Abstract
The electrochemical sensor was fabricated for the simultaneous determination of levodopa and cabergoline using carbon paste electrode (CPE) modified with graphene quantum dots (GQD), 2-chlorobenzoyl ferrocene (2CBF) and ionic liquid (IL). Then, the electrochemical behavior of levodopa alone and simultaneously with cabergoline at the surface of GQDs/2CBF/IL/CPE was investigated in phosphate buffer solution (PBS). Under optimal PBS, pH=7 condition, oxidation peak current has been found proportional to levodopa concentration in the range between 0.07 μM and 500.0 μM, with the limit of detection (LOD) of 0.02 μM (S/N=3). Outputs showed that at GQDs/2CBF/IL/CPE surface, the levodopa and cabergoline oxidation peaks are separated by the potential difference of 200 mV. In addition, it was found that this modified electrode possesses acceptable sensitivity, selectivity, stability and repeatability. All these properties were sufficient to allow simultaneous detection of levodopa and cabergoline in real samples at the surface of GQDs/2CBF/IL/CPE. This was supported by the successful application of this electrochemical sensor electrode for the determination of levodopa and cabergoline in urine, serum, and cabergoline tablets.

Keywords
Electrochemical sensor; chemically modified electrode, levodopa, cabergoline.

Introduction
There is a continuously growing interest in electrochemical sensors and bio-sensors for drugs in environmental, food, and agricultural analyses. This is probably a result of both, electrochemical behavior of biomolecules and advancement in electrochemical testing [1-4]. Hence, merging the rapid, selective, sensitive, precise, affordable, as well as miniaturized electrochemistry-based sensing handhelds with biochemistry, proteomics, nanotechnology, molecular biology, and drug analyses resulted in developing electrochemical sensors [5-9].
It is a well-known fact that the carbon paste electrode (CPE) has a widespread utilization for electrochemical determinations of diverse biological and pharmaceutical species, which resulted from low residual currents and noise, simplified construction, wider cathodic and anodic potential range, fast surface renewal, and low cost. In addition, the CPE surface can be easily chemically modified via the addition of diverse materials to enhance the selectivity, quickness, and sensitivity of determinations [10-13].

Generally, the chemical modification of an inert substrate electrode with mediators offered significant advantages in the development of electrochemical sensors [14]. Redox-active sites shuttle electrons between the analyte solution and the substrate electrode, which is frequently in line with a considerable diminishing of the activation overpotential for the corresponding electrochemical reaction. Other advantages of chemically modified electrodes over the unmodified substrate electrodes are their lower susceptibility to the surface fouling and formation of oxides at their surfaces.

When choosing modifying materials, it is important to know that functional mediators should exhibit lower relative molar mass and possess reversible, rapid and regenerable reaction at low potentials. Furthermore, they should be pH-independent, highly stable in oxidized and reduced forms, unreactive to oxygen, and nontoxic. Beyond the most successful mediators, it was already shown that mediators based on ferrocene and its derivatives met most of the mentioned criteria [14-16].

In recent years, researchers were also focused on designing and synthesizing nanomaterials for various applications due to their unique physical and chemical properties [17-21]. In this context, graphene quantum dots (GQDs) have been introduced as small units of graphene with sizes smaller than 30 nm. GQDs are zero-dimensional substances that combine both carbon dots (CDs) and graphene features [22]. Therefore, researchers considered GQDs in diverse areas because of the confinement of quantum and edge impacts that cause specific electronic, optoelectronic, photoelectric, larger surfaces, and better conductive characteristics. With these unique features, GQDs have been introduced as a useful material for electrochemical sensors. GQDs contributed to larger surface areas in contact with the analyte. As the electroactive surface area is highly significant in electrochemistry, researchers predicted that modifying electrodes by GQDs would increase electrochemical reaction rates [23,24].

Room-temperature ionic liquids (ILs) exhibit encouraging features for electrochemical utilizations, like high ionic conductivity and non-volatility [25,26]. Due to high ionic conductivity, broader electrochemical window, and faster ion mobility, ILs are frequently employed as electrolytes, binders, and solvents in modified electrode electrochemical preparations. According to the outputs, ILs can enhance response sensitivity and simplify direct electron transfer of diverse electroactive compositions [27,28].

In this work, CPE has been modified with all three mentioned materials (ferrocene, GQDs and IL) to form an efficient sensor electrode for detecting and determining levodopa alone or in the presence of cabergoline.

Levodopa is one of the catecholamines with an alkylamine chain bond to a benzene ring with 2-hydroxyl groups. Levodopa that is chosen to treat Parkinson’s disease is metabolized by one enzymatic reaction (dopa-decarboxylase) to dopamine and compensates for diminished dopamine in the brain [29]. Actually, Parkinson’s disease has been considered one of the progressive neurological disorders, which happens in the case of brain failure for the production of sufficient dopamine, which results in tremor, muscles rigidity or stiffness, slow movement (bradykinesia) as well as imbalance. However, it is not possible to directly administer levodopa because it cannot
permeate the blood-brain barrier. Hence, levodopa that could be taken orally will be utilized for providing a resource of dopamine and for treating Parkinson’s disease for relieving the symptoms in a majority of the patients at the early phases of the disease. Consequently, researchers designed various analytical procedures to determine levodopa [30-32].

Cabergoline is one of the ergot alkaloid derivatives for treating Parkinson’s disease as the dopamine agonist. This drug is applied as the only agent for treating early Parkinson’s disease and the adjunct to levodopa in the late phase of the disease. It was confirmed that using this drug delayed the initiation of the levodopa-induced motoric consequences and decreased total levodopa dose crucial for sufficient control over the symptoms of the disease. Finally, its reverse impacts could be compared with the effects of other dopamine agonists like bromocriptine [33,34]. Therefore, the combined treatment of cabergoline and levodopa is usually utilized to treat the disease. In general, the oral administration of cabergoline in a dosage >1 mg/day would be commonly prescribed to treat Parkinson’s disease. Moreover, researchers used radio-immunoassay and indicated that the level of cabergoline in plasma in the healthy participants receiving a single oral dosage equal to 0.6 mg had been ranged between 80 and 800 pg/mL [34].

The present research detected levodopa in the aqueous buffer solution by the developed GQDs/2CBF/IL/CPE sensor. Also, analytical functions of the modified electrode for quantifying levodopa in the presence of cabergoline was assessed. Ultimately, the developed electrochemical sensor has been utilized to determine cabergoline and levodopa in some real samples.

**Experimental**

**Instruments and chemicals**

An Auto-lab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, The Netherlands) was utilized for electrochemical experimentations and monitored with general-purpose electrochemical system software. A traditional three-electrode cell was used at 25±1 °C. A platinum wire, a conventional Ag/AgCl/KCl (3.0 M KCl) electrode, and GQDs/2CBF/IL/CPE were employed as the auxiliary, reference, and the working electrodes, respectively. A Metrohm 710 pH meter was used to measure pH. Cabergoline, levodopa, and all remaining analytical grade reagents were obtained from Merck (Darmstadt, Germany). Finally, ortho-phosphoric acid and its salts (KH$_2$PO$_4$, K$_2$HPO$_4$, K$_3$PO$_4$) were utilized to prepare 0.1 M phosphate buffer solution (PBS) with pH ranging from 2.0 to 9.0.

**Electrode preparation**

Based on the research design, GQDs/2CBF/IL/CPE was prepared by dissolution of 0.01 g 2CBF in 3 mL diethyl ether. Afterward, this solution was mixed into 0.1 g GQDs and 0.89 g graphite powder composed of pestle and mortar. Then, 0.6 mL of paraffin and 0.3 mL of IL (n-hexyl-3-methylimidazolium hexafluoro phosphate) were added to the mentioned mix and shaken for 15 minutes until a uniform paste was achieved. In the next stage, the paste was packed into a glass tube (ca. 3.4 mm i.d. and 10 cm long), with a copper wire located at the carbon paste to ensure electrical contact. Afterward, a fresh surface was attained via impelling an excessive paste outside the tube and polishing with a weighing paper.

It should be noted that in order to compare the materials, bare CPE was analyzed with GQDs/CPE (without 2CBF and IL), 2CBF/CPE (without GQDs and IL), and GQDs/2CBF/CPE (without IL), which were all procured similarly.

The surface areas of GQDs/2CBF/IL/CPE and bare CPE were obtained by CVs of 1 mM K$_3$Fe(CN)$_6$ in 0.1 M PBS, recorded at different scan rates. Using the Randles-Ševčik formula [35] for
GQDs/2CBF/IL/CPE, the electrode surface was calculated as 0.35 cm², about 3.9 times greater than bare CPE.

Preparation of real samples

Urine samples were stored in a refrigerator upon the collection. Then, 10 mL of the specimens were centrifugated for 15 min at 2,000 rpm, and afterward, a 0.45 µm filter was applied to purify the supernatant. In the next step, various contents of the supernatant solution were transferred into 25 ml volumetric flasks and diluted to the marks with PBS (pH 7.0). The diluted urine specimens were spiked with various levels of cabergoline and levodopa. Finally, the recommended process was used to analyze the amounts of cabergoline and levodopa, employing the standard addition technique.

The serum samples were prepared similarly by centrifugation, filtration and dilution with PBS (pH 7.0). Afterward, the diluted specimen of the serum sample has been injected with various contents of cabergoline and levodopa. Next, the newly recommended procedure and standard addition technique were used to determine the contents of cabergoline and levodopa in the tablets.

Results and discussion

Electrochemical features of levodopa at the surface of GQDs/2CBF/IL/CPE

For studying the electrochemical oxidation behaviour of levodopa, which is pH-dependent reaction according to the oxidation mechanism presented in Scheme 1, finding an optimal pH value would be of high importance for achieving acceptable outputs.

![Scheme 1. Electrochemical oxidation mechanism of levodopa at the surface of the modified electrode](image)

Therefore, we used the modified electrode to run experiments using 100 µM of levodopa in 0.1 M PBS of different pH values, ranging from 2.0 to 9.0. According to Figure 1, showing oxidation peak current values of 100 µM levodopa at GQDs/2CBF/IL/CPE in dependence on pH of the solution, the most acceptable output was observed for electro-oxidation of levodopa at pH of 7.0.

![Figure 1. I_p vs. pH curve obtained from DPVs of GQDs/2CBF/IL/CPE in solution containing 100.0 µM of levodopa in 0.1 M PBS of different pH (2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0)](image)
Figure 2 represents CV responses for different electrodes in solutions with and without 100.0 µM of levodopa. CVs of bare CPE in the blank solution and solution containing 100.0 µM of levodopa are presented by curves a and b, respectively. CVs of GQDs/2CBF/IL/CPE in blank and levodopa solutions are presented as curves c and f, respectively. The rest two curves are CVs recorded for partially modified electrodes in levodopa solutions, i.e., GQDs/CPE (curve d), and 2CBF/CPE (curve e).

Figure 2 demonstrates that in solutions containing levodopa, the potential of the anodic peak is approximately 820 mV for oxidizing levodopa at the bare CPE surface (curve b) and 645 mV at the surface of GQDs/2CBF/IL/CPE (curve f), what is a difference of 175 mV.

The highest oxidation currents were observed for the surface of 2CBF/CPE (curve e) and particularly for GQDs/2CBF/IL/CPE (curve f). The significant increase of the anodic peak current for GQDs/2CBF/IL/CPE compared to either GQDs/CPE or 2CBF/CPE, implies the influence of ionic liquids (ILs) present on CPE. IL/CPE has some benefits like quick transfer of electrons, suitable antifouling traits, greater conductivity, and the catalytic nature of ILs. Therefore, IL mass has been inserted into the carbon and paraffin oil that connect the granules. Thus, IL/CPE conductivity has been considerably improved, which is consistent with the electrochemistry outputs of the present study. Finally, GQDs on the IL surface largely enlarged the electrochemical responses likely caused by the potential features of the GQDs, such as the greater surface area, stronger chemical stability, and suitable electrical conductivity.

**Impacts of the scan rate**

The effectiveness of the potential scan rate on the oxidation current of levodopa is presented in Figure 3, showing linear sweep voltammograms of GQDs/2CBF/IL/CPE in 0.1 M PBS, pH 7.0 with 100.0 µM of levodopa. It is obvious from Figure 3 that higher scan rates resulted in enhanced oxidation peak current values. Additionally, it has been found that $I_p$ is linearly related to the square root of the potential scan rate ($\sqrt{\nu}$), demonstrating that levodopa oxidation is the diffusion-controlled process (Figure 3, inset A). In addition, the electrocatalytic mechanism (EC*) has been shown by the plot of the scan rate normalized current ($I_p/\sqrt{\nu}$) against the scan rate (Figure 3, inset B).
ELECTROCATALYTIC DETERMINATION OF LEVODOPA

Figure 3. Linear sweep voltammograms of GQDs/2CBF/IL/CPE in 0.1 M PBS, pH 7.0 with 100.0 μM levodopa, at distinct scan rates (1-9 correspond to 5, 10, 20, 30, 40, 50, 60, 70, and 80 mV s⁻¹). Insets: (A) variation of anodic peak current with ν¹/²; (B) variation of scan rate normalized currents (Ip/ν¹/²) with scan rate.

Chronoamperometric measurements

The analysis of chronoamperometry for levodopa specimens at GQDs/2CBF/IL/CPE was performed at 0.7 V. Figure 4 displays chronoamperometric outputs of diverse concentrations of levodopa in PBS, pH 7.0. In addition, the Cottrell equation was applied for the chronoamperometric analysis of electroactive moieties reaction based on the mass transfer restricted conditions [35]:

\[ I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \]  

(1)

In Eq. (1), \( D \) is diffusion coefficient (cm² s⁻¹), \( C_b \) stands for the bulk concentration of analyte (mol cm⁻³), \( A \) is electrode surface area (cm²), \( n \) is the number of transferred electrons, and \( t \) is time.

Figure 4A shows experimental results of \( I \) vs. \( t^{-1/2} \), reflecting the best fit for distinct concentrations of levodopa.

Figure 4. Chronoamperograms of GQDs/2CBF/IL/CPE in 0.1 M PBS, pH 7.0 for different levodopa concentrations (1-4 correspond to 0.1, 0.6, 1.5 and 3.0 mM). Inset A: \( I \) plot vs. \( t^{-1/2} \) from chronoamperograms 1 to 4. Inset B: slope plot of straight line vs. concentration of levodopa.

Afterward, final slopes relative to the straight lines in Figure 4A were drawn versus levodopa concentration (Figure 4B). Using the Cottrell equation and resultant slopes, the mean value of \( D \) was calculated as 7.45×10⁻⁵ cm²/s.

Calibration plot and detection limit

Considering the oxidation peak currents for different concentrations of levodopa with GQDs/2CBF/IL/CPE, levodopa can be quantitatively analysed in the water solution. The modified
electrode (GQDs/2CBF/IL/CPE) was used as a working electrode in the concentration range of levodopa from 0.07 to 500.0 µM in 0.1 M PBS, pH 7.0, and differential pulse voltammetry (DPV) measurements were performed due to DPV merits like more reasonable sensitivity and more acceptable performance in analytical utilizations. The recorded DPVs (step potential=0.001 V, amplitude=0.02 V) are presented in Figure 5, showing that peak currents are linearly related to levodopa concentration ranging from 0.07 to 500.0 µM, with the correlation coefficient equal to 0.9999. Also, the detection limit, \( C_m \), of levodopa was obtained using the following equation:

\[
C_m = \frac{3s_b}{m}
\]  

In Equation (2), \( m \) is the slope of the calibration plot (0.1003 µA µM\(^{-1}\)) and \( s_b \) is the standard deviation of the blank response obtained from 20 replicate measurements of the blank solution. The detection limit was calculated as 0.02 µM.

**Concurrent detection of levodopa and cabergoline**

No study has been reported on the use of CPE modified with GQDs/2CBF/IL for simultaneous detection of cabergoline and levodopa. In addition, because the electrochemical detection of levodopa in the presence of cabergoline at unmodified electrodes would have a drawback of interference with cabergoline due to the comparative oxidation capacity of both samples, we tried to separate two analyte peaks. This stage has been proceeded by concurrent changes in the analyte concentration and recording DPVs. It is shown in Figure 6 that specific anodic peaks have been observed at 630 and 830 mV for oxidizing levodopa and cabergoline, respectively, which confirms the use of GQDs/2CBF/IL/CPE, by which it becomes possible to detect the analytes with no interference between them.

**Stability of GQDs/2CBF/IL/CPE**

According to the research design, the stability of GQDs/2CBF/IL/CPE was tested via holding it in PBS pH 7.0 for 20 days. Then, the cyclic voltammogram was registered in the presence of 50.0 µM levodopa after cycling the potential fifteen times at 50 mV s\(^{-1}\). The measured CV was then compared with that observed before submersion. According to the findings, the levodopa oxidation peak did not change the peak potential value, while peak current value showed a certain decrease (≤2.5 %) compared to the initial response, reflecting acceptable stability of GQDs/2CBF/IL/CPE.
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Interference study

This study examined the possible effects of various materials as compounds that might have a potential interference with cabergoline and levodopa detection under optimized conditions with 50.0 µM levodopa at pH 7.0. It should be noted that potentially interfering materials have been selected from a group of materials usually observed with levodopa pharmaceuticals and/or biological fluids. The limit of tolerance has been described as the highest concentration of the interfering material, which gives rise to less than ±5 % error in detecting levodopa. As shown by the outputs, glucose, lactose, fructose, sucrose, ethanol, citric acid, methanol, Fe³⁺, Mg²⁺, Fe²⁺, Al³⁺, SO₄²⁻, CO₃²⁻, NH₄⁺, F⁻, Cl⁻, glycine, alanine, methionine, folic acid, phenylalanine, urea, and the saturated starch solution had no interference with cabergoline and levodopa detection. On the other side, ascorbic acid, dopamine, norepinephrine, and epinephrine with the same concentration showed some interference with cabergoline and levodopa detection. Even though ascorbic acid had interfered, it might be omitted if required via the ascorbic oxidase enzyme that has an excellent selectivity to oxidizing the ascorbic acid.

Analysis of real samples

For assessing the usability of the developed modified electrode for determination of levodopa and cabergoline in real samples, GQDs/2CBF/IL/CPE was applied for biological fluids and drugs, i.e., urine, serum and cabergoline tablets. Consequently, a standard addition procedure was employed, and outputs are presented in Table 1. As seen, reasonable recovery of levodopa and cabergoline, as well as reproducible outcomes were obtained based on the mean relative standard deviation (RSD).

Table 1. Levodopa and cabergoline concentrations determined by GQDs/2CBF/IL/CPE in real samples (n=5).

| Sample          | Spiked Concentration, µM | Found Concentration, µM | Recovery, % | RSD, % |
|-----------------|--------------------------|--------------------------|-------------|--------|
|                 | Levodopa | Cabergoline | Levodopa | Cabergoline | Levodopa | Cabergoline | Levodopa | Cabergoline |
| Urine           | 0        | 0           | 5.0      | 7.5        | 98.0     | 102.7       | 3.5      | 1.9       |
|                 | 10.0     | 12.5        | 10.1     | 12.3       | 101.0    | 101.0       | 2.7      | 2.4       |
| Serum           | 0        | 0           | 6.0      | 10.0       | 101.7    | 98.0        | 2.3      | 3.1       |
|                 | 12.0     | 15.0        | 11.7     | 15.5       | 97.5     | 103.3       | 2.9      | 1.7       |
| Cabergoline tablet | 0        | 0           | 5.0      | 5.1        | 102.0    | 98.3        | 3.4      | 1.9       |
|                 | 12.0     | 7.5         | 11.7     | 11.3       | 97.5     | 102.7       | 2.4      | 2.7       |
Conclusions

An electrochemical sensor with high sensitivity, selectivity, and stability based on GQDs/2CBF/IL modified CPE was successfully fabricated. GQDs/2CBF/IL/CPE showed much better performance when compared with bare CPE, GQDs/CPE and 2CBF/CPE. The developed GQDs/2CBF/IL/CPE showed excellent catalyzing effect for levodopa oxidation and found suitable for simultaneous determination of levodopa and cabergoline. The anodic peak currents were linear to levodopa and cabergoline concentrations in the reasonable concentrations ranging from 0.07 to 500.0 µM, respectively. In addition, GQDs/2CBF/IL/CPE sensor can be favorably employed for determining levodopa as well as cabergoline in real samples.

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