

## A novel highly sensitive catalytic determination of selenium using a digital camera

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### ABSTRACT

Simple, lower-cost, selective and highly sensitive methods for the assessment of Selenium (IV) as a critical environmental pollutant are highly desirable, especially under resource limited settings. Therefore, herein we describe the coupling of digital imaging with catalytic analysis for the extremely sensitive determination of Se(IV) using its catalytic effect on the micellar sensitized Methylene blue (MB) – hydrosulfide reduction reaction. The initial rate kinetic measurement was applied by following the decolorization of MB within 60 sec of mixing the reactants using a digital camera or spectrophotometrically at 665 nm. The optimum reaction conditions were: 9.0  $\mu\text{mol L}^{-1}$  MB, 10.0  $\text{mmol L}^{-1}$   $\text{Na}_2\text{S}$ , 6.3  $\text{mmol L}^{-1}$   $\text{Na}_2\text{SO}_3$ , 8.0  $\text{mmol L}^{-1}$  CTAB, 90.0  $\text{mmol L}^{-1}$  borax, 0.15 v/v% HCHO, 30.0  $\text{mmol L}^{-1}$  of EDTA, 3.0 v/v% triethanolamine, at  $\text{pH} = 9.30 \pm 0.10$  and  $30^\circ\text{C}$ . The calibration graphs fitted second order polynomial equations for up to 19.0 and 20.0  $\text{ng mL}^{-1}$  Se(IV) with a detection limit ( $3S_b$ ) of 0.2 and 0.5  $\text{ng mL}^{-1}$  Se(IV), for the spectrophotometric and digital imaging monitoring respectively. The amended methods were successfully applied to various natural waters and bird feed samples. The analytical data were statistically in excellent agreement with those obtained following the official AOAC 996.16 method.

**Keywords:** Digital imaging; Kinetic analysis; Selenium; Methylene blue-hydrosulfide reaction; Natural waters, Bird Feed.

### INTRODUCTION

Selenium as an essential element for humans, animals and plants. At trace levels it participates in crucial biochemical thyroid function, however, excess selenium consumption, whether acute or chronic, can cause selenium toxicity<sup>(1)</sup>. Consequently, the development of low-cost, selective, highly sensitive and precise methods for the determination of very trace levels of Selenium in natural waters is an important task. Current methods for sensitive assessment of Se(IV) include atomic absorption spectrometry, AAS<sup>(2-4)</sup>, inductively coupled plasma optical emission spectrometry, ICP-OES<sup>(5-7)</sup>, inductively coupled plasma mass spectrometry, ICP-MS [8-10], spectrophotometric<sup>(11-14)</sup> and the highly sensitive catalytic spectrophotometric methods<sup>(15-24)</sup>. However, the high capital costs, high running and maintenance costs, the limited sensitivity and selectivity, and/or the high limits of detection are common drawbacks of many of the above methods. Otherwise, the well-established 2,3-Diaminonaphthalene method is well sensitive and was adopted as a standard for the assessment of trace Se(IV) in various food and water samples<sup>(11, 25)</sup>. Yet, low-cost,

straightforward, highly selective, more sensitive and precise methods for assessing Se(IV) are still highly desirable.

Recently, digital image-based analysis (DIBA) was described as a simple, portable, low-cost, highly sensitive and selective analytical tool that favorably compete with hi-tech and high cost devices<sup>(26-30)</sup>. DIBA depends on a color change, wherein the analyte participates in a chromogenic reaction to produce a colored product whose acquired digital images are analyzed to obtain the red, green and blue (RGB) intensity values ( $I_R$ ,  $I_G$  and  $I_B$ ). ), or thereof, and were used as promising and versatile analytical signals<sup>(26-30)</sup>.

However, conventional DIBA methods are of limited sensitivity and its coupling with catalytic methods of analysis would enhance its sensitivity from  $\mu\text{g mL}^{-1}$  to the  $\text{ng mL}^{-1}$  levels. To date and to the best of our knowledge, there is no paper dealing with the use of digital imaging for the catalytic monitoring of Selenium (IV). Thus, herein we describe an inexpensive, straightforward, selective and ultrasensitive catalytic determination of Se(IV) using its catalytic effect on an amended MB-HS<sup>-</sup> reaction as a promising tool for the ultrasensitive assessment of Se(IV) under resource-limited settings. The rate of the amended MB-HS<sup>-</sup> reaction is traced kinetically by following the color fading of MB either by capturing digital images or spectrophotometrically at 665 nm within 60 sec of mixing the reagents, in the absence and the presence of catalytic amounts of Se(IV). The proposed method was conveniently applied to the assessment of down to  $0.2 \text{ ng mL}^{-1}$  Se(IV) in natural waters and bird feed samples. Statistical analysis of assessment data showed excellent agreement with the standard AOAC method for Selenium determination<sup>(25)</sup>.

## MATERIALS AND METHODS

### 1 Instruments and software

Digital Image Based Analysis (DIBA) measurements was performed using a traditional IXUS 185 Canon digital camera mounted in our formerly reported and validated homemade platform<sup>(26-28)</sup>. In brief, the platform consisted of (1) the digital camera as the sensor (2) two 10 mm path-length glass cells and (3) a white paper as a diffuser. The camera and a 3D printed cell-holder were fixed on a 20x20 cm PVC sheet, in a cartoon box placed on the bench of our laboratory. The platform does not require any mirrors, lenses, photodiodes or photomultipliers. Videos were captured on the bench of our laboratory. Using the freeware VLC media player, each captured video was converted to frames (digital images), extracted at predefined time intervals, e.g., every 5 sec, for subsequent rate measurements. Image cropping and RGB processing was performed on a DELL-Inspiron 5570 laptop using Adobe Photoshop CC 2017 and ImageJ 1.52 freeware, respectively.

Alternatively, initial rate measurements were recorded on a Shimadzu UV-1650 PC double beam spectrophotometer (Kyoto, Japan) that is controlled by a UV probe-2.5 software running under Windows® environment. The temperature of the glass cells compartment was controlled at the desired temperature ( $\pm 0.1^\circ\text{C}$ ) by circulating water from a Julabo thermostatic water bath. Eppendorf variable volume pipettes (0.5-10, 10-100 and 100-1000  $\mu\text{L}$ ) and a calibrated EDT pH/mV meter were used to deliver accurate volumes and pH measurements, respectively. For validation studies, the fluorescence measurements were performed on a Shimadzu RF-1501 spectrofluorometer, using excitation and emission slit widths of 10 mm and following the AOAC official method<sup>(25)</sup> No. 996.16.

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### 2 Reagents and solutions:

Chemicals of the ACS grade or equivalent were purchased from Merck or Sigma-Aldrich (Darmstadt, Germany) and were used without further purification. Ultrapure water ASTM grade I (18.2 M $\Omega$ ) and aqueous solutions were used throughout.

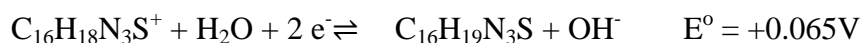
A stock standard solution of 200  $\mu\text{g mL}^{-1}$  of Se(IV) was prepared from selenous acid<sup>(11,25)</sup>. An aqueous stock 0.05 m/v% methylene blue solution was prepared by ultrasonic dissolution of 0.05 gm of the 95% methylene blue hydrate in deionized water in a 100 mL volumetric flask. A 3.0 mL of this solution was further diluted in a 50 mL volumetric flask to give the 0.003 m/v% (or 90  $\mu\text{mol L}^{-1}$ ) working MB solution. The following working freshly prepared aqueous solutions were used: a 0.075 mol L<sup>-1</sup> borax buffer solution of pH = 9.30 $\pm$ 0.10, a 0.30 mol L<sup>-1</sup> Cetyltrimethyl-ammonium bromide (CTAB) surfactant solution, a 34.5 m/v% formaldehyde stabilizing solution, a reducing mixture of 0.30 mol L<sup>-1</sup> Na<sub>2</sub>S and 0.19 mol L<sup>-1</sup> Na<sub>2</sub>SO<sub>3</sub>, and a masking mixture of 0.3 mol L<sup>-1</sup> EDTA and 2.25 mol L<sup>-1</sup> TEA (triethanolamine). The reducing and masking mixtures may affect the buffer capacity of the used borate buffer, and therefore, its pH was adjusted to 9.30 $\pm$ 0.10 prior to use.

### 3 Recommended procedures for Selenium assessment

The working and unknown test solutions were kept at 30°C in the thermostatic water bath. To a conventional 10 mm path-length spectrophotometric cell with a 4.50 mL capacity, the following reacting working solutions were added: 800  $\mu\text{L}$  borax buffer, 50  $\mu\text{L}$  of the working formaldehyde solution, 300  $\mu\text{L}$  of the composite EDTA-triethanolamine masking reagent and 100  $\mu\text{L}$  of the reducing sulfide-sulfite mixture. After shaking well, an X  $\mu\text{L}$  of the standard Se(IV) or the unknown sample solution containing less than 60 ng Se(IV) were added followed by 80  $\mu\text{L}$  CTAB, (1570-X)  $\mu\text{L}$  water, and 100  $\mu\text{L}$  of MB and mixed well. The initial rate was obtained by recording a digital video for the color fading process for 180 sec using our homemade platform as a new sensing tool in catalytic/kinetic analysis. For comparison purposes, the initial rate was also obtained spectrophotometrically by recording the absorbance (change) of the reacting 3000  $\mu\text{L}$  mixture at 665 nm for 120 sec. In both cases, the digital video recording and the spectrophotometric monitoring, the initial rate was obtained from the slope of the initial linear part of the A-t graph within 90 sec of pressing the start button of the camera or the spectrophotometer. It is important to state that, from each frame, a 200  $\times$  200 pixels was cropped and the obtained RGB intensities ( $I_R$ ,  $I_G$ ,  $I_B$ ) were converted into the corresponding color absorbance values from the equation [ $A_{R,G,B} = \text{Log}((I_0/I)_{R,G,B})$ ]. The values of I and  $I_0$  stands for the desired color channel intensity of sample and blank, respectively. However, the change in the red channel color absorbance ( $A_R$ ) was only used as an analytical signal due to its higher analytical response. All measurements were performed against water as a reference. The unknown Se(IV) concentration of the sample was determined from a similarly prepared calibration graph constructed using the standard working Se(IV) solution.

## RESULTS AND DISCUSSION

The reduction of MB with excess sulfide ions in a relatively slow process to give the colorless leuco-methylene blue (LMB) that can be effectively accelerated by the addition of nanogram levels of selenium(IV) as a catalyst<sup>(31)</sup>



### 1. Effects of reaction variables

The absorption spectrum of a 30  $\mu\text{mol L}^{-1}$  MB, recorded following the recommended procedure exhibited one absorption maximum at 665 nm.

In neutral and acidic media, there is an apparent loss of sulfide ions due to the protolytic equilibria involving the volatile  $\text{H}_2\text{S}$ ,  $\text{pK}_{\text{a}1} = 7.04$ ,  $\text{pK}_{\text{a}2} = 11.96$ <sup>(32)</sup>. Therefore, the MB-hydrosulfide reduction reaction is usually performed in alkaline media, 7.80-10.00, to prevent the losses of  $\text{H}_2\text{S}$ <sup>(24, 32-35)</sup>. Induction period, fixed time and fixed absorbance (concentration) measurements have been previously reported for the catalytic determination of Se(IV) using the MB- $\text{Na}_2\text{S}$  reaction in strongly alkaline media of about 1M NaOH<sup>(24, 32-35)</sup>. However, no published report described the application of the simpler initial rate measurements, probably because of the fear of the presence on induction periods. Herein, we investigated, optimized, applied and validated the use of the initial rate mode of measurement for the MB -  $\text{HS}^-$  - Se(IV) system.

Following the recommended procedure, changing the pH in the range of 7.8-10.0 caused minor changes in the initial rates of the uncatalyzed reaction ( $\text{IR}_{\text{B}}$ ); however, it produced a marginal increase in the initial rate of the catalyzed reaction ( $\text{IR}_{\text{S}}$ ) up to  $\text{pH}=9.3\pm 0.2$  and after that  $\text{IR}_{\text{S}}$  decreased again (Fig. 1a).

Therefore, an optimum operating pH of  $9.30\pm 0.10$  was adopted in the recommended procedure due to the high sensitivity ( $\text{IR}_{\text{S}}-\text{IR}_{\text{B}}$ ) and the high buffering capacity of the used borate buffer. At this working pH,  $\text{H}_2\text{S}$  and  $\text{S}^{2-}$  has no practical existence ( $\text{pK}_{\text{a}} = 7.04$  and  $11.96$ )<sup>(32)</sup> and the only contributing form of  $\text{H}_2\text{S}$  is the hydrosulfide ion,  $\text{HS}^-$ <sup>(36)</sup>.

In preliminary experiments, highly organized assemblies of cationic surfactants' micelles exerted a pronounced synergistic effects on the MB- $\text{HS}^-$  reaction; however, cetyltrimethylammonium bromide, CTAB, showed the best performance on the Se(IV)-catalyzed MB- $\text{HS}^-$  reaction. Therefore, the effect of CTAB concentration was thoroughly investigated. The rates of the uncatalyzed ( $\text{IR}_{\text{B}}$ ) reaction increased very slightly with CTAB concentration, However, that rate of the catalyzed ( $\text{IR}_{\text{S}}$ ) reaction and the sensitivity ( $\text{IR}_{\text{S}}-\text{IR}_{\text{B}}$ ) marginally increased with CTAB concentration up to about 7  $\text{mmol L}^{-1}$  CTAB, after that the sensitivity exhibited a near plateau behavior (Fig. 1b). Therefore, an 8  $\text{mmol L}^{-1}$  CTAB was adopted in the recommended procedure due to its high sensitivity and relatively low blank values.

The marginal sensitizing effect of CTAB on the Se(IV) catalyzed reaction revealed the strong interaction between the positively charged CTAB micelles and the negatively charged selenohydrosulfide  $[\text{SeO}_4^{2-} \dots (\text{HS}^-)_n]^{(-n-2)}$  intermediate species that is formed between  $\text{SeO}_4^{2-}$  and  $\text{HS}^-$  ions, *vide infra*. Moreover, CTAB stabilizes and dissolves the so formed colloidal sulfur species that results from the oxidation of  $\text{HS}^-$  by  $\text{SeO}_4^{2-}$  in the catalytic cycle for the regeneration of the selenium catalyst species, *vide infra*.

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The effect of MB concentration is depicted in Figure (1c). The initial rate of the uncatalyzed reaction increased almost linearly with MB concentration. However, the initial rate of reaction catalyzed by Se(IV) and the reaction sensitivity ( $IR_S-IR_B$ ) marginally increased with MB concentration up to about  $9 \mu\text{mol L}^{-1}$  MB, after that the sensitivity exhibited a near plateau behavior. Thus, a  $9 \mu\text{mol L}^{-1}$  MB concentration was adopted in the recommended procedure.

The increase in the concentration of the MB dye can not alone explain the marginal increase in the rate of the catalyzed reaction with the MB concentration. We believe that under the alkaline conditions of the recommended procedure, the positively charged surfaces of CTAB hydrophilic micelles strongly attract the negatively charged hydroxide ( $\text{OH}^-$ ) and selenosulfide ( $[\text{Se}^{4+} \dots (\text{HS}^-)_n]^{-(n+4)}$ ) ions that in turn attract the positively charged  $\text{MB}^+$  cations as secondary binding ions. This increases the proximity and instances of interaction between the reacting  $\text{MB}^+$  and the catalytically active selenohydrosulfide  $[\text{Se(IV)} \dots (\text{HS}^-)_n]^{-(n+4)}$  species that is formed only in the presence of excess  $\text{HS}^-$  ions.

Sodium sulfide is a powerful reducing agent that effectively reduces MB to its colorless LMB; whereas, the  $\text{HS}^-$  ion is oxidized to elemental sulfur ( $\text{S}^0$ ). The reaction requires very large molar excess of sulfide ion over MB and Se(IV). However, in the recommended procedure, we adopted a freshly prepared  $\text{Na}_2\text{S}$  solution sulfide containing  $\text{Na}_2\text{SO}_3$  as a stabilizer to prevent the formation of any less catalytically active polysulfide species<sup>(24)</sup>. The initial rates of the catalyzed and uncatalyzed reactions increased gradually with  $\text{Na}_2\text{S}$  concentration in the range of  $1-45 \text{ mmol L}^{-1}$   $\text{Na}_2\text{S}$ , whereas the sensitivity remained almost constant. This can be attributed to the presence of a trace impurity, in the commercially used  $\text{Na}_2\text{S}$ . Similarly the effect of  $\text{Na}_2\text{SO}_3$  concentration was studied in the range of  $1-30 \text{ mmol L}^{-1}$   $\text{Na}_2\text{SO}_3$  and exerted almost no effect of the uncatalyzed and catalyzed reactions. Therefore, a freshly prepared composite reducing mixture containing  $1.2 \text{ g Na}_2\text{S}$  and  $1.2 \text{ g Na}_2\text{SO}_3$  per  $50 \text{ mL}$  was used as a working reducing solution to furnish a  $10 \text{ mmol L}^{-1}$   $\text{Na}_2\text{S}$  and  $6.3 \text{ mmol L}^{-1}$   $\text{Na}_2\text{SO}_3$  in the final measured reaction cell.

Formaldehyde not only improved the blank's stability, but also almost eliminated the long induction period that progressively decreased as the formaldehyde level increased. The effect of adding  $0.0$  to  $150 \mu\text{L}$  of the  $34.5\%$  HCHO% studied following the recommended procedure (Fig. 1d). The initial rates of the uncatalyzed and catalyzed reactions gradually increased with HCHO% up to  $0.05\%$  due to the removal of the induction period and the increased linearity of the recorded  $A-t$  graphs. However, at  $0.05-0.20$  HCHO%, the sensitivity remained almost constant at an optimum level. Therefore, it was decided to use  $50 \mu\text{L}$  of the working  $34.5\%$  HCHO in  $3.00 \text{ mL}$  reaction cell; that is equivalent to  $0.15\%$  HCHO as it provided the best sensitivity.

A  $5-50 \text{ mmol L}^{-1}$  EDTA and  $1.0-5.0 \text{ v/v}\%$  triethanolamine proved to be remarkable masking agents against various metal ions in solution that may interfere in the MB- $\text{HS}^-$  reaction and can effectively mask Cu(II), Co(II), Mn(II), Pb(II), Fe(II) Sb(III) and Fe(III) among other interferences, without exerting any harmful effect on the assessment of Se(IV). Therefore a  $30 \text{ mmol L}^{-1}$  EDTA and  $3.0 \%$  triethanolamine were adopted in the recommended procedure to provide a good masking action and confers enhanced selectivity to the proposed method, while not interfering in the Se(IV) determination.

The effect of temperature on the MB- $\text{HS}^-$  reaction was studied following the recommended procedure in the range of  $15-50 \text{ }^\circ\text{C}$ . The initial rates of the catalyzed ( $IR_S$ ) and the uncatalyzed ( $IR_B$ ) processes slightly increased; however, the sensitivity ( $IR_S-IR_B$ )

remained almost constant. Therefore, a 30C° was adopted in the recommended procedure, to provide a relatively low blank.

The initial rate of the catalyzed reaction slightly varied with the order of mixing the reagents. Different combinations of the order of addition of the reagents were systematically tested. The best results were obtained when the sample or Se(IV) standard was mixed with HCHO, the composite buffer and the composite masking agent, the composite reducing agent followed by CTAB and MB as the last reagent.

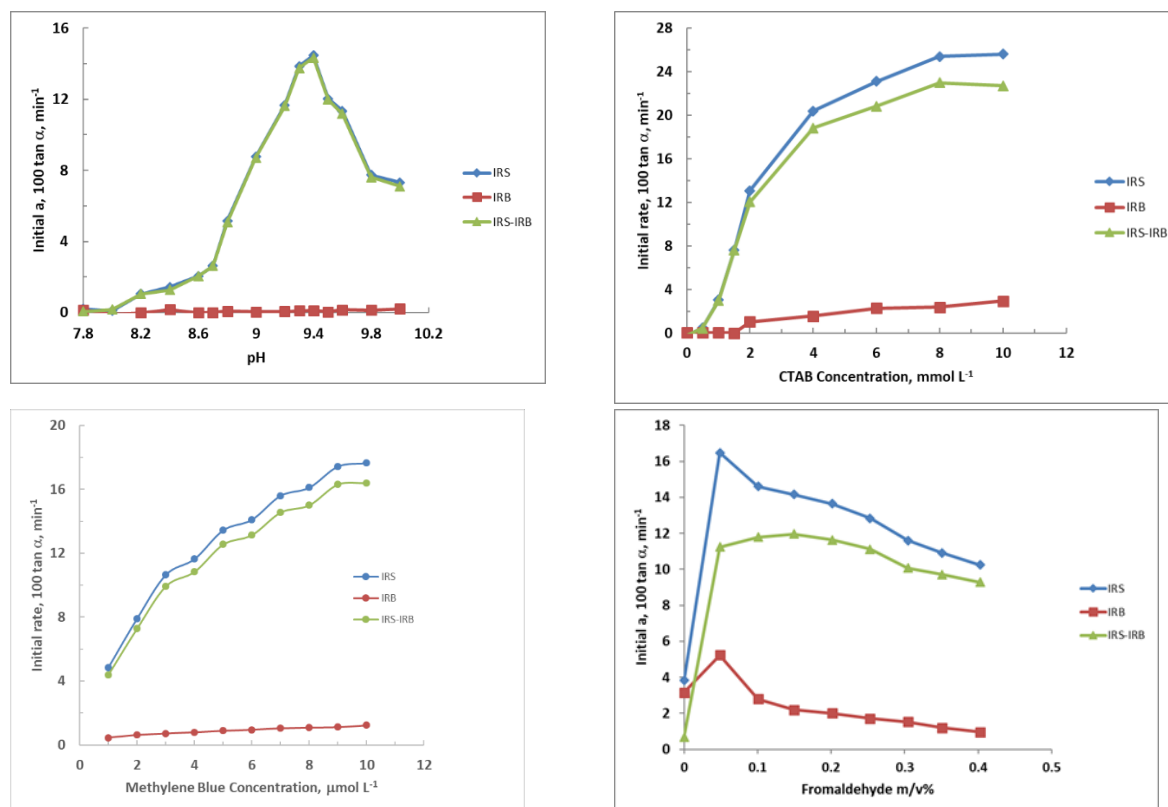


Fig. 1. (a) Effect of pH, (b) Effect of CTAB concentration, (c) effect of MB concentration, (d) Effect of HCHO%. IR<sub>B</sub> is the initial rate of the uncatalyzed reaction; IR<sub>S</sub> is the initial rate of the Se(IV) catalyzed reaction; (IR<sub>S</sub>-IR<sub>B</sub>) is the reaction sensitivity. Except for the abscissa variable, other conditions and symbols were those of the recommended procedure. The catalyst concentrations were (a) 7.0, (b) 12.0, (c) 9.0, (d) 7.0 ng mL<sup>-1</sup> Se(IV).

The effects of potential interfering species that may accompany Se(IV) in natural waters, were studied. The maximum tolerable concentrations of foreign ions are shown in Table (1), where the tolerance limit was defined as the concentration of foreign species that produced an error of ±5% in the rate of the Se(IV) catalyzed MB-HS<sup>-</sup> reaction. Many ions form turbidity/precipitates with sulfide ions at pH 9.30 that require centrifugation and/or filtration; however, the use of the composite masking mixture of EDTA/TEA largely prevented this turbidity/precipitate formation. Moreover, the use of the initial rate measurements, i.e., the change in absorbance with time, is greatly unaffected by minor changes in solution clarity. However, the strong positive interference of Cu(II), Fe(III) and Sb(III) at a 1:1 ratio to Se(IV), was successfully eliminated by the addition of the composite EDTA/TEA masking mixture as described earlier in the recommended procedure.

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Table 1 Tolerance Limits of Foreign species in the determination of  $12 \text{ ng mL}^{-1} \text{Se(IV)}$ \*

Tolerance limit, $\text{mg L}^{-1}$	Interfering species
100	Na(I), K(I), Ca(II), Sr(II), Ba(II), Mg(II), $\text{PO}_4^{3-}$ , $\text{NH}_4^+$ , $\text{Cl}^-$ , $\text{Br}^-$ , $\text{F}^-$ , $\text{HCO}_3^-$ , $\text{CH}_3\text{COO}^-$ , $\text{NO}_3^-$ , $\text{SO}_4^{2-}$
10	Ag(I), Cu(II), Pb(II), Hg(II), Fe(II), Zn(II), Co(II), Cd(II), Ni(II), Mn(II), Pd(II), Bi(III), Al(III), Cr(III), Fe(III), Sb(III), Sn(IV), Zr(IV), As(V), V(V), W(VI),
1**	Hg(II), Fe(II), Zn(II), Co(II), Cd(II), Ni(II), Mn(II), Pd(II), Al(III), Cr(III), Sn(IV), Zr(IV), V(V), W(VI),
0.01**	Ag(I), Cu(II), Pb(II), Fe(III), Bi(III), Sb(III), As(V)

\* Following the recommended procedure

\*\* In the absence of the composite masking mixture

### 2 Digital imaging and kinetic monitoring of the $\text{Se(IV)-MB-HS}^-$ system

Digital videos have been recorded for cuvettes, prepared following the recommended procedure, of the MB-hydrosulfide reaction with various  $\text{Se(IV)}$  concentrations. Each video was converted to digital images using VLC freeware that are then cropped using Photoshop CC2017. Figure (2) shows the original digital images, of representative MB- $\text{HS}^-$ - $\text{Se(IV)}$  cuvettes, captured after 10 and 40 sec of pushing the start button. Note that the collected photos in Figure (2) were randomly compressed to fit within the page margins; however, the original uncompressed images were used for image analysis with ImageJ. The corresponding RGB channel intensities,  $I_R$ ,  $I_G$ , and  $I_B$ , were obtained by analyzing each cropped image that are then converted into the corresponding channel color absorbance  $A_R$ ,  $A_G$ , and  $A_B$ . The linearity of RGB intensities versus time was poor. However, the red channel color absorbance  $A_R$  vs. time graphs exhibited promising linearity for subsequent initial rate measurements.



Fig. 2. Original Digital images and cropped images of MB- $\text{HS}^-$ - $\text{Se(IV)}$  cuvettes captured after 10 and 40 sec of pushing the start button and following the recommended procedure. From upper left to right, 0, 2, 4, 6, 8, 11, 14, 17, and  $20 \text{ ng mL}^{-1} \text{Se(IV)}$ , respectively.

The rate was taken as the change in the initial linear part of the red channel color absorbance within 90 sec of pushing the start button; e. g., between 10 and 40 sec.

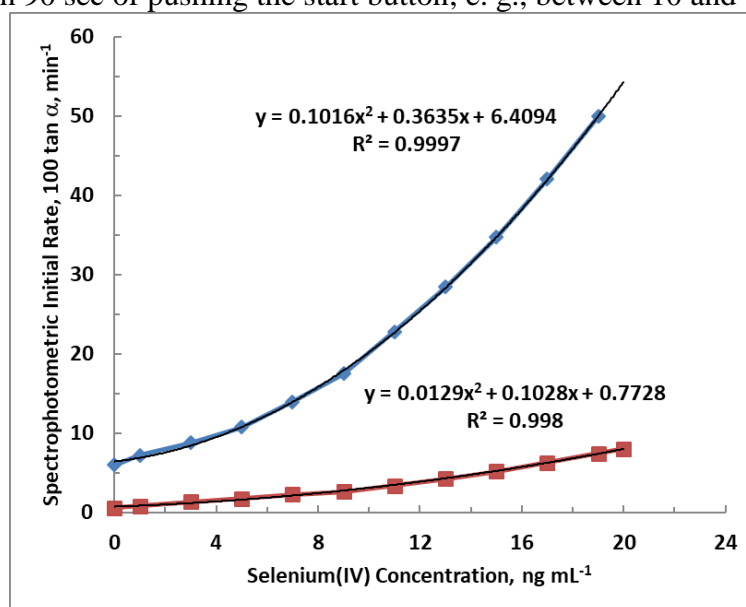


Fig. 3. Spectrophotometric and digital imaging calibration curves of Se (IV). Conditions and symbols were those of Figure (1).

## 2 Calibration graphs and detection limits

Following the recommended procedure with spectrophotometric and digital imaging monitoring, nonlinear second order polynomial calibration graphs were obtained for up to 19.0 and 20.0 ng mL<sup>-1</sup>Se(IV), respectively, as shown in Figure (3a,b). The second order polynomial calibration equations were:

$$100 \tan \alpha (\text{min}^{-1}) = 6.4094 + 0.3635[\text{Se(IV)}] + 0.1016[\text{Se(IV)}]^2 ; \text{ spectrophotometric}$$

$$100 \tan \alpha (\text{min}^{-1}) = 0.7728 + 0.1028[\text{Se(IV)}] + 0.0129[\text{Se(IV)}]^2 ; \text{ digital imaging}$$

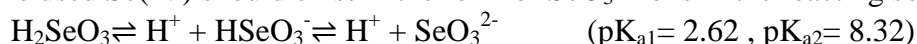
where [Se(IV)] is the selenium concentration in ng mL<sup>-1</sup>. The determination coefficients ( $R^2$ ) were 0.9997 and 0.998 and the detection limits, based on the 3Sb-criterion, were 0.2 and 0.5 ng mL<sup>-1</sup>Se(IV), for the spectrophotometric and digital imaging, respectively. In addition, the use of Se(VI) instead of Se(IV) gave the same calibration equation revealing that both Se(IV) and Se(VI) are analytically equivalent catalysts for the MB<sup>+</sup> - HS<sup>-</sup> reaction.

## 3. The Proposed Reaction Mechanism:

To suggest a possible reaction mechanism, the following should be considered:

[i] In catalytic analysis, a proper catalyst forms a transient state complex with one or more reactants such that this transient state complex is more active than the individual reactants and thereby produce a marked catalytic effect<sup>(37)</sup>.

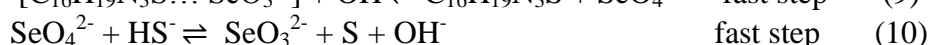
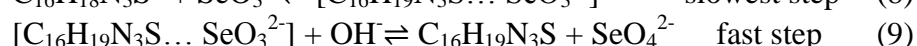
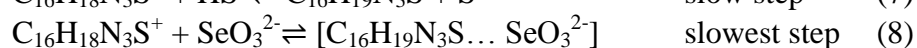
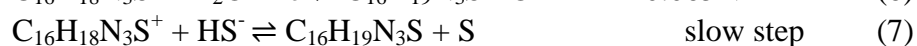
[ii] Based on the pKa values of H<sub>2</sub>SeO<sub>3</sub> (pK<sub>a1</sub>= 2.62, pK<sub>a2</sub>= 8.32), and the work at pH 9.3; therefore, the used Se(IV) should exist in the form of SeO<sub>3</sub><sup>2-</sup> ions in the reacting solution.





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[iii] The standard reduction potentials of S, Se, and MB<sup>+</sup> species in alkaline media are given by equations 1-6<sup>(32, 38)</sup>. However, the entire catalytic cycle can be represented by equations 7-10.



Equation (7) represents the relatively slow uncatalyzed reaction where HS<sup>-</sup> ions reduces the MB<sup>+</sup> to form the colorless LMB and colloidal sulfur. This is confirmed by the low reaction rates of blank solutions shown in Fig. 1c.

However, equation 8 shows the slowest rate-determining step involving the formation of the true catalytically active transient state complex between the oppositely charged MB<sup>+</sup> and SeO<sub>3</sub><sup>2-</sup> ions. Whereas, equation (9) shows the reduction of MB<sup>+</sup> with SeO<sub>3</sub><sup>2-</sup> to yield the colorless LMB and SeO<sub>4</sub><sup>2-</sup> products. After that, the formed SeO<sub>4</sub><sup>2-</sup> ions from equation (9) is rapidly reduced by the large excess of HS<sup>-</sup> ions, eq. 10, to regenerate the primary SeO<sub>3</sub><sup>2-</sup> catalyst in addition to colloidal sulfur that is stabilized by the presence of the highly organized assemblies of CTAB micelles.

Nevertheless, we should not ignore the possible involvement of hydrosulfide, sulfide, or polysulfide ions (eq. 1-4) in a rapid formation of various selenosulfide complexes, e. g., {[HS<sup>-</sup>.....SeO<sub>4</sub><sup>2-</sup>], [(HS<sup>-</sup>)<sub>n</sub>.....SeO<sub>4</sub><sup>2-</sup>], [(S<sup>2-</sup>)<sub>n</sub>.....SeO<sub>4</sub><sup>2-</sup>]}, before the regeneration of the primary SeO<sub>3</sub><sup>2-</sup> catalyst (equations are not given for simplicity).

### 4. Selenium determination in a bird feed and natural waters

The proposed method was successfully applied to the assessment of Se(IV) in a bird feed (fodder corrector), the nominal mineral composition (μg g<sup>-1</sup>) of which, obtained by ICP-OES, was: Se 106.8, Fe 155.2, Mn 454.7, Cu 57.6, Ni 53.9, Co 250.4 and Zn 122.3. Appropriate masses of fodder (ca. 1.000 g) were extracted in several ways, such as continued boiling of sample with (1) water, (2) 2.0 M hydrochloric acid, (3) 2.0 M sodium hydroxide and (4) a 1:1 v/v mixture of concentrated nitric and sulfuric acids. After cooling, the mixtures were filtered, and diluted with water in 100 mL volumetric flasks. The pH values of these extracts were 6.2, 1.0, 12.1 and 0.5, respectively. Appropriate volumes of these solutions were taken and analyzed following the recommended procedure of the proposed MB methods. The analytical results of feed sample leaching's are given in Table 2. Moreover, the proposed methods were applied to two brands of bottled waters in addition to our laboratory tap water. However, natural water samples required a forty-fold preconcentration with evaporation due to its very low content of Se(IV). The analytical results are shown in Table 2. It is obvious that the results obtained by the proposed spectrophotometric and digital imaging

methods agree well with those obtained with the AOAC official method 996.16 based on the spectrofluorimetric assessment of selenium in feeds and premixes using 2,3-diaminonaphthalene ( $\lambda_{\text{ex}}$  375 nm,  $\lambda_{\text{em}}$  525 nm). It is worthy to mention that, in contrast to the AOAC method 996.16, the proposed MB methods determined selenium without the need for any reduction steps to reduce Se(VI) to Se(IV). Statistical treatment of analytical results showed the absence of any systematic error as evidenced by the excellent agreement between the means and variances of the data of the proposed MB and AOAC standard methods.

## Conclusions

A modified methylene blue-sodium hydrosulfide reaction has been proposed for the ultra-sensitive catalytic determination of selenium(IV) in the presence of highly organized assemblies of CTAB micelles. Selenium(IV) exerted a pronounced selective catalytic activity in enhancing the cited reaction. A mono variate optimization was followed to establish the optimum reaction conditions that have been formulated in a recommended operating procedure that has been successfully applied to bird feed and natural water samples. The obtained data has been validated by comparison with the official AOAC method 996.16 with excellent harmony between the means and variances of the standard and proposed MB methods. Statistical treatment of analytical results could not detect any systematic error in the proposed methods based on spectrophotometric and digital imaging monitoring.

Table 2. Determination of Selenium(IV) in a bird feed \*

Se(IV) found, $\mu\text{g/g}$ (n=3)							
Sample dissolution medium	AOAC method $\pm$ SD	Current spectrophotometric MB method $\pm$ SD	$F_{3,3}^{\#}$	$t_{3,3}^{\$}$	Current digital imaging MB method $\pm$ SD	$F_{3,3}^{\#}$	$t_{3,3}^{\$}$
Water	106.0 $\pm$ 0.4	105.9 $\pm$ 0.6	2.25	0.44	106.2 $\pm$ 0.5	1.56	1.13
HCl	108.1 $\pm$ 1.8	108.3 $\pm$ 1.3	1.92	0.09	108.5 $\pm$ 1.5	0.69	0.17
NaOH	107.4 $\pm$ 1.2	106.6 $\pm$ 1.3	1.17	0.59	106.9 $\pm$ 1.4	1.36	0.34
HNO <sub>3</sub> -H <sub>2</sub> SO <sub>4</sub>	107.2 $\pm$ 1.0	106.7 $\pm$ 1.0	1.0	0.57	106.8 $\pm$ 0.9	0.81	0.51
* Certified Se Value is 106.8 $\mu\text{g/g}$							
# Critical value of $F_{2,2}$ at the 95% confidence level = 19.00							
§ Critical value of $t_{2,2}$ at the 95% confidence level = 4.30							

Table 3. Determination of Selenium(IV) in bottled and tap waters

Se(IV) found, $\mu\text{g/g}$ (n=3)							
Sample*	AOAC method $\pm$ SD	Current spectrophotometric MB method $\pm$ SD	$F_{3,3}^{\#}$	$t_{3,3}^{\$}$	Current digital imaging MB method $\pm$ SD	$F_{3,3}^{\#}$	$t_{3,3}^{\$}$
Dasani Water	1.62 <sub>0</sub> $\pm$ 0.04 <sub>5</sub>	1.61 <sub>4</sub> $\pm$ 0.05	1.23	3.06	1.62 <sub>5</sub> $\pm$ 0.04	0.79	3.18
Aquafina water	0.98 <sub>1</sub> $\pm$ 0.02 <sub>7</sub>	0.98 $\pm$ 0.02 <sub>8</sub>	1.08	1.53	0.98 <sub>3</sub> $\pm$ 0.02 <sub>8</sub>	1.23	2.84
Lab. Tap water	1.32 $\pm$ 0.04 <sub>0</sub>	1.32 <sub>2</sub> $\pm$ 0.03	0.56	1.85	1.31 <sub>5</sub> +2 $\pm$ 0.03	1.27	3.19
* After 40 fold preconcentration step. A 1.000 L sample was evaporated in portion-wise steps until its volume is reduced to ca 20 mL and then diluted to the mark in a 25 measuring flask							
# Critical value of $F_{2,2}$ at the 95% confidence level = 19.00							
§ Critical value of $t_{2,2}$ at the 95% confidence level = 4.30							

**Compliance with ethical standards:** the authors declare that they have no competing interests.

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### طريقة حفزية جديدة فائقة الحساسية لتقدير السيلينيوم باستخدام كاميرا رقمية

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#### المستخلص

تعتبر الطرق البسيطة ، منخفضة التكلفة ، فائقة الانقائية والحساسية ، هامة للغاية ومرغوبة لتقدير السيلينيوم كملوث بيئي حرج (IV) ، خاصة في المعامل ذات الموارد المحدودة. لذلك ، تم اقران التصوير الرقمي مع التحليل الحفزي للتقدير فائق الحساسية لـ Se (IV) بناءً على تأثيره الحفزي على تفاعل اختزال الميثيلين الأزرق بواسطة كبريتيد الصوديوم. وتم تتبع التفاعل بقياس حركية إزالة لون الميثيلين الأزرق في غضون 60 ثانية من خلط المتفاعلات باستخدام كاميرا رقمية وكذلك باستخدام جهاز سبكتروفوتوميتر عند 665 نانومتر. وكانت ظروف التفاعل المثلى هي: 9.0 ميكرومولار من الميثيلين الأزرق ، 10.0 مللي مولار من كبريتيد الصوديوم ، 6.3 مللي مولار من كبريتيد الصوديوم ، 8.0 مللي مولار من CTAB ، 90.0 مللي مولار من البوراكس ، 0.15 % فورمالدهيد ، 30.0 مللي مولار من EDTA ، 3.0 % ثلاثي إيثانول أمين ، عند الرقم الهيدروجيني 9.30 ± 0.10 وعند درجة مئوية. وتماشت منحنيات المعايرة مع معادلات متعددة الحدود من الدرجة الثانية حتى 19.0 و 20.0 نانوغرام/مل Se (IV) مع حد كشف (3Sb) مقداره 0.2 و 0.5 نانوغرام/مل باستخدام جهاز الإسبكتروفوتوميتر وباستخدام التصوير الرقمي على الترتيب. وتم تطبيق الطرق المطورة بنجاح على عينات مياه طبيعية مختلفة وكذلك على علف الطيور. وكانت البيانات التحليلية إحصائياً في توافق ممتاز مع تلك التي تم الحصول عليها باتباع طريقة AOAC 996.16 القياسية.