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Synthesis and Characterization of Electrochemical Sensor Based on Polymeric /TiO₂Nanocomposite Modified with Imidizolium Ionic Liquid for Determination of Diclofenac

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A novel, simple and sensitive polymeric nanocomposite electrochemical sensor based on polymeric / TiO₂ nanoparticles mixed with 1-butyl-3-methylimidazolium Chloride [BMIM]Cl ionic liquid and coated with a polymeric layer of poly(3, 4-ethylene-dioxythiophene) (PEDOT) was fabricated for diclofenac sodium (DCF) determination. The morphology and composition of the polymeric nanocomposite was characterized by means of scanning electron microscopy equipped with energydispersive X-ray spectroscopy (SEM-EDX) and transmission electron microscopy (TEM). X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) were also used. The cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance (EIS) spectra were recorded. All results sustained the electrochemical enhancement for the polymeric nanocomposite sensor, which ascribed to the beneficial effect of both TiO₂ nanoparticles and Imidazolium ionic liquid with the conducting behavior of the polymeric layer. Where, the peak currents for (DCF) at PEDOT/TiO₂/[BMIM]Cl/CPE show a great enhancement with a linear response in the concentration range from $5.0 \times 10^{-6} - 1.0 \times 10^{-4}$ mol L⁻¹. The limit of detection (LOD) was found to be 1.17×10⁻⁸ mol L⁻¹. Additionally, high selectivity of the polymeric nanocomposite sensor was noticed in presence of high concentration of ascorbic acid (AA) and uric acid (UA). Finally, the modified sensor was successfully applied for determination of (DCF) in its pure form, pharmaceutical samples of Voltic tablets and in urine samples with good agreement between the added and recovery.

Keywords:TiO₂ nanoparticles; ionic liquid; poly(3, 4-ethylene-dioxythiophene)PEDOT; Diclofenac sodium (DCF); electrochemistry

1. INTRODUCTION

Voltaren (Diclofenac) (DCF) is designated chemically as 2-[(2,6-dichlorophenyl)amino] benzeneacetic acid (Scheme 1). It is a nonsteroidal anti-inflammatory drug (NSAID) taken or applied to reduce inflammation and pain, is widely used for the treatment of symptoms of osteoarthritis or rheumatoid arthritis and chronic pain associated with cancer [1-5]. Various methods have been employed for the analysis of diclofenac, such as spectrophotometry [6-13], high performance liquid chromatography (HPLC) [14-20], and electroanalytical methods. Compared with other methods, electrochemical methods are simple, selective, more economical, and reasonably short analysis time [21-37].

In recent decades, considerable attention has been devoted to nanotechnology and nanoscience, nanomaterial-based electrochemical signal amplifications have great potential of improving both sensitivity and selectivity for electrochemical sensors and biosensors. First of all, it is well-known that the electrode materials play a vital role in enhancing the performance of electrochemical sensor. Furthermore, based on the unique properties of nanomaterials, the biofunctional nanoparticles can produce a synergic effect among catalytic activity, conductivity, and biocompatibility to result in significantly signal amplification for designing a new generation of nanobiosensing device.

TiO₂ nanoparticle has excellent electrocatalytic properties such as large surface area, high chemical and thermal stability, and good conductivity [38,39].

ILs are organic salts, consisting of an organic cation and either an inorganic or organic anion that are liquid below 100°C. Considering their low volatility, good ionic conductivity, excellent thermal and electrochemical stability, low flammability and toxicity, ionic liquids (IL) are widely used in electrochemical analysis.

Among various types of conducting polymers, poly(3,4-ethylenedioxythiophene) (PEDOT) is a widely investigated and can be easily electrodeposited on several surfaces by electro-oxidation of its monomer[40–43]. PEDOT modified electrodes have been extensively reported and showed excellent electrocatalytic effect [44].

Herein, a polymeric nanocomposite sensor based on titanium oxide nanoparticles with ionic liquid [BMIM]Cl and coated by polymer (PEDOT) was fabricated for sensitive electrochemical determination of (DCF). Characterization of the modified electrode was carried out by using different surface and electrochemical techniques in order to evaluate the effect of TiO_2 nanoparticles, ionic liquid, and polymer on enhancing oxidation current peak.Surface analysis (SEM, TEM and EDX) were used to illustrate the morphological structure of the polymeric nanocomposite.FTIR analysis allowed characterization of the molecular interactions occurring after inclusion of TiO_2 nanoparticles. Various parameters were also studied to optimize the sensitivity of the modified paste. The proposed sensor was applied for (DCF) determination in real samples as in tablets and human urine.





Scheme 1. Chemical structure of diclofenac sodium.

2. EXPERIMENTAL

2.1. Materials and Methods

2.1.1. Materials

All chemicals were used as received without further purification. Pure diclofenac sodium (DCF) was obtained from JamjoomPharma, An Afrasia Enterprise, Jeddah, Saudi Arabia. Graphite, titanium (IV) oxide (particle size <100 nm), 1-butyl-3-methylimidazolium chloride, [BMIM]Cl, ionic liquid (IL), 3,4-ethyelenedioxythiophene (EDOT), lithium perchlorate (LiClO₄), acetonitrile (HPLC grade), potassium ferrocyanide, ascorbic acid (AA) and uric acid (UA) were supplied by Aldrich Chem. Co. (Milwaukee, WI. USA). Aqueous solutions were prepared using deionized water. Britton-Robinson (B-R) buffer solutions of pH 2–8 were prepared from 0.1 M Boric acid, 0.1 M acetic acid and 0.1 M orthophosphoric acid and adjusted to the desired pH by 0.5 M NaOH. A stock solution of diclofenac sodium (1×10^{-3} M) was prepared with deionized water.

2.1.2. Electrochemical study

All voltammetric and electrochemical measurements have been performed on MetrohmAutolab electrochemical analyzer (Autolab 73022) using NOVA software. Voltammetric experiments were realized using a conventional electrochemical cell with three electrodes, where modified carbon paste electrode was used as working electrode, Ag/AgCl (3.0 M KCl) as reference electrode. The auxiliary electrode was 10 cm long/2.0 mm diameter platinum wire.

The modified working electrode was prepared by mixing 55.0% (w/w) graphite, 12.0% (w/w) TiO₂ nanoparticles and 18.0% (w/w) [BMIM]Cl ionic liquid thorough hand mixing in a mortar and pestle. Using a syringe, 0.3 mL (15.0% (w/w)) of paraffin oil were added drop by drop to the mixture and mixed well for 30 min until a uniformly-wetted paste was obtained. The unmodified carbon paste electrode (CPE) was prepared in the same way without adding TiO₂ nanoparticles and ionic liquid to be used for comparison purposes (Figure 1).

The electrochemical polymerization of EDOT was carried out by cyclic voltammetric method in non-aqueous solution, containing 0.01 M EDOT and 0.1 M LiClO₄, as supporting electrolyte, in

acetonitrile, from -1.50 to +1.50 V with scan rate of 50 mV/s for many repeated cycles. The PEDOT polymer forms a layer that entirely coats carbon paste electrode surface. The thickness of this polymer coat was varied by repeating the voltammetric step for 1, 2, 3 and 4 cycles in order to get the optimum polymer layer thickness for higher sensitivity (Figure 1).



Figure 1. Fabrication of different types of carbon pastes.

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used to record voltammograms using the modified PEDOT/TiO₂/[BMIM]Cl/CPE by scanning the potential from +0.20 to +1.20 V employing a scan rate of 50 mV/s. Appropriate quantities of DCF standard solution (10^{-3} M) were introduced in the electrolytic cell, diluted with B-R Buffer, pH 6, and the voltammograms were recorded. The peak current, Ip, is found by drawing a baseline for the peak of interest and measuring the height of a line extrapolated from that baseline to the crest of the peak. This is always automatically done by the potentiostat software after recoding the voltammogram. In the meanwhile, the peak potentials, Ep, are also evaluated.

Electrochemical impedance spectroscopy was performed using the Autolab system. The parameters in electrochemical impedance experiment were as follows: potential value of 10 mV studied at frequency range of 100 kHz down to 100 mHz with amplitude of 5 mV.

The impedance diagrams were recorded at the free immersion potential and the real (Z) and imaginary (Z') parts of the impedance are calculated from the overall impedance (Z^*) as in equation (1):

$$\mathbf{Z}^{*}(\boldsymbol{\omega}) = \mathbf{Z}(\boldsymbol{\omega}) + \mathbf{j}\mathbf{Z}^{*}(\boldsymbol{\omega})$$
(1)

Where ω is the angular frequency that equals to $2\pi f$ (f is the frequency in Hz).

2.2. Surface Characterization

Philips XL 30 SEM scanning electron microscope was used for characterization of the morphologyand the homogeneity of the coating for the modified carbon paste surfaces at very high magnification of X5000 and compared with bare carbon paste electrode. The samples were coated with gold before SEM examination.

A SEM is equipped with an Energy Dispersive X-ray Analysis system (EDXA) to enable it to perform compositional analysis on specimens. EDXA analysis is useful in identifying materials and contaminants, as well as estimating their relative concentrations on the surface of the specimen.

A model Philips CM10 TEM transmission electron microscope was used to characterize the morphology and grain size of the samples as well as micro-analytical data such as chemical composition at nm scale. X-Ray Diffractometer Bruker D8 Advance was used for recording XRD patterns of pastes.

3. RESULTS AND DISCUSSION

3.1. Surface analysis

3.1.1. Morphological Characterization (SEM, TEM) and elemental analysis (EDXA)



Figure 2. SEM for (a) bare CPE, (b) TiO₂/CPE, (c) TiO₂/[BMIM]Cl/CPE, and (d) PEDOT/TiO₂/[BMIM]Cl/CPE, X-5000 magnification.

Figure 2 displays the scanning electron microscopy (SEM) represented different carbon paste (CPE) surfaces. It can be seen that CPE (Figure 2a) appears as separated layers of irregular flakes of graphite isolated from each other. After TiO₂ NPs were added to the carbon paste matrix, small granules and a lot of aggregation of TiO₂ nanoparticles were detected (Figure 2b). By addition of [BMIM]Cl, ionic liquid (Figure 2c), to the TiO₂–carbon paste, TiO₂ nanoparticles appear as if they are coated with an oily layer that cement them together and by polymer deposition as seen in Figure 2d, the surface became more dense and compact with small nanoparticles layer over carbon sheets.

Whereas, scanning electron microscopy, SEM, detects electrons which reflected from the surface of the sample, transmission electron microscopy, TEM, detects electrons which went through the sample. Thus, SEM focuses on the sample's surface and its composition whereas TEM provides details about the internal composition. Because of the higher resolution of TEM if compared with SEM, it can see particles in the nano-scale and even determine their sizes.



Figure 3. TEM images for (a) bare and (b) PEDOT/TiO₂/[BMIM]Cl/CPE.

For more clarification of the sample morphology, TEM had been done for the same samples bare as well as modified carbon pastes and represented in Figure 3 a and b.

Figure 3a shows the plates of the carbon, while Figure 3b shows that TiO_2 appears in irregular shape, mostly as hexagonal small particles ranging in size from 64 to 187 nm. Imidazolium-type ILs tends to form a gel when mixed with TiO_2 NPs via physical cross-linking of the nanotube bundles, mediated by local molecular ordering of ILs. Thus, free TiO_2 NPs were not observed in the background of the TEM image indicating a strong affinity of TiO_2 NPs for this gel.

Figure 4 shows EDXA spectra represented by bare carbon paste (a) and the polymeric nanocomposite paste (b). Figure 4a shows that the paste contains only carbon and oxygen with 94.99% and 4.96%, respectively. While Figure 4b emphasizes that the modified paste contains titanium with 7.43%, chlorine with 6.33% and sulfur with 2.01%, in addition to carbon and oxygen. These elements are the main components of TiO₂ nanoparticles, [BMIM]Cl ionic liquid and PEDOT polymer. From the previous results, it was confirmed that TiO₂, ionic liquid and polymer are incorporated inside the carbon paste matrix.



Figure 4. EDXA spectra for (a) bare and (b) PEDOT/TiO₂/[BMIM]Cl/CPE.

3.1.2. X-ray Diffraction (XRD) Studies

The x-ray diffraction pattern of a substance is, therefore, like a fingerprint of the substance. The powder diffraction method is thus ideally suited for characterization and identification of polycrystalline phases.

Figure 5 represents the XRD patterns of a bare carbon paste and PEDOT/TiO₂/[BMIM]Clcarbon paste. The XRD patterns of bare CPE showed the characteristic peaks of graphite phase sited at $2\theta = 26.5^{\circ}$, 44.5°, 54.6° and 77.1° (Figure 5a). Figure 5b showed the XRD results for the modified paste where XRD pattern of TiO₂ nanoparticles appears at $2\theta = 25.5^{\circ}$ along with other smaller peaks at 38.0°, 38.8°, 48.1°, 54.1°, 55.1° and 62.9° and those of [BMIM]Cl seems likely to center at higher 2 θ values of 70.0°, 75.0° and 83.0°. Those peaks identify TiO_2 and [BMIM]Cl as the main crystalline components of the modified paste.



Figure 5.XRD patterns for (a) bare and (b) PEDOT/TiO₂/ [BMIM]Cl-carbon paste.

3.1.3. FTIR Analysis

FTIR spectroscopy is useful to verify the presence of functional groups in synthesized compounds or materials in the wave number region 4000-500 cm⁻¹. Figure 6 shows the FTIR spectrum for bare and modified carbon paste. The spectrum of carbon paste (red line) shows the absorption of

C–H stretching at 2948.81, 2924.91 and 2855.54 cm⁻¹. The peaks at 1460.42 cm⁻¹ and 1375.56 cm⁻¹ attributed to CH bend and rock. While, PEDOT/TiO₂/[BMIM]Cl/CPE (blue line) shows the absorption of O–H stretching at 3430.43 cm⁻¹. The peak at 621.97 cm⁻¹ shows stretching vibrations of Ti–O and peak at 1460.69 cm⁻¹ shows stretching vibrations of Ti–O–Ti. Concerning [BMIM]Cl, the peaks of wavenumbers 2958.31, 2929.82 and 2869.66 cm⁻¹ are the aliphatic asymmetric and symmetric (C–H) stretching vibration of the methyl groups. A broad peak in 3148.28 and 3094.46 cm⁻¹ is due to quaternary amine salt formation with chlorine. Peaks at 1628.29 and 1570.03 cm⁻¹ attributed to C=C and C=N stretching. Peak at wavenumber 754.62 cm⁻¹ is due to C–N stretching vibration. Regarding the IR absorption bands of poly(3,4-ethlyenedioxy thiophene), the bands at 1017.41, 1087.22, 1115.57 and 1166.23 cm⁻¹ correspond to the stretching vibrations of ethylenedioxy group (C–O–R–O–C).



3.2. Optimizing electrode condition

3.2.1. Effect of polymer layer thickness on electrochemical response of Diclofenac Sodium

There are various ways in which the polymeric modified electrodes can benefit analytical applications. These include acceleration of electron-transfer reaction, preferential accumulation, and selective membrane permeation. A very thick mediating layer provides a high amount of redox active centers available for mediation but, on the other hand, it shows bad charge transport characteristics and therefore a decreased sensitivity. Therefore, it is recommended to investigate the effect of the thickness of the PEDOT polymer layer on the electrochemical signal of diclofenac sodium. The thickness of this

polymer coat was varied by repeating the electrochemical polymerization step for 1, 2, 3 and 4 voltammetric cycles in order to get the optimum polymer layer thickness for electrochemical studies of diclofenac sodium.

Figure 7.a) Differential pulse voltammograms (DPVs) of 1.0×10^{-4} mol L⁻¹ diclofenac sodium (DCF)/0.1 mol L⁻¹ B-R buffer, pH 6, at CPE electrode modified with 1, 2, 3 and 4 polymerization cycles with PEDOT polymer at scan rate of 50 mV s⁻¹. **b**) Effect of number of polymerization cycles on electrochemical response (peak current, I_p) of DCF.

The differential pulse voltammograms shown in Figure 7a indicates that the polymeric layer obtained after two polymerization cycles furnished the optimum thickness that gives the highest possible peak current (I_p) with the lowest residual current (baseline plateau). This finding is confirmed by Figure 7b where the number of polymerization cycles, that reflects the polymer layer thickness, is plotted against the electrochemical response (peak current, I_p) of DCF. Therefore, PEDOT/TiO₂/[BMIM]Cl/CPE covered with a polymer coat obtained after two electropolymerized cycles will be used in all coming studies.

3.2.2. Effect of pH

Initially known standard of 1×10^{-4} M DCF was used to optimize the pH of supporting electrolyte that best suited for determination of DCF using differential pulse voltammetry. The influence of pH on the electrochemical behavior of DCF was investigated in the range of 2–8 using B-R buffer.

Figure 8A (a-d) shows differential pulse voltammograms of 1×10^{-4} M DCF at different working electrodes, bare CPE (a), TiO₂/CPE (b), TiO₂/[BMIM]Cl/CPE (c) and PEDOT/TiO₂/[BMIM]Cl/CPE (d) at different pH values where one oxidative peak is obtained regardless of either the pH of the medium or the type of electrode paste. These results suggest that DCF, from the electrochemical point of view, behaves similarly at different pH values at all type of pastes (a-d). A plausible mechanism is presented for oxidation of DCF and the pathway is shown in scheme 1. The electrochemical oxidation of diclofenac at the proposed electrodes can be represented by a two-electron coupled two-proton transfer mechanism [45].

Scheme 1. Probable oxidation mechanism for DCF

The effects of pH of the medium on the anodic peak potential and anodic peak current were investigated, and the results are depicted in Figure 8B. It can be seen that as pH of the solution increases, the anodic peak potential (E_P) shifts to less positive values.

Figure 8. A) Effect of solution pH on the behavior of (a) bare CPE, (b) TiO₂/CPE, (c) TiO₂/[BMIM]Cl/CPE and (d) PEDOT/TiO₂/[BMIM]Cl/CPE in 1×10^{-4} mol L⁻¹ DCF/0.1 mol L⁻¹ B-R buffer: at different pH values of 2, 4, 6, 7, and 8 at a scan rate of 50 mV s⁻¹. B) Effect of pH on peak potential, E_p (left) and peak current, I_p (right) of different pastes in 1×10^{-4} mol L⁻¹ DCF/ 0.1 mol L⁻¹ DCF/ 0.1 mol L⁻¹ B-R buffer: at different pH values of 2, 4, 6, 7, and 8 at a scan rate of 50 mV s⁻¹.

In addition, the graph of the anodic peak potential $(E_P)vs.$ pH at PEDOT/TiO₂/[BMIM]Cl/CPE shows a good linear relation in the range of pH 2–8. The linear regression equation was obtained:

$$E_p = 1.067 - 0.031 \text{ pH}$$
 (*R*²=0.981) (2)

which is indicative of equal number of electrons and protons being involved in the oxidation of DCF at different pH values. On the other hand, Peak current I_p increases gradually upon increasing pH values from 2 to 6 and then a sharp decrease in the current response from 6 to 8 was observed.

The maximum current intensity (I_p) was observed at pH 6 throughout the pH range studied beyond which the peak current started to deteriorate. Thus, B-R buffer of pH = 6 was employed for further studies.

3.2.3. Effect of scan rate on the voltammetric response of Diclofenac Sodium

The oxidation peak currents (I_p), of 1×10^{-4} M DCF solution at bare CPE, TiO₂/CPE, TiO₂/[BMIM]Cl/CPE and PEDOT/TiO₂/[BMIM]Cl/CPE at pH 6, varied with change of scan rate (v) in the range of 10–120 mV s⁻¹ as shown by the voltammograms in Figure 9A (a-d). Generally, it was observed that as the scan rates increases, the oxidation peak potential is shifted to more positive values along with a synchronized increase in current in different types of electrode modification.

Voltammetry is very useful in determining the mode of transport for the electrochemical system. The system is designed so that there are two possible modes of adjoining of the electroactive species to the electrode surface, namely adsorption and diffusion. The system might exhibit either of these or a combination of both.

A quantitative method for determining the mode of transport can be accomplished by varying the scan rate (v). For diffusion-controlled reactions, the peak current (I_p) would be proportional to $v^{1/2}$. For an adsorption-controlled reaction, I_p is proportional to scan rate v. Since the major difference is the exponent on scan rate, it is possible, as an alternative, to plot a graph between log I_p(y-axis) versus log v (x-axis). Thus, the slope of this line will be the exponent on the scan rate. If this graph (log I_p vs. log v) has a slope of 0.5, the process is diffusion-controlled. If the graph has a slope of 1.0, the process is adsorption-controlled. If the slope is between 0.5 and 1.0, both processes are important.

In our study, the logarithm of anodic peak current was proportional to the logarithm of scan rate in the range of 10–60 mV s⁻¹ at TiO₂/[BMIM]Cl/ PEDOT/CPE surface (Figure 9B) with the regression equation:

$$Log I_p (\mu A) = 0.585 log v + 1.002 (mV s^{-1}) \qquad (R^2 = 0.9987)$$
(3)

Figure 9.A) Cyclic voltammograms and differential pulse voltammograms of 1.0×10^{-4} mol L⁻¹ DCF/0.1 mol L⁻¹ B-R buffer, pH 6, at (a) bare CPE, (b) TiO₂/CPE, (c) TiO₂/[BMIM]Cl/CPE and (d) PEDOT/TiO₂/[BMIM]Cl/CPE at different scan rates. B) Plot of logarithm of anodic peak current values (log I_p) as a function of logarithm of the scan rate (log v) at PEDOT/TiO₂/[BMIM]Cl/CPE.

This linear relation indicates that the electrode process was controlled mainly by diffusion. That is confirmed by the slope of 0.585, which is rather close to the theoretically expected 0.5 value for a diffusion-controlled process [46]. In the meanwhile, it is also possible to assume that DCF moiety may to some extent adsorb on the electrode surface.

3.3. Electrochemical Investigations

3.3.1. Electrochemical Impedance Measurements

Figure 10. Impedance data recorded as Nyquist plot of 1.0×10^{-3} mol L⁻¹ K₄[Fe(CN)₆]·3H₂O at (**a**) bare CPE, (**b**) TiO₂/CPE, (**c**) TiO₂/[BMIM]Cl/CPE, (**d**) PEDOT/TiO₂/[BMIM]Cl/CPE.

Electrochemical impedance spectroscopy (EIS) is considered to be an effective tool of investigating the property of electrode interfaces. Nyquist plots for the bare CPE, TiO₂/CPE, TiO₂/[BMIM]Cl/CPE, and PEDOT/TiO₂/[BMIM]Cl/CPE, have shown in Figure 10 (curve a,b,c,d), respectively. In a typical Nyquist plot, EIS consists of two parts: the linear part at low frequency and semicircle part at high frequency. Linear part is diffusion controlled process, and the semicircle part is in conformity to the electron transfer resistance (R_{et}). On the bare CPE, the value of R_{et} was 36.85 KΩ (curve a), attributed to the presence of non-conductive liquid paraffin in the carbon paste, while for TiO₂/CPE, the value of R_{et} was decreased to 7.85 kΩ (curve b), which was smaller than that of CPE. This decrease arises from the presence of conductive TiO₂ in the carbon paste. By addition of IL into TiO₂/CPE to get a TiO₂/IL/CPE, the value of R_{et} was due to the presence of high conductive IL in the carbon paste. On the PEDOT/TiO₂/IL/CPE (curve d), the value of R_{et} was equal to 0.88 kΩ, which has the best current enhancement. All these results demonstrated that PEDOT/TiO₂/[BMIM]Cl has better electrochemical properties and can promote electron transfer process more than that of bare CPE, TiO₂/CPE and

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 $TiO_2/[BMIM]Cl/CPE$. Consequently, DCF can be successfully oxidized on the surface of PEDOT/TiO_2/[BMIM]Cl/CPE with the lowest charge transfer resistance at the interface and hence the faster electron transfer rate [47-49].

3.3.2. Electrochemical behavior of Diclofenac Sodium at different modified carbon paste electrodes

The selection of a working electrode for a certain application depends primarily on two factors; the redox behavior of the target analyte and the background current over the potential region required for the measurement. The microstructure and chemical composition of the electrode surface determines how an electron-transfer reaction proceeds.

The electrochemical behavior of diclofenac sodium at different modified electrodes was studied by differential pulse voltammetry. Figure 11 shows the DPV responses of 1×10^{-4} mol L⁻¹ DCF at the surface of bare CPE (a), TiO₂/CPE (b), TiO₂/[BMIM]Cl/CPE (c) and PEDOT/TiO₂/[BMIM]Cl/CPE (d) in 0.1 M BR buffer solution, pH 6, at a scan rate of 50 mV s⁻¹. At first, it is noticed that modification of carbon paste electrode does not actually affect the redox behavior of diclofenac as one oxidative peak is obtained for diclofenac in all types of pastes.

An oxidation peak of a relatively low activity was observed at both bare CPE (Fig. 11a) and at TiO₂/CPE (Fig. 11b). The DCF peak potentials at bare CPE and atTiO₂/CPE were observed around 0.60 and 0.59 V with oxidation peak currents of 6.0 and 9.7 μ A, respectively. The slight increase in current response of DCF at TiO₂/CPE may be attributed to the good electrical conductivity, high chemical stability and large surface area of TiO₂ nanoparticles.

In contrast, TiO₂/[BMIM]Cl/CPE exhibits a significant oxidation peak around 0.60 V with a peak current of 87.0 μ A (Fig. 11c). The imidazolium ion of [BMIM]Cl could interact with graphite and TiO₂ through π – π and/or π –cationic, hydrophobic, or electrostatic interaction, which resulted in the formation of a stable modified electrode. The dramatic increase in oxidation current peak of DCF indicated that the presence of ionic liquid in TiO₂/[BMIM]Cl/CPE has a great improvement in the electrochemical response, which was partly due to the electronic properties of IL together with TiO₂ nanoparticles that gave the ability to promote charge transfer reactions, provide a relatively higher electrochemically accessible surface area and leads to good anti-fouling properties.

Furthermore, the enhancement in the anodic peak current (100.6 μ A) and the slight anodic shift in the peak potential (0.68 V) at the surface of modified PEDOT/TiO₂/[BMIM]Cl/CPE (Fig. 11d) in comparison to that of bare CPE indicated the role of coating the electrode surface with PEDOT in accelerating electron transfer process and increasing the surface area of the proposed modified electrode. The facility in the electron transfer kinetic of DCF can be also deduced from the high chemical stability, high surface area and good electrical conductivity of PEDOT. In addition, the polymeric thick layer film permits a higher surface coverage and therefore increases the amount of the electroactive DCF moiety at the electrode surface.

Finally, one can deduce that incorporation of TiO_2 nanoparticles and 1-butyl-3-methylimidazolium chloride ionic liquid, [BMIM]Cl, in the carbon paste electrode and coating its surface with poly(3,4-ethyelene-dioxythiophene) polymer enhance tremendously the electrochemical response of DCF if compared with the bare carbon paste electrode (Fig. 11a-d).

Figure 11. Differential pulse voltammograms (DPVs) of 1.0×10^{-4} mol L⁻¹ Diclofenac Sodium (DCF)/ 0.1 mol L⁻¹ B-R buffer, pH 6, at (a) bare CPE,(b) TiO₂/CPE, (c) TiO₂/[BMIM]Cl/CPE, (d) PEDOT/TiO₂/[BMIM]Cl/ CPE, at scan rate of 50 mV s⁻¹. Inset: DPVs of the respective electrode in B-R buffer.

3.3.3. Interference Studies

In biological environments, the main interference of diclofenac is the presence of high concentration of ascorbic acid (AA) and/or uric acid (UA). So it is important to examine the electrochemical response of DCF in the presence of AA and UA. Thus, the DPV technique was used to investigate the interference study in a mixture of 1.0×10^{-3} M AA, 1.0×10^{-3} M UA and 1.0×10^{-4} M DCF as shown in Figure 12.

The electrochemical oxidation of AA and UA at the modified PEDOT/TiO₂/- [BMIM]Cl/CPE, at pH 6, occurs at approximately 0.05 V and 0.4 V, respectively, while the oxidation peak of DCF is observed at 0.68 V. These results clearly indicate that DCF shows a well-defined anodic peak that is largely separated from those of UA and AA by about 0.3 V. This illustrates the good selective

determination of DCF using PEDOT/TiO₂/[BMIM]Cl/CPE in presence of high concentration of AA and UA at pH 6.

Figure 12. Differential pulse voltammograms showing the effect of interference of 1.0×10^{-3} mol L⁻¹ AA, and 1.0×10^{-3} mol L⁻¹ UA on electrochemical signal of 1.0×10^{-4} mol L⁻¹ DCF/0.1 mol L⁻¹ B-R buffer, pH 6, scan rate 50 mV s⁻¹. Inset: in absence of DCF.

3.4. Analytical studies and applications

3.4.1. Calibration Graph

The electrochemical response of DCF was studied by differential pulse voltammetry (DPV). Figure 13 shows the calibration curve constructed using different concentrations of DCF at bare CPE electrode in 0.1 mol L^{-1} B-R buffer solutions, pH 6. As DCF concentration increases, the anodic peak current (I_p) increases and was found to be linearly dependent on DCF concentration in the range of 10.0–100.0 μ M. The linear regression equation is presented as:

 $I_{p}(\mu A) = 0.0083 C (\mu M) + 8.744 \qquad (R^{2} = 0.989)$ (4)

Where *C* is the concentration of DCF. The limit of detection (LOD = $3\sigma/b$) and limit of quantitation (LOQ = $10\sigma/b$) were calculated [50], where σ is the standard deviation of the intercept and *b* is the slope of the calibration graph. LOD and LOQ were found to be 2.77×10^{-6} mol L⁻¹ and 9.23×10^{-6} mol L⁻¹, respectively. The sensitivity was calculated to be 0.132μ A.

Figure 13. a) DPVs for 0.1 mol L^{-1} B-R buffer of pH 6 at bare CPE in different concentrations of diclofenac sodium (10.0 μ M–100.0 μ M). b) Calibration curve of DCF at bare CPE electrode in the concentration range from 10.0 μ M up to 100.0 μ M.

Figure 14a shows differential pulse voltammograms of various concentrations of DCF at PEDOT/TiO₂/[BMIM]Cl/CPE in 0.1 mol L^{-1} B-R buffer solutions, pH 6. With increasing DCF concentration, the anodic peak current increases [51]. The calibration curve, under the same conditions, is shown in Figure 14b.

Figure 14. a) DPVs of 10 mL of 0.1 mol L^{-1} B-R buffer of pH 6 at PEDOT/TiO₂/[BMIM]Cl/CPE in different concentrations of diclofenac sodium (5.0 μ M–100.0 μ M). **b)** Calibration curve of DCF for concentrations from 5.0 μ M up to 100.0 μ M.

The peak current (I_p) was found to be linearly dependent on DCF concentration in the range of 5.0–100.0 μ M. The linear regression equation is presented as:

 $I_{p}(\mu A) = 0.0435 C(\mu M) + 95.53 \qquad (R^{2} = 0.997)$ (5)

The LOD and LOQ were found to be 1.17×10^{-8} mol L⁻¹ and 3.91×10^{-8} mol L⁻¹, respectively. The sensitivity was calculated to be 0.69 μ A

On comparing the performance characteristics of the modified PEDOT/TiO₂/ [BMIM]Cl/CPE electrode with those of the bare carbon paste electrode, it is clear that the modified electrode is far better regarding the determination of diclofenac sodium. The limit of detection for the former is lower by order of 100 while the sensitivity is five times higher than that of bare electrode.

3.4.2. Determination of Diclofenac in Urine

The proposed sensor was used to detect diclofenac sodium in urine samples, which has been obtained from healthy volunteer. No signal was observed for DCF in urine samples; therefore, the urine samples were spiked by different concentrations of DCF standard solution, and then used for further determination.

The urine samples were diluted 10 times in B-R buffer, pH 6, to minimize any matrix effect. The anodic peak current (I_p) obtained in each measurement, with the help of the regression equation 5, is used to calculate the recovery values of DCF in urine. The data are presented in Table 1. The recovery of the spiked samples ranged between 97.20% and 99.94%. The RSD (n=5) was less than 2.3%.

Table	1. Recovery	data fo	r synthesized	l biological	solution	spiked	with	various	amounts	of 1	1.0×10^{-4}
	$mol L^{-1}DC$	F, in fre	sh urine samj	ole taken fro	om health	ıy volun	teers.				

Urine	Spike	Found	Recovery	RSD
sample	(µmol L ⁻¹)	(µmol L ⁻¹)	(%)	(%) ^a
1	10.00	9.72	97.20	1.9
2	30.00	29.68	98.93	2.3
3	50.00	49.97	99.94	2.0

^a Average of five replicate measurements

It is obvious that the results detected by this method are in good agreement with the spiked concentrations, revealing that the proposed method has good accuracy, high precision and promising applications. It is worth to mention that uric acid and ascorbic acid that are commonly present in urine samples don't interfere.

3.4.3. Determination of Diclofenac in Voltaren Tablets

In order to verify the reliability of PEDOT/TiO₂/[BMIM]Cl/CPE for analysis of DCF in a pharmaceutical product, the modified electrode was used to determine DCF in Voltaren tablets (50.0 mg diclofenac sodium per tablet).

The modified electrode was applied for the recovery assessment of diclofenac in tablets using standard addition method by adding different standard concentrations of DCF to the dissolved tablet sample. The results in Table 2 indicate that the amounts obtained by the proposed modified electrode are in good concurrence with the declared specifications on the pharmaceutical samples with recoveries values between 97.98 and 102.05% for five measurements. The results found here indicate that the proposed voltammetric procedure is quite reliable and accurate for assay of DCF in its pharmaceutical formulation.

Table 2. Recovery data obtained for diclofenac in pharmaceutical Voltaren tablets by standard addition method.

Sa	Content	DCF added	DCF found	Recovery
mple	$(\mu mol L^{-1})$	$(\mu mol L^{-1})$	(µmol L ⁻¹)	(%) ^a
1	40.00	3.00	43.86	102.05
2	40.00	5.00	44.28	98.40
3	40.00	7.00	46.86	99.70
4	40.00	10.00	48.99	97.98

^a Average of five replicate measurements

In addition, the results of the proposed sensor were compared with the results of the HPLC reference method [52], by means of Student's t- and F-ratio tests at 95% confidence level [53], and there is no significant difference in either accuracy or precision is observed between the two methods (Table 3).

Table 3. Statistical comparison between the results of Voltaren tablets using the proposed method and the reference HPLC method.

Parameter	Proposed method	Reference method
Mean recovery, %	99.13	98.46
SD	2.375	1.120
RSD, %	2.396	1.138
F-ratio (6.388) ^a	4.497	
t-test (2.132) ^b	0.631	

Average of five determinations for the proposed and reference methods.

^a Tabulated F-value at 95% confidence level (F_{4,4}).

^b Tabulated t-value at 95% confidence level and 4 degrees of freedom.

In Table 4, the response characteristics of the proposed method are compared with those obtained by some reported methods. In comparison with some other electroanalytical methods for diclofenac determination, our method is comparable, or even better in some aspects to those described in the literature. The designed sensor is prepared in simple steps with cheap and simple reagents and no pretreatment needed before the measurements. This gives the sensor more advantages over other modified electrodes used in the literature. This sensor showed high sensitivity and anti-interference ability. The sensor was further utilized to determine diclofenac level in human urine and pharmaceutical formulation and satisfactory results were obtained with low detection limit.

Table 4. Comparison of the proposed method with other electroanalytical methods used for determination of diclofenac.

Composition of the modified electrode	Linear range (mol L ⁻¹)	LOD (mol L ⁻¹)	Reference
Pt Hg Hg2(DCF)2 graphite	$5.0 \times 10^{-5} - 1.0 \times 10^{-2}$	3.2×10 ⁻⁵	[54]
Dropping mercury electrode	$1.2 \times 10^{-7} - 2.6 \times 10^{-6}$	6.0×10 ⁻⁸	[55]
Boron-doped diamond electrode (BDDE)	$4.9 \times 10^{-7} - 4.4 \times 10^{-6}$	1.2×10^{-7}	[56]
edge-plane pyrolytic graphite electrode	$2.5 \times 10^{-8} - 1.5 \times 10^{-6}$	2.2×10 ⁻⁸	[57]
(EPPGE)/SWCNTs			
MWCNTs/[BMIM]PF6/CPE	$3.0 \times 10^{-5} - 7.5 \times 10^{-4}$	9.0×10 ⁻⁷	[58]
MWCNTs/Cu(OH)2/[EMIM]PF6/GCE	$1.8 \times 10^{-7} - 1.2 \times 10^{-4}$	4.0×10 ⁻⁸	[59]
PEDOT/TiO ₂ /[BMIM]Cl/CPE	5.0×10 ⁻⁶ -1.0×10 ⁻⁴	1.17×10 ⁻⁸	our work

4. CONCLUSION

A novel, facile and fast-response sensor based on PEDOT/TiO₂/[BMIM]Cl- carbon paste electrode was fabricated for selective and sensitive electrochemical determination of diclofenac.

The surface morphology and composition of the paste were examined by SEM, TEM, EDXA, XRD and FTIR spectroscopy and the results shows the good incorporation of TiO_2 nanoparticles and its uniform distribution in the paste. Furthermore, surface studies confirm that ionic liquid forms a thin binding gel over TiO_2 particles, in addition to PEDOT polymer which forms a conducted layer that completely cover the carbon paste.

The proposed sensor can be applied for detection of DCF at pH 6, and demonstrated that it is easily to discriminate DCF from AA and UA as common interference in biological fluids. The sensor was further utilized to determine diclofenac in human urine and Voltaren tablets with good recovery values.

The modified electrode showed a great improvement to the electrode process of DCF compared to the traditional carbon paste electrode. The obtained results have assured that the proposed polymeric nanocomposite paste is promising for measurements of diclofenac in biological fluids and pharmaceutical formulations with high sensitivity, selectivity, accuracy and with low limit of detection.

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