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Adsorptive stripping voltammetric determination of imipramine, trimipramine and desipramine employing titanium dioxide nanoparticles and an Amberlite XAD-2 modified glassy carbon paste electrode†

Bankim J. Sanghavi and Ashwini K. Srivastava*

An Amberlite XAD-2 (XAD2) and titanium dioxide nanoparticles (TNPs) modified glassy carbon paste electrode (XAD2-TNP-GCPE) was developed for the determination of imipramine (IMI), trimipramine (TRI) and desipramine (DES). The electrochemical behavior of these molecules was investigated employing cyclic voltammetry (CV), chronocoulometry (CC), electrochemical impedance spectroscopy (EIS) and adsorptive stripping differential pulse voltammetry (AdSDPV). After optimization of analytical conditions using a XAD2-TNP-GCPE electrode at pH 6.0 phosphate buffer (0.1 M), the peak currents were found to vary linearly with its concentration in the range of 1.30×10^{-9} to 6.23×10^{-6} M for IMI, 1.16×10^{-9} to 6.87×10^{-6} M for TRI and 1.43×10^{-9} to 5.68×10^{-6} M for DES. The detection limits ($S/N = 3$) of 3.93×10^{-10}, 3.51×10^{-10} and 4.35×10^{-10} M were obtained for IMI, TRI and DES respectively using AdSDPV. The prepared modified electrode showed several advantages such as a simple preparation method, high sensitivity, very low detection limits and excellent reproducibility. The proposed method was employed for the determination of IMI, TRI and DES in pharmaceutical formulations, blood serum and urine samples.

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1 Introduction

Imipramine (IMI), trimipramine (TRI) and desipramine (DES) are active ingredients of psychiatric drugs widely used in the treatment of depressive disorders.[1] These tricyclic antidepressants (TCAs) are used primarily in the clinical treatment of mood disorders such as major depressive disorder, dysthymia, and bipolar disorder, especially of the treatment-resistant variants. However, their overdose is fatal to the central nervous system and may result in drowsiness, convulsions, respiratory disorders, ophthalmoplegia and finally coma.[2] Hence, their determination in pharmaceutical formulations, urine and blood serum is of tremendous importance.

The most common techniques for the determination of TCAs in the commercial dosage form and biological fluids have been based on capillary electrophoresis,[3,4] high performance liquid chromatography (HPLC)[5,6] and gas chromatography.[7] These methods are time consuming, involve intensive solvent usage, are laborious, and require expensive devices and maintenance. Electrochemical methods, on the other hand, are extremely sensitive, selective and moreover, have an added advantage of no preliminary separation, a step commonly required in chromatographic analysis that increases both cost and analysis time. Due to these advantages, a few methods have been developed for the determination of TCAs employing electrochemical methods.[8–19]

Chemically modified electrodes (CMEs) have been employed for the voltammetric determination of a wide variety of analytes.[20–22] Some modifiers reported recently for the voltammetric determination of various molecules/metal ions are *viz.*, nanomaterials,[23–28] macrocycles,[29–31] rice husk,[32] inorganic complexes,[33,34] *etc.* These electrodes are inexpensive and possess many advantages such as low background current, wide range of potential windows, rapid surface renewal and easy fabrication.

A glassy carbon paste electrode (GCPE) combines the attractive properties of both composite electrodes and glassy carbon and has therefore been employed as an electrode material.[35–37] The literature reveals that Amberlite XAD-2 (XAD2) has been employed as a modifier for voltammetric determination of paraquat.[38] XAD2 preconcentrates the analyte onto the electrode surface and thus enhances the sensitivity for its detection. Titanium dioxide nanoparticles (TNPs), on the other hand, due to their large aspect ratio, optical transparency, good biocompatibility and relatively good conductivity have also been widely employed in voltammetry for analysis of various analytes.[39–41]

Department of Chemistry, University of Mumbai, Vidyanagari, Santacruz (East), Mumbai-400098, India. E-mail: aksrivastava@chem.mu.ac.in; akschbu@yahoo.com; Fax: +91-22-26528547

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In the present work, we investigate the performance of a GCPE doubly modified by XAD2 and TNPs, for sub-nanomolar determination of IMI, TRI and DES employing AdSDPV. The superior performance of the XAD2 and TNPs modified GCPE (XAD2-TNP-GCPE) is demonstrated by the determination and quantitation of these molecules in pharmaceutical formulations, human blood serum and urine samples. The proposed method has been validated by using HPLC. To the best of our knowledge this is only the second instance wherein XAD2 has been employed as a modifier in voltammetry.

2 Experimental

2.1 Chemicals and instrumentation

All chemicals were of A. R. grade and were used as received without any further purification. Imipramine hydrochloride (IMI), trimipramine maleate (TRI), desipramine hydrochloride (DES), glassy carbon powder (2–12 μm, 99.95%), titanium dioxide nanoparticles [TNPs, <100 nm particle size (BET), 97%] and Amberlite XAD2 were procured from Sigma-Aldrich and were used as received without further purification. Mineral oil (Nujol oil) was procured from Fluka. All solutions were prepared using double distilled water of specific conductivity 0.3–0.8 μS. The developed method was employed for the analysis of the following pharmaceuticals: IMI [Tofranil (25 mg), Depsol (75 mg)], TRI [Surmontil (25 mg), Stangyl (100 mg)] and DES [Norpramine (10 mg), Pertofrane (25 mg)]. IMI, TRI and DES were also determined in human urine and blood serum samples.

All voltammetric, chronocoulometric and electrochemical impedance study (EIS) measurements have been performed on Eco Chemie, Electrochemical Work Station, model Autolab PGSTAT 30 using GPES software, version 4.9.005 and Frequency Response Analyser, software version 2.0 respectively. A three-electrode system employing XAD2-TNP-GCPE was used as the working electrode, and platinum wire and Ag/AgCl (sat. KCl) were used as counter and reference electrodes, respectively. The pH measurements were performed using ELICO LI 120 pH meter. HPLC used for validating the method was an Agilent model 1100. The scanning electron microscope employed for surface characterization of the electrodes was a FEI Quanta-200 model.

2.2 Preparation of the modified electrodes

GCPE was prepared with the composition of 70 : 30 (glassy carbon powder : mineral oil) using a mortar and pestle and was allowed to undergo the process of self-homogenization for 24 hours.[42] The paste was then filled in a Teflon micropipette tip. A platinum wire was dissected through the paste, to provide an electrical contact. Smooth and fresh electrode surfaces were obtained by squeezing out 0.5 mm of paste from the syringe, scraping off the excess and polishing it against butter paper until the surface had a shiny appearance. The XAD2-GCPE was prepared in a manner similar to GCPE with glassy carbon powder : XAD2 : mineral oil composition being 67 : 3 : 30. TNP modification of the electrode was done by incorporating (5%) TNP in glassy carbon powder and using mineral oil (Nujol) as a binder with the composition of 66 : 4 : 30 (glassy carbon powder : TNP : mineral oil). XAD2-TNP-GCPE was prepared similarly having a composition of 63 : 3 : 4 : 30 (glassy carbon powder : XAD2 : TNP : mineral oil).

2.3 Experimental procedure

For stripping voltammetric analysis of IMI, TRI and DES, an appropriate quantity of the analyte solution was placed in a 25 mL standard volumetric flask and then diluted to the mark with pH 6.0 phosphate buffer (0.1 M). The solution was then transferred into the electrochemical cell where the measurements were carried out. A magnetic stirrer (Expo Hi-Tech, India) with a stirring bar was used to provide the convective transport of the analyte during its accumulation onto the carbon paste electrode surface. Accumulation potentials (E_{acc}) of −0.2, −0.4 and −0.2 V at accumulation times (t_{acc}) of 100, 120 and 100 s were applied to the XAD2-TNP-GCPE for IMI, TRI and DES respectively while the solution was stirred at 400 rpm with the magnetic stirrer. At the end of the accumulation period, the stirring was stopped, and a 15 s rest period was allowed for the solution to become quiescent. The voltammogram was then recorded by scanning the potential towards the positive direction from +0.45 to +1.1 V for all the three TCAs using the differential pulse mode employing a step potential of 5 mV and a modulation amplitude of 50 mV. The cyclic voltammetric experiments were carried out by scanning the potential in the range of −0.3 V to +1.3 V for IMI, TRI and DES. Double potential step chronocoulometry was carried out with a pulse period of 5 s from +0.6 V to +1.2 V for IMI, TRI and DES *vs*. Ag/AgCl.

The samples for the SEM imaging were prepared as follows: (a) 70 mg glassy carbon powder was sonicated for 30 minutes in toluene, (b) 3 mg of XAD2 and 67 mg of glassy carbon powder were sonicated in toluene for 30 min, (c) the sample for glassy carbon powder modified with TNP was prepared by sonicating 4 mg of TNP with 66 mg of glassy carbon powder, (d) the sample preparation for XAD2 and TNP modified glassy carbon powder were carried out similarly. All the samples were allowed to dry under an I. R. lamp and *ca.* 15 mg was employed for SEM analysis.

2.4 Treatment and determination of samples

The analysis of IMI, TRI and DES was carried out in pharmaceutical formulations, human blood serum and urine samples. Twenty tablets of IMI, TRI and DES were weighed and ground to a fine powder using a mortar and pestle. For all of these experiments, the samples were diluted to 100 mL with pH 6.0 phosphate buffer solution. Recovery tests were performed for determination of IMI, TRI and DES by spiking standard solutions of these molecules into pharmaceutical formulations. The urine and blood serum samples were collected from healthy volunteers. For the determination of the TCAs in urine samples, no pretreatment step was carried out. Blood serum samples were obtained from a local

pathology clinic and stored under refrigeration. To avoid interferences from the serum matrix, a 100 μL serum sample was added to the electrochemical cell containing 25 mL of pH 6.0 phosphate buffer solution. The cleaning of all the samples was done by filtering through a 0.22 μm PVDF syringe filter (Millex, Millipore Corporation) before voltammetric measurements.

3 Results and discussion

3.1 Effect of pH and supporting electrolyte

Standard solutions of IMI (5.02×10^{-6} M), TRI (4.83×10^{-6} M) and DES (5.35×10^{-6} M) were used to find the optimum pH of the supporting electrolyte best suited for their determination by GCPE employing differential pulse voltammetry (DPV). The influence of the pH on the oxidation peak current of IMI, TRI and DES was investigated in the pH range of 2–8 employing the Britton–Robinson (B. R.) buffer. It was observed that as the pH of the medium was gradually increased, the potential shifted towards less positive values, suggesting the involvement of protons in the reaction. From the plot of E_p *vs.* pH for IMI, TRI and DES, it was observed that slopes of −24.9 mV [Fig. S1, line (a)†], −27.7 mV [Fig. S2, line (a)†] and −26.3 mV [Fig. S3, line (a)†] were obtained in the pH range of 2.0–8.0 for the first oxidation peak (O1) of IMI, TRI and DES respectively which is indicative of an unequal number of electrons and protons being involved in the oxidation of these TCAs. Beyond pH 8.0, the molecules were insoluble in the B. R. buffer and thus gave negligible peak current. For the dimer reduction peak, slopes of −55.8 mV [Fig. S1, line (b)†], −56.3 mV [Fig. S2, line (b)†] and −55.7 mV [Fig. S3, line (b)†] were obtained for IMI, TRI and DES respectively indicating that an equal number of electrons and protons are involved in the dimerization step. The effect of pH on the slopes of the peak potentials can be studied based on the following equation:

$$E^{01} = E^0 - \frac{(2.303mRT)}{nF}pH = E^0 - \frac{(0.0592m)}{nF}pH \qquad (1)$$

where m and n are the number of protons and electrons involved in the redox reaction respectively. All other symbols have their conventional meanings. On solving eqn (1), slopes of −59.2 and −29.6 mV/pH are obtained for reactions involving equal and unequal numbers of protons and electrons respectively. These values are in agreement with the values of the slope obtained experimentally.

It was also observed that the peak current reached its maximum value at pH 6.0 for all the three TCAs. Thus, this pH was employed for further studies. Various buffers, such as acetate, citrate, phosphate, citrate phosphate were employed at pH 6.0 (Fig. S4†). Of all these buffers, the pH 6.0 phosphate buffer gave the best response in terms of the peak current and peak shape for all the three TCAs. Hence, this medium was employed for further studies. In the next step, optimization of buffer concentration was carried out by varying its concentration in the range of 0.02–0.2 M. The best peak response was observed for 0.1 M phosphate buffer and therefore this concentration was used for all further studies.

3.2 Effect of amount of XAD2 and TNPs on the oxidation peaks of IMI, TRI and DES

The effect of the amount of XAD2 and TNPs as modifiers in GCPE was first studied. It was observed that the peak currents for IMI increased with increase in the amount of XAD2 up to 3% due to accumulation of IMI onto the electrode surface. Beyond this point, a decrease in the peak current was observed (Fig. S5†). This decrease in peak current of IMI can be attributed to an increased amount of resin onto the electrode surface which results in enhanced resistance to the electron transfer. Thus, 3% of XAD2 was selected as the optimum amount for the preparation of the XAD2-GCPE. The effect on the peak currents of IMI was then investigated using TNPs. Herein, the effect of percentage of TNP on the anodic peak current of IMI was studied. It was found that the oxidation peak current for IMI increased with increase in the amount of TNPs up to 4% beyond which saturation occurred (Fig. S6†). As a result, 4% TNPs was selected as the optimum amount for the preparation of the TNP-GCPE. Similar results were obtained for both TRI and DES.

3.3 Cyclic voltammetry (CV)

In order to understand the whole electrochemical mechanism involved in the reaction of IMI, the voltammetric process was recorded in two repetitive cycles. In the first cycle, only one irreversible peak (O1) is obtained at +0.878 V which is due to the electro-oxidation of IMI. The electrochemical oxidation of TCAs occurs at the nitrogen atom in cyclohexane ring resulting in the formation of a radical which is similar to that for the oxidation of methyliminobibenzyl.[43] On the reverse sweep from +1.2 to −0.4 V, a reduction peak (R2) at 0.104 V is observed due to the formation of a dimer [Fig. 1], which gets subsequently oxidized (O2) in the second cycle at 0.163 V. The appearance of the

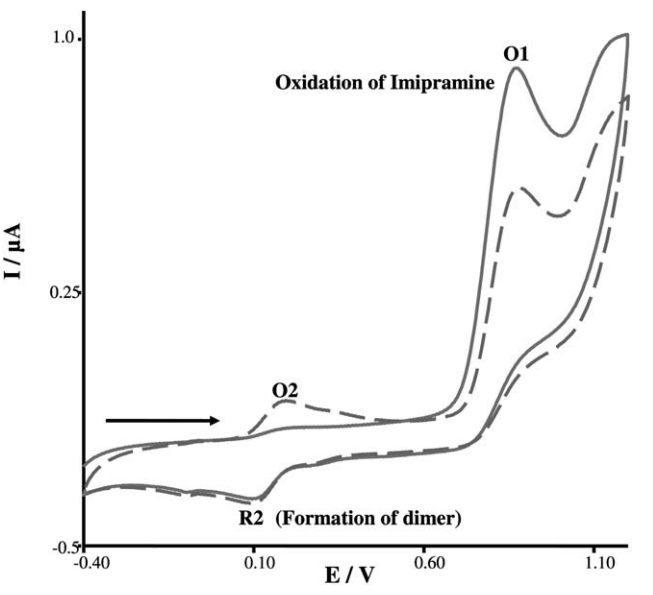


Fig. 1 Two repeated CV scans for 4.14×10^{-6} M IMI at GCPE in 0.1 M phosphate buffer (pH 6.0). First scan (——) and second scan (– – –) at a scan rate of 100 mV s^{-1}.

oxidation peak (O1) is due to a two-electron and one proton irreversible oxidation of the TCA radical and that the R2/O2 redox peak is caused by the reversible one electron and one proton oxidation and reduction of the dimer. The reactions are as depicted in Scheme 1.[15] Similar results were obtained for both TRI and DES.

The cyclic voltammograms of 7.41×10^{-7} M IMI, 5.87×10^{-7} M TRI and 6.91×10^{-7} M DES at all the four electrodes are given in Fig. 2, S7 and S8† respectively. It can be observed from these figures that moving from GCPE to XAD2-TNP-GCPE, for the three TCAs, the peak current increases, the reasons for which are as follows: (a) XAD2 is a nonionic resin which promotes an accumulation of the three TCAs onto the electrode surface and (b) TNPs due to their high aspect ratio (surface area/volume ratio) and relatively good conductivity further enhance the peak current of all the three TCAs. Thus, due to the synergistic effect of both XAD2 and TNPs, the sensitivity of IMI, TRI and DES detection increases on employing XAD2-TNP-GCPE. The surface areas of the electrodes were calculated using the Randles–Sevcik equation [using 1 mM $K_3Fe(CN)_6$]. Employing this equation, the surface areas for GCPE, XAD2-GCPE, TNP-GCPE and XAD2-TNP-GCPE were calculated to be 0.012 cm^2, 0.058 cm^2, 0.073 cm^2 and 0.134 cm^2 respectively.

The effect of potential scan rate on the peak currents of IMI, DES and TRI was further studied. Fig. 3 is a representative plot for the electro-oxidation of IMI (7.75×10^{-7} M). It can be seen that the oxidation peaks (O1 and O2) shifted to a more positive value for IMI with increasing scan rates along with a concurrent increase in current. Similarly, the reduction peak (R2) shifted to more negative values along with an increase in the peak current. The cyclic voltammetry results indicated that the anodic peak currents (I_p) of all the three molecules

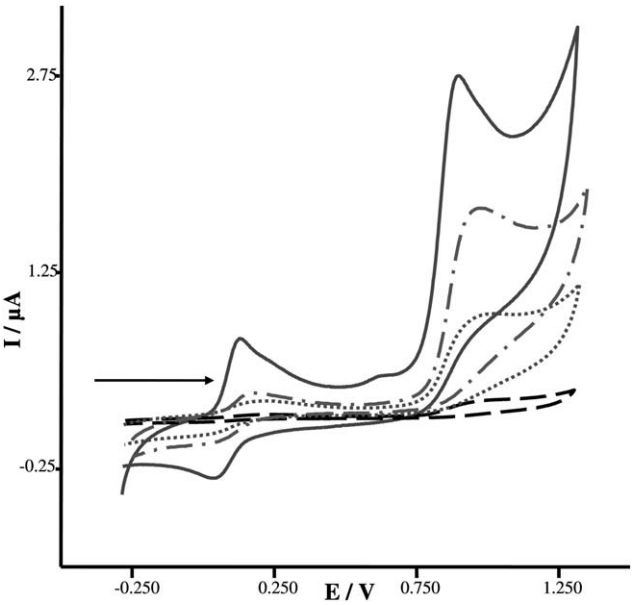
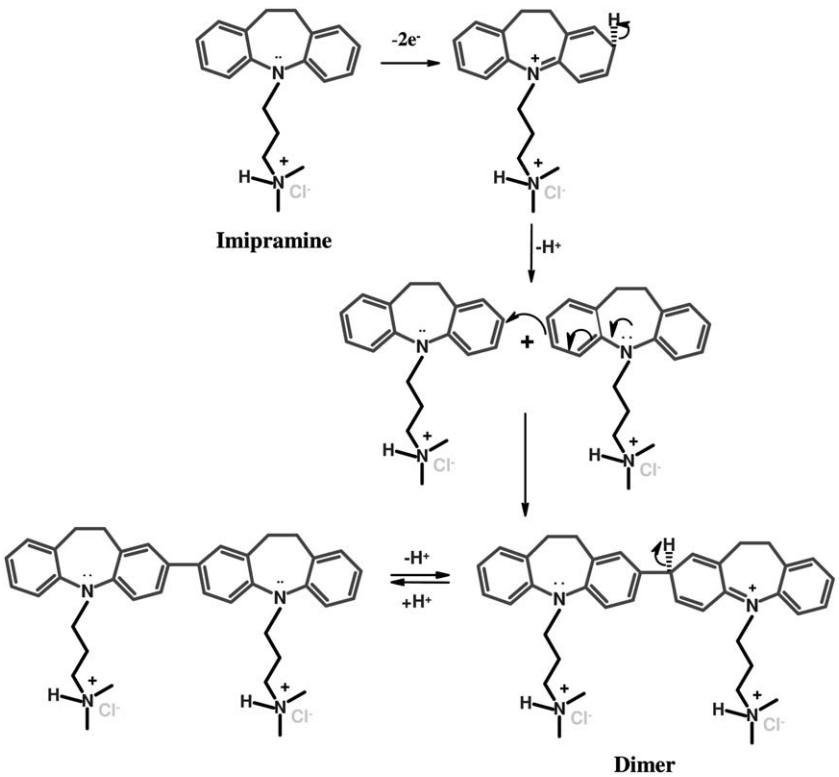


Fig. 2 Cyclic voltammograms of 7.41×10^{-7} M IMI at GCPE (– – –), XAD2-GCPE (• • • • • •), TNP-GCPE (•—•) and XAD-TNP-GCPE (——). Voltammetric conditions: scanning electrode potential between −0.20 and +1.3 V at a scan rate of 10 mV s^{-1} in phosphate buffer (pH 6.0).



Scheme 1 Scheme of the electron-transfer process involving IMI.

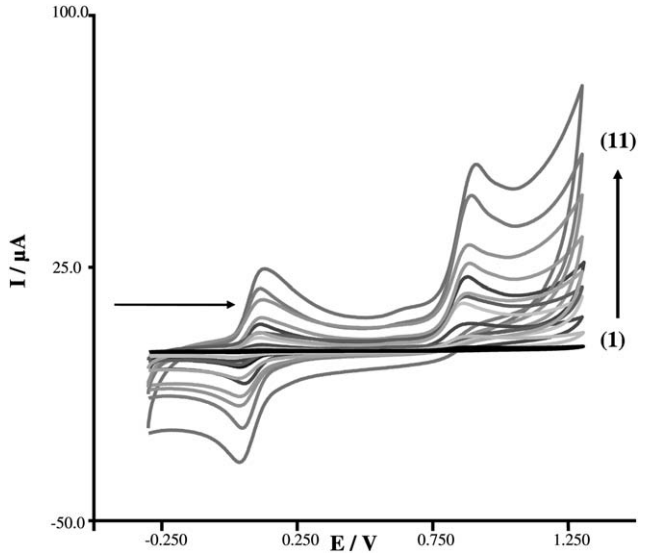


Fig. 3 Cyclic voltammograms of IMI (7.75×10^{-7} M) obtained in phosphate buffer solution (pH 6.0) at XAD-TNP-GCPE employing varying scan rates (mV s^{-1}): 10, 20, 30, 40, 60, 80, 100, 200, 300, 400 and 500. Other conditions as given in Fig. 1.

increase linearly with the scan rate (ν) in the range from 10 mV s^{-1} to 500 mV s^{-1} (Fig. S9†) which implies that the oxidation of all the TCAs is adsorption controlled on XAD2-TNP-GCPE, governed by the following equations:

$$\text{IMI (O1): } I_p\ (\mu A) = 0.0432\nu + 6.9268\ (r = 0.9972) \quad (2)$$

$$\text{TRI (O1): } I_p\ (\mu A) = 0.0438\nu + 4.9812\ (r = 0.9985) \quad (3)$$

$$\text{DES (O1): } I_p\ (\mu A) = 0.0425\nu + 6.1420\ (r = 0.9982) \quad (4)$$

Furthermore, kinetic terms [transfer coefficient (α), number of electrons in the rate determining step (n_α) and order of reaction] were calculated employing the following formulae[44] (varying concentration of IMI in the range of 5×10^{-8} to 25×10^{-8} M):

$$I_{pa} = (2.99 \times 10^{-5})\, n[(1-\alpha)n_a]^{0.5} A C_0^* D_0^{0.5} \nu^{0.5} \quad (5)$$

On solving the equation, the value of $(1-\alpha)n_a$ is calculated to be 0.50. Thus, the number of electrons in the rate determining step is 1. Substituting this value of $(1-\alpha)n_a$ and other relative parameters in eqn (4), the number of electrons involved in the electrode reaction for oxidation of IMI is calculated to be 2.

Also, the number of electrons (n) involved in the reaction was calculated from the cyclic voltammetry. $E_p - E_{p/2}$ values were calculated to be 47.3 mV and 48.0 mV for IMI and TRI, respectively. These values were then substituted in the following equation to obtain 'n':

$$E_p - E_{p/2} = 47.7/(1-\alpha)n_a \text{ mV at } 25\ ^\circ\text{C} \quad (6)$$

The αn_a values are found to be 1.03 and 0.99 respectively for IMI and TRI respectively. Now, for a totally irreversible reaction, the electron transfer coefficient (α) is assumed to be 0.5. Therefore, by substitution of the value of α in the above equation provides the value of n to be *ca.* 2 for both the molecules of interest. Thus by using both the formulae, we obtain the number of electrons involved in the electrochemical reaction of IMI as 2.

Furthermore, the slope of the straight (plot of log I_p *vs.* log C_0^*) for IMI oxidation peak is 0.99. This slope reveals that the oxidation reaction for IMI at XAD2-TNP-GCPE is first order.

3.4 Electrochemical impedance spectroscopy (EIS)

The electrochemical characterization of GCPE, XAD2-GCPE, TNP-GCPE and XAD2-TNP-GCPE was carried out by means of electrochemical impedance spectroscopy (EIS). The Nyquist plots for IMI (7.12×10^{-5} M) show a significant difference in the response for all the four electrodes as shown in Fig. 4. A semicircle with larger diameter is observed for GCPE in the frequency range of 10^2 to 10^6 Hz. However, the diameter of semi-circle diminished with the use of XAD2-TNP-GCPE. The charge transfer resistance (R_{ct}) values obtained from Fig. 4 for IMI at GCPE, XAD2-GCPE, TNP-GCPE and XAD2-TNP-GCPE are 775.97, 376.63, 253.59 and 104.73 Ω respectively. This implies that the charge transfer resistance of the electrode surface decreases and the charge transfer rate increases on employing XAD2-TNP-GCPE.

3.5 Chronocoulometry (CC)

For the determination of the kinetics and mechanism of electrode reactions involved in the oxidation of 4.77×10^{-5} M IMI, 4.23×10^{-5} M TRI and 5.05×10^{-5} M DES at the GCPE, XAD2-GCPE, TNC-GCPE and XAD2-TNP-GCPE, chronocoulometry was employed. By employing double-potential step chronocoulometry, after point-by-point background

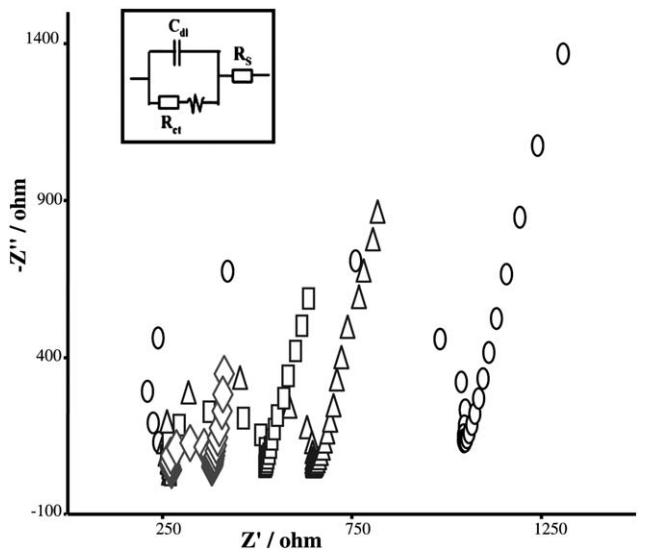


Fig. 4 Nyquist plots for EIS measurements (7.12×10^{-5} M) IMI at GCPE (**ooo**), XAD2-GCPE (ΔΔΔ), TNP-GCPE (□□□) and XAD2-TNP-GCPE (◊◊◊). In the box on the left upper side: equivalent circuit used for data fitting.

subtraction, the plot of charge (Q) *vs.* the square root of time ($t^{1/2}$) shows a linear relationship. According to the integrated Cottrell equation, the diffusion coefficient and Q_{ads} of IMI can be estimated from the slope and intercept respectively from the plot of total Q *vs.* $t^{1/2}$ which is given by the Anson equation.[45] The calculated parameters are presented in Table S1.† As can be seen from Table S1,† the value of slope and Q_{ads} for IMI, TRI and DES employing XAD2-TNP-GCPE are higher as compared to other electrodes, confirming that XAD2 along with TNPs makes the accumulation of all the three TCAs onto the electrode surface more effective. The surface coverage (Γ^0) for all the four electrodes is calculated using eqn (7)

$$Q_{ads} = nFA\Gamma^0 \quad (7)$$

and the results are given in Table S1.† From the values of Q_{ads} it is observed that the surface coverage in case of XAD2-TNP-GCPE is maximum and hence the sensitivity for all the three TCAs is maximum at this electrode.

3.6 Scanning electron microscopy (SEM)

Fig. 5 compares the morphological features of GCPE, XAD2-GCPE, TNP-GCPE and XAD2-TNP-GCPE using SEM. The SEM profile of GCPE [Fig. 5(a)] shows a non-porous spherically shaped glassy carbon powder on the surface. Fig. 5(b) indicates that the GCPE has been modified by XAD2. The SEM image of TNP-GCPE [Fig. 5(c)] depicts that GCPE has been modified by TNPs. Hence, the current increase at XAD2-TNP-GCPE [Fig. 5(d)] as compared to GCPE is probably due to the perfectly spherical structure of GCPE which is synergistically modified with XAD2 and highly conducting TNPs. This synergistic combination of both XAD2 and TNPs results in high sensitivity of the modified electrode for all the three molecules.

3.7 Adsorptive stripping differential pulse voltammetry (AdSDPV)

AdSDPV was employed by studying the influence of accumulation potential (E_{acc}) and accumulation time (t_{acc}) on the oxidation peak current of IMI (Fig. S10†), TRI (Fig. S11†) and DES (Fig. S12†). Keeping t_{acc} as 20 s, E_{acc} was determined by employing a potential window of −1.0 V to +0.5 V. It can be seen from Fig. S10–S12† that the peak current for IMI, TRI and DES reached its maximum at an E_{acc} of −0.2, −0.4 and −0.2 V respectively. Thus, these E_{acc} values were selected for further studies. An increase in the accumulation time improves the sensitivity of determination. Hence, the effect of variation of t_{acc} was studied over a period of 20 s to 200 s, employing optimized E_{acc} values. From 20 to 100 s, there was a linear increase in the peak current of IMI and DES (Fig. S10 and S12†) beyond which the current began to level off while for TRI (Fig. S11†), the current began to level off after 120 s. This implies that the surface saturation occurs at accumulation times above 100 s (for IMI and DES) and 120 s (for TRI). Thus these optimized t_{acc} values were selected as the optimum time at which all the three TCAs can be determined with good sensitivity. Thus, the final conditions for the AdSDPV for IMI: $E_{acc} = -0.2$ V and $t_{acc} = 100$ s; TRI: $E_{acc} = -0.4$ V and $t_{acc} = 120$ s; DES: $E_{acc} = -0.2$ V and $t_{acc} = 100$ s. It can be observed that the accumulation time and accumulation potential for TRI is higher as compared to those of IMI and DES. This may be because TRI has two methyl substituted groups in its structure which makes it more bulkier as compared to IMI and DES.

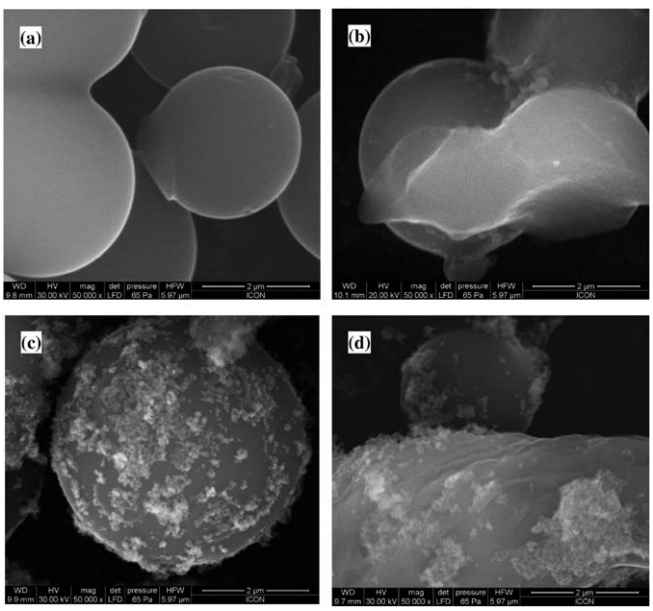


Fig. 5 Scanning electron microscope images of (a) GCPE, (b) XAD2-GCPE, (c) TNP-GCPE and (d) XAD2-TNP-GCPE.

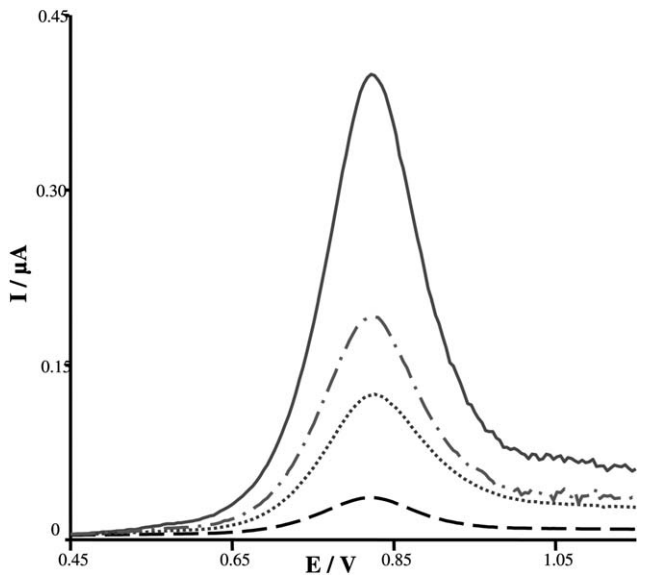


Fig. 6 AdSDPV obtained for 6.33×10^{-8} M IMI at four electrodes: GCPE (– – –), XAD2-GCPE (••••••), TNP-GCPE (•—•) and XAD2-TNP-GCPE (——). Accumulation potential of −0.20 V was applied to all the four electrodes for an accumulation time of 100 s in pH 6.0 phosphate buffer solution (0.1 M).

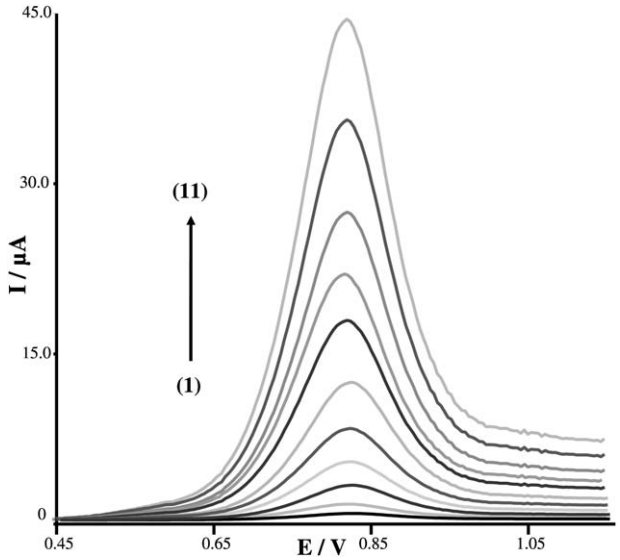


Fig. 7 AdSDPV obtained using XAD2-TNP-GCPE for IMI at different concentrations: (1) 1.30×10^{-9}, (2) 3.57×10^{-8}, (3) 8.33×10^{-8}, (4) 2.45×10^{-7}, (5) 6.18×10^{-7}, (6) 8.29×10^{-7}, (7) 1.52×10^{-6}, (8) 3.15×10^{-6}, (9) 4.23×10^{-6}, (10) 5.15×10^{-6}, (11) 6.23×10^{-6} M. Other conditions as given in Fig. 6.

Employing AdSDPV for 6.33×10^{-8} M IMI (Fig. 6), 4.37×10^{-8} M TRI (Fig. S13†) and 7.44×10^{-8} M DES (Fig. S14†), a comparative study has been carried out on GCPE, XAD2-GCPE, TNP-GCPE and XAD2-TNP-GCPE. It can be observed that, the best results for both peak current and peak shape are obtained in the case of XAD2-TNP-GCPE. Thus it can be concluded that the electro-oxidation of all the three TCAs becomes facile at XAD2-TNP-GCPE.

3.8 Interference studies, validation studies and analytical applications

In order to evaluate the selectivity of the method for the determination of IMI, TRI and DES, the influence of potentially interfering substances on the determination of these compounds was investigated. The tolerance limit for interfering species was considered as the maximum concentration that gave a relative error of less than ±5.0% at a concentration of 1.0×10^{-7} M IMI, TRI and DES. Ascorbic acid, uric acid, citric acid, dopamine and glucose are the most common interferences found along with all the three TCAs in biological fluids. Ascorbic acid interfered when present in a 90-fold excess (Fig. S17†) while uric acid and dopamine interfered beyond 70-fold excess. Glucose and starch did not interfere up to a 300-fold excess. K^+, Ca^{2+}, Na^+, Mg^{2+}, NO_3^-, NH_4^+ and Cl^- have no effect on the I_p of IMI, TRI and DES up to a 500-fold excess. This suggests that the determination of these molecules in the pharmaceutical formulations and biological samples at XAD2-TNP-GCPE is not affected considerably by the common interfering species commonly present along with the molecules of interest.

Validation of the proposed procedure for an assay of standard IMI, TRI and DES was examined *via* evaluation of analytical figures of merit *viz.*, limit of detection (LOD), limit of quantitation (LOQ), reproducibility, precision, selectivity and robustness. A plot of I_p (μA) *vs.* potential for increasing concentrations of the three TCAs is given in Fig. 7, S15 and S16.† The plot of I_p (μA) at E_p *vs.* concentration was linear in the range of 1.30×10^{-9} to 6.23×10^{-6} M, 1.16×10^{-9} to 6.87×10^{-6} M and 1.43×10^{-9} to 5.68×10^{-6} M for IMI, TRI and DES respectively. The LODs ($S/N = 3$) of 3.93×10^{-10} M, 3.51×10^{-10} M and 4.35×10^{-10} M were obtained for IMI, TRI and DES respectively using AdSDPV. Characteristics of the calibration graph for the AdSDPV technique have been reported in Table 1. These results indicate the reliability of the proposed method for the trace analysis of standard IMI, TRI and DES solutions. For validation of the proposed method, various parameters such as repeatability, reproducibility, precision and accuracy of analysis were obtained by performing five replicate measurements for 5.43×10^{-8} M standard IMI, 4.27×10^{-8} M standard TRI and 6.11×10^{-8} M standard DES over a single day (intra-day assay) ($n = 5$) and for five days over a period of one week (inter-day assay). Satisfactory mean percentage recoveries (%R) and relative standard deviations (% RSD) were obtained [Table S2(a)†]. The recoveries obtained confirmed both the high precision of the proposed procedure and the stability of IMI, TRI and DES solutions. The

Table 1 Analytical parameters for electrochemical determination of IMI, TRI and DES in pH 6.0 phosphate buffer (0.1 M)

	Molecule		
	IMI	TRI	DES
Linear working range (M)	1.30×10^{-9} to 6.23×10^{-6}	1.16×10^{-9} to 6.87×10^{-6}	1.43×10^{-9} to 5.68×10^{-6}
Linear regression equation	I_p (μA) = 5.791×10^{-6} C (μA) + 0.691	I_p (μA) = 6.031×10^{-6} C (μA) + 0.589	I_p (μA) = 5.707×10^{-6} C (μA) + 0.996
Correlation coefficient (r)	0.9970	0.9983	0.9945
Standard error of slope	2.27×10^{-4}	3.54×10^{-4}	3.99×10^{-4}
Standard error of intercept	6.90×10^{-4}	6.41×10^{-4}	7.52×10^{-4}
Limit of detection (M)	3.93×10^{-10}	3.51×10^{-10}	4.35×10^{-10}
Limit of quantification (M)	1.18×10^{-9}	1.05×10^{-9}	1.30×10^{-9}
Repeatability of peak current (%RSD)	2.23	2.57	2.04
Reproducibility of peak current (%RSD)	2.97	3.12	2.83

robustness of the proposed procedure [Table S2(b)†] was examined by studying the effect of small variation of pH, accumulation potential, E_{acc} and accumulation time, t_{acc} on the recovery of IMI, TRI and DES. As can be seen from the table, %R for all the three molecules were in the range of 98–102% under all variable conditions and did not show a significant change when the critical parameters were varied and is hence robust in nature. For further evaluation of the validity of the proposed method, the recovery tests for IMI, TRI and DES in pharmaceutical formulations, urine and blood serum samples was carried out as shown in Table S3.† Recovery tests were performed even on pharmaceutical formulations as mentioned in Table S3† giving %R in the range of 97.0–102% for all the three TCAs. Also, recovery tests performed on morning urine samples collected from healthy volunteers and human blood serum samples gave similar %R (Table S3†). Recovery results were not affected significantly and consequently the described method is accurate for the assay of IMI, TRI and DES in pharmaceutical formulations as well as biological fluids. For analytical applications, determination of amounts of IMI, TRI and DES in all samples has been carried out by the standard addition method. The amount of IMI, TRI and DES obtained in pharmaceutical formulations agree well with the label contents. The proposed method was further validated by HPLC. The results are as given in Table 2. It can be seen from the table that the amounts of IMI, TRI and DES obtained by the proposed method agree well with those obtained by the standard methods. Applying paired *F*- and *t*-tests on the results obtained by this procedure and those claimed on the labels, it was found that all results are in agreement at the 95% confidence level. The values of *F*- and *t*-tests (calculated experimentally) were less than theoretical *F*- and *t*-values showing that there was no significant difference between the proposed procedure and the HPLC method. Thus determination of IMI, TRI and DES can be carried out in various matrices by the proposed method.

3.9 Stability and reproducibility of the XAD2-TNP-GCPE

The stability of the XAD2-TNP-GCPE was tested by keeping the electrode in pH 6.0 phosphate buffer for 10 days and then the CVs were recorded and compared with the CVs obtained before immersion. The results indicated that the peak current changed only slightly for XAD2-TNP-GCPE, which indicates that it has good stability. The stability of XAD2-TNP-GCPE over time was checked during a period of 120 days. In between measurements the sensor was kept at room temperature in the dry state. A graph of I_p *vs.* days reveals that a small change in the AdSDPV signal (approx. 5%) was observed after 120 days of storage. These results indicate that XAD2-TNP-GCPE is significantly stable and has good capacity to perform repeated measurement for 120 days. In order to study the repeatability of the electrode preparation procedure, five modified electrodes based on the same fabrication procedure were prepared and used for the determination of 7.12×10^{-7} M IMI, 6.33×10^{-7} M TRI and 8.02×10^{-7} M DES solutions. The RSD for the peak currents for all the five electrodes (average of five determinations on each electrode) was calculated to be 2.91%. The reproducibility of the proposed method was checked by determining the RSD for 10 replicate measurements on a single electrode in 7.12×10^{-7} M IMI, 6.33×10^{-7} M TRI and 8.02×10^{-7} M DES. It was found out to be 2.34%. These results indicate that the modified electrode has high repeatability and reproducibility in both the preparation procedure and the AdSDPV determinations.

3.10 Comparison of the proposed method with literature methods

A comparison of the analytical performance of XAD2-TNP-GCPE developed in this study with other sensors dealing with the analysis of IMI,[12–19] TRI[12,16,19] and DES[17,19] is presented in Table 3. The present method is better in terms of sensitivity as compared to all the other reported literature methods except the one given in ref. 13. This method[13] involves determination of only IMI by employing flow injection analysis using Au microelectrode. However, the present method is simple, cost effective and does not require any pre-treatment procedure for the determination of IMI, TRI and DES. As can be seen from Table 3, the lowest LODs are obtained for both TRI and DES by this method. This reveals that XAD2-TNP-GCPE shows excellent analytical performance for determination of IMI, TRI and DES in terms of wide linear dynamic range (more than three orders of magnitude), very low detection limit, high sensitivity, and very good repeatability and reproducibility over other methods reported in the literature.

Table 2 Comparison between the proposed method and the HPLC method for sample analysis[a]

	IMI		TRI		DES			
Sample	a	b	a	b	a	b	*F*-Test	*t*-Test
Tofranil	24.32 ± 1.14	23.93 ± 1.46	—	—	—	—	0.421	0.289
Depsol	74.71 ± 0.87	74.24 ± 0.92	—	—	—	—	0.550	0.327
Surmontil	—	—	25.12 ± 2.51	23.93 ± 1.34	—	—	0.634	0.532
Stangyl	—	—	99.52 ± 1.39	98.22 ± 1.55	—	—	0.731	0.513
Norpramine	—	—	—	—	10.04 ± 2.15	9.92 ± 2.56	0.367	0.321
Pertofrane	—	—	—	—	24.91 ± 1.23	74.24 ± 1.84	0.442	0.410

[a] Theoretical *F*-value = 6.39 and *t*-test value = 2.77 at 95% confidence limit for $n_1 = 5$ and $n_2 = 5$. a: Amount of TCA (IMI/TRI/DES) obtained by the proposed method (mg) ± %RSD ($n = 5$); b: amount of TCA (IMI/TRI/DES) obtained by the HPLC method (mg) ± %RSD ($n = 5$).

Table 3 Comparison between various electroanalytical methods for the determination of IMI, TRI and DES with the proposed method

Molecule	Modified electrodes	Linear working range (M)	Limit of detection (M)	Samples analyzed	References
IMI	Poly(*N*-vinylimidazole)	6.0×10^{-5} to 8.0×10^{-4}	—	Tablets	11
	Glassy carbon electrode	—	8.20×10^{-9}	Serum	12
	FFT at Au electrode	4.41×10^{-11} to 7.07×10^{-8}	1.42×10^{-11}	Tablets	13
	AuNPs modified ITO	5.0×10^{-6} to 1.0×10^{-3}	1.0×10^{-9}	Tablets	14
	Graphite–polyurethane composite electrode	3.04×10^{-7} to 3.04×10^{-6}	4.60×10^{-9}	Tablets	15
	Boron doped diamond electrode	5.0×10^{-8} to 1.0×10^{-4}	3.0×10^{-9}	Plasma	16
	β-CD modified carbon paste electrode	1.0×10^{-7} to 1.0×10^{-6}	2.0×10^{-8}	Tablets	17
	Boron doped diamond electrode	—	5.0×10^{-10}	—	18
	Glassy carbon electrode	—	1.5×10^{-8}	Urine	19
	XAD2-TNP-GCPE	1.30×10^{-9} to 6.23×10^{-6}	3.93×10^{-10}	Pharmaceutical formulations, urine, blood serum	This work
TRI	β-CD modified carbon paste electrode	1.0×10^{-7} to 1.0×10^{-6}	2.0×10^{-8}	Tablets	17
	Glassy carbon electrode	—	1.4×10^{-8}	Urine	19
	XAD2-TNP-GCPE	1.16×10^{-9} to 6.87×10^{-6}	3.51×10^{-10}	Pharmaceutical formulations, urine, blood serum	This work
DES	Glassy carbon electrode	—	1.52×10^{-8}	Serum	12
	Boron doped diamond electrode	5.0×10^{-8} to 1.0×10^{-4}	3.0×10^{-9}	Plasma	16
	Glassy carbon electrode	—	1.7×10^{-8}	Urine	19
	XAD2-TNP-GCPE	1.43×10^{-9} to 5.68×10^{-6}	4.35×10^{-10}	Pharmaceutical formulations, urine, blood serum	This work

4 Conclusion

In the present work, incorporation of XAD2 and TNPs in the matrix of GCPE is introduced as a new and very efficient method for enhancement of resolution and selectivity in the voltammetric response of XAD2-TNP-GCPE for the determination of IMI, TRI and DES. This AdSDPV method benefits from such advantages as high sensitivity, very low detection limit with high reproducibility, easy handling and low cost. Furthermore, the analytical response was unaffected by the presence of common interfering agents in biological fluids. The proposed method has been successfully employed to analyze IMI, TRI and DES in pharmaceutical formulations, urine and blood serum samples. Consequently, this method is recommended for the analyses of these TCAs in various matrices in clinical as well as quality control laboratories.

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