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Electrochemically selective detection of dopamine over serotonin by ITO/NiO(OH) electrode

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Abstract: Dopamine and serotonin are the two key neurotransmitters that play vital roles in human central nervous system. It is essential to monitor the level of these neurotransmitters in our body. Enzyme-less or electrochemical detection of such bio-analytes are one of the accurate methods for this purpose. However, proper choice of the semiconductor electrode is crucial. To address this, thin films of nickel-oxy-hydroxide [NiO(OH)] was deposited on indium doped tin oxide (ITO) coated glass substrates using chronoamperometry. Thorough characterization of the deposited films were carried out using x-ray diffraction, Fourier transform infrared spectroscopy, atomic force microscopy, field emission scanning electron microscopy and UV-Vis spectroscopy. For the detailed electrochemical sensing of dopamine and serotonin, cyclic voltammetry (CV), steady-state amperometry, differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) were carried out in aqueous phosphate buffer solution (pH ~ 7). Electrochemical analyses for dopamine yielded high sensitivity of 11.12 µAµM⁻¹cm⁻² with a limit of detection of 0.55 µM, i.e., in sub µM level. Whereas, for serotonin, the value of sensitivity and limit of detection were found to be 0.04 µAµM⁻¹cm⁻² and 1.70 µM, respectively; which are inferior compared to dopamine detection. In addition, with the presence of serotonin as an interfering analyte, the performance of the developed electrode did not suppress much and effectively displayed the results in favour of dopamine; making the developed electrode dopamine selective.

Keywords: Nickel-oxy-hydroxide; electrodeposition; neurotransmitters; electrochemical sensing; dopamine selectivity.
1. Introduction:

Dopamine (3,4-dihydroxyphenethylamine) and serotonin (5-hydroxytryptamine, 5-HT) are the two most common neurotransmitters that belong to the family of catecholamines and also known as chemical messengers that establish communication between nerves and other structures in human body. These two coexist in human body but have different effects on mood, memory, digestion and other physiological functions. An imbalance in the concentration of dopamine and serotonin can cause several neurological disorders [1-5]. On the other hand, high level of dopamine is one of the causes of cardiotoxicity which leads to rapid heart rates, heart failure, hypertension, etc. [6]. Whereas, low dopamine level is responsible for some critical illness like schizophrenia, Alzheimer’s disease, and the most common is the Parkinson’s disease. Low dopamine level can also cause stress and depression like mood swings [7-9]. Besides dopamine, low serotonin level can cause anxiety, depression, migraine, bipolar disorder and some other diseases [10-15]. As these two neurotransmitters coexist in human body, selective detection of either of these two plays a crucial role. In this regard, the sensing platform should be developed in such a manner that it should detect either dopamine or serotonin in a sensitive and selective way.

Till date, various methods, like high performance liquid chromatography (with and without electrochemical detection), capillary electrophoresis, fluorimetry, thin layer chromatography, coulometry, mass spectroscopy, reverse-phase ion pair, voltammetry, to name a few, have been used to detect the concentration level of dopamine and serotonin [16-22]. However, such techniques require expensive equipment and suffer from high cost of implementation, complex process, prolonged time with extensive consumption of organic solvents, etc.; which make these routes complex for the analysis. Now a days, electrochemical sensing methods have gained wide attention due to its high sensitivity, fast response, selective and low-cost attributes [23-27] and ease of handling. So, this technique may also be chosen for
the detection of dopamine and serotonin, however, the issue of selective detection remains as oxidation/anodic peak potential for dopamine and serotonin are close enough.

These points have encouraged the researchers to develop various electrochemical methods and electrode materials for the rapid, sensitive and selective determination of these analytes [28]. Till date, variety of materials have been searched and used as the building block to modify an electrode, such as metal-oxides and noble metal nanoparticles [29-33], carbon nanomaterials [34], polymers [35], etc., for the electrocatalytic sensing. Among all, the metal oxide nanoparticles have gained much attention in the field of biosensor [36] due to their excellent electro-chemical properties and cost effectiveness. As a p-type semiconductor with wide band gap, the oxides and hydroxides of nickel have earlier been used as a suitable and stable material [37] for the determination of bio-analytes owing to the favourable electrocatalytic activity and biological compatibility [38-41] of it. In this context, here it has been aimed to develop a dopamine selective sensing platform/electrode for the efficient detection of dopamine over serotonin. For this purpose, a less studied but stable compound of nickel, viz., nickel oxy-hydroxide [NiO(OH)] has been chosen as the sensing platform and developed in the form of a thin film on indium doped tin oxide (ITO) coated glass substrates. The material has been thoroughly characterized and detailed investigations on enzymeless electrochemical detection towards both dopamine and serotonin has been carried out with it. The results open up new avenues for selective and rapid detection of dopamine.

2. Experimental section:

2.1. Materials and methods:

All chemicals were of analytical (AR) grade. NiSO₄·6H₂O, ethylenediaminetetraacetic acid (EDTA) and NaOH were procured from reputed commercial sources. Dopamine hydrochloride (CAS no.: 62317) and serotonin hydrochloride (CAS no.:15398) were used as the source of the analytes. Phosphate buffer solution (PBS) was used as the medium for
electrochemical measurements. All experimental solutions were prepared using de-ionized water (Millipore, 18 MΩ). Properly cleaned ITO (indium tin oxide) coated glass substrates were used as the working electrodes for the deposition of the nickel compound on it.

A three-electrode electrochemical analyser (CHI6145E, CH Instruments, USA) was used to perform all electrochemical depositions and electrochemical sensing applications. Bruker D8 Advanced x-ray diffractometer was used for x-ray diffraction (XRD) studies. The diffractometer was operated with Bragg-Brentano goniometer geometry, θ-2θ scanning mechanism and radiation source of Cu-Kα (λ = 1.540598 Å). JASCO 4700 ATR FTIR (4 cm⁻¹ resolution) was used to carry out the Fourier transform infrared spectroscopy. The micrographs were captured using a field emission scanning electron microscope (FESEM, Zeiss Sigma) and atomic force microscope (AFM, NT-MDT Solver Next). AFM was done in semi-contact mode over 5 µm × 5 µm area. Optical property was evaluated by JASCO V-730 UV-Vis spectrophotometer.

2.2. Film deposition:

The thin film deposition on ITO (indium tin oxide) coated glass substrates was carried out amperometrically, using a three-electrode electrochemical setup, where, the ITO coated glass substrate acted as working electrode, saturated calomel electrode (SCE) served as the reference electrode, and, the counter electrode was a pure platinum wire. These three electrodes were dipped in a solution of 25 ml 0.015 M NiSO₄·6H₂O mixed with 25 ml 0.01 M aqueous EDTA solution, at an adjusted (with dilute NaOH solution) pH c.a. ~7. Mixing was done through continuous stirring at ambient temperature. The colour of the final solution appeared as sky blue by changing from the initial light green colour (before the addition of EDTA solution). At first the deposition conditions were optimized by cyclic voltammetry (CV) technique at ambient temperature. Then, chronoamperometry (i – t) was performed at a fixed
potential of -0.8V (vs. SCE) for 300 sec (without stirring) to deposit the film. The area of the deposition of working electrode (sheet resistance of 10 \( \Omega \)/sq) was 2 cm \( \times \) 1 cm. Initially, the colour of the thin film appears as greenish black shown in Fig. 1.

2.3. Activation of the as-deposited film:

To activate the surface of the as deposited film for sensing applications, cyclic voltammetry (CV) was carried out with the film in only PBS solution as shown in the inset of Fig. 1. After scanning through the positive potential region from -0.1 to 0.6 V (vs. SCE) for 2 cycles in PBS (pH \( \sim \)7), the colour of the film changed from greenish black to pale green. The greenish black film might be due to the presence of the complex compound of nickel, i.e. NiO(OH)-EDTA in the film, which is unstable in positive potential window and gets converted to pale green coloured NiO(OH). Further, this green coloured film, which is highly adherent to ITO substrate and stable, was used for characterization and all electrochemical sensing performances.

![Deposition i-t curve and CV curve (inset).](image)

Fig. 1: Deposition i-t curve and CV curve (inset).

2.4. Electrochemical sensing:

All electrochemical sensing performances were measured by using cyclic voltammetry (CV), chronoamperometry, differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) techniques. The electrochemical measurements were carried out
in 0.2 M aqueous phosphate buffer solution (PBS) of pH ~ 7 using 3-electrode electrochemical set up. Here, the deposited thin film on ITO substrates used as the working electrode or sensing platform with the presence of other two i.e., saturated calomel electrode (SCE) and platinum (pt) wire as the reference and counter electrodes, respectively. Analytical (AR) grade of 0.001 M aqueous dopamine hydrochloride (DA) solution and 0.001 M aqueous serotonin hydrochloride (ST) solutions were used as the stock solution of the analytes for this purpose.

3. Results and discussions:

3.1. Crystalline structure and phase purity:

The crystallinity and phase purity of the as-deposited film (green in colour) were investigated by x-ray diffraction (XRD) technique and the related diffraction pattern is shown in Fig. 2a. The diffraction peaks were found to be centred at the 2θ values of 26.8°, 37.8°, 51.6°, 54.6° and 65.6° that correspond to the diffraction planes of (103), (112), (213), (214), and (022), respectively, for orthorhombic nickel-oxide-hydroxide system and the result matches well with the standard JCPDS card number #841459. The '*' marked peaks appeared at 33.8° and 61.6° are attributed to the presence of SnO\(_2\) in the underlying ITO substrate. To further confirm the presence of nickel-oxide-hydroxide and also to check the stability of the deposited material, the film was annealed at 600°C in air for 10 minutes and the XRD pattern is shown in Fig. 2b. From the diffraction pattern of the annealed sample (Fig. 2b), the peaks were found to be positioned almost at the same 2θ value as that of the as-deposited green sample. The x-ray diffraction analysis indicates that the as-deposited material is nickel-oxy-hydroxide, i.e., NiO(OH). As there was no change in the diffraction pattern and no new peak was found to grow even after annealing in air at 600°C, it could be inferred that the as-deposited film is quite stable and both the as-deposited and annealed films are with good phase purity.
Fig. 2: X-ray diffraction patterns of (a) as-deposited and (b) annealed film.

Fig. 3a and 3b represent the FT-IR spectrum of the deposited green coloured film before and after annealing at 600°C, respectively. The spectrum was recorded within the fingerprint region of 400 to 700 cm$^{-1}$. In Fig. 3a, the bands (i) to (v) are assigned to the Ni-O stretching vibrational modes and Ni-O vibration bonds. These bands are positioned at 437.7, 454.0, 463.7, 476.3 and 517.7 cm$^{-1}$. Whereas, the bands positioned at 557.3, 581.0 and 619.0 cm$^{-1}$ (with numbers (vi) to (viii) in Fig. 2) are attributed to the lattice mode vibration of Ni-OH, bending vibration of Ni-O-H and stretching of Ni-O-H. The ninth band is for Ni-O bond stretching vibration which is found to be centered at 669.0 cm$^{-1}$. These values are well matched with the literature values and are depicted in detail in in the Table – 1.
Fig. 3: FT-IR spectra of (a) as-deposited green film and (b) annealed (at 600°C in air for 10 minutes) film.

The FT-IR spectrum shown in Fig. 3b is for the annealed sample. Each and every band with number (i) to (ix) appeared almost at the same position with that of as deposited green sample. This clearly indicates that severe annealing in air even did not bring any compositional changes in the deposited green material. This again indicates to the stability of the material and also confirms the presence of NiO(OH) in the deposited film.
Table 1: Comparison of FT-IR bands:

<table>
<thead>
<tr>
<th>Band number</th>
<th>For as deposited film (wavenumber cm(^{-1}))</th>
<th>For annealed film (wavenumber cm(^{-1}))</th>
<th>Literature value (wavenumber cm(^{-1}))</th>
<th>Attributed to</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>437.7</td>
<td>440.6</td>
<td>443.5</td>
<td>Ni-O bond stretching vibration</td>
<td>[42]</td>
</tr>
<tr>
<td>ii</td>
<td>454</td>
<td>453</td>
<td>455</td>
<td>Ni-O stretching vibrational mode</td>
<td>[43]</td>
</tr>
<tr>
<td>iii</td>
<td>463.7</td>
<td>464.7</td>
<td>467</td>
<td>Ni-O stretching vibrational mode</td>
<td>[44]</td>
</tr>
<tr>
<td>iv</td>
<td>476.3</td>
<td>470.5</td>
<td>470</td>
<td>Ni-O vibration bond</td>
<td>[45]</td>
</tr>
<tr>
<td>v</td>
<td>517.7</td>
<td>516.8</td>
<td>525, 522</td>
<td>Ni-O vibration bond</td>
<td>[45-46]</td>
</tr>
<tr>
<td>vi</td>
<td>557.3</td>
<td>551.5</td>
<td>558.4</td>
<td>Lattice mode vibration of Ni-OH</td>
<td>[47]</td>
</tr>
<tr>
<td>vii</td>
<td>581</td>
<td>580.4</td>
<td>582</td>
<td>Ni-O-H bending vibration</td>
<td>[48]</td>
</tr>
<tr>
<td>viii</td>
<td>619</td>
<td>619</td>
<td>619</td>
<td>Ni-O-H stretching bond</td>
<td>[45]</td>
</tr>
<tr>
<td>ix</td>
<td>669</td>
<td>677.2</td>
<td>663</td>
<td>Ni-O bond stretching vibration</td>
<td>[42]</td>
</tr>
</tbody>
</table>

3.2. Morphological attributes:

To explore the surface attributes of the deposited films before and after annealing, field emission scanning electron microscopy (FESEM) and atomic force microscopy (AFM) were carried out. Fig. 4a and 4b illustrate the high magnification FESEM images of the as-deposited and annealed (at 600°C in air) nickel-oxide-hydroxide films, respectively. For both cases, the surface was found to be covered with agglomerated fine particles. This gave rise to a very uneven surface composed of randomly distributed particles. Some hollow regions are also evident in both the films. Such uneven surface with hollow regions might be found beneficial
for good adsorption/anchoring of the analyte molecules by the developed materials leading to better sensing performance. It is worth mentioning here, that no major difference in surface morphology is noticeable from the micrographs of these two films, viz., as-deposited and annealed. So, the morphological study was further extended using AFM.

Fig. 4: FESEM micrographs of (a) as-deposited green film and (b) annealed (600°C in air for 10 minutes) film.

Fig. 5a and 5b are representing the 3D – AFM micrographs for the as-deposited and annealed films, respectively. In this case also, the presence of granular particles is quite evident on the surface of the films, however, the particles were not found to be densely packed giving rise to some hollow spaces and valleys between them. From the AFM image of the annealed film (Fig. 5b), some changes in particle size and distribution can be seen and that introduced notable differences between the physical attributes of these two films. The physical parameters obtained from AFM measurements for both types of the films are elaborated in Table – 2.

Table – 2: Comparison of physical parameters obtained from AFM analyses.

<table>
<thead>
<tr>
<th>Film condition</th>
<th>Surface average roughness ($S_a$)</th>
<th>Root mean square roughness ($S_q$)</th>
<th>Maximum peak height ($S_p$)</th>
<th>Maximum pit height ($S_v$)</th>
<th>Kurtosis ($S_{ku}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As-deposited</td>
<td>61.1 nm</td>
<td>75.8 nm</td>
<td>277.0 nm</td>
<td>215.0 nm</td>
<td>2.78</td>
</tr>
<tr>
<td>Annealed</td>
<td>41.0 nm</td>
<td>51.6 nm</td>
<td>238.0 nm</td>
<td>161.0 nm</td>
<td>3.12</td>
</tr>
</tbody>
</table>
Fig. 5: 3D – AFM micrographs of (a) as-deposited green film and (b) annealed (600°C in air for 10 minutes) film.

It is evident from Table – 2 that both surface average roughness and root mean square roughness decrease after annealing. This actually brings the difference between the two types of the film from the point of view of their surface morphology. As better surface roughness facilitates better adsorption/anchoring of the analyte molecules on the sensing material/platform, so, in this case, the as-deposited film could be the better candidate. From the structural and phase analyses (XRD and FTIR), it has been observed that both as-deposited and annealed films bear almost same attributes, however, from the point of view of surface roughness, the as-deposited film is supposed to be better than the annealed one. So, henceforth, the as-deposited film will be considered to investigate its optical, electrical and sensing attributes toward dopamine and serotonin.

3.3. Optical and electrical properties:

As the material under investigation, i.e., NiO(OH), is a relatively less studied material, its optical and electrical properties are worthy to establish and have been done with the help of UV-Vis spectroscopy (Fig. 6a) and Mott – Schottky measurement. The UV-Vis absorption spectrum of the as-deposited film is depicting a sharp rise in absorption band edge from around 550 nm. This corresponds to the band to band transition for the as-deposited NiO(OH) and
represents the optical bandgap energy ($E_{g\text{(opt)}}$). With the help of the empirical formula, $E_{g\text{(opt)}}$ (in eV) = $1239.95/\lambda$ (in nm), the value of $E_{g\text{(opt)}}$ was calculated and was found to be $\sim 2.36$ eV.

Fig. 6: (a) UV-Vis absorption spectrum, and (b) Mott-Schottky plot of the as-deposited film.

Fig. 6b is representing the plot obtained from the Mott-Schottky measurements that has been carried out to study the electronic attributes of the as-deposited film. This experiment was carried out by taking the as-deposited green coloured film as the working electrode in 0.1 M PBS (pH = 7) with 0.1 M Na$_2$SO$_4$ solution at 1000 Hz frequency in the positive potential range. The negative slope of the $1/C^2$ vs. $V$ plot designates the p-type behaviour of the as deposited NiO(OH) film. The carrier concentration and flat band potential were calculated respectively from the slope and intercept to the potential axis of the plot according to the following equation:

$$1/C^2 = (2/N_Ae\varepsilon\varepsilon_0)\times(-E+E_{FB}-KT/e)$$  \hspace{1cm} (1)

Where ‘C’ and ‘$N_A$’ represents the space charge capacitance (in Fcm$^{-3}$) and the charge carrier density, respectively. ‘e’, refers to the electronic charge. $\varepsilon$ and $\varepsilon_0$ denote the dielectric constant and the permittivity of free space, respectively. ‘E’ is the applied potential and ‘$E_{FB}$’ is the flat band potential. ‘K’ refers to the Boltzmann constant, ‘T’ is the temperature in Kelvin. By extrapolating the line to the potential axis (i.e., $1/C^2 = 0$) the flat band potential is calculated and the value was found to be $+2.20$ V vs. SCE. This $E_{FB}$ corresponds to the position of
conduction band edge. Further, the charge carrier density \((N_A)\) was calculated from the slope of the Mott-Schottky plot, i.e., \((2/N_Ae^\phi_0)\), and the charge carrier density value was found to be in the order of \(10^{18} \text{ cm}^{-3}\). So, it can be concluded that, the deposited NiO(OH) film is p-type in nature with a good amount of carrier concentration.

3.4. Electrochemical detection of dopamine:

Detailed electroanalytical activity of the ITO/NiO(OH) electrode for the determination of DA was carried out systematically. At first, a single cycle of cyclic voltammetry (CV) was carried out at a scan rate of 50 mV/s with blank ITO as the electrode in only PBS (pH ~ 7) and in presence of 3 \(\mu\)M DA (Fig. 7a). 3 \(\mu\)M has been chosen as the initial concentration of the analytes. The curves depict that, there is no prominent oxidation / reduction peak for the case of blank ITO as the electrode in presence of both PBS and 3 \(\mu\)M DA. The current response was also not so significant toward both PBS and DA, however, small increment in current density was observed within 0.2 to 0.4 V while 3 \(\mu\)M DA was used as the analyte. In general, it may be inferred that blank ITO is quite nonresponsive to PBS and DA. On the other hand, the current response was found to be better (almost six times) for the ITO/NiO(OH) electrode in PBS as can be seen from the CV curve presented in Fig. 7b. So, it may be inferred that, the ITO/NiO(OH) electrode is more responsive than the blank ITO electrode and former may be subjected for studying in-depth sensing performance.
In order to determine the electrocatalytic behaviour and sensing attributes of the as-deposited NiO(OH) modified ITO electrode, multiple cycles of CV was carried out with gradual addition of DA in PBS and the voltammograms are presented in Fig. 8a. The CV was carried out within the potential window of -0.1 to +0.5 V at a scan rate of 50 mV/s in 0.2 M aqueous PBS (pH ~ 7). A stock solution of 0.001 M aqueous DA was prepared in this regard. With gradual addition of DA solution from 0 to 69.0 μM of concentration, a gradual but steady increase in current density was observed at around +0.25 V. Primarily this indicates to the oxidation of dopamine to dopamine-o-quinone of the ITO/NiO(OH) electrode surface as per the following proposed mechanism [49-50]. This has further been supported by other electroanalytical techniques as a part of this work.

Scheme 1: Proposed mechanism for electro-oxidation of dopamine on ITO/NiO(OH) surface.
Differential pulsed voltammetry (DPV) was further carried out to support the nature of the CV curves obtained for electro-oxidation of DA. DPV was carried out within the potential window of 0 and +0.45 V at the highest concentration of dopamine (69 µM) used in CV. The DPV curve showed a major peak nearly at the potential +0.25 V (Fig. 8b), which is the similar position where electro-oxidation of DA took place during CV. These results primarily validate that the surface of the deposited ITO/NiO(OH) electrode is responsible for the electro-oxidation of dopamine to dopamine-o-quinone as mentioned in Scheme – 1.

Fig. 8: Electrochemical properties towards dopamine sensing of the as deposited ITO/NiO(OH) electrode in PBS solution (a) CV curves with successive addition of dopamine from 0 to 69 µM concentration (b) DPV curve in presence of 69 µM concentration of DA.

Fig. 9a depicts the steady-state amperometric (i-t) response of the deposited material at a constant potential of +0.25 V in 0.2 M PBS, under successive addition of DA solution and slow stirring at ambient temperature. With the successive addition of DA in the concentration range between 0 and 30.0 µM, notable increase in current density was observed with every addition of the analyte. The result indicates that some electron transfer process is happening between the electrode surface and the electrolytic (DA) solution, and this is the reason behind the notable response of the electrode towards DA.
Fig. 9: (a) steady-state amperometric i-t curve obtained after successive addition of DA, (b) plot of current density vs. dopamine concentration, (c) Lineweaver-Burk (L-B) plot, and (d) Nyquist plot (electrochemical impedance spectroscopy).

Fig. 9b represents the linear calibration plot of current density (in $\mu$A/cm$^2$) vs. concentration (in $\mu$M) of the analyte that has been derived from the amperometric i-t curve (Fig. 9a). This plot is typical for estimating the sensitivity and limit of detection (LoD) of the electrode material. It can be seen from Fig. 9a that good linearity in amperometric response has been achieved within the concentration range 0 and 18 $\mu$M. The current response within this range has been taken to draw the linear fit plot (Fig. 9b). The sensitivity was calculated directly from the slope of this straight line and LoD was estimated using the equation $\text{LoD} = (3 \times \text{standard deviation of intercept})/\text{slope}$, where, the value 3 was the signal/noise ratio. The sensitivity was found to be 11.12 $\mu$A$\mu$M$^{-1}$cm$^{-2}$ (where, the correlation coefficient $R^2$ was 0.998),
and the LoD was estimated to be 0.55 µM. These results are quite encouraging for dopamine sensing as can be seen from the comparative data displayed in Table – 3.

Fig. 9c shows the Lineweaver-Burk (L-B) plot or double reciprocal plot of current density vs dopamine concentration that has been derived from the linear calibration plot (Fig. 9b). From this plot the value of maximum current density ($J_{\text{max}}$) toward analyte sensing for any nonenzymatic reaction can be obtained using the following equation:

$$\frac{1}{J_{ss}} = \frac{K_{M_{\text{app}}}}{J_{\text{max}}} \frac{1}{[S]} + \frac{1}{J_{\text{max}}}$$

Where, $J_{ss}$, $J_{\text{max}}$, $[S]$ and $K_{M_{\text{app}}}$ represent the steady-state current density, maximum current density, dopamine concentration and apparent Michaelis-Menten constant, respectively. The $J_{\text{max}}$ and $K_{M_{\text{app}}}$ values were calculated from the intercept and slope of the line and were found to be 2397 µA/cm² sec⁻¹ and 190.7 µM, respectively. This notably high value of maximum current density is one of the reasons behind such a good DA response exerted by the ITO/NiO(OH) electrode.

Fig. 9d illustrates a typical electrochemical impedance spectroscopy (EIS), i.e., Nyquist plot. The plot was found to consist of a semi-circle with a straight line at the tail. From the diameter of the semi-circle, originated at the low frequency range, the values of charge transfer resistance ($R_{ct}$) and solution resistance ($R_s$) at the electrode/electrolyte interface can be obtained. EIS measurement was carried out within the frequency range 0.01 Hz and 10⁶ Hz with 0.005 V amplitude and in presence of 69.0 µM DA solution in 0.2 M PBS at a fixed potential of +0.25 V. The calculated value of $R_{ct}$ and $R_s$ were 131.6 Ω and 25.2 Ω. The notably low value of $R_{ct}$ and $R_s$ with a steep Warburg diffusion line is indicating that the NiO(OH) surface is quite suitable for dopamine detection.
Table – 3: Comparison of electrocatalytic behavior of dopamine with other reports:

<table>
<thead>
<tr>
<th>Electrode</th>
<th>LOD (µM)</th>
<th>Linear range (µM)</th>
<th>Sensitivity (µAµM⁻¹cm⁻²)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosinase/NiO/ITO</td>
<td>1.04</td>
<td>2–100</td>
<td>0.06</td>
<td>[40]</td>
</tr>
<tr>
<td>rGO-Co₃O₄</td>
<td>0.277</td>
<td>0 - 30</td>
<td>0.389</td>
<td>[51]</td>
</tr>
<tr>
<td>NiO/GO</td>
<td>0.10</td>
<td>2 - 60</td>
<td>Data not found</td>
<td>[52]</td>
</tr>
<tr>
<td>Au nanopillars</td>
<td>5.83</td>
<td>1–100</td>
<td>Data not found</td>
<td>[53]</td>
</tr>
<tr>
<td>Pd-NC/rGO/GCE</td>
<td>7.02</td>
<td>20–220</td>
<td>0.943</td>
<td>[54]</td>
</tr>
<tr>
<td>ITO/NiO(OH)</td>
<td>0.55</td>
<td>0 – 18</td>
<td>11.12</td>
<td>This work</td>
</tr>
</tbody>
</table>

3.5. Electrochemical detection of serotonin:

Detailed electroanalytical characteristics of the developed ITO/NiO(OH) electrode toward another catecholamines group based analyte, namely, serotonin; was also carried out so that its performance toward both dopamine and serotonin could be compared. Initially, cyclic voltammetry was used to determine the electrochemical behaviour of serotonin (ST) solution on ITO/NiO(OH) electrode in 0.2 M PBS and the result is shown in Fig. 10a. The CV was performed in the potential range of -0.2 and 0.8 V at a scan rate of 50 mV/s by gradual addition of ST solution up to 12 µM concentration with an interval of 3 µM. The current density was found to increase steadily up to 6 µM of serotonin addition, however, no significant increase in the same was observed on further addition of the analyte up to 12 µM. So, it may be inferred that the saturation limit is achieved at 6 µM concentration of serotonin for this electrode. The oxidation hump, which was prominent for 3 µM of serotonin and occurred between +0.4 to +0.5 V of potential, was also found to be suppressed at the higher concentrations of serotonin. The CV measurements indicate that the surface of the developed ITO/NiO(OH) electrode becomes passive very quickly by the addition of small concentration of serotonin. The possible reaction [55-57] that might be involved for such electrooxidation of serotonin to serotonin quinoneimine is presented in Scheme 2.
Scheme 2: Plausible mechanism for electro-oxidation of serotonin on ITO/NiO(OH) surface.

Fig. 10: Electrocatalytic response of the ITO/NiO(OH) electrode toward serotonin. (a) CV curves for gradual addition of ST, and (b) DPV curves in presence of 3 and 6 μM of ST.

Fig. 10b demonstrates the DPV curves taken at 3 and 6 μM of serotonin concentrations in 0.2 M PBS. This further establishes the occurrence of electro-oxidation process of serotonin on the surface of the developed electrode. Interestingly, the peak in the DPV curve (within +0.4 V to +0.5 V) was prominent for 3 μM of ST and this was found to be diminished significantly for 6 μM of the analyte. This again establishes the passiveness of the ITO/NiO(OH) electrode surface at higher concentration (6 μM) of ST solution. It may also be noted that the current response in both CV and DPV was notably low toward electro-oxidation of serotonin than that has been observed for the case of dopamine.
The electrocatalytic behaviour was further checked by using steady state amperometric (i-t) curve and the result is presented in Fig. 11a. The experiment was carried out at a fixed potential of +0.45 V in 0.2 M PBS, with successive addition of ST solution and slow stirring at room temperature. A 0.001 M stock solution of serotonin was used for this purpose. From the plot it can be observed that, there is no significant steady-state increase in current density up to the addition of 27 µM of ST solution, i.e., the developed electrode material is quite nonresponsive to serotonin, unlike its activeness toward dopamine. Fig. 11b is displaying the linear calibration plot of current density vs serotonin concentration that has been derived from the amperometric i-t curve (Fig. 11a). The curve show linearity in the concentration range of 0 to 21 µM, but the range of current density was notably low in comparison to dopamine. The calculated values of sensitivity and LoD were found to be 0.04 µAµM⁻¹cm⁻² and 1.70 µM (with corelation coefficient R² = 0.99), respectively. Such a low value of sensitivity in turn is indicating that the developed electrode material is not so good for serotonin detection. On the other hand, the LoD value for serotonin is also higher than that of dopamine (0.55 µM), making the electrode material more suitable for dopamine detection.

Another analysis, i.e., Lineweaver-Burk (L-B) plot was further derived to support the detection phenomenon of serotonin on the ITO/NiO(OH) surface and this has been shown in Fig. 11c. According to eq. (2), stated in the description of Fig. 9c, the apparent Michaelis-Menten constant (Kₘₐₚ) and maximum current density (Jₘₐₓ) values were calculated from the plot and were found to be 0.98 µM and 9.26 µA/cm² sec⁻¹, respectively. In this case, the Jₘₐₓ value appears to be very low compared to that of dopamine. Fig. 11d. depicts the Nyquist plot obtained from the EIS spectroscopy for serotonin detection on the electrode surface. At the low frequency range, a semicircle was found to form, and from the diameter of the semicircle the value of Rₑ was calculated and found to be 311.4 Ω. The value of Rₛ was found to be 44.6 Ω, that has been obtained by extrapolating the initial end of the semicircle. The high values of
both $R_{ct}$ and $R_s$ are again referring to the nonresponsive behaviour of the developed electrode toward serotonin.

![Graphs showing amperometric response, linear fit, L-B plot, and Nyquist plot.](image)

**Fig. 11:** (a) steady state amperometric (i-t) response curve, (b) linear fit calibration curve, (c) Lineweaver-Burk (L-B) plot, and (d) Nyquist plot.

### 3.6. Selectivity between dopamine and serotonin:

As both dopamine and serotonin fall under catecholamine group, it is worthy to compare between the electrocatalytic performances of the developed ITO/NiO(OH) electrode toward both the analytes. The CV profiles presented in Fig. 12 are exhibiting the comparative nature of electrocatalytic attributes shown by the electrode in presence of dopamine and serotonin. This has been done at the point of concentration where maximum response was observed for each of the analytes. It is clearly seen from Fig. 12, that the electrode is highly
responsive to dopamine than serotonin as the electro-oxidation of dopamine is happening more prominently with the occurrence of distinct oxidation peak and much higher current response.

It can be inferred from the CV profiles presented in Fig. 12 that electrode material is more inclined or selective to dopamine than serotonin though both of them belong to the catecholamine group. Here, it is worth mentioning that the electrode material is p-type in nature with a carrier concentration in the order of $10^{18}$ cm$^{-3}$ (Section 3.3). This makes the electrode surface enriched with ‘holes’. The randomness of ‘holes’ on the NiO(OH) surface increases in the positive potential window, while performing the CV and/or chronoamperometric scans. This in turn helps the analyte molecules to release one or more electrons on the electrode surface and get oxidized. That means, the detection occurs in this case is mainly through oxidation of the analyte. On the other hand, the molecular structure of both DA and ST include conjugated aromatic rings and delocalization of ring electron clouds through ring resonance happen in both the cases. However, as serotonin has fused six and five membered rings, the delocalization of electron clouds is much higher in it than that occurs in dopamine; as dopamine has only one six-membered aromatic ring. Such stronger delocalisation in serotonin makes the electro-oxidation process less facile on the electrode surface. Whereas, due to lesser electron
delocalization in dopamine, the electro-oxidation process of it on the p-type electrode surface becomes more facile. This might be the reason behind such inclined or selective behaviour of the developed ITO/NiO(OH) electrode toward dopamine over serotonin. The process has been presented schematically in Scheme – 3.

Scheme 3: Proposed electro-oxidation pathways for dopamine on p-type NiO(OH) surface.

4. Conclusion

Aiming to develop a sensing platform for the selective detection of dopamine over serotonin, NiO(OH) thin films on ITO was deposited using an electrochemical technique. The developed electrode material was found to be p-type in nature with good amount of carrier concentration ($10^{18}$ cm$^{-3}$). Structurally, the material was polycrystalline, thermally stable and with moderate surface roughness. The electrode exhibited noteworthy sensitivity of 11.12 $\mu$A$\mu$M$^{-1}$cm$^{-2}$ and low limit of detection of 0.55 $\mu$M toward dopamine. However, for serotonin, notably poor sensitivity (0.04 $\mu$A$\mu$M$^{-1}$cm$^{-2}$) and LoD (1.70 $\mu$M) were observed in comparison to dopamine. This is attributed to the more delocalization of electron clouds in serotonin than that of dopamine. A comparison between sensitivity and LoD value showed that the developed ITO/NiO(OH) electrode can detect dopamine more selectively over serotonin in an enzymeless electrochemical way.
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