Double imprinting in a single molecularly imprinted polymer format for the determination of ascorbic acid and dopamine

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ABSTRACT

A new molecularly imprinted polymer – carbon composite fiber is constructed using reversible addition-fragmentation chain transfer polymerization technique. The fiber was evaluated as a sensor for the simultaneous determination of ascorbic acid and dopamine at ultratrace level, in aqueous samples, without any cross-reactivity. The binding characteristics of ascorbic acid and dopamine were also evaluated by differential pulse cathodic stripping voltammetry. Copyright © 2011 VBRI press.

Keywords: Molecularly imprinted polymer-carbon composite fiber; double imprinting, differential pulse cathodic stripping voltammetry; ascorbic acid and dopamine.

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Introduction

Artificial molecular recognition elements prepared by imprinting techniques attract increasing attention due to their potentials to act as selective sorbents in analytical sample pretreatment, chemical sensing, drug delivery, catalysis, dedicated separations, and finally screening elements in drug discovery. In most of the accounts of molecular imprinting, a single template molecule is used to create specific binding sites in the molecularly imprinted polymer (MIP) motif. However, the imprinting process should not be limited to a single template, and several compounds can also be imprinted simultaneously in a single polymer format. The concept of double imprinting introduces an additional degree of freedom in the material design, because the combination of two different print molecules enables the creation of more suitable hollows for analyte re-inclusion, improved diffusion pathways, and different classes of compounds can be extracted, separated, assayed, detected, or otherwise analyzed at one time [**1, 2**]. In this work, we have fabricated a monolithic fiber of MIP-

carbon composite, where the non-conducting MIP layer was changed to be conducting, in the presence of carbon particles (dispersed as organized strip). Unlike the general MIP-composite electrode preparation protocol [**3**], we have resorted to a fresh approach for an in-situ synthesis of MIP-carbon composite fiber via reversible addition-fragmentation chain transfer (RAFT) polymerization technique. RAFT has recently been emerged as a promising technique due to its versatility and simplicity, and the product (polymer) is free from the contamination of metal catalyst [**4**]. RAFT is a degenerative chain transfer free radical polymerization process. Such living radical polymerization technique is reportedly known to be creating homogeneous binding sites (cavities) within the MIP network.

The proposed MIP-carbon composite electrode was examined for the simultaneous determination of ascorbic acid (AA) and dopamine (DA), in aqueous samples using differential pulse cathodic stripping voltammetry (DPCSV). AA is a soluble vitamin present in many biological systems and in multivitamin preparations, which are commonly being used as supplement to inadequate dietary intake and as anti-oxidants [**5**]. The low concentration of AA in human blood serum results in scurvy and hypovitaminosis. The prevalence and risk factors owing to hypovitaminosis C, consequent upon low serum ascorbic acid level, cause clinical manifestations primarily of hemorrhagic syndrome, edema, stomatologic signs, arthritis, myalgia, anorexia, and depression. Dopamine (DA), a biogenic catecholamine, is among one of the neurotransmitters of central and peripheral nervous systems. Measurement of DA in biological samples is useful for the clinical diagnosis of chronically progressive neurodegenerative diseases, e.g. Parkinson's disease (PD), schizophrenia and Alzheimer's disorder, with low DA levels in serum. Therefore, it is essential to develop a simple and rapid method for the determination of these biological molecules in routine analysis. Although numerous methods have been used for the selective determination of AA [**6-10**] and DA [**11-15**] including high performance liquid chromatography [**6, 11**], capillary electrophoresis [7, 12], soli-phase extraction [**8, 13**], and electrochemical methods [**9, 10, 14, 15**], only a few electrochemical attempts have been made for the simultaneous determination of AA and DA [**16-18**]. However, consequent upon the overlap of their voltammetric responses, the severe electrode fouling, poor selectivity, and reproducibility associated with these methods [**16-18**] make simultaneous determination of AA and DA highly difficult. Furthermore, no MIP-based technique is, hitherto, reported for the simultaneous determination of AA and DA. The present work thus deals, for the first time, with the high selectivity and sensitive simultaneous determination of AA and DA.

Experimental

Materials and reagents

Ascorbic acid (AA), carbon powder (1-2μ), and solvents dimethylsulphoxide (DMSO), methanol, and ethanol were purchased from Loba chemie pvt. Ltd. (Mumbai, India). Ethylene glycol dimethacrylate (EGDMA), dopamine (DA), and benzyl-N, N-diethyldithiocarbamate (BDC,

iniferter) were supplied from Aldrich (Steinheim, Germany). All the reagents were of AR grade and used as received. Phosphate buffer solution (PBS) (pH 7.8, ionic strength 0.1 M) was used as a supporting electrolyte. Standard stock solutions (500 mg L^{-1}) of DA and AA were prepared using deionized triple-distilled water.

Apparatus

Voltammetric measurements were carried out with a polarographic analyzer/stripping voltammeter [model 264 A, EG & G Princeton Applied Research (PAR)] in conjunction with an electrode assembly (PAR model 303 A) and X–Y chart recorder (PAR model RE 0089) (**Fig. 1 A**). Herein a conventional three-electrode system was used where MIP-carbon composite fiber, platinum wire, and saturated Ag/AgCl served as working, auxiliary, and reference electrodes, respectively. 1H NMR characterization was made with the help of JEOL AL 300 FT/NMR (Japan) spectrometer. Morphological images of MIP-carbon composite were recorded on a scanning electron microscope (SEM) (JEOL, JSM, model 840 A, Netherlands).

Preparation of MIP-carbon composite

The monomer, 2,4,6-trisacrylamido-1,3,5-triazine (TAT), was prepared and characterized following a known recipe [**19**]. Accordingly, monomer (TAT, 1.0 mmol, 1.0 mL DMSO) and templates (AA and DA, 0.5 mmol of each dissolved in 1.0 mL DMSO) solutions were mixed together, followed by the addition of EGDMA (5.0 mmol, 1.0 mL).To this mixture iniferter (BDC, 0.5 mL) and an optimized amount (80 mg) of carbon powder were added and stirred for 10 min to obtain a homogeneous but slightly viscous solution. The whole content was degassed gently with N_2 stream for 10 min. Finally, this mixture was filled in glass capillaries (inner diameter 0.8 mm, length 4.0 cm) with the help of a syringe and tightly sealed their openings by teflon tape. The filled-in capillaries were then kept strictly in the horizontal position in an oven, where the polymerization mixture was cured in a UV chamber for 4-5 h. This helped shaping MIP-carbon composite (monolithic) fiber in which carbon particles were organized as strip, under the action of gravity. The fibers were gently pushed out of capillaries by an insertable wire of diameter just close to that of capillary. The templates were retrieved from these fibers by immersing into H_2O–methanol (9:1, v/v) solution for 40 min, with mechanical stirring of the eluent at 600 rpm, until no voltammetric response of the template was observed.

Sensor fabrication and voltammetric procedure

For the sensor fabrication, 5 .0 mm MIP fiber (diameter 0.8 mm) was inserted in another capillary (6 mm length), which was half-filled with an aqueous agarose solution (0.2 g, 10%, 60^0C). This way only 1 .0 mm length of fiber was fixed inside the agarose (for an easy electrical contact) and remaining 4 mm fiber retained out of the capillary. The capillary was housed with one end in a micropipette tip for safe handling of the extruded fiber; while a copper wire was used at agarose end for the electrical connection (**Fig. 1B**). Electrochemical experiments were performed in a three-

electrode cell containing 10 mL PBS (pH 7.8). All DPCSV runs were recorded after purging with N_2 gas for 10 min. After blank run, test solution was added into the cell for subsequent measurement. After analyte accumulation for 90 s at +1.2V vs. Ag/AgCl and 15 s equilibration time, DPCSV runs were recorded in the potential range varying from +0.5 to -1.2V at a scan rate 10mVs^{-1}, pulse amplitude 25 mV, and pulse width 50 ms. All DPCSV runs for each concentration of test analyte were quantified using the method of standard addition [20]. The limit of detection *(LOD)* was calculated by the standard procedure [20].

Fig. 1. (A) Apparatus for voltammetric measurement: (a) stripping voltammeter, (b) X-Y recorder, (c) three electrode assembly. (B) Picture of fabricated MIP-carbon composite fiber sensor: (a) copper wire, (b) micropipette tip, (c) agarose solution, and (d) MIP-carbon composite fiber.

Results and discussion

Spectral characterization and recognition mechanism

The rebinding of AA and DA in their respective molecular cavities, located at the surface or deeper inside the fiber textures, is represented in the structural diagram (**Fig. 2**). This revealed that the recognition occurred as a consequence of multiple hydrogen bonds. The proton NMR suggested binding interactions as follows: all AA peaks
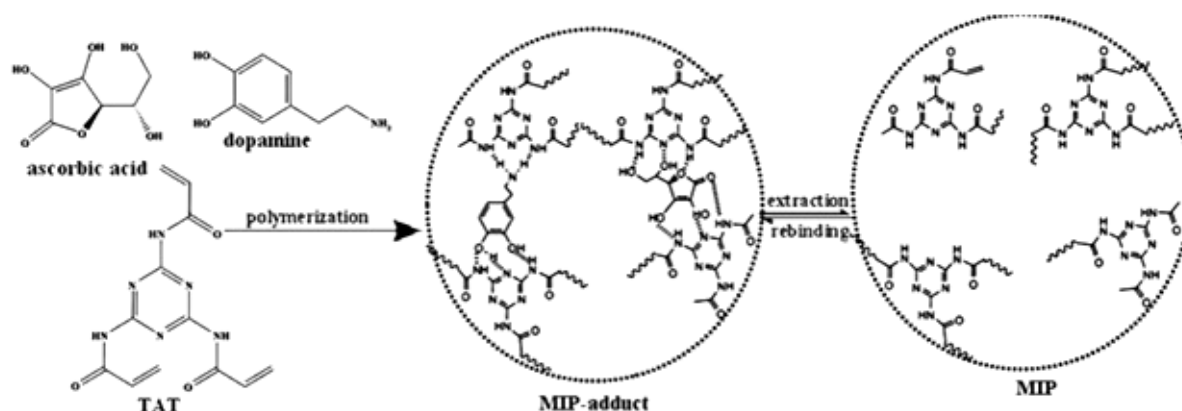
(meta, -OH), 6.2 (para, -OH), and 2.5 ($-NH_2$) ppm] were shifted downfield to 11.4, 3.1 ppm (in case of AA) and 7.9, 6.25, 3.3 ppm (in case of DA), after binding with MIP. These peaks were simultaneously appeared and disappeared after rebinding and extraction, respectively. The porosity of the MIP fiber cross-section could easily be visualized from SEM (**Fig. 3C**). The black strip of ordered carbon particles can be seen in SEM (**Fig. 3B**) and even by the naked eye (**Fig. 3A**). MIP-carbon composite fiber sensors were obtained reproducibly with consistent thickness of 0.8 mm, and with high endurance; these fibers were further regenerated by the method of template extraction for multiple measurements (more than 50 consecutive experiments).
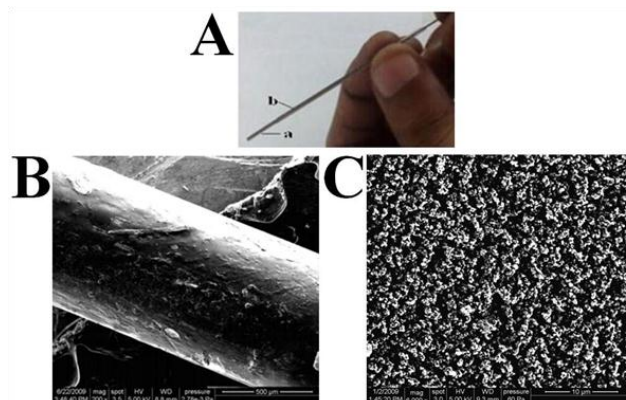

Fig. 3. (A) Picture of MIP-carbon composite fiber: (a) pushed out fiber with a visible black carbon strip, (b) glass capillary. (B) and (C) represent SEM images of MIP-carbon composite fiber at magnifications 200X and 6000X, respectively.

Simultaneous determination of AA and DA in aqueous medium

Typical DPCSV curves for the different concentrations of AA and DA demonstrated very smooth peaks (**Fig. 4**) both

Fig. 2. MIP-carbon composite fiber imprinted with AA and DA.

[10.8 (ring -OH) and 3.0 (-OH) ppm] and DA peaks [7.6

individually (**Fig. 4 d, e** for AA and **Fig. 4 b, c** for DA) and

in their mixtures (AA and DA, **Fig. 4 f, g**). This reveals no interference of either AA or DA in their simultaneous determination. The linear relationships were found for AA and DA, in terms of calibration equations (DPCSV current, *Ipc/μA versus* concentration, *C/ng mL^{-1})* as given below:

● AA, concentration range 8.916-29.126 ng mL^{-1}, *Ipc* = (7.803 ± 0.074) C + (1.758 ± 0.406), ν =0.99, n = 6. *LOD* = 2.635 ng mL^{-1} (*3σ, RSD* = 0.82%), recovery = 92.0-95.1%

● DA, concentration range 8.916-29.126 ng mL^{-1}, *Ipc* = (12.372 ± 0.173) C + (2.589 ± 0.826), ν =0.99, n = 6. *LOD* = 2.247 ng mL^{-1} (*3σ, RSD* = 0.64%), recovery = 93.4-94.7%
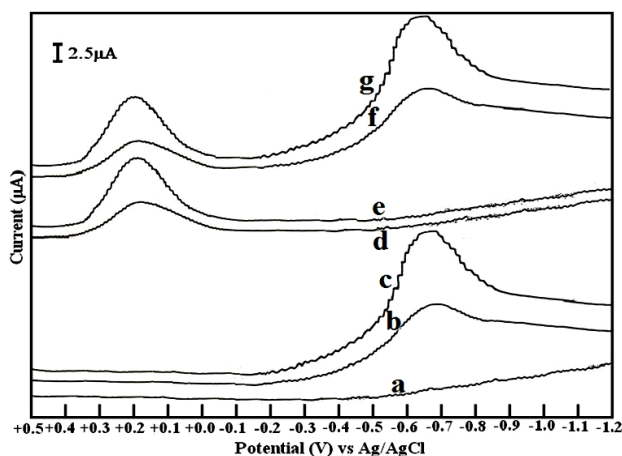

Fig. 4 DPCSV response of MIP-carbon composite fiber sensor: (a) blank, (b) and (d) 10.25 ng mL^{-1}, for DA and AA, respectively; (c) and (e) 16.50 ng mL^{-1}, for DA and AA, respectively; (f) AA and DA mixture (both 10.25 ng mL^{-1}); (g) AA and DA mixture (both 16.50 ng mL^{-1}).

Interference studies

The present method did not reveal any cross-reactivity with interfering species viz, cysteine, tyrosine, histidine, glycine, cystine, phenylalanine, uric acid, epinephrine, urea, and glucose. This showed a selective and specific binding between MIP and template in question, without any false-positive contributions.

Conclusion

This work describes the use of RAFT technique to prepare MIP-carbon composite fiber sensor for the simultaneous determination of AA and DA, in aqueous samples, without any cross-reactivity and false-positives. MIP-fiber sensor has reproducible characteristics for highly sensitive measurements, without any attendant problem of surface fouling.

Reference

1. Sreenivasan, K.; J. Mater. Sci. **2007**, *42*, 7575.
   DOI: 10.1007/s10853-007-1625-7
2. Suedee, R.; Srichana, T.; Chuchome, T.; Kongmark, U.; J. Chromatogr. B **2004,** *811*, 191.
   DOI: 10.1016/j.jchromb.2004.08.044
3. Andrea, P.; Miroslav, S.; Silvia, S.; Stanislav, M.; Sens. Actuators, B **2001,** *76*, 286.
   DOI: 10.1016/S0925-4005(01)00586-X
4. Mccormick, C. L.; Lowe, A. B.; Acc. Chem. Res. **2004**, *37,* 312.
   DOI: 10.1021/ar0302484
5. Yu, A. –M.; Chen, H. –Y.; Anal. Chim. Acta **1997,** *344*, 181.
   DOI: 10.1016/S0003-2670(97)00016-0
6. Gazdik, Z.; Zitka, O.; Petrlova, J.; Adam, V.; Zehnalek, J.; Horna, A.; Reznicek, V.; Beklova, M.; Kizek, R.; Sensors **2008,** *8*, 7097.
   DOI: 10.3390/s8117097
7. Qian, X.; Zhang, Q.; Zhang, Y.; Tu, Y.; Anal Sci. **2010,** *26*, 557.
   DOI: 10.2116/analsci.26.557
8. Legnerova, Z.; Satinsky, D.; Solich, P.; Anal. Chim. Acta **2003,** *497*, 165.
   DOI: 10.1016/j.aca.2003.07.007
9. Nezamzadeh, A.; Amini, M., K.; Faghihian, H.; Int. J. Electrochem. Sci., **2007,** *2*, 583.
   URL: http://www.electrochemsci.org
10. Behfar, A., A.; Sadeghi, N.; Jannat, B.; Oveisi, M., R.; Iranian J. Pharm. Res. **2010,** *9*, 123.
    URL: http://www.ijpr-online.com
11. Hubbard, K., E.; Wells, A.; Owens, T., S.; Tagen, M.; Fraga, C., H.; Stewart, C., F.; Biomed Chromatogr. **2010,** *24*, 626.
    DOI: 10.1002/bmc.1338
12. Zhang, L.; Qvb, S.; Wanga, Z.; Cheng, J.; J. Chromatogr. B **2003,** *792*, 381.
    DOI: 10.1016/S1570-0232(03)00323-4
13. Luliński, P.; Maciejewska, D.; Bamburowicz-Klimkowska, M.; Szutowski, M.; Molecules **2007,** *12*, 2434.
    DOI: 10.3390/12112434
14. Mazloum-Ardakani, M.; Rajabi, H.; Beitollahi, H.; Mirjalili, B., B., F.; Akbari, A.; Taghavinia, N.; Int. J. Electrochem. Sci., **2010,** *5*, 147.
    URL: http://www.electrochemsci.org
15. Manjunatha, J., G.; Swamy, B., E.; Gilbert, O.; Mamatha, G., P.; Sherigara, B., S.; Int. J. Electrochem. Sci., **2010,** *5*, 682.
    URL: http://www.electrochemsci.org
16. Xu, X.; Lin, Q.; Liu, A.; Chen, W.; Weng, X.; Wang, C.; Lin, X.; Chem. Pharm. Bull. **2010,** *58*, 788.
    DOI: 10.1248/cpb.58.788
17. Habibia, B.; Pournaghi-Azar, M., H.; Electrochim. Acta **2010,** *55*, 5492.
    DOI: 10.1016/j.electacta.2010.04.052
18. Tashkhourian, J.; Nezhad, M., R., H.; Khodavesi, J.; Javadi, S.; J. Elecroanal. Chem. **2009,** *633*, 85.
    DOI: 10.1016/j.jelechem.2009.04.028
19. Prasad, B. B.; Madhuri, R.; Tiwari, M. P.; Sharma, P. S.; Biosens. Bioelectron. **2010,** *25*, 2140.
    DOI: 10.1016/j.bios.2010.02.016
20. Skoog, D. A.; Holler, F.T.; Nieman, T.A.; Principles of Instrumental Analysis, 5th ed., Harcourt Brace College Publ., Orlando, FL, **1998**, p. 13.

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