NJC

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/njc

Synthesis of $NiCo_2O_4$/Nano-ZSM-5 nanocomposite material with enhanced electrochemical properties for the simultaneous determination of ascorbic acid, dopamine, uric acid and tryptophan

Balwinder Kaur[a], Biswarup Satpati[b], and Rajendra Srivastava*[a]

[a]*Department of Chemistry, Indian Institute of Technology Ropar, Rupnagar-140001, India*

[b]*Surface Physics and Material Science Division, Saha Institute of Nuclear Physics, 1/AF, Bidhannagar, Kolkata 700 064, India*

E-mail: rajendra@iitrpr.ac.in

Phone: +91-1881-242175; Fax: +91-1881-223395

Abstract

$NiCo_2O_4$/Nano-ZSM-5 nanocomposite materials were prepared by the calcination of the physical mixture of $NiCo_2O_4$ and Nano-ZSM-5. Materials were characterized by the complementary combination of X-ray diffraction, N_2-adsorption and electron microscopic techniques. Electrochemical sensor based on $NiCo_2O_4$/Nano-ZSM-5 nanocomposite was constructed for the simultaneous determination of ascorbic acid, dopamine, uric acid, and tryptophan. Among the materials investigated in this study, $NiCo_2O_4$ (30 %)/Nano-ZSM-5 modified glassy carbon electrode exhibited the highest electro catalytic activity with excellent stability, sensitivity, and selectivity. Under the optimum conditions, a wide linear range was obtained from 1-1200 µM for ascorbic acid, 0.6-900 µM for dopamine, 0.9-1000 µM for uric acid, and 0.9-1000 µM for tryptophan. The detection limit was found to be 0.8, 0.5, 0.7 and 0.7 µM for ascorbic acid, dopamine, uric acid, and tryptophan, respectively. The analytical performance of the developed sensor was demonstrated in the determination of ascorbic acid and dopamine in the commercial pharmaceutical preparations (vitamin C tablets and dopamine injections) and uric acid in human urine samples.

Keywords: $NiCo_2O_4$, zeolite, nanocomposite, electro catalysis, bio-molecule sensing, simultaneous determination.

---

1. Introduction

Nanocomposite materials play important role in the inter-disciplinary research areas for example, electronic device fabrication, energy sector, sensor applications, and green-chemical synthesis.[1-3] Most of these applications are related to electro-catalytic and catalytic activity of nanocomposite materials. Synergistic contribution provided by the constituent components of the nanocomposite materials significantly improves the electro-catalytic and catalytic activity for desired applications. In general, active metal/metal oxide components are supported on the high surface area (active or inert) materials. Zeolites play an important role in catalysis. However, its low surface area and microporous nature restrict their application, when large organic and bio-molecules are involved in the catalytic/electro-catalytic applications. To overcome this limitation, zeolites with interconnected intra or inter-crystalline mesoporosity have attracted much attention.[4, 5] Several attempts have been made for the preparation of nanosized zeolites using soft and hard templates.[6-8] In recent years, zeolites have attracted an increasing interest in the construction of sensors and biosensors because of their high surface area, porous nature and ease of surface functionalization with organic groups.[9-13] Nanocrystalline zeolite significantly improves the sensing capability when compared to bulk zeolite material.[14] Application of the zeolite in the construction of electrochemical sensor is limited because it does not contain any redox metal. Therefore, to introduce the desired activity, either metals/metal oxides are incorporated in the zeolite framework or supported on the external surface of zeolite. Our research group has developed a wide range of structure directing agents for the synthesis of ordered and disordred nanocrystlline zeolites of different framework structures.[15-18]

Transition metal oxides and mixed metal oxides exhibit tunable redox and conducting properties,[19] which are responsible for their wide applications in catalytic and electro-catalytic applications.[20-22] The spinel $NiCo_2O_4$ is currently popular as an electrode material due to its excellent electronic conductivity, multiple convertible valence states, and easily controllable morphologies.[23-25] It has been reported that $NiCo_2O_4$ possesses much better electronic conductivity, at least 2 orders of magnitude higher than nickel oxide and cobalt oxide.[26-28] The high electronic conductivity is beneficial for fast electron transfer in an electrode. $NiCo_2O_4$ adopts a pure spinel structure in which all the Ni ions occupy the octahedral sites and the Co ions are distributed among the tetrahedral and octahedral sites.[29, 30] Very recently, we have shown the application of $NiCo_2O_4$ modified electrode in the non-enzymatic electrochemical sensing of lindane.[31] Furthermore, we have also demonstrated the application of $NiCo_2O_4$ and $Ni(OH)_2$-$NiCo_2O_4$ modified electrodes in the electro-catalytic oxidation of methanol.[32, 33]

The development of a sensitive and selective method for the simultaneous determination of three or four bio-molecules is highly desirable for analytical application and diagnostic research. Co-existence of several molecules in physiological medium makes the accurate simultaneous detection difficult. For example, ascorbic acid (AA), dopamine (DA), uric acid (UA) and tryptophan (Trp) co-exist. These molecules play an important role in physiological function of organisms.[34-36] Though there are numerous reports available in literature for the simultaneous electrochemical determination of two or three such molecules.[37-39] However, only a few reports are available for the simultaneous determination all four analytes.[40-43] Our research group also made attempts to develop electrode materials for the simultaneous determination of these bio-molecules.[44, 45] AA showed very less anodic current response during the simultaneous determination of these bio-molecules at transition metal exchanged mesoporous polyaniline modified electrodes.[44] Furthermore, somewhat merged voltammeteric response was observed for DA and UA at higher concentration of analytes during the simultaneous determination at silver nanoparticle decorated graphene oxide electrodes.[45] Therefore, it is highly desirable to develop electrode materials, which can show highly distinct voltammeteric response with high sensitivity and low detection limit. This study is focused to achieve this objective.

In the present work, a novel $NiCo_2O_4$/Nano-ZSM-5 nanocomposite material was synthesized. Impressively high electro-catalytic activity was observed at $NiCo_2O_4$/Nano-ZSM-5 modified glassy carbon electrode (GCE) in the determination of AA, DA, UA, and Trp when compared with the bare GCE and $NiCo_2O_4$ modified GCE. To the best of our knowledge, this is the first report, which deals with the simultaneous determination of AA, DA, UA, and Trp using $NiCo_2O_4$/Nano-ZSM-5 nanocomposite as an electrode material.

2. Experimental Section

2.1. Materials

All chemicals used in the study were of analytical reagent grade and used as received without further purification. Tetraethylorthosilicate (TEOS, 98%), tetrapropylammonium hydroxide (TPAOH), propyltriethoxy silane (PrTES, 97%), and Nafion® 117 solution (5% a mixture of lower aliphatic alcohols and water) were purchased from sigma Aldrich. Dopamine, ascorbic acid, uric acid, and tryptophan were obtained from Himedia Pvt. Ltd. Nickel chloride ($NiCl_2.6H_2O$), cobalt chloride ($CoCl_2.6H_2O$), and urea were obtained from Spectrochem Pvt. Ltd. Deionized water from Millipore Milli-Q system (Resistivity 18 MΩcm) was used in the electrochemical studies. The standard buffer solutions with different pH values were prepared by adding 7.5 M aqueous NaOH solution to an aqueous solution of ortho phosphoric acid [1L of aqueous solution containing 10.11 mL of H_3PO_4 (85 wt % aqueous solution)] while magnetically stirring until the pH of the aqueous solution reached the desired value (1.0, 3.5, 5.0, 7.0, and 9.0). The electrochemical measurements were performed in the buffer solution (pH 3.5).

2.2 Sample preparation

Nanocrystalline zeolite (Nano-ZSM-5) was synthesized by following the reported procedure.[14] In a typical synthesis, 0.19 g of sodium aluminate (53 wt.% Al_2O_3, 43 wt.% Na_2O) was dissolved in 25 mL of distilled water (Solution A). 2.12 g of PrTES was mixed with 25 mL of TPAOH (1 M aq. solution) (Solution B). Solution A and solution B were mixed, and the resultant solution was stirred for 15 minutes at 300 K, until it became a clear solution. 19.13 g of TEOS was added into the resultant solution and stirring was continued for 6 h. The molar composition of the gel mixture was 90 TEOS/10 PrTES/1 Al_2O_3/3.3 Na_2O/25 TPAOH/2500 H_2O. This mixture was transferred to a Teflon-lined autoclave, and hydrothermally treated at 443 K for 3 days under static condition. The final product was filtered, washed with distilled water and dried at 373 K. Material was calcined at 823 K for 6 h under flowing air. For comparison, conventional ZSM-5 was synthesized at 443 K using the same synthesis composition as mentioned above for the Nano-ZSM-5, but without PrTES additive.

In a typical synthesis of $NiCo_2O_4$, 2.37 g $CoCl_2.6H_2O$, 1.19 g $NiCl_2.6H_2O$, and 2.7 g urea were dissolved in 75 mL distilled water and stirred for about 10 min. The reaction mixture was then transferred into a Teflon-lined stainless steel autoclave, and hydrothermally treated at 393 K for 6 h. Autoclave was cooled to room temperature and the reaction mixture was filtered, washed with distilled water and ethanol, followed by drying at 353 K for 24 h. Finally, the material was calcined at 573 K for 4 h using programmable Furness by maintaining the heating rate of 5 K/min.

For the synthesis of $NiCo_2O_4$/Nano-ZSM-5 nanocomposite materials, $NiCo_2O_4$ and Nano-ZSM-5 with different weight ratios (20, 30, and 40, denoted as $NiCo_2O_4$ (20 %)/Nano-ZSM-5, $NiCo_2O_4$ (30 %)/Nano-ZSM-5 and $NiCo_2O_4$ (40 %)/Nano-ZSM-5, respectively) were grounded uniformly with ethanol using mortar and pastel. Ethanol was slowly removed during the mixing process. The mixture was heated at 473 K in air for 45 min to remove the residual ethanol, followed by calcination at 613 K for 12 h in air to obtain $NiCo_2O_4$/Nano-ZSM-5 nanocomposite.

2.3 Instrumentation

X-ray diffraction (XRD) patterns were recorded in the 2θ range of 5–60° with a scan speed of 2°/min on a PANalytical X'PERT PRO diffractometer using Cu Kα radiation (λ=0.1542 nm, 40 kV, 40 mA) and a proportional counter detector. Nitrogen adsorption measurements were performed at 77 K by Quantachrome Instruments Autosorb-IQ volumetric adsorption analyzer. Sample was out-gassed at 573 K for 3 h in the degas port of the adsorption apparatus. The specific surface area of the material was calculated from the adsorption data points obtained at P/P_0 between 0.05–0.3 using the Brunauer-Emmett-Teller (BET) equation. The pore diameter was estimated using the Barret–Joyner–Halenda (BJH) method. Scanning electron microscopy (SEM) measurements were carried out on a JEOL JSM-6610LV to investigate the morphology of the zeolites. TEM investigations were carried out using FEI, Tecnai G^2 F30, S-Twin microscope operating at 300 kV. High-angle annular dark field scanning transmission electron microscopy (HAADF-STEM) was used here using the same microscope, which is equipped with a scanning unit and a HAADF detector from Fischione (model 3000). The compositional analysis was performed using energy dispersive X-ray spectroscopy (EDS, EDAX Instruments) attachment on the Tecnai G^2 F30. The sample was dispersed in ethanol using ultrasonic bath, and dispersed sample was mounted on a carbon coated Cu grid, dried, and used for TEM measurement.

2.4 Electrode fabrication

Cyclic voltammetry (CV), differential pulse voltammetry (DPV) and chronoamperometry (CA) studies were performed using Potentiostat-Galvanostat BASi EPSILON, USA. A three-electrode electrochemical cell was employed with Ag/AgCl as the reference electrode (3M KCl), $NiCo_2O_4$/Nano-ZSM-5 mounted on glassy carbon (3 mm diameter) as the working electrode and Pt foil as the counter electrode. Before modification, the glassy carbon electrode (GCE) was first polished to a mirror like surface with alumina slurry and then ultra-sonicated in ethanol and deionized water for 5 min, respectively. For the preparation of $NiCo_2O_4$/Nano-ZSM-5 suspension, 2 mg of $NiCo_2O_4$/Nano-ZSM-5 and 10 µL of Nafion were added to 1 mL of distilled water. The resultant mixture was sonicated for 30 minutes to obtain a uniform suspension. 10 µL aliquot of obtained $NiCo_2O_4$/Nano-ZSM-5 suspension was then placed onto GCE surface. The electrode was dried in air leaving the material mounted onto the GCE surface. Nafion is chemically inert and exhibit good conductivity. Therefore, it is used as a binder in the preparation of modified electrodes. Hence, Nafion was used as a binder in the fabrication of $NiCo_2O_4$/Nano-ZSM-5/GCE working electrode. Electrochemical impedance spectroscopy (EIS) was performed using Autolab PGSTAT302N.

2.5 Real sample preparation

Dopamine injections (DOPAA, AAA Pharma; DOMIN, NEON Labs; and DOPRESS, UNITED Biotech) were obtained from Kailash Medical Store, Ropar. 10 mL of 0.1 M stock solution of dopamine hydrochloride injection was prepared using deionized water. Required amount of this solution was added to the electrochemical cell consisting 10 mL of buffer solution (pH 3.5). Vitamin C tablets (Nicholas Paramil, Glaxo Smith Kline Pharmaceuticals Ltd., and CECON ABBOTT) were obtained from Kailash Medical Store, Ropar. Vitamin C tablets (labeled 500 mg vitamin C per tablet) were completely grounded and homogenized before preparing 10 mL of 0.1 M stock solution. Required amount of this solution was added to the electrochemical cell consisting 10 mL of buffer solution (pH 3.5). 500 µL of urine samples were diluted to 10 mL using deionized water. Required amount of diluted urine samples were added to the electrochemical cell consisting 10 mL of buffer solution (pH 3.5) for the determination of UA.

3. Results and Discussion

In this work, $NiCo_2O_4$/Nano-ZSM-5 nanocomposite materials with different weight ratios were prepared by the calcination of $NiCo_2O_4$ and Nano-ZSM-5 at 613 K. Temperature 613 K is chosen because of the instability of $NiCo_2O_4$ in air at a temperature exceeding 673 K.[46] During the calcination process, large $NiCo_2O_4$ particles broke into the small particles and adhered on to the surface of Nano-ZSM-5. The detail of physico-chemical characterization is provided in the following section. $NiCo_2O_4$/Nano-ZSM-5 modified GCE electrodes were constructed for the simultaneous determination of AA, DA, UA, and Trp.

3.1 Physico-chemical characterizations of the materials

Nano-ZSM-5 exhibited MFI framework structure with high phase purity, which was confirmed by XRD (Fig. 1a). Diffraction peaks for $NiCo_2O_4$ can be indexed to a face-centered cubic $NiCo_2O_4$ (JCPDS Card no.73-1702) (Fig. 1a). The XRD pattern of $NiCo_2O_4$ (30 %)/Nano-ZSM-5 shows the diffraction peaks corresponding to both, $NiCo_2O_4$ and Nano-ZSM-5 phases (Fig. 1a). Calcination was favorable in the dispersion process, which appears to cause dispersion of $NiCo_2O_4$ on the surface of Nano-ZSM-5. Broadening in the diffraction peaks corresponding to Nano-ZSM-5 was observed in the XRD pattern of $NiCo_2O_4$ (30 %)/Nano-ZSM-5, which show that during the calcinations process, $NiCo_2O_4$ particles penetrates into the mesoporous void space of Nano-ZSM-5. Further, with the increase in $NiCo_2O_4$ content in $NiCo_2O_4$/Nano-ZSM-5 nanocomposite, increase in the broadening of XRD peaks corresponding to Nano-ZSM-5 was observed (Fig. S1).

The textural properties of the synthesized materials were investigated by N_2-sorption measurements. $NiCo_2O_4$ exhibited type IV isotherm with H3 hysteresis loop, which is the characteristics of mesoporous material. BJH pore size distribution (Fig. 1b, inset) shows a narrow pore size distribution centered at 3.8 nm along with a wide pore size distribution centered at 20.4 nm. The N_2-adsorption isotherms for Nano-ZSM-5 and $NiCo_2O_4$ (30 %)/Nano-ZSM-5 also exhibited a typical type-IV isotherm similar to that of mesoporous materials (Fig. 1b). The distinct increase of N_2 adsorption for Nano-ZSM-5 in the region $0.4<P/Po<0.9$ is interpreted as a capillary condensation in the intercrystalline mesopore void spaces. The mesopores show a pore size distribution in the range of 2-10 nm for Nano-ZSM-5. It is interesting to note that, pore size distribution in the range of 2-10 nm is absent in the case of $NiCo_2O_4$ (30 %)/Nano-ZSM-5, which further confirms that $NiCo_2O_4$ particles penetrate into the mesopore void spaces of Nano-ZSM-5. Textural properties obtained from N_2-adsorption study for different materials investigated in this study are summarized in Table 1. N_2-adsorption investigations clearly show that the total surface area, external surface area, and pore volume of Nano-ZSM-5 is much larger than that of conventional ZSM-5 (Table 1). During the synthesis of Nano-ZSM-5, additive PrTES disfavored the extended growth of large zeolite crystal, which resulted in the formation of very small size Nano-ZSM-5. Mesopores are created by the crystal packing of these zeolite nanocrystals. The BET surface area for $NiCo_2O_4$/Nano-ZSM-5 samples decreased with increase in the $NiCo_2O_4$ content. This decrease in the surface area also indicates that $NiCo_2O_4$ penetrates into the mesopores of Nano-ZSM-5, which resulted reduction in the pore volume and surface area of nanocomposite materials.

SEM was used to investigate the morphology of materials investigated in this study. Urchin-like morphology for $NiCo_2O_4$ was observed, which is composed of nanoleafs radially grown from the center (Fig. S2a). Uniform spherical shaped particles were observed for Nano-ZSM-5 (Fig. S2b). SEM image of $NiCo_2O_4$ (30 %)/Nano-ZSM-5 shows that some nanoparticles are adhered to the surface of spherical Nano-ZSM-5 (Fig. S2c). To obtain in-depth information for nanostructure of $NiCo_2O_4$ (30 %)/Nano-ZSM-5, TEM investigation was made. TEM images clearly show that $NiCo_2O_4$ particles are adhered to the surface of Nano-ZSM-5 (Fig. 2a-c). TEM images also confirmed that large spherical size particles are built with small zeolite nanocrystals of 15-20 nm (Fig. 2d). The HRTEM study of these decorating nanoparticles confirmed them to be of $NiCo_2O_4$. The high resolution TEM image of nanoparticle adhered to the surface of Nano-ZSM-5 in Fig. 2d clearly shows lattice fringes and the measured lattice spacing is 2.77 Å, which correspond to the (311) interplanar spacing of cubic $NiCo_2O_4$ [JCPDS Card no. 73-1704]. The chemical composition of the hybrid nanostructures were investigated using STEM-HAADF-EDX techniques. Fig. 3a depicts the STEM-HAADF image of $NiCo_2O_4$ (30 %)/Nano-ZSM-5 nanocomposite. Energy dispersive X-ray spectroscopy (EDX) data from regions marked by area 1 in Fig. 3a are plotted in Fig. 3b which clearly shows Co, Ni, Si and O. It may be noted that Cu and C signals observed in the EDX mapping are due to the carbon-coated copper grid used in the analysis. The spatial distributions of the atomic contents across $NiCo_2O_4$ (30 %)/Nano-ZSM-5 nanocomposite were obtained using drift corrected EDX imaging. The STEM-HAADF image and the corresponding chemical maps for Co, Ni, Si and O from the area marked by 2 in Fig. 3a are presented in Fig. 3c. EDX maps confirmed the decoration of Nano-ZSM-5 with $NiCo_2O_4$ nanoparticles.

3.2 Electrochemical characteristics of modified electrodes

Electrochemical impedance spectroscopy (EIS) was used to investigate the electrochemical characteristics of various modified glassy carbon electrodes ($NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE, $NiCo_2O_4$/GCE, Nano-ZSM-5/GCE, ZSM-5/GCE) and bare GCE. EIS was carried out in 0.1 M KCl solution containing 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ over a frequency range of 0.1 Hz to 10^5 Hz with the AC signal amplitude of 5 mV (Fig. 4). The Nyquist plot of the EIS includes a semicircular portion featuring electron-transfer-limiting process and a linear portion featuring a diffusion limiting step. The diameter of semicircular portion is equal to the electron transfer resistance, which controls the electron-transfer kinetics of the redox probe at the electrode interface. Fig. 4 shows that bare GCE exhibited a large semicircle portion indicating a high electron transfer resistance. Nano-ZSM-5/GCE and ZSM-5/GCE both exhibited reduced semicircular domain and linear portion indicating the mixed charge transfer and diffusion kinetics controlled reactions. However, a straight line was observed for both $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE and $NiCo_2O_4$/GCE in the whole frequency range without semi-circle indicating the diffusion controlled reaction. The diameter of the impedance arc decreased in the order of bare GCE>ZSM-5/GCE>Nano-ZSM-5/GCE>$NiCo_2O_4$/GCE>$NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE indicating that the electron-transfer resistance decreased in this order. Hence, $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE significantly facilitates the electron transfer rate and improves the diffusion of ferricyanide towards the electrode interface.

3.3 Electrochemical characteristics of AA, DA, UA, and Trp

The electrochemical behavior of AA, DA, UA, and Trp was investigated using CV, DPV and chronoamperometric techniques. In-depth investigations were made using $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE because of its higher electro-catalytic activity. For comparison, the studies were performed using bare GCE.

Fig. 5 shows a comparison of CVs of AA, DA, UA, and Trp, individually, at $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE and bare GCE in buffer solution (pH 3.5). The results show that all four analytes were oxidized with well-defined and distinguishable sharp oxidation peaks with peak potentials at 158, 394, 561 and 820 mV for AA, DA, UA, and Trp, respectively, at $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE (Fig. 5). On the other hand ill defined voltammetric response was obtained for AA and Trp at bare GCE. DA and UA exhibited oxidation peaks at 493 and 615 mV at bare GCE. The current response for all the four analytes was remarkably increased with decrease in the oxidation peak potential (decrease in the over potential) at $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE when compared to bare GCE. This may be attributable to the sluggish electron transfer rate at bare GCE. The increase in oxidation peak current and lowering of oxidation peak potential are an evidence of the catalytic effect of $NiCo_2O_4$ (30 %)/Nano-ZSM-5. Based on the results obtained, it can be concluded that the modification of GCE with $NiCo_2O_4$ (30 %)/Nano-ZSM-5 accelerates the electron transfer rate between the analytes and the electrode.

In order to investigate the kinetics of electrode reaction, CV was used. CV at various scan rates obtained at $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE for AA, DA, UA, and Trp shows that the anodic peak current increases with the increase in the scan rate (Fig. S3). DA exhibited both anodic and cathodic peaks, suggesting the oxidation of DA is a reversible process (Fig. S3b). Further, with the increase in scan rate, the oxidation peak moved to the positive direction and the reduction peak moved to the negative direction, and the peak-to-peak separation was increased. For AA, UA, and Trp, only anodic peak was observed, indicating that the oxidation is irreversible electrode process (Fig. S3a,c,d). The plot of the oxidation peak current against the square root of scan rate (10-600 mV/s) shows an excellent linear relationship for all the four analytes (Fig. S3a-d, inset), suggesting that the oxidation of these analytes at $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE is diffusion controlled process.[47]

Chronoamperometry was used to calculate the diffusion coefficient (D) and rate constant (k) for the electro-catalytic reaction (Fig. S4-7, Supporting information includes experimental details along with a brief discussion). The diffusion coefficients for AA, DA, UA, and Trp, were found to be 2.2×10^{-4}, 8.4×10^{-4}, 4.3×10^{-4}, and 1.4×10^{-4} cm^2/s, respectively. The rate constants for electro-catalytic oxidation of AA, DA, UA, and Trp were found as 7.8×10^3, 8.3×10^3, 3.8×10^2, and 2.8×10^3 1/s M, respectively.

The solution pH affects the electrochemical behavior of AA, DA, UA, and Trp. The effect of the pH on the electro-catalytic oxidation of AA, DA, UA, and Trp was investigated at $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE by varying the pH of the supporting electrolyte in a wider range (1–9) using DPV in a solution containing equal concentrations (10 μM) of AA, DA, UA and Trp. The anodic peak current was found to increase with increase in the pH of the medium, with a maximum anodic peak current at pH 3.5 (Fig. S8a). After pH 3.5, it was further decreased. This behavior was observed for all the four analytes. Peak potentials for the oxidation of AA, DA, UA, and Trp shifted negatively at higher pH value, indicating that protons take part in their electrode reaction processes (Fig. S8b). H^+ ions are the by-product formed in the electro catalytic oxidation of AA, DA, UA and Trp. At very low pH, the high concentration of H^+ hinders the electro catalytic oxidation of these bio-molecules due to common ion effect, thus producing a weak voltammetric response.[48] Perfluorosulfonated polymer Nafion (SO_3^- containing polymer) used as a binder in the electrode fabrication repels anions and facilitates the transportation of cations and neutral species.[49] It may be noted that the pKa_1 values of AA, UA, and Trp are 4.2, 5.4, and 2.4, respectively. By increasing the pH, the anionic form of analyte molecules predominates, which results drop in peak current, probably due to the repulsive electrostatic interactions between anionic species and electrode surface. Therefore, the increasing pH results drop in peak current. DA is protonated in acidic medium and deprotonated in basic medium. Therefore, DA can easily reach to the active sites of working electrode through Nafion resulting high peak current in the acidic medium. The maximum peak current response and separation of peak potentials was observed at pH 3.5. Therefore, in order to obtain high sensitivity and selectivity, pH 3.5 was selected as an optimum pH value for the determination of AA, DA, UA, and Trp.

$$Ep\ (AA) = -0.054\ pH + 0.427 \quad R^2 = 0.933 \qquad (1)$$

$$Ep\ (DA) = -0.059\ pH + 0.643 \quad R^2 = 0.962 \qquad (2)$$

$$Ep\ (UA) = -0.057\ pH + 0.759 \quad R^2 = 0.991 \qquad (3)$$

$$Ep\ (Trp) = -0.056\ pH + 1.060 \quad R^2 = 0.963 \qquad (4)$$

Slopes obtained from the linear behavior between the applied potential and the pH for AA, DA, UA, and Trp were found to be 0.054, 0.059, 0.057, and 0.056 V/pH (Eqs. 1-4), which are close to the anticipated Nernstian value (0.059 V/pH) for a two electrons/two protons reaction.[50] This suggests that the uptake of electrons was accompanied by equal number of protons. It is reported that the electrochemical oxidation of AA, DA, UA, and Trp follow a mechanism with $2e^-$ oxidation in rate-determining step,[45] which is also supported by our pH investigations. The anodic peak corresponds to the oxidation of hydroxyl groups to carbonyl groups of the furan ring in AA, oxidation of catechol to o-quinone in DA, oxidation of bridging double bond to hydroxyl groups in UA, and oxidation of phenyl ring in Trp (Scheme S1).

3.4 Individual electro-catalytic oxidation of AA, DA, UA, and Trp

The individual electro-catalytic oxidation of AA, DA, UA, and Trp was studied using DPV in buffer solution (pH 3.5) at $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE (Fig. 6). The DPV results show that all the four analytes wre oxidized with well-defined and distinguishable sharp oxidation peaks at $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE (Fig. 6). With increase in the concentration of analytes, the anodic peak current increased significantly. A linear dynamic range from 1-1200 μM with a calibration equation of $I_{AA}(\mu A) = 4.38 + 0.05\ C_{AA}\ (\mu M)$ (R^2= 0.998) was obtained for AA (Fig. 6a). A linear relationship in the range of 0.6-900 μM with a calibration equation of $I_{DA}(\mu A) = 7.34 + 0.12\ C_{DA}\ (\mu M)$ (R^2= 0.997) (Fig. 6b) was found for DA. A linear calibration for UA was found to be in the range of 0.9-1000 μM with calibration equation of $I_{UA}(\mu A) = 12.29 + 0.07\ C_{UA}\ (\mu M)$ (R^2= 0.999) (Fig. 6c) and the linear calibration range for Trp was found to be 0.9-1000 μM with a calibration equation of $I_{Trp}(\mu A) = 11.95 + 0.04\ C_{Trp}\ (\mu M)$ (R^2= 0.996) (Fig. 6d).

3.5 Simultaneous electrochemical determination of AA, DA, UA, and Trp

The objective of present work was to determine AA, DA, UA, and Trp, simultaneously as these four analytes co-exists. Applicability of $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE toward the simultaneous determination of AA, DA, UA, and Trp was investigated using DPV (Fig. 7a). DPV was carried out at $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE in 10 mL buffer solution (pH 3.5) (Fig. 7a). Four distinguished and sharp anodic peaks at potentials 158, 394, 561 and 820 mV corresponding to the oxidation of AA, DA, UA, and Trp were obtained during the DPV experiment of a quaternary mixture containing these analytes at $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE (Fig. 7a). The voltammograms for the quaternary mixture containing AA, DA, UA, and Trp were well separated from each other with a potential difference of $\Delta E_{AA–DA}$ = 236 mV, $\Delta E_{DA–UAA}$ = 167 mV and $\Delta E_{UA–Trp}$ = 259 mV, which is large enough for the simultaneous determination of these analytes in their quaternary mixture. When the concentration of these analytes in the electrochemical cell was increased, enhancement in the oxidation peak current was observed. The anodic peak current obtained was found to be linearly dependent on the concentration of analytes in the range of 1-1200 μM for AA, 0.6-900 μM for DA, 0.9-1000 μM for UA and 0.9-1000 μM for Trp with the sensitivity of 0.7, 1.3, 1.0, and 0.6 μA/ μM cm^2 and the lower detection limit (S/N=3) of 0.8, 0.5, 0.7 and 0.7 μM for AA, DA, UA, and Trp, respectively. These results demonstrate that $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE exhibits excellent electro-catalytic activity toward the simultaneous determination of AA, DA, UA, and Trp accurately from their quaternary mixture. The current response and sensitivity for the individual and simultaneous determination of these bio-molecules at $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE was found to be similar, confirming that no mutual interference occurred due to these bio-molecules during their simultaneous determination. The results clearly show that the individual or simultaneous determination of these bio-molecules can be achieved with high sensitivity at $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE. Among $NiCo_2O_4$/Nano-ZSM-5 materials with different weight ratio (20, 30, and 40), $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE exhibited the highest sensing ability. A comparison for the simultaneous electrochemical oxidation of AA, DA, UA, and Trp at $NiCo_2O_4$/Nano-ZSM-5/GCE with different weight ratio is provided in Fig. S9. Low activity of $NiCo_2O_4$ (20 %)/Nano-ZSM-5/GCE compared with $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE clearly show that higher amount of $NiCo_2O_4$ loading in nanocomposite material is better for the high electro-catalytic activity. However, with further increase in $NiCo_2O_4$ content, the electro-catalytic activity was not increased. This can be correlated to the textural properties of nanocomposite materials. Surface area and pore volume for $NiCo_2O_4$ (30 %)/Nano-ZSM-5 was found to be more when compared with $NiCo_2O_4$ (40 %)/Nano-ZSM-5. These results clearly show that the high dispersion of $NiCO_2O_4$ on the surface of Nano-ZSM-5 enhances the accessibility of analytes to the active sites and high surface area and pore volume are favourable for the diffusion of analytes/products from the active sites.

Simultaneous determination studies were also performed at Nano-ZSM-5/GCE, $NiCo_2O_4$/GCE, ZSM-5/GCE, and bare GCE using DPV in buffer solution (pH 3.5). However, in this manuscript, only a comparative electro-catalytic data for the simultaneous oxidation using different electrodes is presented. Bare GCE was unable to detect AA and an ill defined oxidation peak was obtained for Trp at higher potential (Fig. S10). DA and UA exhibited merged oxidation peaks. The oxidation peak current was found to be much lower at bare GCE when compared to $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE (Fig. S10). Nano-ZSM-5/GCE and ZSM-5/GCE were unable to detect AA and the oxidation peaks for DA, UA, and Trp appeared at higher potential with lower oxidation peak current than $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE. $NiCo_2O_4$/GCE was able to detect all the four analytes but the oxidation peak response for Trp was lower than Nano-ZSM-5/GCE and ZSM-5/GCE. The low current response for

$NiCo_2O_4$/GCE further confirms that high dispersion of $NiCo_2O_4$ on Nano-ZSM-5 matrix along with the facile diffusion of analytes/products are responsible for the high elelctro-catalytic activity of $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE. Among all these electrodes investigated, $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE exhibited the highest current response with the lowest oxidation potential (decrease in the over potential). A comparison of different modified GCE investigated in this study toward the simultaneous electro-catalytic oxidation of AA, DA, UA, and Trp is summarized (Fig. 8 and Table S1). Based on the experimental evidence, one can conclude that $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE exhibited superior sensing ability and current sensitivity compared to other modified GCE and bare GCE. The high activity of $NiCo_2O_4$ (30 %)/Nano-ZSM-5 can be attributed to the optimum loading and highly dispersed $NiCo_2O_4$ active centers on Nano-ZSM-5 matrix, along with its unique mesoporous structure, large surface area, and lower electron transfer resistance rate. High electro-catalytic activity of $NiCo_2O_4$ (30%) /Nano-ZSM-5 leads to fast electron transfer rate at the electrode/electrolyte interface and that is responsible for the high sensitivity. The comparison of the results shown in this paper with the published literature is provided in Table S2. This method is able to detect AA, DA, UA, and Trp simultaneously in a wider linear range and lower detection limit, when compared to the literature reports. Hence, $NiCo_2O_4$ (30 %)/Nano-ZSM-5 provides a better platform for the simultaneous and sensitive determination of AA, DA, UA, and Trp.

3.6 Reproducibility, stability, and anti-interference property of $NiCo_2O_4$ (30 %)/Nano-ZSM-5 /GCE

The reproducibility and stability of the sensor was evaluated in the sensing studies. Five $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE were made and their current responses to equimolar (10 μM) concentration of AA, DA, UA, and Trp, in their quaternary mixture were investigated. The relative standard deviation (RSD) was found to be 2.6 %, confirming that the fabrication method was highly reproducible. The long term stability of the sensor was evaluated by measuring its sensitivity towards equimolar (10 μM) concentration of AA, DA, UA, and Trp, in their quaternary mixture for 20 days. The sensor was stored in refrigerator at 298 K and its sensitivity was tested at the interval of 5 days. The DPV response of the electrode to the same concentration of AA, DA, UA, and Trp decreased less than 5.0 % indicating that the electrode has excellent stability. In order to investigate the selectivity of $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE toward the simultaneous determination of AA, DA, UA, and Trp; DPV measurements were performed in the presence of various interfering agents (Na^+, K^+, Mg^{2+}, Zn^{2+}, glucose and citric acid). Fig. S11 shows that the current responses for AA, DA, UA, and Trp oxidation remained almost same even in the presence of higher concentrations of these interfering species, confirming that no interference for these common species occurred. These results confirm the selectivity of $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE toward the determination of AA, DA, UA, and Trp.

3.7 Application of $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE in real samples

The applicability of $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE in real sample analysis was investigated by the direct analysis of AA and DA in commercial pharmaceutical preparations (vitamin C tablets and dopamine injections). The concentration of UA in human urine samples was determined. The real sample analysis data for vitamin C tablets, dopamine injection solution, and urine samples is provided in Table 2. The values of recovery were in the range from 96 to 104 %, suggesting the accuracy of $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE based sensor.

4. Conclusions

In this work, $NiCo_2O_4$/Nano-ZSM-5 nanocomposite materials were successfully prepared by the calcination of $NiCo_2O_4$ and Nano-ZSM-5. Electrochemical sensor based on $NiCo_2O_4$/Nano-ZSM-5 nanocomposite modified glassy carbon electrode was developed for the simultaneous determination of ascorbic acid, dopamine, uric acid, and tryptophan. The results demonstrate that $NiCo_2O_4$ (30 %)/Nano-ZSM-5 modified glassy carbon electrode exhibited the highest electro-catalytic activity toward the oxidation of selected bio-molecules with good stability, sensitivity, and selectivity. The high activity of $NiCo_2O_4$ (30 %)/Nano-ZSM-5 can be attributed to the optimum loading of $NiCo_2O_4$, highly dispersed $NiCo_2O_4$ active centers on Nano-ZSM-5 matrix, along with its unique mesoporous structure, large surface area, and lower electron transfer resistance rate. The analytical performance of the developed sensor was extended in the determination of ascorbic acid, dopamine, and uric acid in vitamin C tablets, dopamine injections, and human urine samples, respectively. High activity and satisfactory recovery of this fabricated sensor may lead to the development of electrochemical sensing device for the detection of several electro-active bio-molecules.

Acknowledgements

Authors thank Department of Science and Technology, New Delhi for financial assistance (DST grant SB/S1/PC-91/2012). BK is grateful to CSIR, New Delhi for SRF fellowship. Authors are also thankful to Prof. M. K. Surappa, Director IIT Ropar for his constant encouragement.

Supporting Information

The Supporting Information contains additional characterization results.

References

1. J. Virkutyte and R. S. Varma, *ACS Sustainable Chem. Eng.*, 2014, **2**, 1545-1550.
2. J. Yang, J.-X. Wu, Q.-F. Lü and T.-T. Lin, *ACS Sustainable Chem. Eng.*, 2014, **2**, 1203-1211.
3. L. Zeng, C. Zheng, C. Deng, X. Ding and M. Wei, *ACS Appl. Mater. Interfaces*, 2013, **5**, 2182-2187.
4. Y. Tao, H. Kanoh, L. Abrams and K. Kaneko, *Chem. Rev.*, 2006, **106**, 896-910.
5. J. Perez-Ramirez, C. H. Christensen, K. Egeblad, C. H. Christensen and J. C. Groen, *Chem. Soc. Rev.*, 2008, **37**, 2530-2542.
6. B. T. Holland, L. Abrams and A. Stein, *J. Am. Chem. Soc.*, 1999, **121**, 4308-4309.
7. M. Choi, H. S. Cho, R. Srivastava, C. Venkatesan, D.-H. Choi and R. Ryoo, *Nat. Mater.*, 2006, **5**, 718-723.
8. C. J. H. Jacobsen, C. Madsen, J. Houzvicka, I. Schmidt and A. Carlsson, *J. Am. Chem. Soc.*, 2000, **122**, 7116-7117.
9. D. Gligor, F. Balaj, A. Maicaneanu, R. Gropeanu, I. Grosu, L. Muresan and I. C. Popescu, *Mater. Chem. Phys.*, 2009, **113**, 283-289.
10. S. N. Azizi, S. Ranjbar, J. B. Raoof and E. Hamidi-Asl, *Sens. Actuators, B*, 2013, **181**, 319-325.
11. B. Kaur and R. Srivastava, *Electrochim. Acta*, 2014, **133**, 428-439.
12. B. Kaur and R. Srivastava, *Electroanalysis*, 2014, **26**, 1078-1089.
13. B. Kaur and R. Srivastava, *Electroanalysis*, 2014, **26**, 1739-1750.
14. B. Kaur, M. U. Anu Prathap and R. Srivastava, *ChemPlusChem*, 2012, **77**, 1119-1127.
15. R. Kore, B. Satpati and R. Srivastava, *Chem. Eur. J.*, 2011, **17**, 14360-14365.
16. R. Kore, R. Srivastava and B. Satpati, *Chem. Eur. J.*, 2014, **20**, 11511-11521.
17. R. Kore, R. Sridharkrishna and R. Srivastava, *RSC Adv.*, 2013, **3**, 1317-1322.
18. R. Kore and R. Srivastava, *RSC Adv.*, 2012, **2**, 10072-10084.
19. Y. Ren, Z. Ma and P. G. Bruce, *Chem. Soc. Rev.*, 2012, **41**, 4909-27.
20. Y. Wan and Zhao, *Chem. Rev.*, 2007, **107**, 2821-2860.
21. E. Kim, D. Son, T.-G. Kim, J. Cho, B. Park, K.-S. Ryu and S.-H. Chang, *Angew. Chem. Int. Ed.*, 2004, **43**, 5987-5990.
22. S. Xiong, C. Yuan, X. Zhang, B. Xi and Y. Qian, *Chem. Eur. J.*, 2009, **15**, 5320-5326.

23. T.-Y. Wei, C.-H. Chen, H.-C. Chien, S.-Y. Lu and C.-C. Hu, *Adv. Mater.*, 2010, **22**, 347-351.

24. L. Yu, H. Wu, T. Wu and C. Yuan, *RSC Adv.*, 2013, **3**, 23709-23714.

25. C. Yuan, J. Li, L. Hou, X. Zhang, L. Shen and X. W. Lou, *Adv. Funct. Mater.*, 2012, **22**, 4592-4597.

26. L. Hu, L. Wu, M. Liao, X. Hu and X. Fang, *Adv. Funct. Mater.*, 2012, **22**, 998-1004.

27. L. Li, S. Peng, Y. Cheah, P. Teh, J. Wang, G. Wee, Y. Ko, C. Wong and M. Srinivasan, *Chem. Eur. J.*, 2013, **19**, 5892-5898.

28. H. Jiang, J. Ma and C. Li, *Chem. Commun.*, 2012, **48**, 4465-4467.

29. O. Knop, K. I. G. Reid, Sutarno and Y. Nakagawa, *Can. J. Chem.*, 1968, **46**, 3463-3476.

30. P. D. Battle, A. K. Cheetham and J. B. Goodenough, *Mater. Res. Bull.*, 1979, **14**, 1013-1024.

31. M. U. Anu Prathap and R. Srivastava, *Electrochim. Acta*, 2013, **108**, 145-152.

32. M. U. Anu Prathap and R. Srivastava, *Nano Energy*, 2013, **2**, 1046-1053.

33. M. U. A. Prathap, B. Satpati and R. Srivastava, *Electrochim. Acta*, 2014, **130**, 368-380.

34. S. Yu, C. Luo, L. Wang, H. Peng and Z. Zhu, *Analyst*, 2013, **138**, 1149-1155.

35. J. Argüello, V. L. Leidens, H. A. Magosso, R. R. Ramos and Y. Gushikem, *Electrochim. Acta*, 2008, **54**, 560-565.

36. X. Tian, C. Cheng, H. Yuan, J. Du, D. Xiao, S. Xie and M. M. F. Choi, *Talanta*, 2012, **93**, 79-85.

37. B. Zhang, D. Huang, X. Xu, G. Alemu, Y. Zhang, F. Zhan, Y. Shen and M. Wang, *Electrochim. Acta*, 2013, **91**, 261-266.

38. J. Yan, S. Liu, Z. Zhang, G. He, P. Zhou, H. Liang, L. Tian, X. Zhou and H. Jiang, *Colloids Surf., B*, 2013, **111**, 392-397.

39. C. Zhou, S. Li, W. Zhu, H. Pang and H. Ma, *Electrochim. Acta*, 2013, **113**, 454-463.

40. H. Li, Y. Wang, D. Ye, J. Luo, B. Su, S. Zhang and J. Kong, *Talanta*, 2014, **127**, 255-261.

41. M. Noroozifar, M. Khorasani-Motlagh, R. Akbari and M. Bemanadi Parizi, *Biosens. Bioelectron.*, 2011, **28**, 56-63.

42. C. Wang, R. Yuan, Y. Chai, S. Chen, F. Hu and M. Zhang, *Anal. Chim. Acta*, 2012, **741**, 15-20.

43. C. Wang, R. Yuan, Y. Chai, S. Chen, Y. Zhang, F. Hu and M. Zhang, *Electrochim. Acta*, 2012, **62**, 109-115.
44. M. U. Anu Prathap and R. Srivastava, *Sens. Actuators, B*, 2013, **177**, 239-250.
45. B. Kaur, T. Pandiyan, B. Satpati and R. Srivastava, *Colloids Surf., B*, 2013, **111**, 97-106.
46. S. Verma, H. M. Joshi, T. Jagadale, A. Chawla, R. Chandra and S. Ogale, *J. Phys. Chem. C*, 2008, **112**, 15106-15112.
47. M. Sharp, M. Petersson and K. Edström, *J. Electroanal. Chem.*, 1979, **95**, 123-130.
48. R. Manjunatha, D. H. Nagaraju, G. S. Suresh, J. S. Melo, S. F. D'Souza and T. V. Venkatesha, *Electrochim. Acta*, 2011, **56**, 6619-6627.
49. S. R. Ali, Y. Ma, R. R. Parajuli, Y. Balogun, W. Y. C. Lai and H. He, *Anal. Chem.*, 2007, **79**, 2583-2587.
50. M. Hosseini, M. Momeni and M. Faraji, *J. Appl. Electrochem.*, 2010, **40**, 1421-1427.

Figures and Tables captions

Figures

Fig. 1 (a) XRD patterns and (b) N_2-adsorption isotherms of $NiCo_2O_4$ (30 %)/Nano-ZSM-5, Nano-ZSM-5, and $NiCo_2O_4$ materials.

Fig. 2 (a-c) Low magnification TEM images of $NiCo_2O_4$ (30 %)/Nano-ZSM-5, (d) HRTEM image of $NiCo_2O_4$ particles.

Fig. 3 (a) STEM-HAADF image of $NiCo_2O_4$ (30 %)/Nano-ZSM-5 nanocomposite, (b) EDX spectra from a region marked by area 1 in (a) and (c) STEM-HAADF-EDX images taken from the area marked by an orange box 2 indicating the locations of different atoms across the structure.

Fig. 4 Nyquist plots of impedance spectra at (a) (i) bare GCE, (ii) $NiCo_2O_4$/GCE, (iii) $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE and (b) (i) bare GCE, (ii) ZSM-5/GCE, (iii) Nano-ZSM-5/GCE in 0.1 M KCl solution containing 10 mM $[Fe(CN)_6]^{3-/4-}$ over the frequency range from 0.1 Hz to 10^5 Hz.

Fig. 5 CVs at a scan rate of 50 mV/s in buffer solution (pH 3.5) containing (a) AA (10 µM), (b) DA (10 µM), (c) UA (10 µM), and (d) Trp (10 µM), at $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE and bare GCE.

Fig. 6 DPVs at $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE in buffer solution (pH 3.5) by varying the concentrations of (a) AA, (b) DA, (c) UA, and (d) Trp. DPV parameters were selected as: pulse amplitude: 50 mV, pulse width: 50 ms, scan rate: 20 mV/s. Inset shows the calibration plot.

Fig. 7 (a) DPVs of the quaternary mixtures containing varying concentrations of AA, DA, UA, and Trp at $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE in buffer solution (pH 3.5). DPV parameters were selected as: pulse amplitude: 50 mV, pulse width: 50 ms, scan rate: 20 mV/s. (b) Calibration plot.

Fig. 8 Comparison of the sensitivity for different analytes at various modified electrodes investigated in this study.

Table

Table 1	Physico-chemical characteristics of various materials investigated in this study.
Table 2	Determination of AA in vitamin C tablets, DA in dopamine injections solution, and UA in urine samples at $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE.

Table 1 Physico-chemical characteristics of various materials investigated in this study.

S.No.	Sample	Total surface area S_{BET} (m^2/g)	External surface area (m^2/g)	Total pore volume (cm^3/g)
1.	$NiCo_2O_4$	71	47	0.23
2.	Nano-ZSM-5	542	364	0.56
3.	$NiCo_2O_4$ (20 %)/Nano-ZSM-5	517	333	0.42
4.	$NiCo_2O_4$ (30 %)/Nano-ZSM-5	486	300	0.39
5.	$NiCo_2O_4$ (40 %)/Nano-ZSM-5	458	276	0.32
6.	ZSM-5	378	63	0.24

Table 2 Determination of AA in vitamin C tablets, DA in dopamine injections solution, and UA in urine samples at $NiCo_2O_4$ (30 %)/Nano-ZSM-5/GCE.

S. No.	Sample	Spiking (μM)	Added (μM)	Detected (μM)	RSD (%)[g]	Recovery (%)
1.	Vitamin C tablet	-	10[a]	9.6	2.6	96.0
2.	Vitamin C tablet	-	10[b]	9.8	2.4	98.0
3.	Vitamin C tablet	-	10[c]	10.1	1.9	101.0
4.	Vitamin C tablet	30	10[a]	40.2	2.7	100.7
5.	Vitamin C tablet	30	20[a]	50.6	1.5	102.0
6.	Vitamin C tablet	30	40[a]	70.3	2.9	101.0
7.	DA injection	-	10[d]	10.4	2.4	104.0
8.	DA injection	-	10[e]	9.9	1.6	99.0
9.	DA injection	-	10[f]	9.7	1.9	97.0
10.	DA injection	30	10[d]	40.6	2.2	102.0
11.	DA injection	30	20[d]	51.0	2.5	103.3
12.	DA injection	30	40[d]	70.7	2.9	102.3
13.	Urine 1	-	-	27.8	2.7	96.0[h]
14.	Urine 2	-	-	32.4	1.8	98.0[h]
15.	Urine 3	-	-	55.2	1.7	104.1[h]

[a] DOPAA, AAA Pharma, [b] DOMIN, NEON Labs, [c] DOPRESS, UNITED Biotech

[d] Nicholas Paramil, [e] Glaxo Smith Kline Pharmaceuticals Ltd., [f] CECON ABBOTT

[g] Average value of five determinations.

[h] Recovery is calculated based on the clinical value.