Electrochemical sensor for uric acid based on a molecularly imprinted polymer brush grafted to tetraethoxysilane derived sol-gel thin film graphite electrode

A.K. Patel, P.S. Sharma, B.B. Prasad *

Analytical Division, Department of Chemistry, Faculty of Science, Banaras Hindu University, Varanasi - 221 005, India

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ABSTRACT

Determination of uric acid in human serum and urine is useful to provide treatment guidelines to hyperuricemic patients. An electrochemical sensor was developed for selective and quantitative recognition of uric acid by using a preanodised sol-gel coated graphite electrode with a molecularly imprinted polymer brush of poly(melamine–co-chloranil) grafted to its exterior surface. During a preconcentration step at (+2.0 V versus saturated calomel electrode), the encapsulated analyte recapture involved hydrophobically induced hydrogen-bondings in outwardly exposed MIP cavities in aqueous environment (pH 7.0), instantly oxidised as dications, and then cathodically stripped off as corresponding lactam responding differential pulse, cathodic stripping voltammetric signal. The uric acid was selectively detected without any cross reactivity in the windows of 14.56–177.42 μg mL^{-1} (aqueous medium), 4.78–106.96 μg mL^{-1} (blood serum), and 7.81–148.42 μg mL^{-1} (urine) indicating detection limits in the range of 3.71–4.10 μg mL^{-1} (3σ, RSD = 1.9%).

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1. Introduction

Our interest in molecular recognition of biomolecules by synthetic receptor is because of the chemistry and topography of receptor surface which effect recognition property to a large extent in biological environment. In order to develop useful materials for many biotechnology applications, suitable interfaces are required that have both enhanced specific binding and reduced non-specific binding to avoid "false positive" results. The surface tailoring by the use of molecularly imprinted polymers (MIPs) over sol-gel matrix is an important avenue for the production of such interfaces. This utilizes sol-gel technology to fabricate a stable, rigid glassy matrix of silica gel via hydrolysis and polycondensation of tetraethyl orthosilicate in acidic medium. It allows low temperature fabrication of a material surface (typically a metal oxide) starting either from a chemical solution (*sol* short for solution) or colloidal particles (*sol* for nanoscale particle) to produce an integrated network (gel). Such surface facilitates surface grafting of MIP, and the resulting hybrid possesses high glass-transition temperature with improved molecular specificity and selectivity. Self-assembled monolayers (SAMs) and polymer brushes have recently attracted considerable attention due to their noble physiochemical surface property and ease of processing [1]. A polymer brush is generally built from an ensemble of polymer chains that are attached with one end either chemically (through covalent bonding) or physically (by physisorption) creating a "grafting to" or "grafting from" surface. Polymer brushes behave very differently from simply

adsorbed polymer chain [2]. The thick polymer brush with high grafting density obtained via "grafting from" synthesis protocol, where polymer chains are grown by the use of initiators on the surface, may involve analyte diffusion impediment particularly through pores of MIP network. Alternatively "grafting to" technique with controlled grafting density by bulk condensation polymerization and minimum overlap appears advantageous in augmenting the slow kinetics of mass-transport of analyte binding, which happens to be a long-due obstacle associated with MIP coating layers. Furthermore, grafting linear oligomeric chains of MIP by one end to a solid surface, possessing all characteristic of SAMs in the form of "polymer brush" is known to produce a protein/cell-resistant surface [3] with minimal non-specific bio-fouling when complex matrices of biological fluids are analyzed without any sample pretreatment (deproteination, ultrafiltration, etc.). The sample pretreatment is unwarranted as it may leads inaccuracies in final results especially in trace analysis.

Uric acid (UA), a primary product of purine nucleotide metabolism, is a key biomarker for several clinical manifestations, such as gout, hyperuricemia, Lesch-Nyhan syndrome, cardiovascular and kidney diseases etc. These diseases are common when UA exceeds the normal range (urine 2 mM, blood 120–450 μM) in human being. Therefore, as a remedial measure, all asymptomatic hyperuricemic patients should be encouraged to make changes in diet and life style. This necessitates to develop a suitable cost-effective monitoring system in order to give adequate feed back and treatment guidelines to hyperuricemic patients.

The detection of UA in physiological samples by using electrochemical techniques [4–8] has been subject of extensive interest because of inherent advantages of simplicity, ease of miniaturization,

* Corresponding author. Tel.: +91 9451954449.
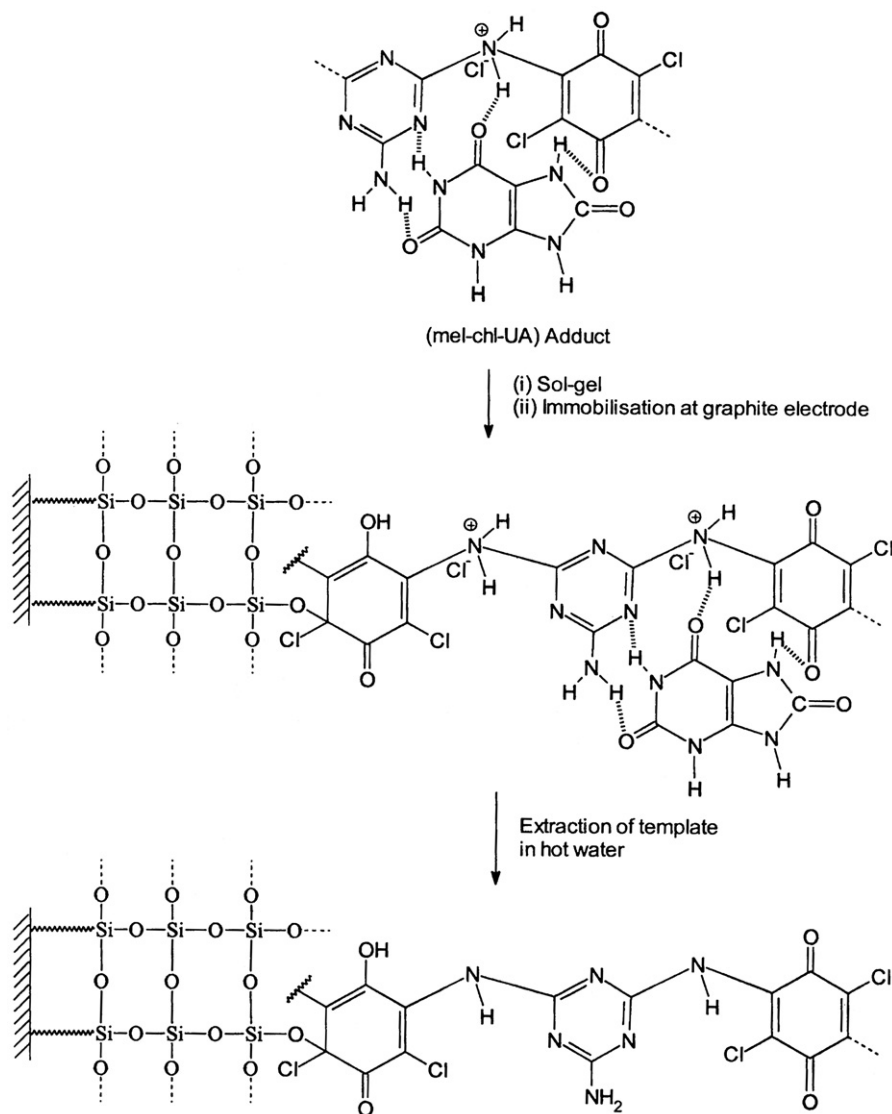 *E-mail address:* prof.bbpd@yahoo.com (B.B. Prasad).

Fig. 1. Schematic representation of the preparation of MIP-sol-gel modified graphite electrode.

high sensitivity, and relatively low-cost as compared to conventional colorimetry and spectrophotometric methodologies [9]. The major problem comes from the massive interference of ascorbic acid (AA) because of overlapping oxidation peaks. A variety of modified electrodes and electrode pretreatment methods have been proposed with the aim to resolve the anodic processes and detecting UA in the presence of excess AA [6,8,10–14]. However, the presence of other coexisting electroactive species (such as cytosine, purine, glucose etc.) may also influence the measurements in biological fluids. Other complications such as electrode fouling and critical regeneration of the electrode pose serious limitations in the practical applications of modified electrode. Carbon-based electrodes are most widely used for the determination of UA but they are either involved the interferences of AA [9,15–19] or have low sensitivity [20–22]. Various enzyme techniques [23] and screen-printed electrodes [24] were also developed to improve the selectivity of the UA measurement, but these methods are very expensive and their detection limits need a major improvement. Insofar as MIP-based sensors are concerned, we have already reported MIP-modified hanging mercury drop electrode (HMDE) for highly selective and sensitive analysis of UA in blood samples [25]; however the HMDE cannot be used as disposable sensing platform owing to pertaining environmental and health risks. Therefore, a robust, cost-effective, and disposable solid-sensor is

required which could be used in consecutive analyses after easy renewal and then disposed off. Till date, a single paper describing an imide type conductive polymer imprinted with UA for clinical uses has been reported with detection limit of 3×10^{-4} mM, without completely resolving AA interferences [26]. Therefore, the present work is a novel attempt in devising solid electrode for sensing hyperuricemia for the first time, exploiting differential pulse, cathodic stripping voltammetric (DPCSV) analysis of UA with MIP based sol-gel modified graphite sensor, without any interferences and cross-reactivities.

2. Experimental

2.1. Materials and reagents

Melamine (mel), chloranil (chl),tetraethoxy silane (TEOS), UA, graphite powder and other chemicals (interferents) were purchased from Loba Chemie, India;Otto Chemie, India; SD Fine, India; and Fluka, Germany. Other chemicals were of analytical reagent grade and used as received. The solvents dimethyl formamide (DMF) and ethanol were of HPLC quality. Graphite rods were procured from Hi-Tech carbon, New Delhi, India. The stock solution of UA was prepared by dissolving 50 mg UA and 23 mg lithium carbonate in about 10 mL water (triple distilled, deionised water, conducting range 0.06–0.07×10^{-6} S cm^{-1})