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Electroanalytical investigation and voltametric quantification of emtricitabine in the biological sample using boron-doped diamond and glassy carbon electrodes

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ABSTRACT: Emtricitabine (EMT) was electrochemically investigated in detail by using boron-doped diamond electrode (BDDE) and glassy carbon electrode (GCE). The measurements were performed via cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. EMT exhibited one irreversible oxidation peak on the BDDE by CV, with dependence of the pH values. It also showed one irreversible oxidation peak on the GCE via CV from pH 0.3 until pH 6.0. Two irreversible peaks were observed at pH 6.0 with phosphate buffer (PB). The aim of this work is to develop a system able to detect EMT in synthetic serum samples using the aforementioned electrochemical techniques. All required validation parameters were examined and computed. The developed sensors were successfully used to analyze EMT in synthetic serum samples. The standard addition technique was applied in the recovery studies with both electrodes. At $1.0x10^{-3}$ M EMT, the highest voltametric peak response was achieved in Britton-Robinson Buffer (BRB) at pH 11.0 by BDEE, and 0.5 M H_2SO_4 by GCE. Good linearity was observed for a concentration range between $1x10^{-3}$ to $4x10^{-5}$ M and $1x10^{-3}$ to $6x10^{-5}$ M, with limits of detection of 1.66 µM and 2.91 µM, for BDDE and GCE, respectively.

KEYWORDS: Emtricitabine; boron-doped diamond electrode; glassy carbon electrode; drug assay; differential pulse voltammetry.

1. INTRODUCTION

Human immunodeficiency virus (HIV), which has caused almost 40 million deaths to date, is one of the critical global public health problems, which still has no cure. In 2021, nearly 1.5 million people were infected with HIV and approximately 650,000 people died from HIV-related causes (*e.g.* acquired immunodeficiency syndrome, AIDS). However, with efficient treatment, protection, diagnosis and an increased access to care, HIV infection has become a manageable chronic health problem, including opportunistic infections, allowing people to live healthful and long lives with the disease. At the end of 2021, almost 38 million people were reported to be living with HIV, of which two-thirds are in the World Health Organization (WHO) Africa Region. HIV can be controlled with treatment regimens consisting of a combination of antiretroviral drugs [1]. Emtricitabine (EMT, Scheme 1) is commonly utilized as part of first-line regimens for the combined modality therapy of human immunodeficiency virus type 1 (HIV-1) infection in adults and as a strong nucleoside reverse transcriptase inhibitor (NRTI). While the pharmacokinetics of other NRTIs, including tenofovir and lamivudine, have been extensively studied, the pharmacokinetics of EMT have been less worked. EMT is eliminated mainly by renal evacuation, with 86 % of the oral dose getting back invariably into the urine [2].

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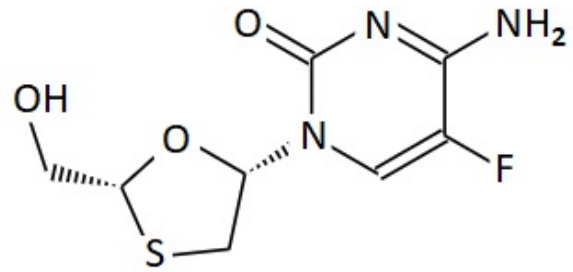

Scheme 1. Structure of Emtricitabine (EMT).

The literature has revealed some analytical methods for the determination of EMT. Among these analytical techniques stand an electrochemical method [3], spectrophotometric [4–9], high-performance liquid chromatographic methods [10–17], liquid chromatography/mass spectrometry [18,19], capillary electrophoresis [20] and colorimetric methods [21].

In the last decades, electrochemical techniques have been raising their attention as drug detection techniques because of their advantages, which include low-cost fabrication, easiness of use and their capability to provide a fast analysis [22]. Mulik et al. [3] published one article devoted to the voltametric study and the determination of EMT. In their work, the electrochemical oxidation of EMT on a gold disk electrode (Au diameter of 3 mm) using cyclic voltammetry (CV) and linear sweep (LSV) was reported.

The selection of the electrodes is a key factor in order to improve the performance of the voltametric technique. The boron-doped diamond electrode (BDDE) has become the most preferred solid and environmentally friendly electrochemical sensor design in many areas, including analytical chemistry, drug analysis and environmental monitoring. The BDDE exhibits remarkable properties over the conventional electrodes (*e.g.* gold, carbon paste, pyrolytic graphite, glassy carbon, platinum, etc.), such as its large electrochemical potential window in aqueous media, a more stable and lower background current, ultra-physical and chemical stability, and a stronger protection against the adsorption of most pollutants on its surface [23–25]. Glassy carbon electrodes (GCE) are the most widely used carbon-based electrodes in most of trendly electrochemical applications. The advantages of GCE are good conductivity, superior stability, smoother surface, high hydrogen over potential, and wide potential application. In addition, GCE is a chemically stable electrode which does not interact with most of matrixes and reagents, making it possible to use it as an inert electrode. Nevertheless, the main drawback of the GCE is that it is not a disposable electrode, making it not suitable for some applications [26,27].

The main objective of this work is to examine the electrochemical behavior and possible oxidation mechanism of EMT on BDDE and GCE, via CV and differential pulse voltammetry (DPV), while determining the optimal experimental conditions. Herein, we compare the BDDE measurements against the ones from GCE. We aim to provide new, affordable, sensitive, specific, fast, fully-validated, easy-to-use and robust voltametric techniques for determining EMT in spiked synthetic human serum samples without time-consuming process for drug analysis.

2. RESULTS AND DISCUSSION

CV method was performed to determine the detailed electrochemical behavior of EMT at the BDDE and GCE. DPV technique was applied to the analysis of EMT for both electrodes.

2.1. Investigation of the electrochemical behavior of EMT on the BDDE and GCE

The electrochemical investigation of EMT was carried out on BDDE and GCE with CV studies shown in Figure 1. To obtain the most optimal experimental parameters, the effect of pH values and supporting electrolytes on the electrochemical current peaks of EMT were performed with DPV and CV between pH 0.30 and 12.00 using BDDE and GCE. Accordingly, CV obtained in BDDE for $1.0x10^{-3}$ M EMT in Britton-Robinson Buffer (BRB) at pH 11.0, and in GCE for the same concentration at 0.5 M H_2SO_4, show one well-defined irreversible anodic peak at a scan rate of 50 mV/s at about 1.7 V (Figure 1). No reduction peak was obtained in the negative scanning data about the irreversible nature of the electrode procedure of EMT on BDDE and GCE.

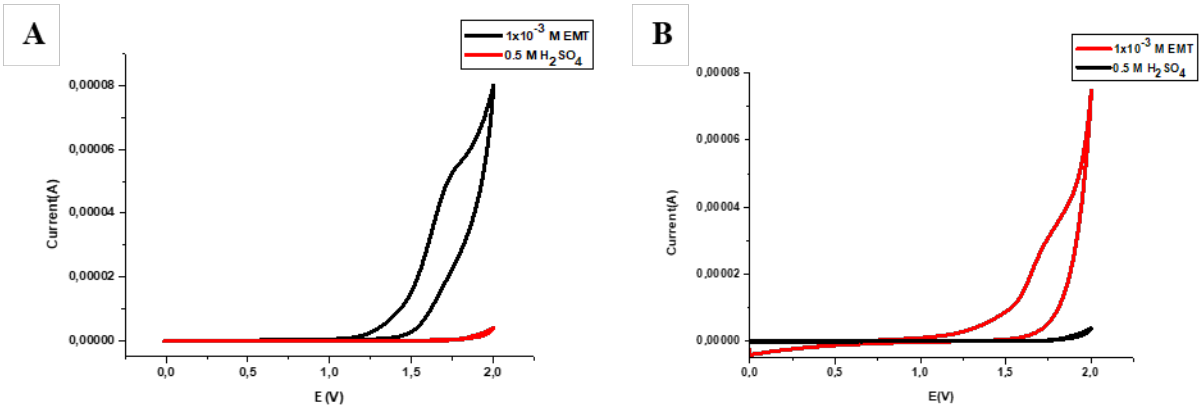

Figure 1. CV of 1.0x10^{-3} M EMT at scan rate 0.5 V/s at A) BDDE in BRB pH 11.0 and B) GCE in 0.5 M H_2SO_4.

2.2. Influence of pH

The pH-dependent oxidation of EMT was examined utilizing 0.1 M and 0.5 M H_2SO_4, Acetate Buffer (AB) (pH 3.7, 4.7, 5.7), Phosphate Buffer (PB) (pH 2.0-8.0), and BRB (pH 2.0–10.0) by DPV, and CV. For pH results, the highest well-defined oxidation currents of EMT were obtained in BRB pH 11.0 and 0.5 M H_2SO_4 at BDDE and GCE, respectively. The voltametric peak potentials for EMT show a negative shift with increasing pH.

The peak current of EMT has reached a maximum of 0.5 M H_2SO_4 and then decreased continuously for GCE. The highest voltametric peak response was observed in BRB pH 11.0 at BDDE. Therefore, 0.5 M H_2SO_4 and BRB pH 11.0 was selected as the optimal working medium for further measurements.

The following equation indicates the influence of pH on the peak potential. For both electrodes, the dependency between Ep and pH can be stated with the following equations:

$$Ep\ (mV) = 1647.6 - 20.2\ pH;\ r = 0.99 \text{ for BDDE (pH 2.0 – 12.0)}$$

$$Ep\ (mV) = 1749.0 - 19.1\ pH;\ r = 0.96 \text{ for GCE (pH 0.3 – 4.7)}$$

The slope values obtained from the above equation show that the number of electrons equals the number of protons in the electrooxidation reaction.

2.3. Influence of scan rate studies

The influence of scan rate on the anodic peak is worked in the range between 10 to 250 mV/s. Scan rate experiments were performed on whether BDDE and GCE procedures were under adsorption or diffusion controlled in 1.0x10^{-3} M EMT. The influence of the scan rate using CV on the peak current and potential was examined in BRB pH 11.0 and 0.5 M H_2SO_4 at BDDE and GCE, respectively, where the maximum peak current was acquired in pH works with a BDDE and GCE. As illustrated in the following equations, the Ip was linear to the square root of scan rate ($v^{1/2}$) for BDDE and GCE in the between of 0.010-0.250 Vs^{-1}:

$$Ip\ (\mu A) = 0{,}9761\ v^{1/2}\ (Vs^{-1}) + 1{,}5659\ (r = 0.9997;\ n=10) \text{ at BDDE in BRB pH 11.0}$$

$$Ip\ (\mu A) = 0{,}1259\ v^{1/2}\ (Vs^{-1}) - 0{,}0591\ (r = 0.9977;\ n=10) \text{ at GCE in 0.5 M } H_2SO_4$$

Considering the linear dependency of the Ip – $v^{1/2}$, the electrooxidation process of EMT is a diffusion-controlled process on BDDE and GCE. There was a linear relationship of the logarithm of Ip (log Ip) versus the logarithm of scan rate (log v) in the following equations:

$$\log Ip\ (\mu A) = 0.3654 \log v\ (Vs^{-1}) + 0.3549\ (r = 0.9911;\ n = 10) \text{ at BDDE in BRB pH 11.0}$$

$$\log Ip\ (\mu A) = 0.6226 \log v\ (Vs^{-1}) - 1.1539\ (r = 0.9956;\ n = 10) \text{ at GCE in 0.5 M } H_2SO_4$$

The slope value of the linear relationship between the log Ip and the log v changed between 0.3654 and 0.6226 for both electrodes in BRB pH 11.0 and 0.5 M H_2SO_4, which is near the theoretical value of 0.5, obviously approving the diffusion-controlled mechanism. For Ep, in BRB pH 11.0 and 0.5 M H_2SO_4, at BDDE and GCE, the linear relationship of the Ep versus log v was revealed as;

$$Ep\ (mV) = 0.0555 \log v\ (Vs^{-1}) + 1.5659\ (r = 0.9999;\ n = 10) \text{ at BDDE in BRB pH 11.0}$$

$$Ep\ (mV) = 0.0991 \log v\ (Vs^{-1}) + 1.456\ (r = 0.9965;\ n = 10) \text{ at GCE in 0.5 M } H_2SO_4$$

With increasing the scan rate, the peak potential was confirmed to be irreversible of the oxidation mechanism of EMT for BDDE and GCE, and the peak potential was shifted to more negative values without the reverse reduction peak. For a 10-fold increase in the scan rate, the Ep shifted to about 30/αn mV more

positive potentials in the irreversible electrochemical process. "α" is the anodic charge transfer coefficient, and "n" is the electron transfer number, respectively [28]. Ep values were shifted to 55.5 and 99.1 mV in BRB pH 11.0 and 0.5 M H_2SO_4 at BDDE and GCE, respectively. The α value can be accepted as 0.5, so the value of n = 1.08 (~1), for BDDE, and 0.61 (~1), for GCE, was determined.

These results showed that the oxidation process of EMT at BDDE and GCE in BRB pH 11.0 and 0.5 M H_2SO_4 undergoes one-electron irreversible oxidation.

2.4. Analytical applications

The relationship between EMT peak current and concentration was examined to assess the analytical applications of EMT using BDDE and GCE. The electrochemical oxidation mechanism of EMT was monitored as a diffusion control mechanism process on both electrodes. The fast and sensitive electrochemical technique, DPV, was applied to determine EMT in BRB pH 11.0 and 0.5 M H_2SO_4 at BDDE and GCE, respectively. Under the optimum conditions, the corresponding characteristics of the linear regression analysis are given in Table 1 for both electrodes. The proposed sensor detected EMT at various amounts under optimum conditions for plotting the calibration graph. The achieved voltammograms, as well as the diagram of peak current vs EMT concentration, are indicated in Figure 2.

Table 1. DPV method validation parameters for standard linearity for assay of EMT in BRB pH 11.0 and 0.5 M H_2SO_4 at BDDE and GCE, respectively, and spiked serum samples.

	Standard Solution (BDDE)	Standard Solution (GCE)	Serum samples (BDDE)	Serum samples (GCE)
Peak potential (V)	1.57	1.61	1.59	1.60
Linearity range (M)	4×10^{-5} – 1×10^{-3}	6×10^{-5} - 1 x 10-3	8×10^{-5} - 8 x 10-4	8×10^{-4} -1 x 10^{-4}
Correlation coefficient	0.99	0.99	0.99	0.99
Slope (μA M^{-1})	9689.87	6898.40	8185.29	1532.56
Interception (μA)	-0.38108835	-0.16210311	-0.292657	0.41952439
LOD (M)	1.66×10^{-6}	2.91×10^{-6}	2.74×10^{-6}	2.30×10^{-5}
LOQ (M)	6.28×10^{-6}	8.81×10^{-6}	8.32×10^{-6}	6.97×10^{-5}
Repeatability of peak current (RSD %) [a]	1.55	1.79	1.66	1.82
Reproducibility of peak current (RSD %) [a]	1.69	1.91	1.73	2.02

[a] Each value is calculated from the average of five measurements.

The limit of detection (LOD) is the lowest amount of analyte detected by an analytical measurement [29–31]. The following equation measures LOD:

$$LOD = 3\ s/m$$

Where m is the slope and s is the standard deviation. In the case of detection, the obtained LODs of EMT for BDDE and GCE are 1.66 μM and 2.91 μM, respectively.

The limit of quantification (LOQ) is the smallest amount of analyte quantifiable. The following equation measures it:

$$LOQ = 10\ s/m$$

The obtained LOQs of EMT detection for BDDE and GCE are 6.28 μM and 8.82 μM, respectively. LOD results proved that the proposed DPV technique, using BDDE, is more sensitive than the proposed DPV technique using GCE.

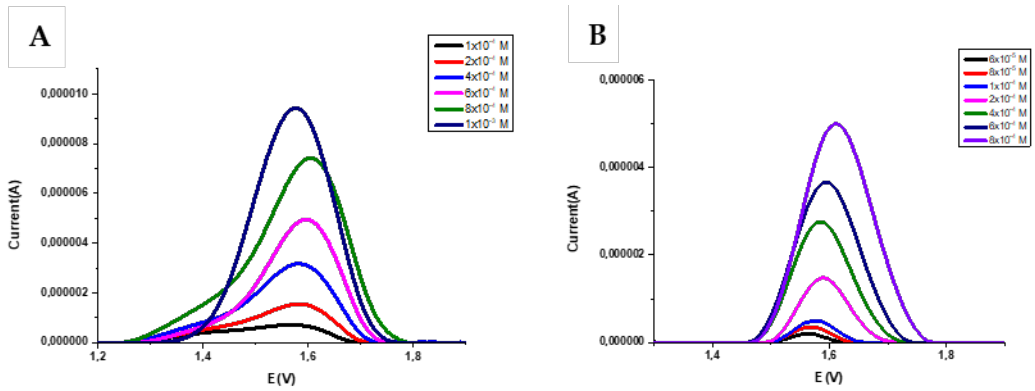

Figure 2. DPV of the developed sensor in different EMT concentrations using A) BDDE in BRB pH 11.0 and B) GCE in 0.5M H_2SO_4.

2.5. Analysis of EMT in biological fluids

The probability of performing the developed techniques for detecting EMT in commercial synthetic human serum samples was studied. Serum samples were spiked with EMT to obtain final amounts of $4x10^{-4}$ M for both electrodes. The concentration of EMT in synthetic human serum was found from the related linear regression equations (Table 1). DPV measurements of EMT studied in synthetic human serum are indicated in Figure 3A and B at BDDE and GCE, respectively. The proposed sensor shows between $8x10^{-5}$ and $8x10^{-4}$ M and $8x10^{-4}$ and $1x10^{-4}$ M linear range, with LOD of $2.74x10^{-6}$ M and $2.30x10^{-5}$ M for BDDE and GCE, respectively. The obtained LOQs are $8.32x10^{-6}$ M for BDDE and $6.97x10^{-5}$ M for GCE.

The percentage recovery of EMT was defined by checking against the peak currents of known concentrations of the drug in synthetic human serum samples with their equivalents in related calibration graphs. The outcomes of these experiments are calculated as given in Table 2. Excellent recoveries of EMT were obtained from commercial synthetic human serum sample matrix for both electrodes.

Table 2. Analytical results for EMT in spiked synthetic human serum samples in BRB pH 11.0 and 0.5 M H_2SO_4 at BDDE and GCE and its recovery results, respectively.

	Serum Samples	
	BDDE	**GCE**
Added(mg)	4.00×10^{-4}	4.00×10^{-4}
Found(mg)	4.01×10^{-4}	3.94×10^{-4}
Average recovery %	100.26	98.44
RSD (%)	1.02	1.24
Bias (%)	0.25	-1.50

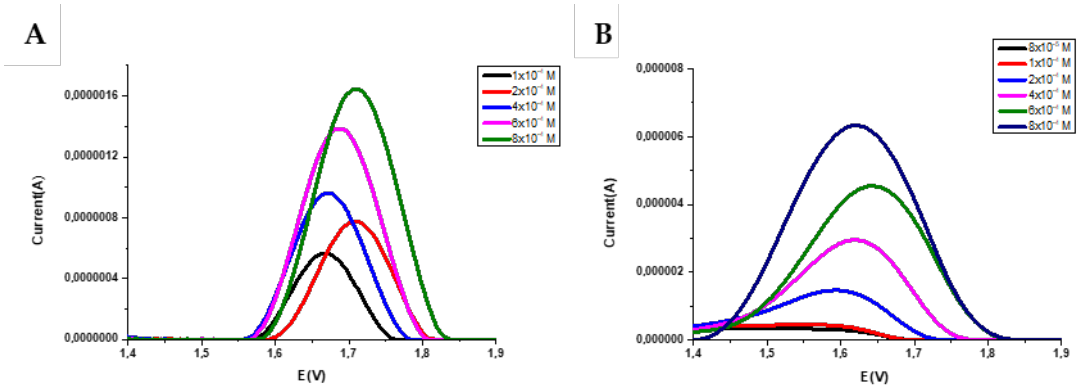

Figure 3. DPV of the developed sensor in different EMT concentrations using A) BDDE B) GCE in serum samples.

2.6. Interferences studies

Some interference agents may influence in the electrochemical peaks of the proposed sensor. To examine the selectivity of the developed sensor, it was studied in the presence of some interference agents such as sodium chloride (NaCl), potassium chloride (KCl), calcium chloride ($CaCl_2$), dopamine, folic acid, paracetamol and ascorbic acid. The interference research results are given in Table 3. No remarkable difference in sensor responses was observed when working with interference agents in 1:1 and 1:10 ratios. The results indicate that the proposed sensor remains robust in the presence of interference agents.

Table 3. Interferences measurements result for EMT in BRB pH 11.0 and 0.5 M H2SO4 at BDDE and GCE, respectively.

Interferences	BDDE				GCE			
	1:1 (%) Recovery	RSD %	1:10 (%) Recovery	RSD %	1:1 (%) Recovery	RSD %	1:10 (%) Recovery	RSD %
NaCl	100.17	0.67	100.63	0.79	100.03	0.84	101.13	2.06
KCl	100.12	0.58	101.23	0.82	100.58	0.63	102.61	1.89
$CaCl_2$	100.35	0.36	100.49	0.74	100.74	0.95	102.42	1.72
Dopamine	101.85	1.25	102.12	1.46	102.79	1.46	103.53	1.84
Folic acid	101.72	1.03	102.46	1.18	102.12	1.59	104.74	1.66
Paracetamol	100.08	0.97	100.62	1.52	100.46	1.06	100.98	1.48
Ascorbic acid	100.89	1.22	101.17	1.44	100.95	0.89	102.06	1.69

3. CONCLUSION

Although chromatographic assay techniques allow simultaneous diagnosis of more than one analyte, they have some disadvantages compared to voltametric techniques [32]. It contains time-consuming sample preparation periods, undefined reaction time, is quite expensive, and less green assay. All these published methods are required highly sophisticated instrumentation. Otherwise, the electroanalytical techniques are low cost, sensitive, rapid analysis times, high accuracy and precision, selective, eco-friendly, practical methods. They are a unique option for detecting pharmaceutically active substances in different matrices. The importance of this technique is that its voltametric peak potential easily identifies each component.

The electrochemical oxidation of EMT on BDDE and GCE progresses in irreversible oxidation steps in a wide pH range. A voltametric technique for EMT detection utilizing DPV with BDDE and GCE was proposed. In this study, a procedure proposed and validated using DPV was speedy, simple, accurate, and precise. CV and DPV were developed to determine EMT with GCE and BDDE. Under the optimum conditions for DPV, the proposed methods offered a LOD of 1.66 µM and 2.91 µM, and a LOQ of 6.28 µM and 8.81 µM for BDDE and GCE, respectively. The developed method offers great advantages, such as low-cost production, eco-friendly, and simple instrumentation, allowing detection from different anions and cations without any interference. An effective analysis has been demonstrated in determining EMT in commercial synthetic serum samples.

Using BDDE, wider linear range values were acquired at 0.5 M H_2SO_4 and serum sample. Also, lower LOD values were acquired in 0.5 M H_2SO_4 and serum samples using GCE. Moreover, recovery results with the serum sample showed the applicability of both electrodes to serum samples. As a result, this work becomes more sensitive, easier to apply, and more environmentally friendly when checked with the other traditional techniques. This work also contributes to sustainable approaches owing to the benefits of electrochemistry, requiring a small number of samples and reagents, reusability of BDDE and GCE, and minimum usage of harmful chemicals. These properties also indicate that it is a suitable technique for the green analytical chemistry approach, which has become very important and popular. The proposed methods are accurate, precise, sensitive, and reliable options for detecting EMT.

4. MATERIALS AND METHODS

4.1. Equipment and Chemicals

Electrochemical measurements were performed by potentiostat AUTOLAB 204 (Eco Chemie, Utrecht, and The Netherlands) with BDDE and GCE. Electrochemical measurements were applied in a three-electrode electrochemical cell. BDDE and GCE were utilized as working electrodes, a saturated Ag/AgCl as a reference electrode, and a Pt wire as a counter electrode. For the cleaning, the BDDE and GCE surface was physically cleaned before each measurement.

Sodium acetate trihydrate, acetic acid, sulfuric acid, phosphoric acid, sodium phosphate monobasic dihydrate, sodium dihydrogen phosphate dihydrate, boric acid, and methanol were supplied by Sigma-Aldrich. Abdi Ibrahim Pharm. Comp. provided the EMT used in electrochemical experiments. The various supporting electrolytes, acetate buffer (AB), Britton-Robinson buffer (BRB), 0.1 M and 0.5 M H_2SO_4, and phosphate buffer (PB), were used for electrochemical experiments. The $1x10^{-2}$ M stock solution of EMT was prepared in bi-distilled water. EMT measurement solutions were prepared and measured daily with appropriate dilution with pH desired buffer solution. They were kept in the refrigerator to protect the freshness of all chemicals and stock solutions used in the experiments.

4.2. Optimizing procedures

CV was performed at a scan rate of 0.05 Vs^{-1}. For drawing the calibration graph of EMT using Britton-Robinson Buffer (BRB) pH 11.0 and 0.5 M H_2SO_4 at BDDE and GCE, respectively, and the DPV parameters were as follows; interval time: 0.5 s, modulation amplitude: 0.05 V; step potential: 0.005 V; modulation time: 0.05 s. All the electrochemical measurements were applied at room temperature.

4.3. Analysis of synthetic human serum samples

Drug-free commercial synthetic human serum was held frozen at −20 °C fridge until examination. A standard process was applied to prepare a stock serum solution. 3.6 mL of synthetic human serum, 5.4 mL of acetonitrile (ACN), and 1 mL of $1.0x10^{-2}$ M EMT were added to a centrifuge tube to obtain a stock serum sample. Firstly, it was centrifuged at 4000 rpm for 20 min, and later, the supernatant was taken. Here, ACN was used to precipitate serum proteins. The supernatant was diluted with selected supporting electrolytes to obtain certain concentrations for the recovery measurements. All the experiments were applied at least five times for calibration and recovery experiments.

Author contributions: Concept – L.K; Design – L.K.; Supervision – S.A.O.; Resources – L.K., S.A.O.; Materials – L.K., S.A.O.; Data Collection and/or Processing – L.K.; Analysis and/or Interpretation – L.K., S.A.O.; Literature Search – L.K.; Writing – L.K., S.A.O.; Critical Reviews – L.K., S.A.O.

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REFERENCES

[1] HIV and AIDS. WHO. https://www.who.int/news-room/fact-sheets/detail/hiv-aids (accessed on 14 August 2023).

[2] Valade E, Tréluyer JM, Bouazza N, Ghosn J, Foissac F, Benaboud S, Fauchet F, Viard JP, Urien S, Hirt D. Population pharmacokinetics of emtricitabine in HIV-1-infected adult patients. Antimicrob Agents Chemother. 2014; 58(4): 2256-61. https://doi.org/10.1128/aac.02058-13

[3] Mulik BB, Dhumal ST, Harale RR, Kharat KR, Sathe BR. Electrochemical Studies of Anti-HIV Drug Emtricitabine: Oxidative Determination and Improved Antimicrobial Activity. Chem Electro Chem. 2018; 5(24): 3926-31. https://doi.org/10.1002/celc.201801228

[4] Choudhari VP, Parekar SR, Chate SG, Bharande PD, Singh RR, Kuchekar BS. Development and validation of UV-visible spectrophotometric baseline manipulation method for simultaneous quantitation of tenofovir disoproxil fumarate and emtricitabine in pharmaceutical dosage form. J Spectrosc. 2013; 1(1). https://doi.org/10.1155/2013/146580

[5] Ghorpade SA, Sali MS, Kategaonkar AH, Patel DM, Choudhari VP, Kuchekar BS. Simultaneous determination of emtricitabine and tenofovir by area under curve and dual wavelength spectrophotometric method. J Chil Chem Soc. 2010; 55(1): 115-7. https://doi.org/10.4067/S0717-97072010000100027

[6] Nagaraju PT, Channabasavaraj KP, Shantha Kumar PT. Development and validation of spectrophotometric method for estimation of emtricitabine in tablet dosage form. International Journal of ChemTech Research. 2011; 3(1): 23-8.

[7] Harini U, Akm P. Development and valıdatıon of stabılıty ındıcatıng sımultaneous uvspectrophotometrıc method for determınatıon of emtrıcıtabıne, tenofovır dısoproxıl fumarate, cobıcıstat, and elvıtegravır ın pure and pharmaceutıcal dosage form. Asian J Pharm Clin Res. 2018; 11(4): 177-84. https://doi.org/10.22159/ajpcr.2018.v11i4.22897

[8] Vinutha K, Kunderu RS. Kinetic spectrophotometric determination of emtricitabine and tenofovir disoproxil fumarate in bulk and pharmaceutical dosage form. Int J Pharm Res. 2020; 12(3): 1142-9. https://doi.org/10.31838/ijpr/2020.12.03.172

[9] Abdelhay MH, Gazy AA, Shaalan RA, Ashour HK. Simple spectrophotometric methods for determination of tenofovir fumarate and emtricitabine in bulk powder and in tablets. J Spectrosc. 2013; 1(1). https://doi.org/10.1155/2013/937409

[10] Gummaluri RK, Parthasarathi TVN, Anjanamadhulika G. Simultaneous Method for Determination of Emtricitabine, Tenofovir Disoproxil Fumarate, Elvitegravir and Cobicistat in Tablets By HPLC. Indian J Pharm Sci. 2016; 78(4): 532-7. https://doi.org/10.4172/pharmaceutical-sciences.1000148

[11] Konidala SK, Samineni R, Rao YV, Chimakurthy J, Kolakaluri CS, Korrapati V. Novel RP-UPLC Method Development and Validation for Simultaneous Quantification of Emtricitabine, Tenofovir and Efavirenz in Bulk and Tablet Dosage Forms. Res J Pharm Technol. 2022; 15(7): 3141-6. https://doi.org/10.52711/0974-360X.2022.00525

[12] Kumar VK, Raju NA. Estimation of Emtricitabine in Tablet Dosage Form by RP-HPLC. Asian J Chem. 2009; 21(8): 5979-83.

[13] Raju NA, Rao JV, Prakash KV, Mukkanti K, Srinivasu K. Simultaneous estimation of tenofovir disoproxil, emtricitabine and efavirenz in tablet dosage form by RP-HPLC. Oriental Journal of Chemistry. 2008; 24(2): 645.

[14] Rao JR, Vidyapeeth B, Gondkar SA, Yadav SS. Simultaneous HPTLC-Densitometric analysis of Tenofovir and Emtricitabine in Tablet dosage form. Artic Int J PharmTech Res. 2011; 3(3): 1430-4.

[15] Kokkirala TK, Suryakala D. RP-HPLC method development and validation for the estimation of Emtricitabine, Bictegravir and Tenofovir alafenamide in bulk and pharmaceutical dosage form. J Taibah Univ Sci. 2019; 13(1): 1137-46. https://doi.org/10.1080/16583655.2019.1689601

[16] Prasad Panigrahy U, Sunil A, Reddy K. A novel validated RP-HPLC method for the simultaneous estimation of Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine in bulk and pharmaceutical tablet dosage forms. Sch Res Libr Der Pharm Lett. 2015; 7(1): 303-14.

[17] Alam P, Haq N, Alqarni MH, Shakeel F. Quantitative Analysis of Emtricitabine in Dosage Forms Using Green RP-HPTLC and Routine NP-HPTLC Methods - A Contrast of Validation Parameters. ACS Omega. 2020; 5(51) :33470-7. https://doi.org/10.1021/acsomega.0c05537

[18] Illamola SM, Valade E, Hirt D, Dulioust E, Zheng Y, Wolf JP, et al. Development and validation of a LC-MS/MS method for the quantification of tenofovir and emtricitabine in seminal plasma. J Chromatogr B Analyt Technol Biomed Life Sci. 2016; 1033-1034: 234-41. https://doi.org/10.1016/j.jchromb.2016.08.011

[19] Srivastava B, Sheeja VK, Haribabu Y, Ebin CJ. Method Development and Validation of Simultaneous Estimation of Emtricitabine and Tenofovir Alafenamide in Bulk and tablet Dosage form using LC-MS/MS. Res J Pharm Technol. 2021; 14(5): 2493-6. https://doi.org/10.52711/0974-360X.2021.00439

[20] Al-Johar HI, Maher HM, Al-Zoman NZ, Al-Shammary DJ, Al-Showiman H. A stability-indicating capillary electrophoresis method for the determination of emtricitabine and tenofovir disoproxil fumarate in pharmaceutical tablets. Acta Chromatogr. 2017; 29(4): 469-76. https://doi.org/10.1556/1326.2017.00057

[21] Ganesh Kumar TNV, Vidyadhara S, Kumar TD, Jaswanth D, Vijetha K, Priyadarshini BG. Development and Validation of a Novel Colorimetric Method for the Estimation of Emtricitabine in Bulk and Tablet Formulation. Indian J Pharm Sci. 2017; 78(6):775-9. https://doi.org/10.4172/pharmaceutical-sciences.1000183

[22] Karadurmuz L, Kurbanoglu S, Uslu B, A. Ozkan S. Electrochemical DNA Biosensors in Drug Analysis. Curr Pharm Anal. 2016; 13(3): 195-207. https://doi.org/10.2174/1573412912666160422152634

[23] Chailapakul O, Siangproh W, Tryk DA. Boron-doped diamond-based sensors: A review. Sens Lett. 2006; 4(2): 99-119. https://doi.org/10.1166/sl.2006.008

[24] Pecková K, Musilová J, Barek J. Boron-Doped Diamond Film Electrodes-New Tool for Voltammetric Determination of Organic Substances. Crit Rev Anal Chem. 2009; 39(3): 148-72. https://doi.org/10.1080/10408340903011812

[25] Luong JHT, Male KB, Glennon JD. Boron-doped diamond electrode: synthesis, characterization, functionalization and analytical applications. Analyst. 2009; 134(10): 1965-79. https://doi.org/10.1039/b910206j

[26] Dekanski A, Stevanović J, Stevanović R, Nikolić BŽ, Jovanović VM. Glassy carbon electrodes: I. Characterization and electrochemical activation. Carbon N Y. 2001; 39(8): 1195-205. https://doi.org/10.1016/S0008-6223(00)00228-1

[27] Zittel HE, Miller FJ. A Glassy-Carbon Electrode for Voltammetry. Anal Chem. 1965; 37(2): 200-3. https://doi.org/10.1021/ac60221a006

[28] Nicholson RS, Shain I. Theory of Stationary Electrode Polarography: Single Scan and Cyclic Methods Applied to Reversible, Irreversible, and Kinetic Systems. Anal Chem. 1964; 36(4): 706-23. https://doi.org/10.1021/ac60210a007

[29] Urcuk A, Karadurmus L, Bakirhan NK, Ozkan SA. Enhancement of graphene oxide through β-cyclodextrin composite to sensitive analysis of an antidepressant: Sulpiride. Open Chem. 2021; 19(1): 228-36. https://doi.org/10.1515/chem-2021-0030

[30] Zahid A, Karadurmuş L, Bakirhan NK, Altay Unal M, Nazır H, Shah A, et al. An electrochemical and theoretical approach for the development of a sensitive flower-like nanosensor as serotonin receptor antagonist tropisetron. Microchem J. 2022; 172:106888. https://doi.org/10.1016/j.microc.2021.106888

[31] Karadurmus L, Kurbanoglu S, Uslu B, Ozkan SA. An Efficient, Simultaneous Electrochemical Assay of Rosuvastatin and Ezetimibe from Human Urine and Serum Samples. Methods Protoc 2022; 5: 90. https://doi.org/10.3390/mps5060090

[32] Gumustas M, Caglayan MG, Onur F, Ozkan SA. Simultaneous determination and validation of emtricitabine, rilpivirine and tenofovir from biological samples using LC and CE methods. Biomedical Chromatography. 2018 Apr;32(4):e4158. https://doi.org/10.1002/bmc.4158