# International Bulletin of Electrochemical Methodology

# Developing and Analytical Application of Modified Electrode for the Electrochemical Determination of Rafoxanide Veterinary Drug

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#### ABSTRACT

Rafoxanide (RFX) drug substance, belongs to the salicylanilide group of anthelmintic drugs. In this work, Electrochemical behaviour and analytical applications of RFX were investigated by the cyclic voltammetry (CV), adsorptive stripping square-wave voltammetry (AdSSWV), and adsorptive stripping differential pulse voltammetry (AdSDPV) on modified titanium dioxide/multi-walled carbon paste electrode ( $TiO_2/MWCNTPE$ ). The RFX showed a well-defined anodic signal, and has an irreversible electrode reaction, at approximately 0.45 V in the optimum pH 6.0 Britton Robinson (BR) buffer solutions. Some parameters of stripping modules such as supporting electrolyte, pH, frequency, deposition time, pulse time and pulse amplitude, etc. were optimized to develop the most sensitive electroanalytical method. Furthermore, the calibration graphs were created using the standard addition technique with AdSSWV and AdSDPV on the optimum condition. It has a linear working range of 2.0-16.0 mg/L with AdSSWV and 2.5-14 mg/L with AdSDPV for the determination of RFX in pH 6.0 BR buffer solution on the TiO<sub>2</sub>/MWCNTPE. The limit of determination (LOD) by AdSSWV and AdSDPV, which are the most important validation parameter, was found to be 0.054 and 0.072 mg/L, respectively. To investigate the selectivity of the method, analysis of 5 mg/L RFX was carried out on  $TiO_2/MWCNTPE$  with AdSSWV in the presence of various heavy metals and some organic substances. The interference agents had no significant effect on the determination of RFX (less than 10% within the tolerance range). Finally, the RFX drug was successfully evaluated in tap water samples by the proposed AdSSWV without any pretreatment with a high percent recovery. This study for RFX determination has been improved to deduce, a fast, inexpensive, reliable, portable, environmentally friendly, selective, and sensitive new electroanalytical method in real samples.

# KEYWORDS

Rafoxanide, detection, carbon pasta electrode, electroanalytical, voltammetry, modified electrode, nanosensor

#### **1. INTRODUCTION**

RFX (RFX), a veterinary drug active ingredient, is in a class of salicylanilide used as an anthelmintic (1). IUPAC name of RFX is N-[3-chloro-4-(4chlorophenoxy)phenyl]-2-hydroxy-3,5 diiodo-benzamide (**Scheme 1**). It is by a majority used to treat adult liver parasites of "*Fasciola hepatica*" and "*Fasciola*  *gigantica*" species found in ruminant animals (1,2). RFX has a long terminal half-life of approximately 16.6 days after the drug is administered. The European Union has identified maximum residual limit (MRL) of RFX in bovine and ovine muscle. The MRL of RFX in bovine muscle is 30 mg/kg, while this value is identify as 100 mg/kg in sheep muscle

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(3,4). Moreover, while it has a low toxicity level when used at the recommended dose, it causes temporary or permanent blindness if used in excessive doses, and also has a serious effect on the central nervous system (CNS). In addition, prolonged exposure to RFX may cause retinal lesions characterized by necrosis, retinal detachment and, loss of photoreceptor layer in sheep and goats. For these reasons, analytical methods are needed for sensitive, accurate, fast, and reliable determination of RFX active ingredient, which is extremely important for human and animal health, in natural and pharmaceutical dosage samples.

Until now, limited studies on the determination of RFX in real dosage samples in the literature have been dominated by chromatographic and spectrophotometric methods (4-10). In these studies, UV-vis spectrophotometric high-performance and liquid chromatography (HPLC). highperformance liquid chromatography/mass spectrometry (HPCL/MS), liquid chromatography/mass spectrometry (LC/MS-MS) methods were preferred in RFX analysis (4-10). However, these methods have shortcomings such as the use of too much organic solvent, long preproceeding, expensive device hardware, and long analysis times (11,12). Therefore,

#### International Bulletin of Electrochemical Methodology

scientists are in search of new analytical methods that are fast. inexpensive. sensitive, portable, and reliable for the analysis of analytes in natural samples. In this respect, electrochemical methods have attracted great attention with the developing technology and sensor platform in recent years (13–15). In recent years, the use of electrochemical methods has gained stands out, especially due to the rapid analysis time, the use of very few organic solvents, the not need for long preprocessing, and cheap. Moreover, the production of miniature sensors enabled the development of portable devices and led to promising developments in online analysis. In addition to these, these methods are located between the environmentally friendly analytical methods (16,17). Due to the extraordinary properties of carbon-based nanomaterials, they are widely used in electrochemical sensor applications. Due to the extraordinary properties of carbon-based nanomaterials, they are widely used in electrochemical sensor applications. materials preferred Carbon are in qualitative and quantitative analyzes of drugs, pesticides and electroactive substances due to their properties such as conductivity, cost, wide potential range, chemical and physical stability (18).

So far, only two studies have been for the available electrochemical determination of RFX by using adsorptive stripping differential pulse voltammetry (AdSDPV) and differential pulse voltammetry techniques (DPV) (19,20). Radi ve El-samboskany, investigated the electrochemical behavior of RFX using a bare glassy carbon electrode and applied it analytically to bovine milk samples (19). Salem and friends, for RFX detection, developed the cadmium sulfide modified carbon paste electrode (CCPE) and applied it analytically to pharmaceutical dosage forms and biological fluids samples by using the DPV technique (20).

In this study, composite electrodes containing multi-walled carbon paste and  $CeO_2$  or  $TiO_2$  nanoparticles ( $CeO_2/MWCNTPE$  and  $TiO_2/MWCNTPE$ ) were developed for the first time to

#### International Bulletin of Electrochemical Methodology

investigate the electrochemical behavior of RFX and its assignment in natural samples. TiO<sub>2</sub>/MWCNTP modified electrode was found to be the sensitive most electrochemical in RFX sensor determination. Then, the electrochemical behavior of the RFX drug substance was investigated in detail by cyclic voltammetry (CV), adsorptive stripping square-wave voltammetry (AdSSWV), and AdSDPV electroanalytical methods on the composite sensor. The interference effects of some cations and organic substances in determination RFX the of were investigated by using the AdSSWV technique with the TiO<sub>2</sub>/MWCNTPE. Finally, the determination of RFX, which is of great importance for human and animal health, was successfully applied in spike tap water samples with high recovery and low relative error.



Scheme 1. Molecular structure of RFX

### **2. EXPERIMENTAL**

#### 2.1. Apparatus and reagents

IVIUM branded Vertex.one model electrochemical device was used to collect

all electrochemical data. This analyzer consists of an electrochemical cell containing three electrodes. These electrodes consist of a platinum wire

(BASi, MW-1032) the as counter electrode, a carbon paste electrode (BASi MF-2010, a diameter  $\varphi=3$  mm) as the working electrode, and an Ag/AgCl electrode (BASi, MF-2052) as the reference electrode. The pH values of the necessary solutions were meticulously adjusted using an ISOLAB-branded pH meter. In addition, an ISOLAB-branded ultrasonic bath was used to homogenize the solutions.

Analytical-grade reagents were purchased to prepare all solutions accurately and RFX analytical precisely. standard substance was obtained from Merck The company.  $CeO_2$ and TiO<sub>2</sub> nanoparticles purchased were from Nanografi A.Ş. (Turkey) to prepare the composite indicator electrode. acid Hydrochloric (37%) boric acid (99.8%), glacial acetic acid ( $\geq$  99%), and phosphoric acid (85%), sodium hydroxide were received from Sigma-Aldrich.

Stock RFX solution is prepared daily in 10 ml ethanol at a concentration of 500 ppm stock solution. The multi-walled carbon nanotube powder (MWCNT) and mineral oil as a binder were preferred in the preparation of bare electrodes. Two different solutions were used as the supporting electrolyte. The first is 0.1 M HCl (pH 1.0), and the second is 0.04 M

#### International Bulletin of Electrochemical Methodology

Britton Robinson (BR) buffer solution from pH 2.0 to pH 10.0. Relevant concentrations of boric acid, acetic acid, and phosphoric acid were used to prepare BR buffer, and then 2.0 M NaOH or 2.0 M HCl solution was added to adjust to the desired pH values. Care was taken to use distilled water for the preparation and cleaning of all solutions. The solutions were stored in the refrigerator when not in use. In addition, all electrochemical data were obtained at room temperature (25 °C $\pm$ 2).

# 2.2. The fabrication of indicator electrodes

For the construction of bare MWCNTPE, 30% mineral oil and 70% MWCNT were used by mass. This mixture was mixed as much as possible homogeneous with the help of a spatula. Then, the obtained paste was filled into the BASi MF-2010 hollow electrode with the help of a syringe needle. Finally, polishing with zero belt sandpaper was applied to obtain a smooth electrode surface. Before using the bare electrode, the electrode surface was washed with pure water and activated by CV.

To produce composite electrodes, 5% of metal oxide nanoparticles (TiO<sub>2</sub> or CeO<sub>2</sub>), 30% mineral oil, and 65% multi-walled carbon nanotube powder (MWCNT) by mass were mixed until homogeneous.

Then, the electrode surface was washed with distilled water and polished with zero belt sandpaper. Finally, sequential cyclic voltammograms were taken in the blank solution to activate the surface of the composited electrode.

### **2.3. Analytical application**

For the accuracy and precision of the proposed method, the developed analytical method was applied to the tap water sample. For this, 1 mL of stock RFX (500 ppm) and 9 mL of tap water were mixed in an ultrasonic bath. The RFX substance was then analyzed directly from the optimum AdSSWV technique without any pretreatment. Results were calculated as percent recovery by standard addition method according to the anodic peak signal of RFX.

# 3. RESULTS AND DISCUSSION3.1. The characterization of the modified electrodes

#### International Bulletin of Electrochemical Methodology

The main of surface purpose characterization studies is to show how binders are attached to the surfaces of bare electrodes, how they affect the surface area, and what difference they make. It provides important information about the surface condition of the modified materials used in the production of composite electrodes. Therefore, the surface analysis of the bare and developed modified sensor was characterized by energy-dispersive Xray analysis (EDX) and scanning electron microscopy (SEM).

According to the SEM morphological image, it is understood that the TiO2 nanoparticles are treated with MWCNTPE, and the bare electrode is coated by the TiO<sub>2</sub> NPs. To support this, elemental analysis of the modified electrode with EDX was carried out. In this analysis, titanium elemental analysis was found to be approximately 7.36%. This result is the most important evidence that TiO<sub>2</sub> is modified with MWCNTs (**Figure 1**).



Figure 1. The SEM images of (A) bare MWCNTPE, (B) TiO<sub>2</sub>/MWNCTPE (C) The EDX analysis of TiO<sub>2</sub>/MWNCTPE

# **3.2.** Comparison of sensing of working electrodes

First, a study to determine the most sensitive working electrode for the determination of RFX was performed. For this purpose, electrochemical measurements were taken by using AdSSWV for the 10 mg/L RFX on the bare and modified electrodes, prepared with bare MWCNTPE and different nanoparticles (TiO<sub>2</sub> or CeO<sub>2</sub>) under the same experimental conditions (**Figure 2**).



**Figure 2.** Comparison of bare MWCNTPE, CeO<sub>2</sub>/MWCNTPE and TiO<sub>2</sub>/MWCNTPE as working electrodes using AdSSWV method in pH 3.0 BR buffer medium containing 10 mg/L RFX ( $\Delta E=120 \text{ mV}$ ,  $t_{acc}=20 \text{ s}$ ,  $E_{acc}=0 \text{mV}$ , f=10 Hz and  $\Delta E_s=4$ )

According to the AdSSW voltammetric measurements carried out by three different sensors, an anodic peak current of RFX was obtained at 702 mV as 1.975 µA on the bare electrode, while the current signal was obtained at 693 mV as 10.088  $\mu A$  and 702 mV as 6.736  $\mu A$  on the TiO<sub>2</sub>/MWCNTPE modified and CeO<sub>2</sub>/MWCNTPE, respectively. Accordingly, the TiO<sub>2</sub>/MWCNTPE composite new generation sensor, which provides the most sensitive measurement for the determination of RFX, was found to be the most ideal. In addition, when looking at the peak potential value, which is an important indicator for its qualitative determination, an anodic signal of RFX was observed in less positive regions by

using the modified TiO<sub>2</sub>/MWCNTPE. This lower potential shift is proof that the modified electrode has a more effective catalytic property compared with bare MWCNTPE. As a result, further studies with were carried out only TiO<sub>2</sub>/MWCNTPE composite electrode in the electroanalytical methodology to be developed, because the TiO<sub>2</sub>/MWCNTPE composite electrode for RFX analysis is sensitive compared to other indicator electrodes.

### 3.3. pH Effect

To examine the supporting electrolyte effect in the determination of RFX, the effect of different pH values between pH 1 and pH 12 was investigated in detail. For

this by using AdSSWV and AdSDPV, the most sensitive among the voltammetric techniques, voltammograms were obtained with composite TiO<sub>2</sub>/MWCNTPE. In this study, with AdSSWV, wasn't obtained in a strongly acidic medium such as pH 1.0 and pH 2.0. The RFX exhibits a single peak at approximately 0.5 V depending on the increasing pH between pH 3.0 and pH 6.0, while at approximately 0.6 V and 0.2 V from pH 7.0 to pH 11.0 two anodic signals

#### International Bulletin of Electrochemical Methodology

obtained the modified were on TiO<sub>2</sub>/MWCNTPE by AdSSWV (Supple. 4a). The peak potential of anodic peak I exhibited two independent slopes was observed between pH 3.0-7.0 and pH 8.0-11.0. Furthermore, the potential of both peaks of the RFX shifted to less positive regions as nearly -60 mV with increasing per unit pH value (Figure 4). This shows that the electrochemical oxidation reaction of RFX contains proton  $({\rm H}^{+}).$ 

 $Ep = -70.7 \text{ pH} + 840.2 \text{ R}^2 = 0.9801 \text{ (pH } 3.0 - \text{pH } 7.0\text{) for the Peak I (4)}$  $Ep = -61.1 \text{ pH} + 798.1 \text{ R}^2 = 0.9832 \text{ (pH } 8.0 - \text{pH } 11.0\text{) for the Peak I (5)}$  $Ep = -59.7 \text{ pH} + 1030.2 \text{ R}^2 = 0.9963 \text{ (pH } 7.0 - \text{pH } 11.0\text{) for the Peak II (6)}$ 



**Figure 4.** The plot of Ep and Ip versus pH on TiO<sub>2</sub>/MWCNTPE for of RFX determination with AdSSWV and AdSDPV

In the study with AdSDPV, however, no oxidation signal was observed in pH 1.0 and pH 2.0 support electrolytes, as in AdSSWV. When a single signal at nearly 0.45 V was obtained between pH 3.0 and pH 6.0 by contrast with the RFX exhibited

two anodic peaks at 0.45 and 0.2 V from pH 7.0 to pH 11.0 (**Supple. 4b**). Due to all increasing pH values, both anodic signals of RFX were shifted to less positive regions and this potential shift is approximate -50 mV. (**Figure 4**).

$$Ep = -47.7 \text{ pH} + 705.1 \text{ R}^2 = 0.9582 \text{ (pH } 3.0 - \text{pH } 7.0\text{) for the Peak I (7)}$$
$$Ep = -67.9 \text{ pH} + 893.7 \text{ R}^2 = 0.9783 \text{ (pH } 8.0 - \text{pH } 11.0\text{) for the Peak I (8)}$$
$$Ep = -51.1 \text{ pH} + 1021.9 \text{ R}^2 = 0.9952 \text{ (pH } 7 - \text{pH } 11\text{) for the Peak II (9)}$$

#### **3.4. Electrochemical Behavior of RFX**

The electrochemical behavior of RFX on the TiO<sub>2</sub>/MWCNTPE electrode was investigated in detail by CV in pH 6.0 BR buffer solutions. For this, the cyclic voltammograms were measured, with potentials between 0 mV and +1000 mV in the anodic and cathodic directions, at different scanning rates ranging from 25 mV/s to 500 mV/s in the presence of 20 mg/L RFX. While two oxidation peaks were obtained in scanning in the anodic direction, no peaks were obtained in the cathodic direction. This event proves the irreversible electrode reaction of RFX on the TiO<sub>2</sub>/MWCNTPE. A parallel increase in RFX anodic signals was observed with increasing scan rate from 25 mV/s to 500 mV/s (Figure 3). Moreover, it was determined that the anodic signals shifted to positive regions with increasing scan rate. This electrochemical process provides

information that electrons (e) are involved in the oxidation electrode reaction to RFX.

The CV provides very critical information about not only electrochemical behavior but also material transport and kinetic patterns. The scan rate by CV gives valuable information about whether the analyte is transported to the electrode surface in a diffusion-controlled or adsorption-controlled manner in velocity research. For this, the graph of the peak currents (Ip) versus the logarithm of the scan rate (log v) is taken and the slope is calculated. If the slope value is 0.5, the material transport process is expressed as diffusion-controlled, if it is 1.0, it is absorption-controlled (21). In this study, the slope of the peak currents (Ip) versus the logarithm of the search rate  $(\log v)$  was

found to be approximately 0.32, and since this value is close to 0.5, we can say that the transport of RFX to the  $TiO_2/MWCNTP$  electrode surface is diffusion-controlled in the pH 6.0 BR buffer solutions.

log Ip ( $\mu$ A) = 0.321 log v (V/s) – 1.442 r= 0.9319 (2)

In order to elucidate the electrochemical behavior of RFX, the square root of the

scanning rate  $(\sqrt{v})$  and the peak currents (Ip) equation was examined (Eq.3). This equation also supports that the effect of the square root of the scan rates on the peak currents is diffusion-controlled, with peak currents (Ip) versus the logarithm of the search rate (log *v*).

Ip (
$$\mu$$
A) = 0.011  $\sqrt{v}$  (V/s) + 0.039  
r=0.9404 (3)



**Figure 3.** The cyclic voltammograms of 20.0 mg/L RFX on the modified electrode at different potential scan rates from 25 mV/s to 500 mV/s

# 3.5. Optimum AdSSWV and AdSDPV modules

Each of the parameters in the AdSSWV and AdSDPV methods, which have a great effect on the peak signal and peak potentials, such as accumulation potential, accumulation time, amplitude, frequency, step potential, and pulse time have been optimized in the presence of 10 mg/L RFX on the modified TiO<sub>2</sub>/MWCNTPE at pH 6.0 BR buffer solution.

Various pulses ranging from 10 mV to 250 mV were enforced to determine the optimal pulse amplitude for the AdSSWV module (Supple. 1a). The peak signal of RFX increased linearly with applied pulse amplitudes up to 150 mV in pH 6.0 BR buffer solution on TiO<sub>2</sub>/MWCNTPE. Depending the increasing pulse on amplitude, the peak shape of RFX started to become broader. Since it was obtained at pulse amplitude of 100 mV, where the smoothest peak shape was obtained, the optimum pulse amplitude was chosen as this value and continued with the pulse amplitude of 100 mV in further studies. The next optimum module is step potential, AdSSW voltammograms were obtained at different step potentials ranging from 1 mV to 6 mV on TiO<sub>2</sub>/MWCNTPE in pH 6.0 BR buffer solution (Supple. 1b). A clear increase in the peak current of RFX was observed with step potential up to 4 mV. However, there was no significant change in the peak current of RFX at step potentials after 4 mV. The 4 mV was chosen as the optimum step potential, where the most sensitive peak current was obtained. In particular, the most important and influential frequency parameter in the potential was investigated peak in AdSSWV. The AdSSW voltammograms of different frequencies between 5 Hz and 30 Hz were obtained to find the optimum frequency value (**Supple. 1c**). While the peak signal of RFX was significantly increased up to 20 Hz, the peak intensity of RFX decreased quite after the frequency of 20 Hz. At frequencies above 30 Hz, AdSSW voltammograms were obtained so that the peak signal could not be measured. As a result, 20 Hz was chosen as the optimum frequency, where the most sensitive and uniform oxidation peak shape was obtained.

The effects of the accumulation time and accumulation potential, which are the most important parameters for the stripping modules in the AdSSWV method, were estimated in detail on the peak intensity of the target material. Firstly, different deposition potentials between -0.4 V and +0.3 V were applied to find the effect of accumulation modules in pH 6.0 BR buffer solution in the presence of 10 mg/L RFX on TiO<sub>2</sub>/MWCNTPE (Supple. 1d). Among the applied accumulation potentials, the RFX exhibited at approximately 0.42 V did not have a significant effect on the peak intensity as the other optimum parameter. In addition, to examine the effect of the deposition time, accumulation was performed between 10 s and 60 s using a TiO<sub>2</sub>/MWCNTPE indicator electrode in

pH 6.0 BR buffer (Supple. 1e). A linear and significant increase in peak intensity of RFX was observed up to 40 s deposition times. After 40 s of deposition time, reductions in the peak current of the analyte were seen in stripping voltammograms. This is an indication that the electrode surface is completely coated by the substance at deposit times of 40 s and longer. The 40 s, in which the best anodic signal was observed, was chosen as the optimum accumulation time.

For the AdSDPV module, different pulses from 10 mV to 70 mV were applied to find the optimal pulse amplitude (Supple. 2a). The anodic peak intensity of RFX increased linearly with applied pulse amplitudes up to 60 mV in pH 6.0 BR buffer solution on TiO<sub>2</sub>/MWCNTPE. The peak signal remained constant after 60 mV pulse amplitudes. The optimum pulse amplitude was chosen as this value, since it was obtained at 60 mV pulse amplitude, where the smoothest peak shape was obtained, and in later studies, 60 mV pulse amplitude was continued. To find the optimum next parameter as step potential, different step potentials ranging from 2 mV 6 mV were applied to on TiO<sub>2</sub>/MWCNTPE (Supple. **2b**). The increase in the peak current of RFX was observed with step potential up to 5 mV.

However, there was no significant change in the peak current of RFX at step potentials beyond 5 mV. 5 mV was chosen as the optimum step potential, where the most sensitive peak current was obtained.

The factors that have the greatest effect on the peak current are the accumulation time and the accumulation potential. For this purpose, various accumulation potentials between -0.4 V and +0.4 V were applied to find the effect on the anodic signal in pH 6.0 BR buffer solution in the presence of 10 mg/L RFX. However, the accumulation potential from applied potentials did not significantly affect the peak signal which RFX exhibited at about 0.42 V as other optimum parameters. The variation between the smallest peak current and the highest peak current is within 10% at applied accumulation potentials between +0.4 V and -0.4 V. However, further studies were carried out at the optimum deposition potential of +100 mV, where the highest peak current was obtained. In addition, in order to estimate the effect of accumulation time. dissimilar accumulation was applied between 10 s and 40 s for the analysis of RFX using TiO<sub>2</sub>/MWCNTPE indicator electrode in pH 6.0 BR buffer. A linear and significant increase in the peak signal of RFX was observed up to 30 s accumulation times.

After an accumulation time of 30 s, reductions of current were observed until it can be said that the peak signal of the analyte remained constant. This proves that the modified  $TiO_2/MWCNTPE$  electrode surface is fully coated by the RFX substance in 30 s and longer deposition times. The optimum deposition time was

decided as 30 s, in which the best anodic peak height was obtained.

As a result, all optimum conditions obtained from AdSDPV and AdSSWV in the pH 6.0 BR buffer solution are summarized in Table 1 for the first time for RFX determination on the modified  $TiO_2/MWCNTPE$ .

Table 1.	The	optimum	modules	of AdSSWV	and	AdSDPV	methods
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Parameters (Unit)	AdSSWV	AdSDPV	
Accumulation potential (mV)	0	+100	
Accumulation time (s)	40	30	
Step potential (mV)	4	5	
Pulse time (ms)	-	12	
Pulse amplitude (mV)	100	60	
Frequency (Hz)	20	-	
Supporting electrolyte solution	pH 6.0 BR buffer	pH 6.0 BR buffer	

# 3.6. Analytical Performance of Proposed Methods

The validation studies of an analytical method developed for the first time are extremely important. For this, analytical performances of AdSDPV and AdSSWV electroanalytical methods for RFX determination were investigated in detail. For this purpose, AdSSWV and AdSDPV methods, which were chosen as the most sensitive electroanalytical methods for analytical studies, were examined for the quantitative determination of RFX in pH 6.0 BR buffer solution on hybrid TiO<sub>2</sub>/MWCNTPE. Calibration plots were created with standard addition methods using anodic peak current values of approximately 0.45 V RFX under the optimum condition determined for both AdSDPV and AdSSWV methods on the TiO<sub>2</sub>/MWCNTPE. While a well-defined anodic peak was obtained in a wide

working range of 2.5-14.0 mg/L with the AdSDPV electroanalytical method, the linear range from 2.0 to 16.0 mg/L was

obtained with AdSSWV in pH 6.0 BR buffer solution.

Ip (nA) = 21.95 C (mg/L) – 39.80; (2.5 –14.0 mg/L, r = 0.9906) for the AdSDPV (10) Ip (nA) = 37.16 C (mg/L) – 50.08; (2.0 –16.0 mg/L, r = 0.9981) for the AdSSWV (11)



**Figure 5.** AdSSWS voltammograms of different RFX concentrations obtained by standard addition in pH 6.0 BR buffer solution on TiO<sub>2</sub>/MWCNTPE ( $\Delta E$ =100 mV,  $t_{acc}$ =40 s,  $E_{acc}$ =0 mV, f=20 Hz and  $\Delta E_s$ =4 mV)

Furthermore, the limit of detection (LOD) and limit of quantification (LOQ) values, which are necessarily validation parameters, were calculated in detail. In this statistical process, the equations "3 m/s" and "10 m/s" were used to calculate the LOD and LOQ values, respectively (22). In these formulas, "m" represents the slope of the calibration graph, and "s" represents the standard deviation of the calibration cutoff point. While the LOD and LOQ values in the RFX assay under optimum AdSDPV conditions were found to be 0.072 mg/L and 0.234 mg/L, respectively, these values were calculated as 0.054 mg/L and 0.180 mg/L with AdSSWV. When comparing the sensitivities of suggested AdSSWV and AdSDPV techniques for RFX determination in natural samples, it is seen

that the AdSSWV method is better than AdSDPV because it has a wider working range and exhibits lower LOD and LOQ detection limits. Therefore, further studies such as interference and analytical application studies were carried out with

#### International Bulletin of Electrochemical Methodology

the AdSSWV electroanalytical method. The validation parameters of the AdSDPV and AdSSWV methods, which are highly sensitive for RFX determination, were summarized in **Table 2**.

Parameters (Unit)	AdSDPV	AdSSWV
Peak potential (mV)	460	450
Linearity range (mg/L)	2.5–14.0	2.0–16.0
Slope (nA.L/mg)	21.95±1.75	37.16±0.76
Intersection (nA)	$-39.80\pm3.36$	$-50.08{\pm}6.76$
Correlation coefficient	0.9906	0.9937
Limit of detection (LOD) (mg/L)	0.072	0.054
Limit of determination (LOQ) (mg/L)	0.234	0.180
Inter-day repeatability (RSD %)*	-	0.772
Intra-day repeatability (RSD %)*	-	1.699

\* Each value is the mean of five experiments.

#### 3.7. Selectivity of the Method

To interpret the feasibility and selectivity of AdSSWV in the determination of RFX, electrochemical measurements were taken for the 5.0 mg/L RFX in the presence of various metal ions and some organic compounds 1:5 and 1:10 by mass at pH 6.0 BR buffer solutions with the TiO<sub>2</sub>/MWCNTPE. The follow-up investigation of the excipient's effect in RFX determination by AdSSWV was calculated as the percent recovery of the

anodic peak signal by taking triplicate measurements with standard deviation. It was observed that heavy metals such as Pb (II), Cu (II), Zn (II), and Fe (III), organic molecules such as neomycin, ketoprofen, and oxfendazole did not have a serious effect on the anodic peak signal of 5.0 5 mg/L RFX at and 10 times concentrations. Over and above, the effect of these interference substances on the determination of RFX was found below the 10% tolerance limit for the RFX peak

#### International Bulletin of Electrochemical Methodology

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current on TiO<sub>2</sub>/MWCNTPE with AdSSWV (**Figure 6**). From these results, it is understood that the selectivity of the

proposed AdSSWV and the TiO<sub>2</sub>/MWCNTP electrode used for RFX analysis is quite high.



**Figure 6.** Interference effects of some interference species on the determination of RFX in pH 6.0 BR buffer solution with AdSSWV on TiO<sub>2</sub>/MWCNTPE ( $\Delta E$ =100 mV,  $t_{acc}$ =40 s,  $E_{acc}$ =0 mV, f=20 Hz and  $\Delta E_s$ =4 mV)

### **3.8 Analytical Application**

The most important analytical performance of a method that is mentioned or developed for the first time is accuracy and precision studies. For the determination of RFX, the analytical application was performed in tap water as the natural samples to demonstrate the validity of the proposed AdSSWV electrochemical method. In line with this goal, 1 ml of 500 mg/L RFX stock solution was added to 9 ml of tap water and mixed in an ultrasonic water bath for 10 minutes. Spiked RFX sample at a concentration of 50 mg/L in tap water was then analyzed by AdSSWV on TiO<sub>2</sub>/MWCNTPE in pH 6.0 BR buffer solution. The proposed electroanalytical method was directly performed for the quantitative analysis of RFX pesticide on TiO<sub>2</sub>/MWCNTPE without any

pretreatment in tap water (**Supple. 7**). The determination of RFX with high percent recovery (%98.80), low relative error (% – 1.20), and low relative standard deviation (RSD, %1.62) was successfully performed

#### International Bulletin of Electrochemical Methodology

(**Table 3**). As a result, it is understood that the AdSSWV method, with the nanosensor developed for RFX determination, has high precision and accuracy.

Table 3. Determination	of RFX in the s	piked tap water sar	nple by AdSSWV
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TiO <sub>2</sub> /MWCNTPE			
	Tap water		
Spiked amount (g/L)	5.00		
Found amount (g/L)	$4.94\pm0.08$		
Recovery (%)	98.80		
RSD (%)*	1.62		
Relative Error (%)*	- 1.20		

\* Each value is the mean of three experiments.

The validation parameters improved the quality of improved methods to determine RFX in tap water samples. In addition, the developed nanosensor showed great stability, reproducibility, and applicability (**Table 2**). As a result, the developed nanosensor has never been mentioned in the literature before. It was applied for the first time for the determination of RFX in tap water samples. (**Table 4**).

 Table 4. Comparison of studies related to RFX detection.

Electrode	Technique	Linear range	LOD	LOQ	Sample	Ref
GCE	AdSDPV	-	1.25x 10 <sup>-3</sup> mg/kg	-	Bovine milk	(18)
ССРЕ	DPV	4.63x 10 <sup>-6</sup> to 4.95x 10 <sup>-4</sup> mg/L	(1.07x 10 <sup>-6</sup> mg/L	3.21x 10 <sup>-6</sup> mg/L	Pharmaceutic al dosage form and biological fluids	(20)
TiO2/MWCNT PE	AdSDPV	2.5 to 14 mg/L	0.072 mg/L	0.234 mg/L	-	This work
TiO <sub>2</sub> /MWCNT PE	AdSSWV	2.0 to 16.0 mg/L	0.054 mg/L	0.180 mg/L	Tap water	This work

# 4. Conclusions

In this study, RFX of electrochemical behaviour, validation, and analytical natural application in samples was investigated by using bare MWCNTPE, TiO<sub>2</sub>/MWCNTPE. modified and CeO<sub>2</sub>/MWCNTPE. Electrochemical methods such as CV, AdSSWV, and AdSDPV were used to carry out these studies. RFX exhibited only an anodic peak and had an irreversible electrode reaction. Among both electroanalytical ways, AdSSWV was more sensitive for the quantitative determination of RFX. The selectivity of the AdSSWV method was investigated with TiO<sub>2</sub>/MWCNTPE sensor in BR buffer (pH 6) in the presence of various cations and some organic substances. Determination of the effects of these substances, which are found together with RFX in nature, on RFX analyzes was made by recovery studies. The percent recovery results obtained by the recovery studies showed that the effect on the detection of RFX was within the tolerance limit. Finally, the determination of RFX without any preprocessing tap water samples with the developed sensor using to AdSSWV method. As a result of this, a simple, fast, inexpensive, reliable, portable, environmentally friendly, selective, and sensitive electroanalytical method was constructed for the analysis of RFX, an

### International Bulletin of Electrochemical Methodology

important veterinary drug active ingredient.

# 5. Ethics approval and consent to participate

This study does not need any Ethics report.

### 6. Consent for publication

The Authors give consent for publication.

### 7. Availability of data and materials

All data and materials of the paper are available to the public.

## 8. Authors' contributions

Ersin DEMİR: Methodology, experimental, validation, writing—original draft preparation, supervisor.

Nida AYDOGDU OZDOGAN: Experimental, writing—review and editing.

Cengiz SARIKURKCU:

Conceptualization, Investigation, review and editing

M. Burak ACIKGUL: Methodology Experimental.

Hulya SILAH: Investigation, review and editing

### **Disclosure statement**

No potential conflict of interest was reported by the author(s).

## REFERENCES

- Saad AS, Hamdy AM, Salama FM, Abdelkawy M. Validated UPLC and TLC-Densitometry Stability Indicating Methods for the Determination of Rafoxanide in the Presence of Its Degradation Products. J Chromatogr Sci [Internet]. 2016 Oct;54(9):1661–9.
- Sweetman SC, Blake PS, McGlashan JM. Martindale: The Complete Drug Reference 35th ed. London: Pharmaceutical Press; 2007.
- Council of the European Union. EU Commission Regulation No. 2377/90. In Off. J. Eur. Commun; 1990. p. 1–8.
- Yeung H, Lee W, Wong Y. Screening of closantel and rafoxanide in animal muscles by HPLC with fluorescence detection and confirmation using MS. J Sep Sci [Internet]. 2010 Feb;33(2):206–11.
- 5. Benchaoui HA, McKellar QA. Determination of rafoxanide and closantel in ovine plasma by high performance liquid chromatography. Biomed Chromatogr [Internet]. 1993 Jul;7(4):181–3.
- Talley CP, Trenner NR, Downing G V., VandenHeuvel WJA. Gas chromatographic determination of rafoxanide [3'-chloro-4'-(4-chlorophenoxy)-3,5-diiodosalicylanilide] in plasma by electron capture detection of its trimethylsilyl derivative. Anal Chem [Internet]. 1971 Aug 1;43(11):1379–82.
- Fink DW. Spectrophotometric quantification of the salicylanilide anthelmintic rafoxanide based on the charge-transfer absorbance of its iron(III) complex. Anal Chim Acta [Internet]. 1981 Nov;131:281–5.
- Horning EC, Maddock KC, Anthony K V., VandenHeuvel WJA. Quantitative Aspects of Gas Chromatographic Separations in Biological Studies. Anal Chem [Internet]. 1963 Apr 1;35(4):526–32.
- Blanchflower WJ, Kennedy DG, Taylor SM. Determination of Rafoxanide in Plasma Using High Performance Liquid Chromatography (HPLC) and in Tissue Using HPLC-Thermospray Mass Spectrometry. J Liq Chromatogr [Internet]. 1990 Apr;13(8):1595–609.
- Saad AS, Attia AK, Alaraki MS, Elzanfaly ES. Comparative study on the selectivity of various spectrophotometric techniques for the determination of binary mixture of fenbendazole and rafoxanide. Spectrochim Acta Part A Mol Biomol Spectrosc [Internet]. 2015 Nov;150:682–90.
- Demir E, Göktug Ö, İnam R, Doyduk D. Development and characterization of iron (III) phthalocyanine modified carbon nanotube paste electrodes and application for determination of fluometuron herbicide as an electrochemical sensor. J Electroanal Chem [Internet]. 2021 Aug;895:115389.
- Inam O, Demir E, Uslu B. Voltammetric Pathways for the Analysis of Ophthalmic Drugs. Curr Pharm Anal [Internet]. 2020 Apr 27;16(4):367–91.
- Isildak I, Attar A, Demir E, Kemer B, Aboul-Enein HY. A Novel all Solid-State Contact PVC-Membrane Beryllium-Selective Electrode Based on 4-Hydroxybenzo-15-Crown-5 Ether

Ionophore. Curr Anal Chem [Internet]. 2018 Jan 8;14(1).

- Karimi F, Demir E, Aydogdu N, Shojaei M, Taher MA, Asrami PN, et al. Advancement in electrochemical strategies for quantification of Brown HT and Carmoisine (Acid Red 14) From Azo Dyestuff class. Food Chem Toxicol [Internet]. 2022 Jul;165:113075.
- Silah H, Erkmen C, Demir E, Uslu B. Modified indium tin oxide electrodes: Electrochemical applications in pharmaceutical, biological, environmental and food analysis. TrAC Trends Anal Chem [Internet]. 2021 Aug;141:116289.
- Sarakhman O, Švorc Ľ. A Review on Recent Advances in the Applications of Boron-Doped Diamond Electrochemical Sensors in Food Analysis. Crit Rev Anal Chem [Internet]. 2022 May 19;52(4):791–813.
- Umapathi R, Ghoreishian SM, Sonwal S, Rani GM, Huh YS. Portable electrochemical sensing methodologies for on-site detection of pesticide residues in fruits and vegetables. Coord Chem Rev [Internet]. 2022 Feb;453:214305.
- Silah H, Demir E, Yıldırım S, Uslu B. Carbon nanomaterial-based sensors for the development of sensitive sensor platform. In: Carbon Nanomaterials-Based Sensors [Internet]. Elsevier; 2022. p. 191–246.
- Radi A-E, El-samboskany H. Anodic Adsorptive Stripping Voltammetric Determination of Rafoxanide on Glassy Carbon Electrode. Comb Chem High Throughput Screen [Internet]. 2020 Dec 28;23(10):1002–9.
- 20. Salem WM, Abdel-Lateef MA, Abdel Hamid MA, Batakoushy HA. Fabrication of ultrasensitive carbon paste electrode with nanocomposite CdS modification for electroanalysis of rafoxanide in dosage form and biological fluids. Rev Anal Chem. 2022;41(1):267–74.
- Laviron E, Roullier L, Degrand C. A multilayer model for the study of space distributed redox modified electrodes. J Electroanal Chem Interfacial Electrochem [Internet]. 1980 Sep;112(1):11–23.
- Gustavo González A, Ángeles Herrador M. A practical guide to analytical method validation, including measurement uncertainty and accuracy profiles. TrAC Trends Anal Chem [Internet]. 2007 Mar;26(3):227–38.