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APPLICATION OF KINETIC CATALYTIC SPECTROPHOTOMETRIC METHOD FOR THE SPECIATION OF SELENIUM IN ENVIRONMENTAL WATER

By

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

Division of Chemistry
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March 2007

DEDICATION

To my wonderful parents, for their love and support

CERTIFICATE OF AUTHENTICITY

I, Vimlesh Chand, hereby declare that this thesis is the result of my own work and the same has not been submitted elsewhere for the award of a degree, and any information obtained from literature or otherwise has been duly and appropriately acknowledged and referenced.

……

Vimlesh Chand

The work presented in this thesis was performed under my supervision.

……

Dr. Surendra Prasad (Thesis Supervisor)

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ABBREVIATIONS

a	- Intercept of linear equation
A	- Absorbance
AAS	- Atomic Absorption Spectrometry
AES	- Atomic Emission Spectrometry
AFS	- Atomic Fluorescence Spectrometry
ANOVA	- Analysis of Variance
AOAC	- Association of the Official Analytical Chemists
APHA	- American Public Health Association
ASV	- Anodic Stripping Voltammetry
AWWA	- American Water Works Association
b	- Slope of linear equation
C	- Catalysed reaction
CCA	- Copper, Chromium and Arsenic
CL	- Confidence Limit
CKM	- Catalytic Kinetic Method
CRM	- Certified Reference Material
CSV	- Cathodic Stripping Voltammetry
CV	- Coefficient of Variation
D1	- First derivative of UV-visible spectra
DEDSe	- Diethyldiselenide
DESe	- Diethylselenide

DMDSe - Dimethyldiselenide

DMSe - Dimethylselenide

DNA - Deoxyribonucleic acid

DPCSV - Differential Pulse Cathodic Stripping Voltammetry

DPP - Differential Pulse Polarography

EAAS - Electrothermal Atomic Absorption Spectrometry

EDTA - Ethylenediaminetetraacetic acid

Fig - Figure

GC - Gas Chromatography

GLC - Gas Liquid Chromatography

GF - Graphite Furnace

h - Hour (s)

HG - Hydride Generation

HGAAS - Hydride Generation Atomic Absorption Spectrometry

HPLC - High Performance Liquid Chromatography

I - Ionic strength

ICP - Inductively Coupled Plasma

ICPMS - Inductively Coupled Plasma Mass Spectrometry

IUPAC - International Union of Pure and Applied Chemistry

KAC - Kinetics in Analytical Chemistry

KMA - Kinetic Methods of Analysis

L - Litre

LOD - Limit of Detection

M - Molar (moles per litre)

MB - Methylene Blue

mg - Milligram

min - Minute

mL - Milliliter

MO - Methyl orange

mol - Moles

MS - Mass Spectrometry

n - Number of replicates

NAA - Neutron Activation Analysis

ng - Nanograms

NIST - National Institute of Standards and Technology

nm - Nanometers

ppb - Parts per billion

ppm - Parts per million

ppt - Parts per trillion

RSD - Relative Standard Deviation

s - Seconds

SAE - Standard Analytical Error

SD - Standard Deviation

Se - Selenium

SPDANS - 4,5-dihydroxy-3-(p-sulfophenylazo)-2,7-naphthalene disulfonic acid

SPREP - South Pacific Regional Environmental Programme

SRM - Standard Reference Material

t - Time of reaction

t - Student's t-value

TBT - Tributyltin

USDHHS - United States Department of Health and Human Services

USEPA - United States Environmental Protection Agency

U - Uncatalysed reaction

UV - Ultraviolet

vs - Versus

WEF - Water Environment Federation

WHO - World Health Organisation

Δ - Change

λ - Wavelength

% - Percentage

~ - Approximately

× - Multiplied by

√ - Square root

°C - Degree Celsius

μ - Micro

ε - Molar absorptivity

ABSTRACT

A simple and sensitive catalytic kinetic spectrophotometric method was optimised, validated and successfully applied for the determination of inorganic selenium at parts per billion levels in synthetic and real water samples. Experimental variables affecting the sensitivity of method were investigated and optimum conditions were established. Two techniques of data treatment, Initial rate and Fixed time method, were used and compared for their sensitivity. The limit of detection of the methods were found to be 1.3 $\mu g\ L^{-1}$ and 14.7 $\mu g\ L^{-1}$ for the Initial rate and Fixed time method, respectively.

Standard reference materials consisting of selenium standards and real water sample were used to validate the method. The accuracy and precision was determined using recovery studies in the lower range of selenium concentration. The reproducibility of the method was investigated using quality control procedures. Different sample digestion techniques were studied for determining the concentrations of different inorganic selenium species in water. The existing standard digestion techniques, HCl method for the determination Se(IV) and American Public Health Association method for the determination of total Se were found to be suitable with minor modifications. Within and between day analysis of selenium standards showed very good reproducibility and precision of the method.

The proposed method was applied to environmental water samples collected from Suva and Labasa areas in Fiji. Overall, it was found that the proposed method is reliable and accurate for environmental monitoring of inorganic Se in the aquatic system. In addition,

water samples tested showed that Se levels were below detection limit of the method, hence within the limits set by the World Health Organisation.

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CHAPTER 1

General Introduction

1.1 Introduction

The demand for a country's economic, agricultural and industrial development has significantly outweighed the demand for a safe, pure, and natural environment for its people (Leiva *et al*. 2004). Consequently, one of the major problems faced by the world in the twenty first century is the degradation of the aquatic environment from contamination by anthropogenic sources (Batley 1996). Every city in the world is facing critical environmental problems today as a result of overloading the aquatic ecosystem with pollutants such as heavy metals, persistent organic pollutants, oils and nutrients (Ferretti *et al*. 2007). In order to monitor these threats, it is imperative that simple inexpensive but sensitive analytical techniques must be available to regulating agencies so that environmental monitoring can be done regularly to detect any threat to the aquatic ecosystems from harmful pollutants (Batley 1996).

One of these hazardous pollutants, the heavy metals, that are gaining considerable attention as evident from the increasing number of publications in this area of research. Though environmental heavy metal contamination has been largely associated with developed countries, studies and experiences now reveal that this problem is no longer a stranger to developing countries, such as the Pacific islands (SPREP 2004). Countries like Fiji, Tonga and Tuvalu are now facing the growing threat of rising contamination of their drinking water sources from anthropogenic activities and hence are at risk of deterioration of the quality of their citizens' lives (SPREP 2004; IWP 2007).

Consequently, there is pressing need to adopt tougher approach by the government and environmental enforcement agencies of the affected countries in order to safeguard their precious natural resource from toxic heavy metal contamination.

Heavy metals are one of the most extensively studied groups of pollutants in the aquatic environment. The term “Heavy Metal” refers to any metallic element that has a relatively high density (> 4.5 times that of water) (Hawkes 1997; Pais and Jones 1997) and is known to have toxic effects at very low concentrations (Gangaiya *et al.* 2001). They are stable in nature, have atomic weights between 63.5 and 200.6 g mol^{-1}, and also include some metalloids, such as selenium (Hawkes 1997; Pais and Jones 1997). However, it should be noted that there are many definitions for heavy metals and none of these have been derived by an authoritative body (Duffus 2002). Since these metals and metalloids occur naturally in the earth's crust, they are released to the hydrologic cycle during physical and chemical weathering of igneous and metamorphic rocks (Garret 2000). Some heavy metals are naturally abundant and have high background concentrations such as Al and Fe, while others are rare and have low background concentrations such as Hg, Cd, Ag, As and Se (Binning and Baird 2001). However, human civilisation has had a profound impact on the heavy metal balance in the nature, which has significantly been disturbed. While the heavy metal inputs to the aquatic system can either be natural or anthropogenic, it is mostly by anthropogenic sources. Research has shown that about 90% of the world’s anthropogenic release of heavy metals has occurred since 1900 A.D. (Nriagu 1996). Hg, Pb, As, Cd, Se, Cu, Zn, Cr and V are some of the most highly disposed heavy metals in the environment by anthropogenic sources (Hassan 2004). The

anthropogenic sources of heavy metals include industrial and municipal waste products, urban and agricultural runoff, fine sediments eroded from catchments, atmospheric deposition, CCA treated wood walkways, antifouling paints from ships (mainly tin and copper), metals from pipes in sewage treatment plants and drainage from acid sulfate soils and mine sites (Ferretti *et al*. 2007).

1.2 Pollution of the Aquatic Environment

Aquatic contamination from heavy metals is of great concern because they cannot be degraded further, their toxic effects can last long and their concentrations can increase through bioaccumulation in the food chain (Gangaiya *et al.* 2001). As heavy metals are contained in all four types of reservoirs in an aquatic environment, namely, the surface water, the pore waters, the suspended sediment, and the bottom sediment, contamination has serious repercussions for aquatic inhabitants. Numerous studies elsewhere have documented elevated heavy metal concentrations in aquatic systems caused by contamination sources and the literature continues to grow continually on heavy metal pollution around the world (Garret 2000).

The challenges of analysing heavy metals in the aquatic environment are complicated further because of the changes that occur in their chemical forms as they undergo biogeochemical cycling (Garret 2000). Unfortunately, there have been only a few published studies of environmental water quality in the Fiji islands (Lee and Brodie 1982; Gangaiya *et al*. 1988) and even the levels of heavy metals in environmental water sources are little investigated (Singh and Mosley 2003). It has been realised that the inability of

many Pacific Island countries to accurately measure parameters such as heavy metals in water has been due to lack of updated labs, technical staff, cost effective analytical methods and lack of funding to send samples elsewhere for analysis (Singh and Mosley 2003). However, with the increasing need for environmental monitoring, effective solutions must be found so that regular sampling and analyses of environmental water samples are carried out without putting much strain on the allocated environmental budget.

1.3 Importance of Elemental Speciation

It is understood that a metal undergoes major chemical transformations as it is recycled in the environment and different species of the metal would have varying degrees of toxicity on living organisms (Caruso and Montes-Bayon 2003). Chemical species refer to the specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure. Speciation analysis involves identifying and/or measuring the quantities of one or more individual chemical species in a sample (Sanz-Mendel 1998).

The oxidation state of an element can profoundly affect its toxicity and the absorption and elimination of an element. In the case of iron(II), it readily diffuses across membranes, while iron(III) is easily hydrolysed in aquatic and biological systems (Templeton *et al*. 2000). Even though Hg(II) gets trapped into cells, a bacteria reduces it to Hg^0 which then diffuses from the cell. In general, metals such as Hg and Sn undergo biomethylation in environment and this generally increases the toxicity of metals

(Cornelis *et al*. 2003). However, biomethylation of Se detoxifies the oxoanions of this highly toxic metalloid (Spallholz 1994).

Toxicity often results from bioaccumulation of metal forms in fatty tissues and penetration of membrane barriers. For example, methylmercury is more toxic than Hg^{2+} and tributyltin (TBT) is a most potent biocide, while Sn(IV) is not (Cornelis *et al*. 2003). While a fractionation (for instance, determining soluble and insoluble forms or the particle size distribution) provides more information than measuring total element only, additional useful information on different species with carcinogenic potential can be obtained by speciation analysis (Templeton *et al*. 2000). Hence, total element concentrations, which have traditionally been reported, no longer provide sufficient information to allow risk-based toxicity assessment. Several diverse fields such as toxicology, clinical chemistry, geochemistry and environmental chemistry have accepted that the behaviour of a particular element is affected by the distribution among its various species (Templeton *et al*. 2000).

1.4 Chemical Analytical Techniques in Pollution Monitoring

In order to place emphasis on the role of chemical analysis to determine the concentration of toxic metals species in the aquatic environments from human impacts, standard, inexpensive and simple analytical methods are required (Sanz-Mendel 1998). While standard methods for heavy metals are usually available, methods capable of detecting individual metallic species in a sample are not readily available due to high costs involved. While the literature is informative on the total metal (As, Cu, Zn *etc*.)

concentration in environmental samples, speciation data is almost non-existent for countries like Fiji (O'Brien *et al.* 2003). Majority of studies on heavy metals have focused only on total metal analysis, which do not give much information on the toxicity and bioavailability of these metals in the environment. Thus, the development of standard analytical techniques for metal speciation study has become important so that methods are readily available to environmentalists, which are suited to local conditions.

While analytical techniques for metal speciation in water samples are being developed at rapid pace, their high sensitivities do not come without high costs (Capelo *et al*. 2005). The instruments involved in these studies are usually very expensive, require skilled operators and need high maintenance costs. Developing countries like the South Pacific islands countries can hardly afford to establish such expensive hyphenated techniques hence continue to lag in environment monitoring exercises.

1.5 Present Project

Consequently, the objective of the present work was to standardise and apply an analytical procedure for Se, an extremely toxic metalloid, in some environmental water samples of Fiji. In particular an effort was made to develop a kinetic spectrophotometric method, which was to be based on the catalytic effect of Se on a redox reaction, to differentiate and determine inorganic Se dissolved species in natural water. It has already been established that Fiji's aquatic system is already threatened with toxic heavy metal contamination from anthropogenic sources (Singh and Mosley 2003). Se has been hardly studied in Fiji or any of the South Pacific countries. Waters with anthropogenic inputs of

Se are often at toxic levels, however, it can still be below the detection limits of the most of the commonly used analytical methods (Wake *et al*. 2004). This leads to difficulties in quantifying individual species. This project aims to invest interest in the study of highly important environmental chemistry of Se as well as promote the use of cheaper and affordable analytical techniques for elemental speciation of Se in the South Pacific.

The proposed method for Se speciation is based on the catalytic effect of Se(IV) on the reduction of bromate by hydrazine dihydrochloride. It is well established that Se(IV) has a catalytic effect on the reduction reaction of bromate by hydrazine dihydrochloride (Afkhami *et al*. 1992). However, the experimental data on bromate-hydrazine-methyl orange indicator reaction are confined to very high reactant concentrations while the dynamic range and detection limit of determination reported by these authors is quite wider and lower, respectively. Based on the literature review of the development of catalytic kinetic methods (CKM) for determination of various species this appears to us to be unlikely. In addition, the choice of experimental conditions such as wavelength maxima and concentration of methyl orange (MO) are neither studied nor supported with literature. The authors' selection of the optimum reaction pH on the basis of the claim that it provided the most stable reaction is in contrast to their experimental data. Consequently, these considerations raised doubt about the reported dependence study for the optimisation of the indicator reaction. This prompted us to investigate the detailed dependence studies on the reactant concentrations to clarify the optimum conditions and discover the feasible dynamic range for selenium determination based on its catalytic effect on indicator reaction between hydrazine and bromate ion and the same is reported

in the present thesis. The reaction is monitored spectrophotometrically by measuring the decrease in absorbance of methyl orange *versus* time for the first six minutes. The proposed method use readily available inexpensive reagents and instrumentation and have the capability to analyse different inorganic Se species and total Se in all kinds of environmental water. The method validation and application was carried out successfully using standard reference materials for selenium.

Water samples included from sources such as drinking, natural, groundwater and polluted, so that the response of the proposed method could be investigated to a range of samples which had different matrices. However, no Se was detected in the real water samples, probably because it was present below the detection limits of the proposed method. This also confirmed that there was no anomalous levels of Se present in the samples tested, which were limited to two sites in Fiji.

CHAPTER 2

Literature Review

2.1 Background

Selenium (Se), an element with atomic number of 34, is an important metalloid with environmental, biological, industrial, and toxicological significance. It is an essential nutrient for humans, animals and plants (Pais and Jones 1997). It was discovered in 1817 by Swedish chemist, Jons Jacob Berzelius, while analysing a red deposit on the wall of lead chambers used in the production of H_2SO_4 (Tinggi 2003). Se occurs in a number of oxidation states (-II, 0, +IV, and +VI) in elemental and combined forms. More than twenty Se compounds have now been identified (Dumont *et al.* 2006). Se consists of six stable isotopes: ^{74}Se (natural abundance fraction, 0.89%), ^{76}Se (9.37), ^{77}Se (7.63), ^{78}Se (23.77), ^{80}Se (49.61), and ^{82}Se (8.73) (Suzuki *et al.* 2006). In the environment, total Se levels range from 0.1 - 400 $\mu g\ L^{-1}$ in natural waters, to 0.06 - 1.8 $ng\ g^{-1}$ in soils and a few nanograms per cubic meter in the atmosphere (Conde and Alaejos 1997).

In spite of being essential for human and animal nutrition, elevated levels of Se in either water or in animal diet can result in acute or chronic poisoning, pathological changes in tissue, impaired reproduction (including mortality of young) of adult animals (Tinggi 2003). Se has the narrowest range of deficiency and toxicity of all elements (Goldberg *et al.* 2006). Se occurrence in natural and environmental waters has received wide attention (Conde and Alaejos 1997). Anthropogenic activities such as combustion of fossil fuel etc. are increasingly delivering Se to surface waters, drawing considerable attention to the behaviour of this element in the aquatic environment (Cutter 1993).

2.1.1 Overview of Se

Se is an essential dietary nutrient for all mammals, and recognised as an important element for many cellular processes (Letavayov *et al.* 2006). The pioneering work of Schwarz and Foltz in 1957 revealed that Se at very low dietary concentrations is an essential nutrient (Schwarz and Foltz 1957). However, in the first half of the 20th century, before any health benefits of Se were known, it was considered an undesirable element for higher organisms, as evident from its toxicity to animals. Toxicity of Se was first confirmed in 1933 in livestock that consumed Se accumulator plants of the genus *Astragalus*, *Xylorrhiza*, *Oonopsis* and *Stanleya* in the western regions of the United States (Letavayov *et al.* 2006).

2.1.2 Se Occurrence in the Environment

Determination of Se concentrations in a variety of materials indicates that Se is widely distributed throughout the environment. It occurs naturally in sedimentary rock (Dietz *et al.* 2004) and in natural deposits as ores containing other elements (USEPA 2007). The processes responsible for its distribution include volcanic activity, the burning of fossil fuels, the weathering of rocks and soils, groundwater transport, precipitation of Se minerals, adsorption, chemical or bacterial reduction and oxidation, and metabolic uptake and release by plants and animals (Dietz *et al.* 2004). Se has been reported on a large scale in some specific geographical locations such as China (Melwanki and Seetharamappa 2000), however, a few locations in the world do not have enough natural Se in soil to support human dietary needs (USDHHS 2003).

2.1.3 Se in Human Health

It is well established that Se has multiple roles in biological systems, including structural and enzymatic roles (Cankur *et al.* 2006). It has capability to act as an antioxidant. Researches over the last twenty years have shown that dietary Se can prevent cancer and cardiovascular diseases (Irwin 1997). Se also occurs in biological specimens as complexes with metals (Hawkes and Kutnink 1996) such as mercury, cadmium, and zinc and hence it has the ability to prevent toxic effects of heavy metals (Frisk *et al.* 2001).

Se resembles sulphur in many of its chemical properties. Its biologically important inorganic and organic forms are in general analogous to sulphur compounds. Se is mostly found in the amino acids selenocysteine ($HSeCH_2CH(NH_2)CO_2H$) and selenomethionine ($CH_3SeCH_2CH_2CH_2(NH_2)CO_2$). It is in this form *i.e.* Se(-II), that Se exerts its major biochemical effects. Se in the form of selenomethnione may be randomly incorporated into proteins in place of methionine (Patching and Gargner 1999). Se is also known to function as the active centre of selenoproteins for some redox enzymes such as glutathione peroxidase (Suzuki and Orgra 2002). At least 15 selenoproteins and/or selenoenzymes have been identified in biological systems (Cankur *et al.* 2006).

Se compounds at low concentration exhibit protective anticarcinogenic properties, whereas at higher concentration they are genotoxic and possibly even carcinogenic (Letavayov *et al.* 2006). The initial US recommended daily allowance in 1989 was 50 - 70 μg per day (this value has recently been lowered to 55 μg per day) for healthy human adults (El-Bayoumy 2001; Whanger 2004). Based on human studies, intakes of 400 μg

per day were established as the maximum safe dietary dose with no observed adverse effects. The low adverse effect of Se supplementation was calculated to be 1540 - 1600 μg per day. An intake of 3200 - 5000 μg per day resulted in definite occurrence of selenosis (Reid *et al.* 2004). On the other hand a level of about 40 μg per day was suggested as the minimum requirement, while an intake of < 11 μg per day results in deficiency problems (Whanger 2004).

Being an essential trace element, Se deficiency can cause a number of diseases. For example, "Keshan disease" and "Kashin-Beck disease" have been reported in humans in Se-deficient populations in China. (ATSDR 2003). Keshan disease is characterised by cardiac enlargement, heart failure and cardiogenic shock while Kashin-Beck disease, which occurs primarily in children between the ages of 5 and 13 years, is characterised by atrophy, degeneration, and necrosis of cartilage tissue (ATSDR 2003; USDHHS 2003). Its deficiency may cause significant increase in number of cancer patients in several countries (Kapolna 2007).

2.1.4 Sources of Dietary Se

Animals normally take up Se from food, water, and air (Irwin 1997), while food being the primary source of intake (ATSDR 2003; Bierla *et al.* 2004; USEPA 2007). For example, the estimates of the average intake of Se from food for the United States population range from 71 to 152 μg of Se per person per day (ATSDR 2003). Se exists in mostly organic forms in normal diets. Organic Se is present in foods mainly in the form of selenomethionine, selenocysteine and Se-methylselenocysteine. Inorganic Se is present

either as selenite or selenate though much less frequently and in very low amounts in food (Letavayov *et al.* 2006). Se content of food varies widely among different regions of the world due to differences in Se content of the soil (Kapolna *et al.* 2007) and also due to variation in the dietary habits of people (Al-Saleh *et al.* 2006).

Drinking water usually contains Se at very low levels (usually < 0.01 mg L^{-1}). For example, Se levels were tested less than 10 ppb in 99.5% of drinking water sources in United States (ATSDR 2003). However, occasionally, higher levels of Se has been found in drinking water, usually in areas where high levels of Se is present in soil (ATSDR 2003). Drinking water contributes to 1 - 6% of Se uptake for typical water concentrations and water consumption of 3 L per day (Robberecht and Grieken 1982). However, should Se concentration in water increase due to some anthropogenic reason, a daily intake of 2 L water containing the USEPA upper limit of Se (10 μg L^{-1}) can make a significant fraction (33%) of Se recommended daily intake (Robberecht and Grieken 1982).

2.1.5 Se Metabolism

Both organic and inorganic forms of Se appear to be utilised with similar efficacy in the body to produce selenoproteins (Shiobara *et al.* 1998) but the Se enters at different points in metabolism depending on its chemical form. A metabolic scheme showing Se metabolism is presented in Fig 1.

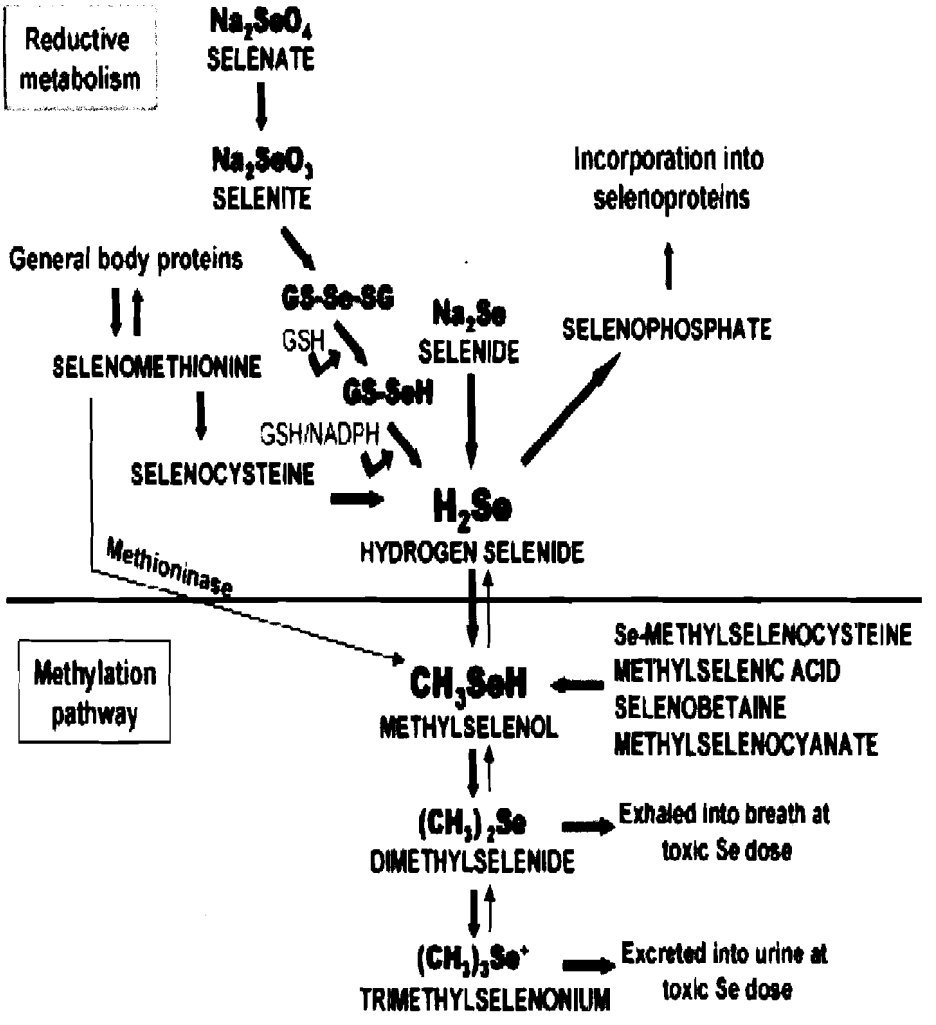


Fig 1 Schematic representation of the Se metabolic pathway (adapted from Letavayov *et al.* 2006)

2.1.6 Toxicity of Se

Depending on the species, oxidation state and concentration (Dietz *et al.* 2004), Se compounds range from being essential to highly toxic to animals, plants, and humans (Jacobs 1989). Levels of Se intake may be useful indicators of healthy and toxic doses of Se (Kobayashi *et al.* 2002). Generally, the methylation pathway is considered to be the detoxification pathway for all forms of Se in the diet or in supplements (Foster *et al.* 1986; Lu *et al.* 1995). The actual metabolism and requirements of Se in the organisms are not yet completely understood, high doses of Se have been known to cause major health problems in livestock and humans for more than a century (Cankur *et al.* 2006).

Elevated levels of Se in either water or an animal diet can result in acute toxicity and chronic poisoning of adult animals, pathological changes in tissue, impaired reproduction (including mortality of young). Toxic Se intake for adults and children has been determined to be 5 mg day^{-1} (Pais and Jones 1997) and 0.1 mg day^{-1} (Gomez-Ariza *et al.* 2004), respectively. Se in feeds exceeding 5 mg dm^{-3} concentrations can cause chronic selenosis in animals (Holak and Spechio 1994). Sodium selenite (Na_2SeO_3), an inorganic Se compound, has been reported to induce deoxyribonucleic acid (DNA) damage, particularly DNA strand breaks and base damage in mammalian cells (Letavayov *et al.* 2006).

Epidemiological studies of chronic human exposure to high levels of Se in food and water have reported discoloration of the skin, pathological deformation and loss of nails, loss of hair, excessive tooth decay and discoloration, lack of mental alertness, and

listlessness (Patching and Gardiner 1999; ATSDR 2003; USEPA 2007). Acute human exposure to Se compounds via the oral route has resulted in pulmonary edema and lesions of the lung; cardiovascular effects such as tachycardia; gastrointestinal effects including nausea, vomiting, diarrhea, and abdominal pain; effects on the liver; and neurological effects such as aches, irritability, chills, and tremors (Patching and Gardiner 1999; ATSDR 2003). These health effects, called selenosis, were seen in several villages in China where people were exposed to foods high in Se for months to years (ATSDR 2003).

In cattle and livestock, poisoning by Se has resulted in loss in fertility, atrophy of hooves, lameness and anemia (Fuavao 1986). Acute animal tests in rats, mice, and guinea pigs, have shown sodium selenite to have extreme toxicity from oral exposure (ATSDR 2003). "Alkali disease" is a disease in livestock resulting from chronic consumption of high levels of Se; it is characterised by hair loss, deformation and sloughing of the hooves, erosion of the bone joints, anemia, and effects on the heart, kidney, and liver (USDHHS 2003). The consumption of high levels of Se in the diet by pigs, sheep, and cattle has been shown to interfere with normal fetal development and to produce fetal malformations (ATSDR 2003).

2.1.7 Industrial Uses of Se

There is a fast growing use of Se in the industrial sectors, such as in microelectronics, semiconductors and optoelectronics, where most of the processed Se is currently being used (Jacobs 1989). It is often used in the glass industry, steel production, as a component of pigments in plastics, paints, enamels, photographic toner, rubber and pharmaceuticals (D'Ulivo 1997). Se salts are used as nutritional supplements for animals to overcome problems of Se deficiency. Se sulphide is commonly used as an ingredient in antidandruff shampoos (Aggarwal *et al.* 2003). The United States Environmental Protection Agency (USEPA) has determined that Se sulfide is a probable human carcinogen (USDHHS 2003). Se sulfide is used in anti-dandruff shampoos by the common trade name Selsun Blue, a prescription dandruff shampoo containing 2.5% Se sulfide. Other industrial applications include its use as an additive in alloys to improve their machinability and corrosion resistance. Se is also used as catalyst in chemical reactions, manufacture of rubber and as a grid hardener in lead-acid batteries (Reilly 1997).

As a result of these anthropogenic activities with Se, which was just a rare and low level constituent in our diet, has become one of the adventitious environmental contaminants of the modern technological world (Reilly 2004). Release of Se in the environment as a result of human activities was estimated to be 79,000 tonnes per year in 1988, with a clear increasing tendency since then (Dietz *et al.* 2004).

2.2 Se in the Aquatic Environment

2.2.1 Fate of Se in the Aquatic Environment

When dissolved Se enters an aquatic ecosystem, it can be absorbed or ingested by organisms, it can bind or complex with particulate matter or surficial sediments or it can remain free in solution (Lemly 1999). Over time, most of the Se is either taken up by organisms or bound to particulate matter (Fig 2). Deposition of the biologically incorporated Se and settling of particulate matter (sedimentation), leads to accumulation

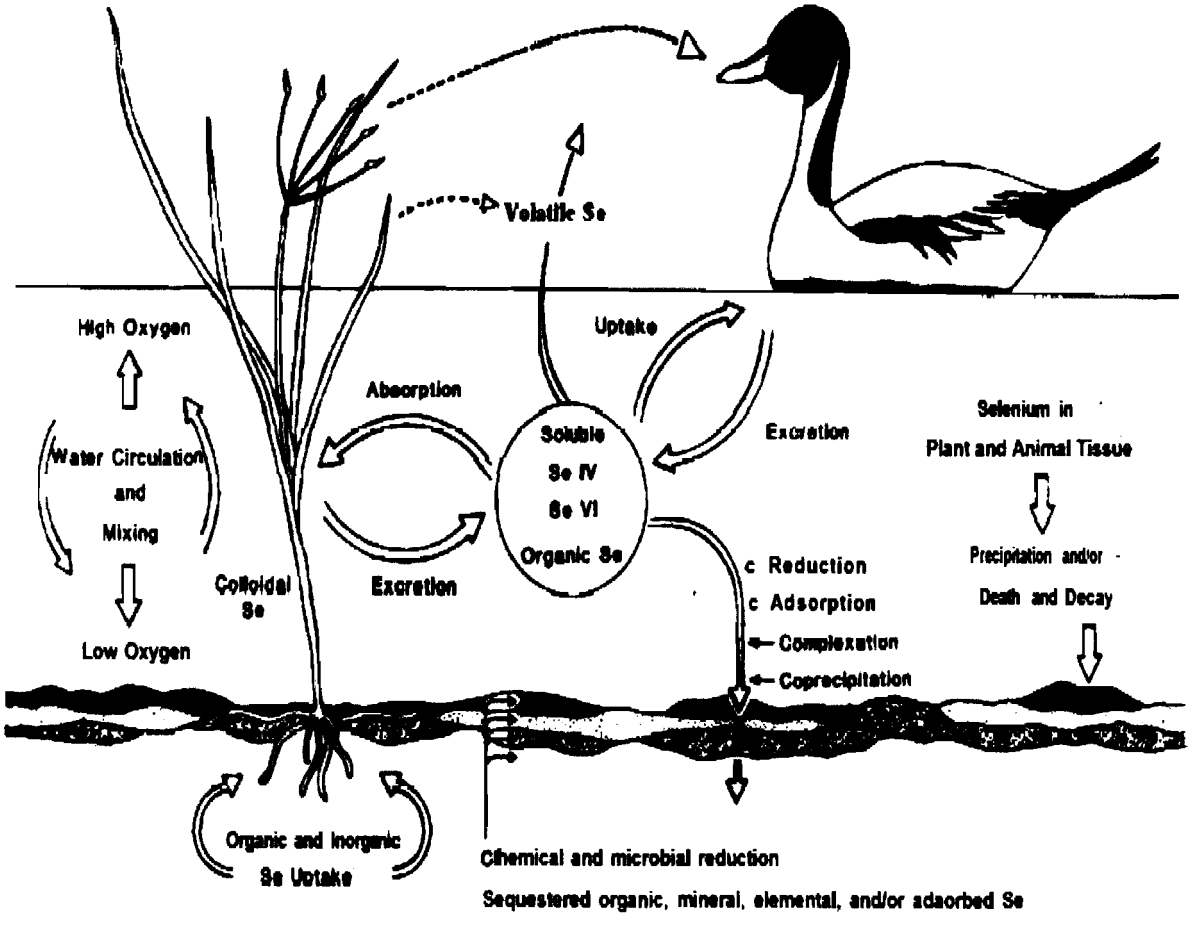


Fig 2 Biological, chemical and physical processes cycle Se into and out of the water, sediments, and biota (adapted from Lemly 1999)

of the Se in the top layer of sediment and detritus. However, because biological, chemical, and physical processes move Se out of, as well as into the sediments, the sediments are only a temporary repository for Se. Aquatic systems are highly dynamic and Se can be cycled back into the biota and remain at elevated levels for years after waterborne inputs of Se are stopped (Lemly 1997a).

2.2.2 Aquatic Contamination by Se

Se contamination of aquatic system has already been demonstrated internationally to lead to bioaccumulation of Se in plants as well as in insects, birds, mammals, reptiles and amphibians at levels which may lead to toxicosis of the animals and those which prey on them (Nriagu and Wong 1983; Nobbs *et al.* 1997). Se gained recognition among research scientists, regulatory authorities, and fisheries managers in the late 1970's when the landmark pollution episode took place at Belews Lake, North Carolina (Lemly 2002). Contamination occurred form Se in wastewater from a coal-fired plant, causing chronic Se poisoning and teratogenic deformities in aquatic life as shown in Fig 3. Thus toxic impacts to the resident fish community were studied for over two decades. In the early-1980s, it was at Kesterson Reservoir in California where Se contamination at high concentration was also observed due to irrigation practices (Jacobs 1989), mass graves of aquatic birds were reported (Nobbs *et al.* 1997). Se contamination of fish from Lake Macquarie in New South Wales, Australia, has been found to be twelve times higher than those recommended by Australian Health Authority, requiring ban of fishing (Nobbs *et al.* 1997). Anthropogenic contamination of Se in this case occurred from industrial sources including lead-zinc smelter and sewage works. Two anthropogenic activities,

disposal of fossil fuel wastes and agricultural irrigation of arid, seleniferous soils, have poisoned fish and wildlife, and threatened public health at many locations in the United States and elsewhere (Lemly 1997a). The residual impacts from Se contamination kept on occurring at these sites for many years even after Se inputs were stopped (Lemly 1985, 1997b, 2002).

Fig 3 Se toxicity causing deformity in fish in a contaminated aquatic environment (adapted from Lemly 1999)

In addition, there are possibilities of contamination from Se occurrence at higher than natural levels in soil. A sudden prevalence of human Se poisoning called selenosis occurred in a province in China in the early 1960s (Zhu *et al.* 2004). A large amount of native Se in rocks was activated, transformed and then enriched in local food via Se-rich water irrigation systems. Research studies of these episodes have generated a database that clearly illustrates the environmental hazard of excessive Se (Lemly 1997a).

2.2.3 Bioaccumulation of Se

As a precaution, the narrow concentration range between deficient and toxic level of Se requires precise knowledge of the Se content in the environment. It has been found that Se accumulates in living tissues (Irwin 1997; Lemly 1997a; USEPA 2007). Parts per billion levels of inorganic Se in water can, through bioaccumulation and bioconcentration in the food chain, lead to lethal consequences for plant and animal to human (Lemly 1999). Plants can take up Se from soil, groundwater, sewage sludge, and polluted air. Therefore, Se is bioconcentrated by a large number of aquatic plants and animals such as algae and zooplankton, which can accumulate Se to concentrations several-hundred times that found in water (Hultberg 2002). Bioconcentration factors in aquatic systems are especially high when Se occurs at very low waterborne concentrations (Seed *et al.* 2000). In oceans, an increase in Se concentration enhances growth rate of alga, which later ends in eutrophication (Erikson 1990) and causes damage to marine life (Koike 1993).

Se in water can be concentrated from 100 to more than 30,000 times in the food organisms eaten by fish and wildlife, which exposes them to a highly concentrated dietary source of contamination (Lemly 1999; Hultberg 2002). Biomagnification may also occur, resulting in a two to six fold increase in Se between primary producers and forage fish. Moreover, if the ecosystem is allowed to reach equilibrium such that recycling of Se from sediment occurs, the detrital food pathway can deliver toxic doses of Se for many years even if waterborne sources are eliminated (Lemly 1985, 1997a). Moreover, aquatic food organisms of wildlife strongly bioaccumulate Se from hundreds to thousands times the waterborne concentration-but are unaffected by tissue deposits that

are high enough to cause reproductive failure when consumed by fish and aquatic birds (Nobbs *et al.* 1997). As an example, a relation between the concentration of Se and degree of reproductive failure in selective fish species is shown in Fig 4.

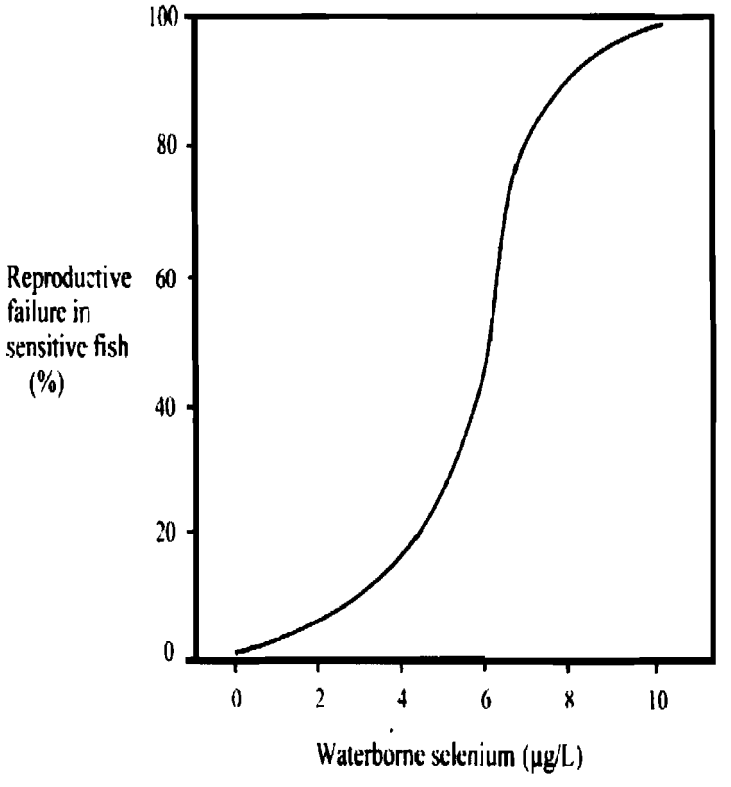


Fig 4 Relationship between the concentration of Se in habitats favourable to bioaccumulation and the degree of reproductive failure in sensitive fish species (adapted from Lemly 1999)

2.2.4 Aquatic Se Standards

Based on the world example of Se contamination and toxicity, the World Health Organisation (WHO) and national environmental agencies have started regulating Se in aquatic environment. Most natural waters tend to have Se concentrations of lower than 0.01 mg L^{-1}. (Krishnaiah *et al.* 2003). Surface waters can receive Se from the atmosphere by dry and wet deposition, from adjoining waters that may contain Se, from surface runoff, and from subsurface drainage. Sewage treatment plants are another source of Se releases to aquatic environment. The WHO has set the standard for drinking water set at 0.01 mg dm^{-3} and a limit of 0.05 mg L^{-1} for irrigation water needs (USDHHS 2003). The USEPA also restricts 0.05 mg L^{-1} Se in drinking water (USEPA 2007). The traditional approach to evaluate waterborne Se concentrations is to compare them to the USEPA's national freshwater criterion of 5 μg L^{-1}. However, some of the water quality agencies in developed countries, such as Canada, have adopted 2 μg L^{-1} as their management or regulatory level (Hamilton and Lemly 1999). They have set this standard on the basis that Se is strongly bioaccumulated by aquatic organisms and even slight increases in waterborne concentrations can quickly result in toxic effects (Lemly 1997). The states of Arizona in 1992 and New Mexico in 1995 have also established a water quality standard for Se for the protection of aquatic life at 2 μg L^{-1}. The critical link in the recommendation of 2 μg L^{-1} as the potentially safe waterborne Se concentration for the protection of fish and wildlife resources is bioaccumulation and biomagnification into the food chain (Hamilton and Lemly 1999).

There is a growing body of literature that continues to document the extensive contamination of aquatic environments with Se, and the adverse effects in aquatic organisms. The majority of this literature demonstrates the need for a national water quality criterion below the current value of 5 $\mu g\ L^{-1}$. Several extensive reviews of the literature have concluded that a criterion of 2 $\mu g\ L^{-1}$ is justified (Hamilton and Lemly 1999). Despite the mounting evidence of toxic effects below 5 $\mu g\ L^{-1}$, there is a controversy over whether the current national criterion is too high or too low. USEPA is currently reevaluating the national water quality chronic criterion for Se, which was set at 5 $\mu g\ L^{-1}$ in 1987 (USEPA 2007). The current standard of 5 $\mu g\ L^{-1}$ was established based almost solely on information from Belews Lake, North Carolina (Hamilton and Lemly 1999). However, majority of literature now supports a lower chronic Se criterion.

2.2.5 Need for Se Speciation in the Aquatic Environment

Knowledge of the chemical speciation of trace elements in natural waters is essential to an understanding of the toxicity and bioavailability of these elements, and so special attention has also been paid to the content of the different forms of Se (Robberecht and Grieken 1982). Se speciation has been attracting much attention in recent years because certain Se compounds have been reported to have anticarcinogenic activity and act as an antidote for mercury, cadmium, arsenic (Afkhami *et al.* 1992). Thus biological effects of Se is dependent upon its chemical form (Afkhami *et al.* 1992; Irwin 1997; Makowska *et al.* 2004). Generally, organic forms of Se are more bioavailable and less toxic than the inorganic forms (selenites, selenates) (Jacobs 1989).

The relative toxicity of various chemical forms of Se generally follows the order (from most to least toxic): hydrogen selenide ~ selenomethionine (in diet) > selenite ~ selenomethionine (in water) > selenate > elemental Se ~ metal selenides ~ methylated Se compounds (Irwin *et al.* 1997). Se oxoanions are highly water soluble, and therefore bioavailable and potentially toxic. Inorganic Se species like selenite are found to be upto 500 times more toxic than common organo-Se compounds (Jacobs 1989). Elemental Se is the least bioavailable followed by selenate, and then selenite, while the most bioavailable form is organic Se, such as selenomethionine. Three dissolved Se species of selenite [Se(IV)], selenate [Se(VI)] and organic Se exist in natural water (Robberecht and Grieken 1982). The most important inorganic Se species in water are selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) (Kucukbay and Demir 2001), however the most mobile and bioavailable inorganic form of Se in waters is selenate (Dietz *et al.* 2004). Selenite is generally considered to be more toxic than selenate (Goldberg *et al.* 2006; Irwin *et al.* 1997). The concentration and dominance of different dissolved forms of Se can also depend on the aquatic environment. For example, dissolved Se in seawater exists as selenate, selenite and dissolved organic selenide, although from the viewpoint of thermodynamics only selenate should be stable on oxic seawaters (Yao and Zhang 2005). Thus, it is very important to quantitate the different Se species in water samples, which is has not been determined in water resources of Fiji.

2.3 Environmental Se Status in Fiji

Recently research has already indicated that Fiji's aquatic environment has been contaminated with arsenic (O'Brien *et al.* 2003) and other toxic heavy metals (Naidu and

Morrison 1994; Fung and Chand 1996; Tamata *et al.* 1996; Deo 2000; Gangaiya *et al.* 2001; Singh 2001; Singh and Mosley 2003). The disposal of Se into the environment from industrial activities such as mining, factory discharges to air and water, leaching from rubbish dumps, sewage sludge, effluent discharges and urban runoff may have increased the concentration of Se in Fiji's environmental water sources. For example, the Emperor Gold Mine in Fiji, until 1980, has recovered significant amounts of Se and tellurium oxides (Lyday 1998). Recently it has been reported in the media that stockpiles of agricultural and laboratory chemicals have contaminated 19 sites in Fiji (The Fiji Times 2003). There is a potential threat of environmental contamination of Se in the South Pacific and it may become a problem in future with the current rapid growth of human population, which will continue to generate more wastes due to use of Se containing products.

The major concern lies in the fact that small increases in waterborne Se can lead to devastating effects on aquatic life. It is generally assumed that free metal ions are more toxic to aquatic biota than metal ions bound to large organic molecules (Wang *et al.* 2001). Once Se bioaccumulation in the aquatic food chain begins it is too late to intervene therefore pre-pollution assessment and management are the keys to preventing impacts. Se is a potential environmental contaminant, however, there is a complete lack of Se data published for the South Pacific region. This shortage of data is of concern given the increasing development and industrial activity on many islands. Even if no sources of anthropogenic contamination exist there is a potential for natural levels of metals to be harmful to human health (Singh and Mosley 2003).

While previous studies have already demostrated that Fiji's aquatic environment has been polluted by other heavy metals to some extent, except for some tributyl tin (TBT) studies (Stewart and Mora 1992; Davis *et al.* 1999, Maata and Koshy 2001), the data obtained has been limited to total metal concentrations. Since it has already been established that total metal concentrations do not provide an insight into the toxicity and bioavailability of the metals in the environment, speciation studies of metals has taken priority in recent years.

Hitherto, only one monitoring work on Se was carried out in Fiji by Fuavao and co-workers in 1986 as an undergraduate project (Fuavao *et al.* 1986). The samples for the determination of Se were collected from Rewa River and analysed by hydride generation atomic absorption spectroscopy (HGAAS). The amount of Se found was 1- 4 μg L^{-1}, which was below the recommended limit set out by WHO for irrigation water (50 μg L^{-1}).

2.4 Analytical Considerations for Se Determination in Environmental Water

The speciation of metals in the environment is not as convenient as determining total metal concentration (Caruso and Montes-Bayon 2003). Since most heavy metals are trace elements in nature, their species concentrations would be even lower. Hence, highly sensitive and selective analytical methods would be necessary to evaluate the concentration of each particular metal species in an environmental sample (Batley 2004). In addition, associated analytical details such as sampling procedures and sample storage

have to be taken in to consideration to maintain the integrity of metal species until their analysis (Heninger *et al.* 1997).

The aquatic environment is one of the most complex chemical systems of the nature, as well as the receiving end of much of anthropogenic inputs. Consequently, it has often been used to determine the extent of contamination in a particular environment (Batley 2004). Water, sediments, animal and plant samples of the aquatic environment have been used to evaluate heavy metal contamination all over the world. From immediate toxicity perspective, the determination of concentration of metals in water would be the most appropriate while sediments, animals and plants are indicators of long term contamination. As compared to sediment, animals and plants, the content of heavy metals and their species is very low in water, even though concentrations are well above contamination levels. This poses an exciting challenge to the analytical scientists, who are always attempting to develop analytical techniques that would be able to detect analytes at the lowest concentration possible in water samples (Batley 1996).

Not surprisingly, the literature is now replete analytical methods, which describe in detail the determination of a Se in a particular sample matrix, with associated quality control procedures. Routine methods are now available which can detect down to sub parts per trillion (ppt) levels of Se species in water samples using state-of-art instruments (Caruso and Montes-Bayon 2003; Batley *et al.* 2004).

Unfortunately, the quest for higher sensitivity, selectivity, reproducibility and reliability of analytical methods has overlooked the ultimate factor, which is the cost (Capelo *et al.* 2005). Most standard methods now available in literature for speciation studies of Se in water use highly expensive instrument as well as highly trained personnel for operation. Alongside these, the storage and maintenance of these costly instruments is another factor that severely limits the routine analysis of environmental samples in places over the world where environmental pollution is significant but resources are scarce to effectively carry out environmental monitoring.

In natural waters, the extremely low concentration of Se (even as total) makes the evaluation of each species very difficult that is further compounded by interference problems (Robberecht and Grieken 1982). Since the toxicological properties of different Se compounds differ, analytical procedures must be available to determine not only total Se but also the various Se species encountered in the aquatic environment. The importance of its determination can be seen by the large number of analytical methods that have been developed and continue to be developed and modified for its analysis in aqueous matrices (Conde and Alaejos 1997).

The low content of Se in water demands a high sensitivity of the analytical method used for its determination as well as sample handling procedures. It is not possible to directly determine the level of Se species like Se(IV) and Se(VI) using traditional techniques such as spectrophotometry and atomic absorption spectrometry (Tuzen *et al.* 2007). Determination of Se in environmental samples is usually difficult due to its trace

quantities, pre-concentration stages, destruction of organic matrices by acid digestion and oxygen plasma combustion followed by separation from interfering metal ions such as ion exchange separation, solvent extraction or hydride generation (Inam and Toprak 2004). These are all time consuming procedures and losses of Se are also possible. It is therefore very important to accomplish methods with minimal interference.

2.4.1 General Analytical Methods for Se

There are various Se speciation study techniques that have been developed over the past few decades, such as atomic absorption/emission/fluorescence spectrometry (AAS/AES/AFS), inductively coupled plasma (ICP), gas chromatography (GC), spectrofluorimetry, neutron activation analysis (NAA), electrochemical methods (DPP/DPCSV/ASV), spectrophotometry, and coupled techniques (D'Ulivo 1997). For the well resourced and advanced laboratories, analytical approach toward Se speciation now is the hyphenation of a powerful separation technique such as HPLC with a sensitive and selective detection method such as ICPMS (Pyrzynska 2002; Kapolna 2007).

The majority of the above methods are tedious, often involving lengthy sample preparation procedures, such as solvent extraction(s) or freeze drying of the element from water analysis and the reagents used are toxic and/or many of these methods suffer from the fact that the reagent has to purified extensively before use. For example the EAAS signal is very sensitive to the matrix components and the ICPMS application to saline waters is restricted due to low tolerance limit to the total dissolved salts (Tang *et al.* 2005). Also, for the determination of nanogram or lower amounts, these techniques can

be applied only after preliminary isolation and preconcentration. Spectrophotometric methods are inexpensive and readily accessible in most laboratories, however, their sensitivities are difficult to match with those of AAS and voltammetric techniques (Melwanki and Seetharamappa 2000; Suvardhan *et al.* 2007). In addition, the spectrophotometric methods are not always sensitive enough for determination of Se at ppb levels (Robberecht and Grieken 1982).

2.4.1.1 Atomic Absorption Spectrometric (AAS) Methods

HGAAS and EAAS are the most commonly used for routine determination of Se in water at ppb levels (Carrero and Tyson 1997). The best absolute detection limit can be observed in an EAAS but HGAAS is preferred as it is faster and relatively cheaper. The hydride generation acts as both, a preconcentration and selective derivatisation of Se(IV), since AAS does not possess enough high sensitivity nor it can differentiate Se forms on its own (Tang *et al.* 2005). Although analysis by AAS has been shown to be a quick and simple method using the hydride vapour generation technique, there are also a number of factors generally overlooked, such as interferences, matrix effects and losses through volatilisation at high temperature. For example, of the two AAS techniques, hydride generation is the technique of choice because it is relatively interference free as compared to EAAS (Tang *et al.* 2005). However, determination of Se as hydrogen selenide by AAS is subject to much interference due to Ag, Cu, Ni, Pd, Pt, Rh, Ru and Sn, which can interfere with the formation of the H_2Se gas (Afkhami *et al.* 1992). The relatively poor precision, losses and interferences are the main problems of both methods (Conde and Alaejos 1997).

2.4.1.2 Inductively Coupled Plasma (ICP) Methods

Inductively coupled plasma atomic emission spectrometry (ICPAES) or mass spectrometry (ICPMS) present poor detection limits and low precision for Se in water (Conde and Alaejos 1997). While effort has been made to improve these techniques and look to be promising, they have still not been applied to routine Se determination in water samples without coupling with interference removal and preconcentration techniques.

2.4.1.3 Gas Chromatographic (GC) and Spectrofluorimetry Methods

In GC methods and fluorimetric methods, digestion and reduction are necessary to determine Se. The sample is then treated with aromatic o-diamines to form piazselenols, which is then extracted in organic solvent and measured by spectrofluorometer or by GC with the sensitive electron capture detector (Pyrzynska 1998). Again, the sensitivity of these methods is not as good as AAS methods (Conde and Alaejos 1997).

2.4.1.4 Neutron Activation Analysis (NAA) Techniques

While the sensitivity and precision of NAA are lower than fluorimetric methods, it can be valuable as a reference for validating alternative analytical methods due to high cost of sample irradiation. In addition, its advantages include nondestructive to sample and minimal losses of Se due to reduced sample treatment (Conde and Alaejos 1997). However, this method has hardly been used for routine Se analysis in water due to its unavailability in the most analytical laboratories.

2.4.1.5 Electroanalytical Methods

Differential pulse polarography (DPP) and differential pulse cathodic stripping voltammetry (DPCSV) are the most applied voltammetric techniques for Se speciation (Pyrzynska 1998). The polarographic technique offers little sensitivity as compared to the DPCSV technique, and suffers form interference problems, especially from elements that form insoluble selenides. While Se(VI) cannot be reduced at the mercury electrode, Se(IV) is electroactive and can be measured directly at the dropping mercury electrode. The formation of Se amalgam on mercury electrodes has been used in determining Se by CSV and related electroanalytical techniques. For total Se analysis, all species has to be oxidised to Se(VI) and then reduced to Se(IV) (Conde and Alaejos 1997). The presence of dissolved organic matter, mostly as humic substances, strongly disturbs the measurement. UV irradiation helps in eliminating this problem (Pyrzynska 1998).

2.4.1.6 Spectrophotometric Methods

This was the most cited technique in the past, however, direct spectrophotometric method has been not sensitive enough for the determination of Se in trace amounts. On the other hand, spectrophotometric methods based on catalytic reactions are generally much more sensitive and selective than those based on stoichiometric reactions. The application of UV-visible absorption spectrometry to the determination of trace metals is still popular in many laboratories, especially in developing countries (Ojeda and Rojas 2005). The technique provides easy determination of many metals from low to high concentrations at affordable cost.

2.4.1.6.1 Kinetic Spectrophotometric Methods

The use of kinetics has taken a more significant role in analytical chemistry in the past few decades, especially in the determination of metal and inorganic ions (Crouch *et al.* 2000). Kinetic method of analysis (KMA) is an analytical method in which the rate of a reaction or a related quantity is measured and utilised to determine concentrations (Svehla 1993). With the IUPAC getting involved in presenting the correct terminology frequently used in the nomenclature of kinetic methods of analysis (Svehla 1993; Muller 1995), it is now being incorporated in most modern analytical chemistry texts. Increasing interest in KMA is also demonstrated by the fact that the International Symposium of Kinetics in Analytical Chemistry (KAC) has become a biannual event. For example, the 8th and 9th International Symposium on Kinetics in Analytical Chemistry were held from 8-10 July 2004 in Rome, Italy (KAC 2004) and 4-6 November 2006 in Marrakech, Morocco (KAC 2006), respectively. In order to meet the challenging needs of analytical chemistry, considerable efforts have been made towards the instrumentation and data processing approaches of kinetic methods (Crouch *et al.* 2000). The aim of this is to reduce the influence of experimental variables on the quality of results. The use of statistical analysis and quality control procedures has greatly given reliability to data from kinetic methods. Efforts have been directed to keep the kinetic analytical methods as simple as possible such as using a minimum amount of mathematics for treating kinetic data from chemical systems.

The earliest methods based on chemical kinetics were based on the catalytic activity of enzymes using fixed time analysis (Christian 2004). By then, the application of catalytic

methods can be directly utilised for many metal speciation studies in different types of environmental samples.

In catalytic methods, the reaction catalysed by the analyte is known as the "indicator reaction" (Svehla 1993; Muller 1995). The change in concentration of only one of the substances participating in a reaction is determined. Since the analyte affects the rate of reaction, it is not monitored. The substance by which the indicator reaction rate is monitored is often called the "indicator substance". The most common indicator reactions are redox in nature and involve various types of oxidants and organic or inorganic reductants (Prasad 2002, 2005; Prasad and Halafihi 2002, 2003). Redox reagents react by electron transfer through their d-orbitals (*e.g.* Fe^{3+}, Cu^{2+}, Se^{4+}, Ce^{4+}) and s, p orbital (*e.g.* Sn^{4+}, Sb^{5+}, Pb^{4+}, Bi^{5+}), with the former being faster reactants. For the catalyst which undergoes changes in oxidation state in reactions, high sensitivities are obtained (10^{-6} - 10^{-11} g cm^{-3}). In addition to redox indicator reaction systems, catalysed ligand exchange reactions have also been frequently used for the development of highly sensitive kinetic methods for many analytes (Prasad 2004, 2005, 2007 and references cited therein).

2.4.1.6.1.2 History

In 1876, Guyard described the determination of vanadium through its catalytic effect on the oxidation of aniline by $KClO_3$ (Muller *et al.* 1995). This was the earliest known kinetic determination of a species on the basis of its catalytic effect. The catalytic methods were also used for the development of spot "glow tests" for metals such as platinum (Philip 1951) with excellent sensitivity. These successful experiments provided

awareness for the importance of catalytic reactions and their development for quantitative applications to elements. The experiments were efficient in showing the excellent sensitivity and selectivity of catalytic methods.

2.4.1.6.1.3 Present Status

Recent years have witnessed an upsurge in kinetic approaches in analytical chemistry (Crouch 2005). Even though immerging trends focus on miniaturisation, biosensing and microfluidic approaches (Palleschi 2005), "classic" direct kinetic determinations of catalysts and other species are still of great interest (Harvey 2000). This is mainly due to their simplicity, speed of determination and relatively low experimental costs. These proposed methods should have appropriate analytical features and be simple enough to be able to carry out in modestly equipped laboratories by personnel of average qualification. It has been shown that trace metal analysis can be performed in a practical and economic way by exploiting relatively simple chemical reactions whose rates are sensitive to the presence of metals (Crouch *et al.* 2000). With careful elucidation of kinetics and mechanistic anatomy, catalysed reactions with spectrophotometric detections are frequently used for environmental monitoring, especially with regard to surface and ground water.

2.4.1.6.1.4 Catalytic Kinetic Spectrophotometric Methods (CKM) for Analysis of Se

The requirement for KMA is that the rate of the chemical reaction must be fast enough that the analysis can be conducted in a reasonable time, but slow enough that the reaction does not approach its equilibrium position while the reactants are mixing. A second

requirement is that the kinetics and mechanistic study of the reaction should be studied so that the rate law for the chemical reaction must be known for the period in which measurements are made. Since some rate laws are too complicated to be analytically useful, pseudo-first-order-kinetics are generally achieved by using a large excess of reactants other than the analyte, so that their concentration remains essentially constant (Crouch 1994). A final requirement for a KMA is that it must be possible to monitor an indicator reaction's progress by following the change in concentration for one of the reactants or products as a function of time (Ojeda and Rojas 2005).

Amongst the possible types of catalytic reactions, the redox reactions have been most widely used (Muller 1995). The reaction ability of a metal ion, and in particular, its catalytic activity is largely dependent on its forms in which the metal exists in solution. Therefore, distinguishing a species of the metal ion, which displays the catalytic effect, may provide a better insight into the mechanism of the catalytic reaction. Given that such a metal species is defined, both the choice of conditions for kinetic determinations, selectivity control and enhancement of the sensitivity would become greatly facilitated (Kawashima and Tanaka 1968).

A major breakthrough in the development for catalytic kinetic methods for Se analysis came when Feigl and West (1947) developed a catalytic method for Se determination. They used redox reactions involving alkali sulphides, where Se was involved as a catalyst in minute amounts. That the reducing power of alkali sulphides could be enhanced by elemental Se was shown by the reduction of several compounds with S^{2-}. The compounds

tested were potassium chromate, picric acid, dichlorophenol indophenol, cacotheline and methylene blue (MB). The authors discussed the mechanism of the MB-S^{2-}-Se reaction mechanism and applied it to determine submicrograms of Se. The colorless leuco methylene blue (HMB) is formed when sulphide reduces MB:

$$2MB + S^{2-} + 2H_2O \rightarrow 2HMB + 2OH^- + S$$

Similar to the formation of polysulphides by sulphur in the presence of excess S^{2-}:

$$S + S^{2-} \rightarrow [S\text{----}S]^{2-}$$

in the presence of Se, selenosulpides are formed:

$$Se + S^{2-} \rightarrow [Se\text{----}S]^{2-}$$

Since $[Se\text{----}S]^{2-}$ has greater activity than $[S\text{----}S]^{2-}$, they react with MB in a similar but more quicker manner than the S^{2-}:

$$2MB + [Se\text{----}S]^{2-} + 2H_2O \rightarrow 2HMB + 2OH^- + Se + S$$

The catalytic activity of Se is evident from its regeneration at the end of the reaction. Se must be in elemental form to be catalytically active, which is achieved by its reaction with S^{2-} in basic medium, which reduces Se to its elemental form:

$$SeO_3^{2-} + 2S^{2-} + 3H_2O \rightarrow Se^0 + 2S + 6OH^-$$

Polysulphides have an interfering effect in this method. The authors proposed that the interference due to the presence of polysulphides is readily eliminated by the addition of sulphide, which reacts with polysulphides to form thiosulphates, which have no interfering effect (Feigl and West 1947). Since then, many researchers have tried to develop kinetic catalytic methods for Se determination based on this reaction.

Kawashima and Tanaka (1968) proposed a procedure for Se determination based on the catalytic reduction of 1,4,6,11-tetraazanaphthacene, which in presence of hypophosphorus acid is reduced to a blue compound, 1,6-dihydro-1,4,6,11-tetraazanaphthacene (DHTAN). The absorbance was measured at 600 nm using reaction temperature of 55 °C for 30 min. Their method was subject to much interference from foreign ions, which could be removed with solvent extraction with oxine or by exchange resin. It also suffers from non-linear calibration and the reagents were also unstable for routine application.

West and Ramakrishna (1968) applied Feigl and West's work and they succeeded in determining Se using color comparison by varying Se from 0.1 - 1.0 μg and recording the time for complete decolorisation of MB. The calibration curve was constructed using the plot of T^{-1} (min^{-1}) *versus* Se amount. Even then Cu was identified as a serious interferon for the method. The mechanism of the reaction, as discussed by Feigl and West (1947) had shown that polysulphides can also interfere (*vide infra*), however addition of Na_2SO_3 overcomes this problem. EDTA was used as a masking agent for other interfering cations. To improve the efficiency of this technique, Mesman and Doppelmayr (1971) developed a photometric device, which would replace the visual end point detection with photoelectric detection and the reduction time to be documented on a recorder. The calibration curve was obtained similarly as West and Ramakrishna (1968) who had tried unsuccessfully to monitor the reaction using a spectrophotometer. The average precision of the method was 2.7%. Gary and Schwig (1972) applied comparison of the end point detection spectrophotometrically and electrochemically to West and Ramakrishna's

(1968) method. Their application permitted the determination of 0.002 - 0.01 μg mL^{-1} Se with precision of 13%.

Fukasawa *et al.* (1976) monitored the MB (λ = 668 nm) and S^{2-} reaction at 668 nm and observed an induction period when HCHO was added. A linear relationship was obtained for 0.05 - 0.9 μg Se using a plot of the reciprocal of the induction period *versus* Se concentration. Interfering cations like Cu^{2+} was masked by a solution containing EDTA, $FeCl_3$ and triethanolamine.

Methylene blue has also been replaced by some other indicators in some cases in an attempt to obtain lower detection limits. Keyvanfard and Sharifian (2006) developed a kinetic spectrophotometric method for Se(IV) based on its catalytic effect on the reduction of 4,5-dihydroxy-3-(p-sulfophenylazo)-2,7-naphthalene disulfonic acid (SPADNS) by S^{2-} in micellar media. The reaction was monitored spectrophotometrically by measuring the decrease in the absorbance of SPADNS at 515 nm with a Fixed time method. The decrease in the absorbance of SPADNS is proportional to the concentration of Se(IV) in the range 0.5 - 100 ng mL^{-1} uaing a fixed time of 2.5 - 7.0 min from the initiation of the reaction. The limit of detection is 0.3 ng mL^{-1} Se(IV). The relative standard deviation for the determination of 0.02 and 0.10 μg mL^{-1} Se(IV) was 2.10 and 1.95%, respectively. The method was applied to the determination of Se(IV) in water. Further works on kinetic catalytic determinations of Se in water are summarised in Table 1.

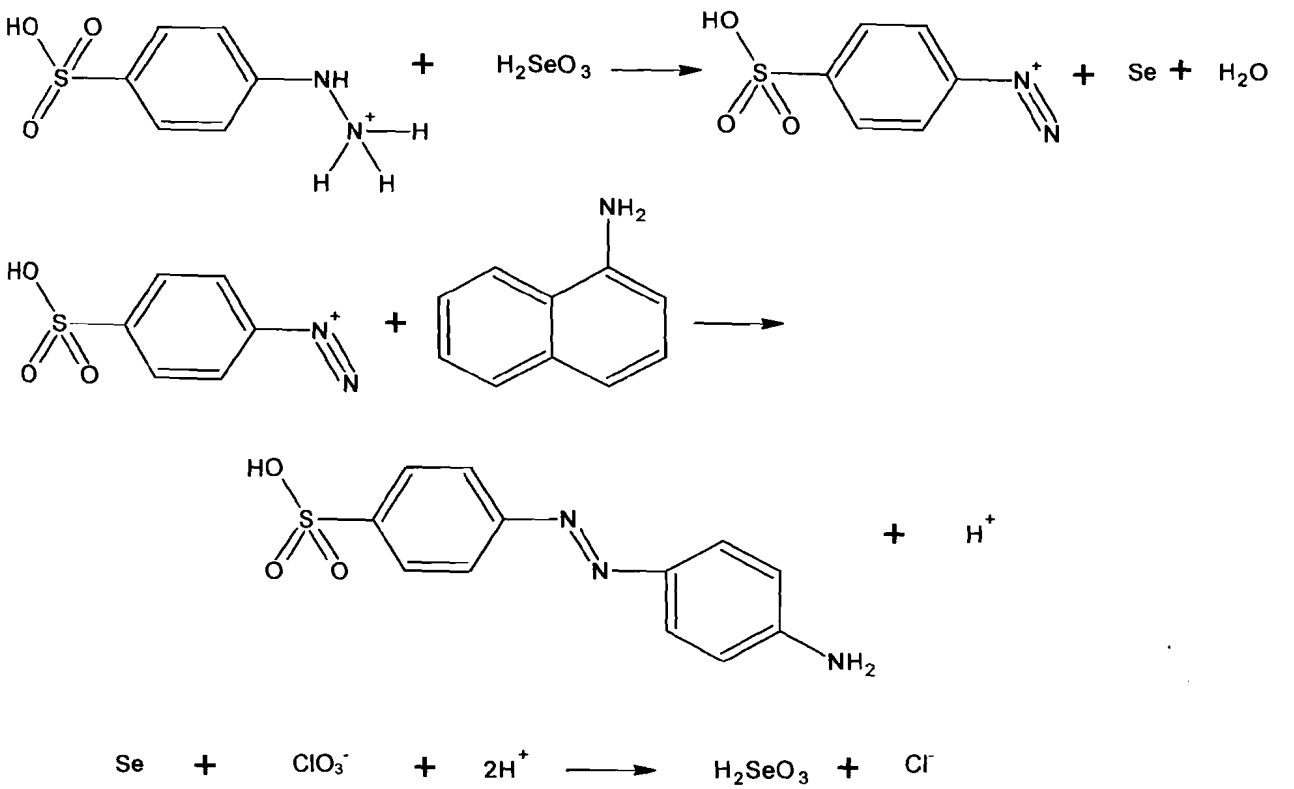
Table 1 Kinetic methods for Se based on catalytic reactions of Se in indicator reactions using sulphide as the reductant

Reagents	Detn. limit, detn. range, molar abs. or Sandell's sensitivity	Remarks	Sample	Reference
Picrate, S^{2-}	2.5 - 30.0 $\mu g\ mL^{-1}$	Picric acid electrode made by modifying the fluoroborate electrode, recovery 91 and 106%, RSD < 3%	wastewater	Hua 1988
Toulidine blue (phenothiazine dye), S^{2-}	8 ng cm^{-3}, 0.03 - 1.5 $\mu g\ mL^{-1}$	605 nm, RSD 1.4% for 1 $\mu g\ mL^{-1}$	water samples	Shamsipur and Mousavi 1993
Resazurin, S^{2-}	8.0×10^{-4} $g\ mL^{-1}$	605 nm, RSD 0.7% for 10 ng	-	Safavi *et al.* 1990
Methylene blue, S^{2-}	15 - 75 $\mu g\ L^{-1}$	monitored at 645 nm, 20 °C, pH 9 or 10.5, RSD 2.8% for 1 $\mu g\ mL^{-1}$ (n = 16)	water, standard sample	Brenal *et al.* 1990
Methylene blue, S^{2-}	0.6 - 6.4 M	use of cation micellar medium, RSD < 4%	-	Arikan *et al.* 1996
Methylene blue, S^{2-}	2.5 - 30 ppb	monitored at 668 nm, room temperature; interference removal with organic solvents, recovery 91.84% and RSD 2.27% for 15 ppb	water	Songsasen *et al.* 2002
Gallocyanine, S^{2-}	0.002 $\mu g\ mL^{-1}$, 0.010 - 0.500 $\mu g\ mL^{-1}$	reaction monitored at 620 nm by fixed time for first 2 min at 30 °C and pH 7.0, interference removal with cation exchange resin, , RSD 2.5% (n = 6) for 50 $\mu g\ mL^{-1}$	synthetic water samples	Ensafi and Dehaghi 1995
Gallocyanine, S^{2-}	1.0 ng mL^{-1}, 2.5 - 500 ng mL^{-1}	flow injection, sampling rate 35 samples h^{-1}, monitored at 620 nm, 30 °C, pH 7.0, interference removal by cation exchange resin, RSD 1.5% for 50 ng mL^{-1} (n = 10)	water	Ensafi 1997
Brilliant cresyl blue, S^{2-}	3×10^{-5} $\mu g\ mL^{-1}$, 0.0001 - 0.500 $\mu g\ mL^{-1}$	monitored for first 4 min at 630 nm, 30 °C, pH 7.0, interference removal by cation exchange resin, RSD 2 - 2.5 % for 0.1 - 100.0 ng mL^{-1}	wastewater	Ensafi *et al.* 1997

Thionine, S^{2-}	1.3 ng mL^{-1}, 2 - 90 ng mL^{-1}	598 nm, fixed time procedure used from first 45s from initiation of reaction, 30 °C, pH 7.0, RSD 2.51% for 40 ng mL^{-1} (n=10)	synthetic water samples	Mousavi and Jahanshahi 1999a
Methyl violet, S^{2-}	42 ng mL^{-1}, 80 - 1800 ng mL^{-1}	fixed time method, pH 8, 590 nm	water	Mousavi and Jahanshahi 1999b
Azure A, S^{2-}	2.5 ng mL^{-1}	600 nm	spiked water	Safavi and Mizaee 1999
Maxilon blue-SG, S^{2-}	0.205 ng mL^{-1}, 0.004 - 0.200 g mL^{-1}	monitored with fixed time method 4.0 min from initiation of reaction at 654 nm, 30.0 °C, pH 6.5, interference removal by cation exchange resin, recovery 91.50 - 99.88%, RSD 0.32 - 2.27% for 0.004 - 0.160 μg mL^{-1}	spring water	Gurkam and Akcay 2003
Sulfonazo, S^{2-}	0.3 ng mL^{-1}, 0.5 - 180 and 50 - 2300 ng mL^{-1}	monitoring decrease in absorbance at 570 nm or increase in absorbance at 680 nm by fixed time method; interference removal with cation exchange resin	natural water, synthetic samples	Ensafi and Lemraski 2004

Kirkbright and Yoe (1963) had developed a spectrophotometric method for Se determination based on the oxidation of p-sulfonic acid by seleneous acid. The diazonium oxidation product of the reaction was coupled with 1-naphthylamine to give a coloured azo dye (λ_{max} = 520 nm). The method was developed with an optimum pH 1.8 - 2.2 and the absorbance of the reaction mixture was measured for absorbance after 10 min. Sandell's sensitivity was obtained as 0.002 μg cm^{-2}. Detection upto 0.04 ppm and standard deviation of < 0.006 was obtained but the method suffered from numerous interferences. Interferences from Cu and Fe were removed by extraction in $CHCl_3$. In another study, vanadium was determined using its catalytic effect on the oxidation of phenylhydrazine p-sulfonic acid by ClO_3^-. On the basis of the above two reactions, Kawashima *et al.* (1970) developed a method for Se(IV) determination using its catalytic

effect on the oxidation of phenylhydrazine p-sulfonic acid in the presence of ClO_3^-. The diazonium oxidation product is coupled with 1-naphthylamine to give a coloured azo dyestuff (λ_{max} = 525 nm). The chlorate ion assists in the regeneration of Se(IV) as shown by the following reaction schemes:



Different reaction variables such as reagent concentration, pH and temperature were optimised and foreign ions were investigated. The reaction mixture was heated at 50.0 °C for 60 min, cooled in ice and monitored at 525 nm within 20 min. The reaction was very unstable in the optimum pH chosen (0.8 - 1.2). Several anions and cations interfered in the method, however the interfering metals could be removed by the extraction of oxinates. Other researchers have also reported kinetic catalytic method for Se analysis in water based on almost similar reactions, which are summarised in Table 2.

Table 2 Kinetic methods for Se based on catalytic reactions of Se in indicator reactions using different types of hydrazine as the reductant

Reagents	Detn. limit, detn. range, molar abs. or Sandell's sensitivity	Remarks	Sample	Reference
Hydrogen peroxide, phenylhydrazine, α-naphthylamine	0.003 ng mL^{-1}, 0 - 0.24 ng mL^{-1}	530 nm	river, well water	Safavi *et al.* 1990
Phenylhydrazine, potassium chlorate, 1,8-dihydroxynaphthalene-3,6-disulfonic acid (chromotropic acid)	0.52 ppm or upto 0.15 ppm, 0.0 - 50.0 ppm	flow injection, 45 samples h^{-1}, 60 °C, 1.2 M HCl, interference removal by EDTA, RSD < 1.5% (n = 7 or 10)	synthetic samples	Shiundu and Wade 1991
Phenylhydrazine hydrochloride, H-acid, potassium chlorate	-	reaction mixture heated for 30 min in steam bath, cooled and monitored at 527 nm with pH 1.4, recovery 104 - 111%	natural and spiked waters	Seung-Hwa *et al.* 1994
Bromate, hydrazine dichloride, Ponceau S	3.3 ng mL^{-1}, 4.5 - 400 ng mL^{-1}	-	water	Safavi *et al.* 1999
Bromate, hydrazine dichloride, Ponceau S	-	use of artificial neural networks for Te(IV) interference removal, monitored at 510 nm, 40 °C, pH 1.2, error < 5%	natural and synthetic water samples	Absalan *et al.* 2001
p-Hydrazinobenzene-sulfonic acid (HBS), N-(1-naphthyl)ethylene-diamine (NED), bromide as activator for catalysis and reducer for Se in acidic medium	0.2 - 6 ng mL^{-1}	flow injection with two schemes, 25 °C and 100 °C, 538 nm, RSD 1.2 and 1.3% for 3 ng mL^{-1} Se(IV) and Se(VI) (n = 10)	natural water	Nakano *et al.* 2004

Klochkovskii and Neimysheva (1973) developed catalytic methods for Se based on the oxidation of Fe(II) by NO_3^-. Se was determined by its catalysis on the oxidation of the Fe(II) salt of Triton B by NO_3^- at pH 2.0 - 2.5. The same authors determined Se based on

Table 3 Kinetic methods for Se based on catalytic reactions of Se in indicator reactions using Fe(II) as the reductant

Reagents	Detn. limit, detn. range, molar abs. or Sandell's sensitivity	Remarks	Sample	Reference
FeL^{2-} (H_4L = EDTA), NO_3^-, use of Greiss reagent for complexation with NO_2^-	0.002 μg mL^{-1}, 2 - 20 mg mL^{-1}	pH 2, 525 nm, RSD 0.06 - 0.13 for (0.5 - 2.0) × 10^{-5} % Se	water	Lebed and Pantaler 1988
Iron(II) ethylenediamine-tetraacetate, NO_3^-, 4-Nitroaniline, *N*-diethyl-*N*-(1-naphthyl)-ethylenediamine	0.1 ng mL^{-1}, 4.5 × 10^4 L mol^{-1} cm^{-1}	540 nm, interference removal by ultrasonic treatment, RSD 6% and 2% for 0.2 and 2 ng mL^{-1}	potable and natural waters	Gudzenko *et al.* 2004
Etheylenediamine tetrecetic acid disodium salt (EDTA), NO_3^-, ammonium iron(II) sulfate hexahydrate	2 × 10^{-9} g mL^{-1}, 5 × 10^{-9} - 2 × 10^{-7} and 2 × 10^{-7} - 2 × 10^{-6} g mL^{-1}	flow injection with 7 samples h^{-1}, monitored at 440 nm in acidic media, recovery 95 - 104%, RSD 3.4% for 5 × 10^{-8} g mL^{-1} Se(IV) (n = 11), 2.7% for 5 × 10^{-7} g mL^{-1} Se(IV) (n = 11)	seawater	Zhengjun *et al.* 2005

the catalysis by Se(IV) on the oxidation of the Fe(II)-EDTA complex, $[FeL]^{2-}$, by NO_3^- at pH 2 and 25 °C (Klochkovskii and Neimysheva 1974). Sensitivity of the method was 3 × 10^{-3} μg mL^{-1} and error was 10%. Serious interference from Rh and NO_2^- was discovered. They also determined Se based on its catalytic activity on the oxidation of the Mn(II)-EDTA complex by H_2O_2 (Klochkovskii and Neimysheva 1974). A higher sensitivity of 3 × 10^{-4} μg ml^{-1} Se was obtained but the method had high level of errors (30 - 50%). Table 3 summarises the work of some other investigators who have tried to use the basis of the same reaction for the development of catalytic method for Se.

Apart from these established and popular methods, some investigators have reported a few other methods for Se based on its catalytic effect on some other redox reactions. Se catalyses the reduction of tetranitro blue tetrazolium by dithiothreitol at 30 °C and pH 8.9, which can be monitored at 600 nm for 4 min. This system was used used for the determination of 1.2 ng as selenite, 21 ng selenocysteine, 150 ng selenocystine (Hawkes 1986). Interferences were masked with organic acids in the determination of Se in synthetic samples with a RSD of 2.7 - 9.1%

Li *et al.* (1992) investigated the catalytic affect of Se(IV) on the reaction between $KBrO_3$ and Vitamin C. Using aminoacetate/HCl medium and $Al(NO_3)_3$ and KCl as activating solution, Se(IV) was determined in river water by monitoring the reaction at 420 nm at pH 2.5. The sensitivity of the system as molar absorptivity was 1.2×10^7 L mol^{-1} cm^{-1} but at an elevated temperature of 90 °C.

Se(IV) catalysed reduction of 3-(4,5-dimethyl-2-diphenyl-2H-tetrazolium bromide was used to determine Se upto 1.3 pmol-1.2 nmol with a detection limit of 0.63 picomol (Aoyama *et al.* 1991). The method was applied to standard samples and interference removal was achieved using an octadecyl silane (ODS) column.

The reaction of Se(IV) catalysed oxidation of Nile Blue A by H_2O_2 was studied by Milovanovic *et al.* (1997). Se was determined in ethanol solution with a detection limit of 1.6×10^{-3} ng cm^{-3} and in a linear dynamic range of 0.95 - 12.6×10^{-2} ng cm^{-3}. The

reaction was monitored at 637.5 nm and pH 10.8. The RSD achieved was below 5.2% in Se analysis in mineral water.

Afkhami and Madrakian (2002) recently proposed a highly sensitive technique for determination of Se(IV), Se(VI) and total inorganic Se in water samples. They achieved the reduction of Se(IV) to Se(0) by L-ascorbic acid and hence preconcentrated Se(0) on activated carbon. Se(0) was then oxidised to Se(IV) by bromate and the reaction was monitored through the oxidation of methyl orange by reaction products. Similarly, Se(VI) was determined after reduction to Se(0) by hydrazine salts. Analysis using fixed time of 30 s after initiation of reaction at 525 nm a detection limit of 0.012 ng $mL^{-1}$and linear working range of 0.02 - 20.0 ng mL^{-1} were achieved. The recovery and RSD were 89 - 105% and 1.0 - 7.1%, respectively in the determination of inorganic Se in environmental water samples.

2.4.1.6.1.5 Overview of CKM methods for Se

It is obvious that many attempts have been made successfully to develop CKMs for Se determination in natural water samples. In many of these papers, information on quality assurance is lacking and it is not easy to fully assess the reliability of the described procedures. For example, the performance of many of the methods has not been compared to reference methods, interlaboratory studies or analysis of reference materials. This could also be attributed to the unavailability of certified reference materials for Se in their species form, which has only been made available recently. Most of these methods are unable to achieve detection limits low enough to determine Se levels in water. Some

researchers have claimed to achieve detection limits of upto sub-ppb or ppt levels using preconcentration or otherwise, however their methods have not been tested in real samples. Some methods are limited to very narrow linear ranges or are subjected to numerous interferons, which need special treatment for their removal (Absalan *et al.* 2001; Ensafi and Lemraski 2004; Gudzenko *et al.* 2004). Few methods were found to employ lengthy analytical procedures which compromise the speed at which analysis can be done for many samples. Though it is common among all methods to determine Se based on its form, which is Se(VI) in all cases, the lack of associated procedures for determination of Se(VI) and total inorganic Se as well is a major drawback of these methods. This is important for any particular analytical technique for Se determination in water samples since both forms of Se occur simultaneously in natural waters, with Se(VI) predominating in most of the cases. Therefore, combined with simplicity and high sensitivity, a validated CKM method can become a very cost-effective technique for the determination of Se(IV), Se(VI) and total inorganic Se in natural water samples.

2.4.1.6.1.6 Data Treatment Methods in Kinetic Analysis

The concentration of all reagents except for the analyte in the indicator reaction is usually arranged to be present at a much higher level, so that their concentration remains effectively constant during an experiment (Muller 1995). A pseudo-first-order reaction in the analyte is observed, hence increasing in the concentration of the catalyst gives rise to a direct increase in the reaction rate (Crouch 1994). Therefore the rate as a function of analyte concentration can be calibrated by using a series of standard solutions. The

sensitivity of the reaction is found to be the difference between the total reaction rate and the rate of the noncatalytic reaction.

Differential and integral methods are most frequently used for catalysed reactions (Muller 1995). Differential methods involve direct evaluation of signal (d(signal)/dt), from initial measurements, in which the initial rate is determined and utilised for the evaluation of concentration. In other words, differential methods use slope measurements, in which the slope of the response curve at a selected point is measured and related to concentration (Svehla 1993). Integral methods are based on the evaluation of the corresponding rate expressions over a finite, constant and normally small time interval, Δt (Muller 1995). This involves fixed time measurements, in which the change of a parameter (*e.g.* absorbance), related to the concentration of reactant or product, is measured over a predetermined interval (Svehla 1993). Fixed concentration or variable time method, in which the period of time, required to bring about the same predetermined change in the concentration (or absorbance) of a reactant or product is also measured. In developing a method, some researchers have used more than one of the above techniques in the determination of analytes as a method of comparison for the most efficient method for data analysis for the particular reaction system (Mihai *et al.* 2005).

2.4.1.7 Hyphenated/Coupled Techniques

The indirect determination of Se(IV) could be overcome by combining chromatographic separation, specifically either ion chromatography or ion-pair reversed chromatography, with sensitive detection. Element specific detectors involving atomic emission,

absorption, fluorescence or mass spectrometry are the most interesting ones in respect to low detection limits and sensitivity to interference (Pyrzynska 2002). Chromatographic techniques such as gas liquid chromatography (GLC) allows for elimination of interference from the matrix when analysing environmental samples (USDHHS 2003). One of the most powerful techniques available to separate and identify elemental species is the interfacing liquid chromatography (LC) with inductively coupled plasma mass spectrometry (ICPMS) but not a cost effective technique (Uden 2002). Separation of elemental species is also achieved by ion-exchange HPLC with detection by AAS (Capelo *et al.* 2005). Selenite and selenate are also commonly separated by ion exchange chromatography and ion-pair reverse-phase chromatography (Pyrzynska 1998).

2.4.1.8 Standard Methods

Some standard methods by various organisations have been listed for the determination of Se in water samples. The APHA has listed the standard methods for Se analysis in water such as EAAS, Manual/Continuous HGAAS, ICP, ICPMS and colorimetric methods (Clesceri *et al.* (Eds) 1998). The EAAS method gives relative error of 12, 9, 6 and 17 - 37% for lab pure water, drinking water, surface water and effluent, respectively, in the determination of 10 $\mu g\ L^{-1}$ Se. The LOD is 2 $\mu g\ L^{-1}$ for the linear working range of 5 - 100 $\mu g\ L^{-1}$. For the Manual HGAAS method, a 2 $\mu g\ L^{-1}$ LOD is reached with the linear working range of determination 2 - 20 $\mu g\ L^{-1}$. Recoveries are 100.6 and 110.8% for Se(IV) and Se(VI), respectively. For the Continuous HGAAS method, the RSD for the determination of 4.3 - 52.8 $\mu g\ L^{-1}$ Se(IV) is 12 - 5%. The colorimetric method is based on the formation of a piazselenol complex from Se(IV) and 2,3-diaminonaphthalene,

followed by extraction of the complex with cyclohexane and measurement of its absorbance at 480 nm. The detection limit is given as 10 μg L^{-1}, with a linear range of 0 - 2 mg L^{-1}. The method has about 90% recovery from Se in a certified reference material consisting water. The USEPA (2007) also uses EAAS, HGAAS and ICPMS methods, while the AOAC lists ICPMS (0.8 - 200 μg L^{-1} Se) as the standard method for Se determination in water (AOAC 2005).

2.4.2 Digestion Techniques for Se in Water

In natural water samples, Se species in three oxidation states (-II, IV and VI) have been determined mainly by analysis of three separate aliquots: (i) direct determination of Se(IV) using specific analytical methods such as voltammetry (ASV, DPP, DPCSV), fluorimetry, HGAAS, spectrophotometry; (ii) after reduction of Se(VI) to Se(IV) with hot HCl, the sum of Se(IV) and Se(VI) is determined, thus Se(VI) content is determined by difference and (iii) after mineralisation of the organic matrix (by UV irradiation or wet digestion) followed by reduction to Se(IV)-all Se species is determined (Pyrzynska, 2002). The most widely used wet digestion methods for total determination of Se involve decomposition with acid or acid mixtures such as HNO_3, HNO_3/HCl, $HClO_4$/HNO_3 and H_2SO_4/$HClO_4$ (Adeloju *et al.* 1984). Wet digestion methods are preferred over dry ashing because of reduced danger of losing Se at low temperature and the simplicity of the apparatus required for wet digestion. There is no significant difference between the results obtained for Se by open digestion and those by closed digestion which utilised a similar acid or acid mixture (Adeloju *et al.* 1983).

2.4.3 Se Stability and Storage - Analytical Considerations for Speciation Studies

The determination of species is a more complex task than the determination of total element concentrations. Since during sampling, storage and analysis of the samples species transformation may occur, appropriate action must be taken to assure that the species to be determined does not change during the interval between sampling and analysis (Rassler *et al.* 1998). Hence, the stability of chemical species in solution during storage is one of the critical aspect that has to be carefully considered *e.g.* for calibration purposes (Quevauviller *et al.* 1995). Se occurs at or below ppb levels in most environmental waters, hence stability of Se species in standards and water samples contribute significantly to the results obtained upon their analysis. Several studies have been devoted to this aspect of Se speciation and continue to be studied extensively in an effort to validate a proper procedure on sample and standards storage.

Robberecht and Grieken (1982) have provided a good review of many of the studies done on this topic. The review shows that Se loss in synthetic and environmental water samples occur due to several factors such as element concentration, chemical form, container material, contact time, pH, salinity, suspended matter and microorganisms. It has been concluded that Se loss by adsorption could be greatly minimised by acidifying samples (pH 2 or less) with a strong acid (HCl or H_2SO_4) in borosilicate glass containers. However, due to the probability that strong acids can also change Se speciation, freezing at 4 ^{o}C is recommended as an alternative using high density polyethylene or polytetrafluoroethylene (Teflon) containers. This also helps preventing the introduction of contaminants and loss of volatile Se compounds, because freezing of samples may not

be practicable during transport over long distances. Even with all these treatment and care, it is still advisable to analyse samples in the shortest time possible to maintain sample integrity.

More such similar studies done recently point out that acidification or freezing is the only solution to Se species preservation. Nevertheless, as Gomez-Ariza *et al.* (1999) have shown, the best results would be achieved using a combination of these best conditions. While many researchers have just confined their research to either of acidification or freezing, they have investigated and proved that a combination of freezing, acidification and use of Teflon containers would provide the longest term solution to inorganic Se species storage.

Filtration of a solution may lower metal concentration through adsorption of metal species to the filter. A study by Weltje *et al.* (2003) using eight types of 0.2 μm membrane filter for testing metal affinity. They concluded that to minimise filtration errors, polycarbonate or nylon filters are to be recommended when dealing with low volumes of high pH and low metal species concentrations.

2.4.3.1 Inorganic Se {Se(IV) and Se(VI)} - Standards and Samples

A study was carried out by Massee *et al.* (1981) to investigate the influence of different container types, pH and storage the sorption of Se. They found that 10^{-7} M level Se in distilled and artificial seawater was not significantly adsorbed on borosilicate glass, high pressure polyethylene and Teflon bottles at pH 1, 2, 4 and 8.5 and from 1 min to 24 days.

The authors recommend shortening storage times, acidification with strong acid and reducing the ratio of inner container surface to sample volume as measures to minimising Se adsorption.

Cheam and Agemian (1980) investigated the stability of inorganic Se(IV) and Se(VI) species at levels of 1 and 10 $\mu g\ L^{-1}$ under different pH levels, type of water, and type of container. Preservation of Se(IV) at the 1 ppb level in 500 mL pyrex or polyethylene bottles at pH 1.5 (0.2% v/v H_2SO_4) was satisfactory for deionised distilled water and unfiltered seawater. Higher pH values were unsatisfactory, but at higher pH, Pyrex was a better container than plastic. A 25-gallon polyethylene barrel was effective for preserving bulk water samples at natural pH for about 4 months. In general, Se(VI) was more stable than Se(IV) in aqueous solutions, and recoveries were satisfactory for both glass and polyethylene bottles.

Wiedmeyer and May (1993) investigated the storage characteristics of selenate, selenite, and selenomethionine in low and high ionic strength water. At 10, 50, and 100 ppb, borosilicate glass and high density polyethylene, and two temperatures (glass: 4 °C; polyethylene: -20 °C) over a period of 120 days, the three Se forms were tested. Selenomethionine was most stable over the duration of the study, with virtually no significant influence from temperature, species concentration, container material, or water matrix. For inorganic Se forms, significant changes in selenite were observed over 120 days, although less change was observed for solutions stored in glass. Selenate losses were observed from solutions stored under conditions of a low ionic strength matrix,

polyethylene container, and freezing. Overall, the least changes among the three Se species were observed from solutions stored in glass at 4 °C.

Wang (1994) studied Se losses in river, ground, snow-melt and tap water samples, as well as the recovery of selenite, selenate and selenomethionine added to purified water. In 1 litre high-density polyethylene bottles, Se concentrations of 44.5 - 138 ng L^{-1} in tap, river and snow-melt water samples could be stored at 4 °C for up to 15 days without Se losses. In similar samples stored at room temperature Se losses of 13 - 25% after 15 days were found, except for groundwater, which showed no Se losses during storage for 13 months at room temperature or at 4 °C. Selenite and selenate added to purified water were recovered without losses after 15 days at 4 °C, while 7.5% of selenomethionine was lost. The stability of different chemical forms of Se during storage followed the order: selenate > selenomethionine > selenite. Wang recommended that unacidified water samples should not be kept in polyethylene bottles at room temperature for more than one week, nor stored at 4 °C for more than two weeks, before analysis for Se.

Heninger *et al.* (1997) have shown that 29% oxidation of Se(IV) to Se(VI) occurs in less than one month in acidic and oxygenated medium in the presence of chloride ions. This may imply that acidification of Se standards and samples with HCl may change Se speciation. However, Gomez-Ariza *et al.* (1999) showed that selenite and selenate were stable in acidified samples at pH 2 with HCl at -20 °C in Teflon containers for the twelve months tested. However, losses of selenite were observed after six months in river and tap water samples. Selenate was more stable than selenite and higher concentrations were

more stable than lower concentrations. The order of decreasing stability with respect to containers, pH and temperature was Teflon > polyethylene > polypropylene, pH 2 > pH 4 > pH 8 and -20 °C > 4 °C > 25 °C > 40 °C.

Lindemann *et al.* (2000) investigated the stability of three Se species (Se(IV), Se(VI), selenomethionine) in water. Best storage of the species was achieved at 3 °C. According to Sigma-Aldrich (2006), the Na_2SeO_3 standard stock may be frozen for stability purposes. Working aliquots are stable for 30 days at 2 - 8 °C.

2.4.3.2 Organic Se – Standards and Sample

The stability of four volatile organic Se species in seawater was studied (Gomez-Ariza *et al.* 1999). Seawater was spiked at concentrations of 50 μg L^{-1} for both dimethylselenide (DMSe) and diethylselenide (DESe) and at concentrations of 0.50 μg L^{-1} for both dimethyldiselenide (DMDSe) and diethyldiselenide (DEDSe), stored at 4 °C and -20 °C, in three different container materials (Teflon, polyethylene and polystyrene). The four species were only stable for 24 h. The order of decreasing stability was DMDSe > DESe > DEDSe > DMSe, Teflon > polyethylene > polystyrene and -20 °C > 4 °C.

2.4.3.3 Optimum Storage Conditions

It is now obvious that the literature now seems confusing and in disagreement over the correct procedure for standards storage. Hence, there is hardly any uniformity in the conditions employed on the preparation of Se standards by researchers who are working in the field of Se speciation (Capelo *et al.* 2005). The trends that could be observed from

their steps are the freezing of inorganic and organic standards after preparation at -20 °C or acidifying standards with strong acids (HCl, H_2SO_4, HNO_3) to a very low pH (0 - 2). For non-volatile organic Se species, conditions such as high ionic strength solutions and weighing under nitrogen are required. Volatile Se organic species are the most difficult to handle and store since they are very unstable at many conditions tested. Generally, selenite is the least stable species followed by organic Se and selenate. For best results, it is recommended to keep bulk samples concentrated, acidify (pH 2 or less) with HCl, freeze in Teflon or polyethylene containers and analyse in shortest time as possible. In present study, Se standards were stored in glass bottle at 4 °C, while water samples were stored in polyethylene bottles at -20 °C.

CHAPTER 3

Methodology

3.1 Apparatus

A Perkin Elmer Lambda 16 UV-visible Spectrophotometer (Model No. 1096) with 10 mm matched quartz cells was used for all spectral and absorbance measurements. A thermostatic water bath (Thermoline, Australia) was used to control the temperature of the reagents and reaction (Fig 5). A Hanna Instruments, pH 211 Microprocessor pH meter was calibrated with standard buffers (pH 4 and pH 7) and used for measuring pH of solutions.

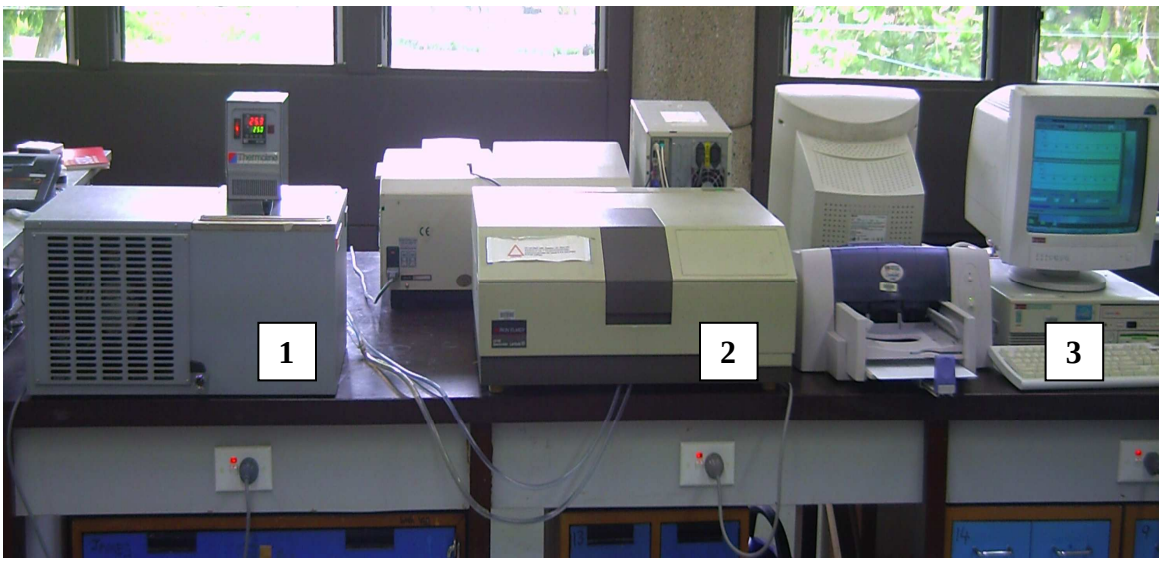


Fig 5 Photo of the Perkin Elmer Lambda 16 UV-visible Spectrophotometer (2) connected to a temperature controlled water-circulating bath (1) and a desktop computer (3)

3.2 Standards and Reagents

All chemicals and reagents used were of analytical grade and used without further purification. Distilled deionised water (DDW) was used to prepare all solutions throughout the study. A 1.0×10^{-2} M (789.6 mg L^{-1}) Se(IV) standard solution was prepared by dissolving 0.1110 g of SeO_2 (Great Western Inorganics, USA) in 100 mL water. This solution, prepared daily, was used to prepare calibration curve and quality control samples. A 1.0×10^{-2} M (789.6 mg L^{-1}) Se(VI) standard solution was prepared by dissolving 0.1889 g of Na_2SeO_4 (Great Western Inorganics, USA) in 100 mL water. A 1000.0 mg L^{-1} methyl orange solution was prepared by dissolving 0.1000 g of 4-(p-[Dimethylamino]phenylazo)benzenesulfonic acid sodium salt ($C_{14}H_{14}N_3O_3SNa$) (Fluka, Switzerland) in 100 mL water. A 5.0×10^{-2} M bromate solution was prepared by dissolving 0.8350 g $KBrO_3$ (Ajax Finechem, Australia) in 100 mL water. A 5.0×10^{-2} M hydrazine solution was prepared by dissolving 0.5249 g $N_2H_4.2HCl$ (Sigma-Aldrich, USA) in 100 mL water. Buffer solutions of glycine-HCl were prepared from 0.1 M glycine (CH_2NH_2COOH) and 1.0 M HCl and solution for range pH 1 - 3. Solutions of ions for interference studies were prepared from their soluble salts in water. Se Standard Solution (NIST SRM No. 3149) and Trace Elements in Natural Water (NIST SRM No. 1640) were used as the standard reference materials for this study.

3.3 Preparation of Buffer Solutions

Previous work on this reaction system have used glycine-HCl buffer. However, the literature mentioned for preparation of such buffer was not accessible, hence an attempt was made to prepare glycine-HCl buffer by experimentation.

The procedure involved adding 0.1 M glycine solution from a burette to a known amount of HCl solution delivered by pipette in a beaker (Table 4). A pH meter was used to monitor the pH of the resulting buffer solution through an immersed pH electrode in the beaker. The glycine-HCl mixture was consistently stirred after every installment of glycine addition.

Once the buffers were ready, 2.0 mL of the buffer solution was added to the other reagents in a 10 mL volumetric flask. The pH of the reaction mixture was monitored over time using a pH meter. No pH change of the reaction mixture was observed over the course of 10 min. However, it was observed that the pH of the reaction mixture was more than the pH of the buffer solution. This was evident for all pH levels tested (Table 5). This was assumed to be attributed to the five fold dilution effect of the buffer in the reaction mixture. A similar change was observed even after increasing the buffer volume to 5.0 mL. In fact, a linear relationship was observed between the buffer pH and the experimental pH. Therefore, on this basis, a desired experimental pH was obtained using the corresponding buffer *versus* experimental pH curve (Fig 6).

Table 4 Buffer concentration for different pH at 25 °C

0.1 M CH_2NH_2COOH solution, mL	1 M HCl solution, mL	pH ± 0.02
3.00	20.0	0.30
27.00	20.0	0.50
24.50	10.0	0.70
43.50	10.0	1.00
35.00	6.0	1.18
35.00	5.0	1.35
33.00	4.0	1.50
29.00	3.0	1.71
32.00	2.5	2.01
30.00	2.0	2.21
33.00	1.5	2.51
35.00	1.0	2.85

Table 5 Results for buffer pH in reaction mixture pH

pH of buffer (± 0.02)	pH of mixture (± 0.02)
0.30	0.91
0.50	1.01
0.70	1.24
1.00	1.44
1.18	1.66
1.35	1.77
1.50	1.88
1.71	2.02
2.01	2.25
2.21	2.38
2.51	2.64
2.85	2.95

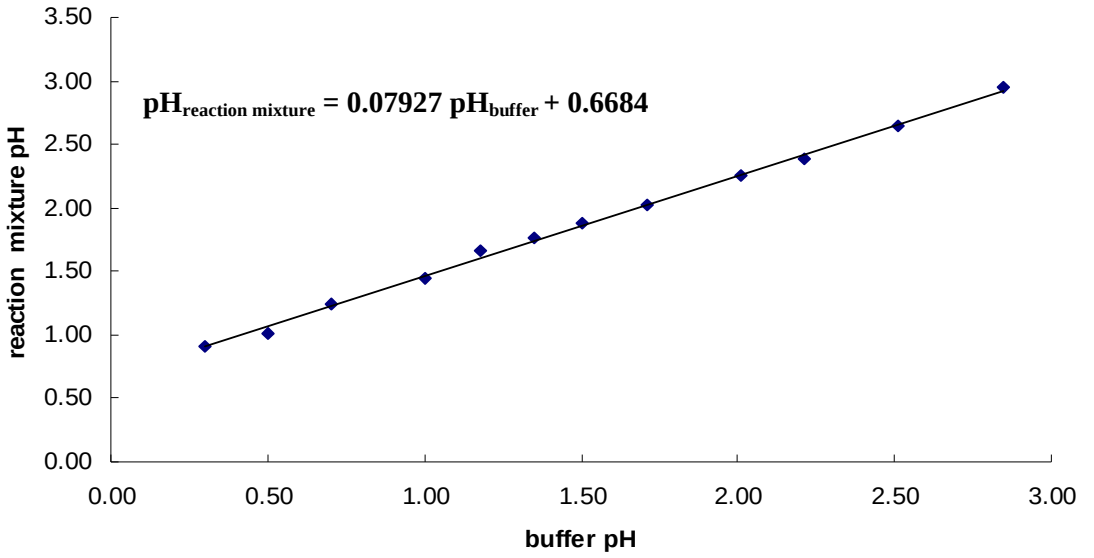


Fig 6 Linear relationship between buffer and reaction mixture pH

3.4 Water Sampling

3.4.1 Sampling Method

Sample bottles (polyethylene) were first cleaned with detergent and then by soaking in acid (10% aqua regia) for at least 24 h (Singh and Mosley 2003). Bottles were then rinsed several times with distilled water, filled with 1% HNO_3 acid and stored in sealed plastic bags. At the sampling sites the acid was removed, the bottles were rinsed three times with the sample prior to filling, capped and returned to the plastic bag. Latex hand gloves were worn during sample collection. Bottom water samples were collected with acid washed Niskin bottles before being transferred into the polyethylene bottles. Samples were taken in duplicate from all sites. Physical parameters such as pH, salinity, dissolved oxygen, temperature and conductivity were also recorded at the sampling sites. All samples were kept in ice until it reached the laboratory. In the laboratory, the water sample was filtered

through a 0.45 µm nylon membrane filter and kept in the refrigerator at 4 °C until further analysis. All samples were analysed within two weeks of sampling.

3.4.2 Sampling Strategy

Drinking, natural and polluted water samples were collected around the Suva and Labasa area. The Marine Studies Programme boat was used for collection of water samples from sea, while the USP vehicle was used for the collection of land based water samples from Suva. The objective was to take ten samples from the sources of each water type: drinking water, natural water and polluted water. Drinking water was sourced from the taps, wells, boreholes and reservoirs located in Suva and Labasa. Natural water sources included river water, creek water, spring water and sea water. Polluted water was collected from rivers and coastal areas close to industrial and waste dumping sources such as rubbish dumps, sewage treatment plants and factory discharges.

3.4.2.1 Sampling Areas

3.4.2.1.1 Drinking Water

Sample bottles were filled with ten random household taps in Suva and Labasa areas. Ten wells and ten boreholes were sampled from Labasa area only since they were not available in Suva. Five brands of commercial bottled water were brought for analysis.

3.4.2.1.2 Natural and Polluted Water

A total of ten sea water samples were collected from Laucala Bay, near Nukulau Island, Kinoya Sewage Treatment Plant outfall, Suva Harbour, vicinity of Lami Rubbish Dump.

Three samples of each river water were collected from Vatuwaqa River, Nubukalou Creek, Tamavua River, Samabula River and Rewa River in Suva and Qawa River and Labasa River from Labasa. Spring and hot spring waters were collected from Labasa.

3.5 Optimisation of Reaction Variables

All reaction variables affecting reaction rate were studied to obtain the optimum conditions for the analysis of Se. The optimisation of the reaction variables was done sequentially. The variable that was to be studied was varied while all the other variables were kept constant. pH, [MO], [N_2H_4.2HCl], [$KBrO_3$], temperature and ionic strength was studied by this method and optimum conditions were established.

3.6 Kinetic Procedure for the Determination of Se(IV)

3.6.1 Initial Rate Method

To a series of 10 mL standard volumetric flasks hanging in the water bath maintained at 25.0 ± 0.1 ºC, the reagents were added in the sequence as: 2.0 mL of buffer solution, 1.0 mL of Se(IV) solution or sample, 1.0 mL of 5.0×10^{-2} M N_2H_4.2HCl solution, 1.0 mL of 50.0 mg L^{-1} MO solution (for higher concentrations of Se(IV), the concentration of MO used should be 100.0 mg L^{-1}), 1.0 mL of 5.0×10^{-3} M $KBrO_3$ solution (for higher concentrations of Se(IV), the [$KBrO_3$] used should be 5.0×10^{-2} M) and diluted to mark with water. The stopwatch was started immediately after the half of the $KBrO_3$ solution was added. The contents of the flask were mixed well. A portion of the reaction mixture was transferred to a 10 mm quartz cell placed in a thermostatic cell component, whose temperature was maintained to a desired value. The decrease in absorbance of MO at 507

nm was monitored as a function of time against a reagent blank prepared similarly. The initial rate of the reaction for different concentrations was obtained from the slope of the tangent to the absorbance-time curve at exactly one min after the start of the reaction. The calibration curve was constructed by plotting the initial rate of reaction *versus* the concentration of Se(IV). The amount of Se(IV) was obtained from the regression equation derived from the calibration graph.

3.6.2 Fixed Time method

The absorbance at 507 nm of each sample solution was measured at a preselected fixed time against a reagent blank prepared similarly. The difference in absorbance change for sample reaction (the reaction in the presence of Se, ΔA_C) and blank reaction (the reaction in absence of Se, ΔA_U) was determined as the net change in absorbance. The calibration curve was constructed by plotting the net change in absorbance at fixed time, t, (ΔA_t) against the concentration of Se(IV). The amount of Se(IV) in each sample was determined from the regression equation obtained from calibration graph.

3.7 Validation

The proposed CKM has been validated for specificity, linearity, precision, accuracy and recovery. Statistical analysis was rigorously carried out according to Miller (1991), Mullins (2003), Christian (2004) and Hibbert (2006) for data analysis and interpretation of results. The mean, SD, standard uncertainty, recovery, RSD, error, SAE and CL were calculated for each analysis according to the following formulae or software application

listed in Table 6. The results were represented graphically using either Microsoft Excel and SPSS Sigmaplot software.

3.7.1 Selectivity

The effect of various cations and anions commonly present in natural water samples was investigated on the determination of 31.6 µg L^{-1} Se(IV). Initially, 1000 mg L^{-1} standard stock solutions were prepared for each ion, while subsequent working standards were prepared as required during the course of interference study. Since NO_3^- was found to be an interferon in this reaction, nitrate salts of metals were avoided. Chloride salts of metals were used as much as possible. In the case where the chloride salt of the metal was not available, other compounds were used. For vanadium(V), vanadium pentaoxide was used. However, such compounds were insoluble in water. Therefore, these metal compounds were first dissolved in concentrated HNO_3 and had their pH adjusted to neutral with solution before making the stock solution.

The procedure for interference studies were followed exactly the same as for the determination of Se(IV), except that 1 mL of the interferon solution was also added just after adding the Se(IV) standard in the reaction mixture. If an error of more than ±3 % was obtained in the determination of 31.6 µg L^{-1} Se(IV), then the interferon working standard was diluted by two fold and the procedure was repeated until the error obtained was within the required limit. Five replicates were run for each concentration level and the average absorbance value was used to determine the amount of Se(IV) recovered. All measurements were carried out against a blank prepared similarly except for Se(IV).

Table 6 Summary of methods used for statistical analysis

Parameter	Calculation	Reference
Regression of the form: $y = a + bx$	Microsoft Office Excel Data Analysis software	Microsoft Office Excel; Miller 1991
Mean ($\bar{x}$)	Microsoft Office Excel Data Analysis software	Microsoft Office Excel
SD of mean	Microsoft Office Excel Data Analysis software	Microsoft Office Excel
Combined SD (addition & subtraction)	$\sqrt{(SD_1)^2 + (SD_2)^2 \ (SD_3)^2 + \ldots}$	Mullins 2003; Christian 2004
Combined SD (multiplication & division)	product or quotient $\times$ $\sqrt{(CV_1)^2 + (CV_2)^2 - (CV_3)^2 + \ldots}$	Mullins 2003; Christian 2004
Standard uncertainty	$t/b\sqrt{\left(SD^2\left(1+1/n+\frac{(y_o-\bar{y})^2}{b^2\sum_{i=1}^{n}(x_o-\bar{x})^2}\right)\right)}$	Mullins 2003; Hibbert 2006
Recovery (%)	(Nominal concentration – theoretical concentration)/100	Christian 2004
RSD (%)	$(SD/\bar{x}) \times 100$	Christian 2004
Error (%)	((Nominal concentration – theoretical concentration)/ theoretical concentration) $\times 100$	Christian 2004
SAE	$SD/\sqrt{n}$	Miller 1991
CL	SAE × Student's t-value at 95% confidence level and $(n-1)$ degrees of freedom	Miller 1991
t	Student's t-value	Eton's Statistical Tables 1980

3.7.2 Linearity

For evaluation of linearity, Se was determined using two calibration ranges, 0 - 126.3 µg L^{-1} (0 - 1 × 10^{-6} M) and 0 - 789.6 µg L^{-1} (0 - 1 × 10^{-5} M) for the initial rate method and 0 - 315.8 µg L^{-1} (0 - 4 × 10^{-6} M) and 0 - 789.6 µg L^{-1} (0 - 1 × 10^{-5} M) for the Fixed time method. Seven concentration levels were used for each calibration. Each concentration was analysed for seven times and the average was use to obtain the linear regression parameters.

3.7.3 Precision and Accuracy

The repeatability and reproducibility of the proposed methods were determined using three concentrations within the lower linearity range: 31.6, 63.2, and 94.8 µg L^{-1}. The purpose for choosing lower concentration levels is that Se usually occurs at very low levels in water. Five sample solutions of each concentration were prepared and analysed within one day. This assay was to be repeated for five consecutive days. The intra and inter precision and accuracy in the analysis of these quality control samples was determined for within and between days (Miller and Miller 1988). Two NIST certified reference material for Se were analysed as well.

3.7.4 Recovery Studies

To study the accuracy of the proposed method and to check the interference from foreign ions in natural water samples, recovery experiments were carried out by the standard addition method (Miller and Miller 1988) for all water samples.

3.8 Procedure for Determination of Inorganic Se in Water Samples by Standard Addition Method

Se determination in environmental water samples and recovery studies were carried out using the standard addition method. Se was found to be below the detection limit of the method in all samples tested. The water samples were spiked simultaneously with Se(IV) and Se(VI) standards at five different concentration levels. For this, 1 mL of 31.6 mg L^{-1} standard Se(IV) solution and 1 mL of 31.6 mg L^{-1} standard Se(VI) solution was diluted to 100 mL in a standard flask with a water sample. This way, the five final concentrations of 0, 31.6, 63.2, 94.8, 126.4 µg L^{-1} were achieved and determined using the recommended procedure.

3.8.1 Analysis of Se(IV)

Since the method has been demonstrated to be specific for Se(IV), the analysis for Se(IV) was carried out directly using the recommended procedure against a blank prepared similarly. A standard addition calibration was obtained using Initial rate and Fixed time methods and the recovery of Se(IV) was determined from the linear regression equations.

3.8.2 Analysis of Total Inorganic Se [Se(IV) plus Se(VI)]

Since the method is only specific for Se(IV), it was necessary to convert all Se(VI) in the sample to Se(IV) prior to analysis. For this conversion, 25 mL of the spiked solution was mixed with 25 mL of 12 M HCl in a 100 mL capped hard glass test-tube and heated in a water bath at 91 ºC for 30 min (Brimmer *et al*. 1987). This procedure converts Se(VI) to Se(IV) by almost 100% according to the following reaction:

$SeO_4^{2-} + 4H^+ + 2Cl^- \longrightarrow H_2SeO_3 + H_2O + Cl_2$

The resulting solution was diluted to 100 mL with 6 M NaOH solution to adjust the pH (Ensafi 1997). The analysis for Se was carried out using the recommended procedure against a reagent blank. A standard addition calibration was obtained using Initial rate and Fixed time methods and the recovery of total inorganic Se was determined from the linear regression equations after correcting for the dilution factor.

3.8.3 Se(VI)

Recovery of Se(VI) was determined as a difference between the recovered total inorganic Se and Se(IV).

3.9 Procedure for Determination of Se in Standard Reference Materials

Since no reference material was available with certified Se(IV) or Se(VI) content, two NIST SRM containing certified total Se content was used for method validation purposes. This required prior treatment of the materials to convert all forms of Se to Se(IV). The certificates of both materials are provided in the Appendix.

3.9.1 NIST SRM No. 3149 (Se Standard Solution)

NIST SRM No. 3149 is reportedly prepared by solubilisation of elemental Se in concentrated HNO_3 followed by dilution (Martens and Suarez 1997). This solution contained 10.11 ± 0.02 mg g^{-1} Se in 10% HNO_3 in an ampoule, which was transferred to a 100 mL standard flask and made up to the mark with water. The concentration of this

solution was calculated as per instructions on the label. Further dilutions were done to achieve a concentration of 8 µg mL^{-1}. Since the form of Se in the SRM was stated as selenic acid (Se(VI)) and elemental Se, it was oxidised to Se(VI) by method 3030F of the Standard Methods for Examination of Water and Wastewater (Clesceri *et al*. (Eds.) 1998).

A suitable amount of sample water (50 mL) was treated with 3 mL of concentrated HNO_3 in a 100 ml beaker and covered with a watch glass. The flask was then placed on a hot plate and cautiously evaporated to less than 5 mL. After cooling, the walls of the beaker and watch glass was rinsed with DDW and 5 mL concentrated HNO_3 was added. The beaker was covered again with the watch glass and heated on hot plate with increased temperature and gentle reflux action. This was continued until no further colour change of the digestate was observed. Upon cooling, 10 mL of 1:1 HCl (v/v) and 15 mL water was added and heated for additional 15 min to dissolve all residues. The contents were then cooled, glassware walls rinsed and filtered. The filterate and washings were transferred in a 100 mL volumetric flask and the pH was adjusted using 6 M NaOH solution. This treatment converts all forms (organic and inorganic) of Se to Se(VI). This is then reduced to Se(IV) by the HCl method as described for the standard addition procedure. Se(IV) was then determined using the recommended procedure (*cf.* 3.8.2). The assay was repeated for five times against a reagent blank prepared in a similar way. Se was determined using regression equations obtained from linear calibration curves. After Se was determined in the digested sample, the respective dilution factors were multiplied to obtain the original concentration of the SRM.

3.9.2 NIST SRM No. 1640 (Trace Elements in Natural Water)

This solution contained a fresh water sample in 0.5 M HNO_3 with a certified Se concentration of 21.96 ± 0.51 µg kg^{-1}. Since the content could not be assayed directly as the Se content was too low as well as of unknown form, the contents were spiked with 1 mL of 31.6 mg L^{-1} standard Se(IV) solution and 1 mL of 31.6 mg L^{-1} standard Se(VI) solution in a 100 mL volumetric flask. This was then treated for oxidation to Se(VI) and reduction to Se(IV) as described for SRM No. 3149, before determination of Se using the recommended procedure against a reagent blank. The amount of Se in the CRM was determined after correcting for dilution and subtracting the spiked amount.

3.10 Se Determination in Environmental Water Samples

This was achieved using the standard addition method after digestion using the method 3030F of the Standard Methods for Examination of Water and Wastewater (Clesceri *et al*. (Eds.) 1998) to convert total Se to Se(VI) and then selective reduction of Se(VI) to Se(IV) using the standard digestion method (Brimmer *et al*. 1987). The determination of Se in environment water samples usually requires the destruction of the matrix and the transformation of the organic Se into inorganic forms. In many studies reporting Se determination, the conventional wet digestion method is used. A mixture of HNO_3 and $HClO_4$ is generally employed to destroy the organic matrix in traditional wet digestion procedures (Clesceri *et al.* (Eds.) 1998), however the use of $HClO_4$ can often result in an explosion or a fire during digestion if the mixture becomes dry (Wang *et al*. 2001). Recently there has been an increasing interest in using microwave digestion method to speed up the dissolution of a variety of environmental samples. The merit of premised

acid digestion in closed vessel with microwave heating, particularly the increased speed, reduced losses of volatile elements and effective prevention of sample contamination resulting from the environment and reagents are widely recognised (Wang *et al*. 2001). Unfortunately, due to the unavailability of a microwave digester, a standard wet digestion method (Clesceri *et al*. (Eds.) 1998) was employed in the present study, followed by a standard procedure for Se(VI) reduction to Se(IV) (Brimmer *et al*. 1987). A schematic diagram for the digestion procedure is given shown Fig 7. The detailed digestion procedures were followed as described for the Se determination in NIST SRM No. 3149 (*cf*. 3.9.1).

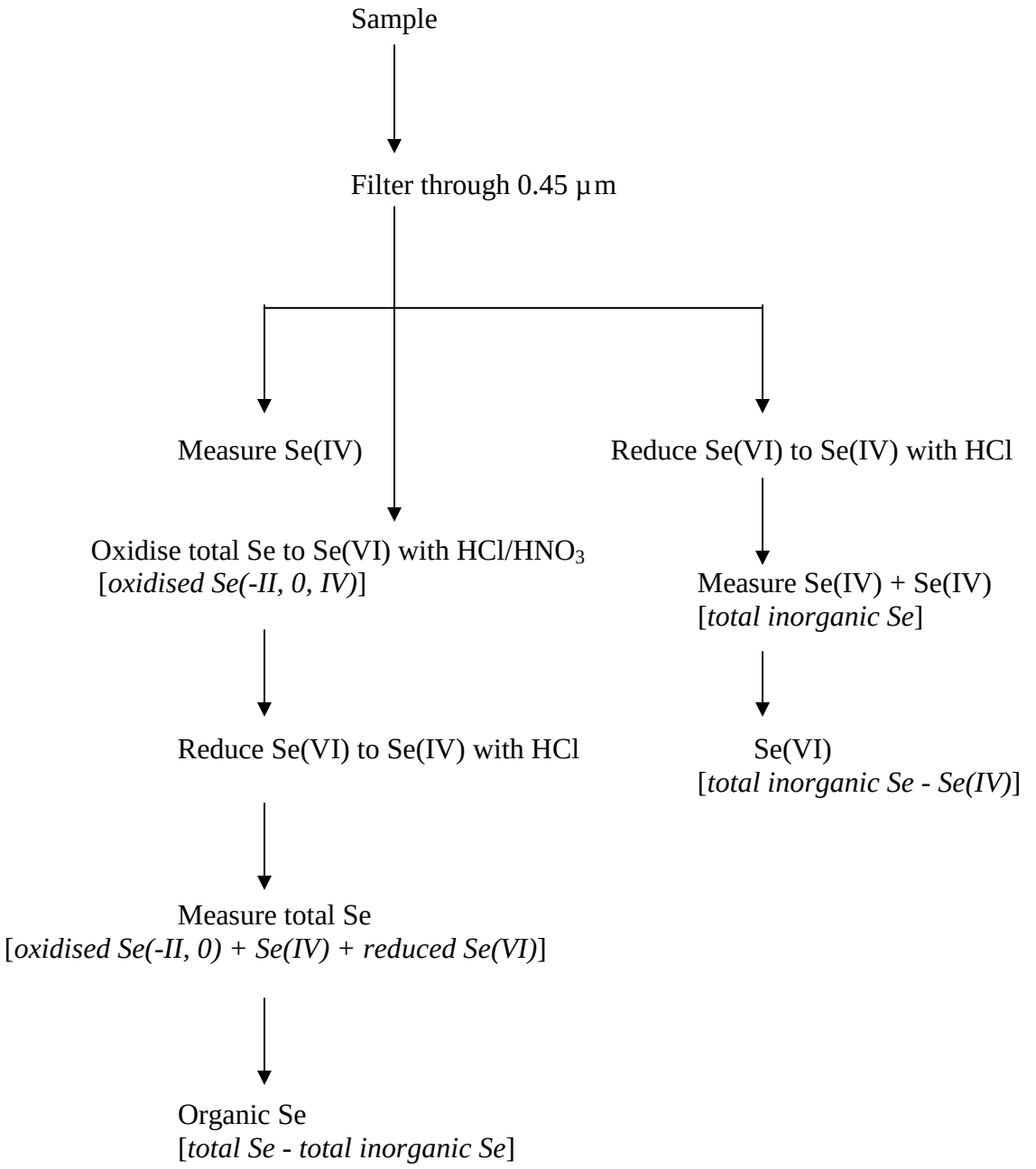


Fig 7 Flow diagram of digestion procedure used for dissolved Se speciation in water samples

CHAPTER 4

Results and Discussion

4.1 Preliminary Spectral Studies

The first phase of method development was to determine which wavelength would be most suitable for recording absorbance change during the course of the catalysed and uncatalysed reactions. In the present case, this wavelength was determined for methyl orange (MO), as it is the only absorbing species in the visible range. The maximum absorption is observed at a wavelength of λ_{max} = 465 nm (Fig 8), which is in good agreement with previously reported values (Tawarah and Abu-Shamleh 1991; Buwalda and Engberts 2001).

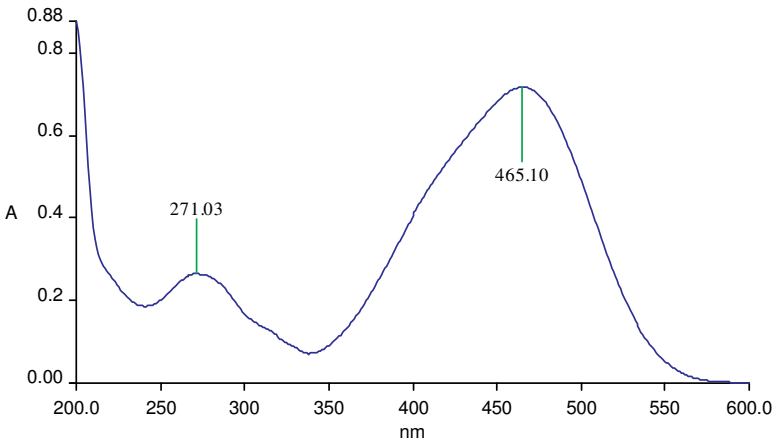


Fig 8 UV-visible spectra of MO in aqueous solution at pH 5.20 ± 0.02

MO is a sulfonated para-susbstitued phenylbenzene containing two protonation sites on nitrogen atoms. Its chemical structure and acid/base equilibria are given in Fig 9 (Tawarah and Abu-Shamleh 1991; Fan *et al*. 1998). The first and second dissociation

constants of MO in water have been measured to be $pK_{a1} = -6.09 \pm 0.13$ and $pK_{a2} = -3.37 \pm 0.01$ (Boily and Seward 2005; Suleimenov and Boily 2006). These values thus imply that MO exists as an anion at high pH (abbreviated MO^-), and as a zwitterions at low pH (MOH), whereas it's positively charged form (MOH_2^+) can never be reached in aqueous solution (Tawarah and Abu-Shamleh 1991).

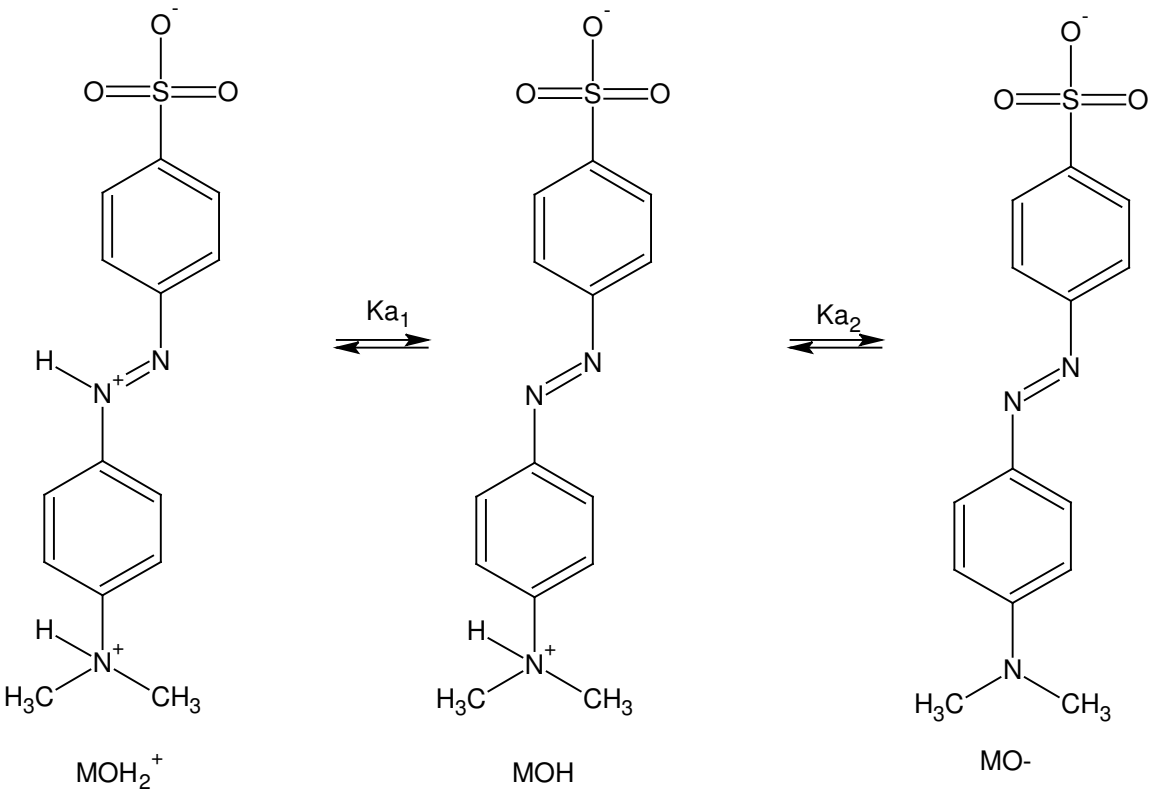


Fig 9 Chemical structure and acid/base equilibra of MO

A pilot study was done to find the optimum wavelength for monitoring absorbance during reaction. It is observed that at different MO concentrations at pH greater than 5, MO exhibits maximum absorption near 465 nm. However, as the pH is lowered, the absorption maxima of MO is shifted to a higher wavelength of 507 nm, with the

appearance of a shoulder on the right side of the peak. The spectral changes occurring from higher to lower pH is shown in Fig 10.

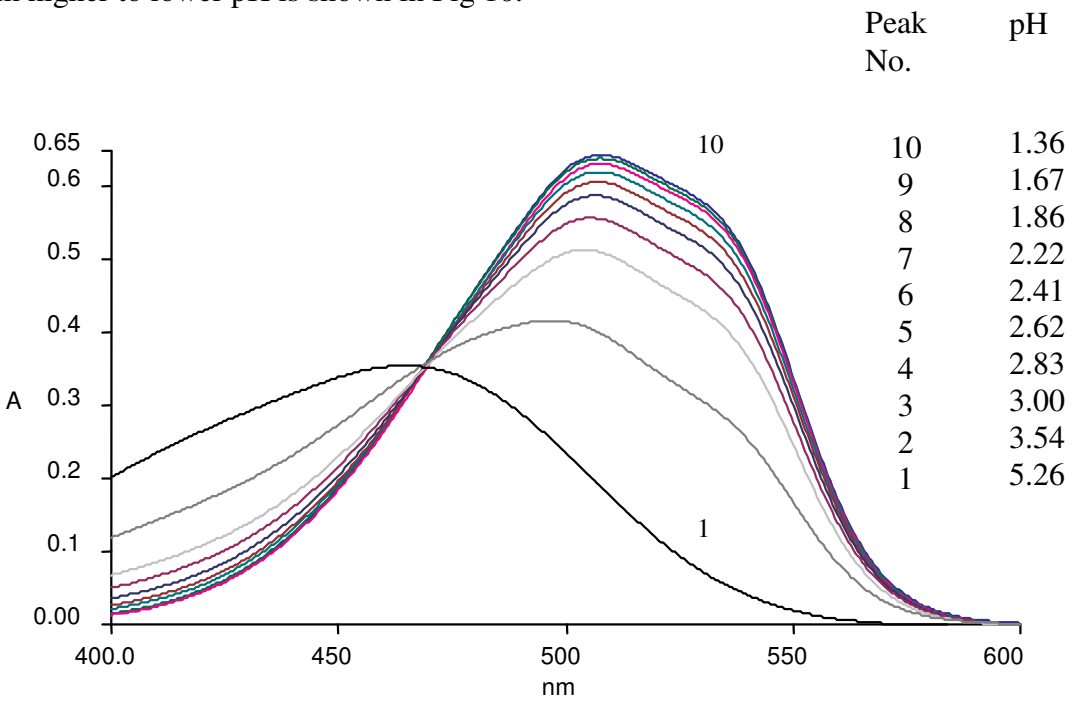


Fig 10 UV-visible spectra of MO (5.0 mg L^{-1}) in aqueous solution at various pH; indicated as peak number *versus* pH: 1 - 5.26, 2 - 3.54, 3 - 3.00, 4 - 2.83, 5 - 2.62, 6 - 2.41, 7 - 2.22, 8 - 1.86, 9 - 1.67, 10 - 1.36

These changes follow from the increase in concentration of zwitterions of MO with decreasing pH, and the colour of the solution has been attributed to the azonium tautomer of MOH (Tawarah and Abu-Shamleh 1991; Fan *et al*. 1998). The λ_{max} of MO corresponding to different pH values are shown in Table 7 while Fig 11 clearly depicts the pH and absorption maxima relationship. The lowering of pH is also accompanied by an increase in absorbance of MO, and the same is shown in Fig 12. With lowering of pH, there is a corresponding increase in λ_{max} and absorbance of MO at its constant concentration. This phenomenon is illustrated in Table 7 and Fig 13.

Table 7 Wavelength maxima and absorbance of aqueous 5.0 mg L^{-1} MO with increasing pH

pH ± 0.02	λ_{max}	Absorbance
0.82	507.89	0.6487
1.10	507.94	0.6342
1.36	507.86	0.6388
1.67	507.93	0.6320
1.86	507.61	0.6313
2.22	507.19	0.6209
2.41	506.26	0.6070
2.62	506.80	0.5884
2.83	505.42	0.5580
3.00	504.25	0.5139
3.54	497.16	0.4174
5.26	464.06	0.3553

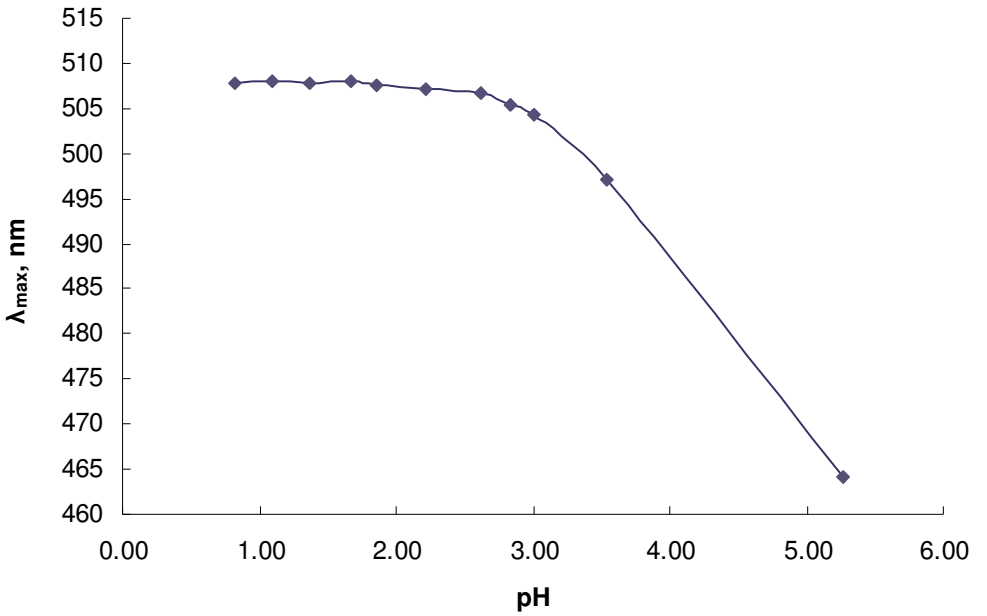


Fig 11 Relationship between pH and the wavelength maxima of aqueous 5.0 mg L^{-1} MO

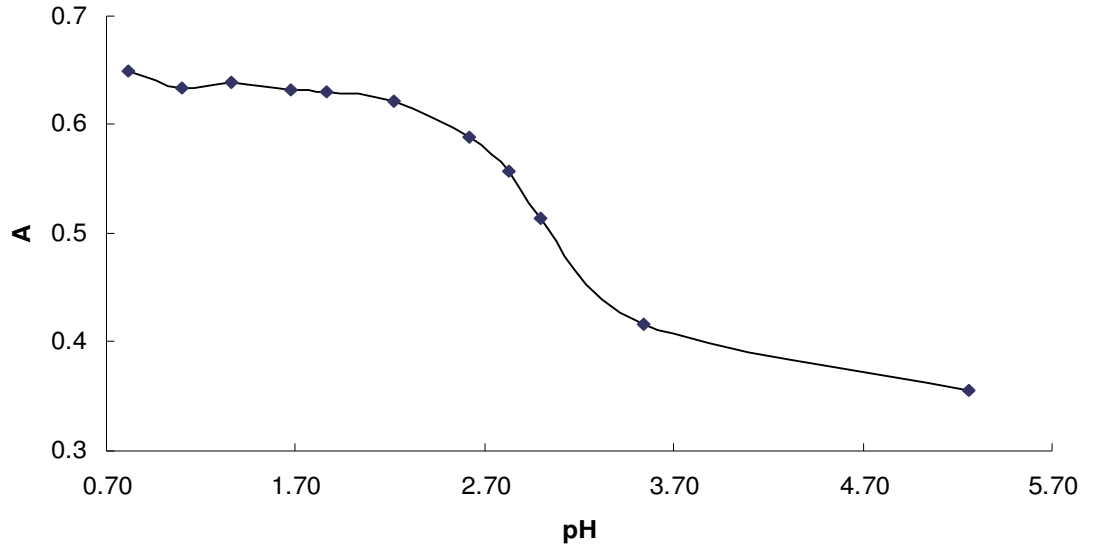


Fig 12 Relationship between the pH and absorbance of aqueous 5.0 mg L^{-1} MO at λ_{max} given in Table 7

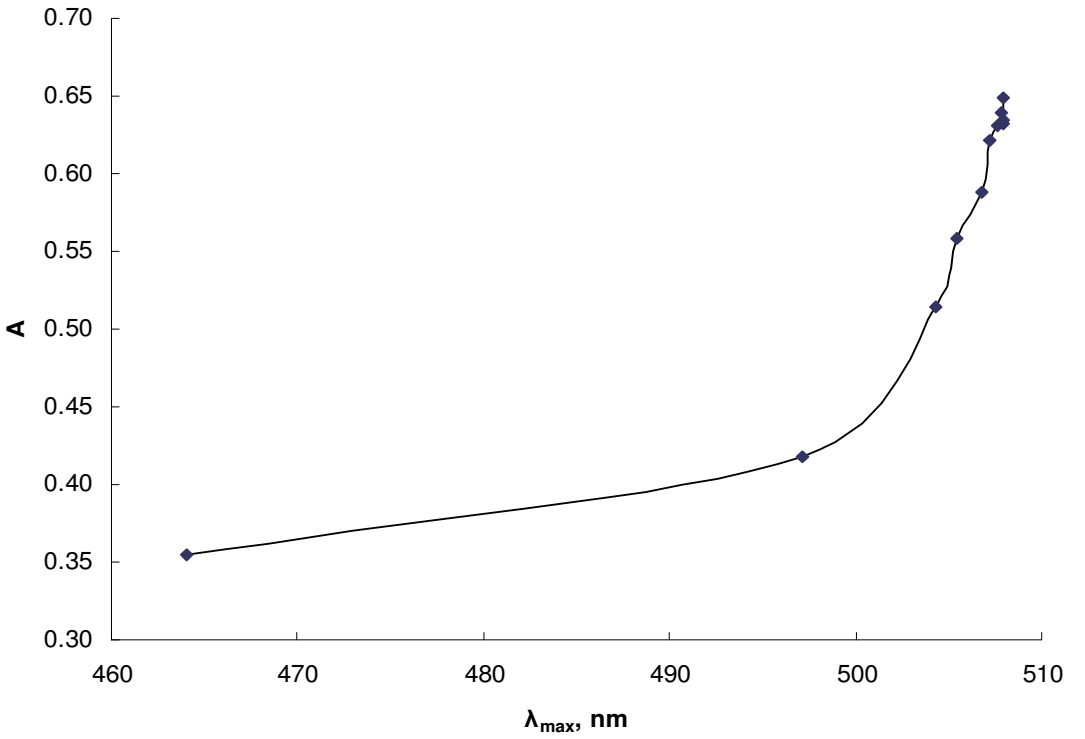


Fig 13 Dependence of absorbance of aqueous 5.0 mg L^{-1} MO at different wavelength maxima (*cf.* Table 7) due to decrease in pH from 5.26 - 0.82

From the initial spectroscopic study results, some important conclusions were derived. The spectrum of MO in aqueous solution shows an absorption band at 465 nm. The addition of buffer and aqueous solutions of Se(IV), $N_2H_4.2HCl$ and $KBrO_3$ to MO causes change in the absorption spectrum with new characteristic bands appearing at about 500 nm. The highest sensitivity was obtained at 507 nm, which is characteristic of acid form of MO (Fan *et al*. 1998; Boily and Seward 2005; Suleimenov and Boily 2006). The reagent blank solution of buffer, Se(IV), $N_2H_4.2HCl$ and $KBrO_3$ show negligible absorbance at 507 nm when measured against distilled water as reference. Therefore, absorbance measurements for the determination of Se(IV) were made at 507 nm (Fig 14), which is significantly different to previous studies, which have reported that reaction system to be monitored at 525 nm (Table 8). The choice for 525 nm by these authors has not been justified experimentally nor referenced to literature.

4.2 The Indicator Reaction

MO is a dye and used as a redox indicator (Tawarah and Abu-Shamleh 1991; Safavi *et al*. 2001). The decolorisation reaction between MO and bromate ion is slow in acidic media (Safavi *et al*. 2001), and it can be kinetically monitored. However, Se catalyses this reaction in acidic media (Safavi *et al*. 2001). In particular, Se(IV) has been reported to catalyse the reduction of bromate in HCl media (Afkhami *et al*. 1992). However, the catalysed reaction is too fast to be able to monitor spectrophotometrically. To overcome this, hydrazine need to be present in the reaction medium. The presence of hydrazine in the medium slows down the rate-determining step, which is fairly fast in its absence or when the medium is very acidic (Linares *et al*. 1986; Afkhami and Afshar-E-Asl 2000). The reactions are represented as follows:

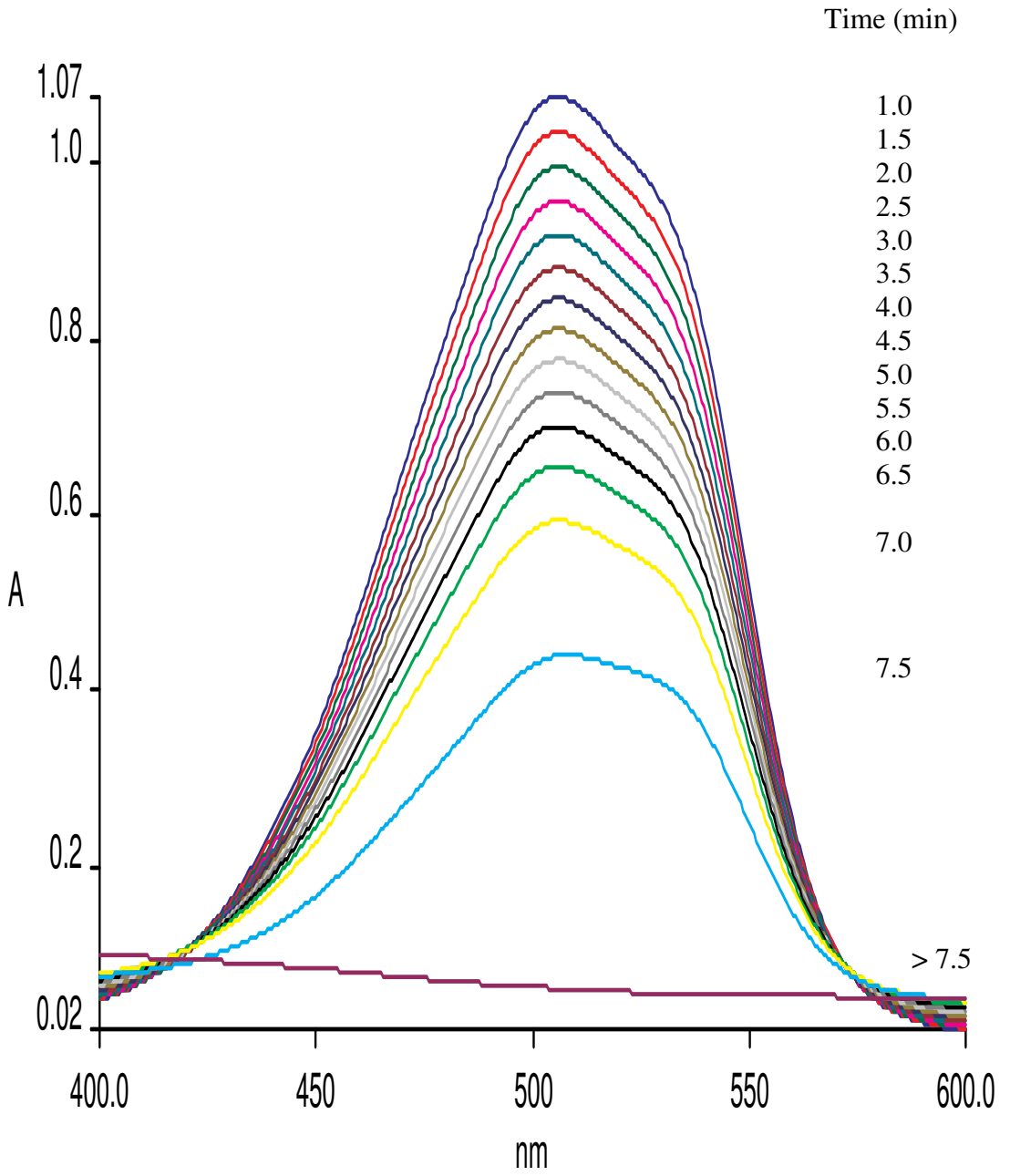


Fig 14 Spectra of catalysed reaction with time for the first seven minutes under conditions: [Se(IV)] = 789.6 µg L^{-1}, [MO] = 10.0 mg L^{-1}, [BrO_3^-] = 5.0 × 10^{-3} M, [N_2H_4.2HCl] = 5.0 × 10^{-3} M, pH = 1.60 ± 0.02, temperature = 25.0 ± 0.1 ^oC, scan speed = 1440 nm min^{-1}

$$2BrO_3^- + 10Cl^- + 12H^+ \rightleftharpoons 5Cl_2 + Br_2 + 6H_2O \qquad \text{(slow)} \qquad (1)$$

$$2X_2 + N_2H_6^{2+} \rightleftharpoons N_2 + 4X^- + 6H^+ \qquad \text{(fast)} \qquad (2)$$

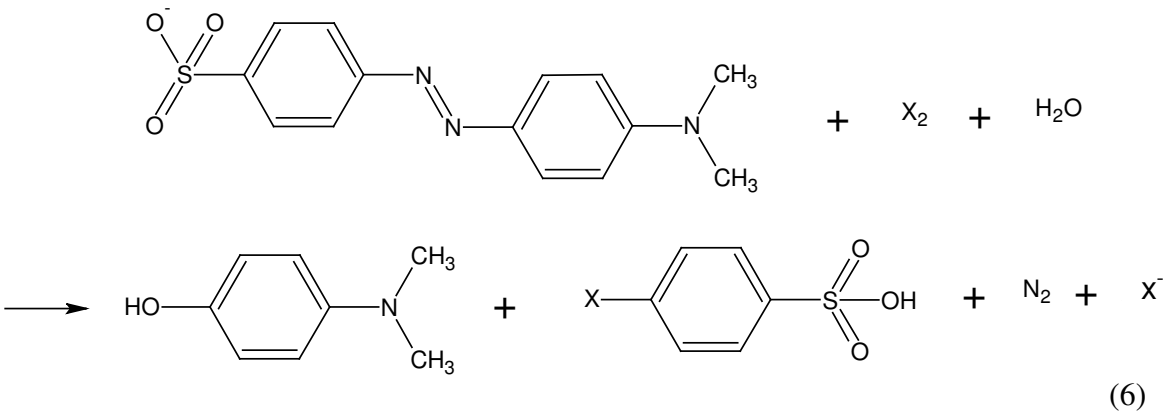
X = Cl or Br

Se(IV) acts as a catalyst for the first step (1) (Afkhami *et al*. 1992). A possible catalytic route of Se is that the hydrazine salts reduce Se(IV) to elemental Se very effectively (99.8%) in acidic medium as shown in equation (3) (Bye 1983; Lee *et al*. 1994). The elemental Se thus formed is oxidised back to Se(IV) by BrO_3^-, generating Br^- in the process {Equation (6)}. The generated Br^- is oxidised by BrO_3^- in acidic medium and forms Br_2 which oxidises MO (Afkhami & Madrakian 2002). Combined with the data reported in literature (Bye 1983; Lee *et al*. 1994; Afkhami & Madrakian 2002), a plausible mechanism of the reaction is shown in equation (3) - (6) as follows:

$$SeO_3^{2-} + 2H^+ + N_2H_4 \longrightarrow Se^0 + N_2 + 3H_2O \qquad (3)$$

$$3Se^0 + 2BrO_3^- + 3H_2O \longrightarrow 3H_2SeO_3 + 2Br^- \qquad (4)$$

$$2BrO_3^- + 5Br^- + 6H^+ \longrightarrow 3Br_2 + 3H_2O \qquad (5)$$



(6)

The oxidation of MO is significantly accelerated in presence of trace quantities of Br_2 (or Cl_2), therefore it is accelerated in the presence of trace quantities of Se {Equation (6)}. The presence and absence of chloride ions is of no importance to this reaction (Bye 1983). A detailed kinetic and mechanistic investigation of this reaction system was beyond the scope of present study. A summary of the work done by other researchers involving BrO_3^--hydrazine-MO indicator reaction system is presented in Table 8.

4.3 Method Optimisation - Experimental Variables

For any analytical method, it is essential to be highly sensitive for the analyte it determines and the same is true in case of CKM. By optimising the different variables which are associated with performance of a method is a good way of optimising a method (Gurkan and Akcay 2003). Kinetic methods involve initial rate determination as the fundamental measurement for analyte determination. Hence all factors which affect rate are optimised to obtain high method sensitivity. These are pH, concentration of reactants, temperature and ionic strength of a reaction. For kinetic measurements, it is desired that little fluctuations on concentration have no effect on initial rate. These conditions must be also chosen in a manner that initial rate will be first order with respect to analyte. Ideally, optimum concentration of each component must give the smallest relative standard deviation and should be zero order with respect to that species except for the catalyst (*i.e.* analyte) (Gurkan and Akcay 2003). A brief summary of the work which has been done using BrO_3^--hydrazine-MO type of reaction systems for the analysis of different analytes is presented in Table 8. Using the optimised conditions reported in previous studies on this indicator reaction system (Afkhami *et al*. 1992), results could not be reproduced,

which are discussed in detail (*vide supra*). Hence, an attempt was made to achieve the optimum reaction conditions suitable for the determination of Se(IV). The effect of different variables affecting the reaction was studied by changing each variable in turn while keeping all others constant. The optimum values of the variables were maintained throughout the experiment.

4.3.1 Effect of Time

The effect of time on the reaction *i.e.* reaction rate was studied for the catalysed as well as uncatalysed reactions. As Fig 15 shows, the decrease in absorbance of the catalysed and uncatalysed reaction with time was linear at 507 nm during the first 5 min (*i.e.* 6 min from initiation of the reaction). Other researchers have reported that the absorbance *versus* time graph was linear upto 2.5, 3 or 4 min (Table 8). Hence, the linearity could differ according to the analyte or the reaction conditions used.

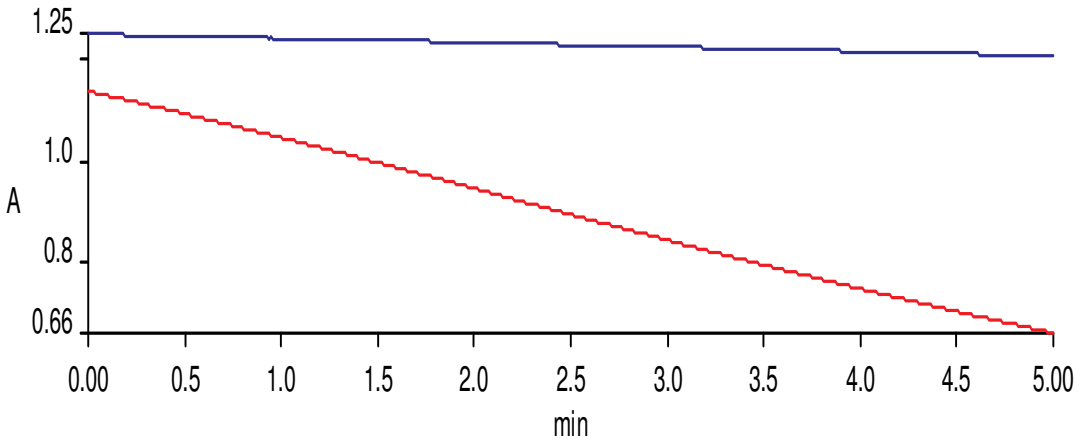


Fig 15 Typical absorbance-time curves of catalysed (in red) and uncatalysed reaction (in blue) under conditions: under conditions: [Se(IV)] = 789.6 µg L^{-1}, [MO] = 10.0 mg L^{-1}, $[BrO_3^-]$ = 5.0 × 10^{-3} M, $[N_2H_4.2HCl]$ = 5.0 × 10^{-3} M, pH = 1.60 ± 0.02, temperature = 25.0 ± 0.1 ^oC

Table 8 Summary of work done on the BrO_3^--hydrazine-MO system for determination of different analytes

Analyte	Experimnetal Conditions	Data Treatment	Inter-feron (s) against analyte (error ≥ 3%)	Linear Range, DL, RSD	Sample, Recovery,	Remarks	Reference
Se(IV)-catalytic action	pH 1, 0.10 M $N_2H_4.2HCl$, 0.024 M $KBrO_3$, 10 mg L^{-1} MO, 25 °C	Absorbance *vs* time graph linear upto 3 min; slope used a measure of initial rate; at 525 nm	La(III), Ce(IV), Fe(III), Cu(II), Pd(II), V(III), Hg_2^{2+}	5-800 ng mL^{-1}; 1 ng mL^{-1}; 0.94 - 0.10 % for 20 - 500 ng mL^{-1} Se(IV) (n = 10)	Se in shampoo; 97.4 % (n=7)	pH choice not good, choice of [MO] and 525 nm not supported by literature	Afkhami *et al*. 1992
Te(IV) - catalytic action	pH 1, 0.10 M $N_2H_4.2HCl$, 0.03 M $KBrO_3$, 10 mg L^{-1} MO, 25 °C	Absorbance *vs* time graph linear upto 4 min; slope used a measure of initial rate; at 525 nm	La(III), Ce(IV), Fe(III), Cu(II), Pd(II), V(III), Hg_2^{2+}	50-2000 ng mL^{-1}; 31.0 ng mL^{-1}; 2.85-0.53 % for 0.10-0.80 µg mL^{-1} Te(IV) (n = 10)	Te (IV) in spiked spring and drinking water; 99 - 102 % for 0.10-0.850 µg mL^{-1} Te(IV) (n = 3)	pH choice not good, [MO] and 525 nm not supported by literature	Safavi *et al*. 1995
Hydrazine - inhibition action	0.35 HCl, 7.2×10^{-5} or 2.4×10^{-4} M $KBrO_3$, 10 mg L^{-1} MO, 30 °C	Induction period (t_{ip}) of absorbance *vs* time; at 525 nm	SCN^-, NO_2^-, I^-, AsO_2^-, SO_3^{2-}	3.1×10^{-6} - 3.2×10^{-5} M; 5.2×10^{-7} M or 3.1×10^{-6} - 3.2×10^{-5} M; 5.2×10^{-7} M; 2.15 - 0.75 % for 6.00×10^{-7}- 2.00×10^{-5} M hydrazine (n = 7)	hydrazine in spiked spring and drinking water; 97.3 - 104.0% for 8.00×10^{-7} - 1.00×10^{-5} M hydrazine (n = 5)	[MO] and 525 nm not supported by literature; effect of *I* not studied	Afkhami and Afshar-E-Asl 2000

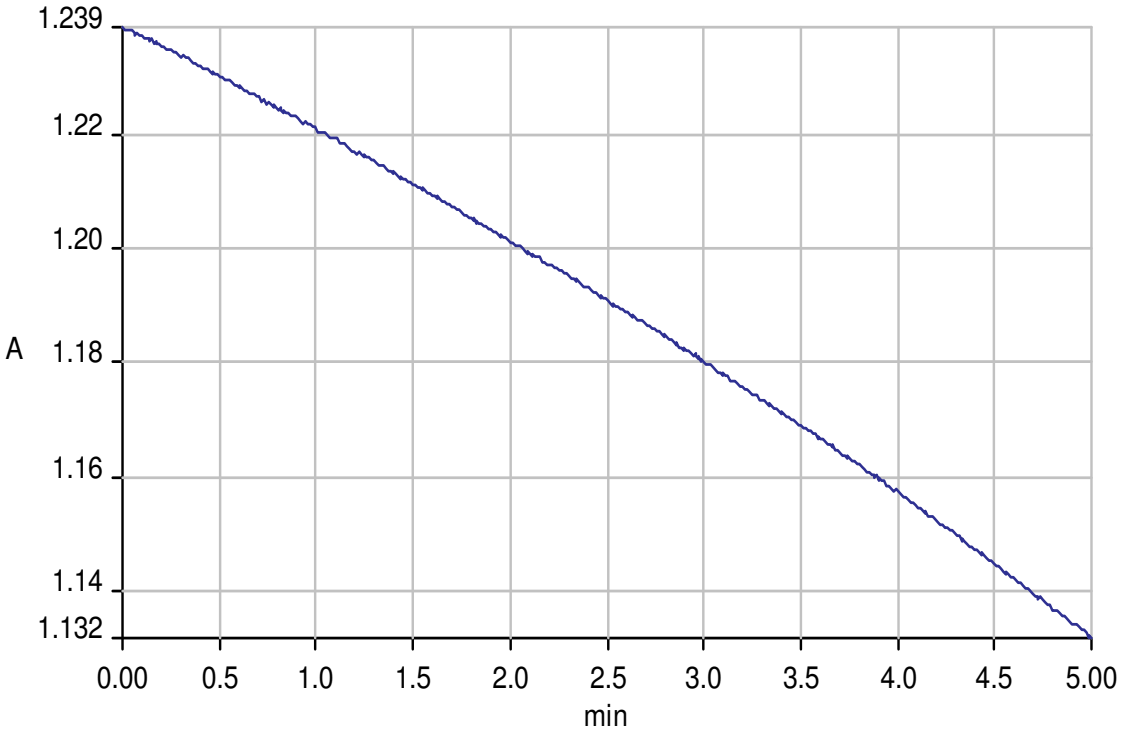
Phenyl-hydrazine - inhibition action	0.35 HCl, 8.04×10^{-5} or 2.4×10^{-4} M $KBrO_3$, 10 mg L^{-1} MO, 30 °C	Induction period (t_{ip}) of absorbance *vs* time graph; at 525 nm	SCN^-, NO_2^-, I^-, AsO_2^-, SO_3^{2-}	4.6×10^{-7} - 1.4×10^{-5} M; 1.85×10^{-7} M or 3.1×10^{-6} - 7.4×10^{-5} M; 2.23×10^{-6} M; 2.72 - 0.86% for 9.25×10^{-7}- 5.55×10^{-5} M phenylhydrazine (n=7)	phenylhydrazine in spiked spring and drinking water; 96.4-103% for 5.00×10^{-7} - 5.00×10^{-5} M phenylhydrazine (n=7)	[MO] and 525 nm not supported by literature; effect of *I* not studied	Afkhami and Assl 2001
IO_3^- (iodate) - catalytic action	0.56 M Cl^-, 0.20 M H_2SO_4, 1.56×10^{-5} M $N_2H_4.2HCl$, 1.92×10^{-4} M $KBrO_3$, 3.05×10^{-5} M (10 mg L^{-1}) MO, 30 °C	Absorbance change measured using fixed time (150 s) from absorbance *vs* time graph; at 525 nm	NO_2^-, Br^-	0.03-1.2 µg mL^{-1}; 0.02 µg mL^{-1}; 2.12-0.92 % for 0.050-1.000 µg mL^{-1} IO_3^- (n=10)	IO_3^- in table salt	525 nm not supported by literature; MO found to have no effect from 1.5×10^{-5} - 7.3×10^{-5} M	Afkhami and Mosaed 2002
IO_4^- (periodate)-catalytic action	0.32 M HCl, 0.5 µg mL^{-1} $N_2H_4.2HCl$, 1.92×10^{-4} M $KBrO_3$, 10 mg L^{-1} MO, 30 °C	Absorbance change measured using fixed time (150 s) from absorbance *vs* time graph; at 525 nm	NO_2^-, Br^-	0.02 - 1.5 µg mL^{-1}; 0.012 µg mL^{-1}; 0.00 - 0.01% for 0.100 -1.200 µg mL^{-1} IO_4^- (n=10)	IO_3^- in spiked spring and drinking water; 97.5 - 102.0 % for 0.080 - 1.200 µg L^{-1} IO_4^- (n=5)	525 nm not supported by literature; MO found to have no effect from 4 - 24 mg L^{-1}	Afkhami and Mosaed 2003

The initial rate was taken as the derivative of the absorbance-time curve at the initial stage of the reaction. The initial rates for a catalysed and an uncatalysed reaction are presented in Table 9. Fig 16 shows the typical analysis for initial rate of an absorbance *versus* time curve for an uncatalysed reaction. In many studies, the slope of the linear range is taken as a measure of initial rate (Afkhami *et al*. 1992; Safavi *et al*. 1995) while some researchers have taken change in absorbance in a time interval as a measure of initial rate *i.e.* Fixed time method (Afkhami and Mosaed 2002; Afkhami and Mosaed 2003). For the Fixed time method, 4 min from the initiation of the reaction was chosen in present study for use on the determination of catalytic Se in order to compromise sensitivity and short analysis time.

Table 9 Absorbance and Initial rate of catalysed and uncatalysed reaction with respect to time under conditions: [Se(IV)] = 789.6 µg L^{-1}, [MO] = 10.0 mg L^{-1}, [BrO_3^-] = 5.0 × 10^{-3} M, [N_2H_4.2HCl] = 5.0 × 10^{-3} M, pH = 1.60 ± 0.02, temperature = 25.0 ± 0.1 ^oC

Time (min)	Absorbance		Initial rate (-dA/dt), min^{-1}	
	Catalysed reaction	Uncatalysed reaction	Catalysed reaction	Uncatalysed reaction
0.0	1.1378	1.2499	0.91159	0.18083
1.0	1.0466	1.2319	0.88705	0.18813
2.0	0.9464	1.2123	0.87676	0.20318
3.0	0.8443	1.1912	0.87104	0.21930
4.0	0.7481	1.1685	0.86866	0.24008
5.0	0.6612	1.1432	0.85246	0.25249

(i)



(ii)

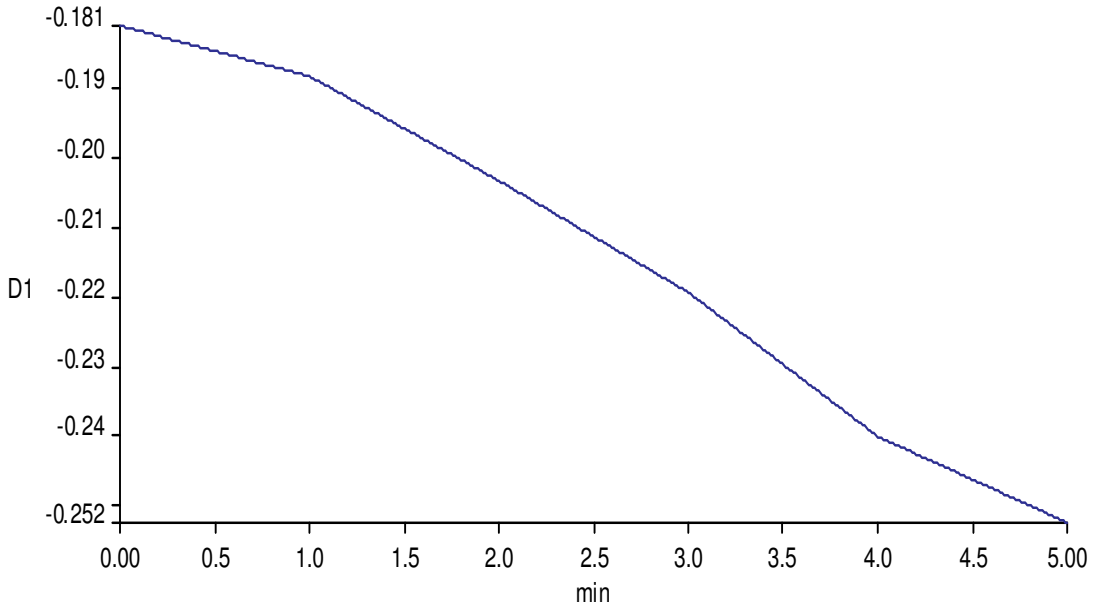


Fig 16 Typical analysis of a absorbance-time graph using the derivative function of the Perkin Elmer Lambda 16 UV-visible Spectrophotometer (i) Absorbance-time graph of an uncatalysed reaction (ii) 1st derivative of the absorbance-time graph under conditions: [MO] = 10.0 mg L^{-1}, $[BrO_3^-] = 5.0 \times 10^{-3}$ M, $[N_2H_4.2HCl] = 5.0 \times 10^{-3}$ M, pH = 1.60 ± 0.02, temperature = 25.0 ± 0.1 ^oC

4.3.2 Effect of pH

According to the stoichiometry of the reaction, the slowest/rate determining steps (1) and (5) of the reaction require an acidic medium {*cf.* equations (1) and (5); (vide infra)}. Thus the hydrogen ion concentration was found to affect the rate of reaction dramatically. Therefore, the effect of pH on the rate of reaction was determined in the pH range 0.91 - 2.95 ± 0.02. The initial rates corresponding to different acidic pH are shown in Table 10. The effect of pH on the initial rate of the catalysed and uncatalysed oxidations of $N_2H_4.2HCl$ is shown in Fig 17.

The present study shows that the reaction is only sensitive below pH 3 (Table 10 and Fig 17). However, at very low pH, especially less than 1, both the catalysed and uncatalysed reaction rates are very unstable as well as give low sensitivity (Fig 17). The rates of both reactions are quite stable from pH 1.4 - 1.9, giving maximum sensitivity. In fact, an ANOVA analysis showed no significant difference between rates in this region. Above pH 2, the rate of catalysed reaction decreases rapidly and since the uncatalysed reaction rate is constant from pH 1.4 onwards; the overall sensitivity is decreased rapidly above pH 2. This implies that the rate of the reaction on $[H^+]$ follows a variable order in the pH range studied.

The decrease in the rate of both uncatalysed and catalysed reaction can be explained in terms of the behaviour of MO in acidic medium. It has been observed that with increasing pH, there is a decrease in the absorbance of MO due to the blue shift, with a corresponding decrease in wavelength maxima. It has also been observed that at different

MO concentrations at pH higher than 5, MO exhibits maximum absorption near 465 nm. However, Fig 10 indicates that as the pH is lowered, the absorption maxima of MO are shifted to 507 nm, with a corresponding increase in absorption. Hence this phenomenon contributes to lower sensitivity of reaction at higher pH for MO, as the absorption also decreases correspondingly. The maximum absorption is observed at a wavelength of λ_{max} = 507 nm below pH 2. It has also been reported that the maximum molar absorptivity (ε = 5.4×10^4 L mol^{-1} cm^{-1}) of aqueous acidic MO occurs at 507 nm (Boily and Seward 2005).

The choice of pH reported by the previous workers for this particular reaction system is questionable. Afkhami *et al*. (1992) and Safavi *et al*. (1995) have preferred pH 1, where they obtain the maximum sensititvity (*i.e.* the difference between the catalysed reaction rate and the uncatalysed reaction rate). However, Fig 17 shows that at pH 1, both the catalysed and uncatalysed reactions are highly unstable, and the uncatalysed reaction rate is the highest at this pH. Hence a slight change in pH of the reaction mixtures could provide unpredicatable results. The present study shows that both reactions are highly unstable at this extremely low pH and may contribute to inconsistent results.

Figure 11 and 12 show that MO has very high molar absorptivity around pH 1.3 - 1.6, at a λ_{max} of 507 nm. The uncatalysed and catalysed reaction rates have been shown to be stable in this pH range, as well as providing maximum sensitivity (Fig 17). Therefore, pH 1.60 ± 0.02 was chosen as optimum for further study.

Table 10 pH dependence study conditions: [Se(IV)] = 789.6 µg L^{-1}, [MO] = 5.0 mg L^{-1}, [BrO_3^-] = 1.0×10^{-3} M, [N_2H_4.2HCl] = 1.0×10^{-3} M, temperature = 25.0 ± 0.1 °C

pH ± 0.02	Initial Rate (-dA/dt), min^{-1}		
	Uncatalysed reaction (U)	Catalysed reaction (C)	Sensitivity
	Average ± SD (*n* = 5)	Average ± SD (*n* = 5)	(C - U) ± SD
0.91	0.176 ± 0.002	0.258 ± 0.017	0.082 ± 0.017
1.01	0.084 ± 0.001	0.236 ± 0.011	0.152 ± 0.011
1.24	0.045 ± 0.003	0.220 ± 0.003	0.175 ± 0.005
1.44	0.025 ± 0.003	0.213 ± 0.002	0.188 ± 0.004
1.66	0.025 ± 0.003	0.211 ± 0.002	0.187 ± 0.004
1.77	0.025 ± 0.003	0.210 ± 0.003	0.185 ± 0.005
1.88	0.024 ± 0.002	0.208 ± 0.003	0.184 ± 0.004
2.02	0.022 ± 0.002	0.186 ± 0.004	0.164 ± 0.004
2.25	0.025 ± 0.002	0.135 ± 0.003	0.110 ± 0.004
2.38	0.027 ± 0.003	0.092 ± 0.002	0.065 ± 0.004
2.64	0.026 ± 0.003	0.055 ± 0.002	0.029 ± 0.004
2.95	0.027 ± 0.003	0.042 ± 0.002	0.015 ± 0.003

4.3.3 Effect of the Concentration of Methyl Orange

An initial study was done with MO to determine the linearity range and data are presented in Table 11. It was found that MO at pH 1.6 obeys the Lambert-Beer law up to 30 mg L^{-1} (r^2 = 0.9996), while at concentrations above 30 mg L^{-1}, deviation from linearity is observed as represented in Fig 18. Hence using this basis, a concentration dependence of MO on reactions rates was done for a concentration range of 0 - 22.5 mg L^{-1} and the initial rate data are presented in Table 12.

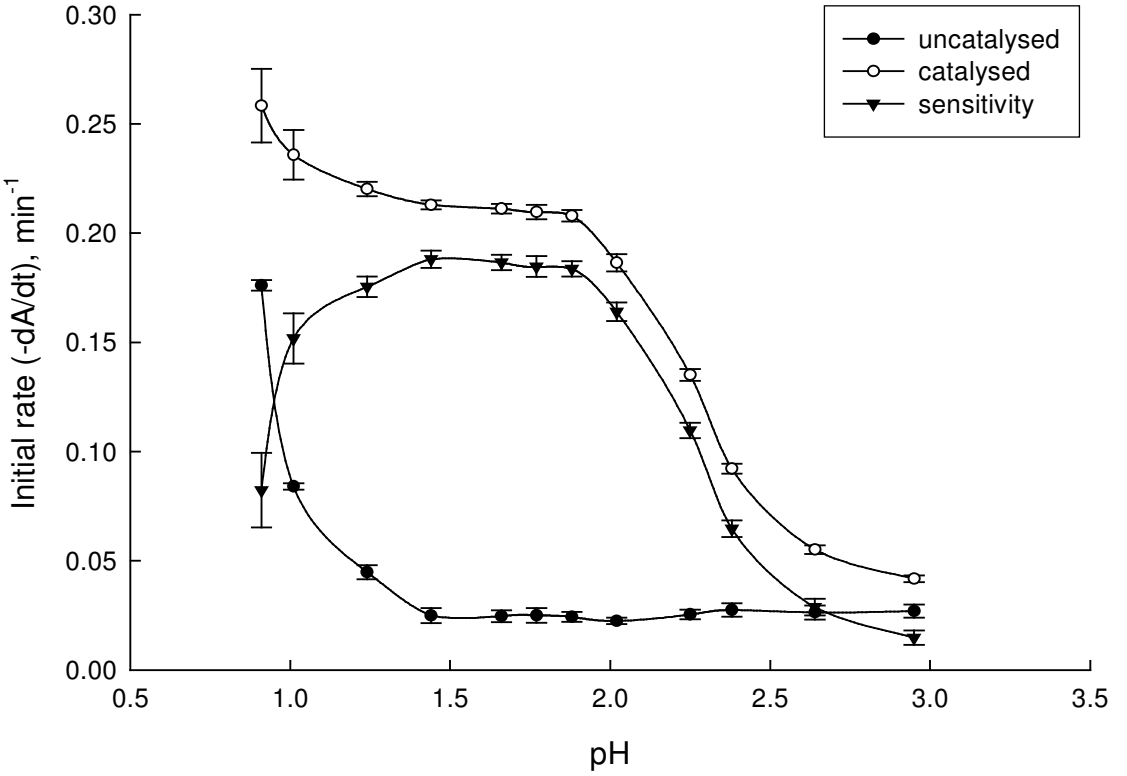


Fig 17 Effect of pH on catalysed and uncatalysed reaction rates with resulting sensitivity under the conditions given in Table 10

Table 11 Absorbance of aqueous acidic MO (pH 1.60 ± 0.02) with increasing concentration

[MO], mg L^{-1}	Absorbance
0.0	0.0000
2.0	0.2534
4.0	0.5153
6.0	0.7617
7.0	0.8896
8.0	1.0156
10.0	1.2783
12.0	1.5507
14.0	1.7971
16.0	2.0292
18.0	2.3037
22.5	2.8055
27.0	3.3451
31.5	3.7224
36.0	3.8273
45.0	3.9500
54.0	4.0386
63.0	4.1203
70.0	4.2669

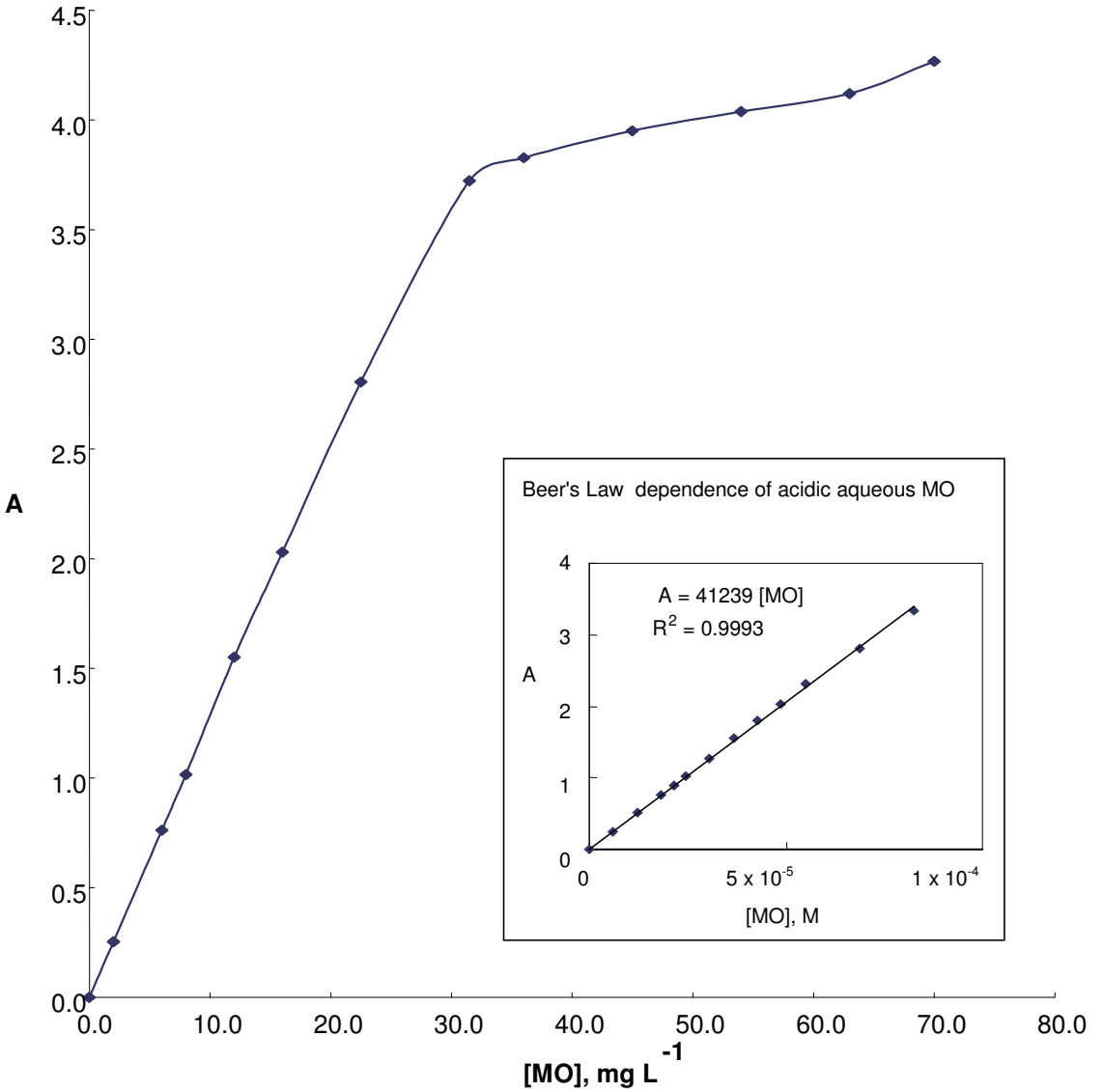


Fig 18 Beer's Law dependence of acidic aqueous MO

Previous studies on this type of reactions have either not reported [MO] dependence study or have reported that MO has no effect on this system (Afkhami *et al*. 1992; Safavi *et al*. 1995; Afkhami and Afshar-E-Asl 2000; Afkhami and Assl 2001). In fact, [MO] 10 mg L^{-1} has been the obvious choice for all workers, without any justification. Afkhami and Mosaed (2002, 2003) reported that 1.5 - 7.3 × 10^{-5} M and 4 - 24 mg L^{-1} MO had no effect on the reaction. On the contrary, the present study has shown that both the catalysed and uncatalysed reaction rates increase with increase [MO] upto 20 mg L^{-1}; and the catalysed rate leveling off at higher concentrations (Table 12 and Fig 19). The catalytic reaction rate showed a more noticeable increase in the lower concentration region. The rate dependence on [MO] is first order for uncatalysed reaction in acceptable concentrations tested, however variable order rate dependence is observed for the catalysed reaction. At low [MO], the reaction is first order while the order decreases from unity at high concentrations (> 14 mg L^{-1}) and finally levels off. Also in our study, it was found that at very high [MO] (> 14 mg L^{-1}), huge error in results was observed due to aggregation of MO, which caused perturbation in absorbance. Thus, though the sensitivity is higher, the results obtained are not consistent in this range. Kendrick and Gilkerson (1987) have reported that the MO undergoes aggregation due to dimerisation of its anion in water. For convenience and reliability, 10.0 mg L^{-1} (3.055 × 10^{-5} M) [MO] was chosen for further studies.

Table 12 [MO] dependence study conditions: [Se(IV)] = 789.6 µg L^{-1}, [BrO_3^-] = 1.0 × 10^{-3} M, [N_2H_4.2HCl] = 1.0 × 10^{-3} M, pH = 1.60 ± 0.02, temperature = 25.0 ± 0.1 °C

[MO], mg L^{-1}	Initial Rate (-dA/dt), min^{-1}		
	Uncatalysed reaction (U)	Catalysed reaction (C)	Sensitivity
	Average ± SD (*n* = 5)	Average ± SD (*n* = 5)	(C - U) ± SD
2.0	0.018 ± 0.000	0.099 ± 0.004	0.082 ± 0.004
4.0	0.030 ± 0.001	0.180 ± 0.001	0.150 ± 0.002
6.0	0.049 ± 0.002	0.251 ± 0.007	0.202 ± 0.007
8.0	0.062 ± 0.001	0.321 ± 0.009	0.259 ± 0.009
10.0	0.073 ± 0.002	0.391 ± 0.007	0.318 ± 0.008
12.0	0.090 ± 0.004	0.457 ± 0.012	0.367 ± 0.012
14.0	0.094 ± 0.004	0.509 ± 0.014	0.415 ± 0.015
16.0	0.119 ± 0.005	0.559 ± 0.015	0.440 ± 0.016
18.0	0.130 ± 0.007	0.602 ± 0.015	0.473 ± 0.017
20.0	0.142 ± 0.009	0.620 ± 0.019	0.478 ± 0.021
22.5	0.153 ± 0.012	0.641 ± 0.020	0.488 ± 0.023

4.3.4 Effect of Concentration of Potassium Bromate

The effect of [BrO_3^-] on reaction rate was studied in its concentration range 2.0 × 10^{-4} - 1.0 × 10^{-2} M. The initial rates for catalysed and uncatalysed reactions are presented in Table 13. Increasing [BrO_3^-] increases rates for both the uncatalysed and catalysed reactions in the range of 2.0 × 10^{-4} - 1.0 × 10^{-2} M. In fact, both reactions are first order; however, the catalysed reaction is variable order at [BrO_3^-] > 1.0 × 10^{-3} M and is shown in Fig 20. At all possible concentrations studied, the rate continued to increase with an

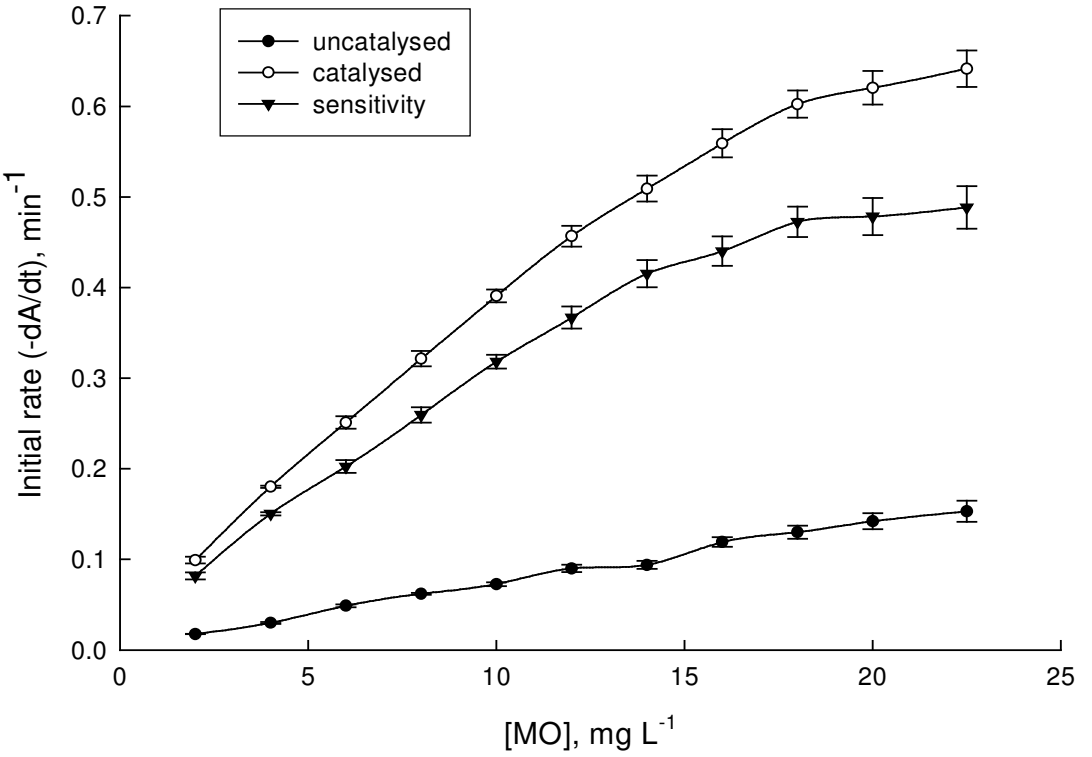


Fig 19 Effect of [MO] on the catalysed and uncatalysed reaction rates with resulting sensitivity under the conditions given in Table 12

increasing [BrO_3^-] (Fig 20). However, [BrO_3^-] > 8.0×10^{-3} M caused the production of nitrogen gas bubbles,which interfered with absorbance measurements of the reaction mixture. As well, high and unstable blank signals were observed at these [BrO_3^-].

A literature review showed that [BrO_3^-] can vary according to the analyte tested (Table 8). The use of 0.024 M (Afkhami *et al*. 1992) or 0.03 M (Safavi *et al*. 1995) was unsuitable for this study since the reactions were unstable at these [BrO_3^-] and the rates obtained were inconsistent. Hence, a workable concentration of 5.0×10^{-3} M was chosen for further studies.

Table 13 [$KBrO_3$] dependence study conditions: [Se(IV)] = 789.6 µg L^{-1}, [MO] = 10.0 mg L^{-1}, [$N_2H_4.2HCl$] = 1.0×10^{-3} M, pH = 1.60 ± 0.02, temperature = 25.0 ± 0.1 ^oC

[$KBrO_3$], M	**Initial Rate (-dA/dt), min^{-1}**		
	Uncatalysed reaction (U)	**Catalysed reaction (C)**	**Sensitivity**
	Average ± SD (*n* = 5)	**Average ± SD (*n* = 5)**	**(C - U) ± SD**
2.0×10^{-4}	0.009 ± 0.001	0.104 ± 0.005	0.095 ± 0.005
4.0×10^{-4}	0.015 ± 0.002	0.164 ± 0.004	0.150 ± 0.004
6.0×10^{-4}	0.021 ± 0.001	0.221 ± 0.005	0.200 ± 0.005
8.0×10^{-4}	0.026 ± 0.003	0.268 ± 0.013	0.242 ± 0.014
1.0×10^{-3}	0.031 ± 0.003	0.320 ± 0.010	0.289 ± 0.010
1.5×10^{-3}	0.042 ± 0.003	0.372 ± 0.005	0.330 ± 0.006
2.0×10^{-3}	0.051 ± 0.002	0.409 ± 0.011	0.358 ± 0.012
3.0×10^{-3}	0.063 ± 0.002	0.445 ± 0.003	0.381 ± 0.004
4.0×10^{-3}	0.085 ± 0.003	0.476 ± 0.003	0.392 ± 0.004
5.0×10^{-3}	0.102 ± 0.004	0.509 ± 0.008	0.408 ± 0.009
6.3×10^{-3}	0.126 ± 0.003	0.551 ± 0.011	0.425 ± 0.011
7.5×10^{-3}	0.146 ± 0.006	0.595 ± 0.017	0.449 ± 0.018
8.8×10^{-3}	0.174 ± 0.007	0.634 ± 0.017	0.460 ± 0.019
1.0×10^{-2}	0.198 ± 0.011	0.678 ± 0.025	0.480 ± 0.027

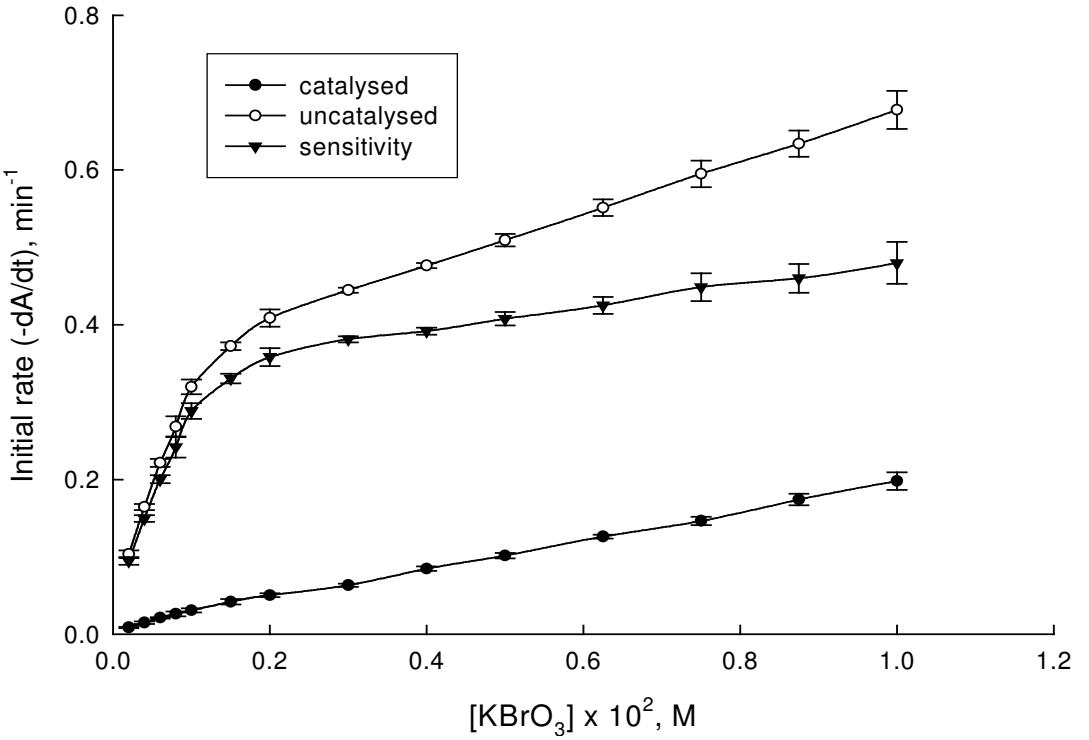


Fig 20 Effect of [$KBrO_3$] on the catalysed and uncatalysed reaction rates with resulting sensitivity under the conditions given in Table 13

4.3.5 Effect of Concentration of Hydrazine Dihydrochloride

The effect of [$N_2H_4.2HCl$] was studied for the concentration range of 0 - 1.0×10^{-2} M and the data obtained are shown in Table 14. The plot of initial rate against [$N_2H_4.2HCl$] is shown in Fig 21, which clearly indicates that the uncatalysed and catalysed reactions are both of variable order in this range. Both rates increase with increasing [$N_2H_4.2HCl$] until 1.0×10^{-2} M, where catalysed rate starts leveling off at further high [$N_2H_4.2HCl$] (Fig 21). At high concentrations ($\geq 7.5 \times 10^{-3}$ M), the production of nitrogen gas bubbles perturb absorbance measurements. Thus the use of 0.1 M [$N_2H_4.2HCl$] (Afkhami *et al.* 1992; Safavi *et al*. 1995) was not appropriate to this indicator reaction because of severe

Table 14 [N_2H_4.2HCl] dependence study conditions: [Se(IV)] = 789.6 µg L^{-1}, [MO] = 10.0 mg L^{-1}, [BrO_3^-] = 5.0×10^{-3} M, pH = 1.60 ± 0.02, temperature = 25.0 ± 0.1 °C

[N_2H_4. 2HCl], M	**Initial Rate (-dA/dt), min^{-1}**		
	Uncatalysed reaction (U)	**Catalysed reaction (C)**	**Sensitivity**
	Average ± SD (*n* = 5)	**Average ± SD (*n* = 5)**	**(C - U) ± SD**
0.0	0.693 ± 0.018	0.696 ± 0.023	0.002 ± 0.029
1.0×10^{-5}	0.121 ± 0.002	0.283 ± 0.005	0.162 ± 0.005
5.0×10^{-5}	0.126 ± 0.003	0.331 ± 0.004	0.205 ± 0.005
2.0×10^{-4}	0.133 ± 0.003	0.374 ± 0.001	0.240 ± 0.003
6.0×10^{-4}	0.136 ± 0.009	0.453 ± 0.007	0.317 ± 0.011
1.0×10^{-3}	0.135 ± 0.008	0.526 ± 0.010	0.390 ± 0.013
2.0×10^{-3}	0.152 ± 0.003	0.674 ± 0.017	0.522 ± 0.017
3.0×10^{-3}	0.161 ± 0.002	0.790 ± 0.010	0.629 ± 0.010
4.0×10^{-3}	0.169 ± 0.001	0.858 ± 0.014	0.688 ± 0.014
5.0×10^{-3}	0.180 ± 0.002	0.909 ± 0.013	0.728 ± 0.013
6.3×10^{-3}	0.190 ± 0.005	0.976 ± 0.018	0.786 ± 0.019
7.5×10^{-3}	0.195 ± 0.006	0.998 ± 0.016	0.803 ± 0.017
8.8×10^{-3}	0.212 ± 0.005	1.031 ± 0.024	0.819 ± 0.024
1.0×10^{-2}	0.231 ± 0.003	1.046 ± 0.025	0.815 ± 0.025

interference from nitrogen bubbles. Other researchers have reported optimum concentration as 1.56×10^{-5} M (Afkhami and Mosaed 2002) and 3.60×10^{-6} M (Afkhami and Mosaed 2003). However, these concentration range did not provide enough sensitivity for the present method. Therefore, 5.0×10^{-3} M was chosen as the optimum [N_2H_4.2HCl], where change in concentration will have minimal or almost no effect.

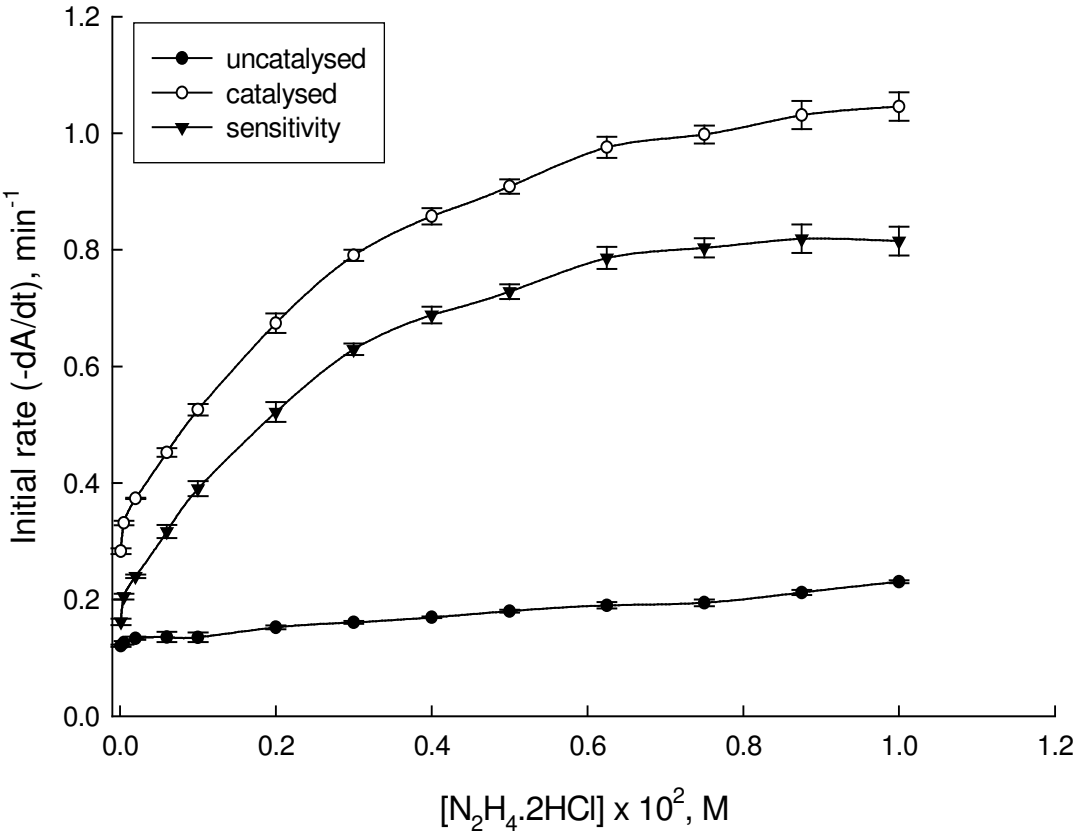


Fig 21 Effect of $[N_2H_4.2HCl]$ on the catalysed and uncatalysed reaction rates with resulting sensitivity under the conditions given in Table 14

4.3.6 Effect of Temperature

Taking the optimum pH and concentration of MO, $KBrO_3$ and $N_2H_4.2HCl$, the effects of reaction temperature on reaction rate was investigated in the range of 15.0 - 42.0 ± 0.1 °C. The rate data corresponding to different temperatures are shown in Table 15 while its plot is shown in Fig 22. It was observed that increasing temperature of the catalysed and uncatalysed reactions accompanied an increase in the rates of reaction (Table 15 and Fig 22). Temperatures greater than 30 ºC contributed to formation of nitrogen bubbles, which interfered with absorbance measurements. The temperature 25.0 ± 0.1 ºC gives stable

Table 15 Temperature dependence study conditions: [Se(IV)] = 789.6 µg L^{-1}, [MO] = 10.0 mg L^{-1}, [BrO_3^-] = 5.0×10^{-3} M, [N_2H_4.2HCl] = 5.0×10^{-3} M, pH = 1.60 ± 0.02

Temperature ± 0.1, ^oC	**Initial Rate (-dA/dt), min^{-1}**		
	Uncatalysed reaction (U)	**Catalysed reaction (C)**	**Sensitivity**
	Average ± SD (*n* = 5)	**Average ± SD (*n* = 5)**	**(C - U) ± SD**
15.0	0.081 ± 0.000	0.622 ± 0.007	0.541 ± 0.007
18.0	0.109 ± 0.003	0.703 ± 0.003	0.594 ± 0.004
22.0	0.141 ± 0.001	0.801 ± 0.011	0.661 ± 0.011
25.0	0.164 ± 0.002	0.890 ± 0.011	0.725 ± 0.011
28.0	0.194 ± 0.007	0.999 ± 0.011	0.805 ± 0.014
30.0	0.209 ± 0.005	1.039 ± 0.014	0.830 ± 0.015
33.0	0.242 ± 0.005	1.170 ± 0.017	0.928 ± 0.018
36.0	0.309 ± 0.014	1.302 ± 0.023	0.993 ± 0.027
39.0	0.362 ± 0.013	1.389 ± 0.024	1.028 ± 0.027
42.0	0.448 ± 0.017	1.583 ± 0.030	1.135 ± 0.034

rates and the uncatalysed rate were also relatively low and stable. At this temperature, there is a possibility of foregoing the use of a thermostatic water bath in case analysis is carried out in a temperature controlled room such as a closed air conditioned room, where the temperature does not vary much over time. Therefore, 25.0 ± 0.1 ºC was the chosen temperature for all subsequent study and analyses.

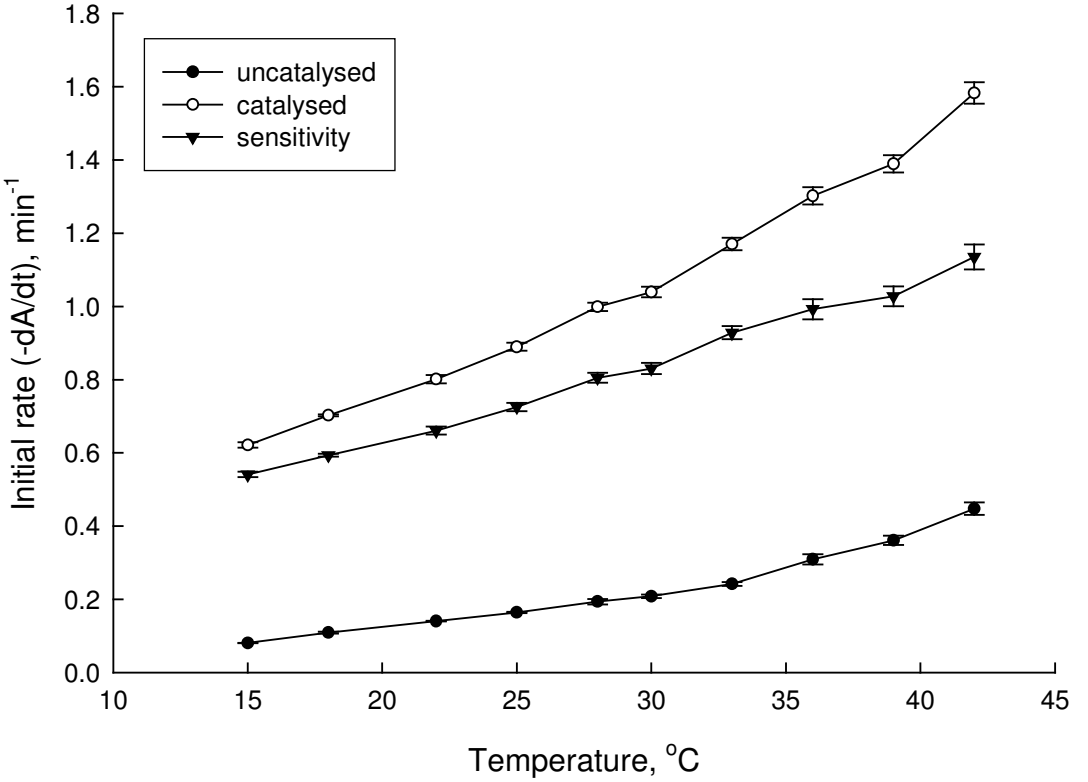


Fig 22 Effect of temperature on the catalysed and uncatalysed reaction rates with resulting sensitivity under the conditions given in Table 15

4.3.7 Effect of Ionic Strength

When KNO_3 was used to study the effect of ionic strength on the rate of reaction, it was found that the presence of nitrate in the concentration range of 0.01 - 0.10 M caused serious interference. Since chlorate, perchlorate, iodate and periodate posed similar interference problems, an attempt was made to use potassium chloride to maintain ionic strength of the reaction system. The ionic strength dependence on the catalysed and uncatalysed reaction was studied in the range of 0.10 - 0.50 M (KCl). The results obtained on ionic strength dependence study are presented in Table 16. A plot of ionic strength in terms of [KCl] *versus* initial rate is shown in Fig 23. The results showed that

Table 16 Ionic strength dependence under conditions: [Se(IV)] = 789.6 µg L^{-1}, [MO] = 10.0 mg L^{-1}, [BrO_3^-] = 5.0 × 10^{-3} M, [N_2H_4.2HCl] = 5.0 × 10^{-3} M, pH = 1.60 ± 0.02, temperature = 25.0 ± 0.1 °C

[KCl], M	Initial Rate (-dA/dt), min^{-1}		
	Uncatalysed reaction (U)	Catalysed reaction (C)	Sensitivity
	Average ± SD (*n* = 5)	Average ± SD (*n* = 5)	(C - U) ± SD
0.010	0.072 ± 0.007	0.736 ± 0.022	0.664 ± 0.023
0.050	0.113 ± 0.004	0.787 ± 0.018	0.674 ± 0.018
0.100	0.118 ± 0.007	0.794 ±0.024	0.676 ± 0.025
0.143	0.116 ± 0.002	0.824 ± 0.022	0.708 ± 0.022
0.188	0.134 ± 0.003	0.829 ± 0.031	0.695 ± 0.031
0.233	0.138 ± 0.003	0.829 ± 0.027	0.691 ± 0.027
0.278	0.182 ± 0.007	0.861 ± 0.024	0.679 ± 0.025
0.323	0.186 ± 0.002	0.891 ± 0.026	0.705 ± 0.026
0.368	0.225 ± 0.008	0.916 ± 0.029	0.690 ± 0.030
0.413	0.255 ± 0.009	0.940 ± 0.033	0.685 ± 0.034
0.458	0.285 ± 0.007	0.946 ± 0.032	0.661 ± 0.033
0.503	0.320 ± 0.008	0.973 ± 0.027	0.653 ± 0.028

both of the reaction rates slowly increased with increasing ionic strength upto 0.50 M. However, the sensitivity of the reaction was found not to change significantly (ANOVA) over the studied range. Therefore, it was concluded that the change in the ionic strength of solution has no considerable effect on the reaction rate for further studies. Also, ionic strength upto 0.70 M (Safavi *et al*. 1995) or 0.81 M (Afkhami *et al*. 1992) has been reported to have no effect on this reaction system. However, the authors have not mentioned the reagent used for ionic strength dependence studies. Hence the present results are conclusive.

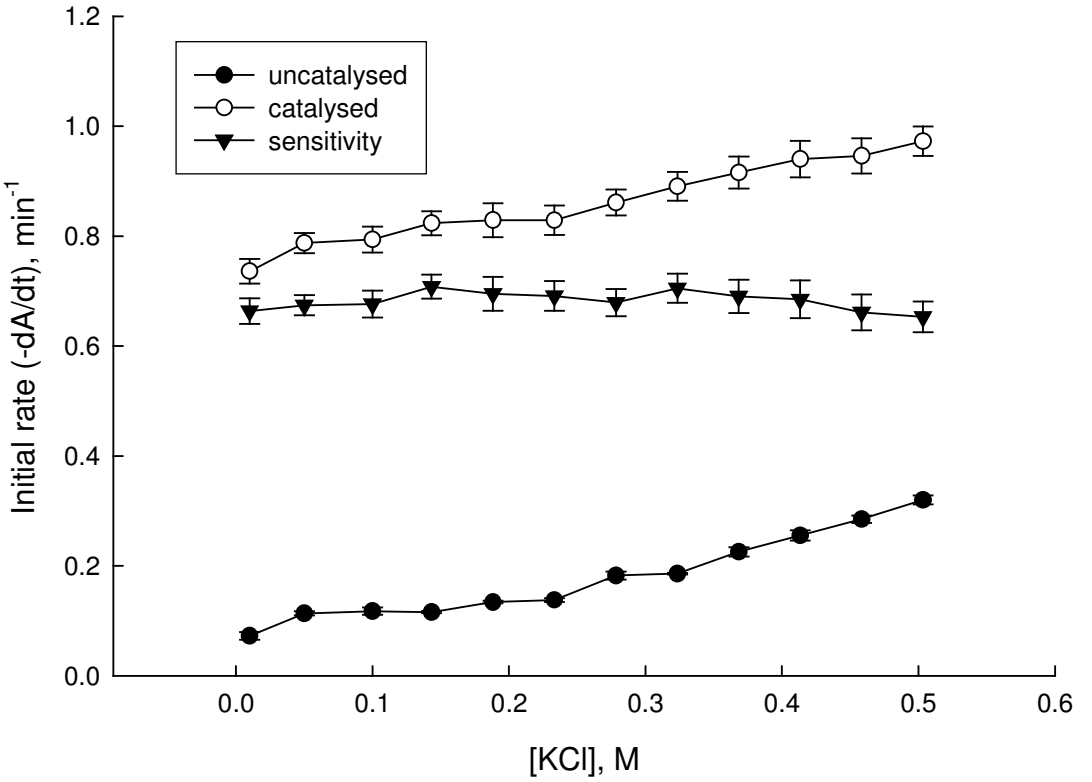


Fig 23 Effect of ionic strength on the catalysed and uncatalysed reaction rates with resulting sensitivity under the conditions given in Table 16

4.4 Analytical Data

Under the optimised experimental conditions, the assay of Se(IV) was carried out in the presence of excess [$N_2H_4.2HCl$], [MO] and [BrO_3^-] at a constant pH of 1.60 ± 0.02 and temperature 25.0 ± 0.1 °C *i.e.* a pseudo-zero-order reaction condition was worked out with respect to the concentration of the reagents. The kinetic plots (absorbance *versus* time) were all linear for 1 - 6 min from the initiation of the reaction. The initial rate of the reaction was obtained by calculating the slopes (tan α = dA/dt) of the initial tangent (t = 1 min) to the absorbance-time curves (Afkhami *et al.* 1992; Safavi *et al*. 1995; Mitic *et al.* 2000) of seven different concentrations of Se(IV) for the linear working range. The

decrease in absorbance for a fixed time from the initiation of the reaction was utilised for measuring change in absorbance for the catalysed reactions (ΔA_C). The measurement in the absence of Se(IV) was prepared to obtain the change in absorbance values for the uncatalysed reaction (ΔA_U). The net reaction rate was obtained from the difference in absorbance change at a fixed time (ΔA_C - ΔA_U) (Afkhami & Madrakian 2002; Gurkan and Akcay 2003). All measurements were repeated seven times, and a calibration curve was constructed by plotting the average of the initial rate of reaction or change in absorbance *versus* the concentration of Se(IV).

4.4.1 Analysis by Initial Rate Method

Under the optimum conditions, a linear relationship between initial rate and [Se(IV)] was obtained for Se(IV) concentration of 0 - 789.6 µg L^{-1} and is shown in Table 17 and Fig 24. A plot of log rate *versus* log [Se(IV)] confirmed that the reaction is first order with respect to Se(IV) in the two calibration ranges (Table 18). The linear regression analysis using the method of least square treatment of the calibration data (n = 7) was made to evaluate slope, intercept and correlation coefficient. The plot Initial rate *versus* [Se(IV)] gave the following linear regression equation:

Initial rate = 9.5613×10^{-4} [Se(IV)] + 2.2176×10^{-3},

with $r^2 = 0.9975$,

where the concentration of Se(IV) is expressed in µg L^{-1}.

Table 17 Initial rate data at different [Se(IV)] in range of 0 - 789.6 µg L^{-1} under conditions of [MO] = 10.0 mg L^{-1}, [BrO_3^-] = 5.0 × 10^{-3} M, [N_2H_4.2HCl] = 5.0 × 10^{-3} M, pH = 1.60 ± 0.02, temperature = 25.0 ± 0.1 °C

[Se(IV)], µg L^{-1}	**Initial Rate (-dA/dt), min^{-1}**	
	Average ± SD (*n* = 7)	**(Average - Blank) ± SD**
0.0	0.190 ± 0.006	0.000 ± 0.008
79.0	0.269 ± 0.004	0.079 ± 0.007
197.4	0.368 ± 0.004	0.178 ± 0.007
315.8	0.496 ± 0.010	0.306 ± 0.011
473.8	0.663 ± 0.008	0.473 ± 0.010
631.7	0.812 ± 0.008	0.622 ± 0.010
789.6	0.927 ± 0.017	0.737 ± 0.018

The limit of detection (LOD), 15.8 µg L^{-1}, was calculated by seven blank measurements using the equation $Y_{LOD} = Y_b + 3S_b$, where Y_{LOD} is the signal for the limit of detection, Y_b is the average blank signal (*n* = 7) and S_b is standard deviation of blank signal (*n* = 7) (Keyvanfard and Rezaei 2005). Linear dynamic range, correlation coefficient, variance, detection limit, standard deviations and confidence limits for slope and intercept of the calibration line are summarised in Table 17 and 18.

Table 18 Spectral and statistical data for the determination of Se(IV) by Initial rate method under the conditions given in Table 17

Parameters	Initial Rate Method
λ_{max} (nm)	507.0
Linear dynamic range (μg L^{-1})	0 - 789.6
Regression equation	Initial rate = 9.561×10^{-4} [Se(IV)] + 2.218×10^{-3}
Log-log plot	log (initial rate) = 0.996 log [Se(IV)] - 3.007 (hence 1^{st} order)
$SD_{calibration}$[a]	1.516×10^{-2}
Intercept	2.218×10^{-3}
$SD_{intercept}$	9.481×10^{-3}
P-$value_{intercept}$[b]	8.243×10^{-1} (> 0.05, hence intercept does not differ from zero)
Slope	9.561×10^{-4}
SD_{slope}	2.126×10^{-5}
P-$value_{slope}$[b]	1.027×10^{-7} (< 0.05, hence slope differs from zero)
Correlation coefficient (r^2)	0.9975
Detection limit (μg L^{-1})	15.8

[a]Standard deviation of the calibration line
[b]Probability value

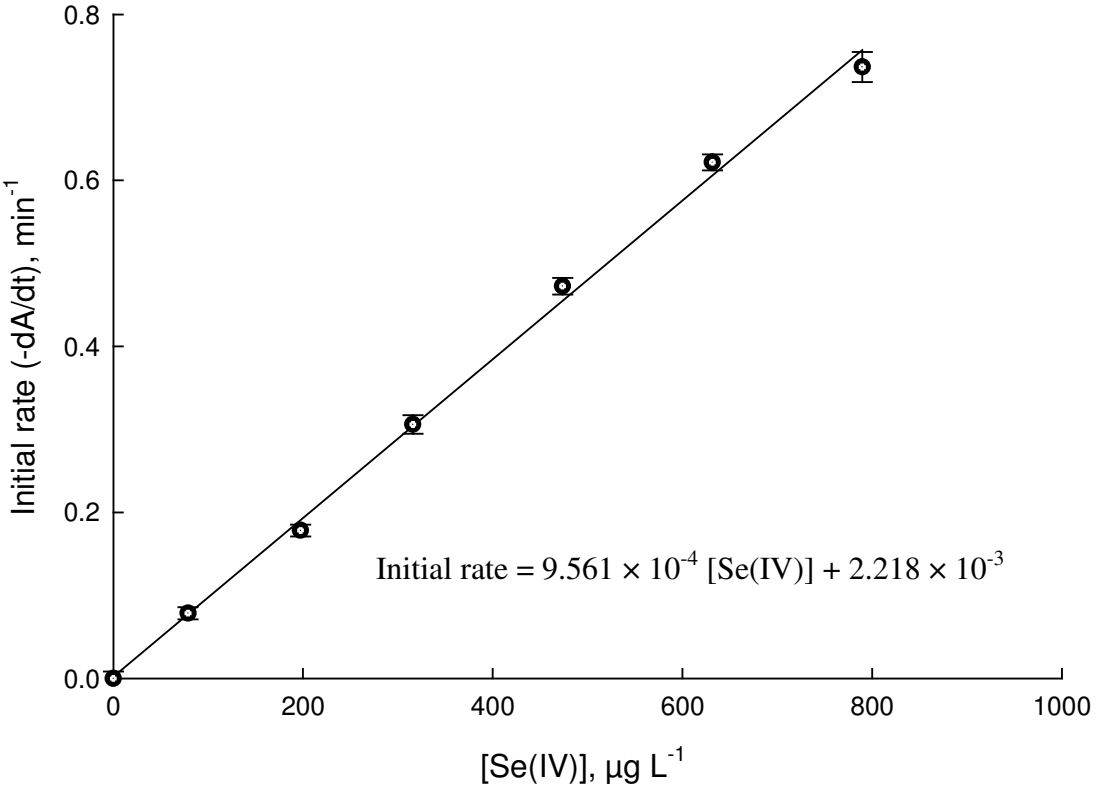


Fig 24 Calibration curve for long range [Se(IV)] of 0 - 789.6 µg L^{-1} using Initial rate method under the conditions given in Table 17

Though a long linear range was obtained using the optimum conditions (Fig 24), the higher sensitivity of the method was compromised by a high blank signal (*i.e.* uncatalysed rate). Since the detection limit was not sufficiently low, an attempt was made to obtain a second working range for lower concentrations of Se(IV). It is obvious from the concentration dependence studies (*vide infra*) that a lower [MO] and [BrO_3^-] could be chosen to give a very low and stable blank. Some previous work on similar reaction systems has also demonstrated that [BrO_3^-] need to be adjusted for obtaining different calibration ranges (Afkhami and Afshar-E-Asl 2000; Afkhami and Assl 2001; Afkhami *et al*. 2005). On this basis, the initial conditions were changed to 5.0 mg L^{-1} [MO] and

Table 19 Initial rate data at different [Se(IV)] in lower range of 0 - 126.3 µg L^{-1} under conditions of [MO] = 5.0 mg L^{-1}, [BrO_3^-] = 5.0 × 10^{-4} M, [N_2H_4.2HCl] = 5.0 × 10^{-3} M, pH = 1.60 ± 0.02, temperature = 25.0 ± 0.1 °C

[Se(IV)], µg L^{-1}	**Initial Rate (-dA/dt), min^{-1}**	
	(Average ± SD) × 10^2 (*n* = 7)	**{(Average - Blank) ± SD} × 10^2**
0.0	0.081 ± 0.008	0.000 ± 0.011
6.3	0.197 ± 0.010	0.115 ± 0.013
15.8	0.380 ± 0.011	0.298 ± 0.013
31.6	0.624 ± 0.013	0.542 ± 0.016
63.2	1.178 ± 0.029	1.097 ± 0.030
94.8	1.680 ± 0.036	1.599 ± 0.037
126.3	2.313 ± 0.043	2.232 ± 0.044

5 × 10^{-4} M [BrO_3^-] to obtain a separate working range of 0 - 126.3 µg L^{-1}. A plot of initial rate versus [Se(IV)] is shown in Fig 25. The linear regression equation in present case is given as:

Initial rate = 1.736 × 10^{-4} [Se(IV)] + 2.537 × 10^{-5},

with r^2 = 0.999,

Where the concentration of Se(IV) is expressed in µg L^{-1}.

In the present case, LOD was calculated to be 1.3 µg L^{-1} (Table 19 and 20).

Table 20 Spectral and statistical data for the determination of Se(IV) by Initial rate method under the conditions given in Table 19

Parameters	Initial rate method
λ_{max} (nm)	507.0
Linear dynamic range (μg L^{-1})	0 - 126.3
Regression equation	Initial rate = 1.736×10^{-4} [Se(IV)] + 2.537×10^{-5}
Log-log plot	log (initial rate) = 0.975 log [Se(IV)] - 3.714 (hence 1^{st} order)
$SD_{calibration}$[a]	2.911×10^{-4}
Intercept	2.537×10^{-5}
$SD_{intercept}$	1.622×10^{-4}
P-$value_{intercept}$[b]	8.818×10^{-1} (> 0.05, hence intercept does not differ from zero)
Slope	1.736×10^{-4}
SD_{slope}	2.469×10^{-6}
P-$value_{slope}$[b]	1.104×10^{-8} (< 0.05, hence slope differs from zero)
Correlation coefficient (r^2)	0.999
Detection limit (μg L^{-1})	1.3

[a]Standard deviation of the calibration line
[b]Probability value

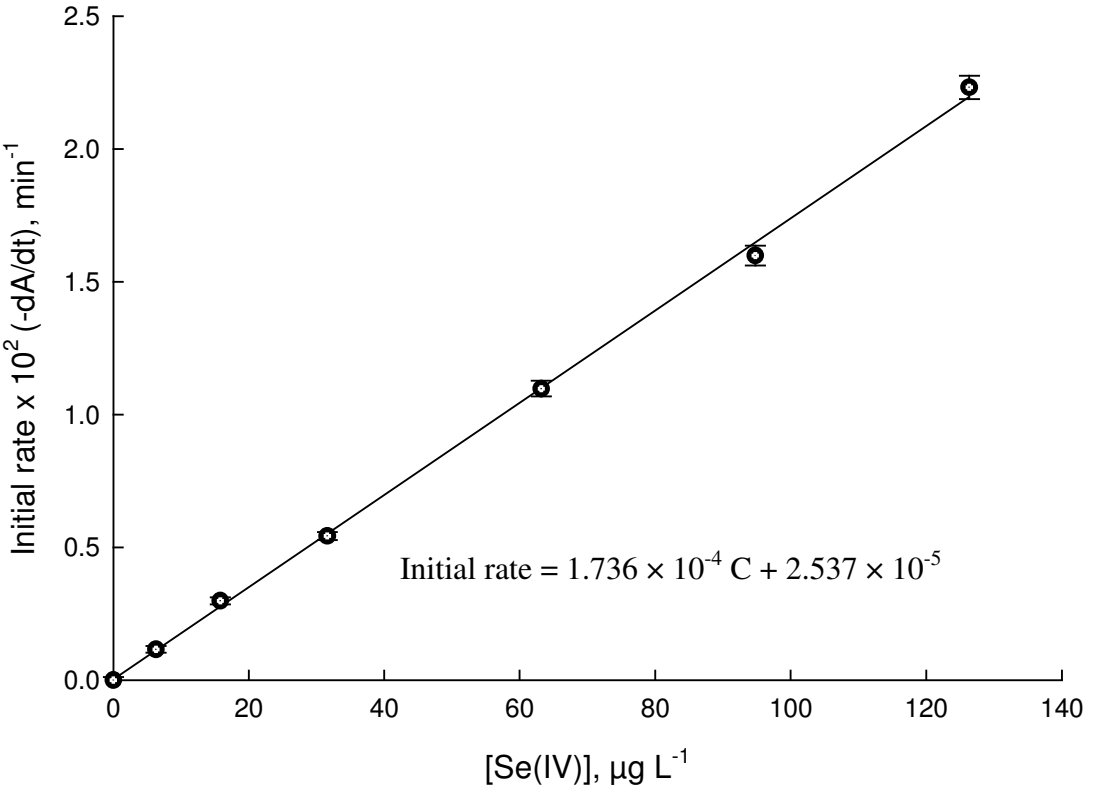


Fig 25 Calibration curve for lower range [Se(IV)] of 0 - 126.3 µg L^{-1} using Initial rate method under the conditions given in Table 19

4.4.2 Analysis by Fixed Time Method

In this method, the absorbance of the indicator reaction (λ_{max} = 507 nm) was recorded at a preselected fixed time, using the same optimum conditions used for initial rate method for the working range 0 - 789.6 µg L^{-1}. Calibration graphs of change in absorbance at a fixed time (ΔA_t) *versus* [Se(IV)] at seven concentration levels were plotted at a fixed time of 2, 3, 4, 5 and 6 min from the initiation of the reaction and is shown in Fig 26. It was desirable to have the longest linear range as well as the lowest possible values of LOD, error and RSD. The combination of these two factors was satisfied by the calibration data for 4 min (Table 21), which was subsequently used as the working calibration curve. The

linear dynamic range was 0 - 789.6 µg L^{-1} with a LOD of 19.0 µg L^{-1}. However, on the other hand, a low detection limit was compromised when using the 4 min results. It was obvious from the fixed time study results that the data for 6 min provided a better sensitivity (*i.e.* slope) than other fixed times, hence a lower detection limit could be achieved using 6 min. The only drawback was that the linear range would significantly become narrower as a result since the calibration curve was only linear for the lower [Se(IV)] (Fig 26). On this basis a second working curve was obtained for a fixed time of 6 min, with a linear working range of 0 - 315.8 µg L^{-1} (Fig 27) and LOD of 14.7 µg L^{-1}.

Error, RSD, Beers Law limit, linear regression equation, coefficient of correlation, detection limit, variance, standard deviation and confidence limits for slope and intercept are summarised and compared in Table 21, 22, 23 and 24 for different fixed time analysis. Test of significance of the intercepts of regression lines of the Fixed time method at different intervals of time showed that these values of intercepts did not differ significantly from the theoretical value, zero (Miller 1991). Thus, the Fixed time methods are free from constant errors independent of the concentration of Se. It is apparent from Table 22 and 24 that the values of error, RSD and detection limit were found to be lowest for fixed time of 4 and 6 min for 0 - 789.6 µg L^{-1} and 0 - 315.8 µg L^{-1} Se(IV), respectively. Therefore, on the basis of lowest values of these parameters, the fixed time of 4 and 6 min was recommended for the assay of Se in water samples.

Table 21 ΔA at different fixed times at [Se(IV)] range 0 - 789.6 µg L^{-1} under conditions of [MO] = 10.0 mg L^{-1}, [BrO_3^-] = 5.0 × 10^{-3} M, [N_2H_4.2HCl] = 5.0 × 10^{-3} M, pH = 1.60 ± 0.02, temperature = 25.0 ± 0.1 °C: (i) with blank and (ii) after subtracting the blank

(i)

[Se(IV)], µg L^{-1}	ΔA of catalysed (C) and uncatalysed (U) reaction				
	2 min	3 min	4 min	5 min	6 min
	ΔA ± SD (*n* = 7)	ΔA ± SD (*n* = 7)	ΔA ± SD (*n* = 7)	ΔA ± SD (*n* = 7)	ΔA ± SD (*n* = 7)
0.0	0.020 ± 0.004	0.043 ± 0.003	0.067 ± 0.003	0.093 ± 0.003	0.120 ± 0.002
79.0	0.025 ± 0.005	0.056 ± 0.005	0.092 ± 0.005	0.127 ± 0.004	0.168 ± 0.004
197.4	0.038 ± 0.006	0.086 ± 0.006	0.131 ± 0.006	0.179 ± 0.005	0.242 ± 0.005
315.8	0.050 ± 0.007	0.111 ± 0.007	0.174 ± 0.006	0.245 ± 0.006	0.334 ± 0.006
473.8	0.068 ± 0.008	0.145 ± 0.007	0.229 ± 0.007	0.315 ± 0.007	0.416 ± 0.006
631.7	0.081 ± 0.008	0.174 ± 0.008	0.271 ± 0.008	0.369 ± 0.007	0.462 ± 0.007
789.6	0.091 ± 0.009	0.201 ± 0.009	0.312 ± 0.008	0.400 ± 0.008	0.477 ± 0.008

(ii)

[Se(IV)], µg L^{-1}	ΔA				
	2 min	3 min	4 min	5 min	6 min
	$(\Delta A_C - \Delta A_U)$ ± SD	$(\Delta A_C - \Delta A_U)$ ± SD	$(\Delta A_C - \Delta A_U)$ ± SD	$(\Delta A_C - \Delta A_U)$ ± SD	$(\Delta A_C - \Delta A_U)$ ± SD
0.0	0.000 ± 0.006	0.000 ± 0.005	0.000 ± 0.004	0.000 ± 0.004	0.000 ± 0.003
79.0	0.005 ± 0.007	0.013 ± 0.006	0.025 ± 0.006	0.035 ± 0.005	0.048 ± 0.005
197.4	0.018 ± 0.007	0.043 ± 0.007	0.065 ± 0.006	0.087 ± 0.006	0.122 ± 0.005
315.8	0.030 ± 0.008	0.068 ± 0.007	0.107 ± 0.007	0.152 ± 0.006	0.214 ± 0.006
473.8	0.047 ± 0.009	0.102 ± 0.008	0.162 ± 0.008	0.222 ± 0.007	0.296 ± 0.007
631.7	0.061 ± 0.009	0.131 ± 0.009	0.204 ± 0.008	0.276 ± 0.008	0.342 ± 0.008
789.6	0.071 ± 0.010	0.159 ± 0.009	0.245 ± 0.009	0.307 ± 0.008	0.357 ± 0.008

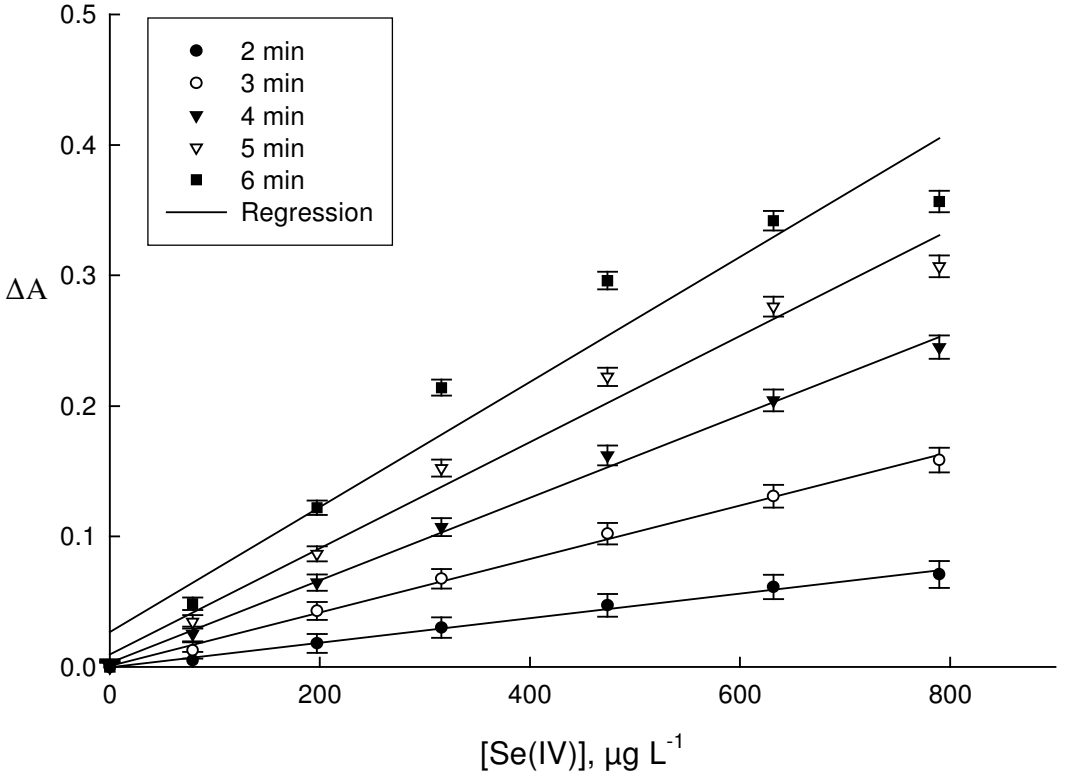


Fig 26 Calibration curves for long range [Se(IV)] of 0 - 789.6 µg L^{-1} using Fixed time method for different fixed times under the conditions given in Table 21 (ii)

Table 22 Spectral and statistical data for the determination of Se(IV) in the range of 0 - 789.6 µg L^{-1} by Fixed time method under the conditions given in Table 21 (ii)

Parameters	Fixed time method				
	2 min	**3 min**	**4 min**	**5 min**	**6 min**
λ_{max} (nm)	507.0	507.0	507.0	507.0	507.0
Linear dynamic range (µg L^{-1})	0 - 789.6	0 - 789.6	0 - 789.6	0 - 789.6	0 - 789.6
Linear regression equation	$\Delta A_2 = 9.429 \times 10^{-5}$ C $- 3.158 \times 10^{-4}$	$\Delta A_3 = 2.054 \times 10^{-4}$ C $+ 8.432 \times 10^{-4}$	$\Delta A_4 = 3.162 \times 10^{-4}$ C $+ 3.109 \times 10^{-3}$	$\Delta A_5 = 4.069 \times 10^{-4}$ C $+ 9.594 \times 10^{-3}$	$\Delta A_6 = 4.791 \times 10^{-4}$ C $+ 2.675 \times 10^{-2}$
Slope of log-log plot	1.1664	1.088	0.999	0.9774	0.9032
$SD_{calibration}$ [a]	2.364×10^{-3}	4.042×10^{-3}	6.009×10^{-3}	1.683×10^{-2}	3.619×10^{-2}
Intercept	-3.158×10^{-4}	8.432×10^{-4}	3.109×10^{-3}	9.594×10^{-3}	2.675×10^{-2}
$SD_{intercept}$	1.479×10^{-3}	2.528×10^{-3}	3.759×10^{-3}	1.053×10^{-2}	2.264×10^{-2}
P-$value_{intercept}$ [b]	0.839	0.752	0.446	0.404	0.290
Slope	9.429×10^{-5}	2.054×10^{-4}	3.162×10^{-4}	4.068×10^{-4}	4.791×10^{-4}
SD_{slope}	3.317 × 10-6	5.670×10^{-6}	8.429×10^{-6}	2.361×10^{-5}	5.077×10^{-5}
P-$value_{slope}$ [b]	1.009×10^{-6}	3.021×10^{-7}	2.535×10^{-7}	1.206×10^{-5}	2.256×10^{-4}
Correlation coefficient (r^2)	0.9939	0.9962	0.9965	0.9834	0.9468
Detection limit (µg L^{-1})	136.4	47.0	19.0	-	-

[a]Standard deviation of the calibration line
[b]Probability value
C in linear regression equation indicates [Se(IV)] in µg L^{-1}

Table 23 ΔA at different fixed times at [Se(IV)] range 0 - 314.8 µg L^{-1} under conditions of [MO] = 5.0 mg L^{-1}, [BrO_3^-] = 5.0 × 10^{-4} M, [N_2H_4.2HCl] = 5.0 × 10^{-3} M, pH = 1.60 ± 0.02, temperature = 25.0 ± 0.1 °C: (i) with blank and (ii) after subtracting the blank

(i)

[Se(IV)], µg L^{-1}	ΔA of catalysed (C) and uncatalysed (U) reaction				
	2 min	3 min	4 min	5 min	6 min
	ΔA ± SD (*n* = 7)	ΔA ± SD (*n* = 7)	ΔA ± SD (*n* = 7)	ΔA ± SD (*n* = 7)	ΔA ± SD (*n* = 7)
0.00	0.020 ± 0.004	0.043 ± 0.003	0.067 ± 0.003	0.093 ± 0.003	0.120 ± 0.002
31.6	0.022 ± 0.004	0.048 ± 0.004	0.077 ± 0.003	0.105 ± 0.003	0.138 ± 0.003
79.0	0.025 ± 0.005	0.056 ± 0.004	0.092 ± 0.004	0.127 ± 0.004	0.168 ± 0.004
138.2	0.033 ± 0.005	0.072 ± 0.005	0.114 ± 0.005	0.158 ± 0.004	0.213 ± 0.004
197.4	0.038 ± 0.006	0.086 ± 0.006	0.131 ± 0.005	0.179 ± 0.005	0.242 ± 0.005
256.6	0.046 ± 0.006	0.100 ± 0.006	0.156 ± 0.005	0.217 ± 0.005	0.293 ± 0.005
315.8	0.050 ± 0.007	0.111 ± 0.007	0.174 ± 0.006	0.245 ± 0.006	0.334 ± 0.005

(ii)

[Se(IV)], µg L^{-1}	ΔA				
	2 min	3 min	4 min	5 min	6 min
	$(\Delta A_C - \Delta A_U)$ ± SD	$(\Delta A_C - \Delta A_U)$ ± SD	$(\Delta A_C - \Delta A_U)$ ± SD	$(\Delta A_C - \Delta A_U)$ ± SD	$(\Delta A_C - \Delta A_U)$ ± SD
0.00	0.000 ± 0.006	0.000 ± 0.005	0.000 ± 0.004	0.000 ± 0.004	0.000 ± 0.003
31.6	0.002 ± 0.006	0.005 ± 0.005	0.010 ± 0.005	0.013 ± 0.004	0.018 ± 0.004
79.0	0.005 ± 0.006	0.013 ± 0.005	0.025 ± 0.005	0.035 ± 0.005	0.048 ± 0.005
138.2	0.013 ± 0.007	0.029 ± 0.006	0.048 ± 0.006	0.065 ± 0.005	0.093 ± 0.005
197.4	0.018 ± 0.007	0.043 ± 0.007	0.065 ± 0.006	0.087 ± 0.005	0.122 ± 0.005
256.6	0.025 ± 0.007	0.057 ± 0.007	0.090 ± 0.006	0.124 ± 0.006	0.173 ± 0.006
315.8	0.030 ± 0.008	0.068 ± 0.008	0.107 ± 0.007	0.152 ± 0.006	0.214 ± 0.006

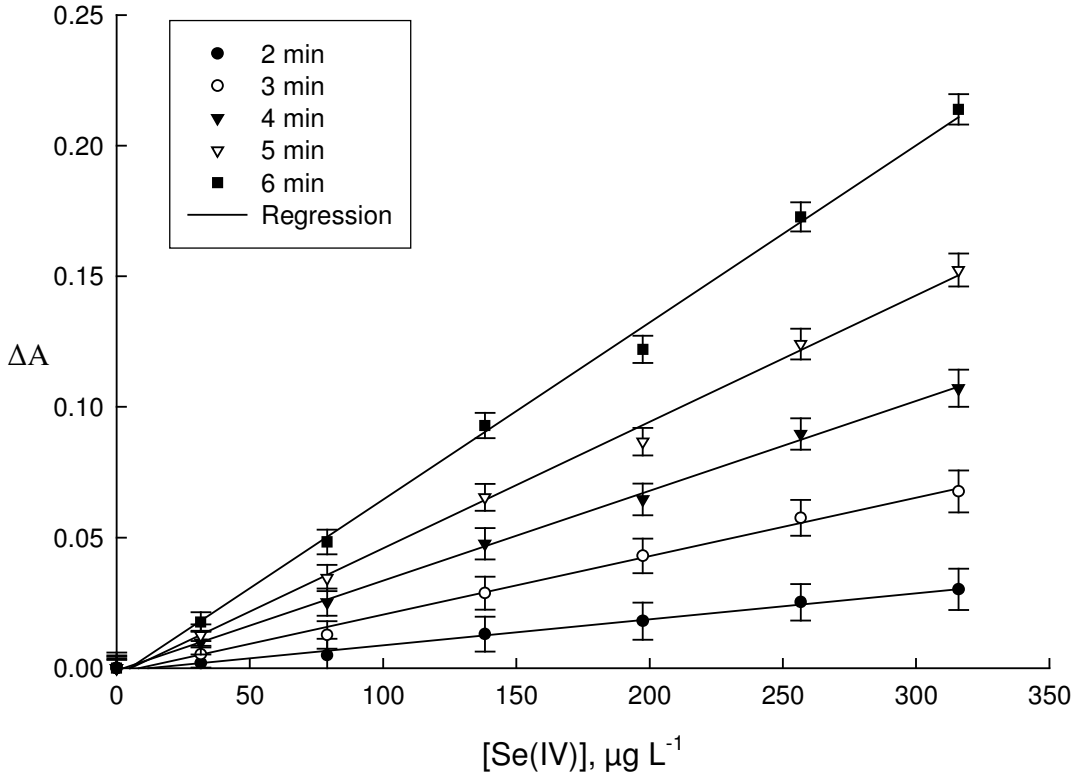


Fig 27 Calibration curves for lower range [Se(IV)] of 0 - 315.8 µg L^{-1} using Fixed time method for different fixed times under the conditions given in Table 23 (ii)

Table 24 Spectral and statistical data for the determination of Se(IV) in the range of 0 - 314.8 µg L^{-1} by Fixed time method under the conditions given in Table 23 (ii)

Parameters	Fixed time method				
	2 min	**3 min**	**4 min**	**5 min**	**6 min**
λ_{max} (nm)	507.0	507.0	507.0	507.0	507.0
Linear dynamic range (µg L^{-1})	0 - 315.8	0 - 315.8	0 - 315.8	0 - 315.8	0 - 315.8
Linear regression equation	$\Delta A_2 = 9.953 \times 10^{-5}$ C	$\Delta A_3 = 2.235 \times 10^{-4}$ C	$\Delta A_4 = 3.437 \times 10^{-4}$ C	$\Delta A_5 = 4.842 \times 10^{-4}$ C	$\Delta A_6 = 6.778 \times 10^{-4}$ C
	-1.186×10^{-4}	-1.859×10^{-4}	-8.864×10^{-4}	-2.514×10^{-3}	-3.302×10^{-3}
Slope of log-log plot	1.2487	1.1505	1.0472	1.0798	1.0796
	-4.6168	-4.0262	-3.5804	-3.5152	-3.3675
$SD_{calibration}$ [a]	1.069×10^{-3}	1.939×10^{-3}	1.678×10^{-3}	3.423×10^{-3}	4.654×10^{-3}
Intercept	-1.186×10^{-4}	-1.859×10^{-4}	-8.864×10^{-4}	-2.514×10^{-3}	-3.302×10^{-3}
$SD_{intercept}$	6.753×10^{-4}	1.225×10^{-3}	1.060×10^{-3}	2.162×10^{-3}	2.940×10^{-3}
P-$value_{intercept}$ [b]	0.139	0.190	0.441	0.297	0.312
Slope	9.953×10^{-3}	2.235×10^{-4}	3.437×10^{-4}	4.842×10^{-4}	6.778×10^{-4}
SD_{slope}	3.718×10^{-6}	6.745×10^{-6}	5.834×10^{-6}	1.190×10^{-5}	1.619×10^{-5}
P-$value_{slope}$ [b]	1.36×10^{-6}	4.710×10^{-7}	2.670×10^{-8}	1.690×10^{-7}	1.460×10^{-7}
Correlation coefficient (r)	0.9931	0.9955	0.9986	0.9970	0.9972
Detection limit (µg L^{-1})	137.9	54.7	29.1	21.4	14.7

[a]Standard deviation of the calibration line
[b]Probability value

C in linear regression equation indicates [Se(IV)] in µg L^{-1}

4.5 Selectivity

In order to assess the application of the proposed method to synthetic samples, the selectivity of the proposed method was evaluated by determining Se concentration (31.6 µg L^{-1}) in the presence of varying amounts of cations and anions which are commonly present in environmental water. The tolerance limit was defined as the concentration of an added ion causing not more than ±3% relative error (Prasad and Halafihi 2003; Prasad 2005). While Afkhami *et al*. (1992) have done a almost similar study, the amount of Se(IV) used was 500 µg L^{-1}. Since Se hardly occurs at this high concentration in natural water, an interference study on a lower Se concentration as well as the realistic foregn ion concentration in natural water was required to be tested. The results of interference study carried out from higher to lower concentration of many ions are summarised in Table 25. It was found that many of these ions did not interfere, even when present in excess of 10000 to 50 fold. Those ions which interfere, if present greater than 10 fold excess, are seldom present at the concentrations levels tested in natural water (Pais and Jones 1997). While iron can cause some interference in the analysis of iron rich waters, this problem can be overcome by addition of 0.5 mL of 0.1 M EDTA (Safavi *et al*. 1995) or by adding a solution of 1 % NaF (Absalan and Alipour 2003). Thus the proposed method is suitable for the determination of Se in environmental waters in presence of its natural constituents.

Table 25 Effect of diverse ions on the determination of 31.6 µg L^{-1} Se(IV) under optimum conditions of [MO] = 5.0 mg L^{-1}, [BrO_3^-] = 5.0 × 10^{-4} M, [N_2H_4.2HCl] = 5.0 × 10^{-3} M, pH = 1.60 ± 0.02, temperature = 25.0 ± 0.1 ^oC and using regression equation based on Initial rate given in Table 20

Interfering ion	**Tolerance limit** [$C_{ion}^*/C_{Se(IV)}$]
Na^+, K^+, Li^+, Ca^{2+}, Mg^{2+} and Ba^{2+}	10000
Ni^{2+}, Cr^{3+}, Zn^{2+}, Cd^{2+}, Al^{3+}, Mn^{2+}, $C_2O_4^{2-}$, NH_4^+, CH_3COO^- and Cl^-	1000
Mo^{6+}, SO_4^{2-}, $S_2O_3^{2-}$, CO_3^{2-}, Ce^{3+} and CrO_4^{2-}	500
Pb^{2+}, Sn^{2+}, Se^{6+}, As^{5+}, NO_3^-, PO_4^{3-} and ClO_4^-	200
SCN^-, SO_3^{2-}, Co^{2+}, F^-, ClO_3^- and Sb^{5+}	100
NO_2^-, IO_3^-, Cu^{2+} and Br^-	50
Fe^{3+}, V^{5+}, I^-, Ce^{4+}, As^{3+}, V^{3+} and Sb^{3+}	10
Te^{4+}	2
Hg^{2+}	1

*C refers to concentration in µg L^{-1}

4.6 Accuracy and Precision of the Proposed Method

The accuracy and precision of the proposed methods was established by measuring the content of Se at three different concentration levels in spiked water (31.6, 63.2, and 94.8 µg L^{-1}) and also in the NIST standard reference materials for Se. The intra day precision

of the proposed methods was performed by carrying out five independent analyses at each concentration level within one day. In the same manner, the inter day precision was also evaluated by measuring the Se content at each concentration level on five consecutive days by initial rate and Fixed time methods. The results of the recoveries by Initial rate and Fixed time methods along with standard deviation and relative standard deviation are presented in Table 26 for intra day assays and Table 27 for inter day assays. Recoveries of Se from NIST SRM No. 3149 and NIST SRM No. 1640 are presented in Table 28, which are well within recommended errors. The recoveries data presented are quite satisfactory. Thus, the proposed method may be very effective in the assay of Se in water samples.

Table 26 Intra day assays: Test of precision of the proposed method for the determination of Se(IV) under conditions of [MO] = 5.0 mg L^{-1}, [BrO_3^-] = 5.0×10^{-4} M, [$N_2H_4.2HCl$] = 5.0×10^{-3} M, pH = 1.60 ± 0.02, temperature = 25.0 ± 0.1 ^oC

[Se(IV)] , µg L^{-1}		Recovery ± RSD (%)	SAE[b]	C.L.[c]
Taken	Nominal ± SD[a]			
	Initial rate method			
31.6	31.65 ± 0.80	100.20 ± 2.53	0.36	0.99
63.2	63.13 ± 1.49	99.94 ± 2.35	0.66	1.84
94.8	94.91 ± 0.77	100.17 ± 0.81	0.35	0.96
	Fixed time method			
31.6	31.72 ± 1.01	100.44 ± 3.20	0.45	1.26
63.2	63.00 ± 1.99	99.73 ± 3.15	0.89	2.47
94.8	94.09 ± 1.57	99.30 ± 1.67	0.70	1.95

[a]Mean for five determinations (n = 5)
[b]SAE, standard analytical error
[c]C.L. confidence limit at 95% confidence level and four degrees of freedom (t = 2.776)

Table 27 Inter day assays: Test of precision of the proposed method for the determination of Se(IV) under conditions of [MO] = 5.0 mg L^{-1}, [BrO_3^-] = 5.0 × 10^{-4} M, [N_2H_4.2HCl] = 5.0 × 10^{-3} M, pH = 1.60 ± 0.02, temperature = 25.0 ± 0.1 ^oC

[Se(IV)] , µg L^{-1}				
Taken	**Nominal ± SD[a]**	**Recovery ± RSD (%)**	**SAE[b]**	**C.L.[c]**
	Initial rate method			
31.6	31.17 ± 1.82	98.67 ± 5.83	0.81	2.25
63.2	62.17 ± 2.17	98.42 ± 3.49	0.97	2.69
94.8	93.82 ± 3.72	99.01 ± 3.96	1.66	4.61
	Fixed time method			
31.6	31.63 ± 1.28	100.14 ± 4.04	0.57	1.58
63.2	62.88 ± 2.00	99.54 ± 3.18	0.89	2.48
94.8	95.11 ± 1.46	100.38 ± 1.53	0.65	1.81

[a]Mean for five determinations (n = 5)
[b]SAE, standard analytical error
[c]C.L. confidence limit at 95% confidence level and four degrees of freedom (t = 2.776)

4.7 Application

The level of Se in natural water samples, which was collected between May to November 2006, was found to be below the detection limit of the proposed method. Hence, the validity of the proposed method was carried out by recovery studies using the standard addition method. For this purpose, known amounts of Se standard was spiked in environmental water samples at five different concentration levels within the lower concentration range and the nominal value of Se was estimated by the proposed method. Each level was repeated five times.

Table 28 Analysis of SRM – Se standard and certified water sample

Method used	NIST SRM No. 3149		
	[Se], mg g^{-1}		
	Certified value ± SD[a]	**Observed value ± SD[a]**	**Recovery ± RSD, %**
Initial rate method	10.11 ± 0.02	10.41 ± 0.62	102.97 ± 5.96
Fixed time method	10.11 ± 0.02	9.62 ± 0.75	95.15 ± 7.80

Method used	NIST SRM No. 1640		
	[Se], µg kg^{-1}		
	Certified value ± SD[a]	**Observed value ± SD[a]**	**Recovery ± RSD, %**
Initial rate method	21.96 ± 0.51	19.25 ± 2.93	87.66 ± 15.22
Fixed time method	21.96 ± 0.51	24.57 ± 2.66	111.89 ± 0.83

[a]Mean for five determinations (n = 5)

4.7.1 Se Recovery From Water Samples

Recovery studies were performed by standard addition technique, whereby a calibration curve was drawn with addition of Se(IV) standard in water samples. When the rate was obtained for each concentration of Se(IV) in sample water, the equation from this calibration curve was used to evaluate the amount of Se(IV) recovered from water samples. It should be noted that the calibration curve obtained from standard Se(IV) solutions is of no use for this purpose because the matrices are significantly different among distilled, tap, well, river, ground, spring and sea water. The result obtained show that the slope of standard Se curve is significantly different from the slope obtained by the standard addition technique. A typical standard addition analysis is shown in Table 29 and 31 for the analysis of a sea water sample. In fact, the slope of standard addition curve was bigger (*cf.* Table 20;30 and 24;32). However, the sample matrix does not affect Se determination since the standard addition curves are very linear with low SD (Table 30 and 32). This calibration method was applied to all other types of water samples. Important steps were taken for a good standard addition technique application, such as noting the amount Se standard to be used against the amount of sample water when spiking. This is greatly preferred to be in the ratio of 1:100, so that the matrix of the water sample is not significantly altered. The intercept of the standard addition curves were statistically shown to be zero (Table 30 and 32), implying that the amount of Se in water samples were below the detection limit of the method. The results of recovery were reproducible with low error and RSD in different kinds of water samples (Table 33 and 34). No interferences from common ions already present in the water samples were observed.

Table 29 Se(IV) recovery from sea water using Initial rate method under conditions of [MO] = 5.0 mg L^{-1}, [BrO_3^-] = 5.0 × 10^{-4} M, [N_2H_4.2HCl] = 5.0 × 10^{-3} M, pH = 1.60 ± 0.02, temperature = 25.0 ± 0.1 ^oC

[Se(IV)] taken, µg L^{-1}	Initial Rate (-dA/dt), min^{-1}		[Se(IV)] found ± error*, µg L^{-1}	Recovery, %	Error, %
	(Average ± SD) × 10 (*n* = 5)	{(Average -Blank) ± SD} × 10			
0.0	0.051 ± 0.002	0.000 ± 0.002	-	-	-
31.6	0.210 ± 0.007	0.159 ± 0.007	31.96 ± 4.51	101.20	+1.20
63.2	0.358 ± 0.011	0.307 ± 0.011	61.58 ± 2.18	97.48	-2.52
94.8	0.531 ± 0.012	0.480 ± 0.012	96.19 ± 3.27	101.52	+1.52
126.3	0.680 ± 0.019	0.628 ± 0.019	125.96 ± 4.32	99.70	-0.30

*uncertainty of result from linear calibration

Table 30 Spectral and statistical data for the determination of Se(IV) in sea water by Initial rate method under the conditions given in Table 29

Parameters	Initial rate method
λ_{max} (nm)	507.0
Linear dynamic range (µg L^{-1})	0 - 126.3
Regression equation	Initial rate = 5.998×10^{-4} [Se(IV)] - 7.600×10^{-5}
Log-log plot	log (initial rate) = 0.999 log [Se(IV)] - 3.300 (hence 1^{st} order)
$SD_{calibration}$[a]	6.394×10^{-4}
Intercept	-7.600×10^{-5}
$SD_{intercept}$	4.952×10^{-4}
P-$value_{intercept}$[b]	8.878×10^{-1} (> 0.05, hence intercept does not differ from zero)
Slope	5.998×10^{-4}
SD_{slope}	6.401×10^{-6}
P-$value_{slope}$[b]	4.630×10^{-5} (< 0.05, hence slope differs from zero)
Correlation coefficient (r^2)	0.9995
Detection limit (µg L^{-1})	1.3

[a]Standard deviation of the calibration line
[b]probability value

Table 31 Se(IV) recovery from sea water using Fixed time method under conditions of [MO] = 10.0 mg L^{-1}, [BrO_3^-] = 5.0 × 10^{-3} M, [N_2H_4.2HCl] = 5.0 × 10^{-3} M, pH = 1.60 ± 0.02, temperature = 25.0 ± 0.1 °C

[Se(IV)], µg L^{-1}	ΔA ($\Delta A_C - \Delta A_U$)		[Se(IV)] found ± error*, µg L^{-1}	Recovery, %	Error, %
	Average ± SD (*n* = 7)	(Average -Blank) ± SD			
0.0	0.153 ± 0.004	0.000 ± 0.004	-	-	-
31.6	0.175 ± 0.005	0.023 ± 0.005	30.66 ± 4.64	97.01	-2.99
63.2	0.199 ± 0.006	0.047 ± 0.006	61.78 ± 2.22	97.80	-2.20
94.8	0.225 ± 0.006	0.072 ± 0.006	94.96 ± 1.45	100.33	+0.33
126.3	0.249 ± 0.006	0.097 ± 0.006	127.03 ± 1.29	100.55	+0.55

*uncertainty of result from linear calibration

Table 32 Spectral and statistical data for the determination of Se(IV) in sea water by Fixed time method under the conditions given in Table 31

Parameters	Fixed time method (6 min)
λ_{max} (nm)	507.0
Linear dynamic range (µg L^{-1})	0-126.3
Regression equation	Initial rate = 7.692×10^{-4} [Se(IV)] - 1.020×10^{-3}
Log-log plot	log (initial rate) = 1.053 log [Se(IV)] - 3.226 (hence 1st order)
$SD_{calibration}$[a]	1.010×10^{-3}
Intercept	-1.020×10^{-3}
$SD_{intercept}$	7.825×10^{-4}
P-$value_{intercept}$[b]	2.834×10^{-1} (> 0.05, hence intercept does not differ from zero)
Slope	7.692×10^{-4}
SD_{slope}	1.011×10^{-5}
P-$value_{slope}$[b]	5.010×10^{-6} (< 0.05, hence slope differs from zero)
Correlation coefficient (r^2)	0.9995
Detection limit (µg L^{-1})	17.4

[a]Standard deviation of the calibration line
[b]probability value

Table 33 Determination of Se(IV) in different water samples by standard addition method using Initial rate method under conditions of [MO] = 5.0 mg L^{-1}, [BrO_3^-] = 5.0 × 10^{-4} M, [N_2H_4.2HCl] = 5.0 × 10^{-3} M, pH = 1.60 ± 0.02, temperature = 25.0 ± 0.1 ^oC

Initial rate method										
Sample	**Concentration, µg L^{-1}**									
	Spiked		**Nominal ± SD[a]**		**Recovery ± RSD (%)**		**SAE[b]**		**C.L.[c]**	
	Se(IV)	Se(VI)	Se(IV)	Se(VI)	Se(IV)	Se(VI)	Se(IV)	Se(VI)	Se(IV)	Se(VI)
Sea water	31.6	31.6	31.88 ± 0.69	31.80 ± 0.62	100.94 ± 2.15	101.92 ± 5.99	0.31	0.28	0.85	0.77
	63.2	63.2	63.35 ± 1.49	63.31 ± 2.99	100.28 ± 2.36	100.20 ± 1.95	0.67	1.34	1.85	3.71
	94.8	94.8	94.12 ± 1.58	95.24 ± 1.55	99.33 ± 1.68	99.70 ± 2.39	0.71	0.69	1.96	1.92
	126.3	126.3	127.83 ± 3.42	127.83 ± 3.42	101.18 ± 2.68	100.08 ± 2.59	1.53	1.53	4.25	4.25
River water	31.6	31.6	31.94 ± 1.23	31.73 ± 1.62	101.13 ± 3.85	100.45 ± 5.11	0.55	0.73	1.52	2.01
	63.2	63.2	63.54 ± 2.20	63.94 ± 1.84	100.50 ± 3.46	101.20 ± 2.87	0.98	0.82	2.73	2.28
	94.8	94.8	94.80 ± 1.59	93.74 ± 2.45	100.05 ± 1.67	98.94 ± 2.62	0.71	1.10	1.97	3.05
	126.3	126.3	126.12 ± 2.44	127.09 ± 2.71	99.83 ± 1.93	100.59 ± 2.13	1.09	1.21	3.03	3.36
Ground water	31.6	31.6	31.28 ± 1.32	31.60 ± 1.52	99.05 ± 4.23	100.04 ± 4.81	0.59	0.68	1.64	1.89
	63.2	63.2	62.86 ± 2.37	62.76 ± 1.82	99.51 ± 3.78	99.36 ± 2.90	1.06	0.82	2.95	2.26
	94.8	94.8	93.57 ± 3.37	94.72 ± 2.64	98.75 ± 3.60	99.97 ± 2.78	1.51	1.18	4.18	3.27
	126.3	126.3	125.13 ± 3.01	125.95 ± 3.53	99.05 ± 2.41	99.69 ± 2.80	1.35	1.58	3.74	4.38
Tap water	31.6	31.6	32.19 ± 1.93	31.77 ± 1.66	101.92 ± 5.99	100.60 ± 5.22	0.86	0.74	2.39	2.06
	63.2	63.2	63.29 ± 1.23	64.05 ± 2.20	100.20 ± 1.95	101.39 ± 3.43	0.55	0.98	1.53	2.73
	94.8	94.8	94.46 ± 2.26	95.78 ± 3.60	99.70 ± 2.39	101.09 ± 3.76	1.01	1.61	2.81	4.47
	126.3	126.3	126.44 ± 3.27	127.70 ± 3.51	100.08 ± 2.59	101.08 ± 2.75	1.46	1.57	4.06	4.36
Spring water	31.6	31.6	31.11 ± 1.70	31.03 ± 1.66	98.49 ± 5.45	98.25 ± 5.35	0.76	0.74	2.11	2.06
	63.2	63.2	63.72 ± 1.56	64.29 ± 2.90	100.87 ± 2.45	101.78 ± 4.51	0.70	1.30	1.94	3.60
	94.8	94.8	94.14 ± 3.79	94.00 ± 4.00	99.36 ± 4.02	99.20 ± 4.25	1.69	1.79	4.70	4.96
	126.3	126.3	127.15 ± 5.05	125.87 ± 4.05	100.64 ± 3.97	99.63 ± 3.22	2.26	1.81	6.26	5.03

[a]Mean for five determinations (n = 5)
[b]SAE, standard analytical error
[c]C.L. confidence limit at 95% confidence level and four degrees of freedom (t = 2.776)

Table 34 Determination of Se(IV) in different water samples by standard addition method using Fixed time method under conditions of [MO] = 10.0 mg L^{-1}, [BrO_3^-] = 5.0×10^{-3} M, [N_2H_4.2HCl] = 5.0×10^{-3} M, pH = 1.60 ± 0.02, temperature = 25.0 ± 0.1 ^oC

Fixed time method										
Sample	**Concentration, µg L^{-1}**									
	Spiked		**Nominal ± SD[a]**		**Recovery ± RSD (%)**		**SAE[b]**		**C.L.[c]**	
	Se(IV)	Se(VI)	Se(IV)	Se(VI)	Se(IV)	Se(VI)	Se(IV)	Se(VI)	Se(IV)	Se(VI)
Sea water	31.6	31.6	32.06 ± 1.57	32.16 ± 1.86	101.52 ± 4.88	101.82 ± 5.80	0.70	0.83	1.94	2.32
	63.2	63.2	63.87 ± 2.58	62.80 ± 3.45	101.11 ± 4.04	99.42 ± 5.49	1.15	1.54	3.20	4.28
	94.8	94.8	93.68 ± 3.26	93.50 ± 3.99	98.86 ± 3.49	98.67 ± 4.26	1.46	1.78	4.05	4.95
	126.3	126.3	126.07 ± 3.05	126.36 ± 5.63	99.79 ± 2.42	100.02 ± 4.46	1.36	2.52	3.78	6.99
River water	31.6	31.6	31.88 ± 1.37	31.57 ± 1.74	100.94 ± 4.30	99.96 ± 5.50	0.61	0.78	1.70	2.16
	63.2	63.2	63.01 ± 2.54	63.32 ± 0.80	99.75 ± 4.03	100.24 ± 3.28	1.14	0.93	3.15	2.58
	94.8	94.8	95.51 ± 2.20	94.08 ± 4.18	100.80 ± 2.30	99.29 ± 4.45	0.98	1.87	2.73	5.19
	126.3	126.3	126.05 ± 5.24	125.25 ± 3.87	99.78 ± 4.16	99.14 ± 3.09	2.34	1.73	6.50	4.81
Ground water	31.6	31.6	31.97 ± 1.56	31.74 ± 1.89	101.21 ± 4.87	100.51 ± 5.97	0.70	0.85	0.70	2.35
	63.2	63.2	63.11 ± 1.69	63.74 ± 2.06	99.90 ± 2.67	100.91 ± 3.24	0.75	0.92	0.75	2.56
	94.8	94.8	94.71 ± 3.72	93.34 ± 3.75	99.95 ± 3.93	98.51 ± 4.01	1.66	1.68	1.66	4.65
	126.3	126.3	126.52 ± 6.42	126.09 ± 4.30	100.15 ± 5.07	99.81 ± 3.41	2.87	1.92	2.87	5.34
Tap water	31.6	31.6	32.14 ± 1.66	31.33 ± 1.65	101.76 ± 5.15	99.18 ± 5.26	0.74	0.74	2.06	2.05
	63.2	63.2	63.00 ± 1.92	62.55 ± 3.63	99.86 ± 3.04	99.02 ± 5.80	0.86	1.62	2.38	4.50
	94.8	94.8	95.18 ± 5.14	95.49 ± 2.66	100.45 ± 5.40	100.78 ± 2.79	2.30	1.19	6.38	3.30
	126.3	126.3	125.79 ± 4.59	126.46 ± 5.02	99.57 ± 3.65	100.10 ± 3.97	2.05	2.25	5.69	6.23
Spring water	31.6	31.6	31.64 ± 1.60	30.76 ± 1.79	100.18 ± 5.07	97.40 ± 5.83	0.72	0.80	1.99	2.23
	63.2	63.2	63.89 ± 3.12	62.52 ± 3.20	101.14 ± 4.89	98.97 ± 5.11	1.40	1.43	3.88	3.97
	94.8	94.8	95.27 ± 4.67	94.79 ± 2.22	100.55 ± 4.90	100.04 ± 2.34	2.09	0.99	5.80	2.76
	126.3	126.3	126.11 ± 4.52	127.19 ± 4.19	99.82 ± 3.58	100.67 ± 3.30	2.02	1.88	5.61	5.21

[a]Mean for five determinations (n = 5)
[b]SAE, standard analytical error
[c]C.L. confidence limit at 95% confidence level and four degrees of freedom (t = 2.776)

CHAPTER 5

Conclusion

The validated kinetic spectrophotometric method employed here proved to be simple, sensitive, selective, inexpensive and hence allows rapid determination of selenium at parts per billion levels in water. Its limit of detection is found to be 1.3 µg L^{-1} Se(IV). The proposed method is suitable for determination of trace amounts of Se(IV), Se(VI) and total selenium in environmental water in presence of other ions at natural levels. However, the method could not be used to evaluate the actual level of Se(IV), Se(VI) or total selenium present in environmental water samples since they were present below the method detection limit. This also confirmed that the selenium levels in Suva and Labasa water samples were much below the regulatory level of 10 parts per billion.

The proposed method can be used as a suitable alternative to the standard hydride generation atomic absorption spectrometric or inductively coupled plasma mass spectrometric methods, which are far more costly and not readily available for selenium speciation studies in the developing countries of South Pacific for routine Se monitoring. A distinct advantage of the proposed method is that it has a long linear working range than the existing standard methods for selenium speciation. With the aid of further detailed studies into the mechanism of the reaction, there is a possibility of increasing the sensitivity of the method.

CHAPTER 6

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APPENDIX

Certificates for Standard Reference Materials for Selenium

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1640

Trace Elements in Natural Water

This Standard Reference Material (SRM) is intended primarily for use in evaluating methods used in the determination of trace elements in fresh water. SRM 1640 is composed of natural fresh water collected from Clear Creek, CO, which has been filtered and stabilized with nitric acid at a concentration of 0.5 mol/L. A unit of SRM 1640 consists of approximately 250 mL of solution in a polyethylene bottle, which is sealed in an aluminized plastic bag.

Certified Values and Uncertainties: The certified values expressed as mass fractions and their expanded uncertainties are listed in Table 1 for 17 elements in SRM 1640. The certified values are equally weighted means of the results of two or more independent analytical methods or a single primary method. Each expanded uncertainty is based on a 95 % confidence interval for the mean, and includes an allowance for differences between the analytical methods used and an allowance for solution stability [1].

Reference Values and Uncertainties: The reference values expressed as mass fractions and their expanded uncertainties are provided in Table 2 for an additional ten elements. The reference values are means from a single method or two or more equally weighted means of results of independent analytical methods for which there is insufficient information to meet NIST certification criteria. Each expanded uncertainty is based on a 95 % confidence interval for the mean and includes an allowance for differences between the analytical method used and an allowance for solution stability but may not include all sources of uncertainty [1].

Information Value: The upper limit information value for thallium, expressed as a mass fraction in Table 3, is an estimate based on the instrumental limit of detection and measurements from a single unit of SRM 1640.

The analytical methods used for the characterization of this SRM are given in Table 4. All values are reported as mass fractions [2].

NOTICE AND WARNINGS TO USERS

Expiration of Certification: This certification of this SRM lot is valid until **01 June 2008**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Use"). However, the certification will be nullified if the SRM is contaminated or modified. Registration (see attached sheet) will facilitate notification.

Use: The SRM should be shaken before use because of potential water condensation. Samples should be analyzed at a room temperature of 22 °C ± 5 °C. To prevent possible contamination of the SRM, pipettes should not be inserted into the bottle. After use, the bottle should be recapped tightly and returned to the aluminized bag, which should be folded and sealed with sealing tape. This precaution will protect the SRM from possible environmental contamination and long-term evaporation.

The mass fractions given in Tables 1 and 2 are expressed as microgram per kilogram or milligram per kilogram. These values can be converted to mass concentrations with units of nanograms per cubic centimeter or micrograms per cubic centimeter, respectively, by multiplying by the density. The density of SRM 1640 at 22 °C was measured to be 1.0015 g/cm^3 ± 0.0005 g/cm^3 (identical to grams per milliliter).

Coordination of the NIST technical measurements was under the direction of J.R. Moody of the NIST Analytical Chemistry Division.

Stephen A. Wise, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Certificate Issue Date: 20 January 2006
See Certificate Revision History on Last Page

Robert L. Watters, Jr., Chief
Measurement Services Division

Statistical analysis of the experimental data was performed by W.F. Guthrie of the NIST Statistical Engineering Division.

The overall coordination of measurements performed by the U.S. Geological Survey National Water Quality Laboratory, Arvada, CO, and by laboratories that participate in the Standard Reference Water Program was under the direction of K. Long.

The support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

Recognizing contamination at the microgram per kilogram level can be a serious problem, labware should be scrupulously cleaned and only high purity reagents employed. Sampling and manipulations, such as evaporations, should be done in a clean environment, such as a Class-100 clean hood.

Table 1. Certified Mass Fractions

Element	µg/kg	Element	µg/kg
Aluminum	52.0 ± 1.5	Iron	34.3 ± 1.6
Antimony	13.79 ± 0.42	Lead	27.89 ± 0.14
Arsenic	26.67 ± 0.41	Manganese	121.5 ± 1.1
Barium	148.0 ± 2.2	Molybdenum	46.75 ± 0.26
Beryllium	34.94 ± 0.41	Selenium	21.96 ± 0.51
Boron	301.1 ± 6.1	Silver	7.62 ± 0.25
Cadmium	22.79 ± 0.96	Strontium	124.2 ± 0.7
Chromium	38.6 ± 1.6	Vanadium	12.99 ± 0.37
Cobalt	20.28 ± 0.31		

Table 2. Reference Mass Fractions

Element	µg/kg	Element	mg/kg
Copper	85.2 ± 1.2	Calcium	7.045 ± 0.089
Lithium	50.7 ± 1.4	Magnesium	5.819 ± 0.056
Nickel	27.4 ± 0.8	Silicon	4.73 ± 0.12
Potassium	994 ± 27	Sodium	29.35 ± 0.31
Rubidium	2.00 ± 0.02		
Zinc	53.2 ± 1.1		

Table 3. Information Mass Fraction

Thallium	<0.1 µg/kg

Source and Preparation of Material: A sample of about 3500 L of natural (fresh) water was obtained by the USGS at Clear Creek, CO. It was filtered through a 0.1 µm ultra filter and acidified with nitric acid. Analysis of the water by inductively coupled plasma mass spectrometry (ICPMS), before and after the stabilization process, showed that arsenic, beryllium, cobalt, selenium, and zinc were decreased in concentration during the stabilization process. These elements were adjusted to their original concentration levels by the addition of salts of the decreased elements. The stabilized solution was then pumped through an ultra filter, past a UV light source (for sterilization purposes), and then to a bottling station. At the bottling station, the bottles were rinsed with the sample and then filled.

Table 4. Methods Used for the Analysis of SRM 1640

Elements	Methods
Aluminum	DCP, ETAAS, ICP-AES, ICPMS
Antimony	ETAAS, Hyd-AAS, ICP-AES, ICPMS
Arsenic	ETAAS, Hyd-AAS, ICP-AES, ICPMS
Barium	DCP, ETAAS, ICP-AES, ICPMS, **ID-ICPMS**
Beryllium	ETAAS, ICP-AES, ICPMS
Boron	COLOR, ICP-AES, ICPMS, ID-TIMS
Cadmium	ETAAS, FAAS, IC, ICP-AES, ICPMS, ID-ICPMS
Calcium	DCP, FAAS, ICP-AES, ICPMS
Chromium	ETAAS, FAAS, IC, ICP-AES, ICPMS
Cobalt	ETAAS, ICP-AES, ICPMS
Copper	ETAAS, FAAS, IC, ICP-AES, ICPMS, ID-ICPMS
Iron	ETAAS, FAAS, ICP-AES, ICPMS, ID-TIMS
Lead	ETAAS, FAAS, IC, ICP-AES, ICPMS, **ID-ICPMS**
Lithium	ETAAS, FAAS, ICP-AES, ICPMS
Magnesium	DCP, FAAS, ICP-AES, ICPMS
Manganese	DCP, ETAAS, FAAS, ICP-AES, ICPMS
Molybdenum	ETAAS, ICP-AES, ICPMS, **ID-ICPMS**
Nickel	ETAAS, FAAS, ICP-AES, ICPMS, ID-ICPMS
Potassium	ETAAS, FAAS, FES, ICP-AES, ICPMS
Rubidium	ID-TIMS
Selenium	EAAS, Hyd-AAS, ICP-AES, ICPMS
Silicon	COLOR, ICP-AES, ICPMS
Silver	ETAAS, FAAS, ICP-AES, ICPMS, ID-ICPMS
Sodium	DCP, FAAS, FES, ICP-AES, ICPMS
Strontium	DCP, ETAAS, ICP-AES, ICPMS, **ID-ICPMS**
Thallium	ICPMS
Vanadium	ETAAS, ICP-AES, ICPMS
Zinc	FAAS, IC, ICP-AES, ICPMS, ID-ICPMS

Methods given in bold indicate that a single NIST primary method was used for certification.

Methods

COLOR	Colorimetry
DCP	Direct current plasma atomic emission spectrometry
ETAAS	Heated graphite atomizer (electrothermal) atomic absorption spectrometry
FAAS	Flame atomic absorption spectrometry
FES	Flame emission spectrometry
Hyd-AAS	Hydride generation-atomic absorption spectrometry
IC	Ion chromatography
ICP-AES	Inductively coupled plasma-atomic emission spectrometry
ICPMS	Inductively coupled plasma mass spectrometry
ID-ICPMS	Isotope dilution-inductively coupled plasma mass spectrometry
ID-TIMS	Isotope dilution-thermal ionization mass spectrometry

Contributing Laboratories and Analysts:

E.S. Beary, M.S. Epstein, K.E. Murphy, P.J. Paulsen, and G.C. Turk; NIST Analytical Chemistry Division, Gaithersburg, MD

Water Resources Division and approximately 70 laboratories participating in the Standard Reference Water Program, under the direction of K. Long; U.S. Geological Survey, Arvada, CO

P. Taylor, L. Van Nevel, I. Lapitajs, A. Kynartren, A. Held, U. Örnemark, and P. De Biévre; Institute for Reference Materials and Measurements, Geel, Belgium

M. Morita; Regional Environmental Division of the National Institute for Environmental Studies, Japan Environmental Agency, Tsukuba, Japan

REFERENCES

[1] ISO; *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9, 1st ed. International Organization for Standardization: Geneva,Switzerland (1993); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurements Results*, NIST Technical Note 1297, U.S. Government Printing Office: Washington, DC (1994); available at http://physics.nist.gov/Pubs/.

[2] Taylor, B.N.; *Guide for the Use of the International System of Units (SI)*; NIST Special Publication 811, U.S. Government Printing Office: Washington, DC (1995).

Certificate Revision History: 20 January 2006 (This revision reflects an extension of the certification period); 17 March 2004 (This technical revision reports a change in the expiration date); 23 January 1998 (Revision reports the addition of an information value for thallium; 02 October 1997 (Original certificate date).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3149

Selenium Standard Solution

Lot No. 992106

This Standard Reference Material (SRM) is intended for use as a primary calibration standard for the quantitative determination of selenium. One unit of SRM 3149 consists of five 10 mL sealed borosilicate glass ampoules of an acidified aqueous solution prepared gravimetrically to contain a known mass fraction of selenium. The solution contains nitric acid at a volume fraction of approximately 10 %.

Certified Value of Selenium: 10.11 mg/g ± 0.02 mg/g

The certified value is based on (1) gravimetric preparation using high-purity selenium metal and (2) inductively coupled plasma optical emission spectrometry (ICP-OES) using three independently prepared primary standards.

The uncertainty in the certified value is calculated as

$$U = (2u_c + B) \text{ mg/g}$$

where u_c is the combined standard uncertainty calculated according to the ISO and NIST Guides [1] and the procedure of Schiller and Eberhardt for combining independent analytical methods [2]. The value of u_c is intended to represent, at the level of one standard deviation, the combined effect of uncertainty components associated with the gravimetric preparation, the ICP-OES determination, and method bias. The quantity, B, is an allowance for between-method differences.

Expiration of Certification: The certification of **SRM 3149 Lot No. 992106** is valid, within the measurement uncertainty specified, until **02 September 2011**, provided the SRM is handled in accordance with instructions given in this certificate (see "Instructions for Use"). This certification is nullified if the SRM is damaged, contaminated, or modified.

Maintenance of Certification: NIST will monitor representative solutions from this SRM lot over the period of its certification. If substantive changes occur that affect the certification before the expiration of certification, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Coordination of the technical measurements leading to the certification of SRM 3149 was provided by G.C. Turk of the NIST Analytical Chemistry Division.

This SRM was prepared by T.A. Butler and analyzed using ICP-OES by M.L. Salit and A.P. Lindstrom of the NIST Analytical Chemistry Division. Primary standards for ICP-OES calibration were prepared by C.M. Beck II of the NIST Analytical Chemistry Division.

Statistical consultation was provided by S.D. Leigh of the NIST Statistical Engineering Division.

The support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

Stephen A. Wise, Chief
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Gaithersburg, MD 20899
Certificate Issue Date: 29 January 2007
See Certificate Revision History on Last Page

Robert L. Watters, Jr., Chief
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TRACEABILITY

Calibration of analytical instruments or procedures for the determination of selenium should be performed using standards whose values are traceable to the certified value of selenium in this SRM. Traceability must be established through an unbroken chain of comparisons, each having stated uncertainties [3]. Comparisons are based on appropriate physical or chemical measurements. These may include various spectroscopic or classical methods of analysis. Gravimetric or volumetric dilution is also a method of comparison, where the mass or volume of a solution before and after dilution is measured. The uncertainties assigned to the traceable values of such standards must include the uncertainty of the certified value of selenium in this SRM, appropriately combined with the uncertainties of all comparison measurements.

INSTRUCTIONS FOR USE

CAUTION: This SRM is an acid solution contained in tip-sealed borosilicate glass ampoules with pre-scored stems. Therefore, all appropriate safety precautions, including use of gloves during handling, should be taken. Unopened ampoules should be stored under normal laboratory conditions in an upright position inside the original container supplied by NIST.

Opening an Ampoule: When an ampoule is to be opened, that area of the stem where the pre-scored band is located (~5 mm below the encircling metallic band) should be carefully wiped with a clean, damp cloth and the body of the ampoule wrapped in absorbent material. Then holding the ampoule steady and with thumb and forefinger grasping the stem at the metallic band, **minimal** thumb pressure should be applied to the stem to snap it. Correctly done, the stem should break easily where pre-scored. Use of a metal file to break the stem is **NOT** recommended.

Working Standard Solutions: After opening the ampoule, the entire contents should be transferred immediately to another container and *working standard solutions* should be prepared. Working standard solutions in the range of 10 mg/kg to 100 mg/kg are recommended, from which more dilute standards can be prepared. The user should establish internal laboratory procedures that specify a maximum shelf life for a working standard solution. Two procedures for the preparation of working standard solutions follow.

Preparation of Working Standard Solutions by Mass: Each working standard solution should be prepared by emptying one or more ampoules of the SRM into an empty, dry, pre-weighed, polyethylene bottle and then re-weighing the bottle. An appropriate dilute acid must be added by mass to bring the solution to the desired dilution. The dilution need not be exact since the mass of the empty bottle, mass of the bottle plus SRM aliquot, and the final diluted mass of the solution will permit calculation of the exact mass fraction (mass of selenium per mass of solution) of the working standard solution. Dilutions prepared gravimetrically as described will need no correction for temperature and no further correction for true mass fraction in vacuum. Volumetric dilutions are **NOT** recommended due to uncertainties in volume calibrations and variations in density. However, for user convenience, a procedure for volumetric preparation that will minimize the major sources of error is given below.

Preparation of Working Standard Solutions by Volume: Each working standard solution should be prepared by emptying one or more ampoules of the SRM into an empty, dry, polyethylene bottle and then weighing the bottle. The solution must now be transferred to a Class A volumetric flask and the polyethylene bottle re-weighed to determine the exact mass of SRM solution transferred. The solution in the flask is then diluted to 99 % + volume using an appropriate dilute acid, mixed thoroughly, and the remaining few drops needed to dilute to exact volume carefully added. The concentration (in mg/mL) of the resulting working standard solution can then be calculated by multiplying the mass (in g) of the SRM solution amount by the SRM certified value (in mg/g) and dividing the numerical product by the calibrated volume (in mL) of the flask used for dilution. If this procedure is followed, no correction for density is needed. Although the concentration of the resulting working standard solution may be an uneven fraction of the original SRM concentration, it will be known as accurately as a volumetric dilution permits.

Possible Presence of Other Elements: Studies conducted by NIST have shown that components of borosilicate glass ampoules may leach into solution. In *undiluted* solutions, Si and Na mass fractions as large as 20 mg/kg, B and La mass fractions in the range 1 mg/kg to 5 mg/kg, and Mg, Al, Mn, As, Ce, Zn, Rb and Ca mass fractions in the range 0.05 mg/kg to 1 mg/kg have been found. When diluted to prepare working standard solutions, the levels of these elements become negligible for most purposes. Nevertheless, possible effects should be considered when this SRM is used.

REFERENCES

[1] ISO; *Guide to the Expression of Uncertainty in Measurement*; ISBN 92-67-10188-9, 1st ed., International Organization for Standardization: Geneva, Switzerland (1993); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297, U.S. Government Printing Office: Washington, DC (1994); available at http://physics.nist.gov/Pubs/.

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[3] ISO; *International Vocabulary of Basic and General Terms in Metrology*; ISBN 92-67-01075-1, 2nd ed., International Organization for Standardization: Geneva, Switzerland (1993).

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