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Declarations under Rule 4.17:

- as to the identity of the inventor (Rule 4.17(i))
- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
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(54) Title: GRAPHENE BASED COMPOSITE AS SOLITARY PLATFORM FOR SENSING DEVICES

(57) Abstract: The invention relates to the preparation of novel Zn- SnO₂ /NG hybrid nanocomposite material and its application in three component electrochemical detection simultaneously. A simple hydrothermal approach is used for preparation and the prepared products characterized by XRD, XPS, Raman, FE-SEM and HR-TEM for their structural, compositional and morphological properties. The multicomponent detection of Zn-SnO₂ /NG hybrid nanocomposite modified GC exhibited good electrochemical redox behavior towards dopamine, cholesterol and glucose on same electrode. Further, the real time capability and reproducibility of hybrid nanocomposite in sensing dopamine, cholesterol and glucose showed exceptional performance with < 2% error.

FIELD OF THE INVENTION

[0001] The present invention relates to graphene based composite, particularly Zn-SnO₂ /NG hybrid composite, and a method of preparation thereof.

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BACKGROUND OF THE INVENTION

[0002] Dopamine, Cholesterol and Glucose are considered as essential component for several body functions. Dopamine, a potential neurotransmitter belonging to catecholamine family is crucial for proper functioning of the central nervous system and facilitating brain-body interactions. Cholesterol is considered as an essential lipid component of human cell membranes. This plays vital role in human body functions like hormone production, vitamin D production, bile production and support to cell membrane. Glucose in human blood serves as principal energy source to drive various cell functions in our body. The human body itself maintains the blood glucose levels neither too low nor high by creating insulin and assist in proper internal functioning.

[0003] The abnormal levels of dopamine (concentrations below 40 nMol L⁻¹), Cholesterol (higher than 200 mg/dL) and Glucose (higher than 140 mg/dL) results in several complicated health diseases. Hence, the production of detection material with more selective and sensitive to human reference range detection of Dopamine, Cholesterol and Glucose becomes crucial.

[0004] Over the past few years, because of its quick response and, low cost, simple operation and high sensitivity use of electrochemical sensors have attracted widespread attention. Graphene is widely used in these applications owing to its exceptional properties and electronic structures. Doping graphene with heteroatom like Nitrogen (N) can significantly alter the band structure and modulate the properties. In addition, decoration of metal oxide nanostructures in the layers of graphene prevents the restacking and aggregation of graphene layers. Several hybrid or composite structures are developed to combine both the advantages of graphene and metal oxide. Xiaojiao et.al reported CeO₂ nanocrystals decorated on nitrogen-doped graphene for luminescence-based biosensing. The ~5nm sized CeO₂ nanostructures decorated on nitrogen doped graphene layers provide increased electrical transport behaviour and prevent aggregation problems ^[1]. Z. H. Sheng et. al reported Nitrogen with their similarity in atomic size and five valence electrons in the outmost shell easily forms of C-N bonds. This formation of bonds facilitates easy doping of nitrogen and thereby modulating the electronic properties and promotes electrochemical reduction of glucose by determining the H₂O₂ level generated from enzymatic reaction in the presence of oxygen ^[2-3].

[0005] Ramendra Sunder et.al developed a hybrid platform with Pt nanoparticles on graphene matrix

for detection of cholesterol enzymes (cholesterol oxidase and esterase). The developed cholesterol biosensor exhibited sensitive and selective detection with fast response time having 0.2 μ M detection limit ^[4]. Though several research reports on various developed new material for single component detection of dopamine, glucose and cholesterol, unavailability of reports for a material to have three-component detection on a single platform kindled our research interest to develop the same.

[0006] Thus, considering all the above significance of intentional incorporation of metal ions to metal oxide lattices, doping graphene, and preparation of hybrid composite it is disclosed here a graphene based composite material which can effectively electrochemically oxidize dopamine, glucose and cholesterol on a single platform.

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OBJECTS OF THE INVENTION

[0007] An object of the invention is to provide a method for preparing a graphene based composite material by hydrothermal approach.

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[0008] It is another object of the invention is to provide a Zn-SnO₂ /NG hybrid compound (graphene based composite) to sense dopamine, cholesterol and glucose on a single platform.

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[0009] It is yet another object of the invention is to provide a graphene based composite material to sense the dopamine, cholesterol and glucose in human blood samples with less than 2% error in reproducibility and can be used in real time.

SUMMARY OF THE INVENTION

[0010] To meet the objects of the invention and overcome the problems associated with the prior art, it is disclosed herein a method for preparing Graphene based composite comprising steps of preparing Zn-SnO₂ nanostructures by hydrothermal treatment using (Zn(CH₃COO)₂.2H₂O and SnCl₂.2H₂O salts as precursor in a solvent containing ethanol and HCl, adding the said Zn-SnO₂ nanostructures, ammonia and hydrazine hydrate to the GO (graphene oxide) dispersion, stirring the resulting dispersion at the room temperature, transferring it to a Teflon-lined autoclave and treating it hydrothermally.

[0011] It is further disclosed that the prepared graphene based composite can effectively detect Dopamine, Cholesterol and Glucose at human reference range. The prepared graphene based composite also hold superior sensing performance in the real time human blood samples for Dopamine, Cholesterol and Glucose The prepared graphene based composite can be used as a single platform to detect Dopamine, Cholesterol and Glucose with subsequent washing with ethanol. Reproducibility of the

materials on sensing three analyte in real time samples remains the same with error percentage less than 2%.

5 **BRIEF DESCRIPTION OF THE DRAWINGS**

[0012] **Fig.1.** shows the XRD spectrum of Zn-SnO₂, NG and Zn-SnO₂ /NG hybrid nanocomposite.

Fig 2. shows the XPS spectrum of Zn-SnO₂ /NG hybrid nanocomposite.

10 **Fig 3.** Raman spectrum of NG and Zn-SnO₂ /NG hybrid nanocomposite.

Fig.4 (a). is a FE-SEM image of Zn-SnO₂ /NG hybrid nanocomposite.

15 **Fig.4 (b).** is a HR-TEM image of Zn-SnO₂ /NG hybrid nanocomposite.

Fig.5. is the Plot of current Vs concentrations of Zn-SnO₂ /NG hybrid nanocomposite modified electrode (a) dopamine, (b) cholesterol and (c) glucose.

20 **Fig.6(a-f)** are the CV graphs obtained for dopamine (40nM), cholesterol (5.5mM) and glucose (5mM) oxidation on three different electrodes in real time human blood samples.

DETAILED DESCRIPTION OF THE INVENTION

25 [0013] Zn-SnO₂ nanostructures is prepared by one step hydrothermal treatment using (Zn(CH₃COO)₂·2H₂O and SnCl₂·2H₂O salts as precursor in solvent containing ethanol and HCl. This is followed by the addition prepared Zn-SnO₂ nanostructures; ammonia and hydrazine hydrate to the GO dispersion. The
30 resulted dispersion is stirred at room temperature

and then transferred to a Teflon-lined autoclave and treated hydrothermally at suitable temperature to obtain graphene-based composite. Prior to modification, the working electrode is polished with 1.0, 0.3, and 0.5 μ m alumina powders and sonicated in ethanol and deionized water for 20 min. Then, graphene based composite are dispersed by sonication in a solvent. The dispersion is then coated by drop casting on a glassy carbon electrode, dried at room temperature and used for further electrochemical sensing measurements.

[0014] The prepared graphene based composite can effectively detect Dopamine, Cholesterol and Glucose at human reference range. The prepared graphene based composite also hold superior sensing performance in the real time human blood samples for Dopamine, Cholesterol and Glucose The prepared graphene based composite can be used as a single platform to detect Dopamine, Cholesterol and Glucose with subsequent washing with ethanol. Reproducibility of the materials on sensing three analyte in real time samples remains the same with error percentage less than 2%.

[0015] In an exemplary case, the XRD patterns of the samples are measured to understand the microstructure properties of prepared nanocomposites. Fig.1 shows the diffraction patterns of Zn doped SnO₂, NG and NG Zn-SnO₂ composites. The diffraction peaks of Zn doped SnO₂

(Fig.1a) can be indexed to tetragonal system of SnO_2 (JCPDS No: 077-0452). As the ionic radius of Zn^{2+} (0.073nm) is close to Sn^{4+} (0.071nm), the increased concentration of dopant ions provoke certain changes in lattice parameters, diffraction peak position and strain without disturbing the crystal system^[5]. The fading of GO peak and the appearance of broader peak at 25.21° (d_{002} , interlay distance of 0.37nm) in prepared NG sheets, indicating that GO was restacked during the hydrothermal reduction process due to van der Waals forces, π - π stacking and electrostatic forces^[6]. The amount of nitrogen doped and the nature of nitrogen can effectively be understood from X- ray photoelectron spectroscopy (XPS) measurements and the respective spectrum is shown in Fig. 2.

[0016] The survey spectrum of graphene based composite show peaks corresponding to Cls at 285.1 eV, N1s at 401 eV, O1s at 531 eV, Sn $3d_{3/2}$ at 496 eV, Sn $3d_{5/2}$ at 487.4 eV, Zn $2p_{1/2}$ at 1046 eV and Zn $2p_{3/2}$ at 1023 eV respectively. The core level Cls spectrum can be deconvoluted to obtain peaks at 284.9 eV, 286 eV and 289.2 eV corresponding to C=C, C-O and O-C=O bonds respectively. Similarly, the N1s spectrum is also resolved into three peaks corresponding to pyridinic N, pyrrolic N and N oxides of pyridinic N positioned at 395.2 eV, 401.2 eV and 402.8 eV respectively^[7-8] and the calculated nitrogen doping percentage is 3.052 at%.

[0017] The Raman spectra showed in Fig 3 for N-graphene have G band and D band located at 1578 cm^{-1} and 1333 cm^{-1} whereas the G band and D band positioned at 1570 cm^{-1} and 1337 cm^{-1} or graphene composite. Further, I_D/I_G of NG was found to be 0.98 whereas for graphene composite it is 1.04. The observed alteration in the of G and D band position, the increased I_D/I_G ratio in the hybrid nanocomposite authenticate the incorporation of nitrogen into the graphene matrix, exclusion of oxygen functional group and more of defect environment in the prepared composite ^[9]. The presence of second order or overtone of D band resulted in appearance of 2D band at 2669 cm^{-1} . This is due to two-phonon vibrational process and the appearance of 2D in the prepared materials indicates the presence of few layers ^[10].

[0018] The peaks observed in the Raman spectrum of the Zn-SnO₂ nanostructures at 242 cm^{-1} and 312 cm^{-1} correspond to the inactive Raman E_u transverse optical mode, while the peaks at 624 cm^{-1} and 777 cm^{-1} are attributed to the A_{1g} and B_{2g} non-degenerative modes ^[11]. The morphological analysis of prepared graphene based composite was characterized using SEM and HR-TEM and their images are shown in Fig 4(a-b). From the SEM images, one can clearly observe folded and wrinkled nature of graphene layers. The graphene layers observed from HR-TEM images exhibit the light and transparent characteristics,

which imply the few layer presence in the prepared hybrid structures. Further, on closer examination of HR-TEM images one can observe the formation of ripples in the graphene layers and this may be due to the defects in the graphene plane or due to the nitrogen atoms incorporation ^[12]. The non- enzymatic electrochemical sensing of graphene based composite for detection of dopamine, cholesterol and glucose was carried out using cyclic voltammetry. The electrochemical sensing of dopamine and cholesterol on the GC electrode modified with hybrid nanocomposites were carried out using PBS buffer (pH 7) and 1M H₂SO₄ for glucose at room temperature with applied 50mV/sec. The electrochemical redox reactions between modified electrode and analyte resulted in well-resolved redox peaks with an oxidation potential of 0.2V for dopamine, 0.6 V for cholesterol and 0.43V for glucose. Further the modified electrode demonstrates a liner relationship with different concentrations of analyte as shown in Fig 5(a-c) and the current obtained are presented in Table 1-3.

Table 1

Concentration (nm)	Current obtained (A)
20	2.984E-4
25	3.124E-4
30	3.246E-4
35	3.326E-4
40	3.466E-4
45	3.631E-4
50	3.753E-4
55	3.899E-4
60	4.114E-4
65	4.302E-4
70	4.529E-4

Table:2

5

Concentration (mM)	Concentration (mg/dl)	Current obtained (A)
1	18.01	9.728E-5
2	36.03	9.905E-5
3	54.05	1.015E-4
4	72.07	1.041E-4
5	90.09	1.061E-4
6	108.10	1.084E-4
7	126.12	1.103E-4
8	144.14	1.113E-4
9	162.16	1.131E-4
10	180.18	1.150E-4

Table:3

Concentration (mM)	Concentration (mg/dl)	Current obtained (A)
1	38.61	7.166E-5
2	77.22	7.337E-5
3	115.83	7.591E-5
4	154.44	7.911E-5
5	193.05	8.284E-5
6	231.66	8.592E-5
7	270.27	8.896E-5
8	308.88	9.126E-5

10

[0019] The electrode has linearity in 20-40nm concentration for dopamine with linear regression equation as, $Y = 3E^{-06} X + 0.0002$ ($R^2 = 0.9855$), 1-8mM concentration for cholesterol with linear regression equation as, $Y = 3E^{-06} X + 7E^{-05}$ ($R^2 = 0.994$) and 1-10mM concentration for glucose with linear regression equation as, $Y = 2E^{-06} X + 1E^{-04}$ ($R^2 = 0.9912$).

EXAMPLE

[0020] To examine the real time usability of the prepared sensor material, electrochemical sensing in human real blood samples were carried out and the respective graphs are shown in Figure 6(a-f). The collected human blood from healthy person is diluted with 99ml of PBS buffer and used for real time experimental purpose. Three different electrodes coated with graphene-based composite is used for real time detection of dopamine, cholesterol and glucose. A known concentration of dopamine (40nm), cholesterol (5.5mM) and glucose (5mM) is added and the potential for each analyte oxidation is noted. The obtained current is kept as reference for 40nM, 5.5mM and 5mM concentration of dopamine, cholesterol and glucose. The known concentrations added are set from human reference level of dopamine, cholesterol and glucose. The obtained currents match well with the current obtained from concentration variation plots.

[0021] In next step, freshly prepared electrode is placed in diluted human sample followed by addition of 40nM dopamine and the current value is recorded. The electrode is then removed, rinsed with ethanol, dried for few min and the same electrode is used for measuring cholesterol in fresh diluted blood with 5.5mM cholesterol addition. After recording the oxidation potential of cholesterol, the washing and drying process is repeated before glucose detection. With the use of same electrode in freshly added diluted human blood and 5mM of glucose the experiment is repeated with corresponding potential recording. The obtained oxidation potentials of dopamine, cholesterol and glucose by using same electrode are then compared with the potentials obtained from the individual electrodes. On comparison, it is found the error percentages are 1.86% for dopamine, 1.53% for cholesterol and 1.90% for glucose. Prepared graphene based composite material exhibit linear detection with different concentration range for dopamine, cholesterol and glucose with > 2% error in real time samples with the use of same electrode. Further the obtained results are correlated with the clinical investigations and the corresponding results are presented in Table 2. Thus, this implies the practical usability of prepared graphene based composite as a non-enzymatic sensor material for effective human risk range detection of dopamine, total cholesterol and glucose.

We Claim:

1. A method for preparing a graphene based composite comprising the steps of: preparing Zn-SnO₂ nanostructures by hydrothermal treatment using (Zn(CH₃COO)₂·2H₂O and SnCl₂·2H₂O salts as precursor in a solvent containing ethanol and HCl, adding the said Zn-SnO₂ nanostructures, ammonia and hydrazine hydrate to the GO (graphene oxide) dispersion, stirring the resulting dispersion at the room temperature, transferring it to a Teflon-lined autoclave and treating it hydrothermally.
2. A graphene based composite for use in sensing of dopamine, cholesterol, and glucose, the said composite prepared by the method as claimed in claim 1.
3. A graphene based composite for use in sensing of dopamine, cholesterol, and glucose in real time with error percentage of less than 2%, the said composite prepared by the method as claimed in claim 1.

1/6

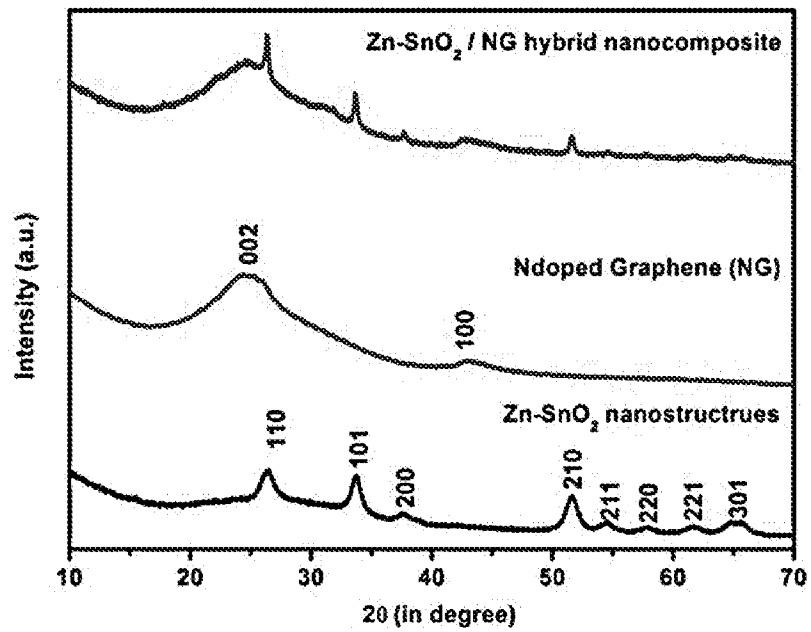


FIG.1

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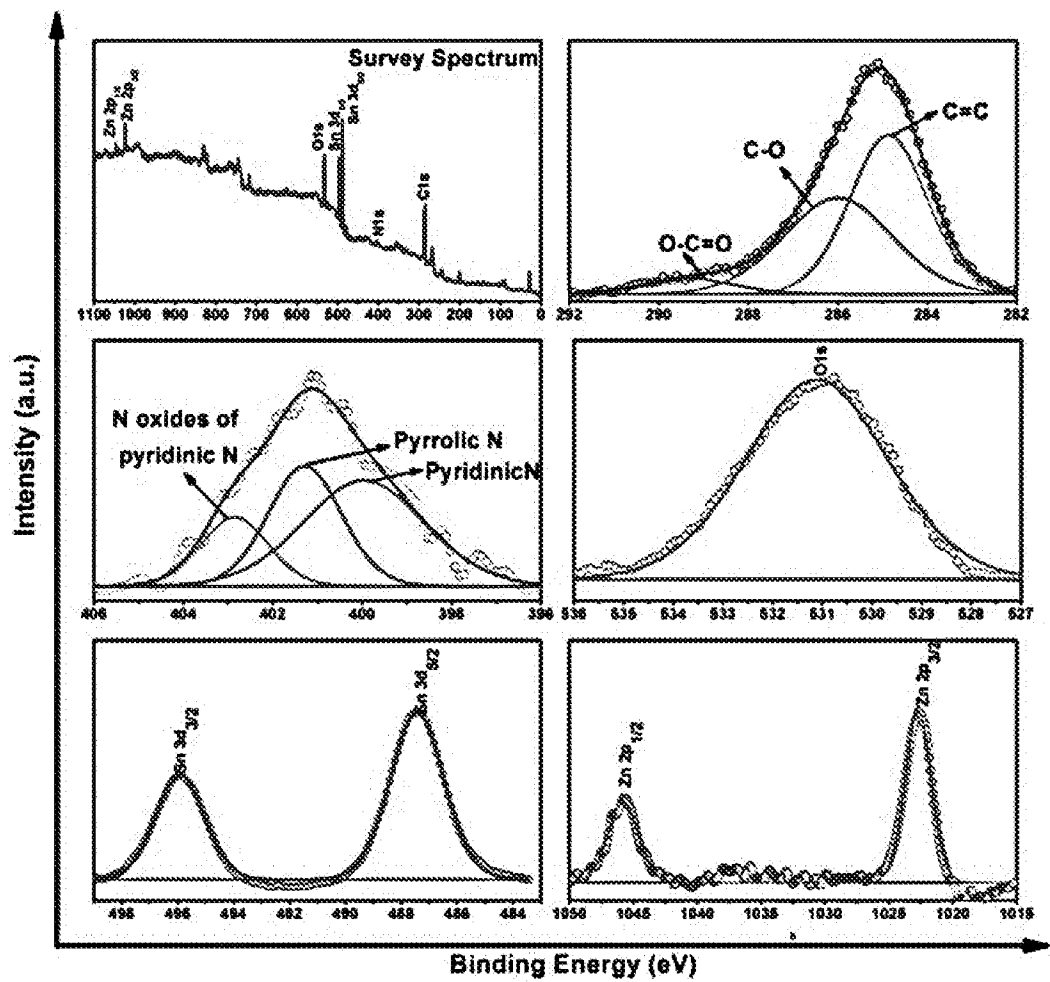


FIG.2

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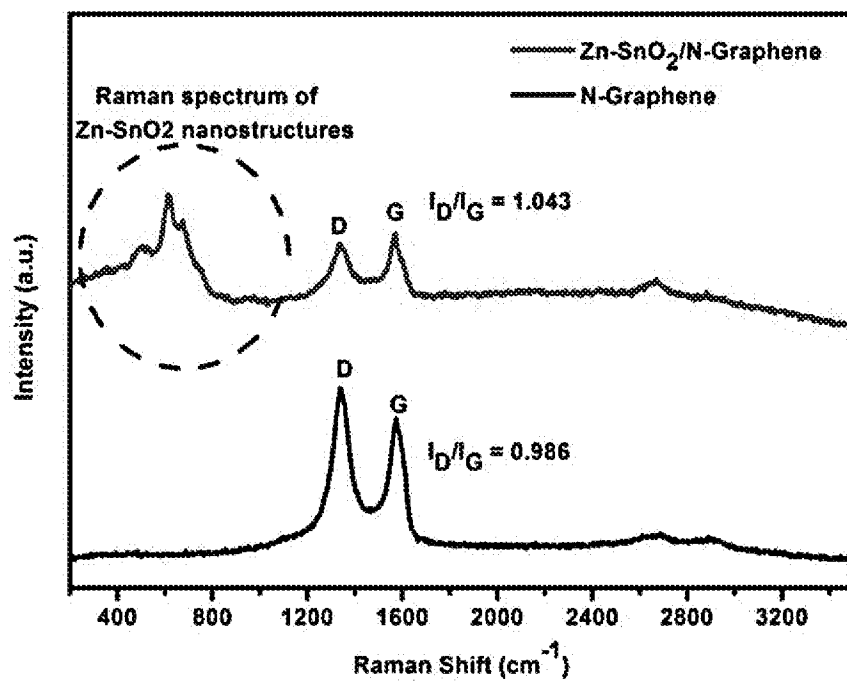


FIG.3

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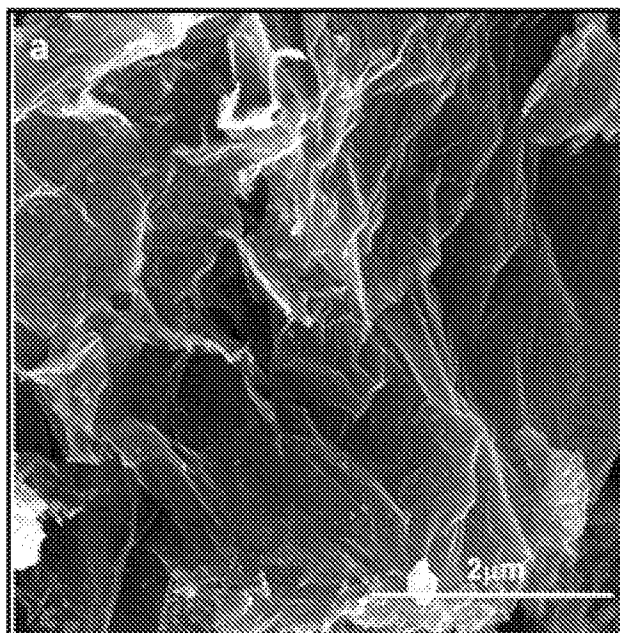


FIG.4(a)

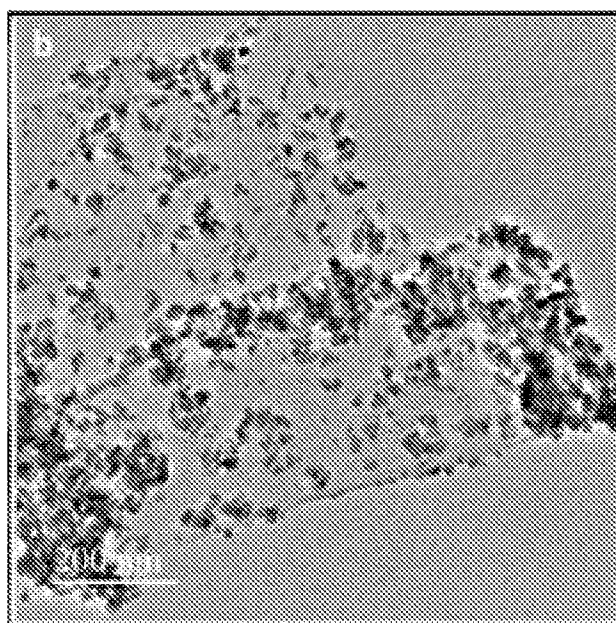


FIG.4(b)

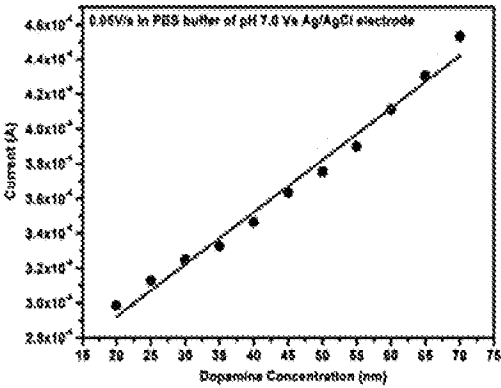


FIG.5(a)

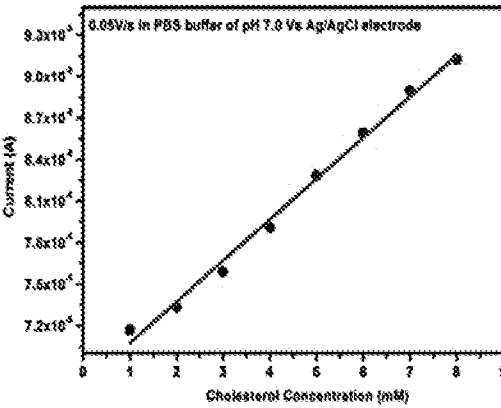


FIG.5(b)

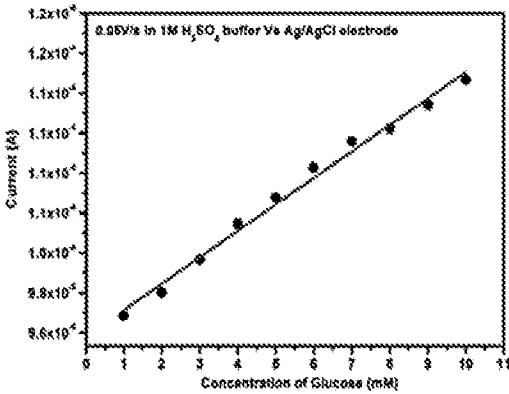


FIG.5(c)

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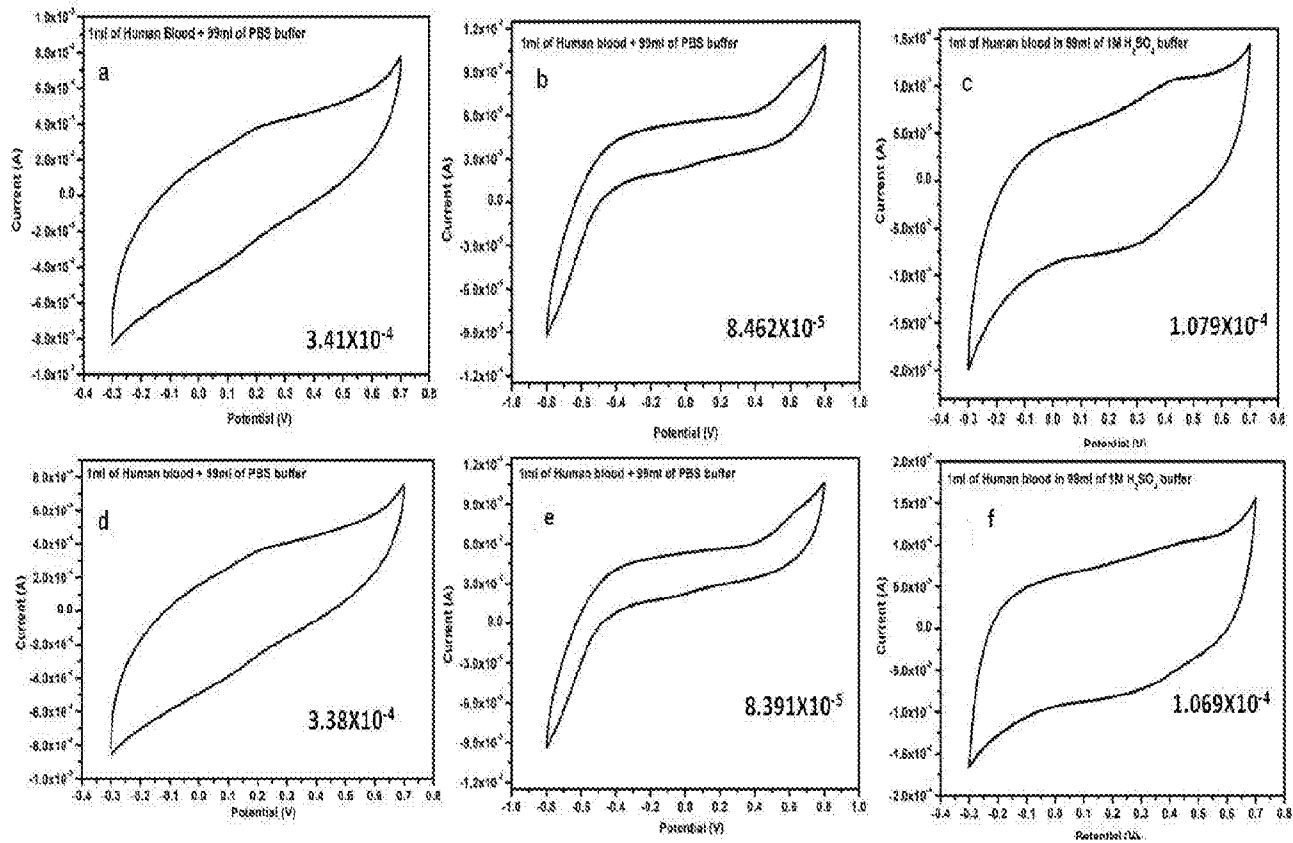


FIG.6(a-f)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2018/050435

A. CLASSIFICATION OF SUBJECT MATTER
C01B32/182 Version=2018.01

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)

C01B

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)

TotalPatent One, IPO Internal Database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MINGJUN JING ET AL., "Zn ₂ SnO ₄ coated reduced graphene oxide nanoribbons with enhanced electrochemical performance for lithium-ion batteries", Journal of Material Research, Volume 31, Issue 23, 14 December 2016, pp. 3666-3674. See pg 3667: II. Experimental Section.	2, 3
Y	..	1
X	PENG DOU ET AL., "Multilayer Zn-doped SnO ₂ hollow nanospheres encapsulated in covalently interconnected three-dimensional graphene foams for high performance lithium-ion batteries", Chemical Engineering Journal, Volume 320, 15 July 2017, Pages 405-415. https://doi.org/10.1016/j.cej.2017.03.076 See abstract.	2, 3
Y	..	1
Y	SUNANDAN BARUAH & JOYDEEP DUTTA, "Zinc Stannate Nanostructures: Hydrothermal Synthesis", Sci	



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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Date of the actual completion of the international search

10-10-2018

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/IN2018/050435

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Technol Adv Mater, 2011 Feb; 12(1): 013004. doi: 10.1088/1468-6996/12/1/11660946. see page 3, 13 -----	1
	WEIGEN CHEN ET AL., "Hydrothermal Synthesis of Pt-, Fe-, and Zn-doped SnO ₂ Nanospheres and Carbon Monoxide Sensing Properties", Hindawi Publishing Corporation Advances in Materials Science and Engineering Volume 2013, Article ID 578460, 8 pages. http://dx.doi.org/10.1155/2013/578460 See page 2: Experimental. -----	1
Y	US2011084252 A1 (Xerox Corp) 14 April 2011 (2011-04-14) See Example 2.	1

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IN2018/050435

Citation	Pub.Date	Family	Pub.Date
US 20110084252 A1	14-04-2011	US 8164089 B2	24-04-2012
		JP 2011082517 A	21-04-2011
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