Voltammetric determination of Diclofenac at a PEDOT modified glassy carbon electrode

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Abstract
In this study, the electrochemical behavior of diclofenac (DCF), a widely used NSAID drug, was undertaken at a modified glassy carbon electrode (GCE). A low-cost, sensitive, stable, and selective electrochemical sensor is proposed for the determination of this analyte by using differential pulse voltammetry in 0.10 M phosphate buffer solution, pH 8.00. At the surface of GCE, EDOT (3,4-Ethylenedioxythiophene) was electropolymerized by chronocoulometry, the charge injected was carefully controlled. The polymerization parameters were 20.0 mM EDOT, H\textsubscript{2}SO\textsubscript{4} pH 1.50, step potential from 0.0 V to 1.1 V vs Ag/AgCl/KCl(sat), for 60 ms. The modified GCE displayed a significant enhancement of the anodic peak current compared to the bare electrode, measured by DPV. A calibration graph of the modified electrode exhibited an increase of the peak current about 63% with respect to the bare. The electropolymerized sensor was applied to DCF quantitation in pharmaceutical preparations with a relative standard deviation of 0.40%. It was also used in urine analysis. The limit of detection of this sensor was 9.06 nM compared to 1.37 µM for the bare electrode.

1. Introduction
Diclofenac 2 -(2,6-Dichloroanilino) phenylacetic acid, (Figure 1), is a non-steroidal anti-inflammatory drug (NSAID) available in different doses and presentations (tablet, capsule, syrup, cream). It is a drug with analgesic and antipyretic properties used to relieve the symptoms of many diseases such as rheumatoid arthritis, osteoarthritis, non-joint rheumatism, post-surgery analgesia in human and veterinary medicine, and sports injuries [1].

Many patients who have been prescribed DCF for arthritis also take additional medications for other chronic health problems such as hypertension, which causes side effects to the ingestion of DCF [2]. Sodium salt is normally used in the form of tablets, capsules, suppositories, intravenous solutions and as gels for dermal application.
Several analytical methods have been implemented for the quantitative determination of DCF in pharmaceutical and biological samples. These methodologies include spectrophotometry, gas chromatography-mass spectrometry, high performance liquid chromatography, and nuclear magnetic resonance [3]. Many of these methods require sophisticated instruments and the analyzes are laborious.

Electroanalytical techniques are now appearing as valid alternatives to determine analytes of pharmaceutical concern. The main advantages of these methods are simplicity, relatively low cost, high sensitivity and low consumption of solvents [4,5]. Mokhtari et al. [6] reported the application of a modified carbon paste electrode with vinylferrocene/multiwall carbon nanotubes, to the analysis of morphine and DCF. They applied different electroanalytical techniques. By the use of square wave voltammetry, the detection limit of DCF was 2.0μmol.L⁻¹. They used this voltammetric sensor to the determination of morphine and diclofenac in real samples. Yilmaz group [7] reported a square wave voltammetric method for DCF determination using a Pt disc electrode in 0.1mol.L⁻¹ TBAClO₄/ACN solution. The limit of detection was 0.50μg.mL⁻¹ (1.69μmol.L⁻¹). A Brazilian group [8] published a paper using modified pencil graphite electrodes to quantify DCF, the limit of detection reached was 0.008μmol.L⁻¹ and a limit of quantification of 0.28μmol.L⁻¹. The electrode displayed good sensitivity, stability, and recovery.

Here, we proposed a voltammetric method for DCF quantitation employing differential pulse voltammetry, DPV. It consisted in a PEDOT polymerized GCE, in 0.10 mol.L⁻¹ phosphate buffer pH 8.00. The detection and quantification limits obtained from calibration plots were 9.06 and 31.7 nmol.L⁻¹, which was much better than other voltammetric approaches. The electropolymerization was performed by chronocoulometry which permitted to control the thickness of the polymer by controlling the concentration of the monomer and the time of the potential step.

2. Methodology

2.1 Instrumentation, electrodes and materials

3,4-Ethylenedioxythiophene (EDOT) was purchased from Aldrich and used as received. All other chemicals were of analytical grade and used without further purification. Electrochemical studies were carried out in 0.10 mol.L⁻¹ phosphate buffer solution, PBS. All solutions were prepared using doubly distilled deionized water and then deaerated by purging with nitrogen (g) for 15 min before performing electrochemical experiments. Experiments were conducted at room temperature.

A 660 E bipotentiostat connected to a PC was used in conjunction with a three - electrode cell. The glassy carbon acted as the working electrode, a Pt wire as the counter electrode, and Ag/AgCl/KCl(sat) as the reference electrode. These electrodes were purchased from BASi®. For the chromatographic analysis a Hitachi La Chrom Ultra L-2455 U was employed coupled to a DAD and a Cadenza CD C18 column 100x4.6 mm, 3μm.

Before modification the GC working electrode was polished with Gamal γ-alumina/water slurry (Fisher) on a micro cloth and then was sonicated first in isopropanol/water for one minute and then in deionized water for 3 minutes. Electrode area was determined by cyclic voltammetry with 0.10mol.L⁻¹ K₃Fe(CN)₆ in 0.10mol.L⁻¹ KCl (Do = 7.63x10⁻⁶ cm².s⁻¹) [9]. For the area calculation the Randles-

![Figure 1. Chemical Structure of Diclofenac.](image-url)
Sevcik equation was used considering a reversible reaction. The calculated area was $0.037 \text{cm}^2$. The Procedure was done thrice.

In experiments were the anodic peak current was measured as a function of sweep rate, $\nu$, the DCF concentration was $1.0 \text{ mmol.L}^{-1}$ in $0.10 \text{ mol.L}^{-1}$ PBS pH 8.00, and the sweep rates were 50, 100, 150, 200, and $250 \text{ mV.s}^{-1}$. The potential window varied from 0.0 to 1.00 V vs Ag/AgCl.

2.2 Characterization of the working glassy carbon electrode

2.2.1 Selection of the pH

In this study, six solutions of $1.00 \text{ mmol.L}^{-1}$ DCF were prepared in phosphate buffer, PBS, at pH values of 6.00, 6.50, 7.00, 7.50, 8.00, and 8.50. The anodic peak current was recorded in each case. The current increased as the pH and at pH 8.00 the sensitivity of the GCE was highest, measured by differential pulse voltammetry, DPV. The pulse amplitude was 50 mV and the pulse width 80 ms.

2.2.2 Calibration curve

With the bare GCE, a calibration curve was prepared. By serial dilution, 10.0mL of solutions 0.75, 2.50, 5.00, 7.50 and $10.0 \mu\text{mol.L}^{-1}$ DCF in $0.10\text{mol.L}^{-1}$ PBS, pH 8.00 were made. DPV was the analytical technique employed from 0.20 V to 0.80 V vs Ag/AgCl/KCl(sat).

2.2.3 Polymer Modification of GCE

The GCE was electropolymerized using EDOT (3,4-Ethylendioxythiophene) by chronocoulometry and by controlling the charge injected. The polymerization parameters were $20.0 \text{mmol.L}^{-1}$ EDOT, $\text{H}_2\text{SO}_4$ pH 1.50, step potential from 0.0 to 1.1V vs Ag/AgCl, for 60ms. These experimental conditions assured a thin layer of the polymer PEDOT and a deposition charge of $35\mu\text{C.cm}^{-2}$, which corresponds to the formation of a mono layer [10]. Different concentrations of EDOT were assayed. The peak current was measured in each case and the best monomer concentration was $10.0 \text{mmol.L}^{-1}$. At higher concentrations the peak current decreased. A calibration plot was prepared with PEDOT/GCE using DCF in the concentration range from 50.0 to $250\text{nmol.L}^{-1}$ in PBS pH 8.00.

2.2.4 DCF in pharmaceutical preparations and spiked urine

Ten tablets of sodium Diclofenac were weighted individually with an average of $0.2110 \pm 0.0072\text{g}$ and mixed thoroughly. 0.2000g was dissolved in 10.0 mL PBS pH 8.00. The standard multiple additions method was employed, DPV was the technique employed.

Urine sample. The experimental Procedure consisted of taking 1.0mL of human urine, 0.50mL $10.0\mu\text{mol.L}^{-1}$ DCF. To this solution 50.0mg of 5-sulfosalicylic acid was added for protein denaturation, the mixture was centrifuged at 8500rpm for five minutes to remove protein residues. The supernatant was carefully filtered and submitted to voltammetric analysis by the multiple additions of the standard method.

2.2.5 HPLC experiments

Since the chromatographic method is widely employed by pharmaceutical companies, we decided to compare both approaches. We employed the method recommended by the 2016 USP pharmacopeia. The mobile phase consisted of a mixture of methanol: PBS 70:30, (v/v), pH 2.50. The mobile phase was previously degassed with nitrogen for 15 minutes and filtered through a $0.22\mu\text{m}$ membrane. The flow rate was 1.0 mL.min$^{-1}$. A calibration plot was constructed from 10.0 to 50.0$\mu\text{g.mL}^{-1}$, prepared in methanol: water (70:30). Measurements were made by triplicate.

2.2.6 Statistical assays

In this part, we assess the stability, repeatability, and reproducibility of the surface modified voltammetric sensor. To a 1.00 mmol.L$^{-1}$ DCF solution, the anodic peak current was measured five times in an 8 hours period. Also, we used this solution for 5 days and repeated measurements were
recorded. The criterion is to have a percent relative standard deviation ≤ 2.00%. From the calibration graph (50.0 to 250 nmol.L⁻¹ in 0.10mol.L⁻¹ PBS, pH 8.00) the dynamic range and the limits of detection, and quantitation were determined.

3. Results and Discussion
The electrochemical parameters at PEDOT/GCE were undergone for DCF determination in 0.10mol.L⁻¹ PBS, pH 8.00, differential pulse voltammetry was the technique employed. The behavior of the modified electrode was superior in comparison to the bare electrode.

3.1 pH effect on electroanalytical response.
The anodic peak current coming from differential pulse of 1.00mmol.L⁻¹ DCF in 0.10mol.L⁻¹ PBS increased gradually until pH 8.00. Then it began to decrease. These peaks were well defined. In the characterization of the electrode and DCF determination, pH 8.0 0 was selected, see Figure 2. At this pH value, the sensitivity was highest.

3.2 Calibration plot of the bare GCE
Peak currents obtained by triplicate measurements of each concentration gave an adjusted graph that follow the equation: \( I_p (\mu A) = 0.171[DCF] + 0.0746 \) where \( R^2 = 0.9846 \). The limits of detection and quantification calculated from the calibration graph were \( 1.37 \) y \( 4.56\mu \text{mol.L}^{-1} \), respectively. The equations used were: \( \text{LOD} = 3.s.m^{-1} \) and \( \text{LQ} = 10.s.m^{-1} \). The standard deviation of the blank is \( s \) and \( m \) the slope of the calibration plot.

3.3 Calibration plot of the PEDOT/GCE
Concentration dependence is linked to the formation of a symmetrical and thin monolayer. Usually, coats or coarse monolayers without contamination. Appropriate electrode /polymer/analyte interactions, enhance electrode response. Figure 3 shows that the current response of 10.0 μM DCF in 0.10mol.L⁻¹ PBS pH 8.00, and 20.0mmol.L⁻¹ EDOT, was optimized. On the modified electrode surface, a DPV of 10.0μmol.L⁻¹ DCF in 0.10mol.L⁻¹ PBS, pH 8.00, showed an Ip response about 60% higher than the bare GCE. In Figure 4 is evident an increase of the peak current of the PEDOT/GCE compared to the bare, indicating a better conductivity, which reflects a decrease of the activation energy for the exchange of electrons at the interface [11,12]. PEDOT has shown better aqueous stability and biocompatibility than polypyrrole and polyaniline and therefore it is considered a promising polymer appropriate for continuous sensing and even in vivo implantation [13]. PEDOT is a superior material due to its low band gap, high electrical conductivity, and stability [14]. The calibration curve exhibited a superior voltammetric behavior as is shown in Figure 5. The \( I_p(\mu A) = 0.0977[DCF] – 3.59, R^2 = 0.9989 \). Taking into account the standard deviation of the blank, 0.047, and the slope of the calibration graph, the calculated detection and quantification limits were 9.06nmol.L⁻¹ (2.90 ppb) and 31.70nmol.L⁻¹ (10.8 ppb).

3.4 DCF in pharmaceutical preparations and in spiked urine
To assess the applicability of the PEDOT/GCE for the determination of DCF in real samples, its utility was proved in DCF determination in tablets (a pharmaceutical preparation) and urine. DPV was the technique used. In figure 4 appears the results obtained by the method of successive standard additions to the tablet sample. The adjusted equation that matches the graph is: \( I_p(\mu A) = 0.030[DCF] + 0.307, R^2=0.9992 \). The Procedure was carried out by triplicate. The outcome was 49.8 ± 1.4mg. The relative error was 0.40%. The DCF content of each tablet is supposedly 50mg.

The pretreated urine sample was analyzed by multiple additions of 10.0 μmol.L⁻¹ DCF standard to the sample. The added volumes were 0.00, 0.10, 0.20, 0.30, and 0.40mL. Differential pulse voltammetry was the technique employed. The graphical treatment of standard additions gave
\[ Ip(\mu A) = 0.026 \ [DCF] + 0.39 \quad \text{with} \quad R^2 = 0.9936. \] The calculated [DCF] in spiked urine was \( 10.9 \pm 0.2 \mu\text{mol.L}^{-1} \).

3.5 HPLC results
A reversed phase HPLC separation of DCF was conducted and a calibration curve was constructed. From the calibration graph, the tablet sample gave \( 49.5 \pm 1.7 \text{mg} \) DCF. The reported value is 50mg. The percent relative error is 1.0\%, referred to 50mg. According to the \( t \)-test there is no significant difference between the two methods for 95\% confidence level. Our voltammetric approach yielded \( 49.8 \pm 1.4 \text{mg} \).

3.6 Statistical assays at PEDOT/GCE
The calibration graph complied the analytical criteria of \( R^2 \geq 0.980 \). For us was 0.9985. The sensitivity of the electrode was higher than the bare electrode. In the analyses of repeatability and reproducibility,
the results are displayed in Table 1. The modified electrode showed good stability. The film did not allow adsorption on its surface.

| Table 1. Repeatability and Reproducibility on PEDOT/GCE. 1.0 mmol.L⁻¹ DCF, PBS pH 8.0 |
|-----------------|-----------------|-----------------|-----------------|
| Repeatability   | Reproducibility | Peak            |
| Time (h)        | Current (μA)    | Day Current (μA)| Potential (V)   |
| 0               | 2.628           | 1               | 2.680           | 0.548           |
| 2               | 2.573           | 2               | 2.762           | 0.542           |
| 4               | 2.571           | 3               | 2.699           | 0.544           |
| 6               | 2.583           | 4               | 2.800           | 0.542           |
| 8               | 2.519           | 5               | 2.780           | 0.544           |
| ⍺              | 2.575           |                 | 2.744           | 0.544           |
| s               | 3.882x10⁻²      |                 | 5.215x10⁻²      | 0.002           |
| %RSD            | 1.51            |                 | 1.90            | 0.450           |

4. Conclusions
Modification of GCE by means of PEDOT electropolymerization (by chronocoulometry) has provided to be efficient to elaborate a voltammetric sensor for the assay of DCF. Optimization of the polymerization parameters such as monomer concentration, potential range, step potential time, and polymer film thickness, yielded excellent analytical performance (in terms of sensitivity, stability, concentration range, and detection limit) in agreement with the concentration of DCF in pharmaceutical preparations and in urine. Our results compete favorably with previous work.

5. References
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