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Phosphomolybdic acid nano-aggregates immobilized nafion membrane modified electrode for selective cysteine electrocatalytic oxidation and anti-dermatophytic activity

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ABSTRACT

Phosphomolybdic acid (PMo12) stabilized Nafion (Nf) membrane colloidal solution ({Nf-PMo12}) modified glassy carbon electrode (GCE/{Nf-PMo12}-CME) has been demonstrated as a new and efficient electrocatalyst for cysteine (CySH) oxidation in pH 7 phosphate buffer solution. Physico-chemical characterizations of the {Nf-PMo12} by TEM, XRD, FT-IR and UV–vis spectroscopy techniques revealed stabilization of the PMo12 as 5–60 nm sized nano-aggregates within the sulphonic acid micro-channels of Nafion without any changes in the crystal structure. Electrochemical characterization of the GCE/{Nf-PMo12}-CME with $Fe(CN)_6{}^{3-}$ and $Ru(bpy)_3{}^{2+}$ indicates metal like surface feature of the working electrode. The GCE/{Nf-PMo12}-CME showed highly stable CySH electrochemical oxidation current signal at 0.58 ± 0.02 V vs. Ag/AgCl in pH 7 with 150 mV reduction in the response and about 8 times higher the peak current than that of the respective unmodified electrode. Amperometric *i*–*t* response of CySH at an applied potential of 0.58 V vs. Ag/AgCl yielded current sensitivity and regression coefficient values of 0.011 μA/μM and 0.9998 respectively without any interference from xanthine, hypoxanthine, nitrite, uric acid and other sulphur containing biochemicals; methionine and taurine, except ascorbic acid. Calculated detection limit value is (signal-to-noise ratio, S/N = 3) 25 μM. In particular, the PMo12 and {Nf-PMo12} systems showed marked anti-dermatophytic activity against *Trichophyton rubrum* organism, and the activity is comparable with standard fluconazole drug.

1. Introduction

Cysteine (CySH) is a thiol-containing α-amino acid playing a key role in biological systems [1] and serves as sulphur source for protein synthesis [2]. CySH is an intermediate for the biochemical synthesis of other amino acids like, glutathione and taurine, which are the most important intracellular antioxidants [3,4]. It is an important biomarker for prostate cancer and for the treatment of skin damages [5]. The altered levels of CySH in plasma and urine can lead to several diseases like, *cystinosis* (genetic disorder), *cystinuria* (metabolic disorder) and *Alzheimer's and Parkinson's* (neurodegenerative disorders). Inadequate dietary intake of CySH may cause several clinical disorders like premature arteriosclerosis, occlusive vascular, leukemia, cervical cancer, diabetes, cataracts and liver disease [6]. CySH is generally found in many foods including egg, oats, soyamilk, dairy products, ricotta and cottage cheeses, lunch meats, red peppers, garlic, broccoli, brussels sprouts, granola, and wheat germ, etc. [7,8]. Note that the oats meal is another healthy source of cysteine, where it lowers high blood sugar and cholesterol levels as well. Hence, selective CySH determination is a significant interest in diverse research fields.

Since CySH does not have any chromophore, derivatization based spectroscopic methodologies were often reported for the analyses. For instance, UV–vis [9,10], flurometric [11,12], electrophoretic [13] detections coupled with or without separation techniques [14,15]. In general, following derivatization agents were often used in the UV–vis detections: fluropyruvic acid [9], 2-chloro-1-methylquinoliniumctetrafluoroborate (CMQT) [16], 1,1-thiocarbonyldiimidazole [17], *p*-bromophenacyl bromide [18] and nanoparticles of gold and silver [19,20]. Similarly some of the fluorescence (FL) tagging reagents reported for the CySH detection are; *N*-(9-acridinyl) maleimide and its derivatives [21,22], iodoacetamide-type reagents [23], halogeno-benzofurazans [24] and monobromobimane [25]. Unfortunately, the above derivatization reagent based techniques have several disadvantages. For example, when the fluropyruvic acid reacts with CySH in the presence of concentric HCl solution liberates equal amount of hydrogen fluoride in the reaction vessel, which is highly toxic and corrosive in nature [9]. Apart from that, the reactions are time consuming (up to 5 h) and require high pure organic solvent for the analyses [21]. In addition, such derivatization methodologies are non-selective; both CySH and cystine (CyS–SCy) compounds are detected [16,25].

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On the other hand, electrochemical techniques have attracted considerable interest recently due to its simplicity, ease of miniaturization, high sensitivity, selectivity and relatively low cost [26]. In this work, a new Nafion/phosphomolybdic acid ($H_3PMo_{12}O_{40}$, PMo12) colloidal solution, designated as {Nf-PMo12}, chemically modified on a glassy carbon electrode (designated as GCE/{Nf-PMo12}-CME; Nf = Nafion) has been demonstrated for sensitive and selective sensing of CySH in the physiological solution.

Some of the conventional working electrodes reported for the CySH oxidation are; Pt at 0.8 ± 0.05 V vs. saturated calomel electrode (SCE) in acidic media [27], GCE at 0.8 ± 0.05 V vs. Ag/AgCl in pH 7 PBS [28], Au at 0.95 ± 0.05 V Ag/AgCl in 0.01 M $HClO_4$ [29]. Unfortunately, the electrode reactions involved with serious fouling of electrode surface, high over-potential and noticeable decrement in the electrochemical activity [27–29]. For instance, on a glassy carbon electrode (GCE) surface, continuous decrease in the CySH oxidation current signal, which may be due to generation of intermediate radical species, $CyS^{\bullet}$ and its destructive interaction with the surface, was noticed [30]. The above problem was solved by replacing the conventional electrodes with highly rigid solid electrode such as boron-doped diamond (BDD) electrode [31]. Meanwhile, several chemically modified electrodes (CMEs) efficient for CySH oxidation were reported in the literature (Table 1). Some of the examples are; Prussian blue (PB) CME in pH ~2 [32], ruthenium(IV) oxide modified carbon past electrode in 0.1 Na_2SO_4 [33], mixed valance ruthenium oxide-ruthenium cyanide CME in 0.2 M K_2SO_4 [34], cobalt phthalocyanine (CoPc) CME in pH 3.2 phosphate buffer [35], metal-phorphyrines and gold nanoparticles modified graphite electrodes in 0.1 M NaOH [36,37], vitamin B12 CME in an acidic pH [38] and pyrroloquinoline quinone CME in pH 8.42 borate buffer [39], etc. Although the above CMEs showed significant electrocatalytic behavior, preparation of stable electrodes and operation in physiological pH are challenging works. Note that the PB based CMEs were often reported to be less stable in neutral pH solution and losses its activity after several electrochemical cycles [40,41]. Nafion®, a perfluoro cationic exchanging polymer with perfluoro back bone, is considered as a rigid matrix to prepare stable chemically modified electrodes and further to reproducible electroanalytical applications [42]. In this regard, Nafion/Graphene [43], Nafion/lead nitroprusside nanoparticles (PbNp) modified ceramic electrode (GCE/Nafion/PbNp) [44], Nafion/Lead oxide-Manganese oxide (PMO) modified screen printed electrode (SPE/Nf-PMO) [45], Nafion/Ruthenium oxide pyrochlore (NPyCME) [46] and Nafion/$Os(bpy)_3{}^{2+}$ [47] were reported. Nevertheless, most of the Nafion modified electrodes involve with either tedious (time consuming) synthetic steps [44–46] or expensive chemicals [44,46]. For instance, 4 days time required for the preparation of NPyCME [46] and the Os and Ru based chemicals were expensive. In 1997, Pan and Lee have prepared a Nafion-PMo12 composite material for electrochromism [48] and in the year 2005, Sauk et al. developed a composite of Nafion/polyphenylene oxide with PMo12 for direct methanol fuel-cell applications [49]. In this work we have prepared a {Nf-PMo12} composite electrode by simple solution phase mixing of Nf and PMo12, which requires 40 ± 2 min time only, and successfully demonstrated for efficient electrocatalytic CySH oxidation and anti-dermatophyte activity.

PMo12 is a keggin type of anionic complex that has several applications in fuel cells, catalysis, molecular materials, electrochromism, super capacitors, medicine and some antibacterial activity against *Escherichia coli* and *Bacillus subtilis* [50,51]. For the first time in this work, we found significant anti-dermatophytic activity of PMo12 and {Nf-PMo12}-CME toward *Trichophyton rubrum* (*T. rubrum*) organism. *Dermatophytes* are a kind of fungus that commonly causes skin disease in animals and humans. Organic and inorganic chemicals such as thymol, pyrithione [52,53], Ni- and Co-N,N′-o-phenylenebis(salicylindeneimine) complexes [54] and Cu-thiosemicarbazone complex [55] have been reported for the anti-dermatophytes activity. This work covers preparation, characterization, electrocatalytic oxidation and antimicrobial applications of the {Nf-PMo12}-CME.

2. Experimental

2.1. Chemicals and reagents

Nafion® 117 perflourinated ion-exchange powder, 5% weight solution in a mixture of lower aliphatic alcohols was obtained from Sigma and Aldrich, India. The following chemicals were of analytical grade purchased from Aldrich, used as received without any further purification; phosphomolybdic acid, CySH, tris(bipyridine)ruthenium(II) chloride and potassium hexacyanoferrate (III). Aqueous solutions were prepared using deionized and alkaline $KMnO_4$ distilled water (designated as DD water). Unless otherwise stated, pH 7 phosphate buffer solution (PBS) of ionic strength = 0.1 M was used as a supporting electrolyte in this study.

2.2. Apparatus

Voltammetric measurements were carried out using CHI model 660C electrochemical work station, USA with 10 mL working volume. The three electrode system consists of a GCE of 0.0707 cm^2 geometrical surface area and its CME as working electrode, a Ag/AgCl with 3 M KCl as a reference electrode and a platinum wire as a counter electrode. The bio-analytical system (BAS, USA) polishing kit was used to polish the GCE surface. The surface of the GCE was cleaned both mechanically (polished with 1 μm alumina powder in the BAS polishing kit, cleaned with acetone and washed with DD water) and electrochemically (by performing cyclic

Table 1
Reported CMEs in the literature for CySH electrocatalytic oxidation with having higher oxidation potentials and interferences.

Reported CMEs for CySH oxidation	pH	E_{pa}/V	Possible interference
1. FTO glass substrate/Prussian blue	2.5	1.2[a] [32]	$NO_2{}^-$ [70]
2. CPE/Ruthenium (IV) oxide	7	1.2[a] [33]	Methionine [72]
3. GCE/Mixed valance ruthenium oxide-ruthenium cyanide	2	0.92[b] [34]	–
4. GCE/CoPc	3.2	0.6[b] [35]	$NO_2{}^-$ [74]
5. CCE/Nafion/PbNp	4	0.78[a] [44]	–
6. SPE/Nf-PMO	7.5	0.8[b] [45]	–
7. GCE/NPyCME	7.2	0.63[b] [46]	Hx [69], $NO_2{}^-$ [42]
8. GCE/Nafion/$Os(bpy)_3{}^{2+}$	4	0.6[b] [47]	$NO_2{}^-$ [75]
9. CPE/Oxovanadium (IV) complex of salen	5	0.66[a] [69]	$NO_2{}^-$ [76]
10. GCE/Indium hexacyanoferrate	2	1.2[b] [70]	$NO_2{}^-$ [77]
11. GCE/{Nf-PMo_{12}} (in this work)	7	0.58[b]	–

[a] 1 = vs. SCE.

[b] 2 = vs. Ag/AgCl.