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One MoNomer doubly imprinted dendrimer nanofilm modified pencil graphite electrode for simultaneous electrochemical determination of norepinephrine and uric acid

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ABSTRACT

One MoNomer dual imprinted hyperbranched polymer (dendrimer) with dispersed gold nanoparticle-functionalized multiwalled carbon nanotubes composite was used for the growth of a nanometer thin film applying 'surface-grafting from' approach on the pencil graphite electrode. The idea of combining dual template imprinting and One MoNomer molecular imprinting for the development of a dendritic nano-film, with homogenously generated molecular cavities (dendritic-boxes), is novel for the simultaneously better ingress-egress of pair of analytes (norepinephrine and uric acid). The differential pulse anodic stripping voltammetric peak potentials for both the analytes were found to be well apart approximately by 200 mV. This enabled simultaneous analysis, one in the presence of other, without any cross reactivity, interferences, and false-positives. The detection limits realized by the proposed sensor, under optimized analytical conditions, were found to be as low as 0.62 ng mL^{-1} for norepinephrine and 0.43 ng mL^{-1} for uric acid (S/N = 3) in aqueous, biological and pharmaceutical samples. Such stringent limits could be considered suitable for the primitive diagnosis of several chronic diseases, in clinical settings.

1. Introduction

Norepinephrine (NE) and uric acid (UA) are compounds of great biomedical interests, playing determining roles in human metabolism. NE is critical in neurological diseases (Parkinson and Alzheimer), heart failure, DNA breakdown in cardiac myoblast cells, diabetes, and HIV replication [1]. The concentrations of NE are elevated (2.86 ng mL^{-1}) in urine and plasma in several diseases and are of clinical interest, especially in the diagnosis of pheochromocytoma, neuroblastoma and ganglioneuroma [2]. On the other hand, uric acid (UA) is the primary end product of purine metabolism. The extreme abnormalities of UA levels in the body are symptoms of several diseases, such as gout, hyper- and hypo-uricemia, Lesch-Nyan, leukemia, and pneumonia. Both NE and UA, being electroactive species, with the ease of oxidation led to the development of appropriate electrochemical procedures for their evaluations at various electrodes [3–17]. In such work, selectivity and sensitivity have been a matter of prime concern. To ensure these aspects many nanostructured synthetically imprinted host systems [molecularly imprinted polymers (MIPs)] have been synthesized by 'imprinting' polymers with template molecules which upon removal leave behind specially arranged functional groups that act as recognition sites. Many molecularly imprinted materials have been used in the recent past for the selective analysis of NE and UA [18–28] present in the samples. However, MIPs have still some limitations such as incomplete template retrieval, broad guest affinities and selectivities, and slow mass transfer. This necessitates evolving some renovations in the imprinting so that desired recognition sites can be molded homogenously in the thin film for an easier ingress and egress of the template molecules in the molecular cavities. The actual obstacle arises when the simultaneous analysis of a pair of templates, concomitantly present together in real samples, is sought for. For instance, both targets NE and UA, as selected for the present study, have very close oxidation potentials and give overlapping voltammetric peaks at bare electrode [29]. This makes their simultaneous electrochemical analysis rather difficult. Although several non-MIP modified electrodes have come up for

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the simultaneous analysis of NE and UA [29–33], they apparently suffered with inferior selectivity and limited endurance. Alternatively, multi-imprinting technology, though scarce, is an upcoming technology which is found to be most suitable for the simultaneous analysis of various analyte mixutes [34–41]. However, simultaneous analysis of NE and UA using dual imprinting technology is not yet attempted. Double template imprinting is attractive because of the fact that diverse classes of analytes can be extracted, separated, assayed, detected, or otherwise analyzed at one time, saving both labor and time. In this context, further improvement has been made involving a new concept to generate homogenous binding cavities in dual imprinted texture employing only a single 'cross-linking monomer' for imprinting the template (s), but in the absence of any external cross-linker. Such materials were called as One MoNomer molecularly imprinted polymers (OMNiMIPs) [42,43]. This resulted in a monolith MIP for better interfacing with the transducer, which allowed more binding events with instantaneous current response. The advantage of OMNiMIP *vis-à-vis* traditional imprinting is apparent as it requires no selection of a crosslinker and thereby optimization of monomer-crosslinker ratio. The first lead compound used earlier was N,O-bismethacryloyl ethanolamine (NOBE) [43] which has bifunctionalities for the chain propagation. However, this is not suitable for the development of a three-dimensional imprinted dendritic nanostructure. Therefore, we have opted for a new trifunctional monomer derived from triamino-triazine (2,4,6-trisacrylamido-3,5-triazine, hereafter abbreviated as TAT). TAT has a compact symmetric and rigid structure (Fig. S1) that can give rise to multiple non-bonding interactions with template molecules in the well-defined three-dimensional preferences. These helped to obtain dendritic growth in the presence of large amount of initiator by an 'initiator fragment incorporation radical polymerization' (IFIRP) technique [44]. Moreover, OMNiMIPs led to higher binding capacities, where the template loading could be enhanced by 20–25% within the homogenously dispersed molecular cavities [36].

To extend the host-guest applicability in monomolecular imprinting, generally two strategies have been adopted: (1) molding an organic receptor around the guest 'template' (traditional imprinting approach) and (2) the approach of the dynamic combinatorial library of hosts, in which one or more members are preferentially bound to and stabilized by the guest molecule, producing a single binding site within the crosslinked MIPs or dendrimers [45,46]. While in both approaches the robust C=C irreversible crosslinkings were feasible, the second approach evolved a dendritic frame-work with lowest 'energy mold' around the template. However, the apprehension of binding sites heterogeneity cannot be ruled out in both approaches. Also, the quantitative retrieval of template could be another obstacle to trace analysis applications. Nevertheless, the second approach could be successfully applied to produce monodisperse dendrimers [46]. However, with this approach the problem of intramolecular crosslinkings might hamper the scale-up of hyperbranching, with template as a core, and thereby restrict the significant quantities of imprinted cavities. In contrast to the aforesaid approaches, we have exploited the synthesis of One MoNomer doubly imprinted dendrimer with duly embedded gold nanoparticle-functionalized MWCNTs (referred henceforth with as OMNiDID) with dynamic imprinting approach. For this, we have selected a trifunctional monomer (TAT) as a core of the hyperbranched (dendrimer) receptor system, in the presence of several molecules of template. This may ensure nearly homogenous binding sites, even in case with dual imprints. Further, OMNiDID protocol to prepare a synthetic host, with monomolecular imprinting inside the dendrimer framework, would be an aided advantage toward the quantitative template removal. This is feasible because the three-diamentional motif of OMNiDID has a surface grafted 'perforated' dendritic infrastructure (in the absence of an extra crosslinker). This would enable facile ingress-egress in the empty spaces (dendritic boxes) prevalent either at surface or deep inside of the coating. This work is quite novel in the sense that our earlier work had used crosslinker generously to yield an apparently thick coating of hyperbranched crosslinked polymer layer [40,44].

2. Experimental

2.1. Materials and methods

Materials and reagents used in this work are detailed in the supporting data Section S.1. All voltammetric measurements were carried out with a portable potentiostat μ-Stat 200 (Drop Sens S.L. Oviedo, Spain). The instrument was connected *via* USB connection to a computer installed with the measurement software Drop View (Drop Sens). A convenient three electrode cell assembly was consisted of OMNiDID/PGE, platinum wire, and Ag/AgCl (3.0 M KCl) as working, counter, and reference electrodes, respectively.

Fourier transform infrared (FT-IR) spectra of OMNiDID, prepared exclusively for this purpose as KBr pellet, were measured on Varian 3100 FT-IR (USA). Morphological images of OMNiDID/PGE surface were studied using scanning electron microscope (SEM, JEOL, JSM, Model–840 A (Netherland)) and atomic force microscope (AFM) (Vecco Instruments. Inc., USA) with a nanoscope IIIa SPM controller (Digital Instruments, USA, tapping mode). Morphological study of GNPs-fMWCNTs composite was made using tunneling electron microscopy (TEM) [Technai-12FEI (Eindhoven, Netherlands)].

2.2. Preparation of GNPs-fMWCNTs composite

Positively charged GNPs were obtained following the known recipe [47] [For details, vide supporting data Section S.2]. MWCNTs were chemically oxidized in a mixture of sulphuric acid and nitric acid (3:1) to yield −COOH group functionalized MWCNTs (fMWCNTs). The colloidal solution (0.5 mg mL^{-1}) of negatively charged fMWCNTs was prepared in triple distilled water, sonicated, and mixed with positively charged GNPs solution (200 μL) [vide, Section 3.1]. This resulted in an immediate decolorization indicating electrostatic deposition of the respective metal on the fMWCNTs structure. The solution was left for a further 18 h with continuous stirring at 40–50 °C. This was finally washed with ethanol and dried at 80 °C. The so-produced GNPs-fMWCNTs composite was characterized using TEM. The purpose of obtaining GNPs-fMWCNTs composite is not only to gain high electroconductivity in the film but also to ensure better dispersion of MWCNTs in GNPs colloid suspension to fabricate a stable GNPs-fMWCNTs-OMNiDID film coated electrode.

2.3. Synthesis of crosslinking monomer

Monomeric precursor, TAT was prepared and characterized following a known recipe [44]. For this, mel (38.5 mmol/25 mL DMF) was reacted with AC (125 mmol), with intermittent stirring for 12 h.

2.4. Fabrication of OMNiDID/PGE

A pencil rod (2B) was first pretreated by dipping in 6 M HNO_3 for 15 min, washing with water, and subsequently smoothening the surface by soft cotton. This was inserted into a Teflon tube where the tip of the pencil rod at one end was gently rubbed with an emery paper (No. 400) to level the pencil surface along the tube