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# One-step selective screening of bioactive molecules in living cells using sulfur-doped microporous carbon

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ABSTRACT

A metal-free electrode using heteroatom-doped microporous carbon was fabricated for the ultrasensitive monitoring of mono-bioactive molecules and the selective signaling of dopamine (DA) secreted by living cells. The constructed electrode based on sulfur-doped microporous carbon (S-MC) shows a high surface area, a spherical construction, numerous carbon chain defects, and microporous structures, which are the key factors of the interactive signaling transducer, fast response, and active interfacial surfaces. The intrinsic features of S-MC with different %S-doping (S-MC-1, and S-MC-2) through the $sp^2$-carbon chain create abundant catalytic active sites, facilitate molecular diffusion through the microporous structure, promote strong binding with the targeted molecules, and induce interactions at electrolyte–electrode interfaces. The S-MC-1 provides selective signaling in a tertiary mixture of DA, ascorbic acid (AA), and uric acid (UA) with a high sensitivity and a wide linear range of 0.01–5, 10–4000, and 1–2000 μM, respectively. The detection limits were set at 3 nM, 1.26 μM, and 0.23 μM for DA, AA, and UA respectively. The S-MC-1 demonstrated a selective screening of DA released from PC12 cells under a $K^+$ ion- stimulator with high sensitivity and promoted high biocompatibility, low cytotoxicity, high stability, and reliable reproducibility (%RSD ranged from 1 to 2.7). Our findings indicated that the S-MC-1 can be utilized as an in-vitro model for simultaneously monitoring extracellular-DA secreted from living cells and sensing mono-bioactive molecules in biological samples.

## 1. Introduction

The control and early investigation of various diseases and genetic problems depend on the level of bioactive molecules, such as dopamine (DA), ascorbic acid (AA), and uric acid (UA), (Chen and Chatterjee, 2013; Li et al., 2013). For instance, DA is a catecholamine neurotransmitter in the mammalian central nervous system, where it controls learning, cognition, locomotion, emotion, and working memory (Zhang et al., 2015a). The investigation of neurological disorders and psychiatric disturbances, such as schizophrenia, cocaine addiction, and Parkinson's disease is depended on the DA level in the brain and/or human blood serum (Farjami et al., 2012; Zhang et al., 2015a). The AA target may act as antioxidants in the human diet, leading to play an important role in the prevention and treatment of several diseases, such as the common cold, mental illnesses, scurvy, and AIDS, as reported elsewhere (Huang et al., 2008). UA is one of the mono molecules present in human fluids, and its level in urine and serum is an indicator of several diseases, such as gout, hyperuricemia, and Lesch-Nyhan syndrome (Jiang and Du, 2014). The successful development of a biosensor for the rapid, cost-effective, and on-site monitoring of a wide linear range of targets is gaining considerable interest. Different techniques, including fluorescence microscopy, high-performance liquid chromatography, capillary electrophoresis, and spectrophotometry were effectively used for monitoring of species (Cheng et al., 2000; Gong, 2013; Lapainis et al., 2007; Wachman et al., 2004). However, on-site, real monitoring, and selective detection of multi-species in one-post screen assay remain challenge.

Electrochemical biosensors have been gaining significant attention for their capability to recognize biomolecules such as DA, AA, and UA because of their high sensitivity, easy operation, low cost, rapid response, handling convenience, qualification for in situ monitoring, excellent compatibility with miniaturization, and good selectivity (Akhtar et al., 2017; Emran et al., 2017, 2018a, 2018b). DA, AA, and UA coexist in the extracellular fluid of the central nervous system and the serum. A reliable analytical method for the one-pot measurement of DA, AA, and UA levels in a biological sample is urgently needed for the in vitro monitoring of DA from its original sources, including the brain or dopaminergic cells, such as PC12, and even in the human blood serum

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(Liu et al., 2014; Qu et al., 2013; Sun et al., 2015a; Wu et al., 2015; Zhang et al., 2013).

The simultaneous detection of DA and UA in the presence of AA was performed based on composite materials (carbon-based materials, such as graphene and multiwall carbon nanotubes and other metal or metal oxides), such as Pt/reduced graphene oxide (Pt/RGO), 3D porous graphene, pDA/(MWCNT), poly(l-methionine)/AuNPs/GCE, 1D carbon nanomaterial, and ZnO-polyaniline/RGO (Ghanbari and Moloudi, 2016; Ojani et al., 2014; Palanisamy et al., 2015; Wang et al., 2016a, 2016b). Previous studies simultaneously detected DA, AA, and UA, such as activated graphene/MWCNT nanocomposite-loaded Au nanoclusters, flexible graphene fiber functionalized by $NiCo_2O_4$ nanowires, $Fe_3O_4$ @ N-doped carbon nanotubes, Pt@GO, AuNPs-GO poly(2,6-pyridine dicarboxylic acid), N,S-dual doping of porous carbon, CTAB-fGO/MWCNT, NiCo@N-doped carbon nanoplates, and graphene-ZnO (Abdelwahab and Shim, 2015; Cai et al., 2016; Fernandes et al., 2014; Gao et al., 2014; Sun et al., 2011; Tığ et al., 2017; Yang and Li, 2014; Zhang et al., 2015b, 2016). These significant developments of electrodes may enable the simultaneous detection of AA, DA, and UA in on-site monitoring assay. However, fabrication of simple, low-cost capital and easy-to-use electrodes yet associated with high-performance sensing assays of biological target species is urgently needed in future design of electrochemical biosensors for clinical application.

The porous materials has received considerable attention in many applications, such as biosensors, catalysis, fuel cells, antimicrobial, adsorption and energy storage (Aboelmagd et al., 2015; Akhtar et al., 2014; Azzam et al., 2017; Derbalah et al., 2015; El-Safty et al., 2013a; Hassen et al., 2016b; Khairy and El-Safty, 2013, 2014, 2015; Khairy et al., 2012). Carbon-based materials have enormous characteristic features, such as a large surface area, numerous edge-plane-like defects, which are directly reflected on its highly electrocatalytic activity, and significant biosensing applications (Inagaki et al., 2016). The addition of a heteroatoms, such as nitrogen, sulfur, phosphorous, and boron, into the graphitic carbon network could enhance the electrocatalytic activity of carbon materials and change the surface characteristics of the carbon matrix, including hydrophobicity, electrical resistivity, charge transfer, and acid/base levels (Chen et al., 2014; Ismagilov et al., 2009; Xiao et al., 2005).

In the present work, the design of metal-free catalysis electrode based on heteroatom-doped carbon material for the ultrasensitive and selective detection of mono-bioactive molecules in the biological fluids and secreted from living cells was achieved. The S-atoms acted as heteroatom dopants and altered the carbon-microspheres for efficient electrocatalysis with high active sites. The S-MC-1 was characterized and provided a high surface area, a dominant microporous structure, a large pore volume, and a microsphere structure, making it an ideal mediator surface for signaling transduction. The microporous structure with efficient doping of S-atoms created highly efficient active sites, facilitated molecular diffusion, and promoted interactions at electrolyte–electrode interfaces. The S-MC-1 showed a real evidence of selective and simultaneous signaling of AA, DA, and UA in the tertiary mixture. The high biocompatibility, low cytotoxicity, high sensitivity, and significant selectivity of the S-MC-1 were ideal as in vitro DA-sensor model in living cells (PC12) and biological samples.

## 2. Experimental section

### 2.1. Synthesis of sulfur-doped microporous carbon (S-MC-1 and S-MC-2)

Sulfur-doped microporous carbon (S-MC) was synthesized as follows: 2 g of D-glucose was dissolved in 50 mL Milli-Q water and sonicated for 30 min. Then, two samples of 0.5 and 1 g of thiourea with a mass ration of D-glucose: thiourea are 4:1 and 2:1. Each one was dissolved in 20 mL Milli-Q water and sonicated for 30 min. The thiourea solution was added to the D-glucose solution and continuously stirred for 4 h. After that, the two solutions were transferred to 100 mL Teflon-sealed autoclaves and maintained at 180 °C for 24 h. After being cooled to room temperature, the black precipitate of the desired materials S-MC-1 (4:1) and S-MC-2 (2:1) were washed several times with water/ethanol and then dried at 60 °C for 24 h. The desired black materials were annealed at 800 °C under $N_2$ atmosphere for 4 h with a step increase in temperature by 2 °C/min.

### 2.2. Cell culture

The PC12 cell line was obtained from PC12 (ATCC® CRL1721™) and was cultured by incubation under 5% $CO_2$ at 37 °C in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS) and 10% heat-inactivated serum. Cells were passaged every five days and the medium was changed two-three times a week throughout the lifetime of all cultures.

## 3. Results and discussion

### 3.1. Micrometric spherical construction of S-doped microporous carbon

The spherical construction growth of carbon material was formed with D-glucose acting as the source of carbon spheres after polymerization and carbonization at high temperature under hydrothermal carbonization (HTC) conditions (Wang et al., 2002). The S-doped carbon spheres from D-glucose were formed by adding thiourea as the S-source under hydrothermal conditions. Two different mass ratios of thiourea (% S) was added to 2 g of D-glucose (glucose: thiourea = 4:1 and 2:1) and heated under HTC at 180 °C for 24 h. Aromatization and carbonization were performed with the formation of carbon spheres as in Scheme S1. The presence of thiourea, which is the source of S-atoms during the polymerization and spherical carbon growth, advanced the S-atom to the carbon chain and formed S-doped carbon with different sulfur contents as its source concentration. The complete carbonization of the S-MC was formed with the microporous network after annealing at 800 °C under $N_2$ flow at an increasing rate of 2 °C/min. After annealing at 800 °C, two varied materials appeared and were named S-MC-1 and S-MC-2 according to the mass ratios of each sample.

### 3.2. Morphological and structural assessments of S-MC

The S-MC hieratical morphology was investigated through field-emission scanning electron microscopy (FE-SEM), as shown in Fig. S1(A-D). As illustrated in Fig. S1A, a high yield of carbon spheres of S-MC-2 was formed with formally aligned spheres. The formation of spherical carbon from D-glucose was grown after successive condensations and polymerization in a spherical shape. High-magnification FE-SEM resulted in micro-spherical carbon structures with size in the range of 3–5 μm, as presented in the inset of Fig. S1A. Fig. S1B shows that in the low-magnification FE-SEM of S-MC-1, symmetrical and formal micro-spheres of S- doped carbon (S-MC-1) were observed, where a high yield and different spheres size were formed. Fig. S1(C & D) illustrates the formation of spherical carbon with a homogeneous spherical distribution, and 3D spheres construction. The self-assembly of spherical carbon-doped materials was realized with a microsphere structure in the range of 1.5–5 μm.

The effective and actual percentage of S-atoms in the carbon microspheres of S-MC-1 and S-MC-2 were investigated through EDX-SEM. Fig. S1(E-i-E-iv) shows the EDX-SEM for S-MC-1 and displays the homogeneity and formality distribution of the C, O, and S atoms on the sphere's surface. Furthermore, Fig. S1(F-i-F-iv) shows the EDX-SEM for S-MC-2 and displays the presence and distribution of C, O, and S. The % mass for C, O, and S of S-MC-1 and S-MC-2 illustrate the higher percentage for S-atoms in S-MC-2 as thiourea concentration was increased. The mass of S increased from 5.27% of S-MC-1–8.45% of the S-MC-2, whereas the mass of C and O were decreased from 74.1% to 73.07% and from 20.63% to 18.74%, respectively. Thus, the decrease in the mass of