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Published version

AL-SAGUR, H., KOMATHI, S., KARAKAŞ, H., ATILLA, D., GÜREK, A.G., BASOVA, T., FARMILO, Nick and HASSAN, Aseel (2018). A glucose biosensor based on novel Lutetium bis-phthalocyanine incorporated silica-polyaniline conducting nanobeads. *Biosensors & bioelectronics*, 102, 637-645.

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A glucose biosensor based on novel Lutetium bis-phthalocyanine incorporated silica-polyaniline conducting nanobeads

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Abstract

The facile preparation of highly sensitive electrochemical bioprobe based on lutetium phthalocyanine incorporated silica nanoparticles ($\text{SiO}_2(\text{LuPc}_2)$) grafted with Poly(vinyl alcohol-vinyl acetate) itaconic acid (PANI(PVIA)) doped polyaniline conducting nanobeads ($\text{SiO}_2(\text{LuPc}_2)\text{PANI(PVIA)-CNB}$) is reported. The preparation of CNB involves two stages (i) pristine synthesis of LuPc_2 incorporated SiO_2 and PANI(PVIA); (ii) covalent grafting of PANI(PVIA) onto the surface of $\text{SiO}_2(\text{LuPc}_2)$. The morphology and other physico-chemical characteristics of CNB were investigated. The scanning electron microscopy images show that the average particle size of $\text{SiO}_2(\text{LuPc}_2)\text{PANI(PVIA)-CNB}$ was between 180-220 nm. The amperometric measurements showed that the fabricated $\text{SiO}_2(\text{LuPc}_2)\text{PANI(PVIA)-CNB/GOx}$ biosensor exhibited wide linear range (1-16 mM) detection of glucose with a low detection limit of 0.1 mM. $\text{SiO}_2(\text{LuPc}_2)\text{PANI(PVIA)-CNB/GOx}$ biosensor exhibited high sensitivity ($38.53 \mu\text{A mM}^{-1} \text{cm}^{-2}$) towards the detection of glucose under optimized conditions. Besides, the real (juice and serum) sample analysis based on a standard addition method and direct detection method showed high precision for measuring glucose at $\text{SiO}_2(\text{LuPc}_2)\text{PANI(PVIA)-CNB/GOx}$ biosensor. The $\text{SiO}_2(\text{LuPc}_2)\text{PANI(PVIA)-CNB/GOx}$ biosensor stored under refrigerated condition over a period of 45 days retains ~ 96.4 % glucose response current.

Key words: Silica nanoparticles, conducting nanobeads, lutetium phthalocyanine, glucose biosensor, PANI(PVIA)

1 **1. Introduction**

2 Diabetes mellitus is a major public health problem, accounting 246 million people worldwide
3 (Tabish, 2007). The human body tightly regulates glucose levels, however, abnormalities in
4 blood sugar levels hyperglycemia (high) or hypoglycemia (low) result in serious, potentially
5 life-threatening complications (Peters et al., 2015). The predictions show that the rate of
6 diabetic people will increase by about 58% by 2025 (380 million) and it is the fourth
7 prevalent cause of death (International Diabetes Federation, 2006; Tirimacco et al., 2010).
8 Factors that limit hospitalisations of diabetic patients include regular/continuous monitoring
9 and control of the glucose level in the body (Shafiee et al., 2012). A variety of unambiguous
10 methods for detecting and quantifying glucose in assorted biological fluids and food matrices
11 exist which include spectrophotometric, calorimetric, chromatographic and electrochemical
12 approaches. Electrochemical biosensors have gained immense acceptance in the field of
13 medical diagnostics due to their attributes of simple, real-time, rapid and economical systems.
14 The device comprises of a synergistic combination of biological recognition element
15 (biotechnology) and a compatible transducer (microelectronics) (Singh et al., 2009). Glucose
16 oxidase (GOx from *Aspergillus niger*) is a homodimer enzyme, which contains one iron atom
17 and one flavin adenosine dinucleotide cofactor which catalyzes the conversion of β -d-glucose
18 to d-glucono-1,5-lactone (Galant et al., 2015). GOx has been widely used in the
19 determination of glucose for its excellent specificity to the analyte and catalyzing activity
20 (Piao et al., 2015; Zebda et al., 2011).

21 Nevertheless, the major challenges in the development of GOx based amperometric
22 biosensors are (i) higher loading of enzyme (sensitivity), (ii) stability of immobilized enzyme,
23 and (iii) reduction in high overpotentials (Singh et al., 2009). Hence the host matrix and the
24 immobilization strategy employed synergistically influence the performance of the biosensors
25 (Li et al., 2000). Several electrodes modifying materials such as carbon based nanomaterials,
26 polymers, metal nanoparticles and silica nanostructures or their hybrids have been widely
27 used for GOx immobilization (Zhu et al., 2014). Among them silica being inert, non-toxic,
28 with tunable porosity and inexpensive to synthesize will suit for this potential application (He
29 et al., 2010; Y. Zhao et al., 2009). Further, silica imparts biocompatibility and hydrophilicity
30 for the immobilized enzyme as well as prevents enzyme leakage (Jaganathan and Godin,
31 2012). However, mere higher loading of GOx alone is not enough; the immobilized enzyme
32 needs to show higher activity too. The relatively poor conductivity of pristine silica makes it
33 difficult to use in practical electrochemical biosensor application (Fang et al., 2015).

1 Phthalocyanines (Pcs) are planar 18 π -electron aromatic compounds with a considerably large
2 π -delocalized surface; they are promising functional materials for diverse applications
3 (Binnemans, 2005). Owing to their excellent electronic properties, rich redox chemistry and
4 high physico-electrochemical stability metal Pc (MPcs) derivatives are widely employed as
5 molecular wires in biosensor applications (Cui et al., 2015; Mani et al., 2014).
6 Nanocomposites of d-block (Co, Cu and Zn) Pcs incorporated graphene/carbon nanotubes has
7 been employed for amperometric glucose biosensor construction (Zhang et al., 2013; Wang et
8 al., 2015; Cui et al., 2013; Devasenathipathy et al., 2015). Olgac et al. reported ZnPc
9 mediated detection of glucose in real samples (Olgac et al., 2017). Double decker lutetium
10 phthalocyanine (LuPc₂) in particular is attractive due to its high intrinsic conductivity redox
11 properties, and chemical stability compared to several other MPcs (Basova et al., 2008a;
12 Basova et al., 2008b). Recently Al-Sagur and coworkers reported on glucose biosensor
13 construction using LuPc₂ as redox mediator decorated in conducting polymer hydrogel (Al-
14 Sagur et al., 2017). Thin films of LuPc₂ have been used for the detection of nicotinamide
15 adenine dinucleotide and volatile organic compounds (Açikbaş et al., 2009; Galanin and
16 Shaposhnikov, 2012; Pal et al., 2011). Physico-chemical properties of LuPc₂ complexes are
17 utilized for the photoconversion of 4-nitrophenol (Zugle and Nyokong, 2012). Literature
18 report reveals that incorporation of MPcs onto a silica support improves the efficacy of its
19 catalytic performance (Armengol et al., 1999). Also MPcs grafted silica gel displayed
20 antibactericidal activity (Kuznetsova et al., 2011). However MPcs incorporated onto silica
21 matrix for electrocatalytic glucose biosensor application has been less studied. To further
22 impart conductivity in bio-sensor construction, conducting polymers especially polyaniline
23 (PANI) as electron transducers due to its excellent conductivity in its doped state, has been
24 employed (Wang et al., 2014). Doping with poly(vinyl alcohol-vinyl acetate) itaconic acid
25 (PVIA) may largely improve the processability, stability and cytocompatibility for
26 biomedical application (Yin et al., 2017; Zeghioud et al., 2015). In this context, we intend to
27 integrate the beneficial properties of silica, MPcs and PANI(PVIA) in the construction of a
28 biosensor for effective GOx immobilization. Bearing in mind the challenges in the
29 preparation of multicomponent based biosensing platforms, a new strategy has been
30 employed for the integration of multicomponents (silica, LuPc₂ and PANI(PVIA)) into a
31 conducting nanobead (CNB) formation. The objective is achieved through the preparation of
32 water soluble LuPc₂; one-step incorporation of LuPc₂ into the porous SiO₂ nanocages
33 (SiO₂(LuPc₂)) during its synthesis; instigating grafting approach for tagging (SiO₂(LuPc₂))

1 with PANI(PVIA) to obtain SiO₂(LuPc₂)-PANI(PVIA)-CNB. We also evaluated the GOx
2 immobilized CNB as a high sensitive glucose biosensor.

3 Herein, we report on a facile preparation of SiO₂(LuPc₂)-PANI(PVIA)-CNB as
4 electrochemical probe for the application of glucose biosensor. Nanoparticles of SiO₂(LuPc₂)
5 were obtained by the Stöber method using TEOS and APTES as a precursor. PANI(PVIA)
6 was obtained by oxidative polymerization of aniline followed by doping it with PVIA in THF.
7 SiO₂(LuPc₂) nanoparticles were grafted with PANI(PVIA) through EDC/NHS chemistry to
8 obtain SiO₂(LuPc₂)-PANI(PVIA)-CNB. The surface morphologies and other physico-
9 chemical characteristics of SiO₂(LuPc₂)-PANI(PVIA)-CNB were investigated. An
10 amperometric glucose biosensor was constructed by immobilization of GOx onto
11 SiO₂(LuPc₂)-PANI(PVIA)-CNB coated screen printed carbon electrode.

12 **2. Experimental**

13 *2.1. Chemicals*

14 Tetraethyl orthosilicate (TEOS, 99.9%), 3-Aminopropyltriethoxysilane (APTES, 99%),
15 ammonium hydroxide solution (NH₄OH) (28.0–30.0 wt% ammonia), Ethanol (≥99.9%),
16 Poly(vinyl alcohol-vinyl acetate) itaconic acid (PVIA), aniline, *N*-(3-dimethylaminopropyl)-
17 *N'*-ethylcarbodiimide hydrochloride (EDC hydrochloride), NHS (N-hydroxysuccinimide),
18 ammonium persulfate (APS), D-(+)glucose, glucose oxidase from aspergillus niger, Type X-
19 S, lyophilized powder, 100,000-250,000 units/g solid (without added oxygen), glutaraldehyde
20 solution (Grade II, 25% in H₂O), Potassium ferrocyanide, Potassium ferricyanide, potassium
21 chloride (KCl), sodium chloride (NaCl), phosphate buffer saline (PBS, pH 7.0), ascorbic acid,
22 uric acid, horse serum and human serum were all purchased from Sigma Aldrich (UK) and
23 used as received. Polyethoxy substituted water soluble LuPc₂ was prepared following a
24 previous method (Ayhan et al., 2013) but with a few modifications. To brief the double
25 decker lutetium (III) compound was synthesised by the reaction of the dinitrile derivative
26 with lutetium acetate in n-pentanol in the presence of DBU as a strong base.

27 *2.2. Apparatus*

28 The morphologies of the as prepared SiO₂(LuPc₂), PANI(PVIA) and SiO₂(LuPc₂)-
29 PANI(PVIA)-CNB were examined by FEI-Nova scanning electron microscopy (SEM) with a
30 low magnification (200,000×) and high voltage (20 kV). A Philips CM20 transmission
31 electron microscopy (TEM) was used to obtain high resolution images operating at a voltage

1 of 200kV. UV–Visible spectrophotometer (Varian 50-scan UV–Visible) was used to measure
2 the absorption spectra of the platform. FT-IR spectra of pristine and integrated CNB were
3 recorded on a Perkin Elmer Spectrum 100 spectrophotometer. The Brunauer–Emmett–Teller
4 (BET) surface area of the platform was investigated through nitrogen adsorption–desorption
5 isotherm measurements and performed on a Micromeritics ASAP 2020 M volumetric
6 adsorption analyzer at 77.34 K. A precision measurement to the platform surface was carried
7 out by using a computer programmed Philips X-Pert X-ray diffractometer to be employed for
8 the X-ray diffraction (XRD) work, using a Cu K α radiation source ($\lambda = 0.154056$ nm for K α 1)
9 working at 40 KV and 40 mA. Electrochemical measurements were performed using a
10 portable multi Potentiostat μ Stat 8000/8 channels purchased from DropSens (Spain) and
11 controlled by PC with DropView 8400 software. Disposable screen-printed carbon electrodes
12 (DRP-C110) from DropSens with 4 mm diameter working electrode (carbon) were used for
13 modification. The auxiliary and reference electrodes are carbon and silver, respectively, while
14 the trager (carrier) is ceramic. The basal carbon working electrodes were modified with
15 pristine SiO₂(LuPc₂) or PANI(PVIA) or SiO₂(LuPc₂)-PANI(PVIA)-CNB for electrochemical
16 purpose. The electroactivity of SiO₂(LuPc₂)-PANI(PVIA)-CNB modified electrode was
17 evaluated by recording cyclic voltammogram (CV) in potassium ferro/ferricyanide solution
18 containing 0.1 M NaCl in the potential range from -0.5 V to +0.5 V. Electrochemical
19 impedance spectroscopy (EIS) measurements were carried out in the frequency range
20 between 10 and 2000000 Hz. The amperometric responses of the fabricated SiO₂(LuPc₂)-
21 PANI(PVIA)/GO_x-CNB biosensor towards glucose detection were recorded under stirred
22 conditions in 0.1 M PBS (pH 7.0) containing 0.1M NaCl by applying a constant potential of
23 +0.2 V at the working electrode. The electrolyte solution was saturated with N₂ gas to remove
24 dissolved oxygen prior to individual measurements. All electrochemical experiments were
25 carried out at room temperature.

26 2.3. Preparation of SiO₂(LuPc₂)-PANI(PVIA)-CNB

27 The preparation of SiO₂(LuPc₂)-PANI(PVIA)-CNB involves two stages: (i) pristine synthesis
28 of SiO₂(LuPc₂) nanoparticles and PANI(PVIA); (ii) formation of CNB. (ia) *Synthesis of*
29 *SiO₂(LuPc₂)*: Monodispersed LuPc₂ incorporated SiO₂-NH₂ nanoparticles (SiO₂(LuPc₂)) was
30 achieved through modified Stober method (Han et al., 2017). Briefly, water soluble LuPc₂
31 (10% V/V) was added to TEOS (3 mL) in NH₄OH/ethanol mixture (7:100 V/V). The mixture
32 solution was allowed to stir for about 12 h followed by quick addition of 4 mL of APTES to
33 the above mixture and continued stirring for another 12 h at room temperature. The resultant

1 colloidal LuPc₂ incorporated SiO₂-NH₂ (SiO₂(LuPc₂)) was obtained by centrifugation and
2 washed with ethanol for three times; (ib) *Synthesis of PANI(PVIA)*: PANI(PVIA) was
3 prepared by doping PANI-EB onto PVIA backbone. PANI-EB was prepared as reported in
4 the literature (Nobrega et al., 2012). Doping was achieved by mixing 1 g of PANI-EB in a
5 THF dispersion with appropriate quantity of PVIA (0.1 M) solution. The suspension was
6 sonicated for about 2 h followed by electromagnetic stirring (6 h) at room temperature to
7 make the dispersion homogeneous. The resultant PANI-PVIA dispersant was filtered through
8 polycarbonate membrane (pore size: 0.2 μm) and washed several times with water till the
9 filtrate became colorless. The precipitate was dried in vacuum oven at 60 °C for 24 h to
10 obtain PANI(PVIA) powder. (ii) *Formation of CNB*: CNB structure of SiO₂(LuPc₂)-
11 PANI(PVIA) was obtained through covalent grafting of COOH groups in PANI(PVIA) with
12 NH₂ groups in SiO₂(LuPc₂) nanoparticles. About 0.05 g of dispersed PANI(PVIA) and 0.05 g
13 of SiO₂(LuPc₂) were redispersed in 80 mL of 0.1M PBS solution (pH 7.0). 20 mL of EDC
14 and NHS solutions (each 25 mM) were added and stirred for about 30 min. The dispersant
15 solution was kept undisturbed at 25 °C for 24 h. The residue (SiO₂(LuPc₂)-PANI(PVIA)) was
16 separated by centrifugation, washed with water and dried at room temperature.

17 *2.4 Fabrication of SiO₂(LuPc₂)-PANI(PVIA)/GOx-CNB biosensor*

18 About 10 mg of as prepared SiO₂(LuPc₂)-PANI(PVIA) was dispersed in 1 mL of isopropyl
19 alcohol/naion mixture (7:3 V/V). 2 μl from the above stock solution was drop casted onto
20 pre-cleaned screen-printed carbon electrode and dried at room temperature. SiO₂(LuPc₂)-
21 PANI(PVIA)/GOx-CNB biosensor was fabricated by simultaneous drop casting GOx (1 μl)
22 (10 mg in 1 mL PBS (pH 7.0)) and glutaraldehyde (1 μl) solution. The modified electrodes
23 were dried at room temperature under N₂ atm for further analysis. Similarly, the other two
24 SiO₂(LuPc₂)/GOx and PANI(PVIA)/GOx biosensors were fabricated.

25 **3. Results and Discussion**

26 *3.1. Preparation of SiO₂(LuPc₂)-PANI(PVIA)/GOx-CNB*

27 The various stages in the formation of SiO₂(LuPc₂)-PANI(PVIA)/GOx-CNB are presented as
28 scheme 1; Stage 1 involves synthesis of SiO₂(LuPc₂) nanoparticles and PANI(PVIA); Stage
29 1a): SiO₂(LuPc₂) nanoparticles were obtained by synthesis of water soluble LuPc₂ as
30 described in 2.3 and subsequent incorporation into SiO₂ nanoparticle through mixed
31 hydrolysis/polycondensation of TEOS and APTES in NH₄OH/ethanol medium. The

1 incorporation of LuPc₂ was achieved through direct encapsulation into SiO₂ nanoparticles
2 through Lu-O-Si bond formation during silanization process (Sorokin et al., 2001; B. Zhao et
3 al., 2009). The colour of the SiO₂(LuPc₂) nanoparticles turns slightly yellow after 24 h of
4 gelation time in contrast to misty white observed in pristine SiO₂ nanoparticle synthesis. This
5 confirms the presence of LuPc₂ in the synthesized SiO₂(LuPc₂) nanoparticles. To further
6 demonstrate the presence of LuPc₂ in the SiO₂ nanoparticles UV-visible spectra were
7 recorded (discussed in section 3.2). Stage 1b): Synthesis of PANI(PVIA) was achieved by
8 polymeric acid doping method (Taşdelen, 2017). The itaconic acid/acetate doping onto the
9 PANI structure was confirmed by the slow colour change of PANI-EB from blue to green
10 (Scheme 1, see: photograph of PANI(PVIA)). Doping was further confirmed by the change in
11 the viscosity of the PANI-PVIA mixture solution. The –COOH/acetate groups of PVIA
12 doped onto nitrogen atoms of PANI are connected to both benzene and quinone rings. It is to
13 be noted that upon PVIA doping the solubility of PANI greatly enhanced (verified by
14 dissolving PANI(PVIA) and PANI-EB in water). The PANI(PVIA) in water remains
15 unsettled over a period of 48 hrs. Stage 2 involves formation of CNB structure from the
16 above synthesized SiO₂(LuPc₂) nanoparticles and PANI(PVIA). The CNB formation was
17 achieved through amide bond formation (amidation) via EDC/NHS chemistry (Booth et al.,
18 2015; Qu et al., 2015). The excess –COOH group in PANI(PVIA) was covalently linked to –
19 NH₂ sites in SiO₂(LuPc₂) by carbodiimide activation with the assistance of NHS, leading to
20 conjugation (Olde Damink et al., 1996; Pattabiraman and Bode, 2011). In this work, we have
21 chosen SiO₂, LuPc₂, PANI, PVIA for CNB formation due to the following reasons. The
22 simultaneous incorporation of LuPc₂ during SiO₂ synthesis leads to the formation of porous
23 cage over LuPc₂ particles. The SiO₂ cage formation over LuPc₂ protects it from leaching and
24 maintains the functionalities at diverse environment. The SiO₂ cage was made conductive by
25 grafting it with PANI(PVIA). The multiple functional groups in PVIA assist grafting PANI
26 onto SiO₂(LuPc₂) nanoparticle. Furthermore PVIA also offers biocompatibility/stability of
27 CNB at different pH (Mishra et al., 2011). Thus, SiO₂(LuPc₂)-PANI(PVIA)-CNB can have
28 the beneficial characteristics of an electron conductive PANI backbone, the electron
29 mediating property of LuPc₂, while SiO₂ to protect leaching of catalyst and PVIA to offer
30 biocompatibility to the CNB structures. The final product was greenish white resulted from
31 the covalent grafting of PANI(PVIA) onto the surface of SiO₂(LuPc₂) nanoparticles.

3.2. Morphology

SiO₂(LuPc₂) nanoparticles exhibited similar spherical morphology (Fig. 1(a)) as that of pristine SiO₂ nanoparticles (Fig. 1(d)), except with the change in the size of the nanoparticles. Fig. 1(a) shows spherical particles of SiO₂(LuPc₂) in different size distribution. The particle size ranges from 150 to 200 nm. However, the as prepared pristine SiO₂ nanoparticles are uniform with an average size of 140 nm (Fig. 1(d)). The variation in the size distribution of SiO₂(LuPc₂) exemplifies the incorporation of LuPc₂ into SiO₂ nanoparticles during the mode of synthesis. Furthermore it could be seen that the particles are slightly tilted to accommodate LuPc₂ in its interior porous structure. The presence of LuPc₂ in SiO₂(LuPc₂) nanoparticles was further confirmed through EDX measurements. The elemental test results confirmed the presence of inorganic ion Lu (24.2 wt%) in the ratio of 1:3 with SiO₂ (Fig.1(b)) within SiO₂(LuPc₂) nanoparticles. Fig. 1(c) shows the morphology of SiO₂(LuPc₂)-PANI(PVIA)-CNB. Upon PANI(PVIA) grafting onto SiO₂(LuPc₂), the size of the nanoparticles transformed between 180 to 220 nm. This ensures the successive grafting of PANI(PVIA) onto the surface of SiO₂(LuPc₂) nanoparticles (Roosz et al., 2017). For further confirmation TEM image of SiO₂(LuPc₂)-PANI(PVIA)-CNB is recorded (Fig. 1g). The dark spots noticed within the SiO₂ nanoparticles ensure the incorporation of LuPc₂ inside the nanocages of SiO₂. However, the TEM image of pristine SiO₂ nanoparticle showed smooth and uniform size distribution of particles (Fig. 1h). On closer analysis, we could notice that the surface becomes coarse due to PANI(PVIA) grafting. For reference the SEM images of PANI(PVIA) and LuPc₂ are shown in Fig. 1(e) and Fig. 1(f), respectively. TEM image of PANI(PVIA) exhibited nanobead like structure with average particle size around 30 nm (Fig. 1i).

The surface area of pristine SiO₂ and SiO₂(LuPc₂) are studied through Brunauer, Emmett and Teller (BET) measurements. The surface area of pristine SiO₂ and SiO₂(LuPc₂) are found to be 48.2889 ± 0.8737 m²/g and 20.4619 ± 0.5225 m²/g respectively. The reduction in the surface area ~57% addresses the incorporation of LuPc₂ well within porous nanocage of SiO₂ nanoparticles. The results are analogous to the significant decrease in the specific surface area noticed in palladium immobilized nanocages of SBA-16 compared to parent SBA-16 (Wang et al., 2013).

3.3. UV-visible spectroscopy

The UV-visible absorption spectra of SiO₂(LuPc₂) (Fig. 2a) show characteristic N, B, and Q bands of LuPc₂ around $\lambda = 315$ nm, sharp band around $\lambda = 390$ nm, and intensive Q absorption band of the macrocycles at $\lambda = 702$ nm (Basova et al., 2008a; 2008b). This

1 features the incorporation of LuPc₂ inside SiO₂ nanoparticles. However the observed
2 variation in peak intensity in addition to small shift in absorption bands compared to pristine
3 LuPc₂ (Fig. 2,inset) may arise due to the interaction of LuPc₂ with host walls of SiO₂ and
4 dimerization of larger aggregates during the gellation process (Holland et al., 1998). Fig. 2
5 b,c shows the absorption spectra of PANI-EB and PANI(PVIA), respectively. The undoped
6 PANI-EB (Fig. 2b) showed absorption bands corresponding to π - π^* transition of benzene
7 ring (310 nm) and excitation of the imine segment on the PANI chain (around 600 nm) (Rahy
8 et al., 2011). Moreover for PANI(PVIA) (Fig. 2c), the disappearance of the band around 600
9 nm indicates that the doping occurs at the imine segment of the emeraldine chain (Wang et al.,
10 2014). The observed bathochromic (red) shift of π -polaron to > 750 nm illustrates that PANI
11 backbone was well doped with -COOH/acetate functional groups in PVIA (Taşdelen, 2017).
12 Additionally, the appearance of the band at 420 nm results from the polaron phenomenon of
13 PANI(PVIA) (Dominis et al., 2002). In the case of SiO₂(LuPc₂)-PANI(PVIA), the polaronic
14 band of PANI(PVIA) exhibits hypsochromic shift to around 360 nm (Fig. 2d) with
15 broadening of the Q band around 700 nm. This ensures that PANI(PVIA) grafting over
16 SiO₂(LuPc₂) (~30 nm thickness calculated from SEM) does insignificantly affect the
17 electronic properties of LuPc₂ (Zhuang et al., 2011). The other physicochemical
18 characteristics such as FTIR and XRD patterns of pristine SiO₂(LuPc₂) and CNB are
19 presented in the Supporting Information (SI) SI-1.

20 3.4. Electrochemical impedance measurements

21 Electrochemical impedance spectroscopy is a powerful tool to study interfacial characteristics
22 of surface modified electrodes as well as gaining information about charge transfer properties
23 of the various compounds incorporated in the electrode to support the function of the
24 modifiers. SI-2(A) shows the impedance measurements (Nyquist plot) of SiO₂(LuPc₂),
25 PANI(PVIA) and SiO₂(LuPc₂)-PANI(PVIA) respectively carried out at the open circuit
26 potential in 5 mM K₃[Fe(CN)₆]/K₄[Fe(CN)₆] containing g 0.1 M NaCl. One could observe
27 distinct differences in the impedance spectra. The charge transfer resistance (R_{ct}) was
28 calculated from the obtained semicircular part at the high frequency region. The results
29 showed that SiO₂(LuPc₂)-PANI(PVIA)-CNB exhibits much lower R_{ct} value (180 Ω)
30 compared to SiO₂(LuPc₂) (1380 Ω) and PANI(PVIA) (1710 Ω) modified electrodes. The
31 electron transfer rate at SiO₂(LuPc₂)-PANI(PVIA)-CNB biosensor was approximately 7.6
32 and 9.5 higher than that at SiO₂(LuPc₂) and PANI(PVIA) electrodes, respectively. The
33 reduction in the resistance to charge transfer in the electrode is possibly accomplished by the

1 LuPc₂, as clearly indicated in SI-2(A) curve (d). The linear part at low frequency region
2 ensures a mixed kinetic and diffusion controlled process at SiO₂(LuPc₂)-PANI(PVIA)-CNB
3 biosensor while surface controlled process prevails at pristine SiO₂(LuPc₂) and PANI(PVIA)
4 modified electrodes (based on tail length). The fast electron transfer rate at SiO₂(LuPc₂)-
5 PANI(PVIA)-CNB informs that the grafted PANI(PVIA) chains electronically wires the
6 electron from the surface through LuPc₂ to the underlying electrode. For comparison the
7 Nyquist plot of LuPc₂ and SiO₂ is also presented. Equivalent circuit model R(Q(R(QR))) for
8 the fabricated biosensor, SiO₂(LuPc₂)-PANI(PVIA)-CNB, where R_s is the uncompensated
9 solution resistance; R_{et} is the electron transfer resistance; R_w is Warburg diffusion element
10 (W) and CPE₁ & CPE₂ standing for the double layer capacitance on the electrode/electrolyte
11 interface and the pseudocapacitance in the polymer film, respectively, is shown in SI-2(B).

12 3.5. Electrochemical behavior of SiO₂(LuPc₂)-PANI(PVIA)-CNB modified electrode

13 The electrochemical behavior of the modified electrodes was investigated by recording cyclic
14 voltammograms (CVs) of modified electrodes using Fe(CN)₆^{3-/4-} as a redox marker. CV
15 obtained at pristine SiO₂ (curve a), SiO₂(LuPc₂) (curve b), PANI(PVIA) (curve c) and
16 SiO₂(LuPc₂)-PANI(PVIA)-CNB (curve d) in Fe(CN)₆^{3-/4-} (5 mM) containing 0.1M NaCl is
17 shown in Fig. 3(A). A pair of one electron quasi-reversible redox peaks corresponding to
18 Fe(II)/Fe(III) transition process was observed at all electrodes. However, the redox peak
19 current (I_{pa}/I_{pc}) and the peak potential separation between anodic (E_{pa}) and cathodic (E_{pc})
20 wave (ΔE_p) differ between the individual electrodes. It is observed that the I_{pa}/I_{pc} value of
21 SiO₂(LuPc₂) increases by ~1.2 times than that of pristine SiO₂ modified electrode. This
22 ensures that the incorporated LuPc₂ within SiO₂ cage enhances the electrochemical activity of
23 SiO₂ (García-Sánchez et al., 2013). Moreover the I_{pa}/I_{pc} redox peak current further increases
24 to 181.4 μA /-168.4 μA (I_{pa}/I_{pc}) at SiO₂(LuPc₂)-PANI(PVIA)-CNB modified electrode
25 (curve d). It should be noted that the Fe(II)/Fe(III) redox peak current is found to be highest
26 at SiO₂(LuPc₂)-PANI(PVIA)-CNB which is ~1.3 and ~1.5 times higher than at SiO₂(LuPc₂)
27 and pristine SiO₂ nanoparticles modified electrodes. The result demonstrates that the presence
28 of PANI(PVIA) as a grafted network onto SiO₂(LuPc₂) augments the electronic conductivity
29 (Gopalan et al., 2010), in addition to the presence of LuPc₂ and SiO₂ that provide three
30 dimensional pathway for the adequate percolation of ions to the electrode surface and
31 facilitate the electron transfer process (Al-Sagur et al., 2017; Gopalan et al., 2009). The
32 I_{pa}/I_{pc} redox peaks of pristine PANI(PVIA) are ~5.1 times lower than that in the case of
33 SiO₂(LuPc₂)-PANI(PVIA)-CNB modified electrode. The ΔE_p value was found to increase in

1 the following order: PANI (PVIA) (145 mV) < SiO₂(LuPc₂)-PANI(PVIA)-CNB (170 mV) <
2 SiO₂(LuPc₂) (172 mV) < SiO₂ (175 mV).

3 CVs of SiO₂(LuPc₂)-PANI(PVIA)-CNB were also recorded for different scan rates (10–
4 100 mV/s) (SI-3). The calibration of $v^{1/2}$ vs I_{pa} or I_{pc} showed linearity with the correlation
5 coefficient of 0.999 (n=10), which confers the diffusion controlled process of Fe(CN)₆^{3-/4-}
6 redox reaction at SiO₂(LuPc₂)-PANI(PVIA)-CNB (Siswana et al., 2006). The diffusional
7 coefficient (D) was calculated to be 8.106x10⁻⁶ cm²/s using Randles–Sevcik equation
8 (Nagarale et al., 2009). With the known value of D and n=1 for reversible redox process, the
9 electrochemical active surface area (A) of the electrode was determined to be 1.184 cm². The
10 value of ‘A’ results from the three dimensional porous structures of SiO₂(LuPc₂)-
11 PANI(PVIA)-CNB. The results from electrochemical activity (CV) demonstrate the
12 importance of individual components (SiO₂, LuPc₂, PANI(PVIA)) in its fabrication design
13 for the further immobilization of GOx for the determination of glucose.

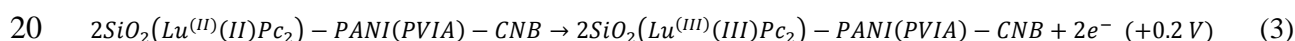
14 3.6. Electrochemical behavior of SiO₂(LuPc₂)-PANI(PVIA)/GOx-CNB biosensor

15 CV response of the GOx immobilized SiO₂(LuPc₂)-PANI(PVIA)-CNB modified electrode in
16 N₂-saturated PBS solution (pH 7.0) containing 0.1M NaCl is shown in Fig. 3B. A well-
17 defined symmetrical redox peaks (0.074 V anodic; -0.212 V cathodic) corresponding to
18 immobilized GOx at scan rate = 100 mV/s could be noticed. The effect of scan rate on the
19 CV response of redox peaks was also studied by varying the scan rate from 100-500 mV/s. It
20 should be noted that even at the higher scan rate of 500 mV/s, the SiO₂(LuPc₂)-
21 PANI(PVIA)/GOx-CNB biosensor showed redox behavior with a slight shift in its ΔE_p. This
22 ensures the stable immobilization of GOx onto SiO₂(LuPc₂)-PANI(PVIA)-CNB in its native
23 configuration (Gopalan et al., 2009). The redox peak current linearly increased with $v^{1/2}$ in the
24 range of 100–500 mV s (R² ≈ 0.999), indicating a diffusion-controlled electrochemical
25 process. The plot of log $v^{1/2}$ vs E_{pa} and E_{pc} (inset Fig 3B) showed straight lines with the
26 correlation coefficient of R² = 0.994 (anodic) and R² = 0.997 (cathodic). The diffusion
27 coefficient (D) of charge transfer was estimated to be 1.47x10⁻⁶ cm²/s using Randles–Sevcik
28 equation (Nagarale et al., 2009). The surface coverage of the modified electrode is calculated
29 to be 7.13x10⁻⁷ mol/cm² which is typically higher than GOx immobilized on SAM modified
30 electrode (4.80x10⁻¹² mol/cm²) (Fang et al., 2003). The higher value of surface coverage
31 admits the increased loading of GOx onto SiO₂(LuPc₂)-PANI(PVIA)-CNB surface. Moreover
32 the immobilized GOx enzymes are well bound on the surface observed from the redox peaks

1 at scan rate = 500 mV/s in 0.1 M PBS. Thus the higher and native loading of GOx could be
 2 achieved by the excess functional groups (from PVIA&PANI) and biocompatible
 3 environment provided by PVIA for the guest enzymes. For comparison CVs of pristine
 4 SiO₂(LuPc₂)/GOx and PANI(PVIA)/GOx were also recorded in 0.1M PBS and presented in
 5 SI-4.

6 3.7. Amperometric response of glucose at SiO₂(LuPc₂)-PANI(PVIA)/GOx-CNB biosensor

7 Amperometric measurements were recorded for varied concentrations of glucose to
 8 demonstrate the functioning of SiO₂(LuPc₂)-PANI(PVIA)/GOx-CNB as a potential glucose
 9 biosensor and the results are shown in Fig. 4. Optimization of experimental parameters for
 10 recording amperometric measurements were presented in SI-5(i-iii). Upon successive
 11 injection of glucose (1 mM) at regular intervals, a rapid and prominent increase in the
 12 bioelectrocatalytic amperometric current (E = +0.2 V) was observed under stirred condition.
 13 The operating principle is based on the enzymatic oxidation of glucose catalyzed by GOx
 14 immobilized onto SiO₂(LuPc₂)-PANI(PVIA)-CNB. The injected glucose are first
 15 enzymatically oxidized to gluconolactone, while GOx(FAD) reduced to GOx(FADH₂).
 16 Thereafter GOx(Red) will be regenerated to GOx(FAD) by electrooxidized SiO₂(LuPc₂)-
 17 PANI(PVIA)-CNB. The plausible mechanism is as follows



21 The current response was linear for glucose concentration in the range of 1–16 mM
 22 (correlation coefficient, $R = 0.997$) (Fig. 4 inset). The responses were saturated when glucose
 23 concentrations were higher than 16 mM that could be attributed to enzyme saturation (Li et
 24 al., 2009). The sensitivity of the SiO₂(LuPc₂)-PANI(PVIA)/GOx-CNB biosensor is
 25 calculated to be 38.53 $\mu\text{A}/\text{mM}/\text{cm}^2$ from the slope of the calibration plot with a RSD of 5.8%.
 26 The sensitivity of the SiO₂(LuPc₂)-PANI(PVIA)/GOx-CNB biosensor is superior than
 27 reported for the glucose biosensor fabricated with other SiO₂ composites for GOx
 28 immobilization. Sol-gel/GOx/copolymer (0.6 $\mu\text{A}/\text{mM}$) (Wang et al., 1998),
 29 PEDOT/PB/MWNT (2.67 $\mu\text{A}/\text{mM}$) (Chiu et al., 2009), Silica/GOx/CNTs (approximately
 30 0.2 $\mu\text{A}/\text{mM}$) (Salimi et al., 2004), and GOx-SWCNT conjugates/PVI-Os bilayers (32
 31 $\mu\text{A}/\text{mM}/\text{cm}^2$) (Gao et al., 2011) are typical examples as reported in the literature. The

1 superior sensitivity results from the judicious design of the fabricated electrode. The presence
2 of thin grafted PANI(PVIA) layer provides excess functional groups (-OH/ -CH₃COO-/-
3 COOH from PVIA and NH₂ sites from PANI) for the bonding of GOx. Also PVIA provides
4 biocompatible environment for the immobilized (GOx) enzyme (biocompatibility of poly
5 itaconic acid for biomolecules). While SiO₂ provides three dimensional porous surface for the
6 grafting process, LuPc₂ in SiO₂ nanoparticles mediates/transfers electrons to the electrode
7 surface. The SiO₂(LuPc₂)-PANI(PVIA)/GOx-CNB biosensor showed a fast response to the
8 changes in glucose concentration and the steady-state response current reached within 2 s.
9 The response time is much lower than in the case of pristine PANI incorporated silica
10 particles (Manesh et al., 2010), SiO₂ grafted with PVA+PVP (Wang et al., 1998), and
11 mesacellular carbon foam (Wang et al., 1998). The instant amperometric current response
12 upon the addition of glucose is attributed to the faster diffusion of glucose at SiO₂(LuPc₂)-
13 PANI(PVIA)/GOx-CNB. The rapid response to glucose was achieved due to the integrated
14 presence of PANI(PVIA) that electronically wires the electron from GOx through LuPc₂ to
15 underlying electrode (Tiwari et al., 2015). The wide linear range (1-16 mM) and high
16 sensitivity (38.53 μA/mM/cm²) of SiO₂(LuPc₂)- PANI(PVIA)/GOx-CNB biosensor made it
17 suitable for human blood glucose detection.

18 The apparent Michaelis–Menten constant (K_M) was calculated as 10.36 mM using the slope
19 and intercept values from the Lineweaver–Burk plot for SiO₂(LuPc₂)- PANI(PVIA)/GOx-
20 CNB biosensor (Mobin et al., 2010). The value is close to that reported for the free GOx
21 enzyme (12.4 mM) (Swoboda and Massey, 1965). This demonstrates that non-denaturated
22 characteristics of GOx immobilized onto SiO₂(LuPc₂)- PANI(PVIA)-CNB. The limit of
23 detection (LOD) for glucose at PAA-rGO/Vs-PANI/LuPc₂/GOx-MFH biosensor was
24 calculated as 0.1 mM (signal to noise ratio=3). The detection limit is estimated as three times
25 of the standard deviation of the background. Comparison of analytical performances of some
26 glucose biosensors based on GOx immobilized onto PANI/pthalocyanine/silica as one of the
27 component in the matrix is presented in SI-6, Table 1.

28 *3.8. Repeatability, Reproducibility and stability of SiO₂(LuPc₂)- PANI(PVIA)/GOx biosensor*

29 To investigate the stability of the SiO₂(LuPc₂)- PANI(PVIA)/GOx biosensor, (preserved in
30 0.1M PBS at 4 °C), amperometric current response was recorded at regular intervals for a
31 period of 45 days (SI-7(i)). After two week time the SiO₂(LuPc₂)- PANI(PVIA)/GOx
32 biosensor retained 98.7% of its initial current response (glucose 4mM). By the end of 45 days,
33 96.4% of the initial current response was restored. These results confirmed that the

1 functioning of GOx immobilized onto SiO₂(LuPc₂)- PANI(PVIA)-CNB was well protected
2 because of the co-presence of PVIA and SiO₂ nanoparticles in the fabricated biosensor
3 (Işiklan et al., 2009). The leaching effect of immobilized GOx from the fabricated
4 SiO₂(LuPc₂)-PANI(PVIA)-CNB/GOx biosensor was investigated by recording cyclic
5 voltammetry after immersion of the test electrode in 0.1M PBS for a period of 1h. From the
6 characteristic redox peaks of GOx, it is confirmed that there is insignificant leaching of GOx
7 from the fabricated biosensor. Also the leaching effect of LuPc₂ in the pristine SiO₂(LuPc₂)
8 electrode was also tested after immersion in Fe(CN)₆^{3-/4-} (5 mM) for the time period of 30
9 min. It was observed from CV (recorded at the scan rate of 50 mV/s) that the redox peak
10 current does not vary before and after immersion. This confirms that LuPc₂ is well
11 incorporated within the host SiO₂ porous cage and hence protected from leaching to the
12 background solution that is usually observed in many mediator based biosensor electrodes
13 (Wang et al., 2015).

14 To examine the reproducibility of SiO₂(LuPc₂)-PANI(PVIA)-CNB/GOx biosensor, seven
15 electrodes were prepared under identical conditions and stored at 4°C. Amperometric current
16 response was recorded in optimized conditions for three different concentrations of glucose
17 (low, normal and high) (SI-7(ii)). The relative standard deviations (RSD) for glucose were
18 2.8 % (2mM), 1.3% (4mM) and 4.9% (9 mM). The relatively low RSD value indicated that
19 SiO₂(LuPc₂)-PANI(PVIA)-CNB/GOx biosensor exhibited good reproducibility in all levels
20 of glucose. The repeatability of SiO₂(LuPc₂)-PANI(PVIA)-CNB/GOx biosensor for 5
21 consecutive measurements of glucose (4 mM) was estimated to RSD = 1.4% under ideal
22 conditions (SI-7(iii)).

23 3.9. Specificity and interference

24 The selectivity of the fabricated electrode is an important criterion for biosensor application.
25 Under the applied potential of +0.2 V, the presence of interfering substances hardly affects
26 the amperometric current response of glucose at SiO₂(LuPc₂)-PANI(PVIA)-CNB/GOx
27 biosensor. Repetitive measurements of glucose (4 mM) in the presence of interfering
28 substances such as dopamine (DA), lactic acid (LA), ascorbic acid (AA) and uric acid (UA)
29 (2 mM each), are shown in SI-8. DA and UA at the concentration of 2 mM produced the
30 relative low response of ~ 2.2% and ~ 5.0%, indicating that these species coexisting in the
31 sample matrix did not affect the determination of glucose. This informs that SiO₂(LuPc₂)-
32 PANI(PVIA)-CNB/GOx biosensor exhibits relatively selective detection of glucose and can

1 be potentially applied for serum samples even in the presence of higher concentration of
2 electrochemically active substances.

3 *3.10. Glucose determination in real samples at SiO₂(LuPc₂)-PANI(PVIA)/GOx-CNB* 4 *biosensor*

5 The suitability of SiO₂(LuPc₂)-PANI(PVIA)/GOx-CNB biosensor in the determination of
6 glucose in real samples was examined. A continuous amperometry was recorded as shown in
7 SI-9(i) at optimized conditions (E= +0.2V) in the presence of diluted (using 0.1M PBS to
8 obtain required concentration) fruit juices and horse serum sample. The results obtained for a
9 typical determination of glucose by standard additions method are presented in SI-9(i) Table
10 2. The results in SI-9 Table 2, indicate that the percentage recovery ranged from 89.72 to
11 105 %, which agrees with other standard spectrophotometric method. The satisfactory results
12 demonstrate the practical usage of the fabricated biosensor. Direct determination of glucose
13 in human and horse serum samples at SiO₂(LuPc₂)-PANI(PVIA)/GOx-CNB biosensor was
14 also carried out at optimized condition (SI-9(ii)). From the amperometric response, it could
15 be understood that the fabricated SiO₂(LuPc₂)-PANI(PVIA)/GOx-CNB biosensor responded
16 well for real samples.

17 **4. Conclusions**

18
19 In this work, we have successfully prepared a multicomponent based conducting nanobead
20 (CNB) comprising lutetium phthalocyanine (LuPc₂), SiO₂ nanoparticle, polyaniline (PANI)
21 and poly (vinyl alcohol-vinyl acetate-itaconic acid) (PVIA). The prepared CNB was utilized
22 as the platform for the immobilization of glucose oxidase (GOx). The new fabricated
23 SiO₂(LuPc₂)PANI(PVIA)/GOx-CNB biosensor has shown good sensitivity (38.53 $\mu\text{A.mM}^{-1}\text{cm}^{-2}$)
24 with wide linear range (1-16 mM) for the amperometric detection of glucose. The
25 SiO₂(LuPc₂)-PANI(PVIA)/GOx-CNB biosensor has exhibited a specific and fast response
26 (~2s) on addition of glucose. The proposed SiO₂(LuPc₂)-PANI(PVIA)/GOx-CNB biosensor
27 showed good accuracy for both juice and serum samples, providing the potential feasibility
28 for its use in Industrial&Clinical analysis. In addition to its use as a glucose sensor, the CNB
29 can be utilized as a platform for the construction of other biosensors in future.

30

31

1 **Acknowledgements**

2 Hadi Al-Sagur acknowledges the financial support provided for his PhD study from the
3 Ministry of Higher Education and Scientific Research (MOHESR) and the College of
4 Medicine/Thi Qar University in the south of Iraq. H. Karakaş, D. Atilla, A. G. Gürek
5 acknowledge the research fund by Gebze Technical University (Project No.: 2017-A-101-01).
6 T. Basova acknowledges the research fund by Nikolaev Institute of Inorganic Chemistry,
7 Russia (basic project).

8

9 **References**

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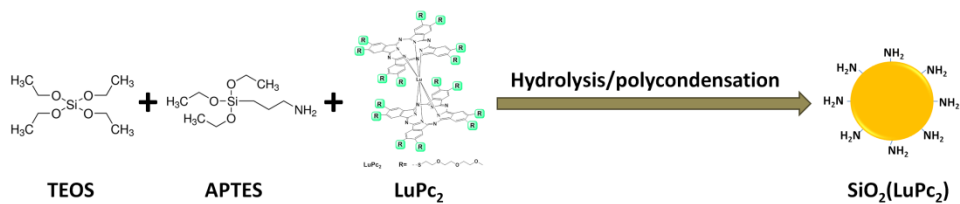
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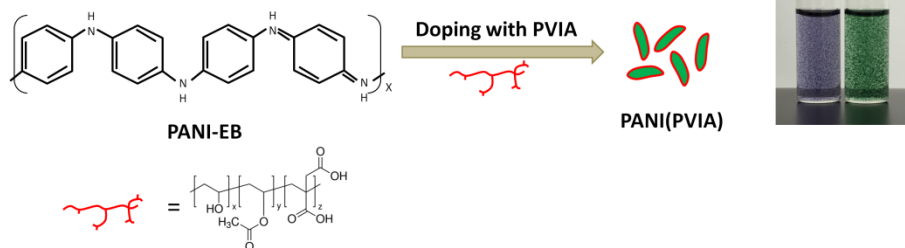
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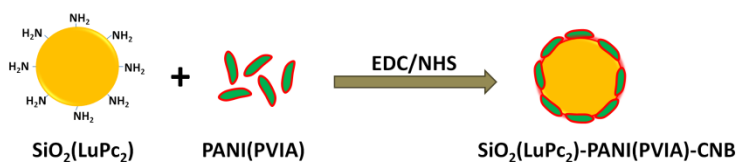
Stage 1: (i) Synthesis of $\text{SiO}_2(\text{LuPc}_2)$ nanoparticles



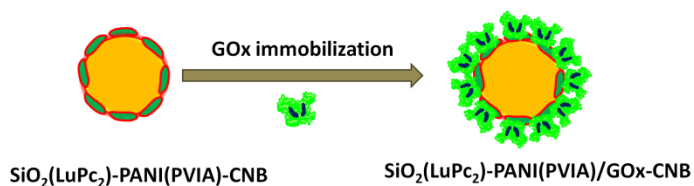
Stage 1: (ii) Synthesis of PANI(PVIA)



Stage 2: Preparation of $\text{SiO}_2(\text{LuPc}_2)$ -PANI(PVIA)-CNB



Fabrication of $\text{SiO}_2(\text{LuPc}_2)$ -PANI(PVIA)/GOx-CNB



Scheme 1 Schematic representation of the formation of $\text{SiO}_2(\text{LuPc}_2)$ -PANI(PVIA)/GOx-CNB

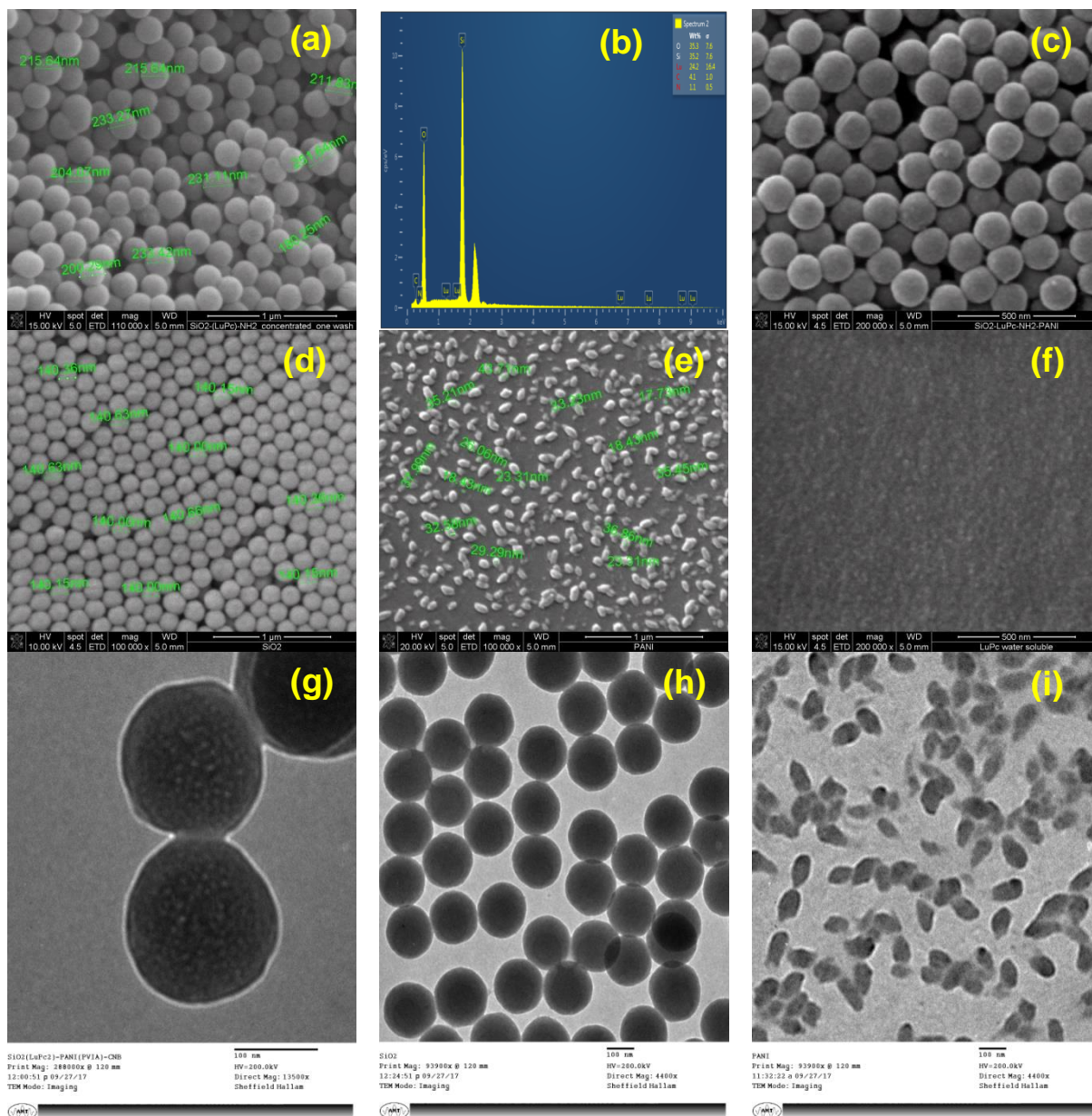


Fig. 1 SEM images of (a) $\text{SiO}_2(\text{LuPc}_2)$, (b) EDX image of $\text{SiO}_2(\text{LuPc}_2)$, (c) $\text{SiO}_2(\text{LuPc}_2)$ -PANI(PVIA)-CNB, (d) SiO_2 , (e) PANI(PVIA), (f) LuPc_2 ; TEM images of (g) $\text{SiO}_2(\text{LuPc}_2)$ -PANI(PVIA)-CNB, (h) SiO_2 , (i) PANI(PVIA)

1

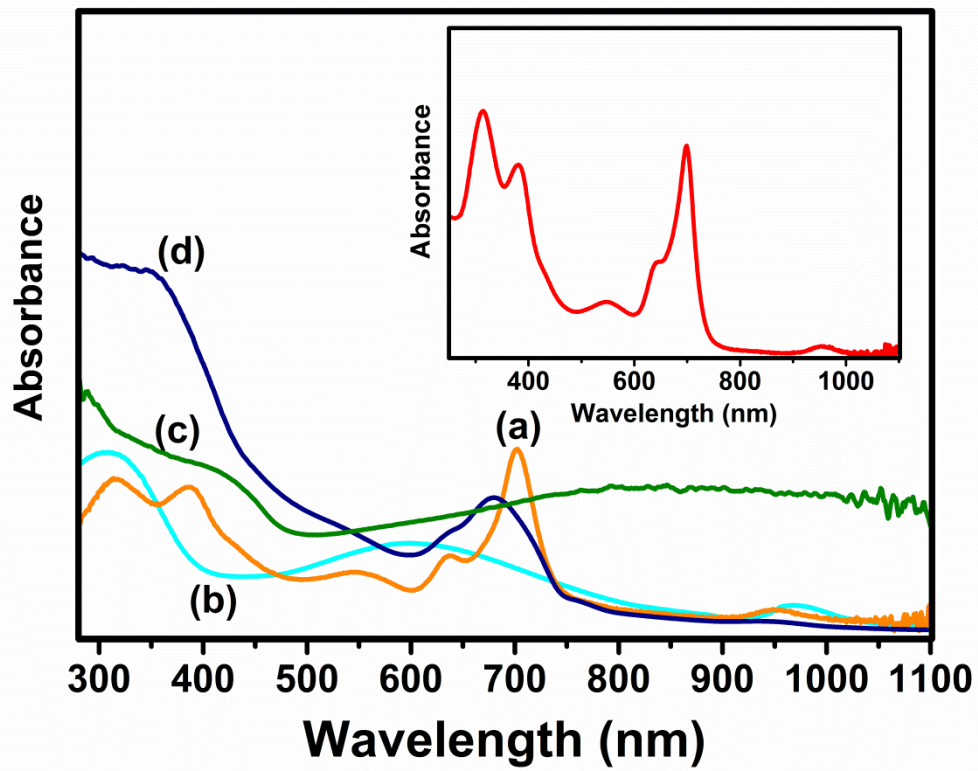


Fig. 2 UV-visible spectrum of (a) $\text{SiO}_2(\text{LuPc}_2)$, (b) PANI-EB (dedoped), (c) PANI(PVIA), (d) $\text{SiO}_2(\text{LuPc}_2)$ -PANI(PVIA)-CNB. Inset UV-visible spectrum of LuPc_2

1

2

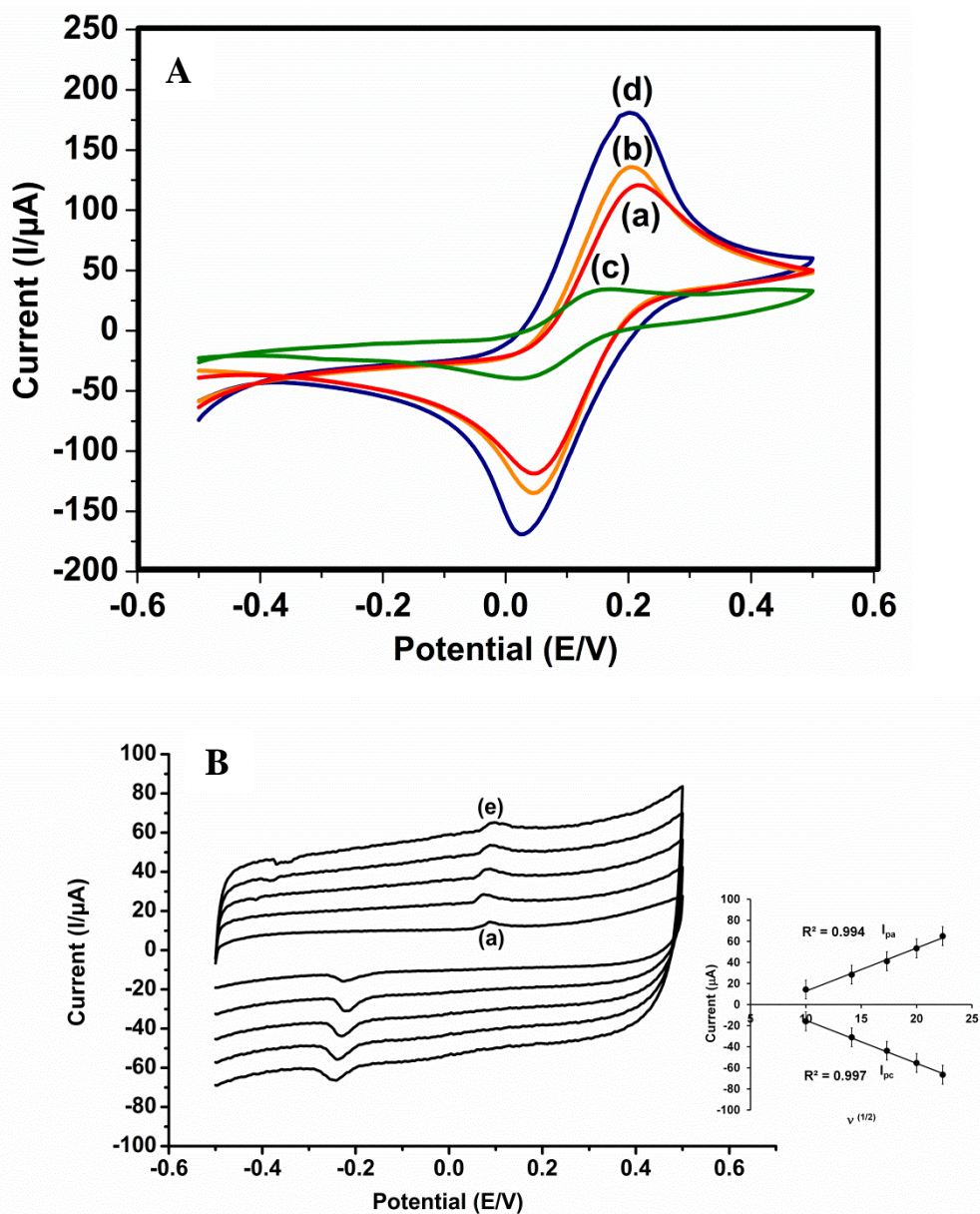


Fig. 3 (A) Cyclic voltammogram of (a) SiO₂, (b) SiO₂(LuPc₂), (c) PANI(PVIA), (d) SiO₂(LuPc₂)-PANI(PVIA)-CNB recorded in 5 mM Potassium ferro/ferricyanide solution containing 0.1 M NaCl; scan rate = 100 mV/s (B) Cyclic voltammogram of SiO₂(LuPc₂)-PANI(PVIA)/GOx-CNB in N₂ saturated 0.1 M PBS (pH 7.0) containing 0.1 M NaCl for different scan rate 100-500 mV/s (a-e); inset: plot of $v^{1/2}$ vs I_p

1

2

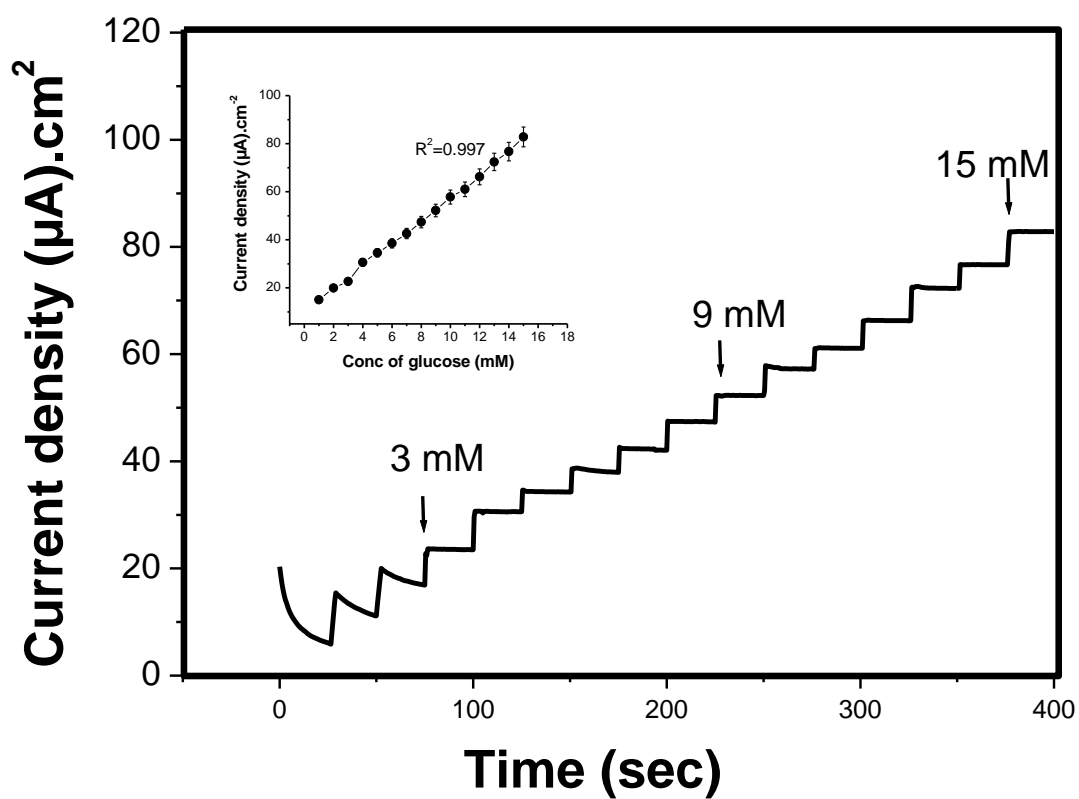


Fig. 4 Amperometry response for successive addition of glucose in 0.1 M PBS (pH 7.0) at SiO₂(LuPc₂)-PANI(PVIA)/GOx-CNB. Inset: calibration plot [glucose] vs peak current density