



# Fabrication of electrochemical sensor biosystems through hexagonal boron nitride Nanosheets for extraction lead in human serum

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## ABSTRACT

Lately a flood of expanded enthusiasm for the peeling of boron nitride (h-BN) has seen because of its energizing electrical, warm, photonics mechanical properties and detecting. A few ways to deal with have risen depicting the peeling, functionalized and solubilization of h-BN. In this investigation, we report on a direct way to deal with alter the surface and its application as another sort of biomedical applications. The readied item is basically described by FTIR spectroscopy, field outflow (FESEM), TGA strategy, XPS range, and BET surface zone estimations. Nano-composites were immobilized on terminals to distinguish the glucose, L-cysteine in cushion medium by cyclic voltammetry (CV), square wave voltammetry (SWV), and electrochemical impedance spectroscopic (EIS). Potential utilization of the covalent functionalization, modest forerunners, biodegradability and multifunctionality of superior composites boron nitride, they could be utilized for a wide scope of things to come biomedical applications. Also, the composites boron nitride was used for extraction lead (Pb<sup>2+</sup>) ions in human serum by micro solid phase extraction coupled with atom trap atomic absorption spectrometry (AT-AAS) by researchers. The LOD and enrichment factor was obtained 2.96 and 9.82, respectively for 10 mL of human serum sample (RSD < 2%). The validation was confirmed by CRM, NIST solution.

## 1. Introduction

Recently, more consideration has seen a flood of enthusiasm for the shedding of graphene, other layered mixes, particularly hexagonal boron nitride (h-BN) [1-4]. The h-BNNs significantly higher compound security and protection from oxidation contrasted with that of graphene. Boron nitride (BN) is the isoelectric and isostructural simple to graphite with rotating boron and nitrogen iotas in the structure [5,6]. Following these underlying reports,

a few different techniques, for example, mechanical shedding by means of drawing, Lithium particle intercalation and low vitality ball processing were utilized to create little amounts of top-notch h-BN nanosheets [7-11]. Substance shedding of h-BN was later proceeded as an option in contrast to the mechanical courses, driving a basic and practical path for the mass peeling of h-BN nanosheets. These were performed by treating the mass h-BN powder in different natural solvents, N,N Di-methylformamide (DMF), methane sulfonic corrosive (MSA), and liquid metal hydroxides. These methodologies yield low-convergence

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of h-BN nanosheets significantly after broad sonication [12-18]. With the expansion of practical gatherings triazine azide an improved scattering and strength of h-BN exists in an assortment of solvents. In this, we report the covalent compound functionalization of h-BN nanosheets utilizing receptive nitrene radicals. Point by point portrayal of the functionalized h-BN was performed to check the covalent idea of the connection [6, 18-22]. Many method was used for triazine determination in liquid phases. The functionalization technique was reached out to covalently unite triazine azide to the outside of h-BN nanosheets by nitrene addition and utilize these nano composites-functionalized BN nanosheets for cooperations with biosystems and improve detecting properties [22-26]. Electrochemical impedance spectroscopy (EIS) has as of late developed as a device to analyze forms that happen at various timescales. The idea of "impedance" is comparable to opposition yet takes into account depiction of complex circuits which have nonlinear current-voltage connections, for example, those which show capacitance, inductance, or mass dispersion. EIS exploits this idea by relating hypothetical circuit components to real electrochemical procedures happening in a material and permitting fitting of current, voltage, and recurrence information to equal circuit models. EIS information is produced by applying an AC potential to an electronic gadget, estimating the AC current reaction, and recording stage move and adequacy changes over a scope of applied frequencies. By analyzing the present reaction over a scope of frequencies permits partition of procedures which happen on various timescales, making it perfect for isolating electronic and ionic procedures in blended conductors [27-30]. This is a key favorable position of EIS making it a well-suited instrument to consider particle dispersion in HOIPs and resolve the substance personality of the particle [3]. In addition h-BN nanosheets was used for separation Pb ions in human biological samples by covalence bonding of BN to Pb (BN:---Pb) by  $\mu$ -SPE coupled to AT-AAS. The absorption capacity was achieved 141.4 mg g<sup>-1</sup> at pH=8.2.

## 2. Experimental

### 2.1. Materials and Instruments

All general materials such as KCl, Fe(CN)<sub>6</sub>, triazine azide, and azine were provided by Merck. h-BNN were purchased from Sigma-Aldrich company. The graphene was provided by Indian Institute for Technology. Other chemicals with analytical grad such as HNO<sub>3</sub>, HCl and NaOH were purchased from Merck, Germany. The pH was adjusted based on 0.32 mol L<sup>-1</sup> of buffer solution (HPO<sub>4</sub>/H<sub>2</sub>PO<sub>4</sub>) from pH of 5.0 to 8.4. The polyoxyethylene octyl phenyl ether as the anti-sticking material was used in human samples (Merck). GBC906 Flame atomic absorption spectrophotometer based on atom trap (AT-FAAS, AUS) measured Pb in serum samples. The air-acetylene based on background correction (LD<sub>2</sub>) tuned by the software of AVANTA of AT-FAAS. The AT-FAAS can be determined Pb with micro liter of the sample with LOD of 0.04 mg L<sup>-1</sup>.

### 2.2 Preparation of h-BNNs

h-BNNs were modified with triazine azide at room temperature with a ratio of 1:2 weight from h-BN nanosheets: triazine azide for giving TN<sub>3</sub>-functionalized BNNs (TN<sub>3</sub>-h-BNNs). After stirring for 24 h, the final product was centrifuged at 5500 rpm per minute. The obtained Tr-h-BNNs sediment was purified by dialysis against DMF for 2 days and dried at 100°C. So., a white powder was obtained for characterization.

### 2.3 Electrochemical characterization of BN-TrAz modified electrode

The electrochemical properties of BN-TrAz/GCE were examined using EIS and CV. The EIS method can be used to study the kinetics of electron transfer between electrolyte and electrode surface. According to Fig. 1, the effect of the modified electrode on the electrochemical response, the EIS spectrum (A) and the cyclic voltammonogram (B) of the 7 mM [Fe(CN)<sub>6</sub>] solution containing 0.1 M KCl for bare glass carbon electrodes (a) BN/GCE (c) and TriAz/BN/GCE (b) was investigated [1-8].

## 2.4. SPE procedure

By  $\mu$ -SPE method, 10 mL of serum were used for speciation and determination of lead ions at pH 8.2. First, 20 mg of *h*-BNNs added to 1-octyl-3-methylimidazolium hexafluorophosphate [OMIM][PF<sub>6</sub>] in ethanol and the suspension of *h*-BNNs in IL were injected to serum sample with concentration of 100  $\mu\text{g L}^{-1}$  at pH=8.2. After shaking with an ultrasound bath for 6.0 min, the Pb (II) ions were efficiently extracted with the nitrate of *h*-BNNs (N:---Pb) as dative bond in pH=8.2. The *h*-BNNs trapped in [OMIM][PF<sub>6</sub>] and separated from the liquid phase in the bottom of the conical tube after centrifuging (3500 rpm). Then, the pb<sup>2+</sup> ions back-extracted from *h*-BNNs by 0.5 mL of nitric acidic (0.2M, up to 1mL) and after diluted determined by AT-FAAS.

## 3. Results and Discussion

### 3.1. Characterization

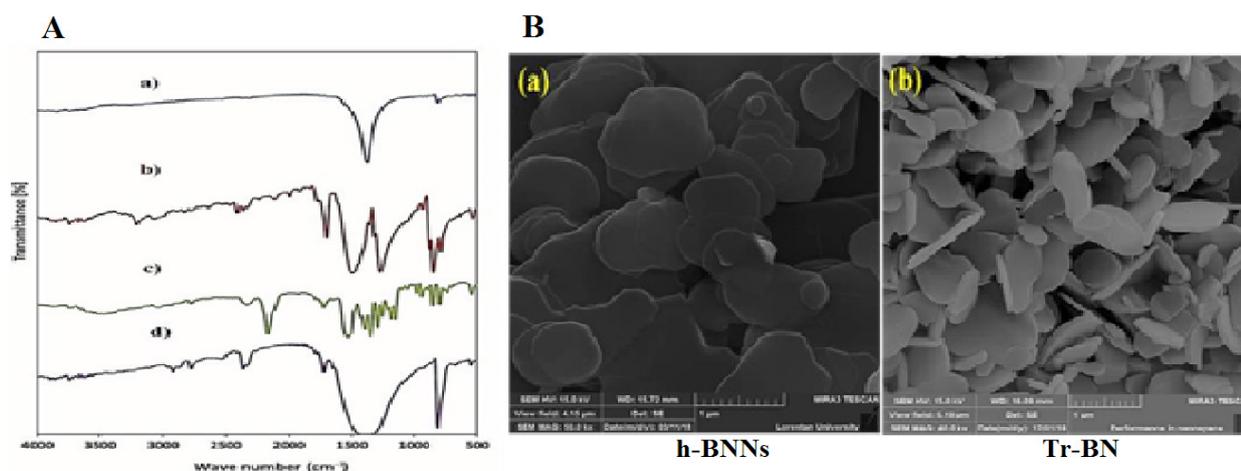
As appeared in Figure 1, the IR range of perfect *h*-BN uncovers a deviated bond at 1384 cm<sup>-1</sup> which compares to B-N stretch and the bond at 818 cm<sup>-1</sup> doled out to B-N-B extending vibration. The IR range of Triazine shows groups at 1701 cm<sup>-1</sup> which were appointed to C=N extending vibration. The morphology and structure of *h*-BNNs and Tr *h*-BNNs was explored by SEM. The shedding procedure brings about not many layer *h*-BNNs

sheets with smooth surfaces and edges and sidelong size in the scope of 1-2  $\mu\text{m}$  (Fig. 1). The Tr-*h*-BNNs sample showed a morphology different from that for *h*-BNNs. Due to the grafted Triazine-azide groups, surface of Nitrene-*h*-BNNs become relatively smooth and compact with porous features with the structure of *h*-BNNPs crystalline produced is preserved [9-11].

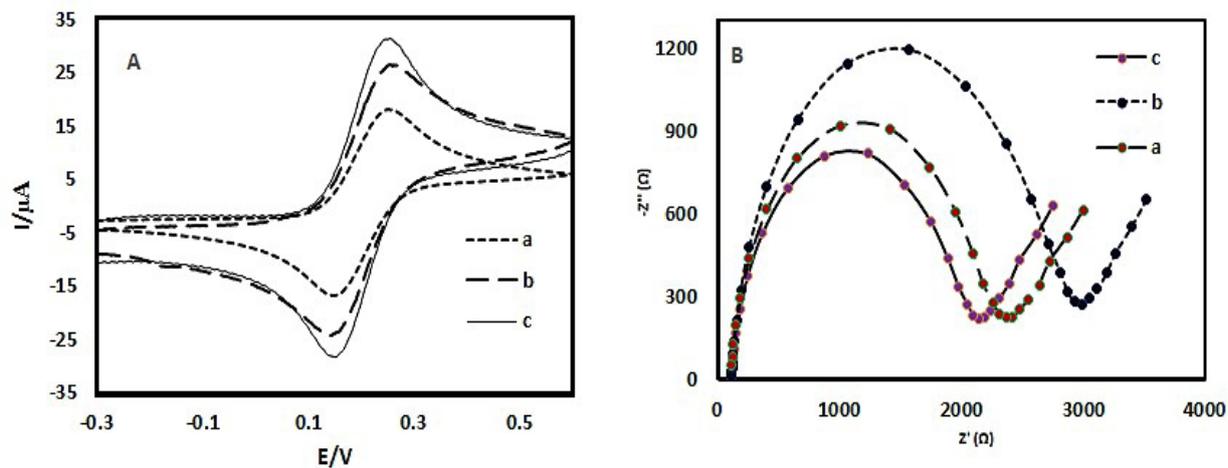
According to Figure 2, the effect of the modified electrode on the electrochemical response, the EIS spectrum (A) and the cyclic voltammonogram (B) of the 7 mM [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup> solution containing 0.1 M KCl for bare glass carbon electrodes (a) BN/GCE (c) and TriAz/BN/GCE (b). The electrochemical behavior of the electrode was investigated after interaction with 200  $\mu\text{M}$  L-cysteine by DPV method was investigated and the results were shown in (Fig.3).

### 3.2. Optimization and Validation

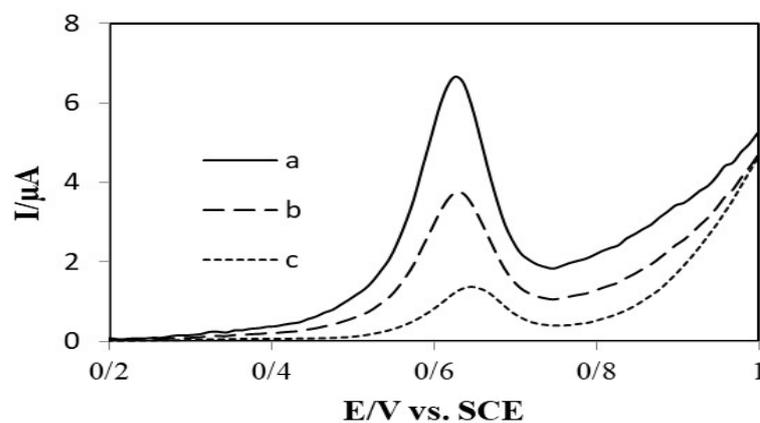
All parameters such as pH, sample volume, mass of sorbent, shaking time for extraction Pb from serum samples was optimized, Based on procedure sample volume from 5-15 mL have good efficiency for lead extraction so 10 mL of sample was used for further study. Also, pH of sample was evaluated between 2-10 and results showed us the pH 7.5-8.2 have high recovery for lead extraction, So, pH of 8 was used as optimum pH (Fig. 4). The amount of



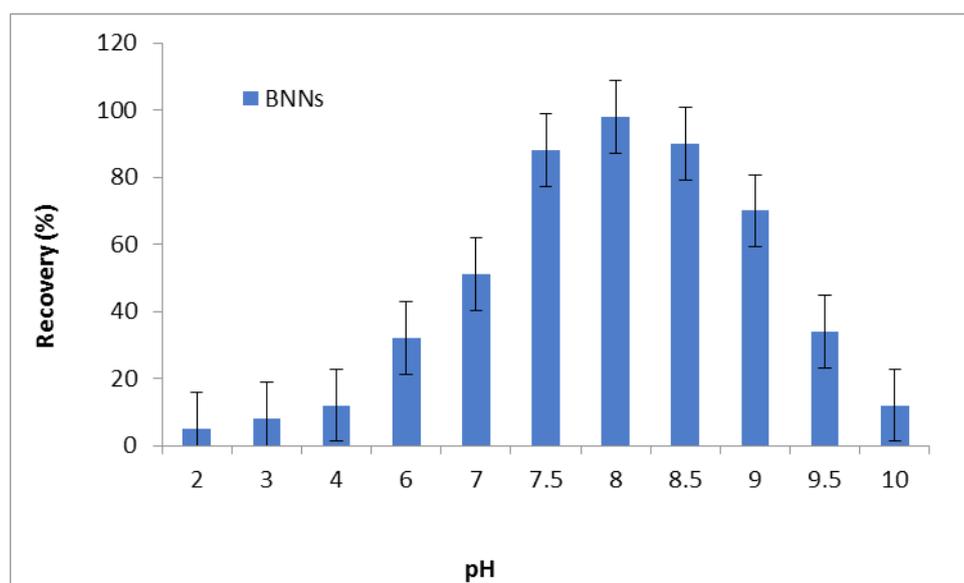
**Fig. 1.** A) FTIR spectra of (a) *h*-BNNs, (b) Triazine, (c) Triazine-azide, (d) Tr- BN. B) Different magnification of SEM images of the a) *h*-BNNs and b) Tr-BN.



**Fig. 2.** The impedance spectra (A) and CV voltammograms (B) of for bare glass carbon electrodes (a) BN/GCE (c) and TriAz/BN/GCE (b) in 7 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  solution containing 0.1 M KCl



**Fig. 3.** Difference pulse voltammogram of 200  $\mu\text{mL}$ - cysteine for different electrodes at pH =7. DPV Voltammograms at the bare glass carbon (b), BN/GCE (a) and TriAz/BN/GCE (c).



**Fig. 4.** The effect of pH on lead extraction by BNNs

**Table 1.** Validation of  $\mu$ -SPE method based on BNNs for lead extraction by certified reference material (CRM, NIST)

Sample	Reference ( $\mu\text{g L}^{-1}$ )	Added	Found* ( $\mu\text{g L}^{-1}$ )	Recovery (%)
<sup>a</sup> CRM	139.5 $\pm$ 0.8	-----	135.8 $\pm$ 8.2	-----
		100.0	231.2 $\pm$ 10.5	95.4
<sup>b</sup> CRM	277.6 $\pm$ 1.6	-----	271.5 $\pm$ 14.4	-----
		200.0	466.4 $\pm$ 22.7	97.3

CRM955c, caprine blood, level 2

<sup>b</sup>CRM955c, caprine blood, level 3

h-BNNs for extraction was optimized and 20 mg of sorbent was selected as optimum mass. The CRM was used for validation samples as Table 1.

#### 4. Conclusions

In present study, nitrene molecules were successfully grafted on the surface of h-BNNs and the resulted compound was used as an L-cysteine sensor of two sulfur of L-cysteine through the formation of Substitution reaction to Chlorine triazine azide. The most important achievement of this study is that L-cysteine is diagnosed in a straightforward manner. In summary, this synthesized modified electrode, could be used as a biosensing interface in the fields of biomedical and clinical diagnostics of Sulfur Protein Amino Acid such as L-cysteine. The modified electrochemical sensor was characterized by SWV, CV, and EIS. Based on results lead (Pb) can be extracted by h-BNNs in pH=8 by  $\mu$ -SPE method at optimized conditions

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