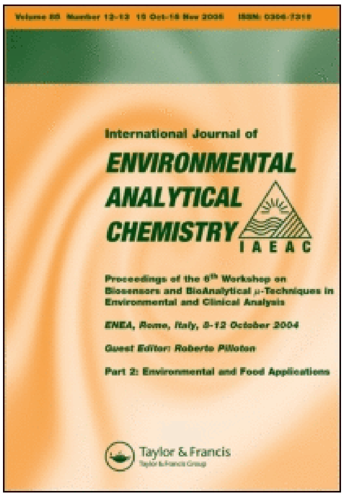


International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Chemical equilibria and sequential extractive spectrophotometric determination of selenium(IV) and (VI) using the chromogenic reagent 4,4'-dichlorodithizone

Mohammad S. El-Shahawi[a]; Abdelhameed M. Othman[b]; Abdulaziz S. Bashammakh[a]; Mervat A. El-Sonbati[c]
[a] Department of Chemistry, Faculty of Science, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia [b] Genetic Engineering and Biotechnology Research Institute, Menoufia University, Menoufia, Egypt [c] Department of Environmental Science, Faculty of Science at Damietta, Mansoura University, Mansoura, Egypt

To cite this Article El-Shahawi, Mohammad S. , Othman, Abdelhameed M. , Bashammakh, Abdulaziz S. and El-Sonbati, Mervat A.(2006) 'Chemical equilibria and sequential extractive spectrophotometric determination of selenium(IV) and (VI) using the chromogenic reagent 4,4'-dichlorodithizone', International Journal of Environmental Analytical Chemistry, 86: 12, 941 — 954

To link to this Article: DOI: 10.1080/03067310600557554

URL: http://dx.doi.org/10.1080/03067310600557554

Intern. J. Environ. Anal. Chem.
Vol. 86, No. 12, 15 October 2006, 941–954

Chemical equilibria and sequential extractive spectrophotometric determination of selenium(IV) and (VI) using the chromogenic reagent 4,4′-dichlorodithizone

MOHAMMAD S. EL-SHAHAWI*†, ABDELHAMEED M. OTHMAN‡,
ABDULAZIZ S. BASHAMMAKH† and MERVAT A. EL-SONBATI§

†Department of Chemistry, Faculty of Science, King Abdulaziz University,
Jeddah, Kingdom of Saudi Arabia
‡Genetic Engineering and Biotechnology Research Institute,
Menoufia University, Menoufia, Egypt
§Department of Environmental Science, Faculty of Science at Damietta,
Mansoura University, Mansoura, Egypt

(*Received 8 September 2005; in final form 20 December 2005*)

The chromogenic reagent 4,4′-dichloro(3-mercapto-1,5-diphenylformazan), $Cl_2H_2D_Z$, forms a yellow–red-coloured complex with selenium(IV). The produced complex species was extracted quantitatively into *n*-hexane, and its absorbance was measured at 416 nm. The chemical composition of the extracted selenium(IV)-$Cl_2H_2D_Z$ chelate and the molar absorptivity at 416 nm were found to be [SeO $(Cl_2HD_Z)_2$] and $9 \times 10^4\,L\,mol^{-1}\,cm^{-1}$, respectively. The values of the extraction constants (K_D, K_{ex}, β) enable a convenient application of the proposed system for the liquid–liquid extraction procedure and sequential spectrophotometric determination of traces of inorganic selenium(IV) and/or selenium(VI) after reduction of the later to selenium(IV) with HCl (6 M). Beer's law and Ringbom's plots were obeyed in the concentration range 0.01–20 and 0.5–19 $\mu g\,mL^{-1}$ of selenium(IV), respectively, with a relative standard deviation of 2.2%. The proposed method has been successfully applied to the determination of selenium(IV) or (VI) and total inorganic selenium(IV) and (VI) in tap and freshwater samples.

Keywords: Chemical equilibria; Speciation; Selenium (IV) and (VI); 4,4′-Dichlorodithizone; Spectrophotometry

1. Introduction

Selenium enters natural waters through seepage from seleniferous soils and industrial waste, and is liberated into the environment (in soil) through a complex biogeochemical reaction forming organoselenium compounds [1]. These compounds are more toxic than inorganic selenium compounds and are absorbed by plants, e.g. cabbage and mustard [2]. However, trace amounts of selenium have been found to be essential

---

*Corresponding author. Fax: +20-502230344. Email: mohammad_el_shahawi@yahoo.co.uk
Permanent address: Chemistry Department, Faculty of Science at Damiatta, Mansoura University, Mansoura, Egypt.

International Journal of Environmental and Analytical Chemistry
ISSN 0306-7319 print/ISSN 1029-0397 online
DOI: 10.1080/03067310600557554

to maintain normal body metabolism [3]. Selenium can also enhance our ability to protect against certain cancer and heart diseases [4]. The maximum allowed tolerance limit of selenium is 0–50 $\mu g\,L^{-1}$ in water, depending on the source, 0.1 $mg\,m^{-3}$ in air, 4–10 $\mu g\,L^{-1}$ in tap water, and 0–80 $\mu g\,g^{-1}$ in soil [5].

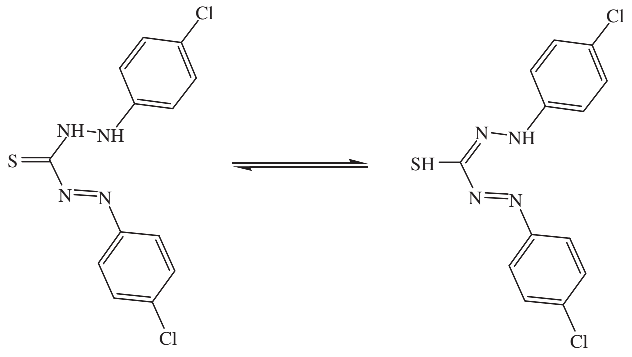
Several methods have been reported for selenium(IV) determination [6–22]. Only a few of the reported spectrophotometric [12] and catalytic [18] methods involving some chromogenic reagents have sufficient sensitivity and selectivity for the trace levels of selenium(IV) in water, polluted water, plant material and steel plant dust. Most of these methods are expensive and unselective, require specific experimental conditions, and are time-consuming [8–11, 15].

Recent years have seen an upsurge of interest in developing novel methods for the chemical speciation of trace amounts of selenium(IV) and (VI) in water [23–27]. The use of the reagent 4,4′-dichloro (3-mercapto-1,5-diphenylformazan) for the chemical speciation, determination, and/or preconcentration of selenium(IV) or (VI) employing polyurethane foams has been reported recently by El-Shahawi and El-Sonbati [28]. However, there have been no studies on the application of the title reagent on the liquid–liquid extractive spectrophotometry for the chemical speciation of selenium(IV) and (VI) or other metal ions in aqueous solutions. Thus, the present paper describes the use of the novel title reagent $Cl_2H_2D_Z$ for the chemical speciation and sequential determination of trace amounts of inorganic selenium(IV) and (VI) in water. The chemical equilibria, chemical composition and characterization of the formed selenium(IV)–$Cl_2H_2D_Z$ chelate were also critically investigated. The method developed not only separates the selenium(IV) and (VI) species but also preconcentrates them quantitatively.

2. Experimental

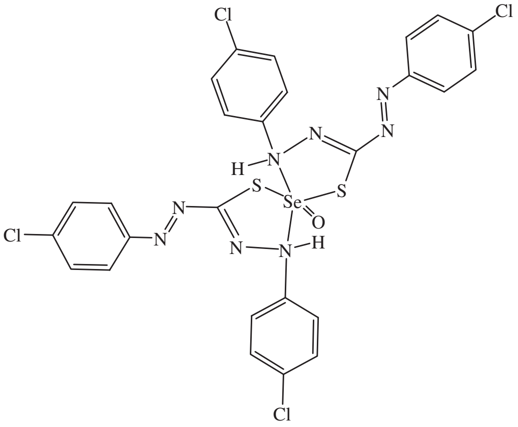
2.1 *Reagents and materials*

All chemicals used were of analytical reagent grade unless otherwise specified. Solvents (BDH) were used without further purification. Deionized doubly distilled water was used for the preparation of stock solutions of sodium hydrogen selenite (Fluka), sodium selenate (Merck), mineral acids (1 M) (BDH), and metal chloride or nitrate salts (BDH). Stock solutions (1 $mg\,mL^{-1}$) of sodium hydrogen selenite and sodium selenate were prepared by dissolving the appropriate weight of the salts in water. The reagent $Cl_2H_2D_Z$ (structure I) was synthesized by the nitroformazyl method reported by Kiwan and Kassim [29]. The chemical structure of the reagent $Cl_2H_2D_Z$ (Mp 125°C) was based on corrected elemental analysis of C, H, N, and Cl and spectral (^{1}H NMR and IR) data. Elemental analysis of $C_{13}H_{10}N_4SCl_2$: required $C = 53.2\%$, $H = 3.4\%$, $N = 19.1\%$ and $Cl = 24.2\%$; found $C = 53.9\%$, $H = 3.5\%$, $N = 19.4\%$ and $Cl = 25.1\%$. The significant IR (KBr disk) bands at 3436 (broad), 1636 (strong, s), 1526 (s) and 1487 (s) cm^{-1} are safely assigned to ν (N–H), ν (N–N), δ (N–H) + (–C = N–) and ν (N–C–S) [12, 29, 30], respectively. The ^{1}H NMR spectrum of the reagent in d_6-DMSO showed signals at δ 7.49–7.6 (m 10 H, Ar, Hs) and δ 15.3 (S^{1}H, NH), confirming the structures proposed in figure 1.



I
Thioketo form

II
Thiol form



III

Figure 1. Proposed chemical structures of the reagent $Cl_2H_2D_Z$, in thioketo(I) and thiol(II) forms and its selenium(IV) chelate(III).

2.2 *Apparatus*

Infrared (IR) spectra were recorded on a Bruker FT-IR spectrophotometer model IFS 66. Microanalyses (C, H, N, Cl) were performed on a Perkin-Elmer 240 C elemental analyser, Plymouth University, UK. The ^{1}H NMR spectrum of the reagent $Cl_2H_2D_Z$ was recorded on a Jeol 270 FT spectrometer, and values in ppm are relative to TMS for $(CD_3)_2SO$. A Shimadzu double beam UV-VIS (UV-160 7pc) spectrophotometer with a 1 cm (path width) quartz cell was used for recording the electronic spectra of the reagent and its selenium(IV) chelate. The absorbance of the organic extracts was measured with a single-beam Digital Spectro UV-VIS (RS Labomed) spectrophotometer with glass cells (10 mm). A pH meter model 3305 (JENWAY) was used for the pH measurement.

2.3 *Recommended procedures*

2.3.1 Determination of selenium(IV). A 10 mL aliquot of an aqueous solution containing selenium(IV) at concentration $\leq 10\,\mu g\,mL^{-1}$ level adjusted to $0 \leq pH \leq 2$

with HCl (1 M) was transferred to a 50 mL separating funnel. The aqueous solution was extracted twice with 10 mL (2 × 5) of *n*-hexane containing the reagent $Cl_2H_2D_Z$ (0.01% w/v) by shaking for 2 min. The *n*-hexane extracts were then collected in a 50 mL beaker containing anhydrous sodium sulphate (1 g), swirled to mix the contents, and transferred to a 25 mL volumetric flask. The residue was also washed with 5 mL (2 × 2.5) of *n*-hexane and transferred to the flask. The free $Cl_2H_2D_Z$ was then removed from the combined extracts by shaking with dilute NH_3 solution (two drops of conc. NH_3 solution in 25 mL of water). The organic extract was finally made up to the mark with *n*-hexane, and its absorbance was then measured at 416 nm against a reagent blank with the aids of calibration curve prepared under the same experimental conditions.

2.3.2 Determination of selenium(VI). An aliquot (10 mL) of the aqueous solutions containing $<5\,\mu g\,mL^{-1}$ of the spiked selenium(VI) was transferred to a 50 mL conical flask and reduced to selenium(IV) with HCl (6 M) after boiling for 15 min in a closed system (to avoid the evaporation of selenium species) and finally cooled to room temperature (25 ± 1°C) as reported earlier [31, 32]. The produced selenium(IV) solution was diluted to 10 mL and adjusted to $pH \simeq 1$ with saturated NaOH. The resulting solution was analyzed as described earlier for selenium(IV) determination by the dithizone H_2D_Z method [10, 33] and also by the proposed procedures *versus* the reagent blank with the aid of standard curves for both methods.

2.3.3 Sequential determination of inorganic selenium(IV) and selenium(VI). An aliquot (10 mL) of a mixture of selenium(IV) and (VI) at a total concentration $\leq 10\,\mu g\,mL^{-1}$ was transferred to a 50 mL separating funnel. The mixture was analyzed according to the described procedure for selenium(IV) determination. Another aliquot (10 mL) was transferred and analyzed as described before for selenium(VI) determination. On the basis of these procedures, the absorbance (A_1) of the organic extract of the first aliquot will be a measure of the selenium(IV) ions in the mixture, while the absorbance (A_2) of the organic extract of the second aliquot is a measure of the sum of the selenium(IV) and (VI) ions. Therefore, the absorbance (A_2–A_1) is a measure of the selenium(VI) ions in the binary mixture.

2.4 *Applications*

2.4.1 Sequential determination of selenium(IV) and total inorganic selenium(IV) and (VI) in freshwater.

- Direct determination method: An aliquot (10 mL) of water sample was treated with 0.5 mL of NaOH (1 M) and 0.5 mL of EDTA, and then pipetted into a 50 mL separating funnel. The solution was centrifuged to remove any formed precipitate, adjusted to pH zero, and 0.5 mL of the reagent $Cl_2H_2D_Z$ (0.01% w/v) added. The reaction mixture was then made up with water to a total volume of 25 mL and swirled well to mix. The mixture was analyzed according to the described procedure for selenium(IV) determination. Another aliquot sample (10 mL) was treated as described earlier for selenium(VI) determination.

The concentrations of selenium(IV) and (VI) and total selenium(IV) and (VI) were then determined from the standard curves of selenium(IV) and (VI).

- Standard addition method: Alternatively, the standard addition (spiking) method was used as follows. A known volume (10 mL) of the test solution adjusted to pH zero and 0.5 mL of the reagent $Cl_2H_2D_Z$ (0.01% w/v) were transferred into a 50 mL separating funnel. The absorbance displayed by the test solution was then measured before and after spiking with various concentrations (1–5 $\mu g\,mL^{-1}$) of selenium(IV), as described for selenium(IV) determination. The change in the absorbance was then used for determining the selenium(IV) concentration in the aliquot sample. Another aliquot of the test sample (10 mL) before and after spiking with various concentrations (1–5 $\mu g\,mL^{-1}$) of selenium(VI) was treated as mentioned for selenium(VI) determination. Selenium (IV) and the total inorganic selenium(IV) and (VI) were then determined.

3. Results and discussion

3.1 *Absorption spectra*

The dithizone analogue $Cl_2H_2D_Z$ under study has the thioketo(I) and thiol(II) forms (figure 1). The absorption electronic spectra of the reagent $Cl_2H_2D_Z$ and its selenium(IV) chelate in *n*-hexane are shown in figure 2. The absorption electronic spectra of the two reagents H_2D_Z and $Cl_2H_2D_Z$ showed two well-defined bands in the region of 429–454 and 615–627 nm, whereas their selenium(IV) chelates showed one well-resolved single band in the range 416–435 nm (table 1). The two chlorine atoms in the para position of the two phenyl nuclei of $Cl_2H_2D_Z$ leads to a slight hypsochromic shift for the observed band at 429 nm and a bathochromic shift for the band at 627 nm

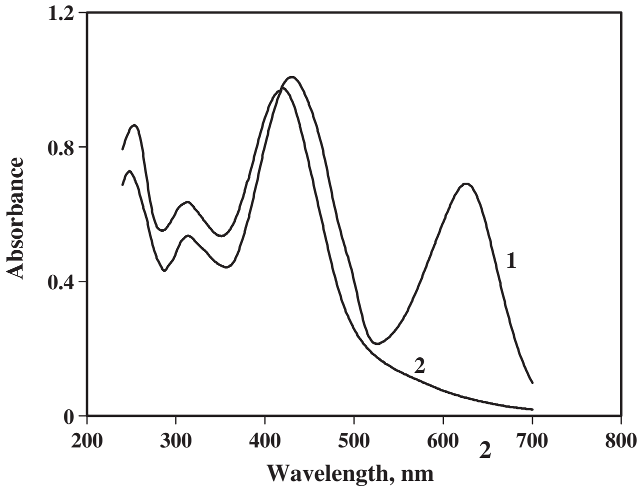


Figure 2. Electronic spectra of the reagent $Cl_2H_2D_Z$ (1) and its selenium(IV) chelate (2). Aqueous phase, 10 $\mu g\,mL^{-1}$ of Se(IV), 0.2 mL of $Cl_2H_2D_Z$ (0.01% w/v), and 10 mL of *n*-hexane.

Table 1. Characteristic absorption IR (/cm) and electronic (nm) spectral data of the reagents H_2D_Z and $Cl_2H_2D_Z$ and their selenium (IV) chelates in KBr disc and in *n*-hexane, respectively[a].

	Wavenumber (/cm)					
Compound	ν (N–H)	ν (N–N)	δ (N–H) + (–C=N–)	ν (N–C–S)	λ_{max} (nm)	$\varepsilon \times 10^4$ L mol^{-1} cm^{-1}
$Cl_2H_2D_Z$	2942 (br)	2374 (w)	1504 (s)	1490 (s)	627	2.43
				1450 (s)	429	2.39
H_2D_Z	2960 (br)	2365 (w)	1515 (s)	1505 (s)	615	3.53
				1465 (s)	454	1.79
$Se(Cl_2HD_Z)_2Cl_2$	3436 (m)	1636	1526	1487	416	9.01
	3280 (s)	1580				
$Se(HD_Z)_2Cl_2$	3215 (m)	1610 (s)	1513 (s)	1501 (s)	435	7.01
	3280 (m)	1580 (s)				

[a] s: strong; m: medium; w: weak; sh: shoulder; br: broad.

compared with H_2D_Z (table 1). The value of the molar absorptivity (ε) at λ_{max} 627 nm decreased to 2.43×10^4, and that at 429 nm increased to 2.39×10^4 L mol^{-1} cm^{-1} for the $Cl_2H_2D_Z$ compared with H_2D_Z in *n*-hexane (table 1).

The reagent $Cl_2H_2D_Z$ reacts with selenium(IV) in strong acidic medium (2 M HCl), to form a yellow–red coloured complex which is insoluble in water and easily soluble in non-polar organic solvents. Based on the electronic spectrum of the produced complex in *n*-hexane (figure 2), the IR spectral data (table 1), and the results reported earlier for the selenium(IV) chelates of the dithizone [10, 30, 33] and bismuthiol II [34], a structure, III (figure 1), is most likely proposed for the formed selenium(IV) chelate. Thus, the overall reaction of selenium(IV) with the reagent $Cl_2H_2D_Z$ in HCl (2 M) most likely proceeds as follows:

$$H_2SeO_{3(aq)} + 2H^+ + 2Cl^- \rightleftharpoons SeOCl_{2(aq)} + 2H_2O \tag{1}$$

$$SeOCl_{2(aq)} + Cl_2H_2D_{Z\,(org)^-} \rightleftharpoons [SeO(Cl_2HD_Z)_2]_{(org)} + 2HCl. \tag{2}$$

The produced complex has two maxima (figure 2) at 313 ($\varepsilon = 2.8 \times 10^3$ L mol^{-1} cm^{-1}) and 416 nm ($\varepsilon = 9 \times 10^4$ L mol^{-1} cm^{-1}) in *n*-hexane which were well resolved and utilized for spectrophotometric determination of selenium(IV). Thus, in the subsequent work, the absorbance data were recorded at 416 nm.

The chemical composition of the extracted selenium(IV)–$Cl_2H_2D_Z$ chelate in *n*-hexane was determined by the slope ratio method [35]. The absorbance of *n*-hexane extracts with a large excess of the reagent and also with a large excess of selenium(IV) solution was finally measured at 416 nm against a reagent blank. Plots of the absorbance *versus* the total concentration of selenium(IV) and also the total concentration of the reagent are shown in figure 3. The slopes of the straight lines obtained on varying selenium(IV) concentrations (S_m) and/or reagent content (S_x) indicated that the selenium(IV) chelate produced has a selenium(IV) to a reagent $Cl_2H_2D_Z$ molar ratio of exactly 1 : 2 ($S_m/S_x = 2.1$).

To fully characterize the formed selenium(IV) chelate, the *n*-hexane extract was evaporated under reduced pressure after shaking with ammonia to remove the unreacted reagent $Cl_2H_2D_Z$ as reported earlier for selenium(IV) chelate with dithizone [10, 30, 33]. Elemental analysis of the proposed selenium(IV) chelate given in figure 1: $[SeOC_{26}H_{18}N_8S_2Cl_4]$ required: 43.6% C, 2.5% H, 15.7% N and 9.6% S; found 42.9%

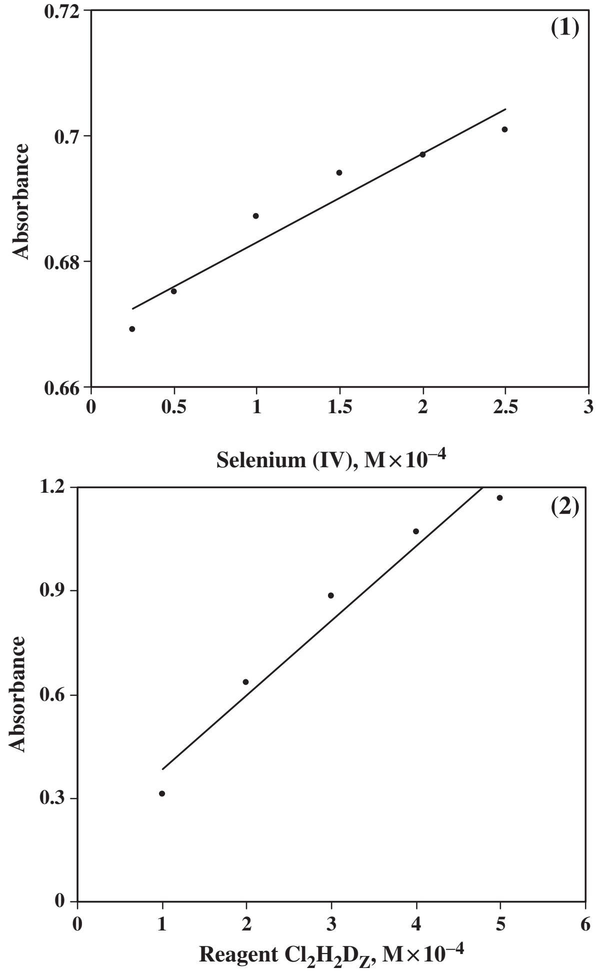


Figure 3. Chemical composition of the selenium(IV) complex by the slope ratio method using excess reagent, 2×10^{-4} M (1) and excess selenium(IV), 2×10^{-4} M (2).

C, 2.7% H, 15.5% N and 8.9% S. The IR spectra of the isolated solid selenium(IV) chelate recorded separately in KBr discs and as a mechanical mixture of selenium(IV) with $Cl_2H_2D_Z$ in a 1 : 2 molar ratio [28] showed the characteristic frequencies at 3436 (br), 1636 (s), 1526 (s), 1487 (s) and 440 cm^{-1} (table 1), which are safely assigned to ν (N–H), ν (N–N), ν (N–H) + (C–N), ν (N–C–S), and ν (Se–Cl) vibrations, respectively [10, 30].

3.2 *Optimal experimental conditions for selenium(IV) uptake by liquid–liquid extraction procedure*

The effect of pH of the aqueous phase on the extraction of the selenium(IV)–$Cl_2H_2D_Z$ complex was studied by measuring the absorbance of the extracted complex in *n*-hexane at 416 nm. The pH of the aqueous solution was adjusted with dilute HCl and/or NaOH, and directly measured before the extraction. Maximum absorbance of the produced complex was achieved at $pH < 2$. In acidic solution of $pH \leq 2$, the protonation of the selenite anions most likely occurred, which enhanced complex formation. In less acidic or alkaline solution, the absorbance decreased, possibly due to the incomplete complex formation, the existence of selenium(IV) as $HSeO_3^-$ or SeO_3^{2-}, which minimizes the chelate formation, and finally formation of non-extractable forms of selenium(IV).

The stability of the produced complex species of selenium(VI) was found to depend considerably on the nature and acidity of the aqueous phase. Thus, the effect of a series of mineral acids, e.g. HCl, HNO_3, H_2SO_4, and $HClO_4$ at 1 M concentration, was examined. Complete and excellent separation in a short time was achieved using HCl (1 M). The influence of HCl concentration (0.5–6 M) was then critically investigated. At an HCl concentration of >2 M, the extraction of the produced complex decreased due to the possible reduction of selenium(IV) to elemental selenium or selenide [31, 32]. The chromogenic reaction was found to be very fast at room temperature, and the selenium(IV) chelate produced was stable for up to 48 h for 2 M hydrochloric acid concentration; consequently, HCl (2 M) was selected in the subsequent work.

The solubility of the produced selenium(IV) complex was investigated in a series of organic solvents, namely: *n*-hexane, dichloromethane, carbon tetrachloride, toluene, chloroform, cyclopentanone, and methyl isobutyl ketone. Of these, *n*-hexane was found to be the best extractant where a maximum apparent molar absorptivity and solubility of the chelate were achieved. Also, the extraction of the developed selenium(IV) chelate in this solvent was completed in a very short time, as a better separation of the layers was obtained. The results also showed that the organic solvents with a low dielectric constant are favoured for the extraction of the chelate produced. However, the extractability of selenium(IV) chelate into $CHCl_3$, which has a dielectric constant higher than *n*-hexane, was more or less the same as the extractability into *n*-hexane. This may be due to the solvation of the complex by $CHCl_3$ molecules. The absorbance of the coloured complex in *n*-hexane was constant for up to 2 h for samples containing $\leq 10\,\mu g\,mL^{-1}$ selenium(IV) in the aqueous solution containing HCl (2 M). Thus, *n*-hexane was chosen as the preferred organic solvent and a shaking time of 1 min was adopted in the subsequent work.

The influence of $Cl_2H_2D_Z$ concentration on the extraction of selenium(IV) complex in *n*-hexane was investigated with $5\,\mu g\,mL^{-1}$ of selenium(IV). The data are shown in figure 4. The absorbance of the formed complex increased on increasing the reagent $Cl_2H_2D_Z$ concentration for up to 5.1×10^{-5} M (0.01% w/v). A large excess of the reagent tends to decrease the absorbance of the organic extract, owing to the increased absorbance of the reagent blank.

The effect of NaCl and/or $CaCl_2$ concentration (1–5% w/v) as a measure of water salinity and hardness on the extraction of selenium(IV) complex with the tested reagent from the aqueous solution was studied. No significant effect of these salts was observed in the absorbance of the organic extract of selenium(IV) chelate.

3.3 *Extraction equilibria*

To calculate the equilibrium constants K_{ex}, β, and K_D for the proposed reaction of selenium(IV) with the reagent $Cl_2H_2D_Z$, the following equilibria are considered [36, 37].

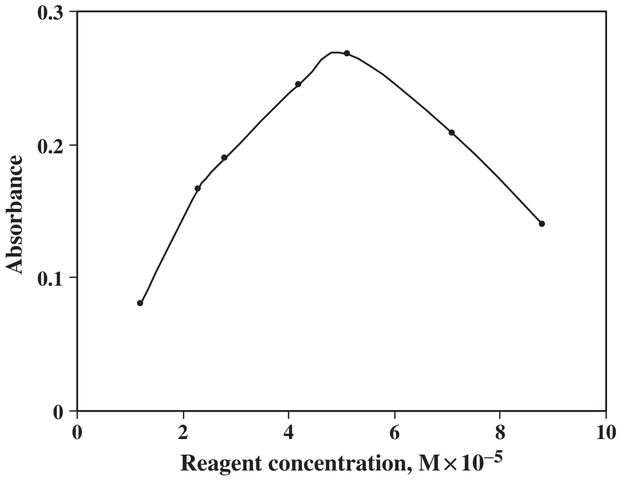


Figure 4. Effect of the reagent $Cl_2H_2D_Z$ concentration [M] on the extraction of selenium(IV) chelate.

Formation of a complex chelate in the aqueous phase containing HCl according to the equation:

$$H_2SeO_{3(aq)} + 2Cl_2H_2D_{Z(org)} \rightleftharpoons [SeO(Cl_2HD_Z)_2]_{(aq)} + 2H_2O, \tag{3}$$

with a corresponding equilibrium constant, β, where

$$\beta = [SeO(Cl_2HD_Z)_2]_{(aq)} \big/ [H_2SeO_3](aq) \cdot [Cl_2H_2D_Z]^2_{(org)}. \tag{4}$$

Distribution of the complex chelate between the aqueous and the organic phase with a distribution constant, K_D

$$[SeO(Cl_2HD_Z)_2]_{(aq)} \rightleftharpoons [SeO(Cl_2HD_Z)_2]_{(org)} \tag{5}$$

$$K_D = [SeO(Cl_2HD_Z)_2]_{(org)} \big/ [SeO(Cl_2HD_Z)_2]_{(aq)}. \tag{6}$$

The overall extraction process is then described by the following equation:

$$H_2SeO_{3(aq)} + 2Cl_2H_2D_{Z(org)} \rightleftharpoons [SeO(Cl_2HD_Z)_2]_{(org)} + 2H_2O, \tag{7}$$

with a corresponding extraction constant, K_{ex}.

$$K_{ex} = [SeO(Cl_2HD_Z)_2]_{org} \big/ [H_2SeO_3]_{(aq)}.[Cl_2H_2D_Z]^2_{(org)}. \tag{8}$$

The distribution ratio, D_{Se}, was determined at a constant initial concentration of selenite in the aqueous phase and various concentrations of the reagent $Cl_2H_2D_Z$ in *n*-hexane. Assuming that one complex species of selenium(IV) is present at equilibrium in the organic phase, the distribution coefficient of the system can be expressed as follows [37]:

$$D_{Se} = [SeO(Cl_2HD_Z)_2]_{(org)} \big/ [SeO(Cl_2HD_Z)_2]_{(aq)} + [H_2SeO_3]_{(aq)}. \tag{9}$$

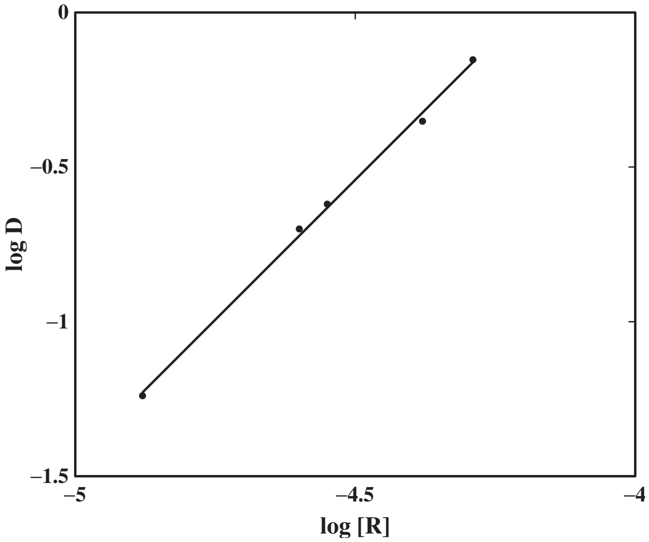


Figure 5. Plot of log $[Cl_2H_2D_Z]$ *vs.* log D of its selenium(IV) chelate in *n*-hexane.

At a low concentration of $Cl_2H_2D_Z$, the term $[SeO\ (Cl_2HD_Z)_2]_{(aq)}$ can be neglected, and equation (9) transforms to:

$$D_{Se^-} = [SeO(Cl_2HD_Z)_2]_{(org)} \big/ [H_2SeO_3]_{(aq)}. \tag{10}$$

After substituting equation (10) into equation (8) and taking the logarithms, equation (11) is obtained as follows:

$$\log D_{Se} = \log K_D \beta + \log [Cl_2H_2D_Z]_{(org)}. \tag{11}$$

The values of β, K_{ex}, and K_D of the extracted species were then determined graphically from the graph (figure 5) of log D_{Se} *versus* $\log[Cl_2H_2D_Z]_{org}$. A slope of 2.2 was obtained, indicating the formation of chelate of 1 : 2 molar ratios of selenite ions to the reagent $Cl_2H_2D_Z$, respectively. These data are in good agreement with the results obtained from the spectral and analytical data. The values of K_{ex}, K_D, and β were found to be equal to 1.2×10^4, 5.6 and 2.1×10^3, respectively. These results suggest the possible application of the proposed reagent for extractive sequential spectrophotometric determination of selenium(IV), (VI), and total inorganic selenium(IV) and (VI) in water after reduction of selenate to selenite.

3.4 *Spectrophotometric characteristics of selenium(IV) chelate*

After adjusting the experimental conditions of the reagent, a linear graph at 416 nm was obtained on recording the absorbance of the produced selenium(IV) chelate in *n*-hexane as a function of the selenium(IV) concentration. Beer's law was obeyed in the concentration range 0.01–20 $\mu g\, mL^{-1}$ selenium(IV) in aqueous solution containing HCl (2 M). The molar absorptivity calculated from the Beer–Lambert plot and Sandell's [38] sensitivity index of the selenium(IV)–$Cl_2H_2D_Z$ chelate at 416 nm were estimated to be

$9 \times 10^4\,\mathrm{L\,mol^{-1}\,cm^{-1}}$ and $0.08\,\mu\mathrm{g\,cm^2}$, respectively. The relative standard deviation (S_r) of five measurements with $5\,\mu\mathrm{g\,mL^{-1}}$ of selenium(IV) is estimated as 2.2%. The detection limit (3δ) based on three times the SD of the blank [39] was $0.01\,\mu\mathrm{g\,mL^{-1}}$ of selenium(IV) employing the proposed reagent $Cl_2H_2D_Z$. Regression analysis of the Beer–Lambert plot of the absorbance at 416 nm (A_{416}) and the concentration (C, $\mu\mathrm{g\,mL^{-1}}$) of selenium(IV) ions in the range 0.01–$20\,\mu\mathrm{g\,mL^{-1}}$ gave the following linear regression equation:

$$A_{416} = 0.0142C + 0.009, \tag{12}$$

with a slope of 0.0142, intercept of 0.009, and a correlation coefficient of 0.9992 for the complex $[SeOC_{26}H_{18}N_8S_2Cl_4]$. The effective concentration range of selenium(IV), as evaluated by Ringbom's plot was found to be 0.5–$19\,\mu\mathrm{g\,mL^{-1}}$ [40].

The plot of the amount of selenium(IV) chelate extracted from the bulk aqueous solution into the organic phase *versus* the amount of selenium(IV) remaining in the bulk aqueous phase varied linearly at low and moderate selenium(IV) concentrations ($\leq 30\,\mu\mathrm{g\,mL^{-1}}$), followed by a plateau at a high level of selenite ion concentration, suggesting a first-order behaviour. A solvent capacity of $2\,\mu\mathrm{g}$ of selenium(IV) ions uptake/mL *n*-hexane was achieved successfully with $Cl_2H_2D_Z$.

3.5 *Effect of foreign ions*

The influence of a series of diverse ions on the determination of selenium(IV) employing the proposed method was investigated. The tolerance limit was defined as the concentration of the foreign ion added, causing a relative error less than ±3%. The selectivity of the developed method at $1\,\mu\mathrm{g\,mL^{-1}}$ of selenium(IV) was critically examined in the presence of a relatively high excess (0.1–$1\,\mathrm{mg\,mL^{-1}}$) of some diverse ions, which often accompany the analyte ion. A percentage recovery of $100 \pm 2.5\%$ of selenium(IV) and a standard deviation of ± 0.38 were achieved in the presence of the ions Li^+, Na^+, K^+, Ca^{2+}, NH_4^+, Al^{3+}, Cl^-, and Br^- at 1 : 100 tolerable concentration of selenium(IV) to the diverse ions, respectively. Positive interferences were observed in the presence of other ions, even at low concentrations, e.g. Cu^{2+}, Fe^{2+}, MnO_4^-, V^{5+}, and NO_3^-. The reason for these interferences is possibly attributed to the ability of $Cl_2H_2D_Z$ to form relatively stable chelates with such metal ions. Elimination of these interferences from the proposed method was achieved successfully by adding EDTA (0.1%) and other reagents to their aqueous solutions (table 2), which promotes unambiguous and sensitive determination of selenium(IV).

3.6 *Sequential determination of selenium(IV) and selenium(VI)*

The procedures developed were applied for the analysis of a binary mixture of inorganic selenium(IV) and (VI) ions at a total concentration of $\leq 20.0\,\mu\mathrm{g\,mL^{-1}}$ in the aqueous media. An aliquot mixture was first determined according to the procedure described for selenium(IV) determination. Another aliquot mixture was reduced to selenium(IV) with HCl (6 M) and determined as described in the recommended procedures. The results obtained are summarized in table 3. Satisfactory recoveries (95.6–101.2%) were obtained, with a good reproducibility and a relative standard deviation in the

Table 2. Elimination of the effect of various ions on the determination of selenium (IV).

Foreign ions	Tolerance limit ($\mu g\,mL^{-1}$)	Note
Cu^{2+}	100	Add 0.5 mL of 0.1 M EDTA
Fe^{3+}	200	Add 0.5 mL of saturated NaF
Mn^{7+}	100	Add a few crystals of NaN_3
V^{5+}	100	Add 0.5 mL of 0.1 M EDTA
NO_3^-	100	Add a few crystals of NaN_3

Table 3. Analytical results of determination of binary mixtures of selenium(IV) and (VI).

Selenium species ($\mu g\,mL^{-1}$)[a]					
Se(IV)		Se(VI)		Recovery (%)	
Taken	Found	Taken	Found	Se(IV)	Se(VI)
10	10.14 ± 0.21	0	0	101.4 ± 0.2	–
10	10.16 ± 0.23	5	4.96 ± 0.2	101.6 ± 0.23	99.2 ± 0.21
10	9.97 ± 0.19	10	9.9 ± 0.2	99.7 ± 0.19	99 ± 0.22

[a] Average ($n = 5$) ± SD.

range of 1.9–2.3 and 2.1–2.2% for selenium(IV) and (VI), respectively, and compared successfully with the results obtained by dithizone method [10, 12]. These data indicate that the method is applicable with excellent accuracy, even in samples with a high content of dissolved salts, e.g. NaCl and/or $CaCl_2$.

3.7 *Application*

The validity of the proposed method for the determination of traces of inorganic selenium(IV) and (VI) in tap and freshwater samples was critically examined. Various amounts ($\leq 20.0\,\mu g\,mL^{-1}$) of selenium(IV) or (VI) and binary mixtures of selenium(IV) and (VI) were spiked to the water samples. Each solution mixture was adjusted to the required acidity, and the selenium(IV) ions were then separated out from the aqueous phase by the reagent $Cl_2H_2D_Z$ in *n*-hexane. The selenium(IV) in the organic extract was then determined with the aid of the standard curve constructed under the same experimental conditions as described earlier. The total inorganic selenium(IV) and (VI) ions were determined after reduction of selenium(VI) to selenium(IV) ions in the samples. Selenium (VI) ions in the water samples were then determined by the difference (A_2–A_1) between the absorbance of the aliquots before (A_1) and after (A_2) reduction. The data obtained are summarized in table 4. On the basis of these results, tap water samples were found to be free from selenium(IV) and/or (VI). The results obtained by the proposed method were compared favourably to those of the atomic absorption spectrometry (AAS) measurements and the spectrophotometric method involving dithizone [12] for selenium(IV) ions in tap water samples utilizing certain statistical evaluations. The results of F (0.075) and t (1.91) tests showed no significant differences (table 4) in accuracy and precision between the proposed and the reference method [12]. The low RSD of the developed method evaluated for a series of replicate analysis

Table 4. Analysis of selenium(IV) and (VI) ions spiked to tap water samples (50 mL) of selenium(IV) and (VI)[a].

Tap-water sample	Se(IV) added ($\mu g\,mL^{-1}$)	Se(VI) added ($\mu g\,mL^{-1}$)	Total Se(IV) and (VI) found ($\mu g\,mL^{-1}$)
1	10	–	10 ± 0.2
2	20	–	20 ± 0.12
3	10	10	20.1 ± 0.3
4	20	10	30.3 ± 0.25

[a] Average ($n = 5$) ± SD and t- and F-tests of significance = 1.91 and 0.075 at $P = 0.05$, respectively.

of samples containing selenium(IV) at different concentrations reflected a good precision and indicated that the proposed method is applicable with a good accuracy.

4. Conclusion

The proposed method for the determination of inorganic selenium(IV) and/or (VI) ions in aqueous via liquid–liquid extraction is accurate, simple, rapid, and inexpensive; does not involve any stringent reaction conditions; and can be compared favourably in both sensitivity and selectivity with the published methods [13, 16–18]. The formed chelate is very stable, and no standing time is needed before determining the ions. The clear advantage of the method is its applicability for the sequential determination of inorganic selenium(IV) and/or (VI) in freshwater. The method has been applied to the determination of traces of selenium in real matrices as well as in synthetic samples. The use of HCl (5–6 M) as a pre-reducing agent permits the quantitative reduction of selenium(VI) to (IV) under relatively simple experimental conditions followed by sequential determination of the produced selenium(IV). Thus, the method can certainly be considered among the most sensitive methods. However, work is still continuing for the sequential determination of total selenium including inorganic and organic bound selenium present in real samples using suitable analytical treatments.

Acknowledgement

The author M. S. El-Shahawi, would like to thank Prof. A. M. Kiwan, for his assistance in the preparation of the reagent $Cl_2H_2D_Z$.

References

[1] M.I. Sitting. *Toxic Metals, Pollution Control and Worker Protection*, Noyes Data Corporation, Park Ridge, NJ (1976).
[2] D.C. Adriano. *Biogeochemistry of Trace Metals*, Lewis, London (1992).
[3] O.A. Levander. *Clin. Biochem.*, **6**, 345 (1982).

[4] L.H. Foster, S. Sumar. *Food Chem.*, **55**, 293 (1996).
[5] G.V. Iyengar, W.E. Kollmer, H.J.M. Bowen. *The Elemental Composition of Human Tissues and Body Fluid*, Verlag Chemie, Weinheim (1978).
[6] H. Robberecht, R.V. Grieken. *Talanta*, **29**, 824 (1982).
[7] W.T. Buckley, J.J. Budac, D.V. Godfrey, K.M. Koenig. *Anal. Chem.*, **64**, 724 (1992).
[8] M.S. Alaejos, D. Romero. *Crit. Chem. Rev.*, **95**, 227 (1995).
[9] R.M. Olivas, O.F.X. Donard, C. Camara, P. Quevauviller. *Anal. Chim. Acta*, **286**, 357 (1994).
[10] A.D. Campbell, A.H. Yahaya. *Anal. Chim. Acta*, **119**, 171 (1980).
[11] S.C. Lavale, M. Dave. *J. Indian Chem. Soc.*, **66**, 974 (1989).
[12] H. Afsor, R. Apak, I. Tor. *Analyst*, **114**, 1319 (1989).
[13] B.L. Osburn, A.D. Shrendrikar, P.W. West. *Anal. Chem.*, **43**, 594 (1971).
[14] K.N. Ramachandran, R. Kaveeshwar, V.K. Gupta. *Talanta*, **40**, 781 (1993).
[15] A. Safavi, A. Afkhami, A. Massoumi. *Anal. Chim. Acta*, **232**, 351 (1990).
[16] R. Gurkan, M. Akcay. *Microchem. J.*, **75**, 39 (2003).
[17] A. Afkhami, T. Madrakian. *Talanta*, **58**, 311 (2002).
[18] K. Pyrzynska. *Anal. Sci.*, **13**, 629 (1997).
[19] H. Khajehsharifi, M.F. Mousavi, J. Ghasemi, M. Shamsipur. *Anal. Chim. Acta*, **512**, 369 (2004).
[20] H.D. Revanasiddappa, T.N.K. Kumar. *Anal. Sci.*, **17**, 1309 (2001).
[21] A. Afkhami, A. Safavi, A. Massoumi. *Talanta*, **39**, 993 (1992).
[22] P. Kuban, V. Kuban. *Anal. Bioanal. Chem.*, **378**, 378 (2004).
[23] P. Kuban, P. Kuban, V. Kuban. *Electrophoresis*, **24**, 3725 (2003).
[24] A. Safavi, M. Mirzaee. *Talanta*, **51**, 225 (2000).
[25] M. Gallignani, M. Valero, M.R. Brunetto, J.L. Burguera, M. Burguera, Y. Petit de Pena. *Talanta*, **52**, 1015 (2000).
[26] P. Kuban, P. Kuban, V. Kuban. *Electrophoresis*, **24**, 1397 (2003).
[27] B.M. Nagabhushara, G.T. Chandroppe, B. Nagappo, N.H. Nagaraj. *Anal. Bioanal. Chem.*, **373**, 279 (2002).
[28] M.S. El-Shahawi, M.A. El-Sonbati. *Talanta*, **67**, 806 (2005).
[29] A.M. Kiwan, A. Kassim. *Anal. Chim. Acta*, **88**, 177 (1977).
[30] H. Mabuchi, H. Nakahara. *Bull. Chem. Soc.*, **36**, 151 (1963).
[31] U. Ornemark, A. Olin. *Talanta*, **41**, 67 (1994).
[32] I.L. Stewart, A. Chow. *Talanta*, **40**, 1345 (1993).
[33] Z. Marczenko. *Separation and Spectrophotometric Determination of Elements*, Wiley, New York (1986).
[34] J.E. Barmey. *Talanta*, **14**, 1363 (1967).
[35] D.T. Sawyer, W.R. Heineman, J.M. Beebe. *Chemistry Experiments for Instrumental Methods*, Wiley, New York (1984).
[36] A. Alexandrov, O. Budevsky, A. Dimitrov. *J. Radioanal. Chem.*, **29**, 243 (1976).
[37] S.M. Kamburova. *Talanta*, **39**, 997 (1992).
[38] E.B. Sandell. *Colorimetric Determination of Trace Metals*, Interscience, New York (1959).
[39] IUPAC. *Pure Appl. Chem.*, **45**, 105 (1976).
[40] A. Ringbom. *Fresenius Z. Anal. Chem.*, **115**, 332 (1939).