

Investigation of Electron Transfer Reaction Between Diphenylbenzidine and Ascorbic Acid

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The kinetics of the reduction of diphenylbenzidine by ascorbic acid were investigated in the pH range 3.5 to 5.0. Diphenylbenzidine was prepared by the oxidation of diphenylamine with cerium(IV) sulfate tetra hydrate. The order of the reaction with respect to each of the reactants, i.e ascorbic acid (ASC), diphenylbenzidine (DPB) and H^+ , was found to be first.

The following rate law is recommended:

$$\text{Rate} = \{k_1 [C_{12}H_{10}N_2C_{12}H_{10}] + k_2K [C_{12}H_{10}N_2C_{12}H_{10}][H^+]\}[C_6H_8O_6]$$

where k_1 and k_2 have the values of $2.0 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ and $6.58 \times 10^3 \text{ dm}^3 \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$, respectively. K is the protonation constant of DPB, having a value of $476 \text{ dm}^3 \text{ mol}^{-1}$. A possible mechanism for the reaction is also proposed.

Introduction

Ascorbic acid, known as vitamin C, is found naturally in a wide variety of plants and animals. It acts as a redox reagent in many biochemical processes¹. It has a relatively low melting point as its intermolecular bonds take less energy or heat to break. It is sensitive to heat and light, and readily oxidizes by reacting with the oxygen in the atmosphere².

In a continuation of our interest in the study of the kinetic behavior of the reduction of DPB reported earlier³, we focused our attention on the electron transfer reaction between DPB and ascorbic acid.

Ascorbic acid is one of the most potent reducing agents for biological systems, as it reacts with trace metal ions to yield active oxygen species⁴⁻⁶. According to Halliwell, in vitro ascorbic acid behaves as an efficient antioxidant in several different ways, such as by scavenging radicals produced by certain drugs. Its action protects lung fluids from malfunctions due to dangerous air pollutants such as O_3 and NO_2 . It reduces lipid peroxidation by cigarette smoke and scavenges peroxy, thiyl, sulfonyl, urate nitroxide and other radicals⁷.

Marchenko and Morosanova have studied the total content of reducing agents in wastewater and of ascorbic acid in fruit juices⁸.

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Recently the University of Virginia issued its free press release to confirm the necessity of ascorbic acid in maintaining the enzyme prolyl hydroxylase in an active form. It does so most likely by keeping its iron atom in a reduced state⁹.

A number of other investigations pertaining to kinetics of ascorbic acid with different ions have appeared in the literature^{10–12}.

Experimental

All chemicals were of Analar grade. Kinetic measurements were carried out on a Shimadzu UV-160, UV/Visible spectrophotometer using quartz cells. pH measurements were carried out on a digital pH meter using a combined glass Ag/AgCl electrode HI-1332.

Preparation of diphenylamine solution

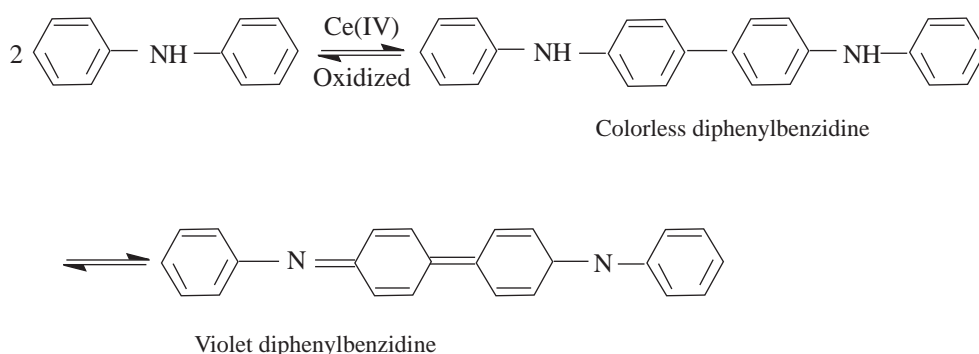
Stock solution of the diphenylamine was prepared at a concentration of 0.1 mol/dm³ by dissolving an appropriate amount in 99% methanol. The pH was maintained at the required value with the help of sodium hydroxide (NaOH 0.5 mol/dm³), and a pH meter was used to validate the pH value.

Cerium(IV) sulfate tetra hydrate

The 0.1 mol/dm³ cerium(IV) sulfate tetra hydrate stock solution was prepared in 0.5 mol/dm³ H₂SO₄. The pH of cerium(IV) sulfate tetra hydrate was maintained at the required value with the help of sodium hydroxide (0.5 mol/dm³).

Violet solution of diphenylbenzidine

Diphenylbenzidine was prepared by mixing diphenylamine and cerium(IV) sulfate tetra hydrate solution in a ratio of 1:2. As a result of oxidation, a violet solution of diphenylbenzidine was obtained. The formation sequence is described below¹³.



Investigation of the molar ratio between diphenylamine and cerium(IV) sulfate tetra hydrate

The molar ratio between diphenylamine and cerium(IV) sulfate tetra hydrate was established through spectral analysis. For this purpose the reaction mixture was maintained in a 3 mL cuvette. The concentration

of diphenylamine was kept constant (1×10^{-4} mol/dm³) and the concentration of cerium(IV) sulfate tetra hydrate (1×10^{-4} , 2×10^{-4} and 3×10^{-4} mol/dm³) was varied in a ratio of 1:1, 1:2 and 1:3, and the ratio of 1:2 was found to be appropriate at a wavelength of 570 nm. Respective spectra of diphenylamine, cerium(IV) sulfate tetra hydrate and diphenylbenzidine are reproduced in Figures 1-3.

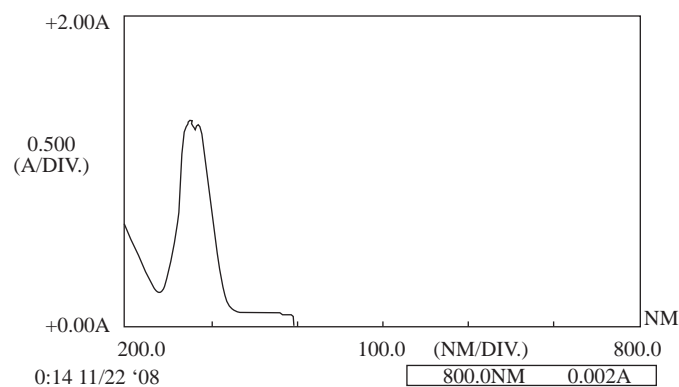


Figure 1. Spectrum of diphenylamine at a concentration of 2×10^{-4} mol/dm³.

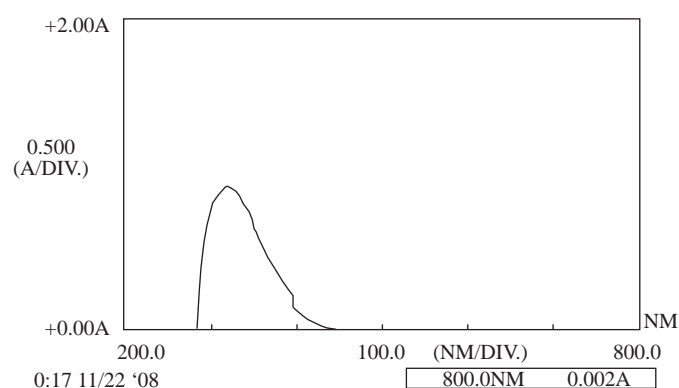


Figure 2. Spectrum of cerium(IV) sulfate tetra hydrate at a concentration of 4×10^{-4} mol/dm³.

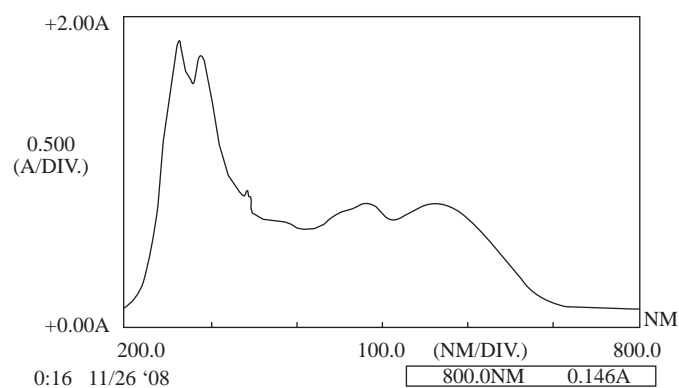


Figure 3. Spectrum of diphenylbenzidine with a ratio of 1:2.

Determination of the molar absorption coefficient

The molar absorption coefficient of diphenylbenzidine was determined at 570 nm. This was established by obtaining a working curve of absorbance as a function of concentrations of diphenylbenzidine. According to Beer's law $A = \epsilon bc$; the slope of A vs. the c plot gives the value of molar absorptivity (ϵ) and path length (b). The value of path length was 1 cm and thus the molar absorptivity (ϵ) can be calculated.

The molar absorptivity of diphenylbenzidine was determined by recording the absorbance of a number of solutions having concentrations of 2×10^{-4} , 3×10^{-4} , 4×10^{-4} and 5×10^{-4} mol/dm³ at 570 nm, respectively, at 32 °C. It was found to be 4920 dm³.mol⁻¹.cm⁻¹.

Determination of the stability of diphenylbenzidine

The reaction between diphenylamine and cerium(IV) sulfate tetra hydrate with a ratio of 1:2 was followed spectrophotometrically at the wavelength maxima (570 nm) of the diphenylbenzidine. The stability of diphenylbenzidine was studied at room temperature by varying the pH between 3.5 and 5.0. The data were collected at 1 min intervals, until the reaction between diphenylamine and cerium(IV) sulfate tetra hydrate was complete. The reaction mixture of diphenylbenzidine was found to be stable for 5 min after its formation at pH 4.0.

Kinetic measurements

The kinetic study of the reduction of diphenylbenzidine by ascorbic acid was carried out under the pseudo first-order condition having [diphenylbenzidine] \gg [ascorbic acid]. Different combinations of both diphenylbenzidine and reductant (ascorbic acid) were prepared by taking a constant concentration of the reductant (3.33×10^{-6} mol/dm³) and varying the concentrations of diphenylbenzidine (3.33×10^{-5} , 5×10^{-5} , 6.66×10^{-5} and 8.33×10^{-5} mol/dm³). Sodium hydroxide base was used to maintain the pH at 3.5-5.0. Various ratios of diphenylbenzidine and ascorbic acid solutions were mixed in a 1 cm quartz cell to a total volume of 3 mL. The optical density was measured spectrophotometrically as a function of time for each set of reaction mixtures at 570 nm.

Results and Discussion

The redox reaction was carried out under the pseudo first-order conditions having diphenylbenzidine \gg ascorbic acid. Kinetic data were collected using an integration method by plotting $\ln \frac{A_{\infty} - A_0}{A_{\infty} - A_t}$ vs. time, where A_{∞} stands for absorbance at infinite dilution, and A_0 and A_t represent initial absorbance and absorbance at time t (s) as shown in Table 1. The plots obtained for a constant concentration of ascorbic acid and a varying concentration of diphenylbenzidine in all 4 ratios (1:10, 1:15, 1:20 and 1:25) produced a straight line passing through the origin. These plots indicate that the reaction is first order in ascorbic acid as shown in Figure 4, which was developed on the basis of the first order rate constant integral equation

$$\ln \frac{A_{\infty} - A_0}{A_{\infty} - A_t} = kt$$

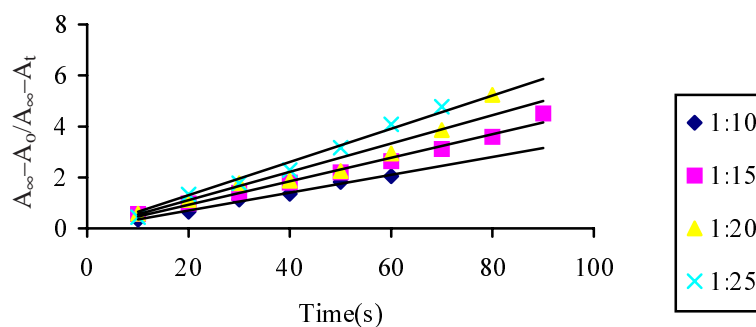


Figure 4. Plot of $A_{\infty}-A_o/A_{\infty}-A_t$ vs. time at 4 different ratios of ascorbic acid and diphenylbenzidine (1:10, 1:15, 1:20, 1:25) at pH 4.0.

Table 1. Values of k_{obs} by plotting a graph between $\ln A_{\infty}-A_o/A_{\infty}-A_t$ vs. time at 4 different ratios of diphenylbenzidine and ascorbic acid $[ASC] = 3.33 \times 10^{-6} \text{ mol/dm}^3$, $\text{pH} = 3.5-5.0$, $\lambda_{max} = 570 \text{ nm}$, $\epsilon = 4920 \text{ dm}^3/\text{mol.cm}$, $T = 32 \text{ }^\circ\text{C}$.

S.No	pH	[diphenylbenzidine] $3.33 \times 10^{-5} \text{ mol/dm}^3$ $k_{obs}\text{s}^{-1} \times (10^3)$	[diphenylbenzidine] $5.00 \times 10^{-5} \text{ mol/dm}^3$ $k_{obs}\text{s}^{-1} \times (10^3)$	[diphenylbenzidine] $6.66 \times 10^{-5} \text{ mol/dm}^3$ $k_{obs}\text{s}^{-1} \times (10^3)$	[diphenylbenzidine] $8.33 \times 10^{-5} \text{ mol/dm}^3$
1	3.5	36.80	51.60	64.60	70.70
2	4.0	35.00	46.20	55.60	65.20
3	4.5	27.30	39.40	40.90	55.30
4	5.0	25.90	31.70	41.30	47.80

Effect of diphenylbenzidine on rate

The plot of $\ln A_{\infty}-A_o/A_{\infty}-A_t$ vs. time for different pH values helped us to compute k_{obs} for each set of experiments. When these values of k_{obs} for different sets were plotted as a function of concentration of diphenylbenzidine, a straight line was obtained, which passed through the origin as shown in Figure 5. From the slope of this, the value of the rate constant for second order was calculated. The order of the reaction with respect to diphenylbenzidine was also found to be first. Thus, the overall order of the redox reaction between diphenylbenzidine and ascorbic acid is 2.

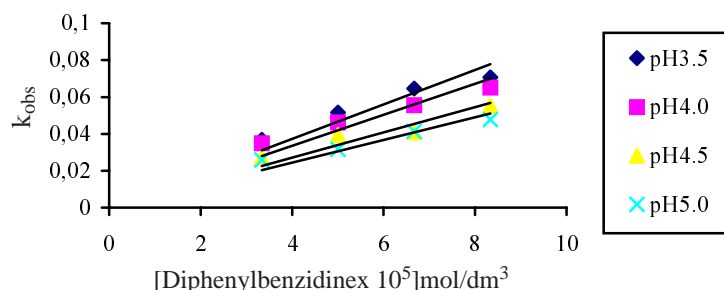


Figure 5. Determination of values of rate for second order of reaction by plotting k_{obs} vs. [Diphenylbenzidine] for pH 3.5-5.0, $[ASC] = 3.33 \times 10^{-6} \text{ mol/dm}^3$.

Table 2. Values of rate for second order of reaction by plotting k_{obs} vs. [Diphenylbenzidine] at specific pH. [ASC] = 3.33×10^{-6} mol/dm³.

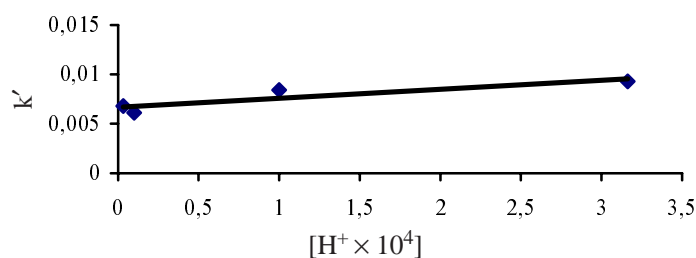
S.No	[Diphenylbenzidine] $\times 10^5$ mol/dm ³	k_{obs} s ⁻¹ $\times 10^3$	k dm ³ .mol ⁻¹ .s ⁻¹
At pH 3.5			
1	3.33	36.80	933.72
2	5.0	51.60	
3	6.66	64.60	
4	8.33	70.70	
At pH 4.0			
1	3.33	35.00	841.16
2	5.0	46.20	
3	6.66	55.60	
4	8.33	65.20	
At pH 4.5			
1	3.33	27.30	681.04
2	5.0	39.40	
3	6.66	40.90	
4	8.33	55.30	
At pH 5.0			
1	3.33	25.90	612.37
2	5.0	31.70	
3	6.66	41.30	
4	8.33	47.80	

Effect of [H⁺] on rate of reaction

To study the effect of [H⁺] on the rate of the reaction, kinetic runs were carried out at different pH values. The remaining reaction conditions were kept unaltered. It was observed that the rate increased with the increase in hydrogen ion concentration. The data of k' vs. [H⁺] were plotted for the pH values of 3.5–5.0. The plot between k' vs. [H⁺] produced a straight line with positive slope (Table 3, Figure 6).

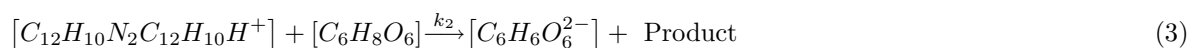
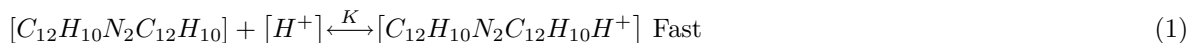
Table 3. Results of effect of H⁺ ion concentration on rate of reaction k' vs. [H⁺], [ASC] = 3.33×10^{-6} mol/dm³, λ_{max} = 570 nm, ε = 4920 dm³/mol.cm.

S.No	[H ⁺] $\times 10^3$	k' dm ³ .mol ⁻¹ .s ⁻¹
1	3.162	933.72
2	1.00	841.16
3	0.0316	681.04
4	0.1	612.37

**Figure 6.** Plot of k_{obs} vs. [H⁺] for the determination of effect of hydrogen ion concentration.

Proposed mechanism

The mechanism is suggested to incorporate the following 3 steps:



The rate of the reaction according to steps 2 and 3 will be

$$\text{Rate} = k_1[C_{12}H_{10}N_2C_{12}H_{10}][C_6H_8O_6] + k_2[C_{12}H_{10}N_2C_{12}H_{10}H^+][C_6H_8O_6] \quad (4)$$

$$\text{Rate} = \{k_1[C_{12}H_{10}N_2C_{12}H_{10}] + k_2[C_{12}H_{10}N_2C_{12}H_{10}H^+]\}[C_6H_8O_6] \quad (5)$$

Assuming that the formation of the protonated species $[C_{12}H_{10}N_2C_{12}H_{10}H^+]$ is quick, the equilibrium constant 'K' for the formation of the protonated species is represented as

$$K = \frac{[C_{12}H_{10}N_2C_{12}H_{10}H^+]}{[C_{12}H_{10}N_2C_{12}H_{10}][H^+]} \quad (6)$$

And therefore

$$K[C_{12}H_{10}N_2C_{12}H_{10}][H^+] = [C_{12}H_{10}N_2C_{12}H_{10}H^+] \quad (7)$$

Substituting $[C_{12}H_{10}N_2C_{12}H_{10}H^+]$ in eq. 5

$$\text{Rate} = \{k_1[C_{12}H_{10}N_2C_{12}H_{10}] + k_2K[C_{12}H_{10}N_2C_{12}H_{10}][H^+]\}[C_6H_8O_6] \quad (8)$$

$$k_{obs} = k_1[C_{12}H_{10}N_2C_{12}H_{10}] + k_2K[C_{12}H_{10}N_2C_{12}H_{10}][H^+] \quad (9)$$

$$k_{obs} = [C_{12}H_{10}N_2C_{12}H_{10}]\{k_1 + k_2K[H^+]\} \quad (10)$$

$$k' = k_1 + k_2K[H^+] \quad (11)$$

For the indirect determination of the order of the reaction for the diphenylbenzidine k' was obtained by plotting a graph between k_{obs} vs. [DPB]. To determine the effect of H^+ concentration the values of k' were plotted against $[H^+]$ and the values of k_1 and k_2 were calculated from the slope and intercept and were found to be $k_1 = 2.0 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ and $k_2 = 6.58 \times 10^3 \text{ dm}^3 \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$, respectively

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