


TECHNICAL NOTE

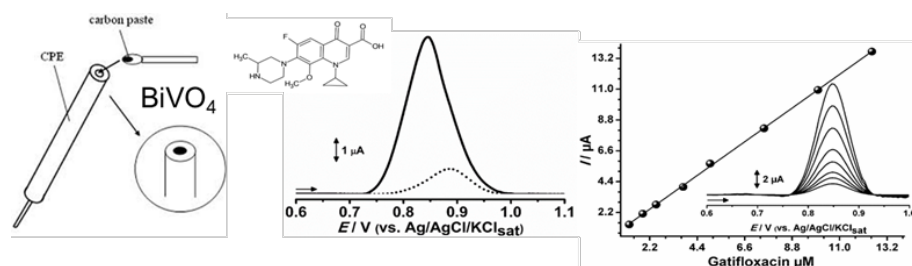
Electrochemical Approach for the Determination of Gatifloxacin Content in Eye Drops using a Carbon Paste Electrode Modified with Bismuth Vanadate

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Gatifloxacin, a fourth-generation antimicrobial agent, requires stringent quality control measures in human medication. Electroanalysis offers a cost-effective method for quality control, showing promise for drug analyses. In this study, gatifloxacin

(GAT) eye drops were analyzed using differential pulse voltammetry (DPV) with a BiVO_4 -modified carbon paste electrode (CPE). The electrochemical characterization of GAT involved a pH study, determining pH 7.0 as optimal with an anodic oxidation process at 0.88 V. The anodic peak suggested equivalence between protons and electrons, with a Nernstian-like slope of around 59 mV pH^{-1} in the E_{pa} vs. pH plot. Subsequent assays using PBS at pH 7.0 showed promising results. The electroanalytical method exhibited precision and accuracy that were suitable for the intended purpose. CPE/ BiVO_4 provided a low-cost, versatile, and rapid alternative for GAT quantification in pharmaceutical formulations, indicating the potential for analyzing other drugs in complex matrices. Subsequent voltammetric assays were carried out using PBS at pH 7.0. Further tests demonstrated a linear correlation between GAT concentration and current intensity ($r^2 = 0.99$), with an LOD of $0.07 \text{ } \mu\text{M}$ and LOQ of $0.23 \text{ } \mu\text{M}$. Precision testing confirmed the method's reliability, showing a relative standard deviation of 1.28% and 1.48% for intra-day and inter-day precision, respectively. The CPE/ BiVO_4 method proved to be accurate, precise, and reproducible, offering a low-cost and versatile alternative for GAT quantification in pharmaceutical formulations. The findings suggest the potential application of this electroanalytical approach for drug analysis in complex biological matrices, showing promise for the quantification of other pharmaceuticals.

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INTRODUCTION

Gatifloxacin (GAT) is a fourth-generation antimicrobial from the fluoroquinolone family. It possesses a broad spectrum of activity, inhibiting the DNA gyrase and topoisomerase IV of both Gram-positive and Gram-negative bacteria.¹⁻⁵ Its chemical formula is $C_{19}H_{22}FN_3O_4$ (Figure 1), and it is available as a semi-dehydrated, crystalline white or slightly yellowish powder.⁵⁻⁷

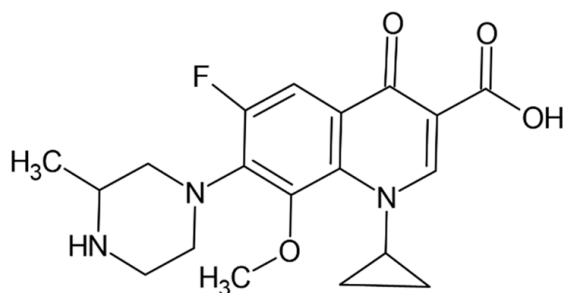


Figure 1. Chemical structure of gatifloxacin.

Eye drops, in general, are prescribed continuously for human use, and among them, GAT stands out when used in treating ocular infections, bacterial conjunctivitis, irritations, and bacterial inflammations.⁵ Thus, medications for human use require quality control parameters to ensure the efficacy and safety of the drugs.⁷⁻¹⁰

Several methods are described in the literature for GAT content analysis, such as high-performance liquid chromatography (HPLC),¹⁰ UV spectrophotometry⁹ and capillary electrophoresis.¹¹ However, these techniques require longer analysis times, and equipment and reagents with higher financial costs.

In this context, electroanalysis is a simple and rapid alternative compared to other methods with a higher cost for application in drug quality control. Additionally, electroanalytical methods provide good sensitivity and versatility and contribute to environmentally friendly analysis.^{12,13} Among the many electrodes used in electroanalysis, sensors based on glassy carbon electrode (GCE) and modified carbon paste electrode (CPE) have been the most used for drug detection. Modified electrodes have advantages for quality control analyses due to their simplicity of application, low cost, and high analytical sensitivity.¹²⁻¹⁵

Various materials can be employed for electrode construction, with the most used being organic modifiers (polymers, enzymes, etc.), inorganic modifiers (oxides, metals, complex ions, etc.), organometallics, nanomaterials, among others.¹⁶

In a study previously conducted by Isecke *et al.* (2023),¹⁷ graphite electrodes impregnated with bismuth vanadate ($BiVO_4$) particles were synthesized and utilized in the present study to enhance the electroanalytical properties of GAT.

Therefore, this work presents the electroanalytical determination of GAT eye drops by differential pulse voltammetry (DPV) using a CPE modified with $BiVO_4$ (CPE $BiVO_4$).

MATERIALS AND METHODS

Materials and reagents

Electrolytic solutions were prepared using high-purity analytical-grade salts, dissolved in Milli-Q water with conductivity $\leq 0.1 \mu S cm^{-1}$ (Millipore S.A., Molsheim, France). The analytical standard of gatifloxacin (GAT) was sourced from the United States Pharmacopeia (USP), while commercial ophthalmic formulations (Zymar® 0.3%, 5 mL) were procured from a local pharmacy in Goiânia, GO, Brazil. Standard and sample solutions were prepared in purified water at a concentration of 0.025 M. Bismuth vanadate ($BiVO_4$) particles were previously synthesized for use as a modifying agent in electrode preparation.¹⁷

Preparation of the carbon paste electrode modified with BiVO_4

The modified carbon paste was prepared by weighing 70 mg of graphite powder, 30 mg of mineral oil, and 7 mg of BiVO_4 . The compounds were mixed until a homogeneous paste was obtained. The unmodified carbon paste was also prepared using the same proportions but without the addition of BiVO_4 . After the pastes were prepared, they were used to fill an electrode with a cavity measuring 2 mm in diameter and 0.5 mm in depth.

Electrochemical assays

Voltammetric analyses were carried out using a PGSTAT[®] 204 potentiostat/galvanostat equipped with the FRA32M module (Metrohm Autolab, Utrecht, Holanda) and operated via NOVA 2.1[®] software. Experiments were conducted in a 5 mL electrochemical cell configured with a three-electrode system, consisting of a carbon paste electrode modified with bismuth vanadate (CPE/ BiVO_4) as the working electrode, a platinum wire as the counter electrode, and a saturated Ag/AgCl/KCl reference electrode (all obtained from Lab Solutions, São Paulo, Brazil).

Differential pulse voltammetry (DPV) measurements were performed within a potential window of 0.0 to 1.3 V, employing a pulse amplitude of 50 mV, a pulse width of 0.5 s, and a scan rate of 10 mV s^{-1} . The assays were conducted in both acetate buffer solution (ACS, 0.1 mol L^{-1}) at pH values of 3.0 and 5.0, and phosphate buffer solution (PBS, 0.1 mol L^{-1}) at pH 7.0, 8.0, and 9.0.

Cyclic voltammetry (CV) experiments were also conducted in the same potential range (0.0 to 1.3 V), evaluating scan rates of 20, 50, 100, 250, and 500 mV s^{-1} to assess the electrochemical behavior of GAT under varying kinetic conditions.

All voltammetric assays for GAT quantification were conducted in phosphate buffer solution (PBS, 0.1 mol L^{-1} , pH 7.0). Differential pulse voltammograms (DPV) were subjected to background subtraction and baseline correction to enhance signal clarity. Each experiment was performed in triplicate, and the resulting data were processed and analyzed using Origin Pro 9[®] software (Northampton, MA, USA).

RESULTS AND DISCUSSION

Evaluation of pH effects on analytical performance

To elucidate the electrochemical behavior of GAT, a pH-dependent study was performed to determine the optimal experimental conditions and to evaluate the influence of proton involvement in the oxidation process (Figure 2).

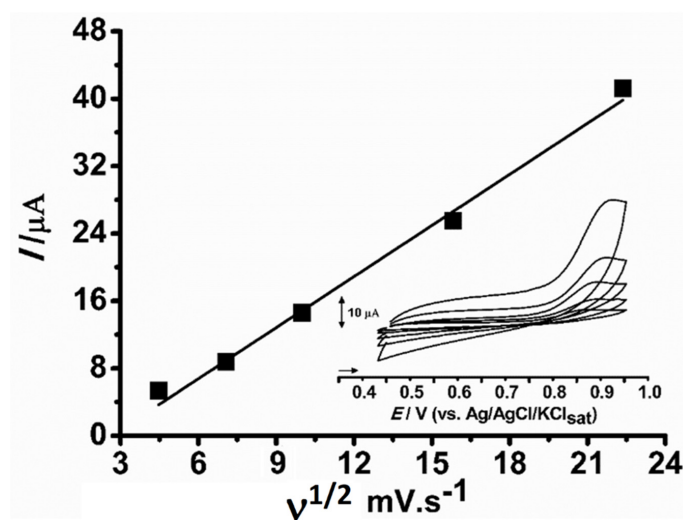


Figure 2. Differential pulse voltammetry results for 0.1 mM GAT at different pH levels (3, 5, 7, 8, 9) in acetate buffer solution (ABS) and phosphate buffer solution (PBS).

The results demonstrated that pH 7.0 provided the highest signal amplitude, with an anodic oxidation peak observed at 0.88 V (vs Ag/AgCl). The electrochemical response indicated a one-to-one ratio between protons and electrons, as confirmed by the E_{pa} vs pH plot, which exhibited a Nernstian slope of approximately 59 mV per pH unit (Figure 2).¹⁸ Based on these findings, phosphate buffer solution (PBS) at pH 7.0 was selected as the optimal medium for subsequent voltammetric assays.

Electrochemical behavior of GAT in CPE/BiVO₄

When subjected to cyclic voltammetry (CV) at different scan rates, GAT displayed a diffusional oxidation process, as evidenced by the linearity ($r = 0.99$) found between the square root of varying scan rates (\sqrt{v}) and the anodic peak current (I_{pa}) (Figure 3A). This result is consistent with the literature, as organic compounds tend to undergo diffusional oxidation processes.^{18,19}

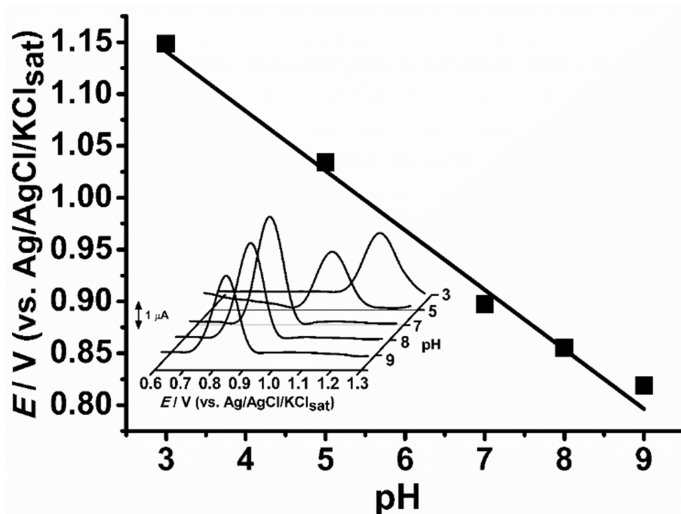


Figure 3. Cyclic voltammetry (CV) profiles of 0.1 mM GAT in PBS (pH 7.0) were recorded at scan rates of 20, 50, 100, 250, and 500 mV s^{-1} .

The electrode surface area was calculated using the Randles–Sevcik equation (Equation 1), where a 0.001 mol L^{-1} solution of GAT was used as a reversible one-electron diffusion-controlled redox system.

$$I_{pa} = 2.69 \times 10^5 \text{ A } n^{3/2} D^{1/2} C v^{1/2} \quad \text{Equation (1)}$$

Where I_{pa} is the anodic peak current; A is the electrode area in cm^2 ; n is the number of electrons transferred; D is the diffusion coefficient, which was previously estimated to be $7.09 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ by our group.²⁰ The values of I_{pa} were obtained from the slopes of the curves presented in Figure 3. The calculated electroactive area of the electrode is approximately 22.3 cm^2 .

Effect of BiVO₄ in CPE performance

Due to the recognized properties of inorganic compounds and their effects as modifying agents,^{16,19,21,22} the use of BiVO₄ was investigated with a focus on the possibility of extending the applications of CPE to samples containing low levels of GAT. Regarding the feasibility of manufacturing procedures, the approach used here was easily conducted and involved a simple homogenization method of the components, followed by filling the electrode. The choice of BiVO₄ was due to its good conductive properties and low susceptibility to leaching processes. Figure 4 shows DPV responses of GCE and CPE/BiVO₄.

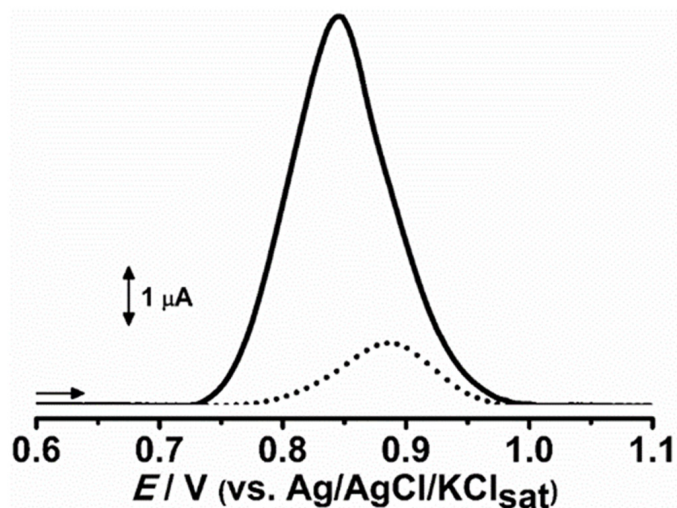


Figure 4. Voltammograms with GCE (···) and CPE/BiVO₄ (-) of 0.1 mM GAT in pH 7.0 PBS.

The analytical signal showed a notable improvement with CPE/BiVO₄ compared to GCE, yielding currents of 6.77 μA and 1.07 μA, respectively—approximately 5.6 times higher the obtained current signal.

Determination of GAT in eye drop solutions

To verify the linearity of the DPV assay using quantitative determinations of GAT in commercial eye drop solutions, calibration curves were conducted at increasing concentrations (Figure 5).

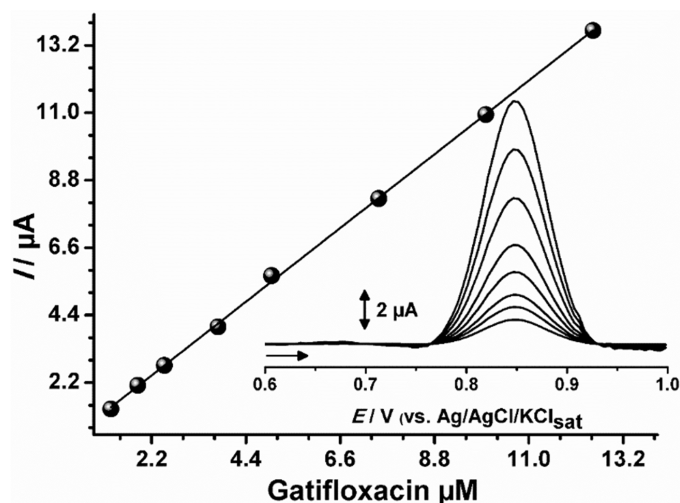


Figure 5. Calibration curve for GAT determination with CPE/BiVO₄ by DPV detection in PBS pH 7.0. Insert shows the differential pulse voltammograms obtained for each CBP concentration 1.25; 1.88; 2.5; 3.75; 5.0; 7.5; 10.0 and 12.5 μM.

A linear relationship was established between the peak current (I , μA) and GAT concentration (μM), yielding a correlation coefficient of $r^2 = 0.99$. The limits of quantification (LOQ) and detection (LOD) were calculated using Equations 2 and 3, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve.

$$LOQ = \frac{10 \times \sigma}{S} \quad \text{Equation (2)}$$

$$LOD = \frac{3 \times \sigma}{S} \quad \text{Equation (3)}$$

The method exhibited a LOD of 0.07 μM and a LOQ of 0.23 μM . To further validate the linearity of the proposed method, statistical analyses were employed to confirm the robustness and reliability of the calibration data (Table S1 in the Supplementary Material).

Precision assays were conducted to complement the validation of the proposed method using the midpoint of linearity (5.0 μM), considered the 100% point on the calibration curve. Thus, Researcher 1 performed repeatability tests, while Researcher 2 conducted intermediate precision tests. Table I shows the results regarding the precision of CPE/BiVO₄. The proposed method was precise and replicable, with a relative standard deviation of 1.28% for Researcher 1 and 1.48% for Researcher 2. The *F*-test indicated no significant difference between the recovery values obtained by the two analysts, as $F_{\text{cal}} = 1.35 < 6.39$; and *P*-value = 0.38 > 0.05, confirming the precision of the proposed method, as shown in Table I.

Table I. Precision and repeatability values of CPE/BiVO₄ by DPV detection for GAT eye drop solutions

Sample	Sample amount (%)	Sample recovery (%) Precision repeatability (<i>n</i> = 6)	Sample recovery (%) Precision intermediary (<i>n</i> = 6)
GAT eye drop solutions	100%	98.11	100.10
		100.40	98.45
		101.18	101.57
		98.55	101.80
		99.470	99.18
		98.11	100.10

After ensuring the replicability of the proposed method, recovery tests were conducted at three different increased concentrations. GAT was determined at three different levels in the sample: 80%, 100%, and 120%, with each analysis performed in triplicate (Table II).

The precision of the proposed method yielded satisfactory results for all GAT concentration levels, with a maximum deviation of 10.0%. Maintaining an appropriate confidence interval between 99.00% and 101.00%, which confirms the method's accuracy, our electrode can accurately quantify GAT by DPV analysis.

Table II. Recovery tests with CPE/BiVO₄ by DPV detection in samples spiked with different GAT concentrations

Sample	Sample amount (%)	Measured amount (%)	Recovery (%)	
GAT eye drop solutions	80 %	81.8	102.3	
		81.1	101.4	
		79.8	99.8	
	100 %	100.1	100.1	
		99.4	99.4	
		98.1	98.1	
	120 %	120.5	100.4	
		Recovery average (%)		100.0
		Recovery relative standard deviation (%)		1.3
Acceptance level (%)		98.00 to 102.00		
RSD acceptance level (%)		≤ 2.0		
Confidence interval 95%		1.00		
Average ± IC95%		99.00% to 101.00%		

As seen in Table III, a comparative summary of our method is presented, showing current analytical approaches for GAT detection, from which the sensitivity and simplicity of this proposal can be inferred. It is employed more quickly and with lower costs compared to other methods. Additionally, the electroanalytical method proposed in this study achieved an LOD of 0.07 µM and LOQ of 0.23 µM, which is sufficient for application in the quality control of GAT in eye drop solutions.

Table III. Analogy between the limit of detection (LOD) and quantification (LOQ) on GAT determination in voltametric (DPV) and some other frequently used methods

Method	LOD	LOQ	Reference
LC-MS/MS	0.11 µg mL ⁻¹	0.42 µg mL ⁻¹	23
FIA-FLUO	22 µg mL ⁻¹	72 µg mL ⁻¹	24
HPLC-DAD	0.7 µg mL ⁻¹	2.3 µg mL ⁻¹	25
HPLC-FLUO	0.01 µg mL ⁻¹	0.03 µg mL ⁻¹	25
CL-ELISA	0.01 ng mL ⁻¹	–	26
LC/ESI-MS/MS	500 pg mL ⁻¹	–	27
DPV	0.26 µg mL ⁻¹	0.86 µg mL ⁻¹	This work

CONCLUSIONS

The electroanalytical method employed with the modified electrode demonstrated suitable precision and accuracy for the developed purpose. Additionally, CPE/BiVO₄ exhibited low cost, versatility, and rapid assay compared to chromatographic methods for GAT quantification reported in the literature. The electroanalytical approach undertaken in this study shows feasibility for its application in the analysis of pharmaceutical forms containing GAT. It holds promise for the quantification of other drugs in biological or complex matrices.

Acknowledgements

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Conflicts of interest

The authors declare that they have no known financial conflicts of interest.

Use of Artificial Intelligence tools

The authors declare that no artificial intelligence tools were used in any aspect of the preparation of this Technical Note.

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SUPPLEMENTARY MATERIAL

Table S1. Statistical analysis of the measurement results obtained

Test	$\alpha = 0.05$ Significance level	<i>p</i> -value
<i>F</i> Test ANOVA slope	< 0.05	4.1329E-41
Student's <i>t</i> -test Linear coefficient	> 0.05	0.1544
Shapiro-Wilk waste normality	> 0.05	0.7112
Breusch Pagan homoscedasticity	> 0.05	0.9607

As the *p*-value (0) from the ANOVA *F*-test is less than 0.05, we reject the null hypothesis (zero slope) at the significance level of 5%, whereas the *p*-value of 0.1544 from the *t*-test is greater than 0.05; therefore, we do not reject the null hypothesis (intercept equal to zero) at the significance level of 5%. The correlation coefficient found of 0.99 is satisfactory, so we conclude that there is an adequate linear relationship, as the *p*-value of 0.9607 of the Breusch Pagan test is greater than 0.05, we do not reject the hypothesis of equality of variances at the level of significance of 5%, therefore, we have a homoscedastic model, finally, as the *p*-value of 0.7112 of the Shapiro-Wilk test is greater than 0.05, we do not reject the hypothesis of normality of residues at the level of significance of 5%.