DEVELOPMENT AND VALIDATION OF DIFFERENTIAL PULSE ANODIC STRIPPING VOLTAMMETRIC ANALYSIS OF SELENIUM (IV) IN BULK AND IN DOSAGE FORMULATIONS AT A GOLD ELECTRODE MULTI-MODIFIED WITH A MIXTURE OF 3, 3′-DIAMINOBENZIDINE.4HCL AND VITAMIN E

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ABSTRACT

Objective: A simple, direct and very easy differential pulse anodic stripping voltammetric analysis (DPASVA) of selenium (IV) in bulk and in dosage formulations using a gold electrode multi-modified with a mixture of 3,3′-diaminobenzidine.4HCl and vitamin E (Vitamin E-Nafion (AuEMD-VN)) has been studied.

Methods: The method involves the study of various parameters (electrolyte, deposition time, pulse duration, pulse amplitude, etc.) affecting the Se(IV) determination. The proposed method was validated for specificity, linearity, precision and accuracy, repeatability, sensitivity (LOD and LOQ), robustness and solution stability with an average recovery of 98.1-100.32%.

Results: Se(IV) was determined in an aqueous HClO₄ (0.2M) medium of pH 0.22 at an accumulation potential of -250 mV and an accumulation time of 200 s for C(Se(IV)) at 1x10⁻⁹ to 1x10⁻⁶ mol/l (0.79 ng/ml to 79 ng/ml) and 350 s for C(Se(IV)) at 1x10⁻⁹ to 1x10⁻⁵ mol/l (0.07896-0.7896 ng/ml) with relative standard deviations (RSD%) 5.2.4% and 4.8% respectively.

Conclusion: This method showed very sensitive results for the determination of Se(IV) using an AuEMD-VN than that obtained using the individual Dₐ or Vₐ. This method could be applicable for the quantitative determination of the bulk Se(IV) as well as dosage formulations.

Keywords: Multi-Modified, Vitamin E, 3,3′-Diaminobenzidine.4HCl, Nafion, Se(IV), Differential pulse anodic stripping voltammetry

INTRODUCTION

Electrochemical sensor based on gold nanoparticle (AuNP) modified glassy carbon electrode for the determination of the Se in water to the limit of 0.64 μg/l using square wave anodic stripping voltammetry (ASV) was studied [1]. A procedure of Se(IV) determination by ASV using two deposition and stripping steps at gold electrodes was proposed. A linear relationship was observed in the range from 5x10⁻⁹ to 1x10⁻⁷ mol/l [2]. A new, simple, and reproducible method is described for the determination of Se (IV) based on differential pulse cathodic stripping voltammetry (DPCSV). The calibration graph is linear up to at least 40 ng/ml of Se(IV) with a relative standard deviation (RSD%) of 2.7% for 20 ng/ml (n=5). Electrochemical sensor with the renewable silver annular band working electrode and deviation (RSD%) of 2.7% for 20 ng/ml (n=5) has been studied. Electrochemical determination of Se(IV) in real water samples was applied [4]. The performance of a poly(1,8-diaminonaphthalene)-modified gold electrode for the determination of the Se(IV) ion in an aqueous medium was investigated with anodic stripping voltammetry. The detection limit employing the anodic stripping differential pulse voltammetry was 9.0x10⁻¹⁰ M for Se(IV) with 4.4% of RSD% [5]. DPCSV determination of Se (IV) from pharmaceutical products was applied. The Se was determined in the range 8 to 64 ng/ml in pharmaceutical products [6]. Electro polymerization of 3,3′-diaminobenzidine (Dₐ) on a gold surface gave an adherent, stable film of poly(3,3′-diaminobenzidin tongues). The optimized method for the continuous flow mode had a detection limit of 5.6x10⁻¹⁰ mol/l of Te(IV) for 10 min preconcentration [7]. Determination of Se(IV) was investigated on 3,3′-diaminobenzidine/Nafion/mercury film modified glass carbon electrode. The analytical signal using cathodic square wave stripping voltammetry (SWSV) was linear from 1 to 300 μg/l with 5 min accumulation [8]. Determination of Se by pulse anodic stripping voltammetry with constant amplitude of negative polarity [9] and differential pulse anodic stripping voltammetric analysis (DPASVA) [10] using a Vitamin E-Nafion modified gold electrode in an aqueous HClO₄ medium (pH=1.1) at an accumulation potential (Pₐ) of -240 mV and an accumulation time (tₐ) of 300 s has been studied. The analytical signal was linear from the concentration ranges of 1x10⁻⁹ to 8x10⁻⁸ mol/l with RSD% 5.2% for 200 s. Liner calibration curve was obtained in the concentration ranges of 5x10⁻⁶ M to 2x10⁻⁴ M with RSD% 5.4% respectively.

In the present work, Differential pulse anodic stripping voltammetric analysis (DPASVA) of Se(IV) using an AuEMD-VN has been applied. This method showed very sensitive results for the determination of Se(IV) than that obtained using the individual Dₐ or Vₐ. This method could be applicable for the quantitative determination of the bulk Se(IV) as well as dosage formulation.

MATERIALS AND METHODS

Chemicals

Nafion perfluorinated ion-exchange resin in ethanol (3% v/v) was purchased from Aldrich. Vitamin E, molecular weight 430.72 g/mol (Scheme 1) was from BASF/Germany. 3,3′-diaminobenzidine.

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Scheme 1: Vitamin E(DL-α-tocopherol), C₂₉H₄₀O₄₂V₇

A stock solutions (a) and (b) of Se(VI) 789.6 µg/ml [0.01 mol/l] and 7.896 µg/ml [0.1 mmol/l] were prepared using HClO₄ solution 0.20M (pH=0.22). All working solution for voltammetric investigations were prepared by dilution of the stock solutions of Se(VI) (a or b) with HClO₄ solution.

Scheme 2: 3,3'-Diaminobenzidine.4HCl.2H₂O, C₇H₈N₂·4HCl·2H₂O, Da

Effect of pH solution
Effect of pH on differential pulse anodic stripping voltammograms of Se(VI) using AuEMDₐVₛN was studied. It was found that the best pH solution 2.22.

Effect of modified electrode composition
The effect of the Nafion, Dₐ and Vₛ concentrations in modified solution for formation AuEMDₐVₛN shows that the peak potential shifted slightly from 995 mV to 1010 mV and the sensitivity increased (CₐVₛN≥1x10⁻⁹ mol/l). While the sensitivity by using modified electrode with Dₐ or Vₛ only not reached less than 5x10⁻¹⁰ mol/l.

Effect of the accumulation potential
The dependence of the differential pulse anodic stripping peak current on the accumulation potential was examined. It was found that the maximum response for Se (IV) occurs with accumulation potentials equal to -0.250 V.

Effect of accumulation time (tₐ)
The dependence of the peak current on the accumulation time for Se(VI) concentrations was studied. The peak current increases with increasing accumulation time. The current is nearly linear from 50 to 450 s. The best time was 200 s for Se(VI) concentrations 1x10⁻⁶ -1x10⁻⁸ mol/l and 350 s for Se(VI) concentrations 1x10⁻⁵ -1x10⁻⁴ mol/l.
Various parameters (electrolyte, accumulation time, accumulation potential, pH solution, scan rate, waiting time, stirring speed of electrode, initial potential, final potential and composition of modified solution) affecting the Se(IV) determination were examined. The optimum parameters for DPASV determination of Se(IV) were selected and presented in the table 1.

### Table 1: The optimum parameters established for differential pulse anodic stripping voltammetric determination of Se(IV) using an AuEMD

| Parameters                          | Operating modes                                                                 |
|-------------------------------------|---------------------------------------------------------------------------------|
| Accumulation (deposition) time \(t_{ac}\) | 200 s for \(C_{Se(IV)}\) 1x10^{-6}-1x10^{-5} mol/l and 350 s for \(C_{Se(IV)}\) 1x10^{-6} mol/l       |
| Accumulation potential \(P_{ac}\)   | -250 mV                                                                         |
| Supporting electrolyte              | 0.20 M HClO₄                                                                    |
| Indicator electrode                 | rotating disk gold electrode \(\text{RD}AuE\)                                   |
| pH solution                         | 0.22                                                                            |
| Modified electrode composition      | 0.63 mg/ml of \(Dab\)+0.30 mg/ml of \(Ve\)+3% v/v Nafion in Ethanol             |
| Drop modified size                  | 5 µl                                                                            |
| Initial potential                   | +400 mV                                                                         |
| Final potential                     | +1250 mV                                                                        |
| Scan rate                           | 10 mV/s                                                                         |
| Stirring speed                      | 1000 rpm                                                                        |
| Temperature of solution             | 25 ±0.5 °C                                                                      |

### Analytical results

The analytical curves, \(I_p = f(C_{Se(IV)})\) for the determination of Se(IV) in presence of 0.20 M HClO₄ using a AuEMD\(ab\)\(Ve\)N by DPASVA showed linear proportionality over the concentration range from 0.07896 ng/ml \((1\times10^{-9} \text{ mol/l})\) to 0.7896 ng/ml \((1\times10^{-8} \text{ mol/l})\) of Se(IV) with accumulation (deposition) time 350 s and 0.7896 ng/ml \((1\times10^{-8} \text{ mol/l})\) to 78.96 ng/ml \((1\times10^{-6} \text{ mol/l})\) of Se(IV) with accumulation time 200 s (fig. 1 and 2). Regression equations and correlation coefficient were as follows: \(y=1.6415x+0.0353 \quad (R^2=0.9973)\) and \(y=0.3178x+0.0598 \quad (R^2=0.9995)\), respectively. In this method a very low concentration 0.07896 ng/ml \((1\times10^{-9} \text{ mol/l})\) of Se(IV) with relative standard deviation not exceed ±4.8% was determined (table 2).

This method showed a very sensitive results for the determination of Se(IV) than that obtained using \(Dab\) or \(Ve\) modified gold electrode \([10, 13]\); because the effect of multi-modified a gold electrode with a mixture of \(Dab\) and \(Ve\)-Nafion making the electrode is more selective for Se (IV) compared to the modified electrode with the individual \(Dab\) or \(Ve\)-Nafion.

### APPLICATIONS

Many applications for the determination of Se(IV) in some pharmaceutical preparations by DPASVA on an AuEMD\(ab\)\(Ve\)N using the optimum parameters were proposed. Standard addition curves for determination of Se(IV) in different pharmaceutical preparations (Cenvite, Supratech and Se) were used. Regression equations and correlation coefficients were included in table 3. Standard addition curves for determination of Se(IV) in different pharmaceutical preparations were used. The amount \((m)\) of Se(IV) in one tablet by mg/tab calculated from the following relationship:

\[
m = h \cdot m',
\]

where: \(m'\) is the amount of Se(IV) in tablet, which calculated from the standard additions curve according to the following regression equation: \(y=a \cdot x+b\); when \(y=0\); \(m' = b/a = \text{intercept/slope}\) (ng/ml) and \(h\) conversion factor is equal to 1.0, 2.2 and 4.0 for all pharmaceuticals content 25, 55 and 100 µg/tab, respectively. The results of the quantitative analysis for Se(IV) in the pharmaceutical preparations using this method included in table 4. The proposed method was simple, very sensitive and successfully applied to the determination of Se(IV) in pharmaceuticals. The results obtained agree well with the contents stated on the labels.
Fig. 2: $I_p = f (C_{Se(IV)})$ for the determination of Se(IV) in presence of 0.20 M HClO$_4$ by DPASVA using a AuEMD, $V_{E N}$ (a) 350 s and (b) 200 s, $P_{ac} = -250$ mV, $pH = 0.22$, scan rate 10 mV/s, temperature 25 ±0.5 °C, $n = 5$; $y$: $I_p$, μA and $x$: $C_{Se(IV)}$, ng/ml

Table 2: Determination of Se(IV) by DPASVA on a AuEMD, $V_{E N}$ $(t_{ac}$: 350 s for $C_{Se(IV)}$ 0.07896-0.7896 ng/ml and 200 s for $C_{Se(IV)}$ 0.7896-78.96 ng/ml, $P_{ac} = -250$ mV, $pH = 0.22$, scan rate 10 mV/s, temperature 25 ±0.5 °C, $n = 5$ and $t = 2.776$)

| $x$, ng. ml$^{-1}$ (taken) | $t_{ac}$, s | $\bar{x} \pm SD$, ng/ml | $SD$, ng/ml | $\frac{SD}{\sqrt{n}}$, ng/ml | RSD % | $\bar{x} \pm SD$, ng/ml (found) |
|---------------------------|-------------|--------------------------|-------------|-------------------------------|--------|--------------------------------|
| 0.07896                   | 350         | 0.0777±0.0062            | 0.0017      | 0.0772±0.0046                 | 4.8    | Not determined                |
| 0.15792                   |             | 0.1555±0.0073            | 0.0033      | 0.155±0.0081                  | 4.7    | =                              |
| 0.31584                   |             | 0.3075±0.014             | 0.0063      | 0.3075±0.017                  | 4.7    | =                              |
| 0.47376                   |             | 0.4902±0.023             | 0.0103      | 0.4902±0.029                  | 4.6    | 0.487±0.023                   |
| 0.63168                   |             | 0.6486±0.029             | 0.0130      | 0.6486±0.036                  | 4.5    | 0.6326±0.029                  |
| 0.78960                   |             | 0.770±0.034              | 0.0152      | 0.770±0.042                   | 4.4    | 0.78±0.034                    |
| 0.7896                   | 200         | 0.803±0.034              | 0.0152      | 0.803±0.042                   | 4.2    | 0.7692±0.034                  |
| 1.974                     |             | 2.014±0.082              | 0.037       | 2.014±0.103                   | 4.1    | 1.997±0.085                   |
| 3.948                     |             | 3.902±0.153              | 0.068       | 3.902±0.189                   | 3.9    | 3.95±0.155                    |
| 5.922                     |             | 5.948±0.226              | 0.101       | 5.948±0.281                   | 3.8    | 5.98±0.236                    |
| 7.896                     |             | 7.836±0.290              | 0.130       | 7.836±0.360                   | 3.7    | 7.83±0.337                    |
| 15.792                    |             | 15.86±0.587              | 0.263       | 15.86±0.730                   | 3.7    | 15.9±0.627                    |
| 27.636                    |             | 26.87±0.967              | 0.432       | 26.87±1.200                   | 3.6    | 26.89±1.02                    |
| 39.480                    |             | 40.71±1.47               | 0.657       | 40.71±1.825                   | 3.6    | 40.24±1.83                    |
| 55.292                    |             | 54.24±1.90               | 0.849       | 54.24±2.357                   | 3.5    | 55.25±1.90                    |
| 67.116                    |             | 68.09±2.38               | 1.066       | 68.09±2.959                   | 3.5    | 68.02±2.32                    |
| 78.960                    |             | 78.47±2.75               | 1.228       | 78.47±3.410                   | 3.5    | 78.03±2.76                    |

Validation of proposed method

The developed method for simultaneous estimation of Se(IV) has been validated in accordance with the International Conference on Harmonization guidelines (ICH) [14].

Selectivity

Selectivity test determines the effect of excipients on the assay result. To determine the selectivity of the method, a standard solution of Se(IV), commercial product solution and blank solutions
were analyzed. The results of the tests proved that the effect of the presence of common excipients such as; starch, lactose, glucose, sucrose, and gum acacia no interference was introduced by any of them.

### Table 3: Regression equations and correlation coefficients for determination of C_{Se(IV)} in pharmaceutical preparations using DPASV on a AuEMD_vN (t_\text{ac}=200 s, P_\text{ac}=-250 mV, pH=0.22, scan rate 10 mV/s, temperature 25 °±0.5 °C and n=5)

| Pharmaceutical preparations | C_{Se(IV)} in tab., µg | Operating modes | Regression equations | Correlation coefficients | Amount of Se^{IV}(m), µg/tab. |
|-----------------------------|------------------------|-----------------|----------------------|-------------------------|-----------------------------|
| Cenvite tablets, Pharmasyr Co., Damascus–SYRIA | 25 | y=0.3180x+7.950 | R^2=0.9989 | 25.00 m =1.0m'=25.00 |
| Supratech tablets, MPI-Damascus–SYRIA, Licensed by: Roche Consumer Health-SWIZERLAND | 55 | y=0.3164x+7.973 | R^2=0.9986 | 25.20 m =2.2m'=55.44 |
| Se tablets, Jamieson Laboratories, CANADA | 100 | y=0.3214x+8.061 | R^2=0.9992 | 25.08 m =4.0m'=100.32 |

'y = n A, x= C_{Se(IV)} (ng/ml)= m’ = intercept/slope.'

### Table 4: Determination of Se(IV) in pharmaceutical preparations using DPASV on a AuEMD_vN (t_\text{ac}=200 s, P_\text{ac}=-200 mV, pH=0.22, scan rate 10 mV/s, temperature 25 °±0.5 °C and n=5)

| Commercial name | Contents, µg/tab. | \text{x}±SD, µg/tab. | RSD % | Assay % |
|-----------------|-------------------|----------------------|-------|--------|
| Cenvite tablets, Pharmasyr Co., Damascus–SYRIA | 25 | 25.00±1.00 | 4.0 | 100.00 |
| Supratech tablets, MPI-Damascus–SYRIA | 55 | 55.44±2.16 | 3.9 | 100.80 |
| Se tablets, Jamieson Laboratories, CANADA | 100 | 100.32±3.81 | 3.8 | 100.32 |

### Linearity
In the proposed methods, linear plots (n= 5) with good correlation coefficients were obtained in the concentration ranges of 0.07896–0.78960 and 0.78960-78.960 ng/ml for Se(IV), linearity equations obtained were y=1.6415x+0.0353 (R^2=0.9973) and y=0.3178x+0.0598 (R^2=0.9995) using accumulation time 350 s and 200 s, respectively.

### Precision and accuracy
The precision and accuracy of proposed method were checked by recovery study by addition of standard Se(IV) solution to pre-analyzed sample solution at three different concentration levels (80%, 100% and 120%) within the range of linearity for Se(IV). The basic concentration level of sample solution selected for spiking of the Se(IV) standard solution was 27.636 ng/ml. The proposed method was validated statistically and through recovery studies and was successfully applied for the determination of Se(IV) in pure and dosage forms with percent recoveries ranged from 98.0% to 101.0% (table 5).

### Repeatability
The repeatability was evaluated by performing 10 repeat measurements for 7.896 ng/ml of Se(IV) using the studied method under the optimum conditions in two concentration ranges. The found amount of Se(IV) (x±SD) was 7.836±0.29 ng/ml and the percentage recovery was found to be 99.24±3.67 with RSD of 0.037. These values indicate that the proposed method has high repeatability for Se(IV) analysis.

### Table 5: Results of recovery studies (n=5)

| Level | % recovery |
|-------|------------|
| 80%   | 98.0       |
| 100%  | 100.2      |
| 120%  | 101.0      |

### Table 6: Robustness of the proposed DPASVA method

| Experimental parameter variation | Average recovery (%)* C_{Se(IV)}=27.636 ng/ml |
|--------------------------------|-----------------------------------------------|
| Temperature 15 °C               | 99.9                                          |
| 25 °C                          | 100.1                                         |
| pH 0.21                        | 99.8                                          |
| 0.23                           | 99.7                                          |
| Accumulation potential -245 mV | 100.2                                         |
| -255 mV                        | 100.1                                         |
| C_{HClO4} 0.19 mol/l           | 99.7                                          |
| 0.21 mol/l                     | 99.9                                          |

* n=5.
Sensitivity (LOD and LOQ)

The limits of detection (LOD) and quantitation (LOQ) were determined using the formula: LOD or LOQ = \( \frac{3.3 \times SD}{b} \), where \( \gamma = 3.3 \) for LOD and 10 for LOQ, SD is the standard deviation of the intercept, and \( b \) is the slope. The values of LOD and LOQ for Se(IV) are 0.0075 and 0.023, respectively.

Robustness

The robustness of the method adopted is demonstrated by the constancy of the current peak \( I_p \) with the deliberated minor change in the experimental parameters such as the change in the concentration of excipients, temperature \( (25\pm5 ^\circ \text{C}) \), pH \( (0.22\pm0.01) \), accumulation potential \( (-250\pm5 \text{ mV}) \), and \( \text{C}_{\text{HClO}_4} \) \( (0.20\pm0.01 \text{ mol/l}) \). Table 6 indicates the robustness of the proposed method. \( I_p \) was measured and the assay was calculated for five times.

Specificity

The specificity of the method was ascertained by analyzing standard Se(IV) in presence of excipients. There was no interference from the common excipients.

CONCLUSION

Differential pulse anodic stripping voltammetric analysis (DPASVA) of Se(IV) using Au:EMD4V3N has been studied. Se(IV) was determined in an aqueous HClO\(_4\) \((0.2M)\) medium of pH 0.22. Under the optimum conditions this method showed that the effect of multi-modified of gold electrode with a mixture of Dab and V3-Nafion making the electrode is more selective for Se (IV) compared to the modified electrode with the individual Dab \([10]\) or V3-[13]-Nafion.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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