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Pretreatment of whole blood using hydrogen peroxide and UV irradiation. Design of the advanced oxidation process

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ABSTRACT

A new process to pretreat blood samples has been developed. This process combines the Advanced Oxidation Process (AOP) treatment (using H_2O_2 and UV irradiation) with acid deactivation of the enzyme catalase in blood. A four-cell reactor has been designed and built in house. The effect of pH on the AOP process has been investigated. The kinetics of the pretreatment process shows that at high $C_{H_2O_2,t=0}$, the reaction is zeroth order with respect to $C_{H_2O_2}$ and first order with respect to C_{blood}. The rate limiting process is photon flux from the UV lamp. Degradation of whole blood has been compared with that of pure hemoglobin samples. The AOP pretreatment of the blood samples has led to the subsequent determination of chromium and zinc concentrations in the samples using electrochemical methods.

1. Introduction

Chemical analysis of metals in blood samples is often used and actively studied [1–4]. The analysis of some metals in whole blood such as lead and cadmium by established methods require minimal preconcentration or pretreatment prior to analysis [5]. Some metals such as chromium, however, are often bound or complex with macromolecules and pretreatment of the sample is required prior to their detection and analysis [6–7]. The pretreatment process can vary in complexity, ranging from simple extractions [8] to online separations [9]. Many pretreatment processes such as microwave [10–11] and HPLC [9] are effective yet require costly instrumentation. Depending on the matrix and the specific metal to be analyzed, pretreatment can take multiple steps, requiring hours and even days to complete. There is an increasing need for fast and convenient pretreatment and detection of heavy metals in various samples [6,12–23].

The Advanced Oxidation Process (AOP) is a combination of hydrogen peroxide (H_2O_2) and ultraviolet (UV) radiation (or ozone) to generate hydroxyl radicals (•OH), and it has been used both for the decomposition of organic compounds [24–27] and in sample pretreatments [28–30]. This process decomposes organic matter in environmental and biological samples prior to chemical analysis [6,31–35]. It has also been demonstrated in the pretreatment of water samples [27,36–39].

There have been few studies of pretreatment of blood samples by AOP for chemical analysis. In a paper that reported pretreating a blood sample through oxidation processes, potassium persulfate ($K_2S_2O_8$) and UV irradiation were used after plasma and erythrocytes were separated and "de-proteinized" [35]. Another paper *recommended* the use of long UV irradiation and H_2O_2 in blood pretreatment but did not disclose an exact time or elaborate on details [28]. Blood is a complicated matrix and is often separated into its serum and cellular components. This, however, requires more tedious effort and time. Examination of whole blood is faster and easier. Catalase is an enzyme that decomposes hydrogen peroxide and protects the body from hydroxyl radicals [40]. The enzyme is, however, detrimental to the AOP treatment of blood samples and leads to vigorous foaming of the sample. Our group has recently used for the first time H_2O_2 and UV irradiation in an AOP process to pretreat whole blood in the detection of trace biological chromium [7].

AOP of blood may offer a new pretreatment method, simplifying the detection process and reducing cost for the analysis of metals in blood. Our earlier study of blood pretreatment was targeted toward chromium detection and limited in scope [7]. We have studied the pretreatment of whole blood by the AOP process in detail. A four-cell reactor has been designed and built to optimize the AOP process that uses 80% less blood in comparison to a single cell reactor [7]. Finally, the pretreated blood sample was examined using electrochemical analysis to detect the metals chromium and zinc. We report here our results and observations.

2. Experimental

2.1. Reagents and analytical instrumentation

Sodium hydroxide (NaOH, Certified ACS, Fisher), potassium oxalate ($K_2C_2O_4$, Certified ACS, Fisher), ferrous sulfate heptahydrate

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($FeSO_4 \cdot 7H_2O$, Acros), acetic acid (HAc, glacial, 99.9%, Fisher), sodium acetate (NaAc, anhydrous, Certified ACS, Sigma-Aldrich), potassium chloride (KCl, 100%, Mallinckrodt), porcine hemoglobin (Sigma), and HNO_3 (70%, Trace Metal Grade, Fisher) were used as received. Zinc AA standard (1000 mg L^{-1}, Certified ACS, Fisher) was diluted prior to use. Porcine blood samples were obtained from Wampler's Farm (Lenoir City, Tennessee). The blood was collected in 1-L bottles each containing 2 g of $K_2C_2O_4$ to prevent coagulation. Deionized water (18 MΩ cm) was used in the preparation of aqueous solutions.

UV–visible spectra were collected using an Agilent 8453 photodiode array spectrophotometer and a 2.0- or 10.0-mm quartz cuvette. Blank spectra of water were used as references. All glassware was soaked in 1 M HNO_3 and rinsed several times with deionized water prior to use. **Caution**: UV light can be dangerous. Wear eye protection.

Anodic stripping voltammetric (ASV) measurements were conducted using an Electrochemical Workstation 440/650A (CH Instruments, Inc.). The working electrode systems have been described previously, and Ag/AgCl and a platinum wire (CH Instruments, Inc.) were used as the reference and counter electrodes, respectively. Buffer solutions were prepared and used as described previously.

2.2. Exp. 1–4 — Pretreatment of whole blood by a single-cell reactor

The tests were carried out in a 20-mL reactor in Scheme 1a. The photoreactor consisted of an outer vessel containing the sample, water-jacketed quartz immersion well, and a UV lamp. The reactor was designed and built in house, except for the quartz immersion well (Ace Glass). The UV lamp was a 5.5-W quartz low-pressure cold cathode mercury gaseous discharge lamp (Ace Glass/Pen-Ray 12132-08) [41].

Samples were prepared either by adding 500 μL of whole blood to 15 mL of deionized H_2O or by diluting 32.5 mL of blood with 975 mL of H_2O, followed by adjusting pH to 3 by HNO_3. It was transferred into a plastic 1 L bottle and stored in a refrigerator until use. For each individual reaction, 15 mL of the solution was transferred into the reactor. H_2O_2 (30%) was then added so that $C_{H_2O_2,t=0}$=0.5–15.0 g L^{-1}.

Twenty samples from the 1 L bottle were used. The first ten samples were irradiated for 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 min, respectively. The remaining samples were irradiated for 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 min, respectively. After the irradiation, the samples were diluted by 12 fold before UV–visible analysis.

Exp. 2-4, Fenton processes with or without UV irradiation, are given in Supplementary data.

2.3. Exp. 5—The effect of irradiation on the sample with no added H_2O_2

The procedure was performed as in Exp. 1 except that no H_2O_2 was used and only the spectra at the beginning and the end of the irradiation were recorded.

2.4. Exp. 6—Pretreatment of pure hemoglobin by AOP

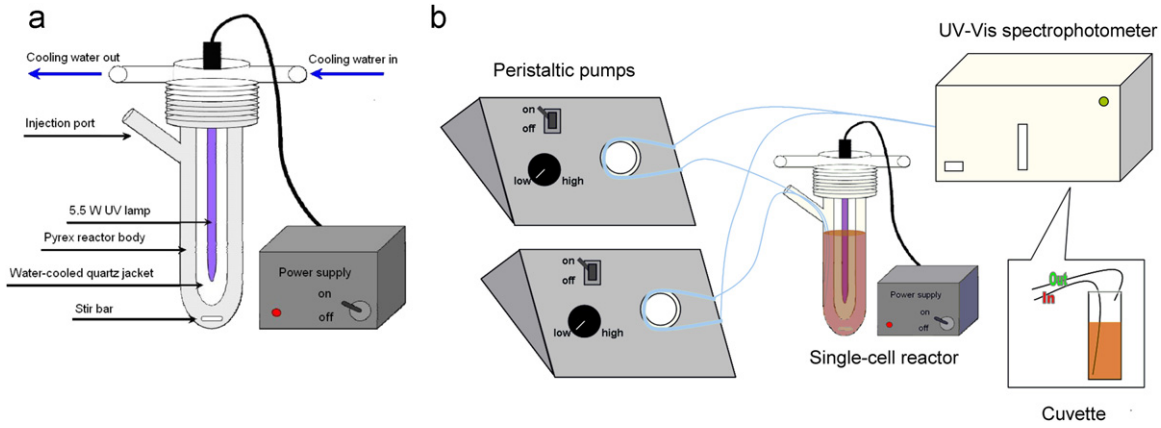
The solution was produced based on calculations of how much hemoglobin is in whole blood and in a diluted sample. Porcine hemoglobin (0.12 g) was dissolved in water (15 mL) and pH was adjusted to 3 using HNO_3. The experiment was conducted as in Exp. 1, except that only the spectra at the beginning and the end of the irradiation were recorded.

2.5. Kinetic studies of the pretreatment

The kinetics of AOP was studied using the same batch stock solution as described in Exp. 1, except that the setup in Scheme 1b containing a dual peristaltic pump system was used to continuously monitor the reaction and collect data by the UV–visible spectrophotometer. The following concentrations were studied in triplicate with $C_{H_2O_2,t=0}$=0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 7.5, 10.0, 13.0, and 15.0 g L^{-1}, respectively. The UV–visible data were collected every minute for a total of 60 min. In separate triplicate tests, samples with $C_{blood,t=0}$=250, 500, and 750 μL in 15 mL of solution, respectively, were used, while $C_{H_2O_2,t=0}$ varied from 3.0, 5.0, and 7.0 g L^{-1} for each blood concentration.

2.6. Four-cell reactor [41]

The reactor is made of a base that contains four stirrers fashioned from DC motors (Jameco Electronics). The stirrers were placed in a custom-made box and controls were added. The top of the box was designed to allow a water-cooled quartz immersion well (Ace Glass) to be inserted horizontally over the sample stirrers. A 100-W UV lamp (Hanovia) was then inserted into the immersion well that was placed inside an elliptical shaped cylinder. A wooden box was used to host the setup to contain any dangerous UV light. A power supply for the 100-W UV lamp was kept outside of the box. Cells for the reactor were made from square borosilicate tubing. The volume of each cell is 5 mL with a surface area of 6.45 cm^2. A photo and details about the four-cell reactor are provided in the Supplementary data.



Scheme 1. . Schematic of: (a) The single-cell AOP reactor for Exps. 1–6. (b) The setup using a UV–visible spectrophotometer to conduct the kinetic studies.

In a typical test, 3 mL of the 1-L batch stock solution was placed into each of the four cells. A stir bar was placed in each cell and all cells were transferred carefully into the reactor.

2.7. Analysis of Zn in an AOP-treated blood sample

Chromium analysis in the AOP-treated blood sample was conducted earlier [7], and the result is cited here.

Zinc analysis was carried out using a bimetallic mercury–bismuth (Hg–Bi)/single walled carbon nanotubes (SWNTs) composite electrode that we developed recently [42]. After AOP 10 mL of the sample was added to 10 mL of a buffer solution (pH 6.0) containing 0.1 M KCl and 0.1 M NaAc/HAc. ASV experiments were performed as described previously [42].

3. Results and discussion

The AOP reaction produces •OH radicals, a very potent oxidant, by Eq. 1 [24]. Subsequent attack of organic/biological species by the radicals gives water, CO_2, and free metal ions. A combination of the following processes contribute to the generation of •OH radicals and the overall efficiency of the method [43–49]. If $Fe^{2/3+}$ ions are present, they may catalyze the generation of the •OH radicals by the Fenton process as well as the photo-Fenton process [41].

$$H_2O_2 + h\nu\ (\leq 255\ \text{nm}) \rightarrow 2 \bullet OH \quad (1)$$

3.1. Pretreatment of blood—A comparison of different processes.

In blood, iron is part of proteins and there are generally no free $Fe^{2/3+}$ ions as catalyst [40]. UV irradiation of a blood solution containing H_2O_2 leads to decomposition of the proteins using the •OH radicals generated in Eq. 1. This initial step is a non-catalytic process. The decomposed proteins, however, release $Fe^{2/3+}$ ions that may then catalyze the subsequent reactions through the photo-Fenton reactions [41]. In other words, the AOP pretreatment of blood is an auto-catalytic process.

We have conducted studies to compare a direct photodissociation, Fenton process conducted without photodissociation, and the photo-Fenton process with an added iron catalyst. In Exp. 1, H_2O_2 was added to the sample and irradiated in a photochemical reactor designed in house (Scheme 1a) for a total of 60 min. A Pen-Ray 5.5-W UV lamp was used in the reactor. This lamp gives an intense peak at 254 nm that accounts for ca. 45.6% of the total intensity of the lamp [41]. This irradiation and possibly others in the 200–300 nm range leads to direct dissociation of H_2O_2 into •OH radicals [46,51–53]. In Exp. 2, the same amount of H_2O_2 was added, and the sample was kept in dark in the reactor for 60 min [41]. Exp. 3 was similar to Exp. 2 except that a small amount of $FeSO_4$ was used [41].

The results of Exp. 1 are shown in Fig. 1. The 300–500 nm range of the spectra reveals the peak of hemoglobins (ca. 380 nm) which essentially disappeared in 60 min. When the UV irradiation was removed in Exp. 2, little decomposition of the blood in dark was observed (Fig. S1) [41], indicating that auto-catalytic Fenton process relying on the release of iron from decomposed proteins was very slow. Subsequently, $FeSO_4$ was added to the sample to provide Fe^{2+} ions (Exp. 3), with no UV irradiation. Over a period of 60 min, the degree of decomposition in Exp. 3 was very small (Fig. S2) [41], in comparison to that in Exp. 1 (Fig. 1).

Exp. 4, with UV irradiation, was conducted as in Exp. 1 except that a small amount of $FeSO_4$ was added as catalyst. To our surprise, this process was slower than that in Exp. 1 [41]. After 60 min, the peak at 380 nm was still significant (Fig. 2 and Fig. S3). Perhaps the added Fe^{2+} ions bind to the organic/biological species in blood, reducing their catalytic capacity. A control test (Exp. 5) using direct UV irradiation *without* H_2O_2 (Fig. S4) showed that the decomposition of the blood by UV alone is negligible [41].

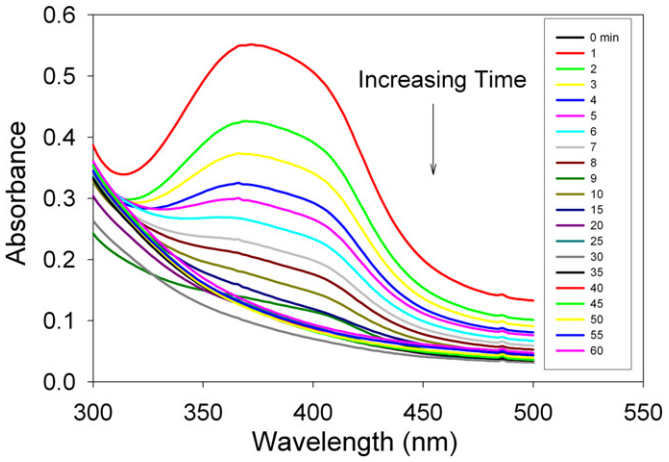


Fig. 1. UV–visible spectra of blood in the AOP pretreatment in Exp. 1, as shown by the peak at 380 nm. $C_{H_2O_2} = 1.5\ g\ L^{-1}$.

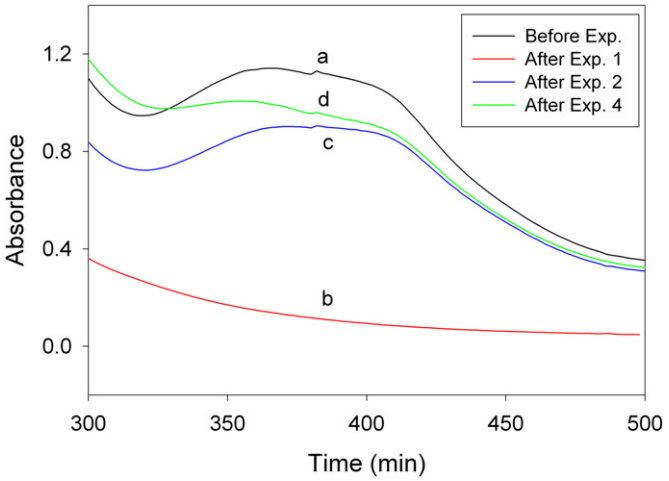


Fig. 2. A comparison of the results of (a) before experiments; (b) after Exp. 1; (c) after Exp. 2; (d) after Exp. 4. $C_{H_2O_2} = 1.5\ g\ L^{-1}$.

The results from Exps. 1–5 clearly show that Exp. 1 is the best process. The observation that the added Fe^{2+} ions in Exp. 4 did not increase the rate suggests that, except at very high $C_{H_2O_2,t=0}$ discussed below, Fenton or photo-Fenton process does not play a leading role in the AOP of blood using UV irradiation at room temperature. The principal pathway is apparently the generation of •OH radicals from H_2O_2 in the mixture by Eq. (1) which then decompose the blood. The reasons for the lack of the Fenton catalysis at the early stage of the AOP process in the current work are not clear. We speculate that the chemical and biological species in fresh blood bind to the $Fe^{2/3+}$ ions, preventing them from catalyzing the conversion of H_2O_2 to •OH radicals. At this early stage, the AOP process perhaps relies on the •OH radicals generated by the direct photolysis of H_2O_2 in Eq. 1. After the AOP process has proceeded for a while, the chemical and biological species in the blood have significantly decomposed, and their abilities to bind to $Fe^{2/3+}$ ions have weakened. The newly released $Fe^{2/3+}$ ions will then catalyze subsequent blood decomposition.

In all the tests, HNO_3 was added to the samples to lower pH to 3 prior to the addition of H_2O_2 [7]. This step inhibited the enzyme catalase so that, when H_2O_2 was added to a diluted sample, no

vigorous foaming due to the decomposition of H_2O_2 would occur. The selection of HNO_3 over other compounds to inhibit catalase has been discussed previously [7]. H_2SO_4 and HCl have been studied as well, and both performed similarly to HNO_3, although it appeared that HCl was not quite as effective. Some initial foaming was observed.

Studies were conducted to confirm that the peak at 380 nm which was monitored in Exps. 1-5 was indeed that of hemoglobin [54]. The confirmation was also important to the kinetic studies below. In Exp. 6, a solution of solely porcine hemoglobin was treated with H_2O_2 and UV irradiation, as in Exp. 1. The results (Fig. S5) show that the 380 nm peak is indeed essentially that of hemoglobin, and its decomposition is similar to that of whole blood in Fig. 1 [41]. Blood is a complex mixture of many chemical and biological components, and there is no good method to follow the decomposition of every component in blood. Hemoglobin is a major component of blood, and its decomposition provides a simple, direct means to follow the blood decomposition.

3.2. Kinetics of blood pretreatment by AOP

An understanding of the kinetics of the pretreatment process would provide basic kinetic parameters such as rates and orders with respect to reactants, and help to better design the process itself. The kinetics of AOP processes for numerous organic compounds has been studied, including the development of a model by Crittenden and coworkers [36]. Zeroth-order [24,27], first-order [55–57], pseudo-first order [58], and second-order [43,59–61] kinetics have been reported.

The H_2O_2 photolysis itself is the initial step in an AOP process with a quantum efficiency of 0.98 at 254 nm [46,59–62]. The analysis in the Supplementary data shows that, if the concentration of H_2O_2 is high and the absorption by water in the UV region is ignored, the limiting reagent is the photon flux of the UV light source (rate of photon emission). Such photochemical dissociation of H_2O_2 follows zeroth-order kinetics. If the concentration of H_2O_2 ($C_{H_2O_2}$) is not high, such photochemical dissociation of H_2O_2 follows first-order kinetics [41]. Thus the limiting kinetic orders for the H_2O_2 photolysis itself are zero when $C_{H_2O_2}$ is high and first when $C_{H_2O_2}$ is not high.

There are challenges in studying the kinetics of the AOP pretreatment of blood by H_2O_2. Blood is a very complex matrix. At any given time of the AOP process, numerous species within the blood could be decomposing. For the sake of simplicity, hemoglobin is used to represent the components in blood (C_{blood}). A decrease in absorbance of hemoglobin at 380 nm indicates the blood decomposition, as shown in Exp. 6. However, using kinetic laws designed for specific reactants for a complex mixture of numerous species in blood may lead to significant deviation from the kinetic laws. In addition, blood absorbs in the UV range (Figs. 1 and 2). Although Exp. 5 revealed that the blood decomposition by direct UV irradiation is negligible [41], the absorption of blood in the UV range reduces the number of photons for the photolysis of H_2O_2, affecting the kinetics of the process.

In order to monitor the process, the solution in the photochemical reactor was circulated through a cuvette in a UV–visible spectrophotometer by a dual peristaltic pump system (Scheme 1b). When $C_{H_2O_2}$ is sufficiently high, the rate law can be expressed as

$$-(dC_{blood}/\,dt) = Rate = kC_{blood}^{\,y} \quad (2)$$

where y = order with respect C_{blood}; k = rate constant.

In order to determine y, ln$A$ vs. t are plotted (Fig. 3). When $C_{H_2O_2,t=0}$ = 1.0–2.5 g L^{-1}, the plots significantly deviate from being linear, suggesting that $C_{H_2O_2,t=0}$ here is perhaps too low to make the reaction zeroth-order with respect to the concentration of H_2O_2. At $C_{H_2O_2,t=0}$ = 3.0, 4.0, and 5.0 g L^{-1}, the ln A vs. t plots are essentially linear with nearly identical slopes of −0.0230 (R^2 = 0.996), −0.0241 (R^2 = 0.996), and −0.0235 (R^2 = 0.990), respectively. These results suggest that the reactions have reached a rate-limiting stage at these high $C_{H_2O_2,t=0}$. In addition, at this rate-limiting stage, the blood decomposition itself follows first-order kinetics. In other words, y = 1 and the rate law in Eq. (2) becomes

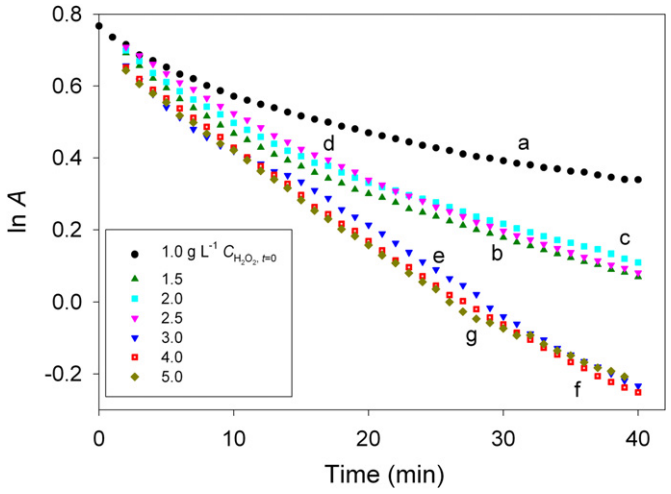


Fig. 3. Plots of ln$A$ vs. t for the pretreatment of the blood samples.

Table 1
Additional rate constants k.

$C_{H_2O_2,t=0}$ (g L^{-1})	$C_{blood,t=0}$		
	250 μL*	500 μL*	750 μL*
3.0	0.0203 (18)	0.0221 (7)	0.0204(12)
5.0	0.0232 (65)	0.0236 (11)	0.0200 (4)
7.0	0.0201 (23)	0.0195 (21)	0.0222 (7)

* $C_{blood,t=0}$ here refers to the volume of the blood in 15 mL of solution.

$$-(dC_{blood}/\,dt) = Rate = kC_{blood} \quad (3)$$

where the observed rate constant k = 0.0230–0.0241 min^{-1}.

Additional kinetic studies were performed in which the $C_{blood,t=0}$ were changed from 250, 500, and 750 μL in 15 mL of solution, respectively, while $C_{H_2O_2,t=0}$ was changed from 3.0, 5.0, to 7.0 g L^{-1}. First-order kinetic relationships were again observed. k from these studies (Table 1), with the average k = 0.0216(18) min^{-1}, are close to those obtained in Fig. 3. The fact that the rate reaches a rate-limiting stage at $C_{H_2O_2,t=0}$ = 3.0–7.0 g L^{-1} indicates that, at the high concentrations of H_2O_2, the reaction rate is limited by the photon flux reaching the solution that decomposes H_2O_2 to the •OH radicals. In other words, the decomposition of H_2O_2 has reached a steady-state with a constant rate of the formation of the •OH radicals such that Eq. (2) appropriates to Eq. (3).

Additional tests using $C_{H_2O_2,t=0}$ = 7.5–15 g L^{-1} show that the treatment by Exp. 1 is faster than that at $C_{H_2O_2,t=0}$ = 3.0–7.0 g L^{-1}. At such high concentrations of H_2O_2, the catalytic role played by the iron ions released from the decomposition of proteins is perhaps more significant. In other words, both direct AOP and the photo-Fenton process are involved in the decomposition of the blood here. It should be pointed out, however, too high H_2O_2 concentrations tend to scavenge the •OH radicals, rendering the process less effective [63].

3.3. Four-cell reactor

After extensive studies using the original reactor, our group designed and built a four-cell reactor, well suited for small sample volumes. Additionally multiple samples can be run simultaneously

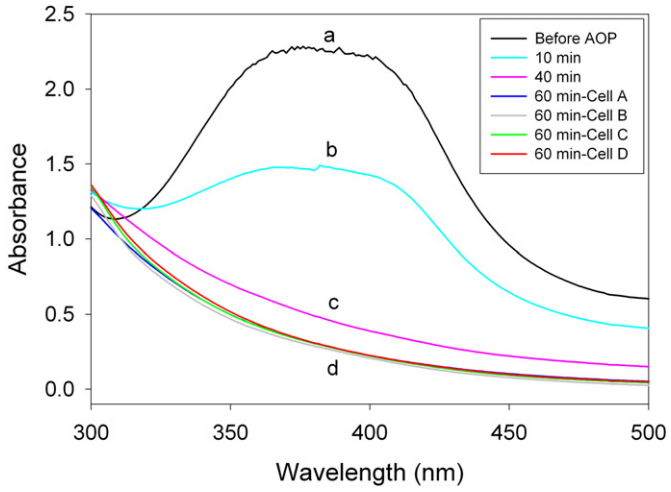


Fig. 4. A comparison of the four cells in the reactor: (a) Before AOP; (b) 10 min; (c) 40 min; (d) 60 min. NaOH as a base was added at 40 min which is discussed below.

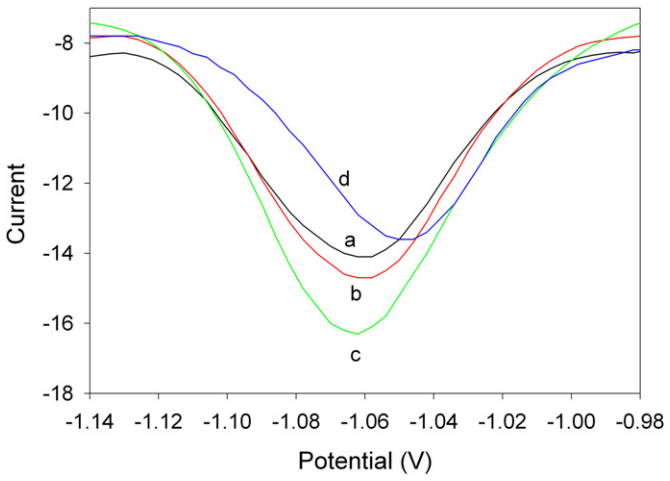


Fig. 5. ASV of Zn in standard solutions (a) 5, (b) 7, and (c) 10 ppb; (d) represents detection in a blood sample after AOP.

[41]. The reactor uses a 100-W UV lamp and four 5-mL cells. An aluminum mirror in the shape of an elliptical cylinder was made with a portion of its bottom open to focus the light from the lamp to the four cells underneath [41].

The spectrum of the lamp reveals several emission lines in the 200–300 nm range that together account for 14.2% of the total intensity [41]. Thus each cell on average receives ca. 3.6 W irradiation in 200–300 nm, the range leading to direct H_2O_2 dissociation into the •OH radicals [45, 50–52]. In comparison, UV irradiation of the 5.5-W lamp in the single-cell reactor is centered at 254 nm [41], accounting for ca. 45.6% (2.5 W) of the total irradiation with 100% quantum efficiency. [The irradiation in the 200–300 nm range accounts for 46.3% (or 2.5 W) of the total.] Thus the reaction rates in the single- and four-cell reactors are not expected to be significantly different.

Results of tests using the four-cell reactor are given in Fig. 4. First, only 97 μL of blood was needed per cell, and the solution volume was 3 mL. In comparison, 500 μL of the blood was needed for the single-cell reactor, and the solution volume was 15 mL. Second, the reactions in the four cells (Fig. 4) proceeded at rates comparable to that in the single-cell reactor, and the process was nearly complete in 40 min, as expected from the analysis of the irradiation from both lamps in the 200–300 nm range. Third, the reactions in each of the four cells proceeded at similar rates (Fig. 4).

3.4. Effect of pH

The AOP of blood needs to start at pH 3 in order to inhibit catalase in blood [40]. Our earlier studies show that AOP is optimal at higher pH [7]. The AOP treatment of chromium(III) propionate, for example, produces protons from both decomposition of the ligand and the Cr(III) to Cr(VI) conversion [6,41]. It is expected that degradation of biological/organic species in blood, generating CO_2 and protons, also lowers pH [41]. Thus, after the AOP process has proceeded sufficiently to decompose catalase, raising pH of the solution at this point to neutralize the acids speeds up the process. After reactions had proceeded for 40 min, raising pH to 9.5 led to additional degradation of the sample (Fig. S6) [41]. If NaOH is added before 40 min, foaming occurs indicating that catalase has not fully decomposed.

3.5. Electrochemical analysis of Cr and Zn in AOP-treated blood samples

Chromium has been studied for its use as a dietary supplement for the treatment of diabetes and its complications, but since levels are extremely low in biological tissues and fluids (<10 ppb in blood of mammals), many difficulties arise during detection. Current analytical techniques with the adequate sensitivity require expensive instrumentation and are thus not widely available. Our group developed a method to use adsorptive stripping voltammetry (CAdSV) for Cr analysis in the AOP-treated blood sample. In the porcine blood sample, the Cr concentration was 6.0(0.3) ppb [7].

It was well established over 100 years ago that zinc is an essential nutrient [64]. Zinc deficiency has been found detrimental in physical growth and bodily function [64]. Low zinc dietary intake has been shown to effect one-third to one-half of the world's population [64]. High zinc levels, however, can be toxic [64]. Zinc level has been analyzed by various methods including electrochemistry which has been shown to be highly sensitive [42,65].

Zn concentration in our AOP-treated blood sample was found to be 282(8) ppb, and the result was consistent with that from the standard dry ashing, followed by an ICP analysis which has been published elsewhere [66]. The results of the Zn detection using ASV are shown in Fig. 5. A calibration range of 5–10 ppb (correlation coefficient $R=0.995$) in a buffer solution (pH 6.0) containing 0.1 M KCl and 0.1 M NaAc/HAc was used to obtain the Zn concentration in the AOP sample [42]. The Zn peak shifted slightly more positive, most likely due to matrix effects of the residues in the AOP-treated sample which has been witnessed other detections [7,67]. While other metals are found in blood they are either at a much lower concentration or are reduced at a different potential and therefore not interfering with zinc [42,66]. Additionally, the electrode used in this analysis found minimal interference regarding zinc detection [42]. While Ru(III) and Rh(III) showed slight interference, neither of these metals are normally found in whole blood [66].

4. Conclusions

The decomposition of blood by AOP has been studied in detail. The studies give an insight into the processes taking place during the pretreatment. The rate limiting process is photon flux from the lamp when $C_{H_2O_2,t=0}$ is sufficiently high. The reaction is zeroth-order with respect to $C_{H_2O_2}$ and first-order with respect to C_{blood}. The four-cell reactor uses much less blood and allows several samples to be treated simultaneously. The blood pretreatment is interdisciplinary in nature involving, e.g., photochemistry of the inorganic chemical H_2O_2, degradation of biological/organic species in blood by •OH radicals, kinetics of the reactions, and the design of new reactors for the pretreatment. To confirm the success of the procedure, Cr and Zn

in blood samples were detected. This procedure developed in the current work leads to the successful decomposition of biological/organic species in blood.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.04.004.

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