Electrochemical oxidation of provitamin B₅, D-panthenol and its analysis in spiked human urine

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Abstract

Background: The behavior of biomolecules and the advancements in the electrochemical techniques play a tremendous role in the development of voltammetric sensors. Redox reactions of biologically active molecules can be studied by using different voltammetry techniques which guide us to understand the metabolic fact of the targeted drug. In the present work, we describe the electrochemical oxidation of D-panthenol (DP) by using a versatile glassy carbon electrode (GCE).

Methods: Experimental section was carried out by using cyclic voltammetry and square wave voltammetry.

Results: Under the optimized conditions (pH 4.2), the square wave voltammetric peak current of D-panthenol increased linearly with its concentration. The detection limit was found to be 5.0 × 10⁻⁷ M. The number of protons and electrons involved in the oxidation process were calculated. The heterogeneous rate constant was found to be (3.67 × 10⁻³ s⁻¹).

Conclusions: The method detects the trace level of the analyte with low detection limit which imparts the development of a sensor for selective and sensitive determination of D-panthenol. This sensor was successfully applied to determine the D-panthenol in spiked urine samples.

Keywords: D-panthenol, Voltammetry, Glassy carbon electrode, Electroanalysis, Oxidation

Background

D-panthenol (2, 4-Dihydroxy-N-(3-hydroxypropyl)-3, 3-dimethylbutanamide) is the more stable alcohol form of pantothenic acid (Vitamin B₅). Therefore, this cosmetic drug is known as provitamin B₅. The structure of D-panthenol (DP) is shown in Scheme 1. DP has been used for years in hair-care products like shampoos and conditioners, because its activity as a humectant increases the water content of hair and improves its elasticity. It coats the hair and seals its surface, lubricating the hair shaft and giving it a shiny appearance. DP is inactive but is readily converted to pantothenic acid in the skin. Pantothenic acid is then incorporated as an important component in the energy cycle of the cell. Panthenol can also attract water into the upper layer of the skin and is thus effective as a moisturizer and softener. Moreover, Panthenol can promote epithelization thereby enhancing the regeneration of the skin. DP is also used as an anti-aging drug.

Voltammetric methods gathered much attention of researchers owing to their rapidity of analysis, no requirement of sample pretreatment, fairly high sensitivity, and inexpensive instrumentation. In addition, application of electroanalytical techniques includes the determination of electrode mechanisms [Malode et al. 2012; Bukkitgar et al. 2015]. The performance of the voltammetric techniques is purely affected by the characteristic feature of the working electrode material such as chemical and physical properties. An overview of the improvement in electroanalytical chemistry demonstrates that solid metal electrodes represent the most rapidly rising class of electrodes [Shetti et al. 2009; Jorge et al. 2010; Wudarska et al. 2013]. Among all those electrodes, the utilization of glassy carbon electrode for the electrochemical measurements has increased in recent years as they provide good
sensitivity, negligible porosity, and superior mechanical rigidity [Nayak and Shetti 2016]. Oxidation and reduction property of drugs can give insight into its metabolic providence or their in vivo redox process or pharmaceutical activity [Kumar et al. 2008; Diculescu et al. 2006].

Panthenol has been determined by chromatographic methods [Bui-Nguyen 1984; Kulikov and Zinchenko 2007; Prosser and Shreppad 1969; Nagamallika and Arunadevi 2013]; thin-layer chromatographic determination of panthenol with spectro-fluorometric detection [Shehat Mostafa et al. 2004], fluorimetry, and colorimetric methods for the detection of panthenol [Shehat Mostafa et al. 2002].

A review of the literature exposes that, till date, there is only one report on the electrochemical behavior of DP. In earlier work [Wang and Tseng 2001], authors used carbon paste electrode (CPE), cobalt oxide-modified carbon paste electrode (CoO/CPE) and cadmium oxide-modified carbon paste electrode (CdO/CPE). Looking at the oxidation mechanism and the linearity range, we have undertaken this work at glassy carbon electrode. The pharmaceutical and cosmological importance of D-panthenol necessitates a sensitive method to be developed. The plan of the present study is to establish the suitable experimental conditions to explore the oxidation mechanism of D-panthenol and its determination in spiked human urine samples using cyclic, square wave voltammetric techniques.

Methods

Apparatus and chemicals

Electrochemical analyzer (CHI Company, D630, USA) was used to study the electrochemical activities of the drug under investigation at an ambient temperature of 25 ± 0.1 °C. A three-electrode system consisting of GCE as working electrode, platinum wire as counter electrode, and Ag/AgCl (3 M KCl) as reference electrode were used in a 10-ml single compartment. D-panthenol (Sigma-Aldrich, USA) was used to prepare 1.0 mM stock solution in double-distilled water. The phosphate buffer solutions ranging 3.0–11.2 pH with ionic strength 0.2 M were prepared according to literature [Christian and Purdy 1962; Bukkitgar and Shetti 2015], and pH of the solutions was measured by pH meter (Elico Ltd., LI120, India). Double-distilled water, analytical-grade chemicals, and reagents without further purification were used throughout the experiments.

Pretreatment of electrode

Prior to use, the GCE was carefully polished using a 0.3-μm Al2O3 slurry on a polishing cloth before each experiment. The GCE was first activated in phosphate buffer (pH 4.2) by cyclic voltammetric sweeps between −2.0 and 3.0 V until stable cyclic voltammograms were obtained. Then, electrodes were transferred into another 10 ml of phosphate buffer (pH 4.2) containing proper amount of DP.

The Randles-Sevcik equation was used to calculate the active area of the electrode using cyclic voltammetric technique and K3Fe (CN)6 1.0 mM as a probe at different scan rates in 0.1 M KCl as supporting electrolyte [Nayak et al. 2015]. At T = 298 K and for a reversible process, the equation is as follows:

\[
I_p = \left(2.69 \times 10^5\right) n^{3/2} A_0 D_R^{1/2} v^{1/2} C_0.
\]

In Eq. (1), \(I_p\) refers to the anodic peak current and \(n\) is the number of electrons transferred during the electrode reaction = 1. \(A_0\) is the surface area of the electrode, \(D_R\) is the diffusion coefficient, i.e., \(7.6 \times 10^{-6}\) cm² s⁻¹ [Adams 1969; Gosser 1994], \(v\) is the scan rate, and \(C_0\) is the concentration of K3Fe (CN)6. From the slope of the plot of \(I_p\) versus \(v^{1/2}\), the area of the electrode surface was calculated to be 0.04 cm².

Preparation of spiked human urine sample

Human urine was obtained from four healthy volunteers of similar sex and age. Aliquots were centrifuged at 7000 rpm for 5 min at room temperature (25 ± 0.1 °C). These urine samples were analyzed immediately or they were stored at low temperature until analysis.

Results and discussion

Voltammetric behavior of D-panthenol

In order to understand the voltammetric behavior of DP, cyclic voltammetry method was utilized. Between the pH ranges 3.0 and 11.2, the electrochemical behavior was studied, and one well-defined irreversible oxidation peak at pH 4.2 was observed. In Fig. 1, voltammetric behavior of DP in phosphate buffer solution (pH = 4.2, \(I = 0.2\)) is represented, curve (a) anodic peak corresponding to DP oxidation appeared at 0.369 V and (b) corresponds to the buffer solution. There was no peak observed on the reverse scan; therefore, electrode process is supposed to be irreversible. Since successive cyclic voltammogram showed decrease in the peak current due to adsorption of DP or its oxidation product, the oxidation peak corresponding to first sweep was only recorded.
Fig. 1 Cyclic voltammograms of 1.0 mM DP on glassy carbon electrode in pH 4.2, phosphate buffer (I = 0.2 M) at 0.05 Vs\(^{-1}\) (a) DP, (b) blank.

Fig. 2 Cyclic voltammograms obtained for 1.0 mM DP in buffer solution at (a) pH 3.0, (b) pH 4.2, (c) pH 5.0, (d) pH 6.0, (e) pH 7.0, (f) pH 8.0, and (g) pH 9.2. A Influence of pH on the peak potential \(E_p/V\) of DP. B Variation of peak currents \(I_p/\mu A\) of DP with pH.
Effect of solution pH
Proton is always involved in the electrochemical reaction of organic molecules and exerts significant impact on the reaction rate. By optimizing the pH conditions, sharper response accompanied with higher sensitivity can be obtained. In earlier work [Wang and Tseng 2001], authors studied different buffer solutions of different pH solutions and they have selected phosphate buffer of pH 6.08. Therefore, we studied phosphate buffer solution over the pH range 3.0–11.2 for the oxidation of DP (Fig. 2). It was observed that, as the pH of the supporting electrolyte increases, the peak potential shifted to less positive values up to pH 9.2 (Fig. 2a). From the plot, it is evident that there are three slopes in the range of 3.0 to 9.2 pH.

\[
E_P = 0.062 \text{pH} + 0.657; \quad R^2 = 0.891 \text{ (pH = 3.0–5.0)} \\
E_P = 0.020 \text{pH} + 0.474; \quad R^2 = 0.843 \text{ (pH = 5.0–8.0)} \\
E_P = 0.049 \text{pH} + 0.686; \quad R^2 = 0.990 \text{ (pH = 7.0–9.2)}
\]

The slope of \(E_P\) versus pH being close to the theoretical value 0.059 suggested that the number of electrons transferred is equal to that of hydrogen ions taking part in the electrode reaction [Bukkitgar et al. 2016]. From the plot of \(I_P\) versus pH (Fig. 2b), it is clear that the best result with respect to sensitivity and sharper response was obtained at pH = 4.2, hence it was selected for further work. The peak current depends on the deprotonation and protonation form of the electro active species in electrochemical cell. Further, the magnitude of current is directly proportional to rate of the electrochemical reaction. Hence, it is apparently concluded that the oxidation of DP is very high at pH 4.2.

Scan rate variation
The relationship between peak current and scan rate gives beneficial information about electrochemical mechanism. At different scan rates, the electrochemical behavior

![Fig. 3 Cyclic voltammograms of 1.0 mM DP in buffer solution of pH 4.2 (I = 0.2 M) at scan rates of (a) blank, (b) 0.006, (c) 0.02, (d) 0.03, (e) 0.05, (f) 0.07, (g) 0.09, and (h) 0.1 Vs⁻¹. A Dependence of peak current \(I_P/\mu A\) on the scan rate \(u/Vs⁻¹\). B Plot of logarithm of peak current \(\log I_P/\mu A\) versus logarithm of scan rate \(\log u/Vs⁻¹\). C Plot of variation of peak potential \(E_P/V\) with logarithm of scan rate \(\log u/Vs⁻¹\).]
of DP was studied by using cyclic voltammetric technique (Fig. 3). The linearity of the peak intensity $I_p$ ($\mu$A) upon the scan rate $\nu$ (Vs$^{-1}$) (Fig. 3a) suggests that the electrode reaction is a surface-controlled process [Gosser 1994; Brycht et al. 2015].

$I_p = 50.06 \nu + 1.238; \ R^2 = 0.980$

From the plot of logarithm of anodic peak current versus logarithm of scan rate, a straight line was obtained with a slope of 0.54 (Fig. 3b) which is closer to the theoretical value of 0.5 for a purely diffusion-controlled process [Gosser 1994; Brycht et al. 2015]. The dependence log $I_p$ versus log $\nu$ is used as criterion to determine whether the reaction is reversible or irreversible, based on the shift in the peak. In our study, the forward peak was shifted, indicating an irreversible electrode process. Gosser also quoted that in an irreversible system, both the electrode kinetics and chemical kinetics are slow [Demir et al. 2014, 1997].

log $I_p = 0.545 \log \nu + 1.310; \ R^2 = 0.986$

As the scan rate increases, the peak potential shifted to more positive values, which also confirms the irreversibility of the oxidation process, and a linear relationship between peak potential and logarithm of scan rate (Fig. 3c) can be expressed by the following equation:

$$E_p = 0.057 \log \nu + 0.387; \ R^2 = 0.957$$

For an irreversible electrode process, according to Laviron [Laviron 1979], $E_p$ is calculated by the following equation;

$$E_p = E^0 + \frac{2.303RT}{anF} \log \frac{RTk^0}{anF} + \frac{2.303RT}{anF} \log \nu$$

(2)

Where $a$ refers to the transfer coefficient, $k^0$ is the standard heterogeneous rate constant of the reaction, $n$ is the number of electrons transferred, $\nu$ the scan rate and $E^0$ is the formal redox potential. Other symbols have their usual meaning. “an” value is calculated from the difference between the peak potential ($E_p$) and half wave potential ($E_{p/2}$) using Eq. (3). Taking $T = 298 \text{ K}, \ R = 8.314 \text{ JK}^{-1} \text{ mol}^{-1}$, and $F = 96480 \text{ C mol}^{-1}$, the value of an was calculated to be 1.037. According to Bard and Faulkner, [Bard and Bard 2004], $\alpha$ can be calculated as

$$\Delta E_p = E_p - E_{p/2} = \frac{47.7}{an} \text{ mV},$$

(3)

where $E_{p/2}$ is the potential where the current is at half the peak value. From the above equation, the value of $\alpha$ was to be 0.56. The number of electrons transferred in electrode oxidation was calculated to be 2.07 $\approx$ 2. Hence, DP may be assumed to undergo a two-proton and two-electron transfer in the electrode reaction. The $E^0$ value is known, i.e., 0.269. It is obtained from the intercept of peak potential ($E_p$) versus scan rate ($\nu$) curve by extrapolating to the vertical axis at $\nu = 0$ [Yunhua et al. 2004]. From the intercept of $E_p$ versus log $\nu$, $k^0$ was calculated to be $3.67 \times 10^3 \text{ s}^{-1}$.

**Oxidation mechanism**

The authors [Wang and Tseng 2001] has undertaken the electrochemical behavior of D-panthenol and proposed

![Scheme 2](image-url)
the reaction mechanism. A two-electron reduction was used to form α-amino alcohol, followed by elimination of water molecule in an acid-catalyzed process to form iminium ion, and finally, they proposed a protonated form of the amine as a product. But, in the present irreversible system, the results recommend a two-electron and two-proton transfer process in the reaction mechanism of oxidation of D-panthenol. From the results obtained above, the following mechanism can be presented for the oxidation of DP at the surface of GCE. The reaction mechanism is given in Scheme 2.

Concentration variation
In view of the fact that square wave voltammetry technique gave sharper and well-defined peaks even at low concentration of analyte, it was preferred to develop a voltammetric technique for the determination of DP in trace level. The phosphate buffer solution of 4.2 pH was selected as the supporting electrolyte for the quantitative determination of DP. Square wave voltammograms obtained with increasing concentration of DP (Fig. 4). The linear equation was expressed as follows:

\[ I_p(\mu A) = 2.504C + 0.887 \quad (R^2 = 0.979, \ C \text{ is in } \mu M). \]

The adsorption of DP or its oxidation product on the electrode surface diverge the linearity for more concentrated solution [Shetti et al. 2012]. Five successive determinations were recorded to develop the statistical data related to calibration curve (Table 1). Limit of detection (LOD) and limit of quantification (LOQ) were been calculated to be \( 5.0 \times 10^{-7} \) and \( 17.0 \times 10^{-7} \) M, using following equation, respectively [Swatz and Krull 1997], which shows good results as compared with previously reported analytical methods.

\[ \text{LOD} = 3S/m \quad ; \quad \text{LOQ} = 10S/m \]

\( S \) is the standard deviation of the peak currents and \( m \) is the slope of calibration curve. The calculated LOD and LOQ values were better as compared with the previously reported work Table 2.

**Table 1** Characteristics of D-panthenol calibration plot using square wave voltammetry at glassy carbon electrode

| Analytical parameters | Values |
|-----------------------|--------|
| Linearity range (M)   | \(1.0 \times 10^{-3}\)–\(8.0 \times 10^{-6}\) |
| Slope of the calibration plot (\(\mu A\ M^{-1}\)) | 2.501 |
| Intercept (\(\mu A\)) | 0.887 |
| Correlation coefficient (\(r\)) | 0.979 |
| RSD of slope (%)      | 0.052 |
| RSD of intercept (%)  | 0.488 |
| Number of data points | 90 |
| LOD (M)               | \(5.0 \times 10^{-7}\) |
| LOQ (M)               | \(17.0 \times 10^{-7}\) |
| Repeatability (RSD %) | 0.182 |
| Reproducibility (RSD %) | 0.084 |

*Average of five determinations*
Table 2 Comparison of linearity range and detection limits of D-panthenol by different reported methods

| Method                     | Linearity range (µg/mL) | LOQ (µg/mL) | Reference                           |
|----------------------------|-------------------------|-------------|-------------------------------------|
| Reversed phase HPLC method | 13–130                  | 2.10        | Kulikov and Zinchenko 2007          |
| Spectro-fluorometric method| 0.3–3.0                 | 1.95        | Shehat Mostafa et al. 2004          |
| Colorimetric method        | 50–500                  | 10          | Shehat Mostafa et al. 2002          |
| Differential pulse voltammetry | 2.0–40                | –           | Wang and Tseng 2001                 |
| Square wave voltammetry    | 1.0–0.008               | 0.5         | Present work                        |

Effect of interfering substances

To evaluate the effect of possible co-existing species on the determination of DP, interference study was carried out. Thus, some biological molecules like citric acid and gum acacia were chosen with concentrated amounts. The tolerance limit was taken as the maximum concentration of the foreign substances, which caused an approximately ±5 % relative error in the determination. Depending on the considerable influence of excipients on the height of the peak current and potentials of voltammograms with reference to only analyte (0.1 mM DP) voltammogram, this study was undertaken. Table 3 shows that 100-fold of citric acid, gum acacia, oxalic acid, sucrose, and urea did not interfere with the voltammetric signal of DP.

Detection of DP in spiked urine samples

The urine analysis was carried out by using square wave voltammetric technique. The recoveries from urine were measured by spiking drug-free urine with known amounts of DP. The calibration graph was used to determine the spiked DP in urine samples. Table 4 illustrates the recovery studies of urine samples, and it was detected in the range from 97.1 to 99.3 % with relative standard deviation (RSD) of 1.13 %.

Table 3 Influence of potential interferents on the voltammetric response of 1.0 × 10⁻⁴ M D-panthenol

| Interferents   | Concentration | Signal change (%) |
|----------------|---------------|-------------------|
| Oxalic acid    | 0.1           | 0.57              |
| Citric acid    | 0.1           | 2.36              |
| Glucose        | 0.1           | 2.47              |
| Lactose        | 0.1           | -1.60             |
| Dextrose       | 0.1           | 2.05              |
| Sucrose        | 0.1           | -0.85             |
| Gum acacia     | 0.1           | -2.32             |
| Starch         | 0.1           | 1.95              |

Table 4 Application of square wave voltammetry for the determination of D-panthenol in spiked human urine

| Sample         | Declared (µg/mL) | Detected* (µg/mL) | Recovery (%) | RSD (%) |
|----------------|------------------|-------------------|--------------|---------|
| Urine sample 1 | 0.1 × 10⁻³       | 0.099 × 10⁻³      | 99.3         | 1.11    |
| Urine sample 2 | 0.2 × 10⁻⁴       | 0.197 × 10⁻⁴      | 98.5         | 1.13    |
| Urine sample 3 | 0.5 × 10⁻⁴       | 0.485 × 10⁻⁴      | 97.1         | 1.14    |

* Average of five determinations

Conclusions

The developed voltammetric method provides a simple and quick tool for the direct determination of provitamin B₅ using glassy carbon electrode. The investigation clearly reveals that the electrochemical oxidation of D-panthenol was found to be irreversible and diffusion-controlled, and it involves a two-electron two-proton electrode mechanism. From the results obtained, a probable electrochemical mechanism was proposed. A square wave voltammetric technique was developed for the determination of D-panthenol in human urine samples quantitatively. As compared to other methods, the proposed method offered an improvement in simplicity and accuracy.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

DSN and NPS planned the experiment and outlined the manuscript. NPS is the corresponding author. Experimental segment and calculation part was carried out by DSN. Both authors have read and approved the final manuscript.

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