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Original Article

Electrochemical behavior and differential pulse voltammetric determination of budesonide in suspension ampoules

Orkhan Yolchuyev[1,2], Zeynep Aydogmus[1]

[1]Istanbul University, Faculty of Pharmacy, Department of Analytical Chemistry, Istanbul, Turkiye
[2]Istanbul University, Institute of Graduate Studies in Health Sciences, Istanbul, Turkiye

ORCID IDs of the authors: O.Y. 0000-0001-9357-1053; Z.A. 0000-0002-6310-1197

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ABSTRACT

Background and Aims: Budesonide (BUD) is a broad-spectrum anti-inflammatory and anti-allergic glucocorticosteroid agent. It is used in the treatment of chronic obstructive pulmonary disease (COPD), Crohn's disease, and ulcerative colitis. The aim of the study was to investigate the electrochemical properties of BUD for the first time and to develop a sensitive, easy, and selective new differential pulse voltammetry (DPV) method for its determination in drug formulation.

Methods: The electrochemical behavior of BUD was investigated using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) with a carbon paste electrode (CPE) in various electrolyte and buffer solutions with pH ranging from 2 to 9. An irreversible, well-defined reduction current peak of about -859 mV was obtained. A DPV method was developed and validated for the determination of BUD in suspension ampoules using a CPE electrode in a 0.1 M HCl electrolyte solution containing 13% KCl and 8% methanol.

Results: The cathodic peak was found to be adsorption-controlled. The calibration curve was linear between 1.65- 35.35 µg/ml. The limit of detection (LOD) and limit of quantification (LOQ) values were found to be 0.52 µg/mL and 1.57 µg/mL, respectively. The developed method offered an effective capability for the determination of BUD in suspension ampoules, with a recovery rate of 98.47%.

Conclusion: The DPV method developed in this study could be used for routine quantitative analysis of BUD in pharmaceutical preparations due to its fast, accurate, inexpensive, and environmentally friendly nature.

Keywords: Budesonide, determination, pharmaceutical preparation, validation, voltammetry

Address for Correspondence:
Zeynep AYDOĞMUŞ, e-mail: aydogmus@istanbul.edu.tr

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INTRODUCTION

Budesonide [BUD, 16,17-Butilidenebis(oxy)-11,21-dihydroxy-pregna-1,4-diene-3,20-dione, Figure 1], a potent glucocorticoid, is an anti-inflammatory agent. BUD mainly treats asthma, COPD, Crohn's disease, ulcerative colitis, catarrh, and inflammatory conditions of the upper respiratory tract and intestines by preventing and reducing swelling and inflammation. Prolonged use of high doses of corticosteroids may cause hypercriticism and suppression of the adrenal axis. BUD is commercially available in inhalation, tablet, nasal spray, and rectal forms.

Patients given BUD should be monitored for symptoms and respiratory function to ensure effective therapy and dose adjustment. Since BUD is mainly metabolized in the liver, it may cause impaired hepatocyte function and accumulation in the blood. In addition, the simultaneous use of thiazide and thiazide-like loop diuretics may increase the risk of developing hypokalemia. Medication, therefore, needs to be monitored (Hofer 2003; Szefler 2001).

For all these reasons, easy, cheap, fast, and reliable detection methods are needed for the determination of BUD. Various high-performance liquid chromatography (HPLC) methods utilizing ultraviolet (Hryniewicka, Starczewska, & Gołębiewska, 2019; Peng et al., 2022), fluorescence (Ahmed & Atia, 2019), mass spectrometry, and tandem mass spectrometry (Gazzotti et al., 2016; Rower et al., 2019; Szeitz, Manji, Riggs, Thamboo, & Javer, 2014), as well as gas chromatography-mass spectrometry (Krzek, Czekaj, Rzeszutko, & Jończyk, 2004; Matabosch et al., 2012) and spectrophotometric (Prasad 2006; Sanap, Sisodia, Patil, & Janjale, 2011) methods have been reported for the determination of BUD in human body fluids and pharmaceutical formulations. While these methods offer sufficient sensitivity and selectivity, they are often expensive, time-consuming, and require multi-step processes such as derivatization and pre-separation, along with excessive use of organic solvents. Therefore, there is still demand for new analytical methods that allow selective, accurate, inexpensive, and environmentally friendly determination of BUD from pharmaceutical and biological samples. According to the literature search, no electrochemical method has been reported for the determination of BUD so far. Electrochemical methods, especially voltammetric methods, have been increasing in recent years because they are easy, fast, sensitive, and selective in the determination of drug analysis. The low capacitive current of the differential pulse voltammetry (DPV) greatly increases the sensitivity of the method. Also, the pulse technique with small step sizes in DPV assists in symmetrical sharp voltammetric peaks, which increases the selectivity of the DPV method (Scott & Yu, 2015).

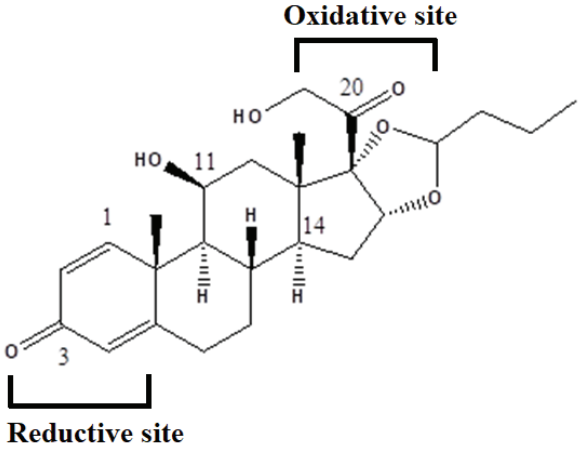

Figure 1. Structure of the budesonide.

Therefore, there is still a need for new analytical methods that offer selective, accurate, inexpensive, and environmentally friendly determination of BUD from pharmaceutical and biological samples. To our knowledge, no electrochemical method for the determination of BUD has been reported so far. In recent years, electrochemical methods, particularly voltammetric methods, have become increasingly popular because of their ease of use, speed, sensitivity, and selectivity in drug analysis. The DPV method is especially advantageous due to its low capacitive current, which significantly enhances its sensitivity. Furthermore, the pulse technique with small step sizes in DPV leads to symmetrical sharp voltammetric peaks, which increases the selectivity of the DPV method (Scott & Yu, 2015)

In this study, the electrochemical behavior of BUD on a simply prepared, unmodified carbon paste electrode (CPE) was investigated, and a fast and accurate DPV method was developed and validated for the determination of BUD in inhalation preparations.

MATERIALS AND METHODS

Apparatus and reagents

Electrochemical measurements were performed using a computer-controlled BASi Epsilon-EC version 2 potentiostat system (Bioanalytical Systems, Inc., West Lafayette, IN) and a three-electrode system with a BASi C-3 Cell Stand. Ag/AgCl (saturated KCl) and platinum wire were used as reference and auxiliary electrodes, respectively. The working electrode was a carbon paste electrode (electrode body BASi CF-1010 carbon paste). pH measurements were employed with a pH ion meter (Mettler Toledo) and pure water was obtained by an ultra-pure water device (Purelab Option).

BUD was kindly provided by DEVA Holding pharmaceutical company. Methanol and sodium hydroxide (NaOH) were purchased from Riedel de Haen. *Hydrochloric acid* (*HCl*), sulfuric acid (H_2SO_4), glacial acetic acid (CH_3COOH), boric acid (H_3BO_3), orthophosphoric acid (H_3PO_4), and potassium chloride (KCl), were used as electrolyte solutions, and were purchased from Merck. All the reagents were of analytical purity. Graphite powder (< 20 µm) was obtained from Sigma-Aldrich. Multi-walled carbon nanotubes (MWCNTs) and graphene were obtained from the Nanografi Company (Turkiye).

BUD was accurately weighed, and 1.0 mg/mL stock solutions were prepared by dissolving it in methanol. The standard solutions at 0.1 µg/mL and 0.01 µg/mL were made by diluting BUD stock solution with methanol. The stock and standard solutions were stored at +4 °C and remained stable for at least one month.

To prepare the 0.1 M phosphate buffer solutions (PBS), phosphoric acid was used to create solutions with pH values between 2.0 and 4.0, while disodium hydrogen phosphate and sodium dihydrogen phosphate were used to create solutions with pH values between 5.0 and 9.0. Britton Robinson (BR) buffer solutions (0.1 M) at pH values between 2 and 9 were prepared using a mixture of phosphoric acid, boric acid, and acetic acid. The pH of the solutions was adjusted to the desired values using NaOH and phosphoric acid solutions.

Preparation of electrode

To obtain a homogeneous carbon paste electrode, 70% graphite and 30% silicone oil were continuously mixed in a small mortar for approximately 10 minutes. The resulting carbon paste was then filled into a hole (0.3 cm deep, 0.7 cm diameter) at the end of a 7.5 cm electrode body. The surface of the paste was smoothed and polished by rotating it on a slippery paper surface. Before measurement, the paste was removed from the electrode cavity and regenerated with fresh carbon paste.

Measurement procedure

A mixture of 0.1 M HCl solution containing 13% KCl and 8% methanol was used in the measurements. The solutions of BUD prepared in 5 different concentrations between 1.65 and 35.35 µg/mL with a final volume of 10 mL were taken into an electrochemical cell. Nitrogen gas was passed for 2 minutes before the measurements. In each series, voltammograms of the empty solutions were recorded first. A new surface was created before each measurement set. differential pulse voltammograms at CPE (against Ag/AgCl) were recorded in the potential range from 0.0 (initial) to -1400 mV(final) (scanning rate =20 mV/s; pulse amplitude =50 mV; pulse width = 50 ms; step E= 4 mV) (Aydoğmuş, Aslan, Yildiz, & Senocak, 2020). Well-defined reduction peak currents at a potential of about -859 mV were recorded in the DPV analysis. In constructing the calibration curve, at least six serial runs for each concentration were performed. A calibration curve was created by plotting the current values measured by DPV against the corresponding concentration, and the regression equation was calculated.

Determination in drug formulation

The plastic PULMICORT® Nebulizer Suspension ampoules containing 1 mg/2 mL of BUD were used to apply the developed DPV method. 0.2 mL of the suspension was taken directly from the ampoules using an automatic pipette to achieve a final concentration of 10 µg/mL and analyzed according to the "measurement procedure" section. Three separate analyses were performed using 2 different suspension ampoules, and the averages were calculated. The concentration of BUD in the drug formulation was determined by substituting the obtained current values in the regression equation prepared for the standard substance.

RESULTS AND DISCUSSION

Selection of the working electrode and electrochemical behavior of BUD

Carbon paste electrodes (CPEs) have high surface activity, and their surface can easily form bonds with various functional groups such as hydrogen, hydroxyl, and carboxyl groups. CPEs are widely used in drug analysis because they are easy and fast to prepare, have low construction costs, can be regenerated, have a wide potential range, have low residual currents, and can contain many electrode materials at the same time. In addition, CPEs can be easily modified to improve their selectivity and sensitivity toward specific analytes (Speranza 2019).

Carbon-based CPE, GCE, 10% graphene-modified CPE, and 10% MWCN-modified CPE electrodes were tested as working electrodes for the sensitive and selective determination of BUD by CV and DPV methods (Figure 2). Initially, CV and DPV analyses were carried out in BR buffers with pH values of pH=2 and pH=7, selected as acidic and basic electrolyte solutions, using a 10 µg/mL standard BUD solution. The electrochemical behavior of BUD at the tested electrode surfaces showed a reduction peak at approximately -900 mV potential in voltammograms taken in the potential range of 0.0 to -1400 mV (Figure 2). The same measurements revealed no peaks in the reverse scan, indicating an irreversible reduction process of the BUD solution. The study found that the reduction peak of BUD was not significantly different between the modified and unmodified CPE electrodes in terms of obtaining a sharp, highly sensitive peak. Therefore, unmodified CPE was chosen as the working electrode since it does not require any additional modification steps and is simpler to use.

Selection of electrolyte solution and pH effect

Cyclic and differential pulse voltammograms were recorded in BR and phosphate buffers (pH 2 - 9), 0.1 M HCl, and 0.1 M H_2SO_4 solutions in the potential range from 0.0 to -1400 mV to select the optimum electrolyte solution in the BUD analysis and investigate the effect of the pH of the buffer solution on the electrochemical process. Since BUD has very low solubility in water, 1.0 mL of methanol was added to each tested electrolyte solution. Depending on the pH, an irreversible reduction peak between -854 and -1088 potentials was obtained in electrolyte solutions. Analysis results showed that BUD solution at CPE gave the highest current peak in 0.1 M HCl solution, very slightly at pH 5, and did not give any reduction peak at pHs above 5.0. Then, in order to increase the intensity of the peak current, 0.1 M KCl *supporting electrolyte* and methanol were added in certain proportions to the 0.1 M HCl solution

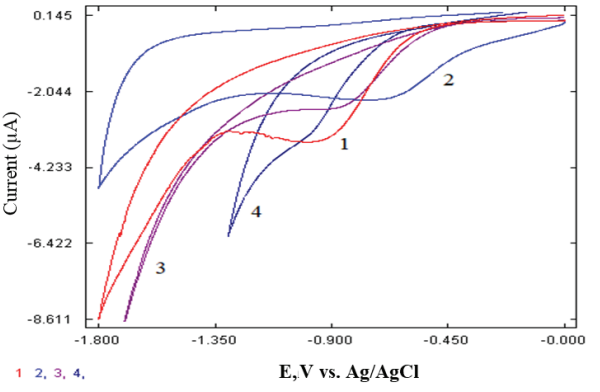

Figure 2. CV voltammograms (100mV/s) of 10 µg/mL BUD with CPE (1), GCE (2), 10% MWCN modified CPE (3), and 10% Graphene modified CPE (4).

and investigated by CV. Experiment results exhibited that the reduction peak of the drug increased significantly in 0.1 M HCl solution containing 13% 0.1 M KCl electrolyte solution and 8% methanol, and further studies were continued with this solution (Figure 3).

Investigation of the pH effect on the peak potential and peak current of BUD was evaluated in the selected 0.1 M HCl solution (pH ~1) containing 0.1 M KCl-methanol, in BR buffer (pH 2-4), and in phosphate buffers (pH 2-5) separately using the CV technique with 10 µg/mL BUD (Figure 3). The shift of the reduction peak potential to a more negative value with increasing pH indicates that the BUD reduction on CPE is pH dependent and protons are involved in the electrode reaction.

The regression equation of the graph drawn between the peak currents (Ip) and the peak potentials (Ep) obtained in the BR buffer system was found to be Ep (pH 1-4) = -52.2pH - 841.5 mV versus Ag/AgCl with a correlation coefficient R^2 = 0.9194. In the phosphate buffer system, the regression equation was found as Ep (pH 1- 4) = [-76.6pH - 772.5] mV versus Ag/AgCl with a correlation coefficient of R^2=0.9904. The negative Ep-pH slopes obtained in two different buffers were found to be -52,2 pH and -76.6 pH, respectively. These slope values are very close to the theoretical 59 mV/pH value at 25 °C, indicating that the number of protons and electrons involved in the reduction reaction is equal (Alimohammadi, Kiani, Imani, Rafii-Tabar, & Sasanpour, 2019).

Effect of scanning rate

The effect of the scanning rate on the reduction peak of the current of BUD (10 µg/mL) on the CPE surface was investigated by CV in the selected solution in the range of 20-200 mV/s. It was observed that the reduction peaks current of BUD increased with increasing scanning rate, and its potential shifted towards a more negative scale (Figure 4). In order to construe whether the electrochemical reaction of the drug is adsorption and diffusion-controlled, calibration curves namely, the logarithm of peak current versus the logarithm of scan rate (log v – log I_p) and peak current versus logarithm square root of the scan rate (I_p - $\upsilon^{1/2}$) values were prepared using the scanning rate and related current values, and the regression equations were log I_p = 1.0412logυ - 1.2531 (R^2 = 0.9887) and I_p(µA) = 1.198 $\upsilon^{1/2}$- 4.6946 (R^2 = 0.9952), respectively (Figures 5). Here,

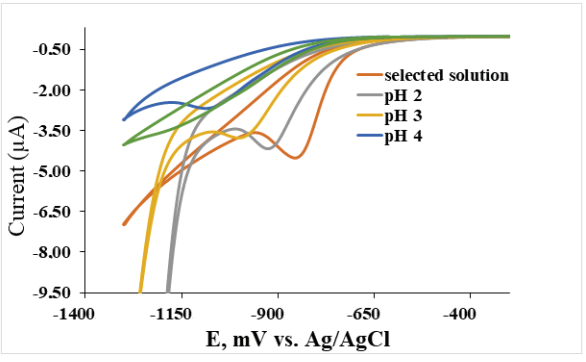

Figure 3. CV-associated voltammograms of 10 µg/mL BUD solution in mixtures of HCl solution (pH~1) and in phosphate buffers of pH 2.0-5.0.

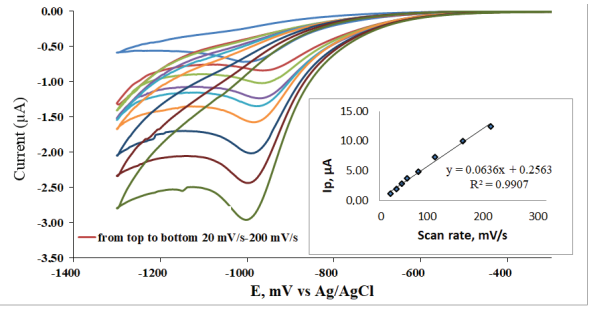

Figure 4. CV of 10 µg/mL BUD at CPE in selected solution in various scan rates. From top to bottom: blank, 20, 30, 40, 50, 70, 100, 150, and 200 mV/s. Inset: Plot of Ip vs. υ.

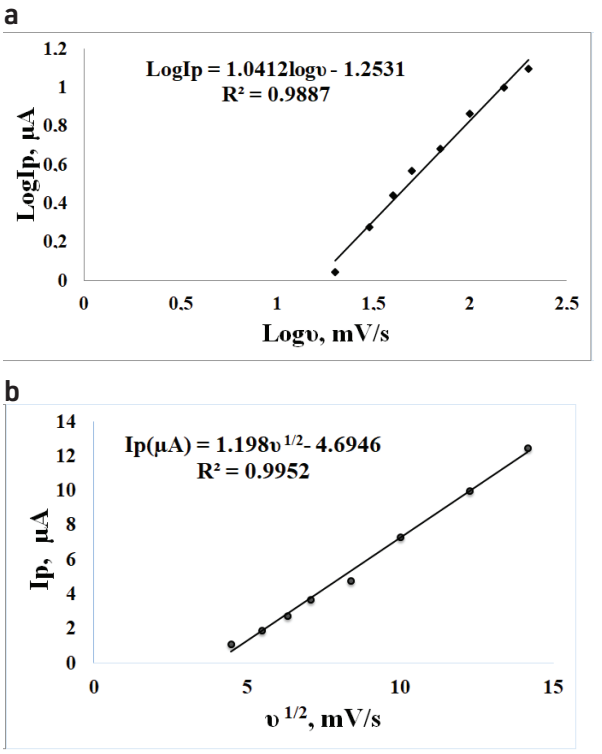

Figure 5. Curve of change of logarithm of peak current versus logarithm of scan rates (logIp - log υ)(a); the curve of variation of the peak current versus the square root of the scan rates (Ip - $\upsilon^{1/2}$) (b).

the slopes of log v – log I_p and I_p - $v^{1/2}$ are between 1.04 and 1.19, indicating that the electrochemical reaction is strongly adsorption controlled. For the irreversible process, Ep can be defined by the Laviron equation (Laviron 1979) given below:

Ep (mV/s) = E^0 + (2.303RT/ αnF) log (RTk^0/ αnF) + (2.303RT/ αnF) logυ (mV/s),

Where α is the electron transfer coefficient, k^0 is the standard heterogeneous rate constant, υ is the scan rate, n is the number of electrons transferred per molecule, Ep is the peak potential and E^0 is the formal potential that can be found from the intercept of the Ep vs scan rate (υ) curve, by extrapolating to the vertical axis at υ = 0. (T = 298 K, R = 8.314 J/K mol and F = 96.485 C/mol) (Aydoğmuş, Aslan, Yildiz, & Senocak, 2020). The regression equation of the linear curve between the peak

potential and the logarithm of the scan rate was obtained as Ep(mV) = -42.385logυ(mV/s) - 905.45 (R^2 = 0.9718). The value of αn is calculated from the slope of Ep against the log v plot by Laviron's equation (Laviron 1979). It was calculated as 1.39 (slope=2.303RT/αnF). In the non-reversible electrode process, α is considered to be between 0.3 and 0.7 (Bond 1980; Guidelli et al., 2014). Assuming α is 0.7, the number of electrons in the reaction was found to be 1.98≈2.

In addition, in the pH-dependent BUD/CPE reaction, the n value was calculated from the equation [E_p-$E_{p/2}$ = (47.7/ αn) mV, 25°C], where E_p is the peak potential and $E_{p/2}$ is the half-wave potential where the current is half of its peak current using CV at 100 mV/s [Sartori, Clausen, Pires, & Salamanca-Neto, 2017). From this equation, αn was calculated as 1.41 and the n value was found to be 2.01≈2 when α is taken as 0.7. These two n-value calculations confirmed each other and were in good agreement with the values reported in the reduction reaction of some corticosteroids (Alimohammadi et al., 2019; Hammam 2007; Vedhi, Eswar, Prabu, & Manisankar, 2008).

Possible reaction mechanism

Although there is no research on the electrochemical properties of BUD in the literature, there are some voltammetry methods developed for the determination of corticosteroid drugs with the molecular skeleton of BUD, such as betamethasone (Alimohammadi et al., 2019; Ghoneim, El-Attar & Ghoneim, 2009; Goyal, Chatterjee & Rana, 2010), triamcinolone acetonide (Goyal, Gupta & Chatterjee, 2009; Hammam 2007; Vedhi et al., 2008), and prednisolone (Rezaei & Mirahmadi-Zare, 2011). In these studies, glassy carbon electrode (GCE) (Vedhi et al., 2008), modified GCE (Alimohammadi et al., 2019), edge-plane pyrolytic graphite electrode (Goyal, Chatterjee & Rana, 2010; Goyal, Gupta & Chatterjee, 2009), hanging mercury drop electrode (HMDE) (Ghoneim, El-Attar & Ghoneim 2009; Hammam 2007), and molecularly imprinted polymer-multiwalled carbon nanotube paste electrode (Rezaei & Mirahmadi-Zare, 2011) have been used as working electrodes.

In general, corticosteroids have two electroactive sites that act separately as reducing and oxidative (Figure 1). Nevertheless, in a few studies, corticosteroids have been found to be reduced from carbonyl groups at unconjugated C-20, which are activated by neighboring hydroxyl groups at C-17 and C-21 (Alimohammadi et al., 2019; Goyal, 2009). In the other studies, it was shown that the C-3 carbonyl group adjacent to the double bonds in drug molecules was reduced more easily than the C-20 carbonyl group. Also, some studies have shown that these molecules can be oxidized depending on the electrode and pH (Rezaei & Mirahmadi-Zare, 2011). For the reduction process, studies exhibited that two hydrogens (+2H) and two electrons (2e-) were added to the C=O groups in drugs, which is consistent with the data found for BUD in the present work. Considering the literature and data from the currently proposed DPV study, it is predicted that BUD is reduced by adding +2H and 2e- to a carbonyl group at C-3 or C-20 under selected acidic analysis conditions.

Method validation

The developed method has been validated with regard to linearity, linear range, detection and detection limits, accuracy, selectivity, and stability.

Linearity and sensitivity

The determination of BUD was performed on a simple, unmodified CP electrode at about -859 mV (against Ag/AgCl) with the DPV method, which is much more sensitive and has a lower background current than CV. The calibration curve was obtained by plotting the peak currents of the BUD against the concentration under the determined optimum conditions. The calibration curve for BUD was determined to be linear between 1.65 and 35.35 µg/mL and the corresponding regression equation was calculated as Ip (µA) = 0.4957C(µg/mL) + 7.3563 (Figure 6). The correlation coefficient (R^2) value of this equation was found to be 0.9999, indicating perfect linearity (Table 1).

The limit of detection (LOD) and limit of quantification (LOQ) values were calculated using the equations LOD = 3.3 SD/m and LOQ = 10 SD/m) (Guideline, ICH Harmonised Tripartite

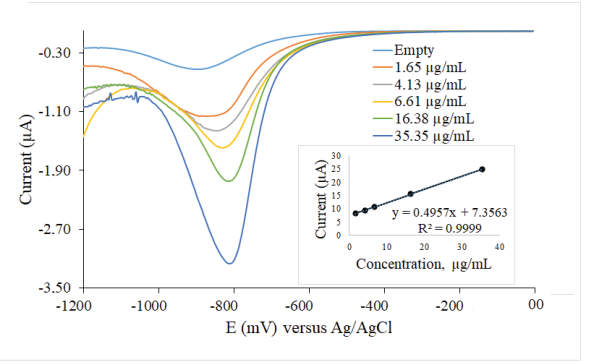

Figure 6. DPV of BUD at various concentrations (blank solution, 1.65, 4.13, 6.38, 16.38 and 35.35 µg/mL). Inset: Calibration curve for BUD from these data.

Table 1. Statistical parameters for analysis of BUD by DPV in standard solution.

Parameter	Value
Measured potential, mV	- 859
Linearity range, µg/mL[a]	1.65-35.35
Regression equation, y = mc + b	I_p (µA) = 0.4957C (µg/mL) + 7.3563
Slope	0.4957
Intercept	7.3563
Coefficient of determination (R^2)	0.9999
SD[b] of m	0.003
SD of b	0.078
LOD, µg/mL	0.52
LOQ, µg/mL	1.57

[a] Average of six determinations for the drug in standard solution (n = 6); 0.1 M HCl containing 13% KCl and 8% methanol medium; working electrode: CPE; potential window: between 0.0 and -1400 mV (amplitude: 50 mV, pulse width: 50 ms and scan speed: 20 mV/s.
[b] Standard deviation.

2005), respectively. Here, SD and m were the standard deviation of the intercept and the slope of the calibration curve, respectively. The LOD and LOQ of BUD were 0.52 µg/mL and 1.57 µg/mL, respectively (Table 1).

Accuracy and precision

In order to evaluate the intraday and interday accuracy and precision of the developed method, three different concentrations of BUD solution (3.0, 10.0, 20.0 µg/mL) were tested under selected conditions. Analyses were performed on the same day (intraday) and on five different days (interday) within two weeks. Five separate analyses were performed for each concentration. The concentration was found by substituting the peak current values in the regression equation obtained for the standard BUD solution. Accuracy was given as the percent recovery values of the concentrations found, while precision was expressed as the relative standard deviation (%RSD) from the determined concentrations. The mean intraday and interday recovery values between 100.38% and 100.05% (SD = 0.18 - 0.09) showed that the accuracy of the method was excellent. The mean relative standard deviation (%RSD) values of the experiments performed intraday and interday were found to be between 1.31-3.18% and 0.33-3.38%, respectively (Table 2).

Selectivity and effect of excipients

The prepared electrolyte solution was analyzed by CV and DPV under conditions determined in the presence and absence of BUD and drug samples. The blank solution gave a current peak at the reducing potential of the drug well below the LOD, indicating that the method is selective. In addition, the potential interaction of excipients conventionally found in pharmaceutical preparations or biological fluids in the determination of BUD with the developed DPV method was investigated. The substances that may cause interference were added 100 times to the BUD solution (30 µg/mL) and it was analyzed whether it caused interference with the developed DPV method under the optimized same analysis conditions. The currents were recorded by making 3 readings before and after adding the substance whose interference effect was examined, and these currents were compared and the % current difference values were calculated separately for each substance. Results given in Table 3 exhibited that a hundred-fold excess of hydroxypropyl methylcellulose, citric acid, lactose, saccharose, and Na^+ did not show any significant interaction in DPV current response. However, ascorbic acid and glucose negatively affected the DPV current response by 14.76% and 7.2%, respectively.

Electrode stability and reproducibility

The stability of the CPE was investigated using three freshly prepared CPEs to determine BUD (10 µg/mL) using CV. These electrodes showed good stability, with a relative standard deviation of 5.6% as a result of voltammograms recorded once a week for 1 month. Prepared CPE was stored in tightly sealed glass containers at 25°C and stayed stable for at least 3 months.

Determination of BUD in ampoules of inhalation suspension

The applicability of the developed DPV method was tested to determine BUD in nebulizer suspension plastic ampoules containing 1 mg/2 mL of Pulmicort Respules (BUD inhalation suspension). Samples corresponding to 10 µg/mL were taken and studied as described in the "measurement procedure" sections (Figure 7). Sample contents were calculated using the measurement curve equation prepared for the standard substance. The recovery was between 91.1% and 100.7% (mean= 98,47),

Table 2. Inter-day and intra-day and accuracy and precision of BUD determination by DPV method (*n* = 5).

	Intraday		Interday	
Concentration (µg/mL)	**Recovery (%)[a] ± SD[b]**	**% RSD[b]**	**Recovery (%)[a] ± SD[b]**	**% RSD[b]**
3.0	100.70 ± 0.10	3.18	99.33 ± 0.10	3.38
10.0	99.80 ± 0.13	1.31	100.30 ± 0.11	1.08
20.0	100.65 ± 0.31	1.56	100.51 ± 0.07	0.33
Mean	100.38 ± 0.18	0.34	100.05± 0.09	1.60

[a]Mean of five determinations (n = 5). [b]SD is standard deviation and RSD is relative standard deviation.

Table 3. Influence of potential excipients on the voltammetric response of 30 µg/mL budesonide.

Excipients	Ip of BUD in the absence of Excipient	Ip of BUD in the presence of Excipient	Signal change (%)
Na+	22.01	22.49	2.19
Glucose	21.61	20.05	-7.2
Ascorbic acid	21.48	18.31	-14.76
Citric acid	21.51	20.90	-2.86
Saccharose	20.64	20.32	-1.52
Lactose	20.89	20.43	-2.2
Hydroxypropyl methylcellulose	21.62	21.52	-0.47

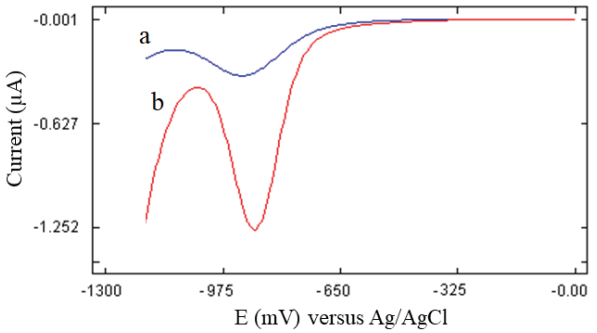

Figure 7. Empty electrolyte solution (a) and BUD inhalation suspension (10 µg/mL) (b).

and the RSDs were found to be 3.73% on average. This shows that the developed method is sensitive and selective enough.

Comparison of the proposed method with some reported methods

In this study, the determination of BUD was performed by the DPV method using unmodified carbon paste electrodes. Various HPLC and spectrophotometric methods have been published for the determination of BUD (Kolsure, Daniel, & Bhat, 2021). However, an electrochemical method has not been reported. Table 4 presents a comparison of some of these methods for determining substance concentration in terms of linear range and LOD with previous findings reported in the literature. The current approach is simple and does not require the use of pretreatment procedures or time- and chemical-consuming reactions such as derivatization. In addition, although the sensitivity and separation power is high in analyses with HPLC or HPLC-mass spectrometry instruments, they are expensive, often requiring very time-consuming processes such as derivatization and requiring the use of large amounts of solvents. However, as can be seen in the Table, the newly developed voltammetric method provides superiority to some HPLC studies in terms of both linear range and sensitivity. Compared with previously published studies on the determination of BUD, the current voltammetric procedure offers a sufficiently wide linear range for drug determination. Working with unmodified CPE is a very inexpensive, simple, fast, and selective method. These evaluations showed that the method developed in this study will be an important alternative to other published methods in terms of a wide linear dynamic range, relatively low detection limit, selectivity, and excellent reproducibility in the determination of the substance.

CONCLUSION

In the study, the electrochemical properties of BUD were investigated and developed a novel, efficient, and reliable new DPV method for its determination in pharmaceutical samples. The effect of scanning rate and pH were investigated to obtain the highest response for DPV analysis of BUD. Electrochemical studies show that the reaction of BUD on CPE was irreversible and adsorption-controlled, involving the transport of two protons and two electrons. The experiments were conducted using 0.1 M HCl solution containing 13% KCl and 8% methanol as the supporting electrolyte with a pH of about 1.0 for CPE. The dynamic linear range was between 1.65 and 35.35 µg/mL with

Table 4. Comparison of linear range and detection limits for BUD with published methods.

Method	Analysis Conditions	Linear range (µg/mL)	LOD (µg/mL)	Application	Ref.
HPLC-UV	C18, 0.05 M Sodium acetate buffer/ acetonitrile (40:60, v/v)	0.5- 50.0	0.187	Inhaler medicine	Salem et al., 2017
HPLC-UV	Hypersil C18, Ethanol/acetonitrile phosphate buffer pH 3.4; 25.6mM) (2:30:68, v/v/v)	2.5 - 25.0	0.30	Pulmicort Turbuhaler	Hou, Hindle, & Byron,2001
HPLC-UV	Kromasil C8 (150 mm x 4.6 mm) Acetonitrile/ phosphate buffer (pH 3.2-0.025 M) (55:45 v/v)	1-50	0.1	Pharmaceutical form	Gupta, & Bhargava,2006
HPLC-UV	Bondapak RP- C18 Acetonitrile/ monobasic potassium phosphate (55:45, pH 3.2) (28)	1-20	0.05	Drug formulation	Varshosaz et al., 2011
UV Spectrophotometry	pH 6.8 buffer	1.4 - 25	0.01	Drug formulation	Bharti et al., 2011
DPV on CPE	0.1 M HCl solution containing 13% KCl and 8% methanol	1.65 - 35.35	0.52	Inhalation preparations	Current study

a low LOD value of 0.52 µg/mL.

The use of unmodified CPE as the working electrode has made the method more accessible and cost-effective, while the wide linear range and low detection limit make it suitable for both quality control and research applications. The validation of the method has also shown its accuracy, selectivity, and stability, making it a promising alternative to existing analytical methods for BUD determination. In addition, the developed voltammetric can be considered a green chemistry approach to drug analysis as it avoids the use of hazardous reagents or solvents that may pose risks to human health or the environment. The use of unmodified CPE is also advantageous in terms of cost-effectiveness and simplicity, making it a promising alternative to other more complex and expensive methods for routine analysis of BUD.

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