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Comparison between Amperometric and Chemiluminescence methods in detection and dosimetry of cobalt-60 gamma ray emission

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▸ ***Technical note***

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ABSTRACT

Background: Hydrogen peroxide (H_2O_2), produced by gamma ray irradiation to watery solution, was used as an analytical parameter for dosimetry of cobalt-60 (^{60}Co) radiation. ***Materials and Methods:*** Detection of the produced H_2O_2 was carried out using two methods: an amperometric biosensor fabricated by immobilization of anthraquinone 2-carboxylic acid modified horseradish peroxidase on glassy carbon electrode, and chemiluminescence technique using luminol as a fluorophore and diperiodatocuprate as a catalyst. ***Results:*** In the first method, at the applied potential of -550 mV (vs. Ag/AgCl) the biosensor showed the sensitivity of 74.46 nA/Gray and detection limit of 0.061 Gray (R.S.D=3.67% for N=3) towards H_2O_2 produced by ^{60}Co. In the second method, the sensitivity and detection limit for ^{60}Co gamma ray was determined to be 2.34 (luminescence intensity/Gray) and 0.069 Gray (R.S.D=4.16% for N=3), respectively. ***Conclusion:*** The obtained results revealed that both proposed techniques, with leaner range from 0.25 to 5 Grays are applicable for detection and dosimetry of gamma ray from ^{60}Co.

Keywords: *Cobalt-60, gamma ray, hydrogen peroxide, electrochemistry, chemiluminescence.*

INTRODUCTION

Gamma ray can be detected when it caused ionization (1). Gamma rays are highly energetic and cause breaking of chemical binds of molecules (2). They have ability to produce reactive oxygen species (ROS). As a result, ROS in biological medium causes oxidative damage to biomolecules (3). This behavior is related to radiolysis of water generating free radicals and related products (4). In recent years, there has been much interest in determination of hydrogen peroxide (H_2O_2) as a stable form of ROS (5). Therefore determination of H_2O_2 is important in both radiobiology and medical investigations (6). ROS were early investigated by expensive method of electron spin resonance (7). Then, they were analyzed based on determination of H_2O_2 using spectrophotometry (8), fluorimetry (9), amperometry (10) and chemiluminescence (7).

Recently, a series of H_2O_2 electrochemical biosensors was developed via immobilization of horse radish peroxidase (HRP), which catalyzes the reduction of H_2O_2 (11). These devices show several advantages such as low cost, high specificity and reaction rate but their sensitivity is lower than that offered by chemiluminescence and ESR methods. Chemiluminescence and amperometry can provide the limit of detection for H_2O_2 at nanomolar or subnanomolar (12, 13) level.

In the present work the H_2O_2 produced by gamma emitter radioisotope of cobalt-60 (^{60}Co)

was detected by both electrochemical and chemiluminescence methods. Then, correlation between the dose rates of ^{60}Co measured by electrochemistry and chemiluminescence methods was established.

MATERIALS AND METHODS

Chemical reagents

HRP (EC 1.11.1.7), sodium 4-(2-hydroxyethyl) -1-piperazine ethansulfonate (Na -HEPES), AQ 98%, H_2O_2 30% (w/w) solution, 4-aminoantipyrine 98% (4-AAP), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride 98% (DEC) were purchased from Sigma (St. Louis, USA). Superfine Sephadex G-25 was obtained from Pharmacia LKB (Uppsala, Sweden). The other chemicals were of analytical grade and prepared from Merck (Germany).

Radioisotope source

H_2O_2 was produced by ^{60}Co source (supplied by Atomic Energy Organization of Iran). In each step, 3 vials each contain 3 ml of deionized-double distilled water were exposed to ^{60}Co radiation. Totally 11 series of triple vials was arranged and each series were exposed to a certain dose of ^{60}Co as follows: 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 Gray (Gy), respectively. The samples were irradiated at a position of 80 cm from the source. At the time of irradiation the total exposure or Air Kerma rate, was 0.238 Gy/min as determined by ^{60}Co radiotherapy radiation source (Picker V9).

Electrochemical measurement

Electrochemical biosensor was prepared using anthraquinone 2-carboxylic acid (AQ) modified HRP (AQ-HRP) according to our previous reports (11, 14).

All cyclic voltammograms (CVs) were obtained in a single-compartment cell, equipped with a platinum auxiliary electrode, an Ag/AgCl reference electrode (Metrohm) and the fabricated biosensor as working electrode. The amperometric measurements were done at constant potential of -550 mV vs Ag/AgCl at room temperature. The electrochemical measurements were carried out using a Potentiostat/Galvanostat (model 263-A, EG&G, USA) equipped with Power Suite software package and a rotating disk electrode (Model 616, PerkinElmer, USA).

Chemiluminescence measurement

Preparations of chemiluminescence solutions were carried out based on reference number 12. Briefly, the chemiluminescence intensity of background electrolyte containing sodium carbonate buffer (470 µl, 0.1 M, pH 11), luminal (10µl), pure water (10µl) and diperiodatocuprate (III) (DPC, 10µl, as catalyst), was recorded as baseline at 425 nm. Then, to prepare the calibration curve, the same experiment as previous one was carried out except that instead of pure water, 10µl H_2O_2 with certain concentration was added. Thereafter, to measure the concentration of produced H_2O_2 by ^{60}Co gamma ray, 10µl sample solution which was irradiated by different dose rates of ^{60}Co was assayed using the same procedure. Chemiluminescence measurements were carried out using a Carry Eclipse Fluorescence Spectrophotometer (Varian Co, Australia) at 4 °C. In all data, each point represents the mean value of three independent measurements.

RESULTS

Dosimetry of ^{60}Co gamma ray by AQ-HRP based biosensor

Using the AQ-HRP based biosensor a pair of well defined quasi reversible cyclic voltammogram (CV) was obtained (Figure 1 A, Curve a). As seen, a significant current response was recorded in the presence of H_2O_2 (1.31 µM). The cathodic peak current (ΔI_{pc}) was increased linearly by changing H_2O_2 concentration. (According to our previous report [19] the biosensor response towards H_2O_2 was linear in the concentration range from 70 nM to 1.31 µM, with the sensitivity of 0.35 nA/nM, detection limit of 7.15 nM and the linear regression of equation 1)

$$\Delta I_{pc} = 0.353\,[H_2O_2] + 11.094 \qquad (1)$$

Consequently, in the present work we used the AQ-HRP based biosensor for quantification of H_2O_2 produced by gamma irradiation of ^{60}Co and applied the result for dosimetry of ^{60}Co. As shown in figure 1(B) (Curve a) the immobilized AQ-HRP on glassy carbon (GC) electrode showed a pair of well defined quasi reversible CV. Upon addition of the sample solutions irradiated with different dose rate of ^{60}Co, the cathodic peak currents were increased while anodic peaks currents were reduced. This behavior is related to the formation of H_2O_2 due to gamma irradiation of ^{60}Co into watery medium. In the inset of figure 1(B), the proportionality of cathodic peak currents produced by H_2O_2 and ^{60}Co gamma ray was shown.

In order to quantify the dose rate of ^{60}Co, irradiated to the buffer sample, amperometric method was used. The amperometric response of the biosensor toward the dose rates of ^{60}Co was evaluated in figure 2(A). As seen, at a constant voltage of -550 mV (vs. Ag/AgCl), after a transient decay a steady state current was achieved in a reasonable response time of 30 s. Figure 2(B) shows the increments in the current (ΔI) against the addition of the certain dose rates of ^{60}Co. As seen, the increasing of current is directly proportional to H_2O_2 produced by ^{60}Co in the solution. As shown in figure 2 (Inset), increasing in gamma emitter radioisotope dose rate causes more production of H_2O_2 and consequently enhances the electric current with a R.S.D value lower than 3.67% (N=3).

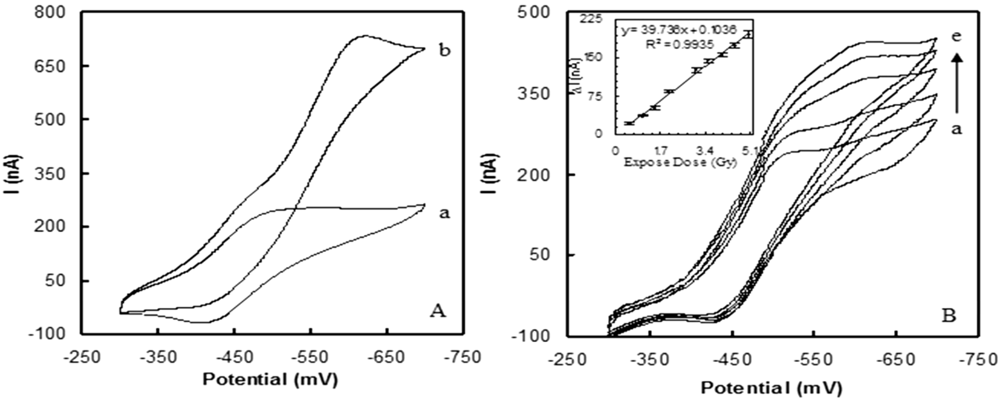

Figure 1. CVs of HRP-AQ/GC electrode in PBS. (A) CVs of HRP-AQ/GC electrode in the absence (a) and presence (b) of H_2O_2 (1.31 µM), respectively. (B) CVs of AQ-HRP/GC electrode in the absence (a) and presence (b to e) of H_2O_2 produced in the solution exposed to ^{60}Co. The CVs (a-e) were obtained by the electrode inserting in the electrolyte containing: PBS (3 ml) and sample solutions (50 ml) exposed to different dose rates of ^{60}Co: 0, 1, 3, 4.5, and 5 Gy, respectively. The scan rate was 20 mV/s. Inset B shows the calibration curve for ^{60}Co dose rates determination. Each point represents the average value of three different measurements.

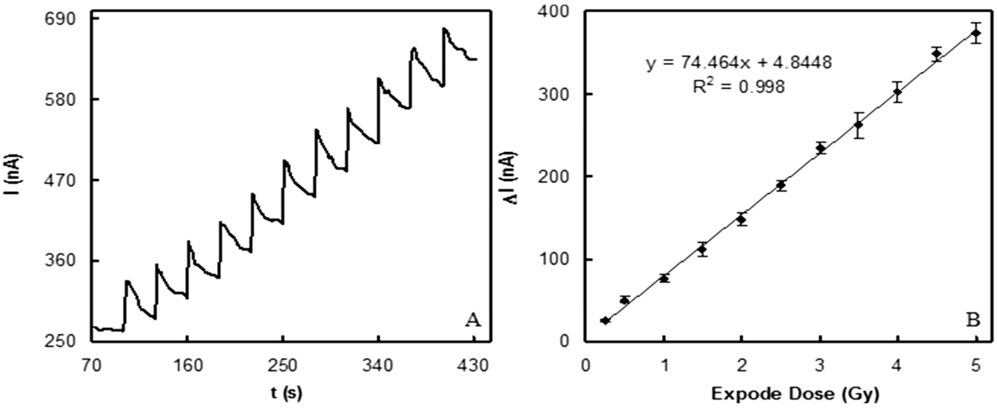

Figure 2. Amperometric responses of the biosensor toward the dose rates of ^{60}Co. (A) Amperogram of AQ-HRP/GC electrode in the presence of different dose rates of ^{60}Co in PBS. Each step (from down to up) shows an amperogram obtained for 3 mL of PBS in which 50 ml of sample solutions exposed to different dose rates of ^{60}Co (0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 Gy) were added. (B) The calibration curve for ^{60}Co dose rates determination. The experiments were done at constant potential of -550 mV (vs. Ag/AgCl). Each point represents the average value of three different measurements.

Dosimetry of ^{60}Co gamma ray by chemiluminescence

Chemiluminescence is the second method which was used to quantify the dose rate of ^{60}Co irradiated to the buffer sample. The total output of the chemiluminescence system was determined by the standard luminol reaction. In an optimal experimental condition of luminol (2×10^{-7} M), DPC (2×10^{-5} M) and H_2O_2 (different concentrations) in sodium carbonate buffer 0.1 M (pH 11, at 4 °C), the changes in peak height were plotted against H_2O_2 concentration (12). The peak height was calculated as the difference between chemiluminescence intensities in the presence and absence (background level at zero concentration) of H_2O_2 (figure 3).

In our recently published work we designed a similar work for determination of H_2O_2 by chemiluminescence method. The calibration curve prepared by this method was used for the direct determination of H_2O_2 (16). In order to make the H_2O_2 sample solution more stable, the measurements were carried out at 4 °C. The intensity of light emission was proportional to the H_2O_2 concentration with the linear regression equatoin (2).

$$\Delta I = 0.01\,[H_2O_2] + 0.20 \qquad (2)$$

Therefore, using chemiluminescence method it is possible to quantify the concentration of H_2O_2 and dosimetry of ^{60}Co, as well. Figure 3 shows a typical oxidation chart of luminol, in the presence of H_2O_2 produced by irradiation of different dose rates of ^{60}Co in the solution. As seen, the light emission is appeared three seconds after the injection of DPC into the mixture of luminol and H_2O_2 then, the chemiluminescence signals would decrease into baseline within 1 minute. Light intensity increases with raising the radioactivity of samples from 0.25 to 5.0 Gy at 425 nm. As shown in figure 3 (Inset), increasing in dose rate causes the enhancement in chemiluminescence emission intensity due to the production of H_2O_2 by gamma emitter radioisotope at different dose rates with a R.S.D lower than 4.16% (N=3).

Evaluation of electrochemical and chemiluminescence methods for ^{60}Co dosimetry

To evaluate the ability of electrochemistry and chemiluminescence methods in ^{60}Co dose rates determination, the linearity and slope of ^{60}Co dose rates vs H_2O_2 concentration produced by the ^{60}Co gamma rays emission were compared. The correlation between H_2O_2 concentrations versus the exposed dose of ^{60}Co obtained by electrochemical method was calculated based on equations 1 and 3. In fact, equation 3 is the linear regression equation of figure 2(B). By combination of these equations equation 4 with the linearity of R^2 = 0.998 and slope of 210.65 was obtained.

$$\Delta I_{pc} = 74.46\,(\text{Exposed dose of } {}^{60}\text{Co}) + 4.84 \qquad (3)$$

$$[H_2O_2] = 210.65\,(\text{Exposed dose of } {}^{60}\text{Co}) + 24.79 \qquad (4)$$

The same calculations were done to obtain the correlation between the exposed dose of ^{60}Co and H_2O_2 concentration for chemiluminescence method. equation 6 with a linearity of R^2 = 0.998 and slope of 220 was resulted by combination of the regression equation 2 and the equation 5 which is obtained from the inset in figure 3.

$$\Delta I = 2.34\,(\text{Exposed dose of } {}^{60}\text{Co}) + 0.28 \qquad (5)$$

$$[H_2O_2] = 220.96\,(\text{Exposed dose of } {}^{60}\text{Co}) + 26.95 \qquad (6)$$

Then, based on the obtained equations of 4 and 6 a plot showing the linear correlation between H_2O_2 concentrations and gamma emitter dose rates were established (figure 4).

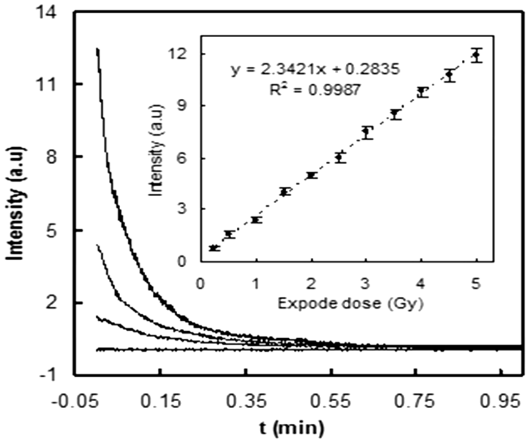

Figure 3. ^{60}Co dose rate determination by chemiluminescence. The experiment was carried out in the presence of sodium carbonate buffer (470 ml, 0.1 M, pH 11), luminol (10 ml, 10^{-5} M), DPC (10 ml, 10^{-3} M) and 10 ml sample solution which was irradiated by different dose rates of ^{60}Co. The curves from down to up shows the dose rates of: 0, 0.5, 1.5 and 5 Gy, respectively. The inset shows the calibration curves for H_2O_2 produced by different dose rates of ^{60}Co. Each point in the inset represents the mean value of three independent assays.

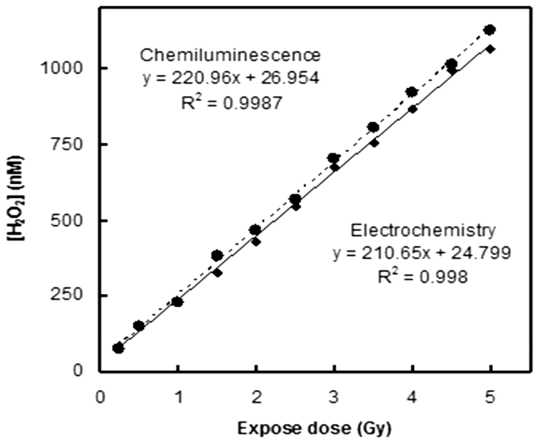

Figure 4. Correlation between the dose rates of ^{60}Co measured by electrochemistry (squares) and those determined by chemiluminescence (circles) methods. Data were extracted from figures 2(B) and 3, respectively.

DISCUSSION

As shown in figure 4, the gamma ray emitted from ^{60}Co can be detected using both electrochemical and chemiluminescence methods in the linear dose rate range from 0.25 to 5 Gy. However, while the detection limits of electrochemical method (0.061 Gy) is slightly lower than that of chemiluminescence method (0.069 Gy); the sensitivity, in terms of H_2O_2 concentration per Gy, for chemiluminescence (220.96 nM/Gy) is slightly more than that obtained by electrochemistry (210.65 nM/Gy). This difference in sensitivity (4.66%) was compared with the RSDs of two methods:

$$4.66\% \approx R.S.D_{chemiluminescence}\ (4.16\%\ \text{for}\ N=3) > R.S.D_{electrochemistry}\ (3.67\%\ \text{for}\ N=3).$$

One of the most probable limitations for HRP based biosensor is HRP suicide inactivation via over production of H_2O_2 (11). But, regarding the H_2O_2 concentration produced at highest dose rates of ^{60}Co (5 Gy), one can estimate that such a low concentration range of H_2O_2 (77.5 nM) is not enough to suicide the immobilized AQ-HRP on GC electrode. Nevertheless, a slight decrease in the slope of electrochemical method (figure 4.) could be attributed to a trivial suicide of the immobilized AQ-HRP.

Comparison of the introduced electrochemistry and chemiluminescence technique and the commercial method of liquid scintillation counters (LSC) revealed some advantages of these methods over conventional LSC. In some conventional LSC, usually radioactive source is added to a chemical solution called scintillation fluid or cocktail so that the source is not re-coverable (17). But, the present methods, without the needs for addition of radioactive source to sample solution, have ability to be used for indirect detection of gamma rays in watery solutions. These approaches make it possible to measure the gamma rays from an external source. This means that in the present methods neither radioactive source is added to the sample nor cocktails is used but the dose rate of rays irradiated (E_γ=1173.2 KeV) to watery substances is evaluated by detection of H_2O_2 produced through radiochemistry of water.

CONCLUSIONS

Bringing all these results together, one comes to the conclusion that both electrochemistry and chemiluminescence techniques have the ability to be used for indirect detection of gamma rays, and evaluating the dose rate of rays irradiated to watery substances. Comparing with the commercial methods these showed some advantages such as lower-price, simplicity, higher sensitivity, and lower detection limit. Therefore, the proposed methods would have such potency to be used as novel commercial detectors for reliable monitoring of gamma ray emitted from source ^{60}Co in aqueous samples.

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Conflict of interest: *Declared none.*

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