparticles (12.5 mg) and DMSO solution of MUA (1 ml, 62.5 mg) and CA (1 ml) of were mixed and sonicated overnight at room temperature under N₂ protection. The modified Fe₃O₄ nanoparticles were washed by dichloromethane 3 times, dried under nitrogen gas, and dispersed in water. The dispersion was dialyzed to remove any residual surfactants using a dialysis bag (MWCO = 10 000) for 2 days in water. A 200 nm syringe filter was used to remove any precipitation, and the final concentration of thiol functionalized Fe₃O₄ nanoparticles dispersed in water was determined by ICP-MS analysis. TEM showed that the particles did not aggregate after ligand exchange, and hydrodynamic sizes were found in the range of 22–47 nm.

4.3 Peroxidase activity of MoS₂/Fe₃O₄

[0053] Peroxidase-like activity of MoS₂/Fe₃O₄ nanocomposite was tested by mixing 0.2 ml of TMB (1 mM), 0.2 ml of MoS₂/Fe₃O₄ ([Mo] = 8 µg/mL, [Fe] = 22 µg/mL) and 0.2 mL H₂O₂ (100 mM). TMB (3,3',5,5'-tetramethylbenzidine) solution was made in 0.1 M citrate-phosphate buffer (pH 5.0). For ABTS (2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic

acid]-diammonium salt) and OPD (*o*-phenylenediamine dihydrochloride) reaction, a similar method was used. OPD was dissolved in 0.1 M citrate-phosphate buffer (pH 5.0) while ABTS was dissolved in 0.1 M citrate buffer (pH 5.0).

Characterization of Fe₃O₄, MoS₂, and MoS₂/Fe₃O₄

[0054] Transmission electron microscopy (TEM), energy dispersive X-ray (EDX), and elemental mapping of the nanostructures were observed using a Hitachi HD2300. The hydrodynamic diameters of the nanostructures were measured by a Malvern Zeta Sizer Nano S-90 dynamic light scattering (DLS) instrument. Raman spectra were collected on the HORIBA LabRAM HR Evolution Confocal Raman System equipped with a solid-state laser. The laser power used was 8.8 uW with a co-condition of 2. X-ray photoelectron spectroscopy (XPS, Thermo Scientific ESCALAB 250Xi) was used for binding energy analysis.

Detection of H₂O₂ and glucose in solution

[0055] For H₂O₂ detection, first 0.2 ml of TMB (1 mM) in pH 5.0 citrate phosphate buffer and 0.2 ml of MoS₂/Fe₃O₄ ([Mo] = 8 µg/mL, [Fe]=22 µg/mL) were mixed. Then, different concentrations of 0.2 mL H₂O₂ were added. A reaction rate of 652 nm was recorded immediately after the addition of H₂O₂, while for the absorbance values, the solutions were kept at room temperature for 5 mins before recording. The absorbance change was recorded in BioTek Synergy 4 Multimode plate reader with onboard dispenser and monochromator.

[0056] For glucose detection, a mixture of 0.1 mL GOx (20 mg/mL) and 0.1 mL of glucose solution of different concentrations in 0.1 M Na₂HPO₄ buffer (pH 7.0) was incubated at 37 °C for 30 min. Then, 0.2 ml of TMB (1 mM) in pH 5.0 citrate phosphate buffer and 0.2 mL of MoS₂/Fe₃O₄ ([Mo] = 8 µg/mL, [Fe]=22 µg/mL) were added. The solutions were kept at 45 °C for 10 mins, and then the absorbance change was recorded in the plate reader. For fructose, lactose and maltose, a similar method was used, except glucose was replaced with one of fructose, lactose or maltose.

Preparation of test strips

[0057] A desktop inkjet color printer Epson Workforce 30 was used to print glucose test strips. The standard cyan, magenta and yellow ink cartridges were replaced with cartridges filled with GOx, chromogenic substrate (ABTS) and MoS₂/Fe₃O₄ solutions. The main reason for this choice of printer was its piezoelectric print heads that use pressure rather than heat, since heat can potentially denature GOx. Test strips were developed by printing layers of each

solution in the form of a square on the paper and drying at room temperature. Finally the strips were stored in 4 °C until further usage.

Detection of glucose via test strips

[0058] Test strips were dipped into glucose solution of different concentrations for 1 min. After that, they were kept in an oven set at 45 °C for 10 minutes, and the color change was observed with naked eye.

Results and Discussion

[0059] TEM images of MoS₂/Fe₃O₄ nanocomposites showed that Fe₃O₄ NPs were decorated on the surface of MoS₂ nanosheets (FIG. 1A). The size of the Fe₃O₄ nanoparticles used was about 8 nm. The Fe₃O₄ NPs acted as stabilizers for MoS₂ sheets and helped them disperse in water. FIGs. 1B and 1C show an elemental map and EDX patterns, respectively, of MoS₂/Fe₃O₄. The Fe, Mo, and S signals from the same area confirm the presence of Fe₃O₄ nanoparticles on exfoliated MoS₂ nanosheets.

[0060] The integration of MoS₂ nanosheets and Fe₃O₄ NPs was also confirmed by X-ray photoelectron spectroscopy (XPS) and Raman spectroscopy. XPS was used to determine the chemical composition and chemical states of the MoS₂/Fe₃O₄ nanocomposite. As shown in full line scans in FIG. 2A, the XPS spectrum of MoS₂/Fe₃O₄ nanocomposite showed characteristic peaks of both MoS₂ (100-400 eV) and Fe₃O₄ (700-900 eV) NPs, confirming the existence of both MoS₂ and Fe₃O₄ in the nanocomposite.

[0061] FIGS. 2B-2D show the detailed scan of the MoS₂/Fe₃O₄ nanocomposite with characteristic peaks of Fe, Mo, and S. The Fe spectrum in the 2p_{3/2} region can be deconvoluted into two main peaks and a satellite peak (FIG. 2B). The same pattern was repeated, with almost half intensity for the 2p_{1/2} component. Fe₃O₄ comprises two oxidation states, Fe²⁺ and Fe³⁺. The lowest binding energy peak at 710.2 eV was attributed to Fe²⁺, with a corresponding satellite at 718.0 eV. The Fe³⁺ peak was found with a binding energy of 713.3 eV. The peaks position of Fe 2p_{3/2} and Fe2p_{1/2} are comparable to the reported values in the literature.²⁰⁻²²

[0062] FIG. 2C displays Mo 3d peaks at 233.04 eV and 229.87 eV, corresponding to the 3d_{5/2} and 3d_{3/2} doublet. The S 2p peak can be deconvoluted into two peaks at 163.88 eV and 162.70 eV (FIG. 2D), attributing to the 2p_{1/2} and 2p_{3/2} orbital. These binding energy values are consistent with those reported in previous studies and confirm the expected charge states of Mo⁴⁺ and S²⁻ in the MoS₂ nanosheets.²³⁻²⁴ It is also worth noting that additional peaks of

metallic Mo or MoO_x were not observed in higher/lower binding energy regions, which confirms the quality of exfoliated MoS₂ nanosheets similar as CVD grown MoS₂.

[0063] Raman spectroscopy can assess the crystallinity and layer thickness of two-dimensional MoS₂ in terms of the position and frequency difference of two characteristic vibrational modes, E_{2g} and A_{1g}.²⁴⁻²⁵ The E_{2g} mode is attributed to the in-plane vibration of Mo and S atoms, while the A_{1g} mode is related to the out-of-plane vibration of S atoms.²⁵ FIG. 3A shows the Raman spectra of MoS₂/Fe₃O₄ nanocomposites, MoS₂ nanosheets and Fe₃O₄ NPs dispersed on a Si/SiO₂ substrate. The measurements were conducted using a solid-state laser (532 nm) with power of 8.8 μW to eliminate the effect of optical heating. FIG. 3B shows the two vibrational modes centered at 380.7 cm⁻¹ and 405.6 cm⁻¹, while the multilayer MoS₂ sheet exhibits modes at 383.8 cm⁻¹ and 408.6 cm⁻¹. Both spectra gave similar E_{2g}-to-A_{1g} frequency differences of ~25 cm⁻¹. This value was smaller than that of bulk MoS₂, but higher than single layer MoS₂, indicating their exfoliated few layer structure.²⁶ Raman spectroscopy has also been utilized to investigate the effects of lattice strain, doping levels, and the van der Waals interaction at the interface of 2D crystals.²⁵ The in-plane Raman mode, E_{2g}, is sensitive to the built-in strain of 2D MoS₂, while the out-of-plane mode, A_{1g}, is a reflection of interlayer van der Waals interactions. Thus, it is reasonable to predict from the A_{1g} shift of 3.9 cm⁻¹ from MoS₂ to MoS₂/Fe₃O₄ that integration of Fe₃O₄ nanoparticles caused in-plane strain in MoS₂ nanosheets.

[0064] The peroxidase-like catalytic activity of MoS₂/Fe₃O₄ nanocomposites was tested in solution of chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB) and H₂O₂.^{17, 27} As shown in FIG. 4A, a solution of H₂O₂ + TMB + MoS₂/Fe₃O₄ turned blue within seconds, while a solution of H₂O₂ + TMB showed negligible color change, confirming the Fe₃O₄ accelerated the color change. As a negative control, a solution of H₂O₂ + MoS₂/Fe₃O₄ was also prepared that remained colorless due to the absence of TMB. (FIG. 4B). TMB is oxidized in the presence of H₂O₂, and the reaction was catalyzed by the MoS₂/Fe₃O₄ nanocomposites, resulting in quick formation a blue complex.²⁷ Similar to TMB, the other chromogenic substrates such as 2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt (ABTS) and *o*-phenylenediamine dihydrochloride (OPD) were also mixed with MoS₂/Fe₃O₄ and H₂O₂ and resulted in green and orange colors, respectively.²⁸ FIG. 4B shows absorbance plots and a picture (inset) of colored (oxidized) substrates (ABTS, OPD, and TMB) in the presence of H₂O₂. These results demonstrate that MoS₂/Fe₃O₄ nanocomposite demonstrates

peroxidase-mimic properties and can be used as an inorganic and robust catalyst for various peroxidase reactions.

[0065] The catalytic reaction was monitored by noting the change in absorbance of converted TMB at 652 nm.²⁷ FIG. 5A shows the time-dependent absorbance changes when different concentrations of MoS₂/Fe₃O₄ nanocomposite were added to H₂O₂ and TMB solutions. The incremental change in the reaction rate with increasing MoS₂/Fe₃O₄ concentration shows that the catalytic behavior follows typical Michaelis–Menten kinetics.²⁹ To compare with MoS₂/Fe₃O₄ nanocomposite, peroxidase activity of Fe₃O₄ nanoparticles and MoS₂ nanosheets was analyzed (FIG. 5B). The kinetic analysis showed that the reaction rate of MoS₂/Fe₃O₄ nanocomposite was significantly higher than MoS₂ nanosheets and Fe₃O₄ nanoparticles with similar Mo and Fe concentrations, respectively.²⁷ Though Fe₃O₄ nanoparticles and MoS₂ nanosheets alone were peroxidase active, the MoS₂/Fe₃O₄ nanocomposites showed superior catalytic activity over their individual components. The integration of Fe₃O₄ NPs on large area 2D MoS₂ nanosheets increased the catalytic sites.

[0066] These synergistic effects in the MoS₂/Fe₃O₄ nanocomposite contributed to the superior peroxidase activity.³⁰

[0067] Based on the intrinsic peroxidase-like activity, a colorimetric detection method for H₂O₂ was designed.¹⁶ Oxidation of TMB was observed at different H₂O₂ concentrations in the presence of MoS₂/Fe₃O₄ and TMB. The concentration of MoS₂/Fe₃O₄ was kept constant and the change in absorbance values was observed at 652 nm (FIG. 6A). The changed in absorbance was detected down to 1.56 μM of H₂O₂. The kinetic analysis suggested that the reaction rate was also dependent on H₂O₂ concentration (FIG. 6B).

[0068] H₂O₂ is the key component in the conventional electrochemical glucose sensors that are based on oxidation reaction of glucose catalyzed by glucose oxidase.³¹⁻³² Since H₂O₂ can be colorimetrically detected using MoS₂/Fe₃O₄, a colorimetric method was designed to detect glucose. When glucose solution was added in a mixture of GOx, TMB, and MoS₂/Fe₃O₄, the solution turned blue (or orange with OPD, or green with ABTS). The reaction is shown in the FIG. 7A. The freshly produced H₂O₂ from glucose-GOx reaction oxidized TMB that resulted in a blue color. FIG. 7B shows absorbance value obtained when solutions of different glucose concentration were mixed with GOx, TMB, and MoS₂/Fe₃O₄. The change in absorbance was detected down to 25 μM of glucose.

[0069] The glucose detection ability of MoS₂/Fe₃O₄ nanocomposite was compared with Fe₃O₄ nanoparticles and MoS₂ nanosheets with 1 mM glucose solution (FIG. 8A). The highest absorbance values showed that the catalytic efficiency of MoS₂/Fe₃O₄ nanocomposite was significantly higher than MoS₂ nanosheets and Fe₃O₄ nanoparticles with similar Mo and Fe concentrations, respectively. To show the selectivity of this glucose detection method, a set of control experiments was performed in the presence of glucose analogues (FIG. 8B). Solutions of fructose, lactose, and maltose were added in a mixture of GOx, TMB, and MoS₂/Fe₃O₄, and their absorbance was observed. The negligible change in absorbance in all of the fructose, maltose, and lactose solutions demonstrates the highly selective nature of this glucose detection method.

[0070] Point-of-care glucose diagnostics is still an early concept and has not been widely accepted. Most of the glucose sensing is done by a conventional glucometer where a drop of blood is deposited on a test strip and the glucose concentration is electrochemically detected. Glucose urine test strips are available, but concentration of glucose in urine does not match the accurate blood glucose concentration. Hence, strips that required no blood pricking and can tell accurate blood concentration very quickly can achieve non-invasive POC glucose diagnostics.

[0071] This glucose detection method and MoS₂/Fe₃O₄ catalyst can accomplish this goal. Based on the colorimetric detection of glucose using MoS₂/Fe₃O₄, GOx, and a peroxidase substrate, test strips have been developed that show quick change in color in glucose solution (FIG. 9). A desktop inkjet color printer (Epson Workforce 30) was used to print glucose test strips. The standard cyan, magenta and yellow ink cartridges were replaced with cartridges filled with GOx, chromogenic substrate (ABTS), and MoS₂/Fe₃O₄ solutions. Test strips were developed by printing layers of each solution in the form of a square on the paper. After dipping into glucose solution for 1 minute and heating at 45°C for 10 minutes, the square block's color on the strip changed from colorless to green. The intensity of color was dependent on the concentration of glucose solution. It could be observed from the naked eye that the test strips allowed glucose detection as low as 12.5 mM or 225 mg/dL, which was sufficient to distinguish blood glucose concentration in healthy (3–8 mM) and diabetic persons (9–40 mM).³³ Therefore, the colorimetric detection of glucose from test strips made of MoS₂/Fe₃O₄ composites can be used for quick determination of glucose concentration. In addition to blood glucose detection, this is a powerful tool for non-invasive glucose detection using saliva, since direct correlation between saliva and blood glucose have been reported.³⁴⁻

³⁶ The strip can be used in developing world areas with low or no diagnostic resources. In an advanced version, a process may be used to digitize the results to be able to read on a portable device.

Summary

[0072] In summary, a facile one-step scalable method to fabricate 2D/0D nanocomposites by exfoliating 2D materials via functionalized nanoparticles is reported here. MoS₂/Fe₃O₄ nanocomposites were developed and their peroxidase-like catalytic activity was explored to colorimetrically detect H₂O₂ and Glucose with LOD 1.5 μM and 25 μM. Glucose test strips were developed that changed their color in presence of glucose solutions as low as 225 mg/dL. MoS₂/Fe₃O₄ nanocomposites may be used for affordable, portable, and point-of-care diagnostics. A variety of 2D/0D nanocomposites may be formed by changing 2D materials and NPs.

[0073] References

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[0074] The word "illustrative" is used herein to mean serving as an example, instance, or illustration. Any aspect or design described herein as "illustrative" is not necessarily to be construed as preferred or advantageous over other aspects or designs. Further, for the purposes of this disclosure and unless otherwise specified, "a" or "an" means "one or more".

[0075] The foregoing description of illustrative embodiments of the disclosure has been presented for purposes of illustration and of description. It is not intended to be exhaustive or to limit the disclosure to the precise form disclosed, and modifications and variations are possible in light of the above teachings or may be acquired from practice of the disclosure. The embodiments were chosen and described in order to explain the principles of the disclosure and as practical applications of the disclosure to enable one skilled in the art to utilize the disclosure in various embodiments and with various modifications as suited to the particular use contemplated. It is intended that the scope of the disclosure be defined by the claims appended hereto and their equivalents.

WHAT IS CLAIMED IS:

1. A method of making a nanocomposite, the method comprising combining a first type of nanostructure with a bulk material in water or an aqueous solution, the first type of nanostructure functionalized with a functional group capable of undergoing van der Waals interactions with the bulk material, whereby the first type of nanostructure induces exfoliation of the bulk material to provide a second, different type of nanostructure while inducing association between the first and second types of nanostructures to form the nanocomposite.
2. The method of claim 1, wherein the bulk material is unfunctionalized.
3. The method of claim 1, wherein the first type of nanostructure is selected from 0D nanostructures, 1D nanostructures, 2D nanostructures and combinations thereof and the second, different type of nanostructure is a 2D nanostructure.
4. The method of claim 1, wherein the first type of nanostructure is a 0D nanoparticle and the second type of nanostructure is a 2D nanosheet, and further wherein the nanocomposite comprises 0D nanoparticles distributed on the exposed surfaces of 2D nanosheets.
5. The method of claim 1, wherein the compositions of the first and second types of nanostructures are independently selected from noble metals, quantum dots, graphene, transition metal chalcogenides, transition metal oxides, nitrides, and combinations thereof.
6. The method of claim 1, wherein the functional group is selected from a thiol, a sulfate, a carboxylate, a cholate, a sulfonate, and trimethyl ammonium.
7. The method of claim 1, wherein the composition of the first type of nanostructure is a transition metal oxide and the composition of the bulk material and the second type of nanostructure is a transition metal chalcogenide.
8. The method of claim 7, further wherein the functional group is a thiol.
9. The method of claim 7, wherein the transition metal oxide is Fe_3O_4 and the transition metal chalcogenide is MoS_2 .
10. The method of claim 9, further wherein the functional group is a thiol.

11. The method of claim 1, further comprising depositing the nanocomposite on a substrate to form a sensor.
12. The method of claim 11, further comprising depositing a chromogenic material on the substrate, the chromogenic material capable of exhibiting a color change when oxidized.
13. The method of claim 12, further comprising depositing an oxidoreductase on the substrate.
14. The method of claim 13, wherein the oxidoreductase is glucose oxidase.
15. The method of claim 14, wherein the composition of the first type of nanostructure is a transition metal oxide and the composition of the bulk material and the second type of nanostructure is a transition metal chalcogenide.
16. The method of claim 15, wherein the transition metal oxide is Fe_3O_4 and the transition metal chalcogenide is MoS_2 .
17. A sensor for a target analyte, the sensor comprising:
 - a nanocomposite comprising a plurality of a first type of nanostructures and a plurality of a second type of nanostructures, the first type of nanostructures in association with the second type of nanostructure, wherein the nanocomposite exhibits intrinsic catalytic activity in a reaction involving a target analyte; and
 - a chromogenic material capable of exhibiting a color change in the presence of the target analyte.
18. The sensor of claim 17, wherein the intrinsic catalytic activity is intrinsic peroxidase activity, and the target analyte is H_2O_2 .
19. The sensor of claim 17, further comprising glucose oxidase and wherein the intrinsic catalytic activity is intrinsic peroxidase activity, and the target analyte is glucose.
20. A method of using the sensor of claim 17, the method comprising exposing the sensor to a sample and measuring the absorbance of the chromogenic material.

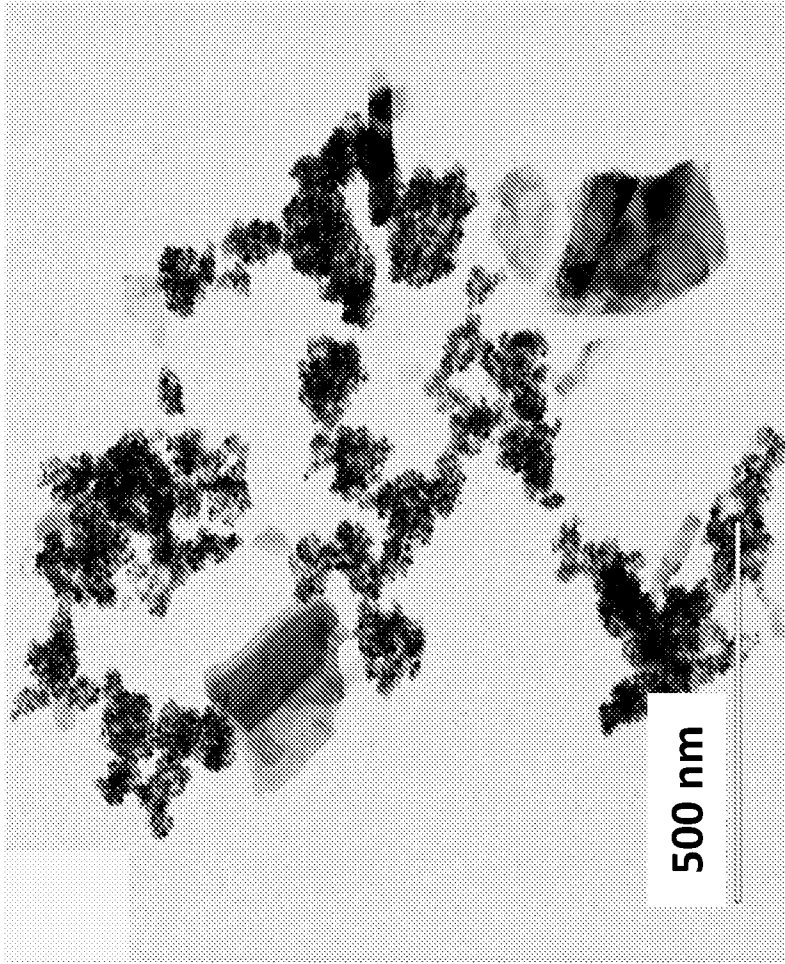


FIG. 1A

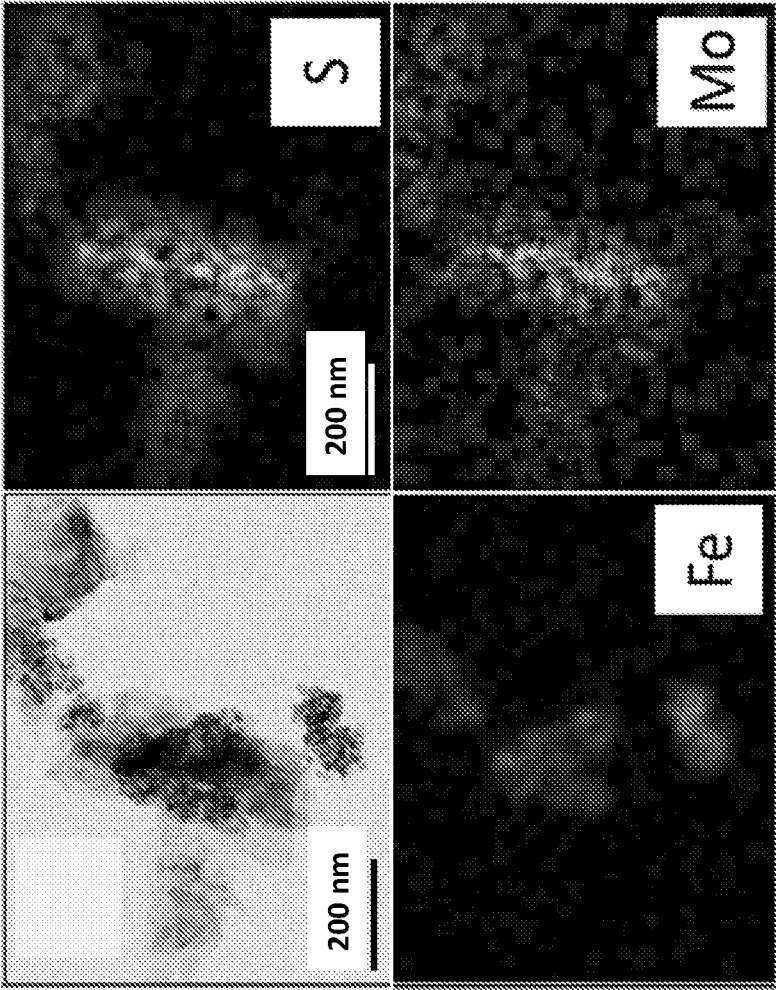


FIG. 1B

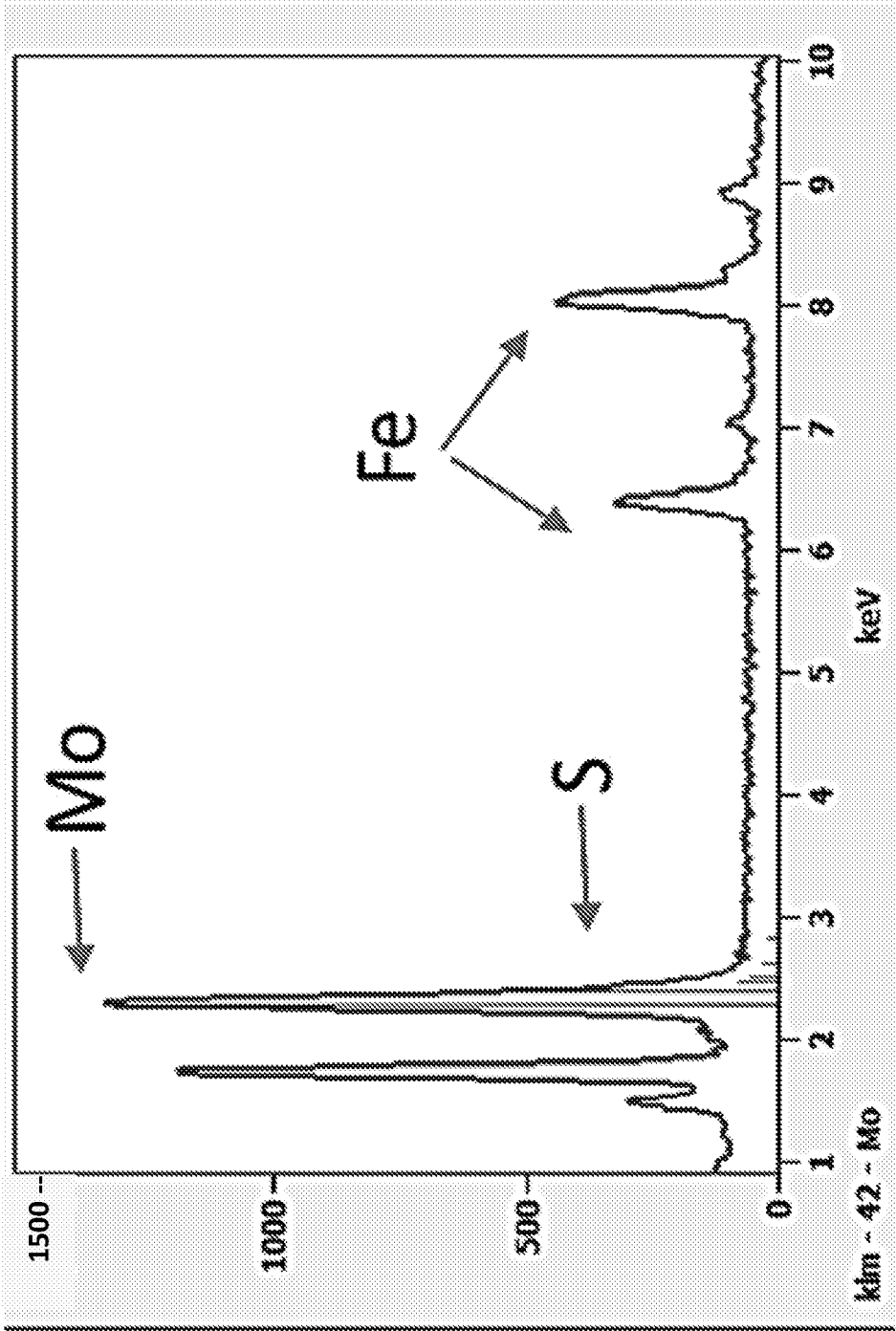


FIG. 1C

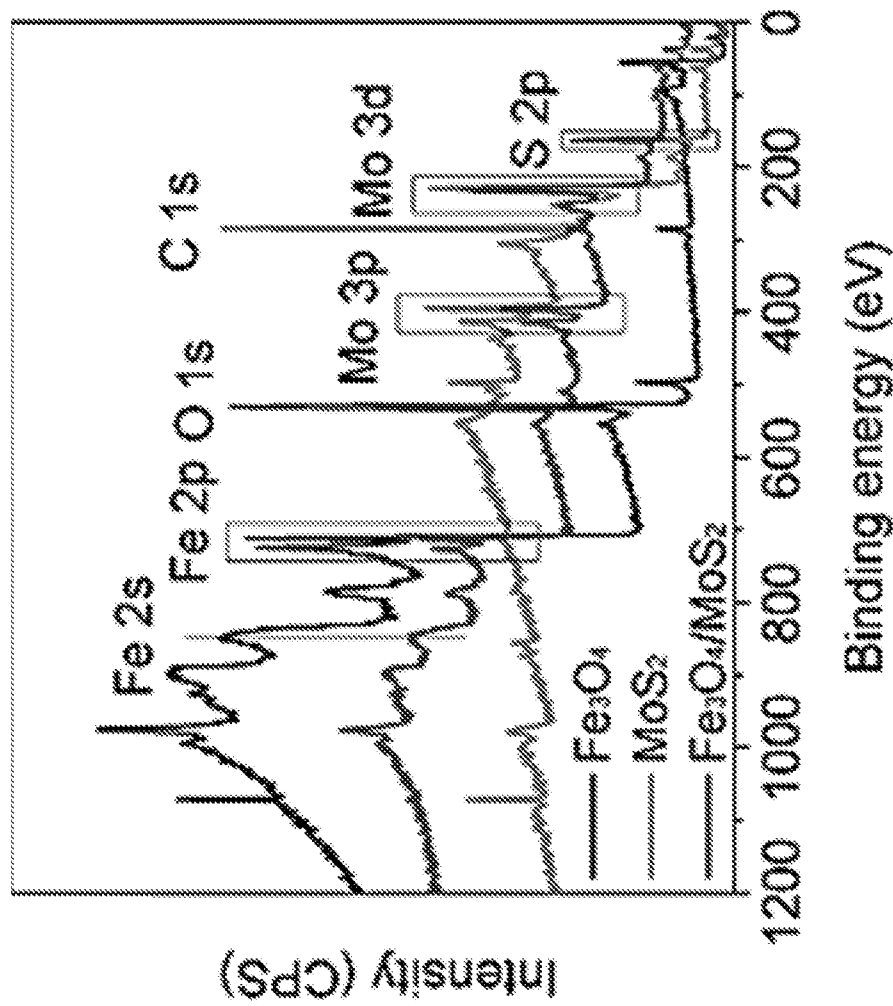


FIG. 2A

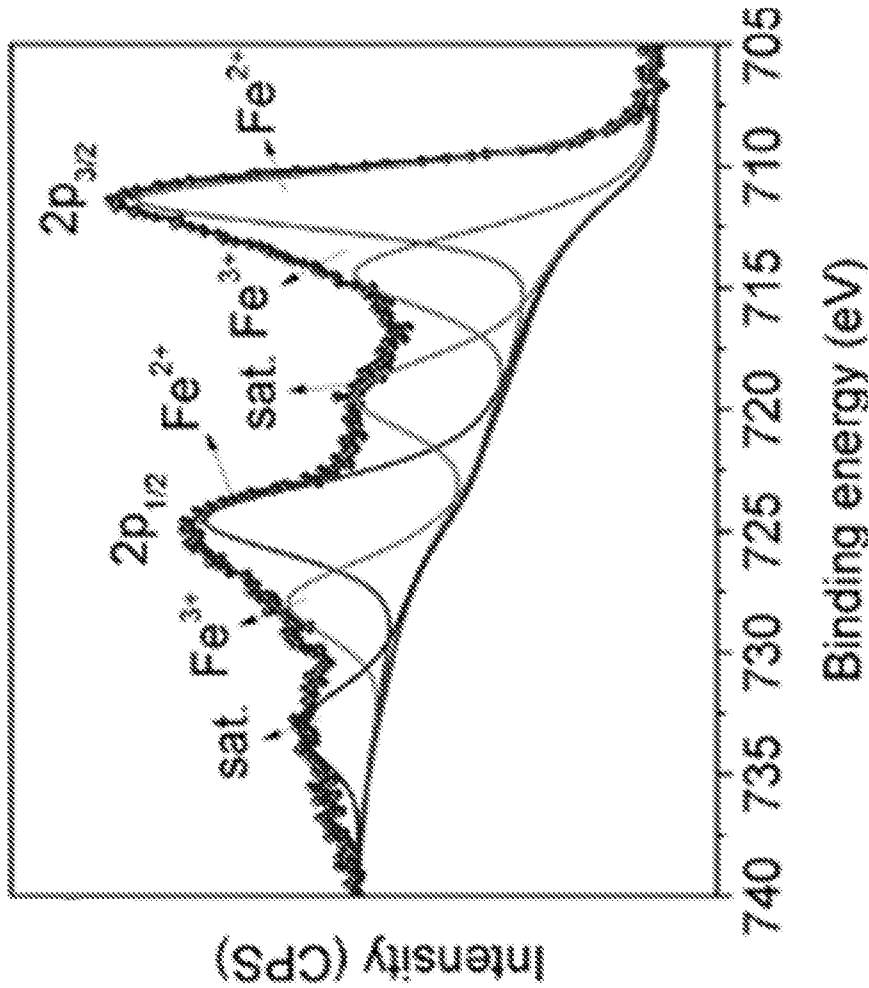


FIG. 2B

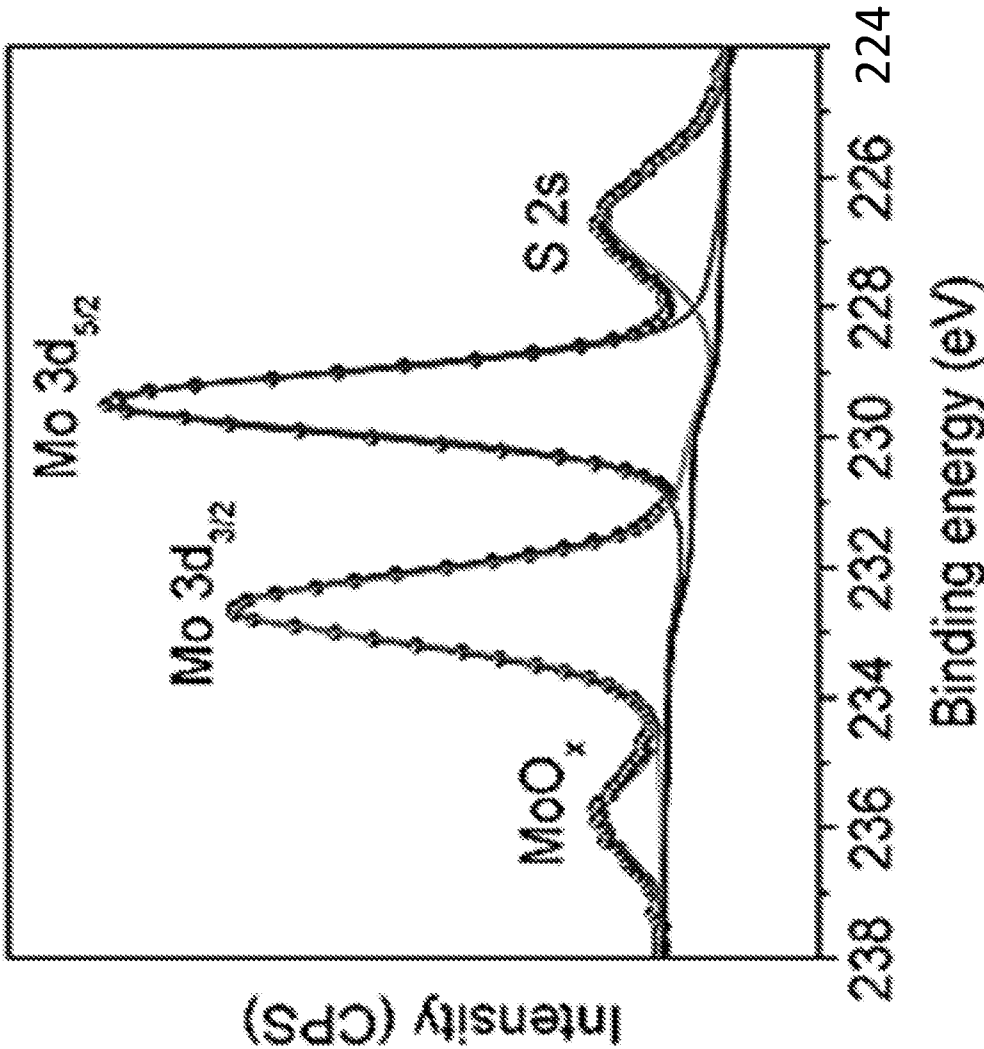


FIG. 2C

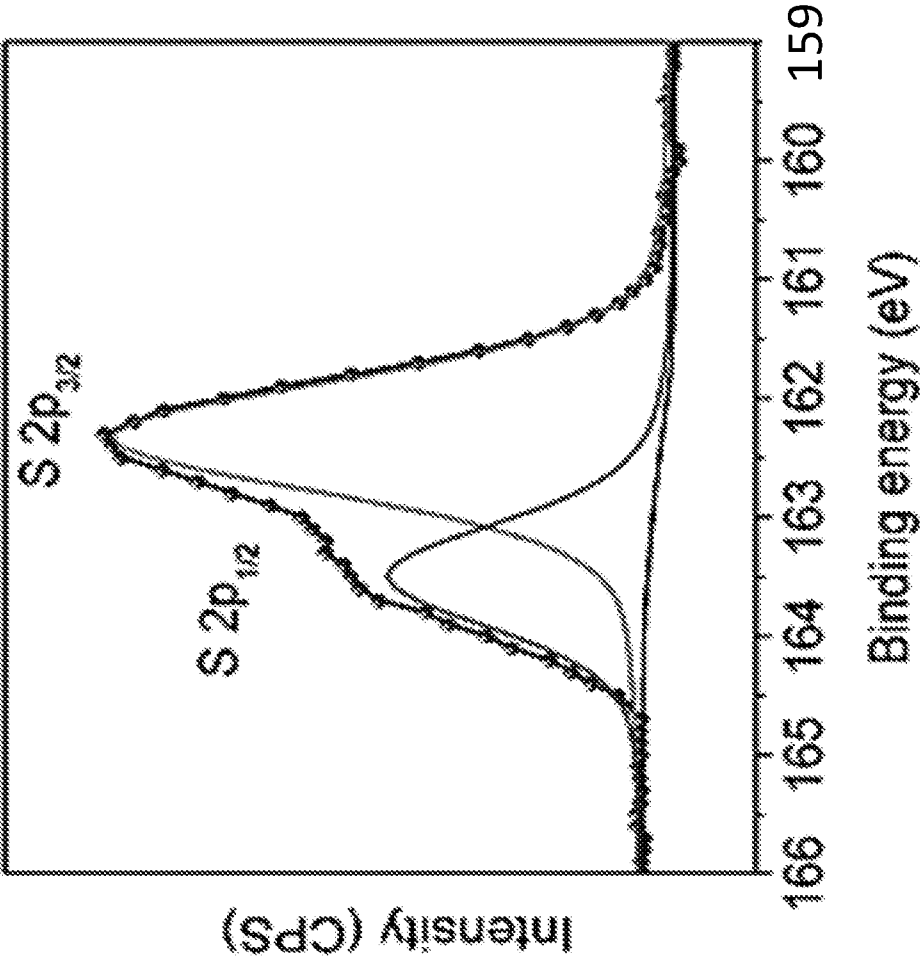


FIG. 2D

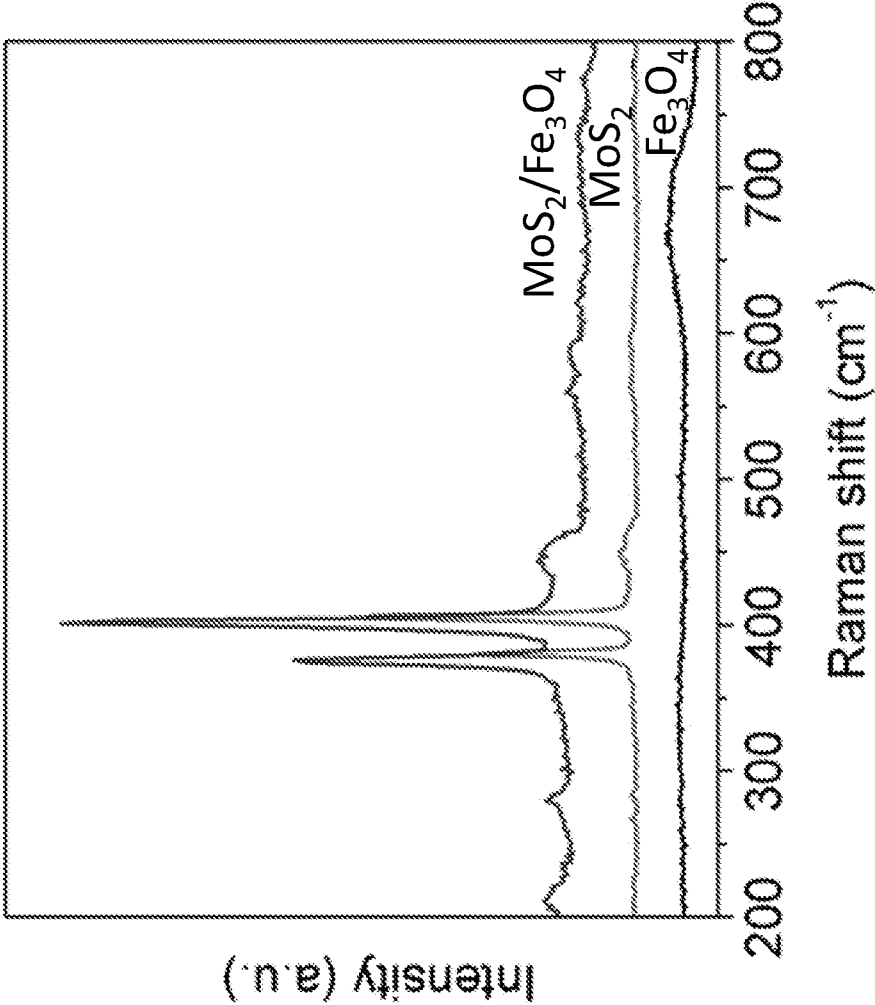


FIG. 3A

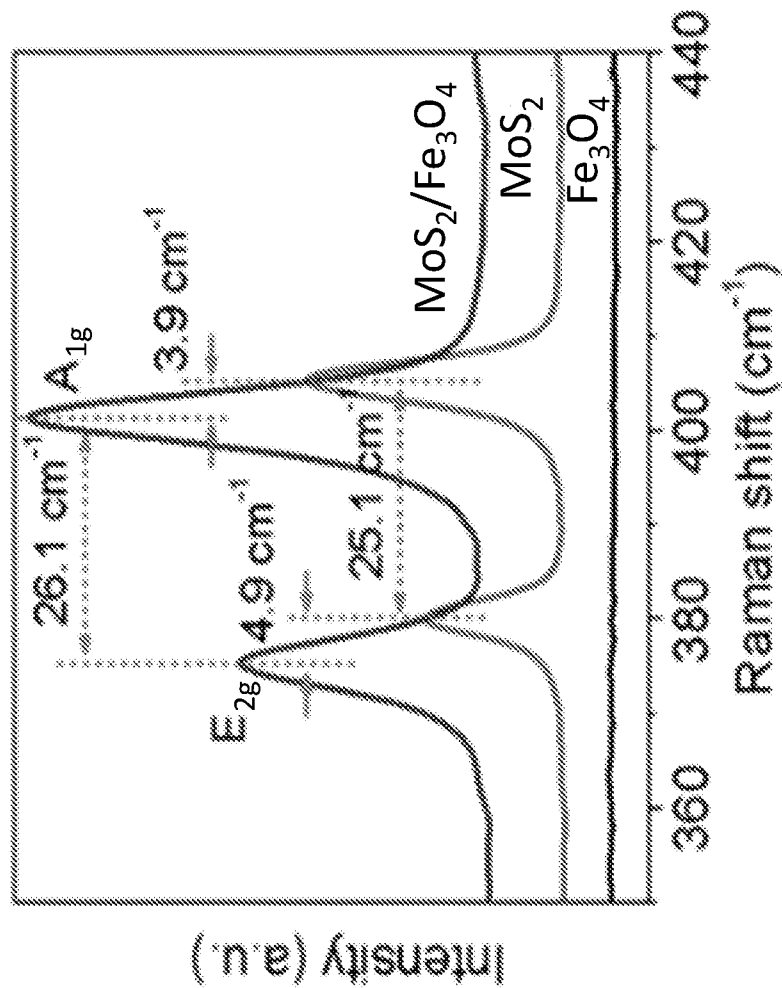


FIG. 3B

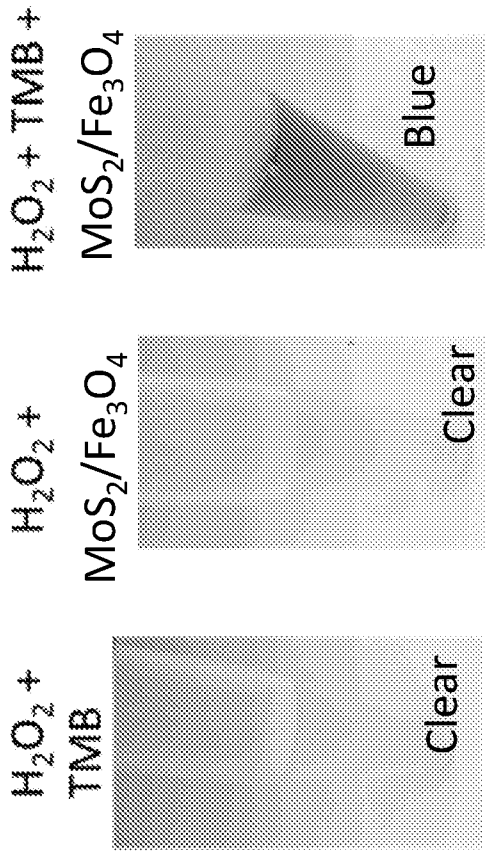


FIG. 4A

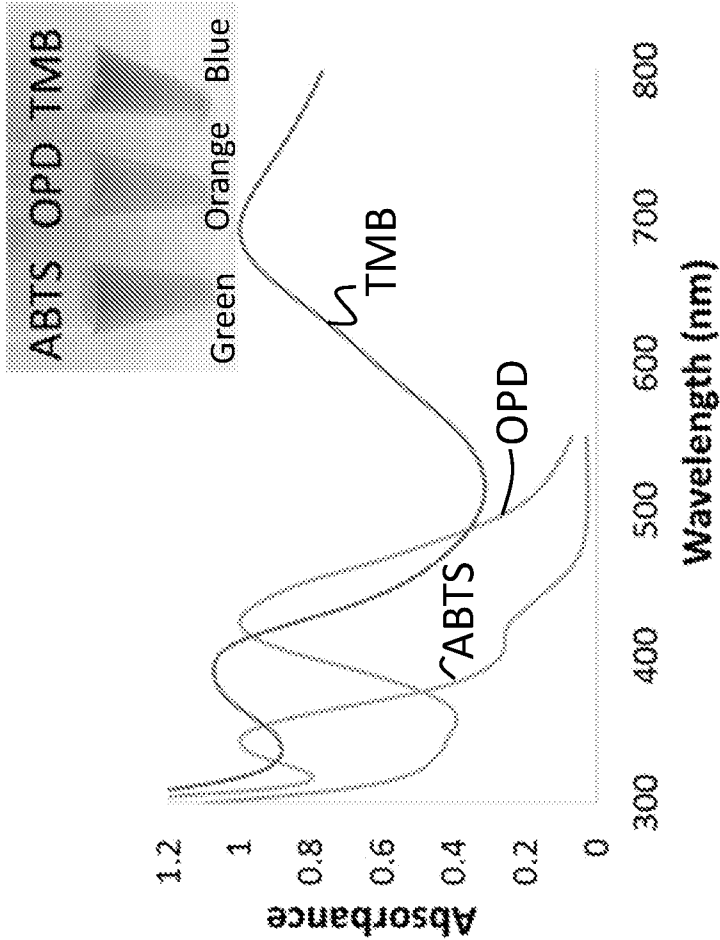


FIG. 4B

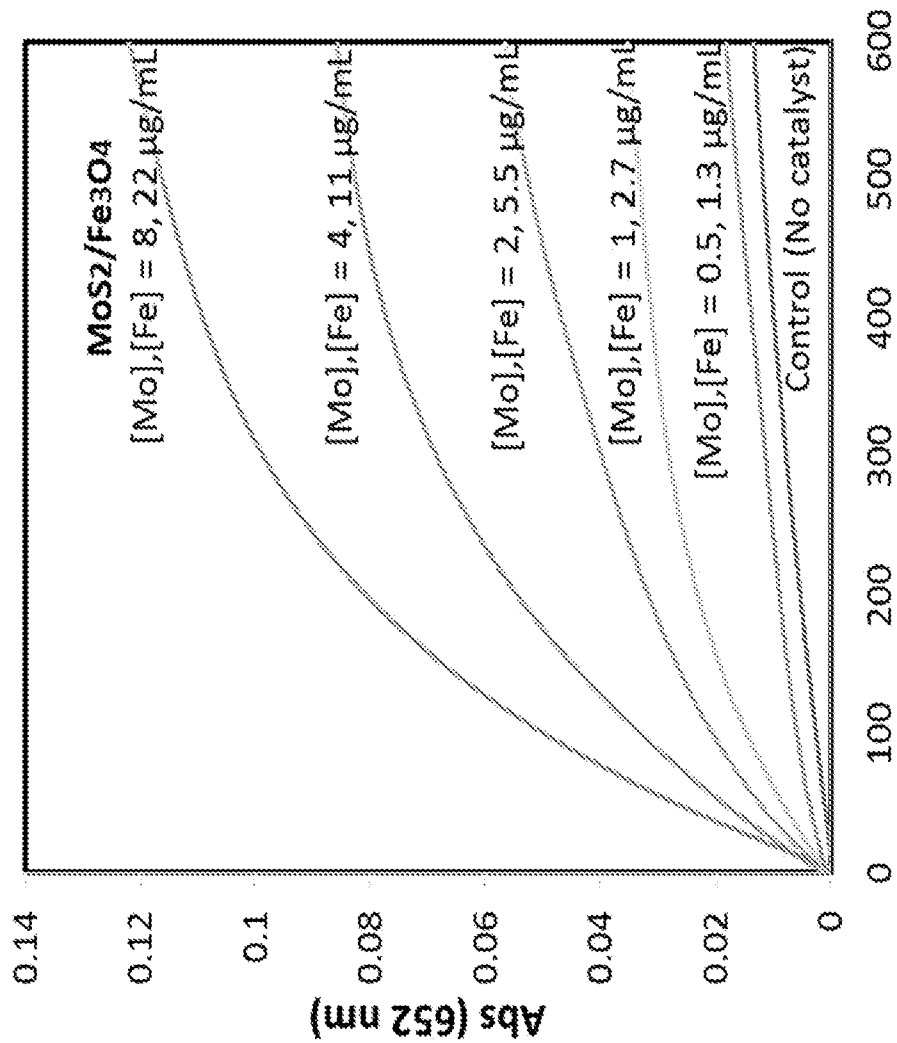


FIG. 5A

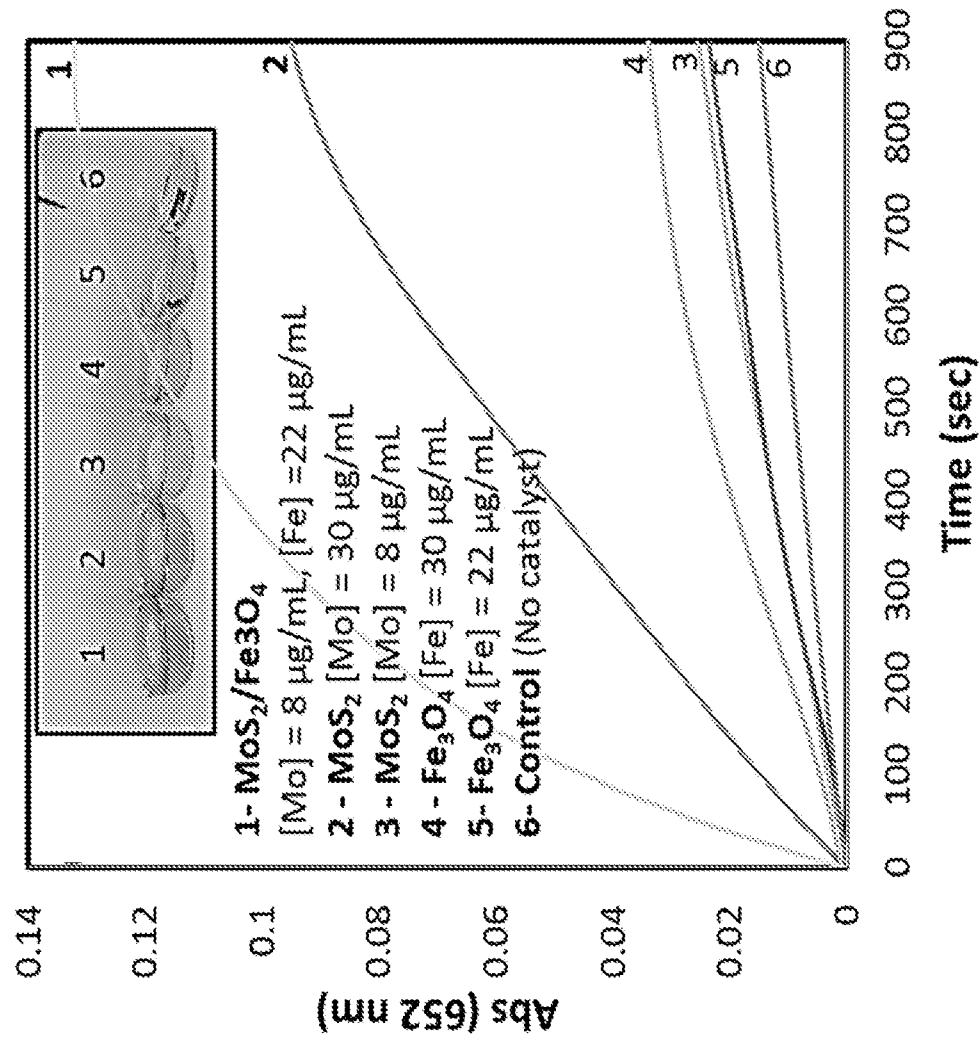


FIG. 5B

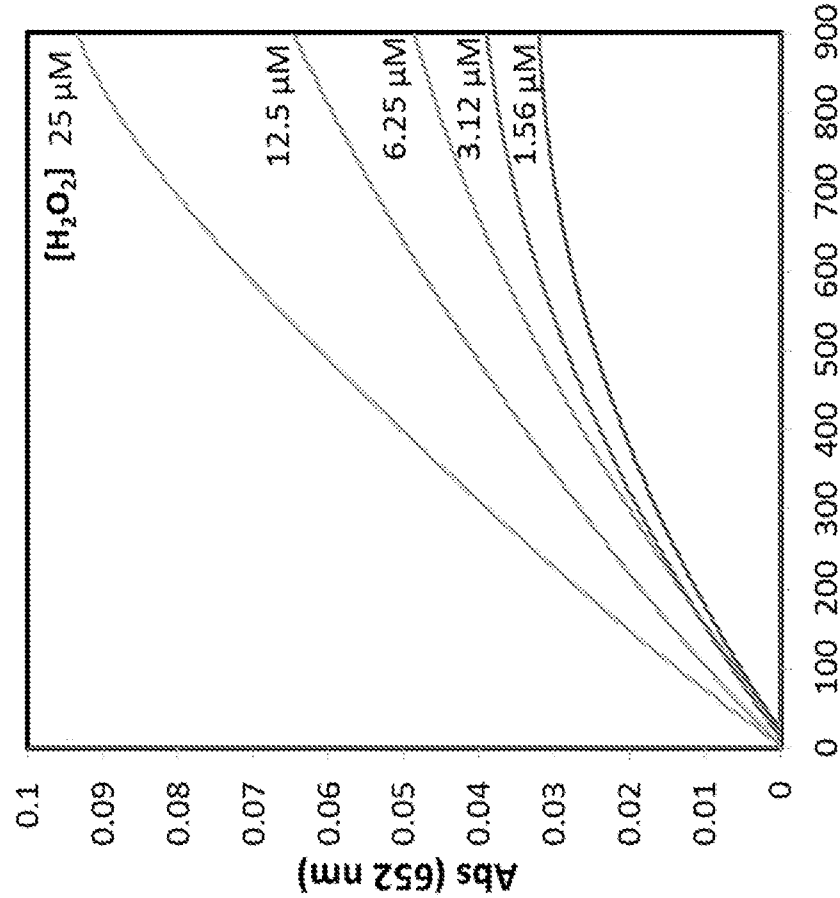


FIG. 6A

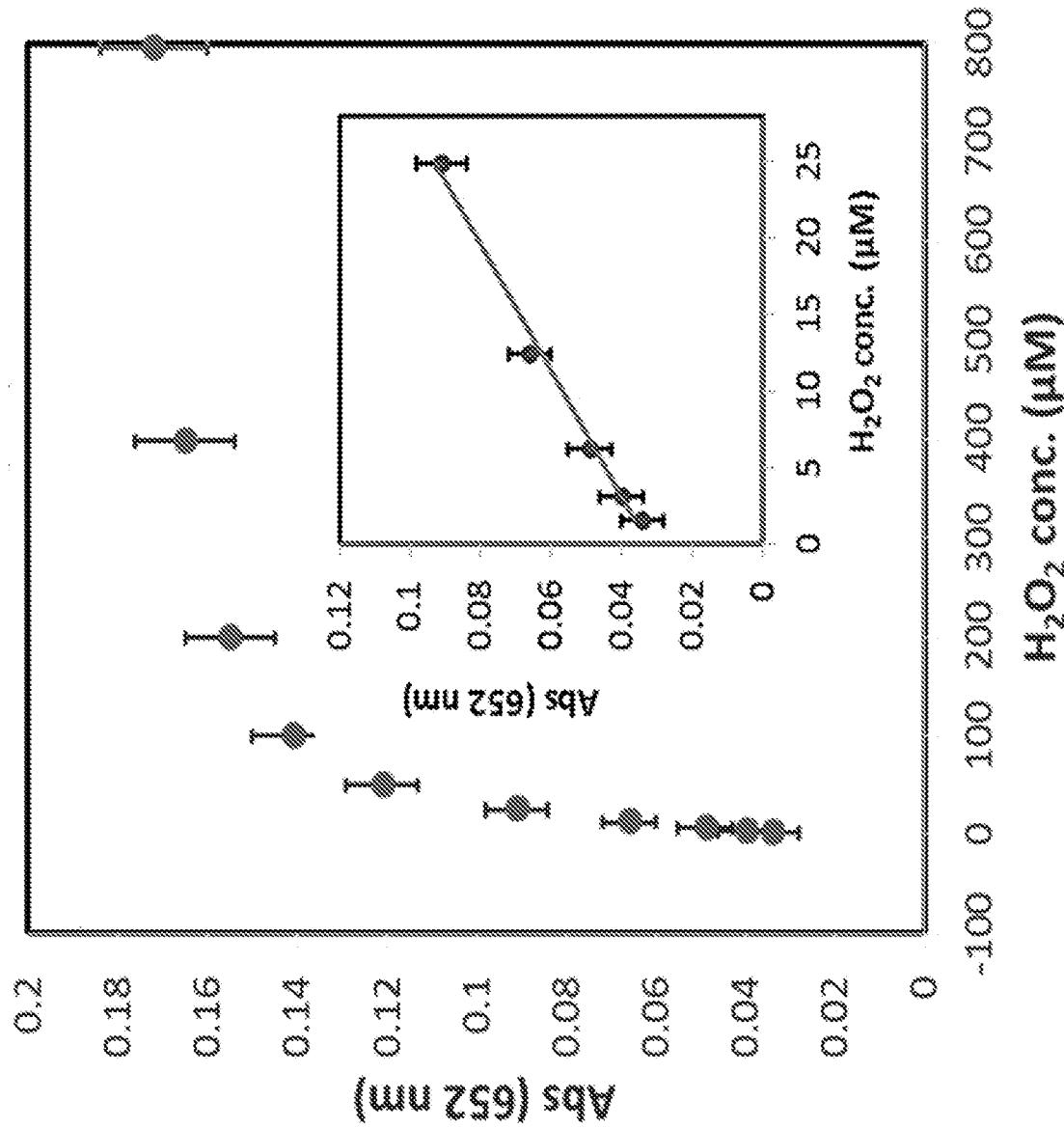


FIG. 6B

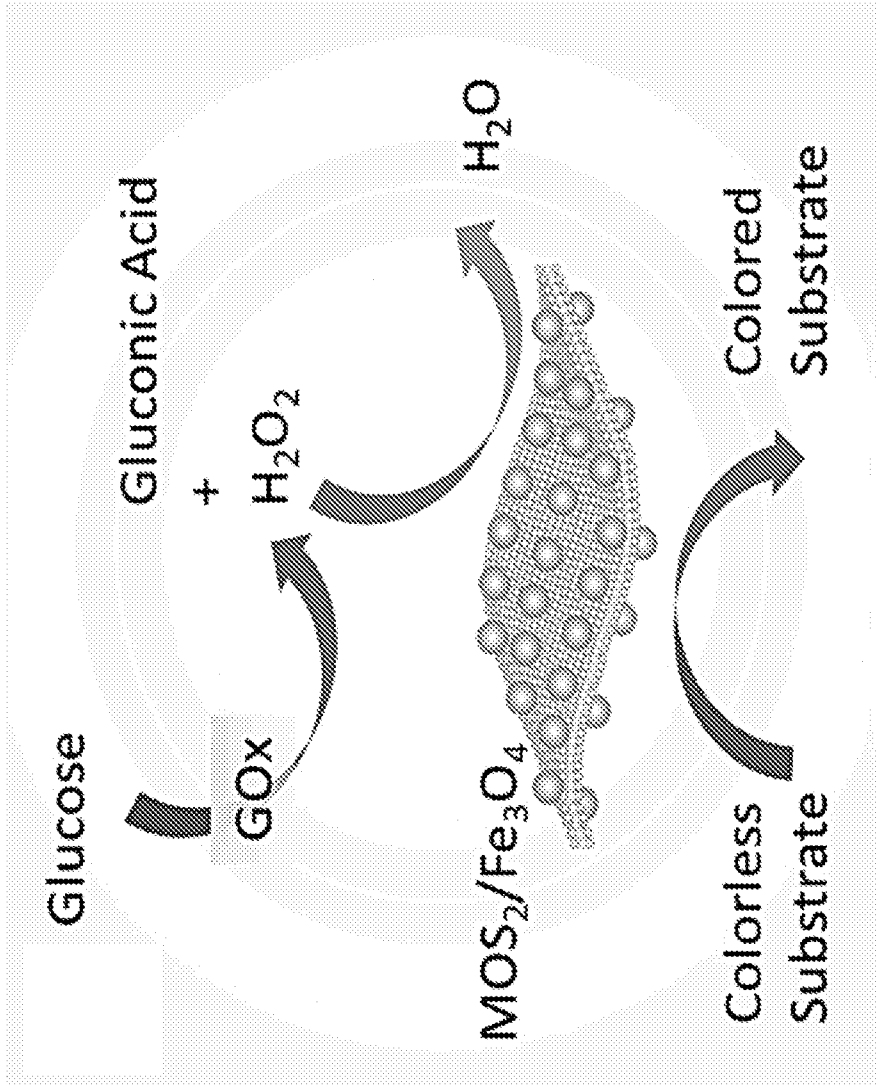


FIG. 7A

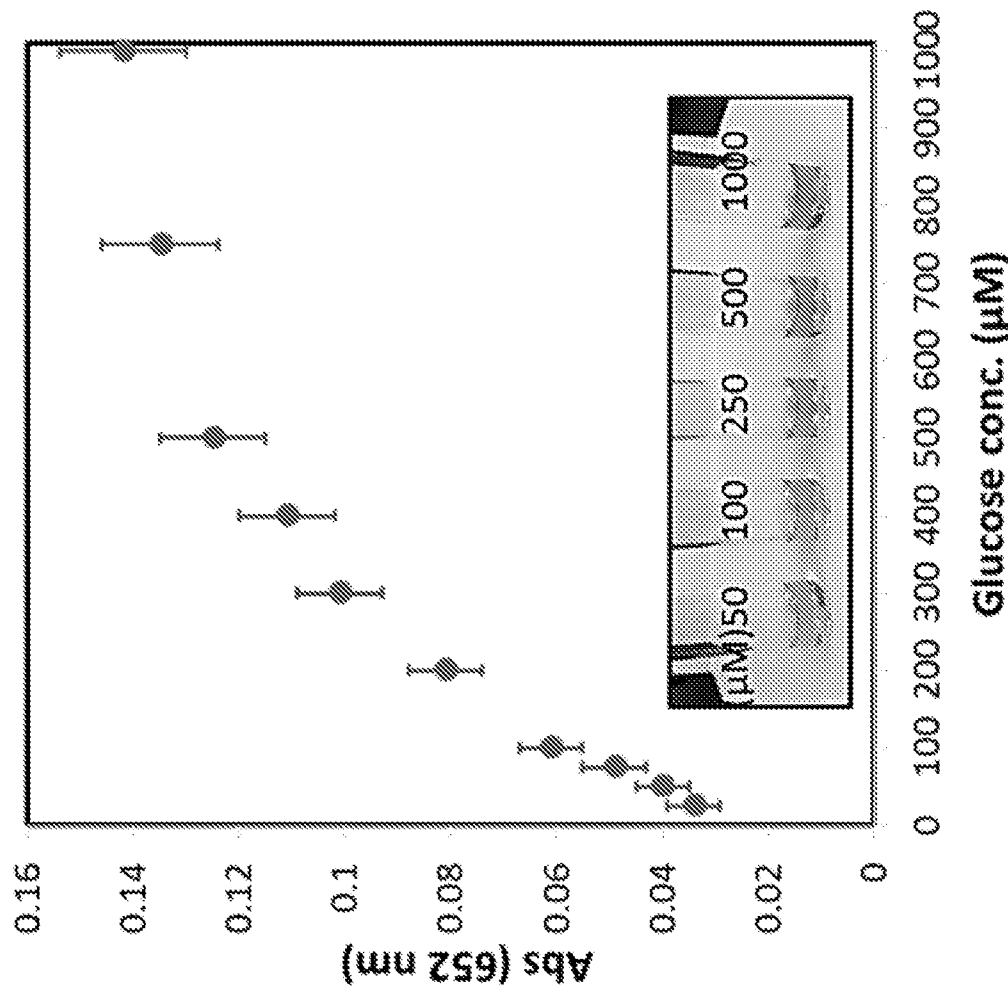


FIG. 7B

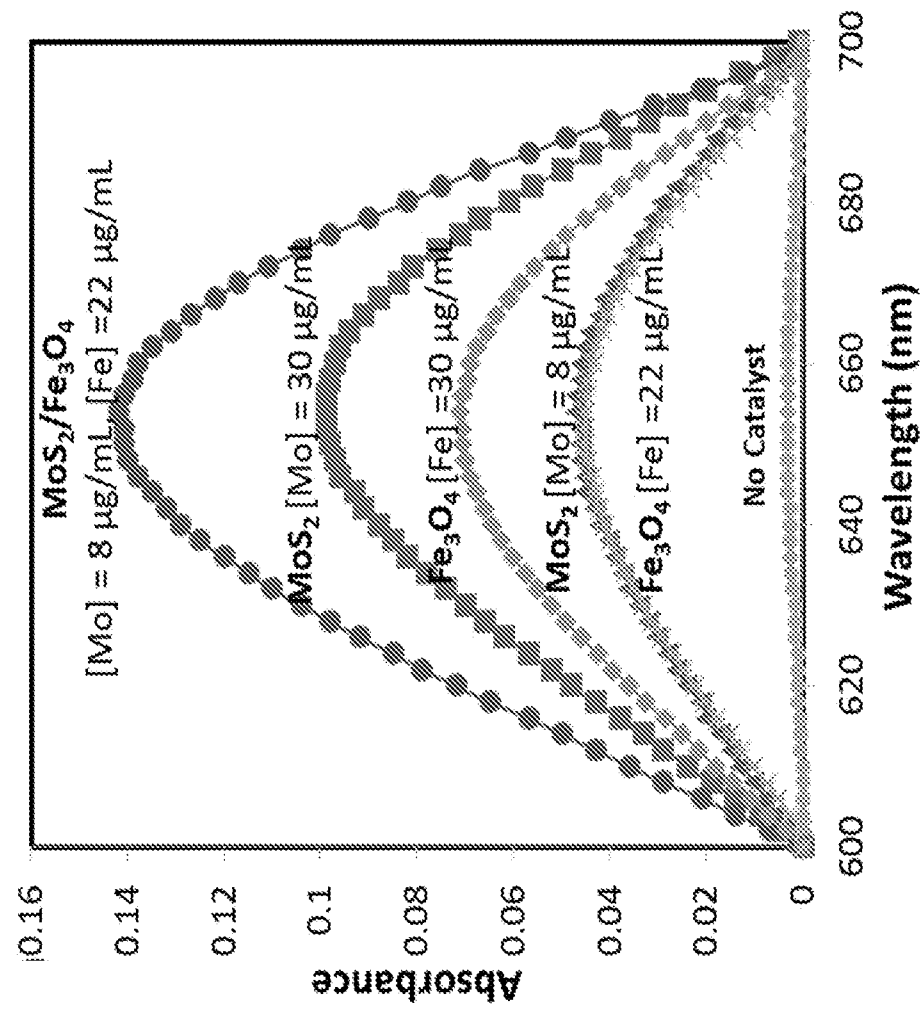


FIG. 8A

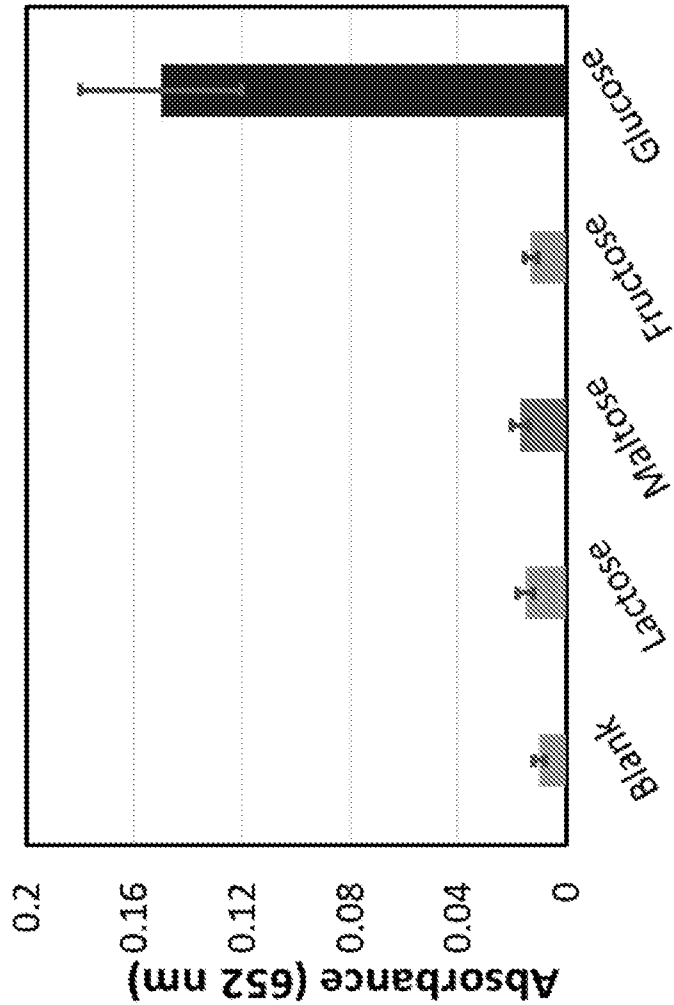


FIG. 8B

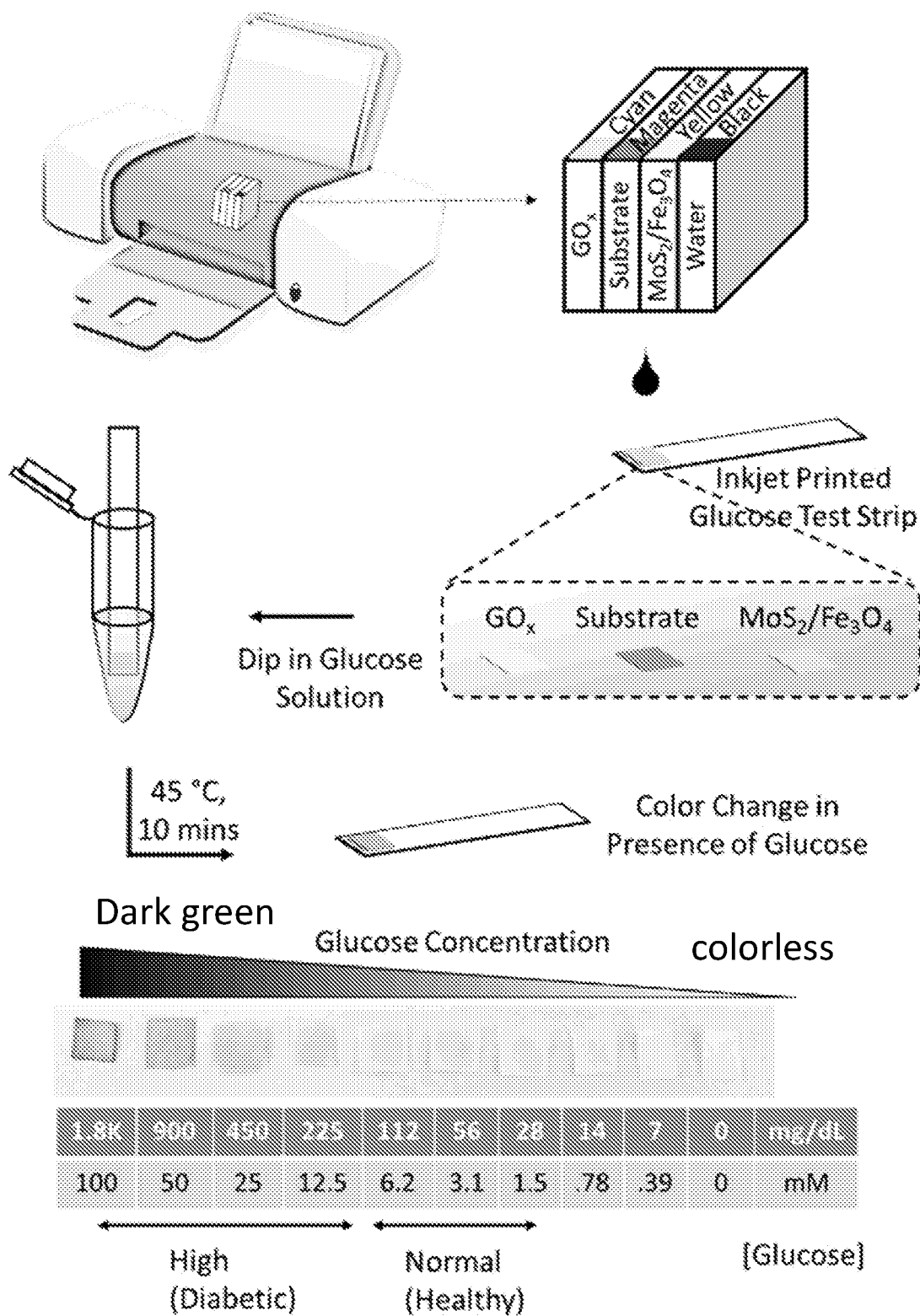


FIG. 9

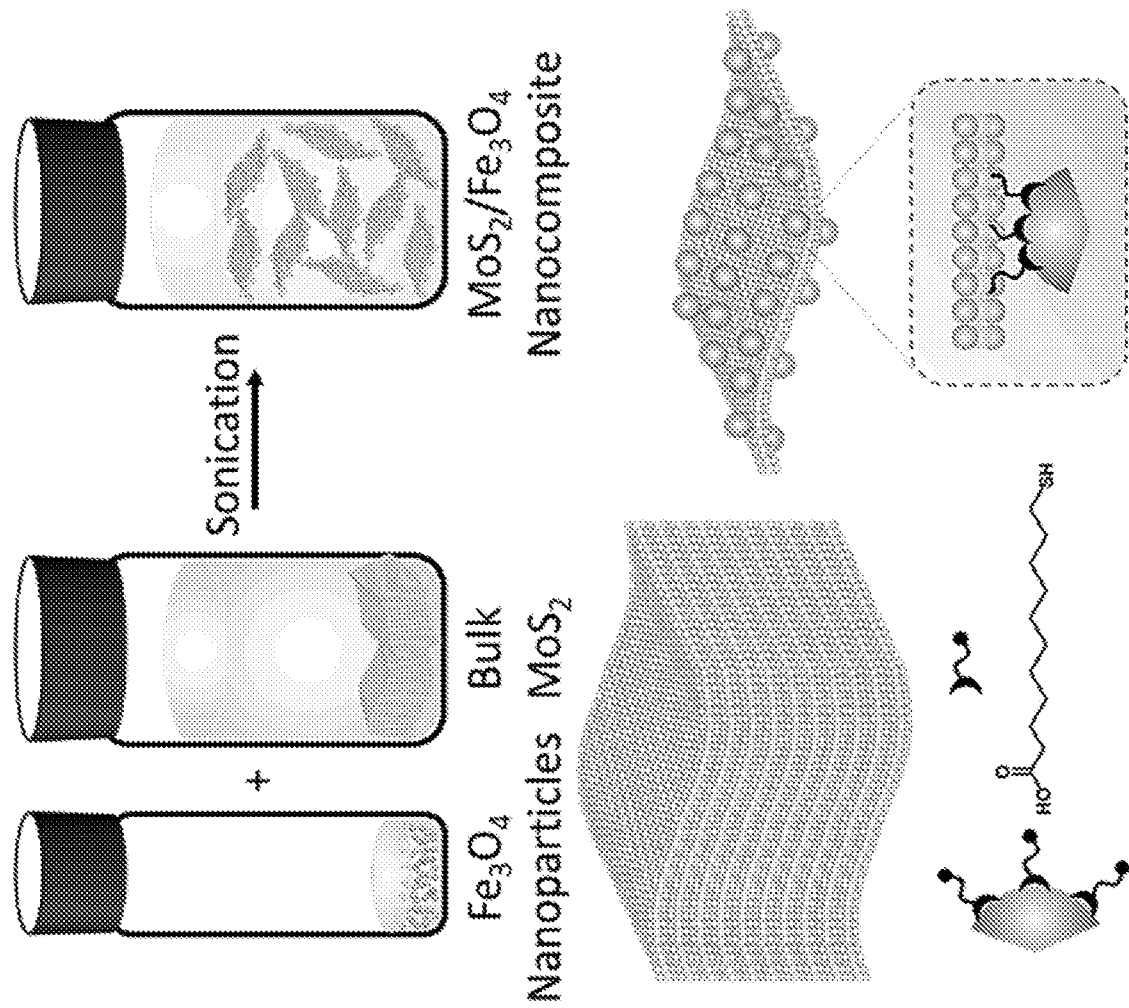


FIG. 10

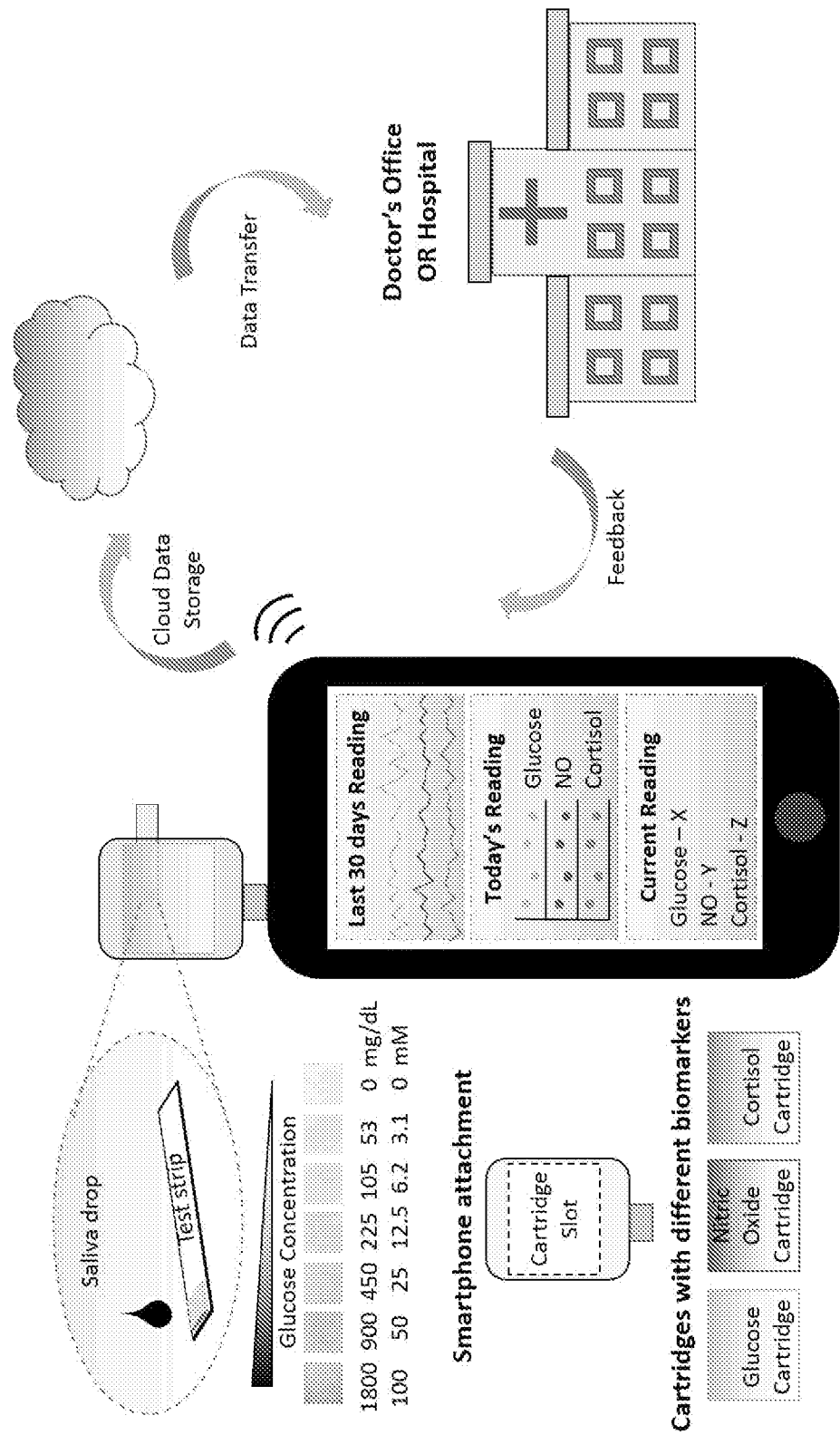


FIG. 11

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/46674

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - B82Y 15/00; B82Y 40/00; C01B 32/19; C01G 39/06 (2018.01)

CPC - B82Y 15/00; B82Y 40/00; C01B 32/19; C01G 39/06; C01P 2004/00; Y10S 977/70

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)

See Search History Document

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

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Liu et al. "Iron Oxide Decorated MoS ₂ Nanosheets with Double PEGylation for Chelator-Free Radiolabeling and Multimodal Imaging Guided Photothermal Therapy" ACS Nano, Vol 9 Issue 1 (06 January 2015): pages 950-960; abstract, page 951 col 2 para 1, page 951 col 2 para 2, page 957 col 2 para 1, page 957 col 2 para 2	1-16
A	Peng et al. "Ultrathin Two-Dimensional MnO ₂ /Graphene Hybrid Nanostructures for High-Performance, Flexible Planar Supercapacitors" Nanoletters, Vol 13 Issue 5 (16 April 2013): pages 2151-2157; abstract, page 2153 col 1 para 2, page 2153 col 2 para 1, page 2153 col 2 para 2	1-16
A	US 2003/0224168 A1 (Mack et al.) 04 December 2003 (04.12.2003); para [0014], para [0050], para [0051]	1-16
A	Peng et al. "Supporting Information for Ultrathin Two-Dimensional MnO ₂ /Graphene Hybrid Nanostructures for High-Performance, Flexible Planar Supercapacitors" Nanoletters, Vol 13 Issue 5 (16 April 2013): pages 2151-2157; page 2 para 3	1-16
X, P	Nandwana et al. "One-Pot Green Synthesis of Fe ₃ O ₄ /MoS ₂ 0D/2D Nanocomposites and Their Application in Noninvasive Point-of-Care Glucose Diagnostics" ACS Applied Nanomaterials, Vol 1 Issue 4 (26 March 2018): pages 1949-1958; entire document	1-16

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

04 October 2018

Date of mailing of the international search report

17 JAN 2019

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/46674

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Wang et al. "Biosensor Based on Ultrasmall MoS ₂ Nanoparticles for Electrochemical Detection of H ₂ O ₂ Released by Cells at the Nanomolar Level" Analytical Chemistry, Vol 85 Issue 21 (25 September 2013): pages 10289-10295; abstract, page 10290 col 2 para 2	1-16

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/46674

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
---See Supplemental Page---

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-16

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Lack of Unity Invention:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-16 directed to a method of making a nanocomposite.

Group II: Claims 17-20 directed to a sensor for a target analyte.

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

SPECIAL TECHNICAL FEATURES

The invention of Group I includes the special technical feature of a method comprising: combining a first type of nanostructure with a bulk material in water or an aqueous solution, the first type of nanostructure functionalized with a functional group capable of undergoing van der Waals interactions with the bulk material, whereby the first type of nanostructure induces exfoliation of the bulk material to provide a second, different type of nanostructure while inducing association between the first and second types of nanostructures to form the nanocomposite, not required by the claims of Group II.

The invention of Group II includes the special technical feature of a sensor comprising: a nanocomposite comprising a plurality of a first type of nanostructures and a plurality of a second type of nanostructures, wherein the nanocomposite exhibits intrinsic catalytic activity in a reaction involving a target analyte, and a chromogenic material capable of exhibiting a color change in the presence of the target analyte, not required by the claims of Group I.

COMMON TECHNICAL FEATURES

Groups I-II share the common technical feature of a nanocomposite comprising a first type of nanostructure and a second type of nanostructure, where the first and second types of nanostructures are associated with each other. However, this shared technical feature does not represent a contribution over prior art as being anticipated by the article entitled "Iron Oxide Decorated MoS₂ Nanosheets with Double PEGylation for Chelator-Free Radiolabeling and Multimodal Imaging Guided Photothermal Therapy" to Liu et al. which discloses of a nanocomposite comprising a first type of nanostructure and a second type of nanostructure, where the first and second types of nanostructures are associated with each other (abstract: "...functionalized iron oxide nanoparticles (IONPs) could be self-assembled on the surface on two-dimensional MoS₂ nanosheets via sulfur chemistry, forming MoS₂-IO nanocomposites..."; page 951 col 2 para 2: "During drastic intercalation and exfoliation, some of the sulfur atoms would be lost from the sandwich surface of MoS₂ nanosheets, forming defects available for binding by sulfur-terminated molecules. IONPs... functionalized with DMSA were then mixed with as-made MoS₂ nanosheets... the binding of thiol groups on DMSA-coated IONPs to the defect sites on MoS₂ nanosheets"; the iron oxide nanoparticles (IONPs), the first nanostructure, is bonded to the sulfur-defected sites in the MoS₂ nanosheets, the second nanostructure).

Therefore, Groups I-II lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.