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The effects of UV radiation on anodic wave of human serum

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Abstract

Polarographic anodic oxidation wave that can be correlated to total antioxidant capacity (TAC) in Human serum corresponds to $2H^+$ and $2e$ process. The limiting current of the wave, which is proportional to TAC, is strongly influenced by UV light intensity and irradiation time. A mechanism has been proposed to explain the effects of UV on anodic wave related to antioxidant behavior. Results show that decomposition processes follow to the excitation a transitory product at three different light intensities at 340 nm. The number of decomposition products increase with increasing light intensity. The rate of oxygen radical capture ability of the serum antioxidants might be estimated by comparing the changes of anodic wave in the absence and presence of naturally dissolved oxygen.

Keywords: Human serum; Anodic wave; UV radiation; Antioxidant; Reaction rate

1. Introduction

Antioxidants protect cells against the damaging effects of reactive oxygen species consisting of free radicals such as $O^{2\cdot}$, $OH^{\cdot}$, and other non-radical oxygen derivatives such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and singlet Oxygen ($^1O_2$). An imbalance between antioxidants and reactive oxygen species results in oxidative stress, which has been linked to cancer, aging, atherosclerosis, ischemic injury, inflammation and neurodegenerative diseases [1]. Biological fluids contain numerous compounds having antioxidant activity, including urate, ascorbate, bilirubin, and thiols in the aqueous phase and α-tocopherol, carotenoids, and flavonoids in the lipid phase [2]. Tryptophan, tyrosine, cysteine and homocysteine also show antioxidant ability [3]. Cooperation of all the different antioxidants provides greater protection against attack by reactive oxygen radicals, than any single compound alone. Thus, the TAC may give more relevant biological information compared to that obtained by the measurement of individual components, as it considers the cumulative effect of all antioxidants present in plasma and body fluids. Because of difficulty in measuring each antioxidant component separately and interactions among antioxidants, methods have been developed to assess the TAC of serum or plasma such as Trolox-equivalent antioxidant capacity (TEAC), the oxygen radical absorbance capacity (ORAC), and the ferric reducing ability of plasma (FRAP), the crocin bleaching and the CUPRAC assays as well as high-throughput fluorescence screening, enhanced chemiluminescence and cyclic voltammetry (CV) techniques are commonly used and have been extensively evaluated [1–13].

By using the CV technique, the reducing power of antioxidants is characterized from the peak potential (E_p) or inflection point of current wave (half wave potential, $E_{1/2}$) of the anodic peak current (I_p), which provides information on the ability of antioxidants to donate electrons. The TAC in plasma and other biological fluids has been determined from the anodic peak current using cyclic voltammetry (CV) [10–13]. The anodic peak having the E_p of ~0.45 V and $E_{1/2}$ of ~0.3 V in human plasma has been attributed to TAC of the plasma [11].

Some compounds such as vitamin C, uric acid and bioflavonoids, which contribute largely to TAC level also give a polarographic wave around similar potentials [14–19]. These biomolecules can absorb UV-A (320–400 nm) and UV-B (280–

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320 nm) radiation leading to intermediates that can remove photodynamically generated reactive oxygen [17–20]. The electrochemical oxidation of uric acid has been studied in NaCl and phosphate buffers [20]. The kinetic investigations show that a UV-absorbing intermediate is produced during electrooxidation of uric acid (7,9-dihydro-1*H*-purine-2,6,8-(3*H*)-trione). On the basis of pseudo first order rate constants observed in different media, it has been concluded that the diimine species formed from the 2e, $2H^+$ oxidation of uric acid is attacked by water to give diol, which then decomposes in a series of reactions to give allantoin as the major product. Several investigators have also studied action of UV radiation on human blood serum [21–24].

In this study, a mechanism has been proposed for antioxidant activity in human serum by following the changes of polarographic anodic wave during UV irradiation.

2. Materials and methods

The freshly taken blood samples from healthy volunteers were centrifuged at 3000 rpm, by using Hettich centrifuge and the serum was separated as supernatant liquid. A 0.075 cm^3 of the serum was added into 25 cm^3 of 0.1 M KNO_3 solution served as a supporting electrolyte. The solution pH was 8.08. This aliquot was placed in a Pyrex polarographic cell with a thermostatic jacked covered with carbon black having a small circular area of 1.0 cm^2 to irradiate the reaction mixture. A two-electrode system was used for obtaining a polarographic wave. Double distillated mercury dropping electrode (DME) was used as a working electrode and a saturated calomel electrode (SCE) served as the reference. The SCE was separated from the cell by a glass frit. The dropping time of DME was adjusted as 2.7 s. per drop. The solution was deaerated by bubbling nitrogen gas for 15 min. Polarograms were recorded using a Radiometer Copenhagen PO-4 DC Polarograph. Thus, a reproducible well-defined anodic oxidation wave was obtained at a scan rate of 13.3 mV s^{-1}.

The solutions were irradiated using a high-pressure mercury vapor lamp (Edison 400 HQL) as UV source. Monochromatic UV light was obtained using a Kodak glass filter of 340 nm. Absorbing light intensities (I_a) were adjusted by changing the distance of the UV source to the system. The values of I_a were determined as 0.66×10^{15}, 1.50×10^{15} and 3.70×10^{15} quanta s^{-1} cm^{-2} using ferrioxalate actinometer. The anodic waves were recorded in regular time intervals during irradiation at varying light intensities. During irradiation, the temperature of the solution was kept constant at 298 K.

Representative polarograms recorded at various irradiation time intervals at 0.66×10^{15} quanta s^{-1} cm^{-2} light intensity are shown in Fig. 1.

The experiment was also conducted in the presence of naturally dissolved oxygen at medium light intensity of 1.50×10^{15} quanta s^{-1} cm^{-2}.

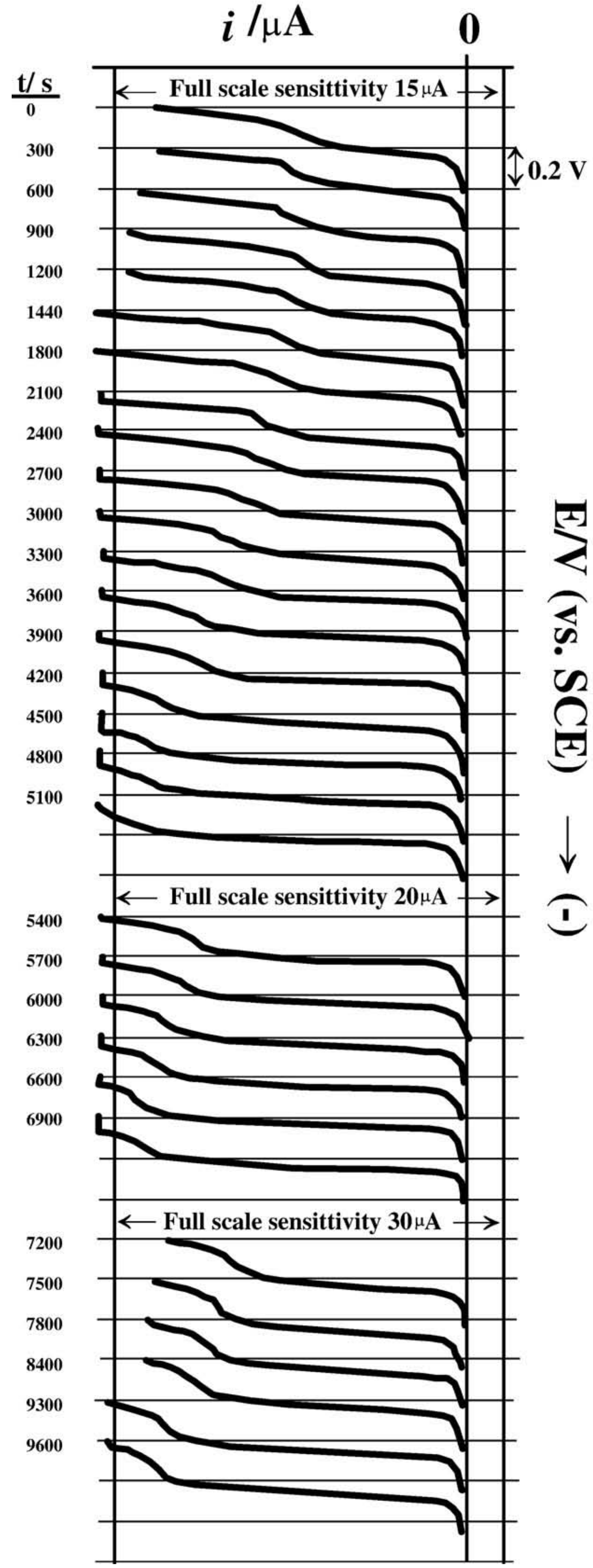

Fig. 1. Representative polarograms for irradiation with UV light of low intensity at 340 nm wavelengths depending on time (Starting potential is 0.5 V and full scale sensittivity of diffusion currents is shown at the beginning of each series).

3. Results and discussion

Serum solution yields a reproducible well-developed anodic wave at the half wave potential of 0.28 V. The limiting current of the wave is ~5 μA in deaerated solution. This value was measured for an anodic wave at $t=0$ when there is no irradiation. Since the residual current increases with the applied e.m.f the initial point of the diffusion current is determined from the intersection point of extrapolated lines of residual-and diffusion currents. The true limiting current is determined by measuring the distance between the total diffusion current and its initial point. The estimated uncertainty of the measured limiting currents is ±3%.

An anodic wave corresponding to a reversible electrode process can be analyzed according to following Heyrovsky-Ilkovich equation [25,26]:

$$E_{\mathrm{d.e.}} = E_{1/2} + \frac{0.059}{n}\log\frac{(i_{\mathrm{d}} - i)}{i} \qquad (1)$$

where $E_{\mathrm{d.e.}}$ and i are corresponding voltage and current values at any point on the wave, $E_{1/2}$ is half wave potential, i_{d} is limiting current and n the number of electrons transferred in the electrode process. It is evident from the Eq. (1) a plot of $E_{\mathrm{d.e.}}$ vs. log $(i_{\mathrm{d}}-i)/i$ plot should produce a straight line with a slope 0.059/n at 298 K, and potential where the log term becomes zero should be the half wave potential. If the electrode reaction is reversible but the reaction product formed by electrolysis is unstable and is irreversibly transformed another compound, the net reaction becomes irreversible. In such a case, it has been theoretically proved that the shape of the anodic wave corresponding to 2e, 2H^+ process at a given pH is identical with that of a reversible reaction, which involves the transfer of two electrons [25,26]. Similar mechanism has been proposed for the anodic waves of some antioxidants such as ascorbic acid [14], hesperidin [17], rutin [18], morin [19] and uric acid [20].

By plotting the values of $E_{\mathrm{d.e.}}$ vs. log $(i_{\mathrm{d}}-i)/i$ a straight line was obtained with a slope of 0.031(not shown here, $r^2=0.9998$), in good agreement with theoretical slope of 0.0296 corresponding to 2e, 2H^+ process. The value of $E_{1/2}$ of 0.28 V is not oxidation potential of the reversible electrode reaction it also involves the rate constant of the irreversible reaction.

Consequently, the anodic wave of serum can be attributed to many species present in human serum that are simultaneously oxidized with the same mechanism and the electrode reactions of these compounds may be generalized in the following form [14,17–20,25,26]:

$$AO \leftrightarrow 2e + 2H^+ + DA^* \ \text{(Transitory)} \qquad (2)$$

$$\mathrm{DA}^* \xrightarrow{k} \mathrm{DA} \qquad (3)$$

Here, AO denotes antioxidant and DA is its dehydro-form, and k is the rate constant.

Limiting current of the anodic wave (i_{d}) is proportional to [DA] concentration according to Ilkovic equation [25]. The changes in limiting diffusion currents depending on irradiation time at varying light intensities were depicted in Fig. 2. The values of i_{d} show an increase at earlier irradiation time at the light intensity of 0.66×10^{15} quanta s^{-1} cm^{-2} but they slow down as the time increases (triangles). The increase in i_{d} values for medium light intensity is higher than at the lowest intensity initially whereas a gradual decrease is observed following a maximum value (diamonds).

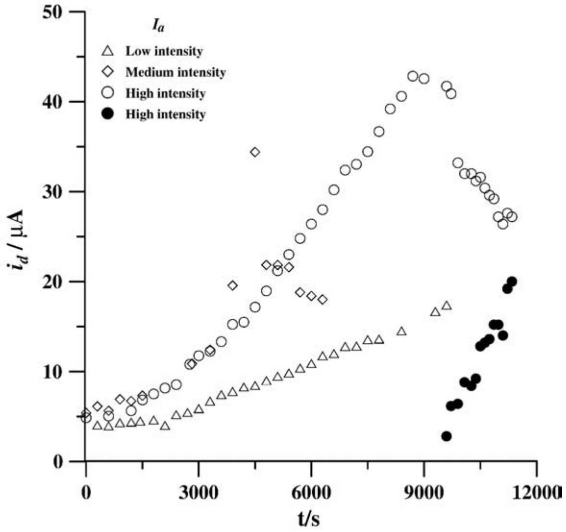

Fig. 2. The influences of UV radiation with different intensities on limiting current of anodic wave of Human serum.

A slower increase is observed in the i_{d} values at the highest absorbing intensity of 3.70×10^{15} quanta s^{-1} cm^{-2} and the maximum value of i_{d} is higher than that of medium light intensity (open circles). When i_{d} values start to decrease another polarographic wave is observed at the half wave potential of 0.38 V. This wave also increases by irradiation (solid circles).

In order to explain the effects of UV light on anodic wave, which can be correlated to antioxidant behavior, following mechanism has been proposed to account for the observed results based on electrode process [14,17–20,25,26]. Each step can play a role in the overall mechanism, depending on light intensity and irradiation time.

$$\mathrm{AO} + h\nu \xrightarrow{k_1 I_a} 2e + 2\mathrm{H}^+ + \mathrm{DA}^* \qquad (4)$$

$$\mathrm{DA}^* \xrightarrow{k_2} \mathrm{AO} \qquad (5)$$

$$\mathrm{DA}^* \xrightarrow{k_3} \mathrm{DA} \qquad (6)$$

$$\mathrm{DA} \xrightarrow{k_4} \mathrm{B} + \mathrm{C} \qquad (7)$$

$$\mathrm{DA} \xrightarrow{k_5} \mathrm{D} + \mathrm{E} \qquad (8)$$

$$\mathrm{DA} \xrightarrow{k_6} \mathrm{F} + \mathrm{G} \qquad (9)$$

$$\mathrm{DA} \xrightarrow{k_7} \mathrm{H} + \mathrm{I} \qquad (10)$$

$$\mathrm{DA} \xrightarrow{k_8} \mathrm{J} + \mathrm{K} \qquad (11)$$

Here, the Eqs. (4)–(6) are elementary steps of electrode process while the Eqs. (7)–(11) are decomposition steps of the dehydro-form of antioxidants which can give B, C, D, E, F, G, H, I, J and K decomposition products. The rate constant for a respective rate process is shown on the arrow.

When serum is irradiated by UV light, the changes in the DA concentration with irradiation time may be written as follows:

$$\frac{d[\mathrm{DA}]}{dt} = k_3\left[\mathrm{DA}^*\right] - k_4[\mathrm{DA}] - k_5[\mathrm{DA}] - k_6[\mathrm{DA}] \quad -k_7[\mathrm{DA}] - k_8[\mathrm{DA}] \tag{12}$$

where, the brackets indicate the concentrations of the various species. The concentration of the transitory product [DA*] in Eq. (12) can be determined from the stationary or steady state principle [27]:

$$k_1 I_a = k_2\left[\mathrm{DA}^*\right] + k_3\left[\mathrm{DA}^*\right] \tag{13}$$

Consequently,

$$\left[\mathrm{DA}^*\right] = \frac{k_1 I_\mathrm{a}}{k_2 + k_3} \tag{14}$$

The rate equation can be obtained in terms of the measurable [DA] concentration by substitution of [DA*] in Eq. (12):

$$\frac{d[\mathrm{DA}]}{dt} = \frac{k_1 k_3 I_a}{k_2 + k_3} - k_4[\mathrm{DA}] - k_5[\mathrm{DA}] - k_6[\mathrm{DA}] - k_7[\mathrm{DA}] - k_8[\mathrm{DA}] \tag{15}$$

This equation is rearranged and integrated:

$$\int \frac{d[\mathrm{DA}]}{[\mathrm{DA}]} = \int \left(\frac{k_1 k_3 I_a}{k_2 + k_3} \times \frac{1}{[\mathrm{DA}]} - k_4 - k_5 - k_6 - k_7 - k_8\right) dt \tag{16}$$

$$\ln[\mathrm{DA}] = \left(\frac{k_1 k_3 I_\mathrm{a}}{k_2 + k_3} \times \frac{1}{[\mathrm{DA}]} - k_4 - k_5 - k_6 - k_7 - k_8\right) t + I \tag{17}$$

The value of integral constant, I, is calculated as:

$$I = \ln[\mathrm{DA}]_0 \tag{18}$$

for initial irradiation time (t=0).

$$\ln\frac{[\mathrm{DA}]}{[\mathrm{DA}]_0} = \left(\frac{k_1 k_3 I_\mathrm{a}}{k_2 + k_3} \times \frac{1}{[\mathrm{DA}]} - k_4 - k_5 - k_6 - k_7 - k_8\right) t \tag{19}$$

$$k' = \frac{k_3}{k_2 + k_3} \tag{20}$$

$$\ln\frac{[\mathrm{DA}]}{[\mathrm{DA}]_0} = \left(\frac{k_1 k' I_a}{[\mathrm{DA}]} - k_4 - k_5 - k_6 - k_7 - k_8\right) t \tag{21}$$

Here, $[\mathrm{DA}]_0$ and [DA] are concentrations of dehydro product at initial and irradiation time t, respectively. The ratio of [DA]/$[\mathrm{DA}]_0$ corresponds to the ratio of limiting current at irradiation time t to that of its initial value. The values of k_1, k_4, k_5, k_6, k_7 and k_8 could be determined from the slopes of $\ln[\mathrm{DA}]/[\mathrm{DA}]_0$ vs. t plot in Fig. 3 for a known I_a and the standard concentration of [DA]=1 mol dm^{-3}.

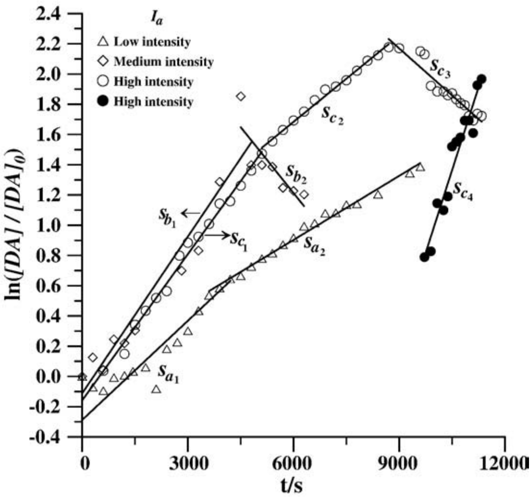

Fig. 3. The plot for determination of rate constants of photochemical reactions depending on irradiation time and light intensity (s denotes the slope of the straight line, subscripts a, b and c represent low, medium and high light intensity, respectively.).

3.1. Determination of the rate constants

The rate constants can be determined by analyzing of $\ln[\mathrm{DA}]/[\mathrm{DA}]_0$ vs. t curves at different light intensity and irradiation times as follows:

3.1.1. Low light intensity

When serum solution is irradiated by UV light with low intensity $\ln[\mathrm{DA}]/[\mathrm{DA}]_0$ vs. t plot gives two straight lines with decreasing slopes of $s_{\mathrm{a}_1}$and s_{a_2}, respectively. By assuming the slope of the second straight line corresponds to rate constant (k_4) of decomposition reaction in Eq. (7) slope of the first straight line can be derived from Eqs. (4)–(7) as follows:

$$s_\mathrm{a} = \frac{k_1 k_3 I_\mathrm{a}}{k_2 + k_3} - k_4 \tag{22}$$

Since electrode reaction becomes irreversible when irradiated by UV light k_2 can be neglected. Thus Eq. (22) can be rearranged as follows:

$$s_{\mathrm{a}_1} = \frac{k_1 k_3 I_\mathrm{a}}{k_3} - k_4 \tag{23}$$

$$s_{\mathrm{a}_1} = k_1 I_\mathrm{a} - k_4 \tag{24}$$

The value of k_4 is calculated as1.40×10^{-4} s^{-1} from the slope of the second straight line (r=0.99). In the kinetic calculations the unit of I_a is converted from quanta s^{-1} cm^{-2} into quanta mol dm^{-3} s^{-1} by dividing it by Avogadro's number and the volume of the solution. Therefore, the value of k_1 is calculated as 7.69×10^3 s^{-1} by using the I_a value of 4.38×10^{-8} mol quanta dm^{-3} s^{-1} and the s_{a_1} value of 1.97×10^{-4} s^{-1} (r=0.96).

3.1.2. Medium light intensity

As shown in Fig. 3, two straight lines are observed the first having a positive slope (s_{b_1}) and the latter negative (s_{b_2}) depending on increasing irradiation time at medium light intensity. These results suggest that reaction mechanism follows the order of Eqs. (4)–(8) and the slopes of the straight lines correspond to:

$$s_{b_1} = k_1 I_a - k_4 - k_5 \tag{25}$$

$$s_{b_2} = -k_5 \tag{26}$$

The value of k_5 is calculated from the observed slope of the second straight-line as $2.90 \times 10^{-4}\ \mathrm{s}^{-1}$ (r=0.94). The s_{b_1} value is computed as $3.37 \times 10^{-4}\ \mathrm{s}^{-1}$ by using the known value of I_a of 9.97×10^{-8} mol quanta $\mathrm{dm}^{-3}\ \mathrm{s}^{-1}$ and k_1, k_4 and k_5 values. This value is in agreement with the observed slope of the first straight line of $3.45 \times 10^{-4}\ \mathrm{s}^{-1}$ (r=0.97).

3.1.3. High light intensity

The ln[DA]/[DA]$_0$ vs. t plot derived from the main polarographic wave at $E_{1/2}$ of 0.28 V gives three straight lines with two positive (s_{c_1} and s_{c_2}) and one negative (s_{c_3}) slopes. At longer irradiations, another straight line having a positive slope (s_{c_4}) corresponding to $E_{1/2}$ of 0.38 V is obtained when the main wave starts to decrease. The observed slope of the first straight line is well consistent with the calculated slope by assuming following equations:

$$s_{\mathrm{c}_1} = k_1 I_\mathrm{a} - k_4 - k_5 - k_6 - k_7 - k_8 \tag{27}$$

$$s_{\mathrm{c}_2} = k_6 \tag{28}$$

$$s_{\mathrm{c}_3} = -k_7 \tag{29}$$

$$s_{\mathrm{c}_4} = k_8 \tag{30}$$

The $s_{\mathrm{c}_1} < s_{\mathrm{b}_1}$ values suggest that additional decomposition reactions occur at the highest light intensity of 2.46×10^{-7} mol quanta $\mathrm{dm}^{-3}\ \mathrm{s}^{-1}$. The rate constants correspond to additional decomposition reactions in Eqs. (9)–(11) could be calculated from the slopes of the straight lines according to Eqs. (28)–(30). The value of the rate constant k_6 is $1.90 \times 10^{-4}\ \mathrm{s}^{-1}$ (r=1.00). The reaction products generated according to Eq. (10) are not polarographically active and the rate constant k_7 can be calculated from the negative slope of the third straight line as $2.10 \times 10^{-4}\ \mathrm{s}^{-1}$ (r=0.95). On the other hand, a polarographically active product can be followed only after the main wave starts to decrease. The rate constant k_8, which corresponds to Eq. (11), can be determined from the positive slope of the fourth straight line as $7.30 \times 10^{-4}\ \mathrm{s}^{-1}$ (r=0.98). Using the known values of the constants involved in Eq. (27), the slope of the first straight line is calculated as $3.31 \times 10^{-4}\ \mathrm{s}^{-1}$. This value is in accordance with its observed value of $3.22 \times 10^{-4}\ \mathrm{s}^{-1}$ (r=1.00).

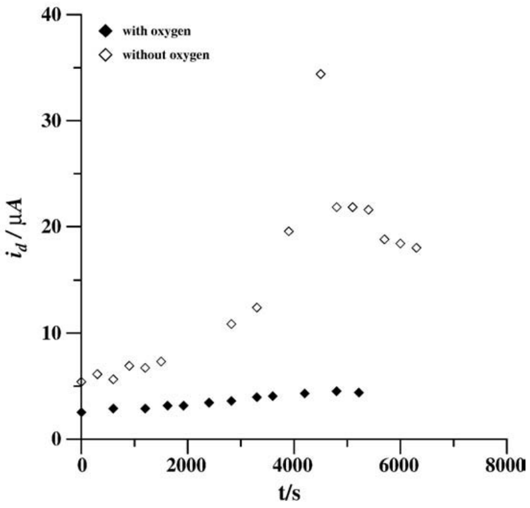

Fig. 4. A comparison of the limiting currents of anodic waves irradiated at the medium light intensity in the presence and in the absence of oxygen.

3.2. Effect of UV irradiation on anodic wave in the presence of oxygen

The attack of antioxidants on oxygen radical species may be better understood by comparing the behavior of anodic wave of serum in the presence of naturally dissolved oxygen to in its absence. A comparison of the limiting currents for irradiations with medium UV light intensity is presented in Fig. 4. As it is seen from Fig. 4, the height of anodic wave in the presence of oxygen is lower than that of its absence under dark conditions (i.e., irradiation time t=0). This suggests that antioxidants present in Human serum are loosely bounded with molecular oxygen, which can be interrupted during nitrogen bubbling under pressure. After removal of oxygen the wave height increases by about two folds.

When serum irradiated under oxic conditions the increase of the wave height is very slow suggesting that light-induced antioxidant species capture the reactive oxygen species, which are produced upon UV radiation. The rate of oxygen radical capture of the serum antioxidants can be estimated as $2.96 \times 10^{-4}\ \mathrm{s}^{-1}$ from the difference of the slopes of ln[DA]/[DA]$_0$ vs. t plots obtained in the absence and in the presence of oxygen.

4. Conclusions

Polarographic technique has provided valuable information about electron transfer mechanism and decomposition reactions of serum antioxidants under UV exposure without any separation procedure.

The polarographic anodic wave of human serum related to TAC has been interpreted on the basis of a mechanism involving a reversible electrode reaction followed by the irreversible conversion of an unstable intermediate to its stable form. When serum is irradiated by UV light reversible electrode process becomes irreversible and decomposition products are generated depending on light intensity and irradiation time.

The reactions initiated with different light intensities are completed at different irradiation time which are reflected by the straight lines having different slopes on ln[DA]/[DA]$_0$ vs. t plot. Results show that one, two and five decomposition reactions appear by irradiation at low, medium and high intensities, respectively. The rate constant k_1 of the irreversible electrode process and k_4 corresponding to the first decomposition reaction can be obtained by irradiations at low intensity. At medium intensity, the first decomposition is completed at earlier time of irradiations and rate constant of the second decomposition (k_5) can be calculated from the slope of the second straight line. Similarly, the first and the second decomposition processes are completed at initial irradiations with high intensity UV light and the corresponding rate constants of three decompositions (k_6–k_8) are calculated from the slopes of the three straight lines, which appear depending on irradiation time.

A comparison of the anodic waves irradiated under oxic and anoxic conditions confirmed that light-induced antioxidant species capture light-induced oxygen species, which are highly reactive and damaging to humans at the molecular level.

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