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Lab on paper chip integrated with Si@GNRs for electroanalysis of diazepam

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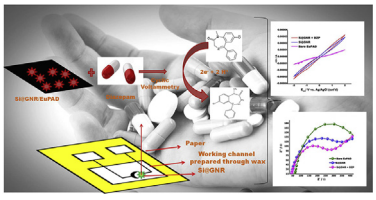
HIGHLIGHTS

- EμPAD offers many advantageous features such as facile approach, economic and have potential for commercialization.
- Extensive development can be made for industrial translation of this fabricated device.
- Si@GNR modified EμPAD showed wide linear range of 3.5 nM to 3.5 mM.
- The detection limit was as low as 1.5 x 10^{-9} M for diazepam detection.
- The developed sensor was tested in real time samples like injection, tablets and found good correlation.

GRAPHICAL ABSTRACT



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ABSTRACT

We describe herein the fabrication of an electrochemical microfluidic paper based device (EμPAD) for the detection of diazepam, a sedative, anxiety-relieving and muscle-relaxing drug. To achieve it, silica coated gold nanorods (Si@GNRs) were synthesized and drop casted on an electrochemical microfluidic paper based device (EμPAD) for the detection of diazepam. The synthesized composites were characterized by recording its images in scanning electron microscope (SEM) and transmission electron microscope (TEM). The experimental results confirmed that Si@GNRs had good electrocatalytic activity towards diazepam. The modified paper based electrode showed a stable electrochemical response for diazepam in the concentration range of 3.5 nM to 3.5 mM. EμPAD offers many advantageous features such as facile approach, economical and have potential for commercialization. Si@GNRs modified EμPAD was also employed for determination of diazepam in spiked human urine samples. Reported facile lab paper approach integrated with Si@GNRs could be well applied for the determination of serum metabolites.

1. Introduction

Lab on chip device provides potential to eliminate the need of complex three electrodes system made up of glass or metals. These

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devices also present many advantageous features such as minimal sample requirement, non-tedious and facile approach, cost-effective lab on a chip and fast response. Captivating features of paper-based devices make them suitable to be used in determination of various serum metabolites, drug analysis in even resource restricted settings. Point-of-care testing (POCT) should be cost effective, require minimal sample, should have high mechanical strength and autonomous operating system to meet the requirements of developing world [1–5]. The present work employed an electrochemical microfluidic paper-based analytical device (EμPAD) that substitute bulky three electrode setup in electrochemical cell with two electrode system grafted on a paper [6]. Paper based devices has any advantages over conventional methods, one of them being high surface area to volume ratio that improves detection limits [7]. EμPADs can be fabricated with a lot of methods like screen-printing, stencil-printing, painting, pencil/pen drawing, inkjet printing, sputter/evaporation deposition, wire application or nanoparticle growth/deposition [8]. Adkins et al. [8] described a number of environmental applications starting from detection of metals, pesticides, insecticides in food and water samples, bioanalytical applications like detection of metabolites, DNA or protein. This methodology entails fabricating electrodes on paper device integrated with silica coated gold nanorods (Si@GNRs) for electroanalysis of diazepam. Nowadays, nanotechnology is also incorporated with electrochemical devices for amplification of sensing signals. The properties such as high surface area, high stability and moreover biocompatibility make nanoparticles as a boon to improve the analytic performance of sensors [7–10]. Thus, we have employed Si@ GNRs for determination of diazepam. The silica shell was coated around gold nanorods to enhance the stability of GNRs. The encapsulation of GNRs by oxide such as silica is to protect the GNRs from aggregation, from thermal coalescence and from thermal rod-to-sphere shape transition [11]. This Si@GNRs also helps in providing a high surface area on that small chip for efficient sensing of the drug diazepam. Si@GNRs have been utilized by many researchers in various areas of electrochemical sensing like colorimetric sensing, electro analysis of hemoglobin and many more. This work also describes them to be as efficient in biosensing fields as in the above areas.

Diazepam (DZP) [7-chloro-1, 3-dihydro-1-methyl-5phenyl-2H-1, 4-benzodiazepin-2-one] is a benzodiazepine drug which is used for administration of acute seizures, insomnia, acute alcohol withdrawal anxiety attacks and panic attacks [12]. Diazepam increases the effects of a gamma-aminobutyric acid (GABA), a neurotransmitter chemical that use to communicate with one another nerves [13]. It is usually intravenously administered for anaesthetic purpose prior to any medical and dental procedures, anticonvulsant, muscle spasms, alcohol withdrawal, opiate withdrawal, and hypnotic medication [14]. An optimum dosage of this drug is normally safe and effective but the protracted usage of diazepam can cause adverse effects such as blurred vision, cyanosis, and dizziness, falling into a senseless state, stomach upset, rashes, nystagmus, weakness and even lethal [15]. Earlier techniques employed for determination of DZP are high performance liquid chromatography (HPLC) [16–20], spectrophotometry [21–24], thin layer chromatography [25], gas chromatography [26–28], polarography [29–31], fluorimetry [32–34], IR spectrometry [35], capillary zone electrophoresis (CZE) [36,37] and potentiometry [38]. Though, these methods are sensitive and refined, yet suffer from one or the other setbacks like some are complicated and require time-consuming sample pre-treatment. Some of them need expensive instrumental set-up and skilled person to operate. The present work eliminates the need for the bulky experimental set up and also diminishes the size of the sensing chip. DZP is generally used in clinical and forensic cases; therefore a fast, non-tedious, economical and facile approach is required for its quantification in biological fluids and commercial pharmaceutical products [39].

In the present study, we have fabricated lab on chip device for sensing DZP. This approach helps untrained users to rapidly provide qualitative and semi-quantitative results. Compared with the conventional microfluidic and other analytical devices which are constructed by silicon, glass and metal as their substrates, the μPADs, fabricated by paper offers advantageous features such as cost-effective, user-friendly, and ubiquitous and non-requirement of complex fabrication processes. Hence, this developed device is providing a common platform for prototyping new POCT for DZP drug. The developed sensor also employs Si@GNRs which helps in amplification of sensing signal. EμPAD integrated with Si@GNRs provided better results in terms of linearity, detection limit and response limit.

2. Materials and methods

2.1. Apparatus

The electrochemical sensing of the diazepam was carried out using cyclic voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS) on an Autolab-PGSTAT-10, Eco Chemie, Utrecht, Netherlands electrochemical analysis system with Nova software package. The two electrode system consists of the EμPAD integrated with silica coated gold nanorods (Si@GNR) as the working electrode and a platinum wire as the auxiliary electrode. The morphologies of the synthesized nanoparticles and nano-composites were studied with a JEOL, JSM-7600F Field Emission scanning electron microscope (FE-SEM; JEOL, JSM-7600F) and High-Resolution Transmission Electron Microscope (HR-TEM) was used for the nano-range size analysis of the Si@GNR. Both FE-SEM and HR-TEM were available at the University Sophisticated Instruments Facility (USIF) at the Aligarh Muslim University (AMU), India.

As the present work involves the fabrication of electrodes patterned on paper and was exploited for electrochemical detection of DZP. Herein this approach, we have employed simple and facile technique by using conductive carbon ink for prototyping the two electrodes on the paper [6]. Solid wax was patterned on the paper. The inks were dried on a hot plate at 65 °C for 20 min so that it infiltrates through the thickness of paper forming hydrophobic barriers surrounding hydrophilic channels on paper. We evaluated the quality of μPAD based electrochemical cells by cyclic voltammetric measurements.

2.2. Synthesis of gold nanorods (GNRs)

Gold nanorods were synthesized using the seed-mediated method [11]. Gold seeds (5 mL) were prepared by reducing $HAuCl_4$ (250 mM) with freshly prepared ice-cold $NaBH_4$ (3 mM) in the presence of cetyl trimethylammonium bromide (CTAB; 75 mM). After mixing, the mixture developed into light-brown colored solution indicating the formation of gold nanoseeds. These nanoseeds were used for subsequent preparation of nanorods after 2 h. To prepare nanorods, growth solution (10.0 mL) was prepared by the reducing $HAuCl_4$ (0.2 mM) with freshly prepared ascorbic acid (L-AA; 7 mM) in the presence of CTAB (1.6 mM). The solution was mixed and kept until its color changes from orange to colorless. The prepared gold nanoseed solution was added to this growth solution. The gradual appearance of red color in the mixture confirmed the formation of GNRs.

2.3. Synthesis of Si@GNRs

The pH of the clean GNRs@cTAB solution was adjusted to 10.5 in