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A Voltammetric Sensor Based on Iodine-Coated Platinum Electrode for Determination of Iron in Blood Serum

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Abstract- A novel, simple, convenient, inexpensive and green voltammetric sensing for iron in human blood serum was developed. The method is based on linear sweep voltammetry at an iodine-coated polycrystalline platinum electrode. A miniaturized home-made small scale 0.5-mL cell was used for the analysis. Oxidation of iron at the iodine-coated platinum electrode was manifested by a peak centered at 0.5 V. The established calibration curve for the relationship between iron (II) concentration and current extracted from the linear sweep voltammograms revealed an excellent linearity(R^2=0.9923). The calculated lowest detection limit, LOD, is 0.26 ppm and the limit of quantification is 0.87 ppm. Recovery studies for a concentration of 1.0 ppm yielded a value of 1.02 ppm (102% percent recovery). No interference with other common essential elements in blood serum was observed which attests to the suitability and accuracy of the method. The developed method was applied to analysis of real blood samples. The results of analysis were compared with the results from conventional standard methods used in medical laboratories where an excellent agreement between the two analytical methods was observed.

Keywords- Voltammetric sensors, Iodine-coated platinum electrode, Serium analysis, Iron determination in blood

1. INTRODUCTION

Various methods for iron analysis in blood serum have been developed including atomic absorption spectrometry [1-5], spectrophotometry [6], colorimetric [7] flow injection [8], ICP-MS [9,10] , ratiometric fluorescence [11], and gold nanoclusters sensing[12]. The quest, however, for a simple, rapid, accurate, precise, user-friendly and low-cost method in terms of reagents and instrumentation for determination of iron in blood serum is timely in the light of expansive use of home analysis devices.

In this regard, electrochemical analysis techniques offer simpler instrumentation, low dependence on special reagents, simplicity of procedures and sample pretreatment [13]. Iodine-coated platinum electrodes offer all the advantages of a typical modified electrode since the monolayer of adsorbed iodine suppresses molecular and ionic adsorption on the highly reactive platinum electrode.

For these reasons iodine-coated platinum electrodes lend themselves for several analytical applications [14-19].

The present work aims at developing a voltammetric sensor for determination of iron in blood serum based on iodine-coated platinum electrode in a miniaturized electrochemical cell. Miniaturization of the cell and the electrode is a requirement in the present work since the provided blood samples for analysis are usually small.

2. EXPERIMENTAL

2.1. Instruments, cell and materials

A PAR potentiostat (model 362, EG & G) interfaced to a computer via GPIB interface (IEEE) data acquisition was used. Locally modified Labview® (IEEE) software was used for data acquisition. A home-made one-compartment small scale (≈0.5-cm^3) electrochemical cell with one inlet/outlet for gas purging and blanketing with oxygen-free nitrogen was used. The working electrode, the reference and the auxiliary electrodes were installed in a PTFE cover which can be easily placed and removed on the miniaturized cell containing the sample. The working electrode was a 0.5 mm polycrystalline platinum wire purchased from Aldrich (99.99% minimum purity certified reagent). The immersed end of the platinum electrode was curved at the end to a U-shape to make a mark for consistent surface area of the immersed part of the electrode. A silver/silver chloride electrode was used as a quasi-reference electrode. The auxiliary electrode was a 0.5 mm polycrystalline platinum wire (Aldrich, certified 99.99% minimum purity).

All reagents used were analytical grade and used as received from the suppliers without further purification. Sulfuric acid (95-97%) was supplied from Merck, Nitric acid (69.5%) was supplied from Scharlau and potassium iodide was purchased from Sigma-Aldrich. Iron(II) sulfate heptahydrate was purchased from AppliChem (USA). Hydroxylammonium

chloride was an AnalaR Normapur®ACS reagent (BDH). The nitrogen gas was a G5 grade (99.999% minimum purity) supplied from The International Industrial & Medical Liquid gases Company (Sahab, Jordan).

2.2. Procedures

2.2.1. Preparation of iodine-coated platinum electrode

Verification of the cleanliness of the platinum electrode is very critical step in any modification of the electrode surface. Cleanliness of the electrode surface was verified by reproducing the well-known voltammogram of polycrystalline platinum electrode. The distinct peaks attributed to hydrogen adsorption and desorption at one hand and the distinct features attributed to oxygen adsorption and desorption attest to the cleanliness of the electrode surface.

Platinum surface was potentiostatically coated at 0.20 V (in the double layer region) with iodine by immersion of the platinum electrode in 0.5 M H_2SO_4 containing 1×10^{-2} M KI for five minutes. The electrode was extensively rinsed with iodine–free 0.5 M H_2SO_4. The cyclic voltammogram for iodine–coated platinum electrode was recorded between -0.25 V and 0.80 V. The absence of all voltammetric features is a manifestation of absence of molecular adsorption and passivity of the electrode (Figure 1).

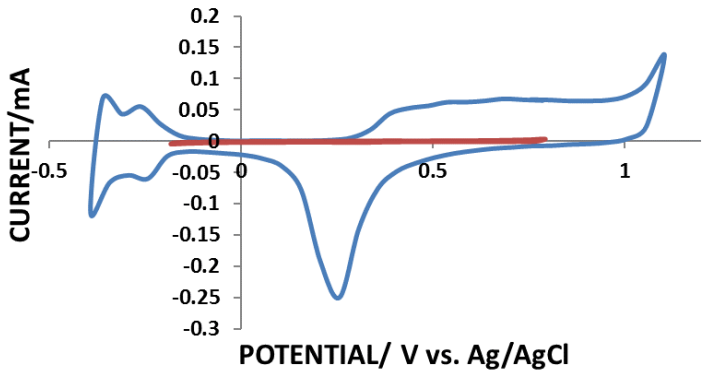

Fig. 1. The cyclic voltammogram of (A) polycrystalline platinum electrode and (B) the same electrode after adsorption of iodine from 0.50 M+1.0×10^{-2} M KI solution. Both i-E scans were recorded in iodine-free 0.5 M H_2SO_4 at a scan rate of 50 mV/s

2.3. Sample preparation

Blood serum samples of different iron concentration were brought from Princess Haya Bint Al- Hussein Military hospital, Jordan. The samples were stored in a refrigerator at 2-8 °C until analyzed.

A volume of 0.1 mL of human blood serum was injected in the above-mentioned electrochemical cell. A 0.10 mL of 1.0 M HNO_3 solution was added to liberate ferric ions. The solution was allowed to rest for 3 min to complete protein digestion. Few crystals of hydroxylamine (NH_2OH) were added to reduce the ferric to ferrous ions. The cell was manually swirled to homogenize the contents of the cell followed by application of a potential scan between -0.2 and 0.80 V *vs*. the quasi-reversible electrode (QRE).

3. RESULTS AND DISCUSSION

3.1. Method analytical parameters

Figure 2 shows a set of voltammograms for a 0.5 M H_2SO_4 solutions with different Fe(II) concentrations. The anodic peak nearly centered at ca. 0.5 V is attributed to oxidation of iron(II) ions. The calibration curve was constructed by plotting the anodic peak currents extracted from the linear sweep voltammograms against the concentration of Fe(II) in the solution. The concentrations involved in establishing the calibration curve ranged from 0.5 to 50 ppm established calibration curve for Fe(II) which exceeds the expected concentration of Fe(II) in blood serum (Figures 3).

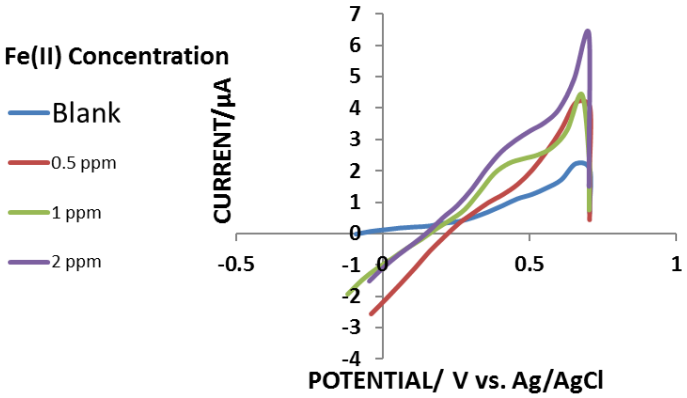

Fig. 2. The cyclic voltammogram of iodine –coated electrode recorded in 0.5 M H_2SO_4 solution containing: (—) blank, (—) 0.5 ppm Fe(II), (—) 1 ppm Fe(II), and (—) 2 ppm Fe(II). Scan rate=50 mV/sec

The calibration curve shows an excellent linearity (R^2=0.992) and the calibration equation is

$$i\ (\mu A)=0.6363C_{Fe(II)}+0.1838$$

Where i is the anodic peak current attributed to oxidation of iron(II) in microampere, C_{Fe} is the concentration of iron expressed in parts per million. The instrumental precision was estimated by recording the voltammograms for ten aliquots taken from 1.0 ppm Fe(II)

solution. The coefficient of variation of these runs was 2.78% which attests to the high precision of the developed method.

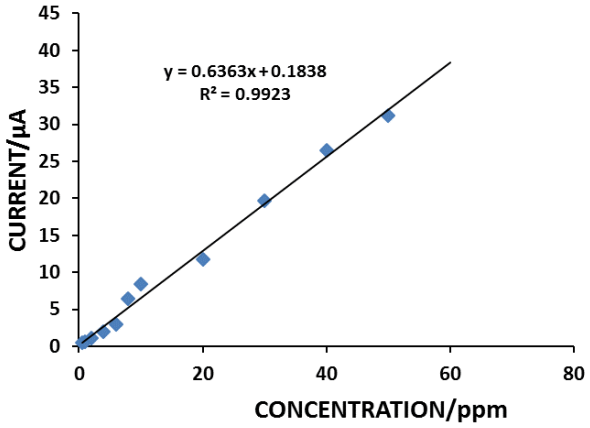

Fig. 3. An extended calibration curve shows the relationship between Fe(II) concentration and the anodic peak current measured from the voltammograms for Fe(II) in 0.50 M H_2SO_4 at iodine -coated platinum electrode. Scan rate=50 mV/s

The limit of detection (LOD) based on S/N=3 and limit of quantitation (LOQ) based on S/N=10 were calculated and there numerical values are 0.26 and 0.87 ppm respectively.

3.2. Recovery

Recovery study was performed on 1.0 ppm of iron(II) standard solution following the same procedure for analysis blood serum. The recovered concentrations were 0.93, 1.06, and 1.07 with average of 1.02 ppm (102% percent recovery) with a standard deviation of 0.08 ppm. The 95% confidence limits are 1.02±0.2 and the confidence interval is 0.82–1.22. Since the true value falls within the confidence interval, no determinate error is indicated at the 95% confidence level which supports the accuracy of the developed method.

3.3. Interference

The above-mentioned recovery experiment can be taken also as an evidence for the absence of interference. The recovered value was very close to the real value despite the fact that blood serum contains a large number of ions including sodium, magnesium, zinc, selenium, copper, and calcium which are common essential minerals in blood [20]. Copper is the only ion which shows a response at the iodine-coated platinum electrode but fortunately at different peak potential.

3.4. Analysis of real blood serum samples

The developed method was applied for determination iron concentration in blood serum samples. Figure 4 shows a representative cyclic voltammogram for human blood serum. Standard addition method was used for determination of iron(II).

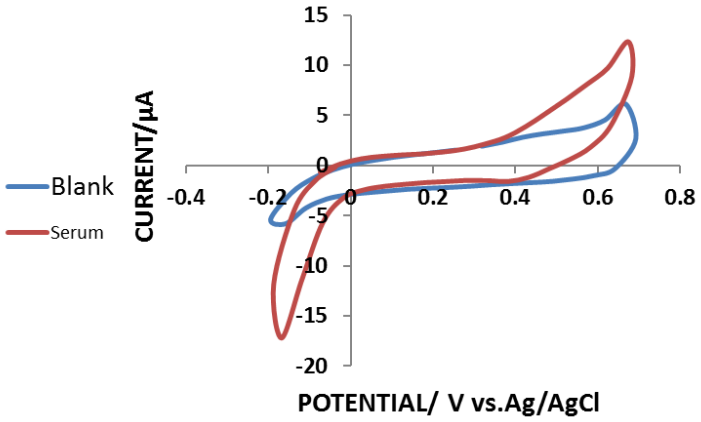

Fig. 4. Cyclic voltammograms of an iodine-coated platinum electrode in human blood serum sample after the treatment with 1 M HNO_3 and a few crystals of hydroxylamine

Concentration in the collected bloodserum samples and compared with the standard clinical results obtained by using Cobas C 311 diagnostic analyzer (Table1).The calculated confidence limits and confidence interval show that the analysis results using the standard clinical method fall within the confidence interval around the mean Table 1 The results of assaying blood serum samples for their iron content by voltammetric analysis at iodine-coated platinum electrode compared with the results of a standard clinical method.

Table 1. The results of assaying blood serum samples for their iron content by voltammetric analysis at iodine-coated platinum electrode compared with the results of a standard clinical method

Human blood serum samples	Iron Concentration (ppm)		Standard deviation	95% Confidence limits	95% Confidence interval	Percent bias	Coefficient of variation
	standard clinical method*	Linear Sweep Voltammetry**					
A	1.06	1.04 ±0.044	±0.044	1.04 ±0.109	0.931–1.149	- 1.9%	4.23%
B	0.26	0.258±0.017	±0.017	0.258±0.042	0.216–0.30	-0.8%	6.60%
C	0.80	0.805±0.035	±0.035	0.805±0.087	0.718–0.892	+0.6%	4.35%

*Samples analyzed using the standard methodology for blood analysis using Cobas c 311 diagnostic analyzer
**The average of three linear sweep experiments at iodine-coated platinum electrode (The developed method)

of results obtained by linear sweep voltammetry at the iodine-coated platinum electrode indicating the absence of determinate errors in the analysis results. The low values of the coefficient of variation (4.23-6.60%) are an indication of the high precision of the developed method. The application of paired t- test for a significant difference between the means of the two analytical methods resulted in a calculated t value of -0.02. The critical t value (for two degrees of freedom from t tables is 4.30 at p=0.05 [21]. Since the calculated t value is less than the $t_{critical}$ at p=0.05 the null hypothesis is true at (p=0.05) and no significant difference between the two means for the results obtained by application of the two methods which attests to the accuracy and applicability of the developed method to real blood serum samples.

The advantages of the developed voltammetric method are the simplicity of analysis procedures, inexpensive instrumentation, and short time of analysis. A comparison between the developed method and some common methods for analysis of iron is given in Table 2.

Table 2. Analytical parameters of the developed method compared to some common method for analysis of iron in blood serum

Method	**Linear Range**	**LOD**	**Matrix**	**Recovery**	**Pretreatment**	**Ref.**
Atomic Absorption Spectrometry	Up to 60 µg/L	4 µg/L	Serun	95-107%	yes	[5]
Inductive coupled plasma-mass spectrometry	10-50 µg/L	4.3×10^{-1} µg/L	Serum	>%90	yes	[9]
A fluorescence ratiometric	0-12.12 mg/L	48.48 µg/L	Serum	-	Yes	[11]
Capillary electrophoresis	0.2-2.35 mg/L	1.96×10^{-6} µg/L	Serum	-90.64 %110.57	Yes	[22]
Cyclic voltammetry	0.5-50 mg/L	0.26 mg/L	Serum	107.0-93.0%	Yes	This work

4. CONCLUSION

The present work is proved on the notion that iodine-coated platinum electrode can be used as a basis for a voltammetric sensor for determination of iron in blood serum. Another advantage of the present work is the use of a very small cell adapted for small samples which proved the applicability of using electrochemical sensor as a chemical sensor for serum iron

determination. A simply prepared iodine–coated polycrystalline platinum electrode combined with cyclic voltammetry technique with its well-known simple instrumentation and simple digestion procedure was successfully applied for determination of iron content in human blood serum samples. As well, the absence of any interference, and the lack of need for expensive reagents may pave the road towards hand-held devices for measurement of blood iron.

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