SPECTROFLUORIMETRIC DETERMINATION OF SELENIUM AFTER CLOUD POINT EXTRACTION

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ABSTRACT

SPECTROFLUORIMETRIC DETERMINATION OF SELENIUM AFTER CLOUD POINT EXTRACTION

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As compared with the concentration in sample when the detection limit of analyte is low, a preconcentration method can be used. In this study, cloud point extraction (CPE) was used as the preconcentration method. The aqueous solutions of nonionic and zwitterionic surfactant materials become cloudy when its temperature reaches the cloud point temperature and analyte collapses with surface active material. The volume of surfactant rich phase is much smaller than the solution volume and therefore a way high preconcentration factor was obtained.

For the cloud point extraction of selenite, a fluorimetric ligand, 2,3-diminonaphthalene (DAN) was used and the hydrophobic Se(IV)-DAN complex formed (4,5-benzopiazselenol) was extracted with Triton X-114. The effects of pH,

complexation period, reaction temperature, DAN concentration and surfactant concentration on the extraction efficiency were investigated. The extraction efficiency at the optimized conditions was 98 percent. Spectrofluorimetric determination of selenium was performed at excitation and emission wavelegths of 379 nm and 582 nm, respectively. The detection limit, established as 3*s* /slope where s is the standard deviation of 9 measurements of 0.020 mg/L Se (IV)-DAN complex after 10 fold preconcentration was 3.7 µg/L Se. By using solid surface fluorescence measurements detection limit could be reduced down to 1.2 µg/L. The obtained detection limits (3.7 and 1.2 µg/L) were sufficiently low for detecting selenite in diverse samples. The accuracy of the method was confirmed by the analysis of trace elements in waste water Standard reference material (EnviroMAT- Waste Water LOW EU-L-1). The interference effects of some anions and cations were also tested.

Key Words: Cloud Point Extraction, Preconcentration, Selenium, Spectroflorometry, 2,3-diaminonaphthaline (DAN), Organized Media, Solid Surface Fluorescence

ÖZ

BULUTLANMA NOKTASI EKSTRAKSİYONUNDAN SONRA SELENYUMUN SPEKTROFLUORİMETRİK OLARAK TAYİNİ

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Tayin edilecek elementin derişimi, gözlenebilme sınırının altında olduğu zaman bir önzenginleştirme işlemi uygulanmaktadır. Bu çalışmada, önzenginleştirme yöntemi olarak bulutlanma noktası ekstraksiyonu (BNE) kullanılmıştır. İyonik olmayan ve zwitteriyonik yüzey aktif maddelerin sulu çözeltileri, bulutlanma noktası sıcaklığının üzerinde bulanıklaşır ve analit yüzey aktif madde ile birlikte çökelir. Yüzey aktif madde bakımından zengin olan fazın hacmi, toplam hacimden çok daha düşüktür ve böylece yüksek bir önzenginleştirme oranı elde edilir. Son yıllarda, bulutlanma noktası ekstraksiyonu metal tayinlerinde geniş uygulamalar bulmuştur.

Selenyumun bulutlanma noktası ekstraksiyonu için fluorimetrik bir ligant olan 2,3-diaminonaftalin (DAN) kullanılmıştır ve oluşan hidrofobik Se(IV)-DAN kompleksi (4,5-benzopiazselenol) Triton X-114 ile ekstrakte edilmiştir. pH, kompleksleşme süresi, reaksiyon sıcaklığı, DAN derişimi ve yüzey aktif madde derişiminin ekstraksiyon verimi üzerindeki etkileri incelenmiştir. Optimize edilmiş koşullarda

ekstraksiyon verimi % 98' dir. Selenyumun spektroflorimetrik tayini sırasıyla 379 nm ve 582 nm uyarma ve emisyon dalga boylarında yapılmıştır. Yöntemin 3s/eğim olarak tarif edilen gözlenebilme sınır 3.7 µg/L dir. Burada s, 10 kat önzenginleştirilmiş 0.020 mg/L Se(IV)- DAN bileşiğinin 9 kez ölçümünden elde edilen standart sapma değeridir. Katı yüzey fluoresans ölçümleri kullanılarak gözlenebilme sınırı 1.2 µg/L' ye düşürülmüştür. Elde edilen gözlenebilme sınırları (3.7 ve 1.2 µg/L), farklı numunelerdeki selenyum derişimlerinin belirlenmesi için yeterince düşüktür. Yöntemin doğruluğu atık sulardaki eser elementler standard referans madde (EnviroMAT- Waste Water LOW EU-L-1 standard reference madde) analizi ile irdelenmiştir. Farklı anyon ve katyonların girişim etkisi test edilmiştir.

Anahtar Kelimeler: Bulutlanma Noktası Ekstraksiyonu, Önzenginleştirme, Selenyum, Spektroflorometri, 2,3-diaminonaftalin (DAN), Organize Ortam, Katı Yüzey Fluoresans

To My Family

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INTRODUCTION

1.1 Essential Trace Elements

Selenium is an essential element. Definition of being an essential element is that its absence causes death or a severe malfunction of the organism and a low but definite concentration level is necessary for the maintenance of life.

Classifying elements as essential or toxic, on the other hand may not be convenient since toxicity depends on concentration to which human beings are exposed. Actually all essential elements may also be toxic in animals and humans if ingested at sufficiently high levels for a sufficient long period [1].

There is a relatively narrow range between the selenium adequate and toxic dietary levels. The recommended dietary allowance (RDA) is 50-70 μg Se per day for healthy adults with 40 μg Se as minimum requirement. Less than 11 μg Se will definitely put people at the risk of deficiency that would be expected to cause genetic damage. Daily doses of 100-200 μg Se above the RDA are needed to inhibit genetic damage and cancer development in humans. About 400 μg of Se per day is considered an upper limit [2]. Selenium toxicity (chronic selenosis) is characterized by loss of hair and nails, skin injury, tooth decay and nervous system abnormalities [3].

Selenium can be present in the following species in environmental and biological systems (In Table 1.1):

Table 1.1: Selenium species in environmental and biological systems [4].

Inorganic species
Elemental selenium, selenide [Se(−II)], selenate [SeO_4^{2-}], selenite [SeO_3^{2-}]
Simple organic species
Methylselenol (MeSeH), dimethylselenide (Me_2Se), dimethyldiselenide (Me_2Se_2) trimethylselenonium cation (Me_3Se^+), dimethylselenone (Me_2SeO_2), dimethylselenoxide (Me_2SeO), methylseleninic acid anion ($MeSe(O)O^-$ dimethylselenosulfide (MeSSeMe), selenocyanate ($SeCN^-$,), selenourea ($Se^{=}C(NH_2)_2$
Amino acids and low molecular mass species
Selenomethionine, selenocysteine, selenocystine, Se-methylselenocysteine, selenocysteic acid, Se-methylselenomethionine, *S*-(methylseleno)cysteine, selenomethionine selenoxide hydrate, selenohomocysteine, γ-glutamyl-Se-methylselenocysteine, Se-adenosylselenohomocysteine, selenocholine, selenobetaine, selenoglutathione
Other compounds
Selenopeptides, selenoproteins, selenoenzymes, selenosugars, Se-metal metallothionines

1.2 Micelles in General: Properties, Characteristics and Uses

When a substance has the property of adsorbing on the surface or interfaces of the system and altering to a marked degree the surface or interfacial free energies of those surfaces resulting a decrease in surface tension when it is present at low concentration, it may be called a surfactant, an abbreviation for **surf**ace-**act**ive **a**ge**nt** [5].

In aqueous solution surfactants can aggregate to form colloidal-sized clusters referred to as micelles (normal micelles). For this phenomenon the minimum concentration of surfactant needed to form is called the critical micelle concentration (CMC). Depending upon solution conditions and surfactant type, micelles have a variety of shape ranging from roughly spherical to ellipsoidal (oblate or prolate). The interior region of the normal micelle belongs the hydrophobic moieties of the surfactant molecules and the outer surface belongs of the hydrated hydrophilic groups along with any bound water molecules. A schematic representation of a typical nonionic surfactant micelle aggregate is shown in Figure 1.1. Micellar shape can be changed, sometimes dramatically, by altering the solution conditions (concentration of surfactant and additives, nature of additive, temperature, etc.) [6].

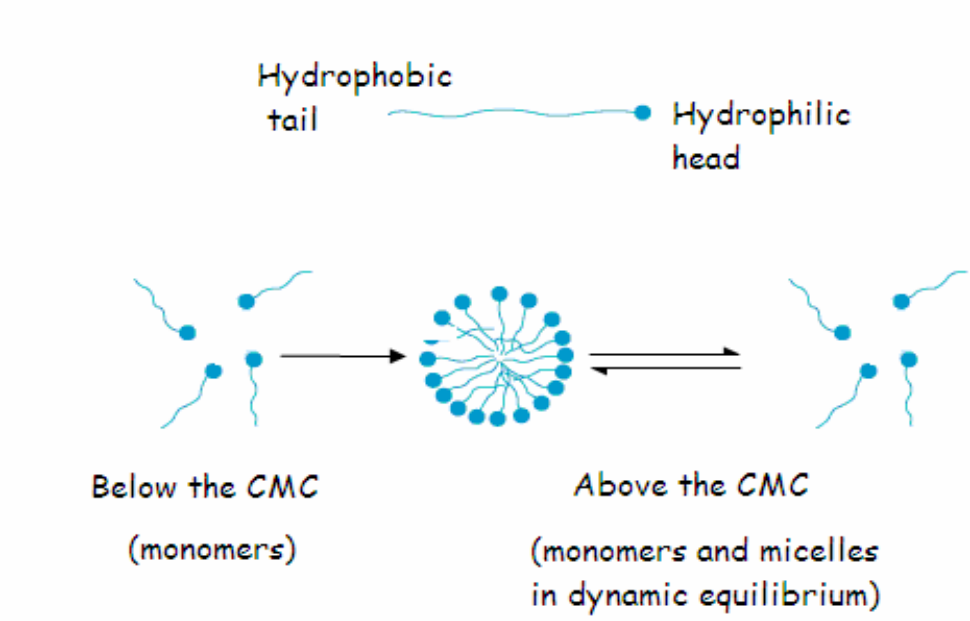


Figure 1.1: The aggregation of monomers to form a normal, aqueous micelle.

Surfactants are amphiphilic molecules. Amphiphilic means that the monomer contains both polar and hydrophobic groups. These molecules contain a polar group (head) at the end of a long hydrophobic carbon chain (tail). In contrast to purely polar or non-polar molecules, amphiphilic molecules exhibit unique properties in water. Their polar group forms hydrogen bonds with water molecules, while the hydrocarbon chains aggregate due to hydrophobic interactions. These properties allow surfactants to be soluble in water. Because of their amphiphilic nature, surfactants are able to solubilize hydrophobic compounds in water.

Solubilization, one of the most important properties of surfactant, is directly related to micelle formation. Both bulk and solvent- soluble and solvent-insoluble species can reversibly interact with and bind to the micellar assembly. Sparingly-soluble or non-water-soluble materials can be solubilized in water due to their binding to the micelles in solution [6].

Solute + micelle $\leftrightarrow$ micelle-bound solute

The most intensely studied and discussed type of microscopically ordered molecular aggregates are the micelles. Micelles are supramolecular structures of colloidal dimensions formed by surfactants molecules that aggregate in a spontaneous way in aqueous solution when critical micelle concentration (CMC) is attained. The CMC of a surfactant is based on several factors including its molecular structure, and experimental conditions such as temperature, ionic strength, counterions, etc. Below the CMC, the surfactant is usually in a nonassociated monomer form. However, when the CMC is reached, the formation process is favored. Micelles are not static structures. An important micelle characteristic is its dynamic equilibrium with the dissolved surfactant monomers, which remain at an approximately constant concentration after reaching the CMC. Micelles are thermodynamically stable and easily reproducible, but they can be destroyed by water dilution when the surfactant concentration becomes lower than its CMC [7, 8].

The formation of a micelle implies a decrease in the entropy of the system. Such free energy will be used up in its formation. In an aqueous solution, the water molecules attract themselves by hydrogen bonds. Thus, in the dissolution of an ionic or polar substance, the necessary energy to break the hydrogen bonds is compensated by the hydration of dissolved species. However, aliphatic chains of surfactants are not appreciably hydrated. Then, Van der Waals forces act to their reciprocal attraction by decreasing the contact area among the surfactant molecules and water. Moreover, the hydrophilic head group of surfactants tends to solubilize in water. All these factors become the surfactant molecules agglomeration a spontaneous phenomenon. When the surfactant is dissolved in aqueous solution above its CMC, the hydrophobic group distorts the water liquid structure and this causes increase of free energy. This increase of free energy compensates for the necessary work to create a surface area permitting the micelle formation [7].

Micelles are able to dissolve chemical species, which can present different polarities and sizes. The solubilization site varies with the nature of the solubilized species and the surfactant. The solubilization sites of micelles can be recognized following ways:

(a) on micelle surface,
(b) among the hydrophilic head groups,
(c) the space among the poly-oxy-ethylene groups (for nonionic surfactants)
(d) the interface between the head groups and the core, and
(e) the hydrophobic core.

The charged chemical species are captured in the sites (a), (b), and (c). The amphiphilic species are extracted into the site (d), and the hydrophobic species, in the core (e). The binding site in a micelle more extensively utilized in cloud point extraction (CPE) is the hydrophobic core. Hence, the stable chelate generation is the main step for the majority of developed methods [7].

Micelle characteristics vary with the nature of the hydrophilic group, surfactants are categorized as follows[7, 9]:

1. Anionic: the hydrophilic group carrying a negative charge such as carboxyl ($RCOO^-$), sulfonates (RSO_3^-), or sulfate ($ROSO_3$). For example; Sodium dodecyl sulfate (SDS), $RCOO^-Na^+$ (soap)
2. Cationic: the hydrophilic group carrying a positive charge, for example, $RNH_3^+Cl^-$ (salt of a long-chain amine), RN $(CH_3)_3^+Cl^-$ (quaternary ammonium chloride). This type of surfactants usually contains quaternary nitrogen head groups due to the stability and commercial availability of these materials.
3. Non-ionic: the hydrophilic group has no charge but derives its water solubility from higly polar groups, for example, Polyoxyethylene(23)dodecanol(Brij35) $(CH_3(CH_2)_{11}(OCH_2CH_2)_{23}OH)$. The polar head groups of nonionic micelles generally consist of polyoxyethylene ($-OCH_2CH_2O-$) or polyoxypropylene groups.

4. Zwitterionic: its molecules present both the cationic groups and anionic and, depending of pH, its prevalence the anionic, cationic, or neutral species, for example, Dodecyldimetyl ammonium butirate (DAB)($CH_3(CH_2)_{11}N^+(CH_3)_2$.

The nature of the hydrophobic groups may be significantly more varied than for the hydrophilic. Generally they are long-chain hydrocarbon residues [9].

The critical micelle parameters, i.e., CMC, cloud point and aggregation number (the number of surfactant molecules per micelle), for different nonionic and zwitterionic surfactants that have been utilized in cloud point extraction are summarized in Table 1.2 [6].

Table 1.2: Summary of the aqueous solution properties of some of the nonionic and zwitterionic surfactant systems that have been employed in Cloud Point Extraction [6].

Surfactant	CMC, mM	Aggregation Number(N)[a]	Cloud point, °C
Triton X-100 (TX-100)	0.17-0.30	120-140	64 - 65
Triton X-114 (TX-114)	0.20-0.35	–	23 - 25
PONPE-7.5	0.085	–	5 - 20
PONPE-10	0.07-0.085	100	62 - 65
Igepal CO-630	–	–	48 - 52
C_8E_3	5.9-7.5	–	10.6
$C_{10}E_4$	0.6-0.8	30	19 - 21
Genapol X-80	0.05	–	42
Brij-30($C_{12}E_4$)	0.02-0.06	40	2 - 7
Brij-56($C_{16}E_{10}$)	0.0006	–	64 - 69
octylβ-D-Thioglucoside(OTG)	9.0	–	10 - 20
octylβ-D-Glucoside(OG)	20.0-25.0	84	2 - 20
C_8-lecithin	–	500	45
C_9-$APSO_4$	45.0	–	65

[a] aggregation number that is the number of monomers per micelle can vary from 50 to 2000.

1.3 Micelles in Analytical Chemistry

In analytical chemistry the micellar media application can be described according to two aspects: the first one refers to the exploitation of micellar media properties such as water solubilization of hydrophobic substances, enhanced detection of spectroanalytical methods by changes of physical and chemical properties of the sample solution, transport and nebulization efficiency improvement, and reactions catalysis. The second aspect refers to the separation and pre-concentration by phase separation phenomenon in the cloud point [10].

1.3.1 Micelles in Analytical Separation and Preconcentration

A number of processes are used in separation and preconcentration with micelles: particle transfer through a liquid interfacial membrane, cloud point extraction, coacervation, micellar ultracentrifugation, foam separation, and solubilization of substances in the adsorption layers of surfactants. The process of choice depends on the nature of analyte and extraction system. The areas of application of separation and preconcentration with the use of organized systems are very diverse. These are the separation and preconcentration of metal ions, metal chelates, many organic compounds, proteins, and other biologically active substances.

Depending on the nature of solvent, two main groups of organized extraction systems can be recognized.

1. Direct (normal) micelles and microemulsions, which occur in an aqueous dispersion medium, belong to one of them.
2. Inverted micelles and microemulsions dispersed in a nonaqueous solvent.

Almost all of the well-known types of surfactants (cationic, anionic, nonionic, and zwitterionic) are used in preconcentration and separation processes with micelles and microemulsions. Surfactant ions can also play an independent role acting as agents that facilitate phase transfer in the water-organic solvent system and increase the rate

of extraction of substances. Thus, they can serve as ion pair reagents or biphasic chelating agents for both metal ions and organic compounds.

In addition to extraction and preconcentration, surfactant ensembles that occur as liquid membranes, such as bilayers, are used for the separation and purification of substances and as models of biological membranes. Micellar ultracentrifugation is another version of separation.

Aqueous systems of nonionic surfactants, which can be separated into two isotropic phases upon heating, have the widest practical application. This process is known as Cloud Point Extraction [11].

1.3.2 Cloud Point Extraction

Determination of analytes at low concentrations has often been a problem for analytical chemists. One of the popular solutions to this problem has been the use of extraction/separation techniques; this approach offeres not only the ability to isolate the target analytes from the matrix solution, thus reducing, controlling or even eliminating the interferences originally present, but also the opportunity for these analytes to be pre-concentrated and determined at trace levels.

The use of organic solvents is the most popular technique applied for extraction of target analyte from its original matrix. It is usually called as liquid extraction. It capitalizes on the tendency of target analytes to be favorably partitioned into an organic solvent. Thus, the analyte can be selectively separated and pre-concentrated into a matrix suitable for the desired application. Extraction into solid substrates, called solid-phase extraction (SPE) or micro-extraction (SPME) as well as some more elaborate techniques, such as electro deposition, pre-concentration on polymeric

membranes and supercritical fluid extraction (SFE) are continuously developing in order to overcome the problems arising from the use of organic solvents.

In 1976, Watanabe and co-workers introduced CPE as new separation and extraction technique and an alternative to organic solvent extraction. Although CPE was initially introduced for the pre-concentration of metals in the form of their hydrophobic complexes [12], it was extensively used as a primary isolation step for the purification of proteins. From this point on, scientists all over the world developed its potential, adding more applications. Thus, in a couple of decades, numerous studies were published covering its theoretical background and especially proposing extraction and pre-concentration schemes for the determination of organic and inorganic analytes [13].

The cloud point extraction is based on the well-known phase phenomenon in micellar solutions of non-ionic surfactants. For example [12, 14], a micellar solution of Triton X-114 becomes turbid when heated above its cloud point. The micellar solution separates into two distinct phases. One phase (surfactant-rich phase) contains most of Triton X-114 and the other phase (aqueous phase) in which the concentration of Triton X-114 is equal to the critical micelle concentration (CMC). Hydrophobic species originally present that bind to micelles in solution can thus be extracted from the micellar solution into a small volume element of the surfactant-rich phase. The cloud point extraction has also been termed as temperature-induced phase separation or micelle-mediated extraction [15-17].

Aqueous solutions of zwitterionic surfactants (at concentrations above the critical micelle concentration, CMC) or β-cyclodextrin [6] also which behave like non-ionic surfactants are homogeneous and isotropic. In Figure 1.2 is shown the structure of β-Cyclodextrin. β -cyclodextrin which is made of seven glucosyl groups. The molecular configuration is like a circular cone. In the cavity of -cyclodextrin, hydrogen and

oxygen atoms of glucoside bonds are situated in screening effect of carbon hydrogen bonds. So the cavity shows hydrophobicity like non-ionic surfactant.

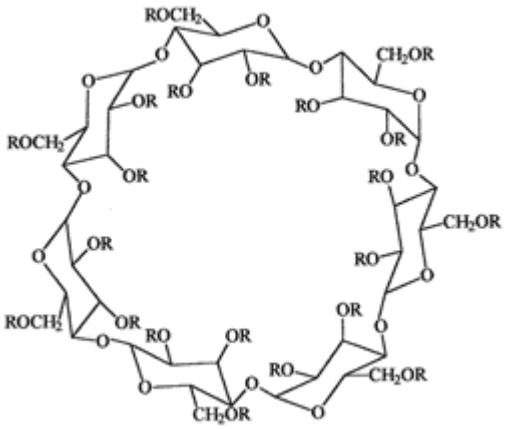


Figure 1.2: The representation of β-Cyclodextrin [18].

The most experimentally modified condition to get the cloud point is the temperature. The temperature at which the phase separation (cloud point temperature or only the cloud point) occurs is a function of the surfactant concentration and its variation can be visualized through a plot. Figure 1.3 is shown phase diagrams for aqueous solutions of Triton X-114, for analytical extraction of metal chelates, Triton X-114 has been frequently utilized, because its dilute micellar solution phase separates easily at room temperature. Figure 1.3 is divided into two regions. For that identified by L, the aqueous solution presents only an isotropic phase. However, in the region identified by 2L, a micellar phase is seperated from an aqueous phase, thus forming an anisotropic mixture that, after some time, separates itself into two isotropic phases [7].

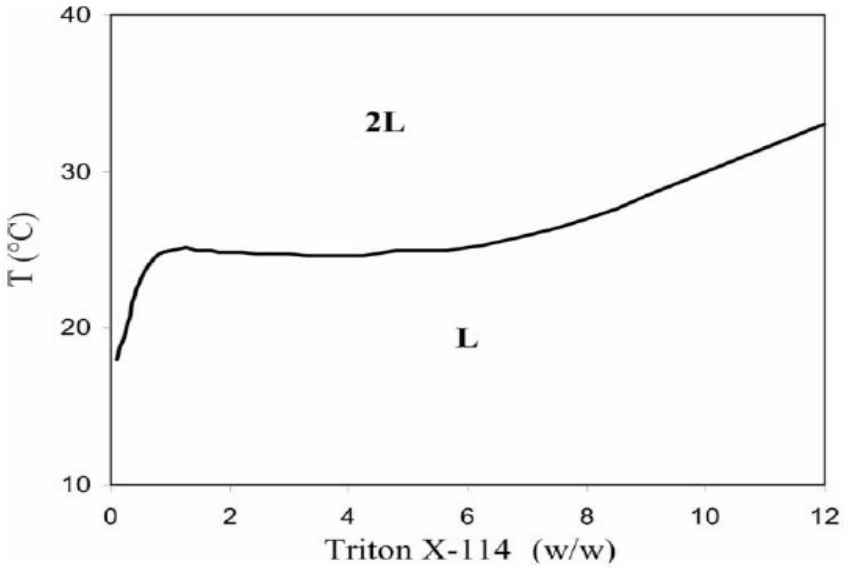


Figure 1.3: Phase diagram of Triton X-114 non-ionic surfactant in aqueous solution [7].

The cloud point phenomenon occurs when a non-ionic or amphoteric surfactant above its CMC promote the separation of the original solution into two phases when heated at the proper temperature. Another cloud point possibility for metal preconcentration is related to anionic surfactants when employed with high acid concentration [19].

In Table 1.2 is summarized some of the most recent applications for cloud point extraction by different methods. Spectrophotometry continues to enjoy wide popularity. The common availability of the instrumentation and simplicity of procedures, as well as speed, precision and accuracy of the technique make spectrophotometric methods an attractive alternative.

Table 1.3: Some Cloud Point Extraction Applications

Metal Ions	Complex formation	Micellar system	Detection	LOD	P[a] F[b]	Ref
Er^{3+}	3,5-diCIDMPAP	PONPE 7.5	UV-Vis, 584 nm	1.48 x 10^{-7} mol/L	3.33[a]	[20]
Gd^{3+}	3,5-diCIDMPAP	PONPE 7.5	UV-Vis, 592 nm	5.8 x 10^{-9} mol/L	25[a]	[21]
Al^{3+}	CAS-BDTAC	PONPE 7.5	UV-Vis, 554 nm	1.12 x 10^{-7} mol/L	50[a]	[22]
V^{4+} V^{5+}	8-quinolinol	Triton X-114	Fluo[b]	0.0007 µg/L	50[a]	[23]
Cr^{3+} Cr^{6+}	8-HQ	Triton X-114	Fluo[b]	0.2 µg/L	75[a]	[24]
Ge	quercetin	Triton X-114	FAAS	0.59 µg/L	200[a]	[25]
Ag	dithizone	Triton X-114	FAAS	0.56 ng/mL	43[a]	[26]
Bi	dithizone	Triton X-114	ETAAS	0.02 ng/mL	196[a]	[27]
Pb	-	PONPE 7.5	ICP-OES	0.077 µg/L	>300[a]	[28]
Se(IV)	DDTP	Triton X-114	ICP-MS	0.02 µg/L	37	[29]

[a]Preconcentration factor = vol. aqueous phase vol. / surfactant-rich phase
[b]Fluorometric determination

1.4 Analytical Methods Used for Selenium Determination

In recent years, selenium analyses become important because of its health aspects. Selenium found naturally in biological materials that change from a few parts per billion to a few per cent. Methods for its determination, therefore, cover a wide range of concentrations. As a result a number of procedures and methods for selenium analysis have been developed.

The analytical methods to determine selenium generally seperated into two groups:
(1) those that do not require the destruction of materials in the sample and,

(2) those that require the elimination of interfering matter before the selenium content can be measured.

X-ray fluorescence and some of the Instrumental Neutron Activation Analysis (INAA) techniques do not require sample destruction, however Gas Chromatography (GC), atomic absorption spectrometry (AAS), polarography, titration, Mass spectrometry (MS), spectrophotometry, fluorimetry, and neutron activation analysis (NAA) techniques require some degree of sample destruction. Nowadays fluorimetry, atomic absorption spectrometry, and neutron activation analysis are the most frequently used methods for Selenyum determination.

For total Se determination, all the Se species must be converted into one form, usually Se (IV) using modifiers and a suitable reduction step. On the other hand, only Se (IV) provides a good signal for Hydride Generation-AAS (HG-AAS); conversion of other species into this form must be accomplished in sample solutions prior to analysis. HG-AFS is also occasionally used [1]. In Table 1.4 are shown some methods and their detection limits to determine Se in different matrices.

Table 1.4: Examples of selenium determination in various materials by different methods.

Materials	Detection Limit	Reagent	Method	Ref
Food, plants, tissue	$1x10^{-8}$ g/g	1,2-Diamino-4,5-dichlorobenzene	GLC[a]	[30]
Blood, urine, river water	$5x10^{-10}$ g	2,3-Diaminonaphthalene	GLC[a]	[31]
Tomato juice	25 ng/g	-	FAAS[b]	[32]
Water	10.1 µg/L	-	HG-AAS	[33]
Serum	2 ng		NAA	[34]

[a] Gas –liquid chromatography

[b] Flame Atomic Absorption Spectrometry

1.5 Fluorometric Determination of Selenium

Because of selenium's toxicological and physiological importance there has been increasing interest in the determination of selenium. Fluorimetric method is one of the simplest, least expensive and most versatile of all the methods to determine selenium. Fluorescence technique is not a direct method so it requires analyte derivatization. In general Fluorimetry has a low detection limit, high sensitivity and precision, but less widely applicable than the other techniques based upon absorption, because many species do not fluoresce [35, 36].

The use of complex forming reagents has been the main requirement for the determination of inorganic selenium by fluorimetry. It is based on piazselenol complex formation between the reagent and selenium. The common reagents are used; o-diamines, 3,3-diaminobenzidine [37], dithizone, o-phenylenediamine [38], 2,3-diaminonaphthalene [39], 8-hydroxyquinoline [40, 41], 2,3-diamino-1,4dibromonaphthalene [42]. 2,3-diaminonapthalene (DAN) is a widely used reagent because of its less toxicity and high availability [43].

The reactions with 2, 3-diaminonaphthalene compounds are specific for Se (IV). So, Se (VI) must be reduced to Se (IV) before complexing reaction. In recent studies [43, 44], the Se(IV)-DAN complex formed in aqueous medium must be extracted with organic solvent, in order to improve the sensitivity of the determination of Se (IV). The use of organic solvents can increase the intensity of fluorescence in aqueous medium due to changes in viscosity, micropolarity and reduction of quenching [44].

In this study, Fluorescence Spectrometry seemed to be a convenient technique to follow up the performance of the cloud point precocentration technique because of the fluorescence enhancement effect of surfactants due to their organized structure. Therefore; 2, 3-diaminonaphthalene was chosen as a complexing reagent because of

its selectivity and sensitivity for selenite Se (IV) in the fluorescence measurements and producing hydrophobic complex, 4,5-benzopiazselenol.

1.6 Aim of This Study

The aim of this study is the determination of selenium at trace levels by means of cloud point extraction and fluorescence spectrometry. In the cloud point extraction process, selenium (IV) is taken into a hydrophobic complex and then solubilized within the nonionic micelle. In this study, Triton X-114 was used as the nonionic surfactant, and 2, 3-diaminonaphthalene (DAN) was used as the complexing agent.

CHAPTER 2

EXPERIMENTAL

2.1 Chemical and Reagents

i) Se (IV) stock solution (1000 mg/L Se): Prepared by dissolving 0.219 g sodium selenite; Na_2SeO_3 (Ventron), in 100 mL de-ionized water.

ii) Se (IV) solution (1 mg/L Se): Prepared from Se (IV) stock solution (1000 mg/L) by dilution with de-ionized water. Working standard solutions were prepared from this solution by appropriate dilution using 0.10 M HCl.

iii) Se (VI) stock solution (1000 mg/L Se): Prepared by dissolving 0.239 g sodium selenate; Na_2SeO_4 (Vetron), in 100 mL de-ionized water.

iv) Se (VI) solution (1 mg L^{-1}): Prepared from Se (VI) stock solution (1000 mg L^{-1}) by dilution with de-ionized water. Working standard solutions were prepared from this stock solution by appropriate dilution using 0.1 M HCl.

v) 2, 3-diaminonaphthalene (DAN) solution (0.10 %, w/v): Prepared by dissolving 1.0 mg of 2, 3-diaminonaphthalene (Aldrich) and 0.050 g Hydroxylamine hydrochloride (Aldrich) in 1.0 mL of 0.10 M HCl. To provide dissolution, the contents were heated at 50 °C for 25 min in a water bath. Due to its sensitivity to light, this solution was freshly prepared each day and stored in dark.

vi) 5.0 % (w/v) Triton X-114: Prepared by diluting 1.0 g of Triton X-114 (Sigma) in 20 mL cold de-ionized water. Triton X-114 (octylphenoxypolyethoxyethanol), has been used as nonionic surfactant from Sigma. The critical micellar concentrations of Triton X-114 is 2.1×10^{-4} M (0.0113 (w/v). Cloud point temperature of Triton X-114 in aqueous solution is 22-24 $^{\circ}$C, its molecular weight is 537 g/mol [45].

vii) 0.30 % (w/v) Sodium tetrahydroborate solution (Merk) prepared daily and stabilized by addition of sodium hydoxide (Carlo Erba).

viii) Concentrated Hydrochloric acid, 37% (w/w), J. T. Baker, was used throughout the hydride generation.

ix) 6.0 M Hydrochloric acid: Prepared by diluting concentrated HCl 1:1(v/v) with de-ionized water.

x) Cyclohexane, Merck, was used as organic solvent for extraction.

xi) Ethylenediamine-N,N,N,N-tetra-acetic acid di-sodium salt, EDTA, SURECHEM PRODUCTS LTD.

xii) Antifoam-A, Sigma

All other reagents were of analytical-reagent grade. De-ionized water obtained from a Millipore water purification system was used for sample and standard preparations. All the glassware and plasticware were cleaned by soaking them in 10.0 % HNO_3 for at least 24 hours and then rinsing three times with distilled water and with deionized water.

2.2 Instrumentation

2.2.1 Fluorescence Spectrometer

A Perkin-Elmer LS-50 B Luminescence Spectrometer (Figure 2.1) equipped with a xenon lamp was used for fluorescence measurements. A micro fluorimeter glass cell (Light Path: 10 mm, Cell Volume: 700 µL) was used for fluorescence measurements and fluorescence intensity versus wavelength was recorded. A Front Surface Accessory was used for solid surface measurements (Figure 2.2). Spectrometer has two grating monochromators, one for the excitation wavelength and the other for the emission wavelength. With the instrument, both excitation and fluorescence spectra can be obtained.

Wavelength of 379 nm was set for excitation and the emission intensity as function of wavelength 582 nm was recorded for organized media. The excitation and emission slits were 15 nm.

Figure 2.1: Perkin Elmer LS-50 B Luminescence Spectrometer

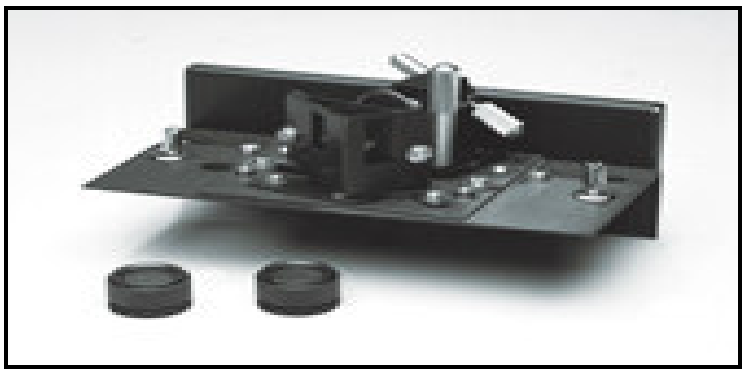


Figure 2.2: Front Surface Accessory for the LS 50 B

2.2.2 HG-AAS

VARIAN AA140 Atomic Absorption Spectrometer was used for measurements at 196 nm resonance line of selenium with band pass of 1.0 nm. Ultra lamp with (Varian) a maximum current of 15 mA was used as radiation source. Gilson Miniplus peristaltic pumps were used for the carrying of the reagents.

In the hydride generation system, all parameters were firstly optimized to improve sensitivity of selenium. The optimization results are given in Table 2.1.

Table 2.1 Instrumental Parameters Used for the Determination of Selenium with HG-AAS.

Parameter	Optimization results
Concentration of $NaBH_4$	0.3 % (w/v) in 0.5 % of NaOH
Concentration of HCl	6.0 M
Flow rate of carrier solution	6.5 mL/min
Flow rate of reduction solution, $NaBH_4$	4.0 mL/min
Flow rate of argon	75 mL/min
Loop volume	500 µL
Length of reaction coil	30 cm
Length of stripping coil	35 cm

In the HG-AAS system, linear range was found between 2-50 ng/mL of Se (IV).

2.3 Procedure

Steps of complex formation and preconcentration of Se (IV)-DAN complex are shown on the Figure 2.3. First Se (IV) reacts with appropriate amount of DAN in the optimized conditions, and then Cloud Point Extraction (CPE) is applied. Experimentally, Cloud Point Extraction procedure is quite simple as shown in the Figure 2.3. First the non-ionic surfactant (optimized quantity) is added to the aqueous solution containing analyte. The final surfactant concentration must be greater than the critical micelle concentration. If temperature of the solution is increased, the surfactant solution becomes turbid because it attaints the cloud point. At this point, the original surfactant solution separates into two phases. One of the phases is a surfactant phase of small volume and rich in the surfactant and containing the analyte trapped by micellar structures and the other one is aqueous phase containing at the critical micelle concentration values of surfactants. After centrifugation two phases are easily separated and aqueous phase is decanted. So analyte can be extracted into a small volume (50-400µL).

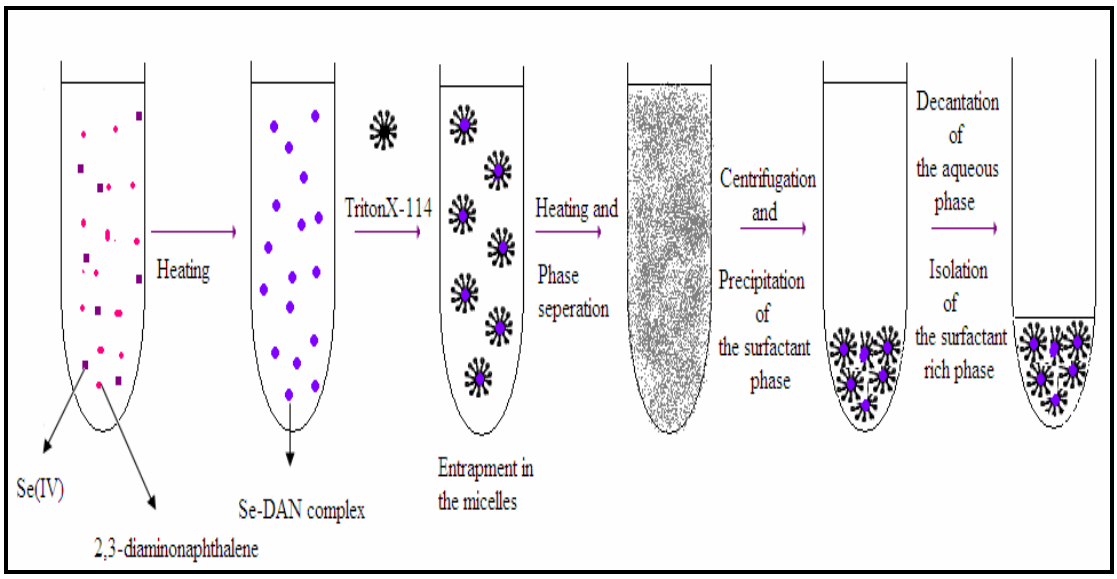


Figure 2.3: Procedure steps of complex formation and preconcentration of complex by surfactant.

2.4 Optimization of the Parameters for the Formation of Se (IV)-DAN Complex in Cyclohexane

In order to increase the sensitivity of the fluorimetric method, different experiments were performed to optimize the experimental parameters. The studied parameters were pH, reaction time and reaction temperature for the formation of Se (IV)-DAN complex, ligand concentration, effect of pH to CPE, surfactant concentration. These optimizations except surfactant concentration were followed by measuring the fluorescence signal of Se-DAN complex after extraction into the organic phase (cyclohexane).

2.4.1 pH Dependency of Complex Formation

To investigate the effect of pH, some solutions containg 0.10 mg/L Se (IV) were prepared the final pH values of solutions varied from 1.0 to 5.0. The pH values of the solutions were adjusted with NH_3 or dilute HCl by using pH meter. After pH

adjustment, 50 μL of 0.10 % (w/w) DAN were added to each solution. Each test tube was covered by aluminium foil because of light sensitivity of DAN and than was kept for 45 min at 70 °C water bath. After Se (IV)-DAN complex formed, it was extracted into 5.0 mL cyclohexane. Following these steps, the phase containing analyte is separated, dissolved in cyclohexane and measurement is made by using fluoresence technique. The emission fluorescence signals were measured at 520 nm. Conditions: λ_{ex}= 379 nm, λ_{em}= 582, slit width= 15 nm, scan rate= 500 nm/min.

2.4.2 Reaction Period and Reaction Temperature for Complex Formation

There are different temperatures given in the literature for the formation of Se (IV)-DAN complex. So, in this study different temperatures were tested for finding appropriate temperature for the formation of Se (IV)-DAN complex.

For that purpose the following experiments were performed: 10 mL of 0.10 mg/L standard Se (IV) solution was reacted with 50 μL of 0.10 % (w/w) DAN solution ([DAN] / [Se(IV)] =50) at pH 1.5 in the centrifuge tubes, the contents were covered with aluminium foil and placed in a water bath at specified temperatures. The temperature was varied from 50°C to 90°C. The period of waiting for complex formation varied from 10 min to 60 min for each temperature.

At the end of heating period, Se (IV)-DAN complex was extracted into 5.0 mL cyclohexane and fluorescence intensity was measured using conditions given in Section 2.3.

2.4.3 Effect of 2, 3-Diaminonaphthalene (DAN) Concentration

In order to find the necessary amount of DAN solution to be used in the formation of Se (IV)-DAN complex, similar procedure as before was applied for preparation of Se-DAN complex. 6 solutions were prepared in which the concentration ratio of DAN to

0.10 mg/L Se (IV) were varied from 5 to 1000. For that 5, 25, 50, 100, 500, 1000 µL of 0.10 % (w/w) DAN were added to 10 mL solutions containing 0.10 mg/L Se(IV) standard. The pH of solutions was adjusted before adding DAN. The tubes were covered with aluminium foil and they were placed in water bath. The solution temperature was kept constant at 70 °C in the water bath for 45 min. After heating period, Se (IV)-DAN complex was extracted into 5.0 mL of cyclohexane and fluorescence intensity was measured using conditions given in Section 2.3.

2.5 Optimization of the Parameters for Cloud Point Extraction of Selenite

2.5.1 Effect of pH to Extraction Efficiency

To investigate the effect of pH to CPE a set of solutions containing 0.10 mg/ L Se (IV) standard and 50 µL 0.1 % of DAN were prepared, before adding DAN pH of the solutions were adjusted by using 1.0 M NH_3 or 0.10 HCl. Final pH of the solution varied in the range of 1.0 - 5.0. The final volume of the solutions was 10.0 mL. The tubes were covered with aluminium foil and placed in water bath. Then complex formation conditions were applied; the solutions placed the water bath at 70 °C for 45 min. After complex was formed, the surfactant solutions added and the cloud point extraction was applied. Surfactant rich phase was diluted to 1.0 mL and fluorescence intensity was measured using conditions given in Section 2.3.

2.5.2 Effect of Surfactant Concentration

To determine the effect of surfactant percentage, a set of solutions was performed. 0.150 mg/L Se (IV) was allowed to react with 75 µL of 0.1 % (w/v) DAN solution ([DAN]/ [Se (IV)] = 50) at pH 1.5 in the centrifuge tubes. The final volume of the solutions was 10.0 mL. After the complex formed in the optimized conditions for complex formation Triton X-114 was added to the solution where the surfactant

concentration was varied from 0.1% (w/v) to 0.5% (w/v), and the contents were mixed. Then 30 minutes was allowed at refrigerator for micelles formation and also to entrap analyte to the micelle. After the period of waiting samples firstly were heated for 10 min at 50 °C and then centrifuged 5.0 min at 4500 rpm. Then the centrifuge tube was removed and kept in an ice bath to effect and see the phase separation easily. Then aqueous phase was discarded by pipet and surfactant rich phase was diluted to 1.0 mL and mixed. The emission signal was measured using conditions given in section 2.3.

2.6 Calibration Plot in Surfactant Rich Phase

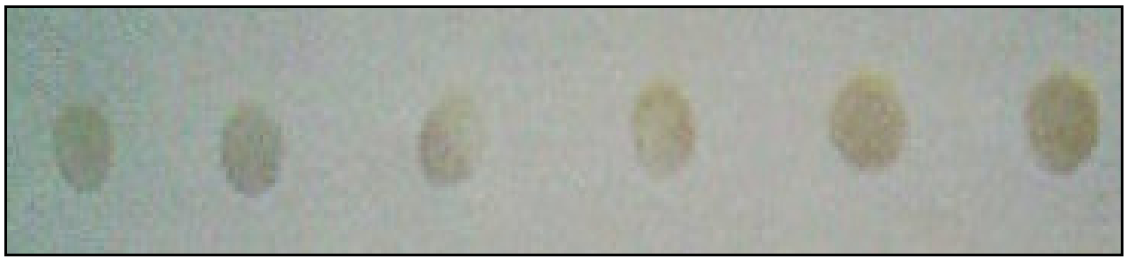
Se (IV) standards with concentrations varing from 0.02 mg L^{-1} to 0.70 mg L^{-1} were used to obtain the calibration curve.

Se was allowed to react with 0.10 % of DAN solution. Firstly stated amount of Se (IV) was added into the tube. The solution pH was adjusted to 1.5 by dropwise addition of 0.1 N HCl by using pH meter and then DAN solution was added to the solutions. The concentration ratio of DAN to Se (IV) was equal to 50 in the final solution. The volume of the solution was completed to 10.0 mL. In order to form Se (IV)-DAN complex contents were kept at 70 °C in a water bath for 45 min. The formed Se (IV)-DAN complex was CP extracted into surfactant phase using 0.25 % (w/v) of Triton X-114. After adding Triton X-114, 30 minutes was allowed at refrigerator for micelle formation and also to entrap analyte. After the period of waiting samples firstly were heated for 10 min at 50 °C and then centrifuged for 5 min at 4500 rpm. Then the centrifuge tube was removed and kept in an ice bath to effect and see the phase separation easily. Aqueous phase was separated from surfactant rich phase by a pipette and discarded. Then the volume of the surfactant rich phase remaining in the centrifuge tube was diluted to 1.0 mL with de-ionized water and the fluorescence emission signal was measured using conditions given in

section 2.3. Fluorescence intensity versus concentration of Se standards was plotted using the initial Se (IV) concentration.

2.7 Solid Surface Fluorescence

In order to see fluorescence intensity of Se (IV)-DAN complex on solid surface, a set of solutions where Se (IV) concentration ranging from 0.01 mg/L to 0.35 mg/ L were prepared. After CPE 25 µL (17 mg) of surfactant rich phase was taken by the help of automated pipet and dropped into glass surface and dried for 30 min at room temperature. In the Figure 2.4 the shapes of the dropped complex that their concentrations are 0.01 mg/L, 0.02 mg/L, 0.05 mg/L, 0.1 mg/L, 0.15 mg/L and 0.2 mg/L from left to right on filter paper were seen. The emission signal was recorded by using solid surface apparatus (Figure 2.2) and using conditions given in section 2.3.



(a) **(b)** **(c)** **(d)** **(e)** **(f)**

Figure 2.4 Representation of Se-DAN complex on filter paper.

2.8 Interference Studies

In order to see the effect of foreign ions to the Se(IV)-DAN complex formation and micellar extraction of Se(IV)-DAN complex 0.070 mg/L Se (IV) was reacted with 50 µL of 0.10 % DAN in the presence of potential interfering ions 1.0 mg/L Cu^{2+}, Sn^{4+}, Al^{3+} and Fe^{3+} to complex formation. To remove interference effect of these ions

EDTA was used. Four different EDTA concentrations; 0.00250 M, 0.003750 M, 0.0050 M and 0.00750 M were studied. In order to adjust EDTA concentration 0.050 M stock EDTA solution was prepared. 0.50, 0.75, 1.00 and 1.50 mL of 0.05 M of EDTA were added to the each solution. Before adding EDTA, the pH of solution was adjusted to 1.5 by using dilute HCl. The volume of the solution was made up to 10.0 mL. For complex formation each tube was kept for 45 min at 70 °C in water bath. The formed Se (IV)-DAN complex was CP extracted into surfactant phase using 0.25 % (w/v) of Triton X-114. After addition of Triton X-114, the solutions were kept at refrigerator for 30 minutes. Then the samples were heated for 10 minutes at 50 °C and then centrifuged 5 min at 4500 rpm. Samples were preperad as mentioned in Section 2.6. Then the volume of the surfactant rich phase remaining in the centrifuge tube was diluted to 1.0 mL with de-ionized water and the fluorescence emission signal was measured using conditions given in Section 2.3.

2.9 Analysis of Real Sample (SRM)

0.1 mL EnviroMAT – Waste Water LOW EU- L – 1 was taken and diluted to 5.0 mL with deionized water and 5.0 mL concentrated HCl were added. By this way the final HCl concentration was equal to 6.0 M. For the reduction of Se (VI) to Se (IV), each sample was heated gently until one drop of sample remains at the bottom of Teflon beaker; vapor was swept by the help of hair dryer. Remaining sample solution was diluted with deonized water and the pH of the solution was adjusted to 1.5 by using di 0.1 M HCl or 1.0 M NH_3 if necessary. For the preparation of the standards exactly the same procedure was followed: Se (VI) standards were prepared in 6M HCl with the final concentrations of 0.0250, 0.0500, 0.0750, 0.100 and 0.1250 mg/L and then Se (VI) was reduced to Se (IV) through gentle heating produced vapor of HCl was swept by hair dryer. Remained sample was diluted with deonized water and the pH of the solution was adjusted to 1.5 by using 0.1 M HCl or 1.0 M NH_3 if necessary. In the final solution EDTA concentration should be 0.0025 M to remove the effect of

interfering ions effects. After this 50 μL of 0.1% DAN were added to each standard and sample solution. And then complex formation and CPE procedure were applied respectively. Aqueous phase was separated from surfactant rich phase by a pipette and discarded. Then the volume of the surfactant rich phase remaining in the centrifuge tube was diluted to 1.0 mL with de-ionized water and the fluorescence emission signal was measured using conditions given in section 2.3.

CHAPTER 3

RESULTS AND DISCUSSION

In this study the cloud point extraction of selenite with DAN was carried out and formed fluorescing Se (IV)-DAN complex was determined by spectrofluorometry. Throughout the study, the spectral characteristics of DAN and Se (IV)-DAN complex were investigated, the parameters affecting the complex formation in organic and aqueous media, the cloud point extraction, the fluorescence measurement for selenite were discussed, the importance and convenience of solid surface fluorescence measurements in cloud point extraction were explored, the interference effects were studied and figures of merit for the determination of selenite with this methodology were measured and compared with the ones given in literature.

3.1 Reaction between DAN and Selenium (IV)

The main requirement of cloud point extraction is that extracted complex should be in the hydrophobic form [13]. Harrison et al. investigated the structure of the Se(IV)-DAN complex by using FTIR, NMR, mass spectrometric and elemental analysis techniques [46]. The reaction of selenite with DAN is believed to produce the hydrophobic compound 4, 5-benzopiazselenol (Se(IV)-DAN) having the following structural formula, Figure 3.1 [47].

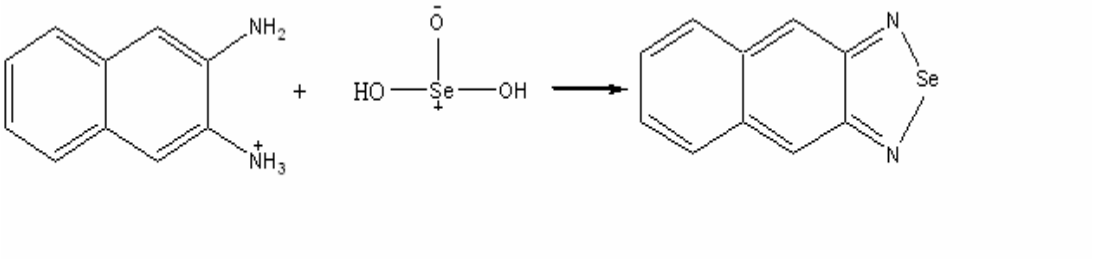


Figure 3.1: The structural formula of Se(IV)-DAN complex proposed by Cukor et.al. [48].

3.2 Fluorescence Spectra of 2, 3-diaminonaphthalene (DAN) and Se (IV)-DAN Complex

2,3-diaminonaphthalene (DAN) is a fluorescent material. In Figure 3.2, excitation and emission spectra of DAN are shown; the excitation and emission wavelength maxima are at 343 and 400 nm, respectively.

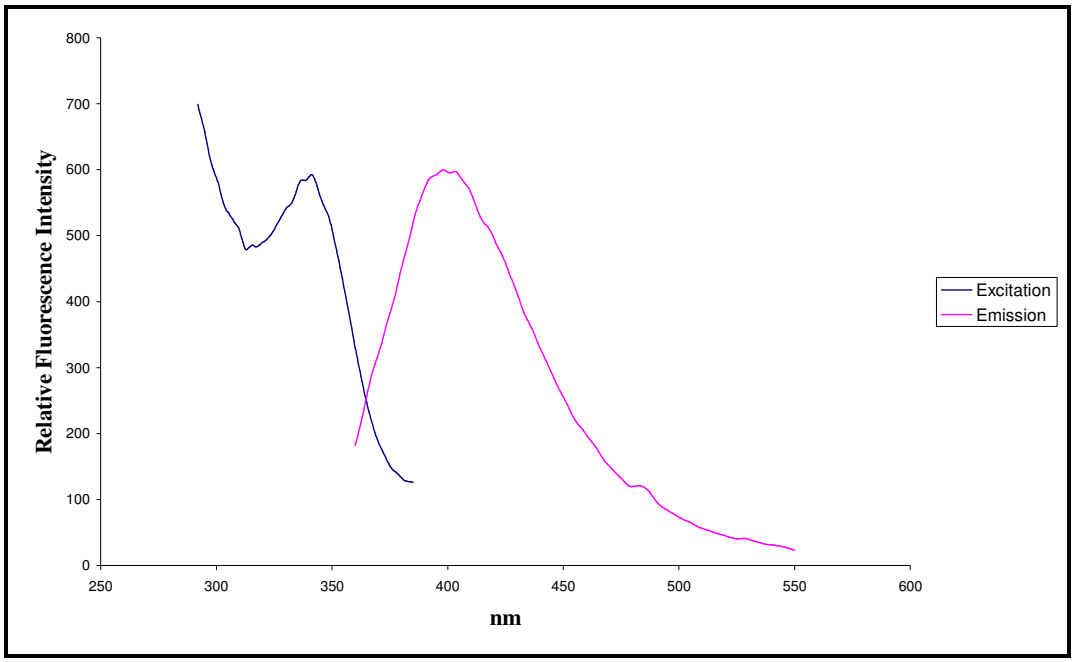


Figure 3.2: Excitation and emission spectra of 10 mg/L of 2,3-diaminonapthalene (DAN) in cyclohexane.

The excitation and emission spectra of Se(IV)-DAN complex are given in Figure 3.3. As can be seen from the figure, after the formation of Se(IV)-DAN complex the excitation and emission wavelengths of Se (IV)-DAN observed to be different from DAN are 379 nm and 582 nm respectively. The difference in wavelength particularly in the emission spectrum is the basis of selenium determination using DAN as a complexing agent. During complex formation excess amount of DAN is required for the completion of the reaction. Therefore it is very important to be able to differentiate the fluorescence signal of DAN from that of Se (IV)-DAN complex. In Figure 3.3, the blank signal is corresponding an example to the emission spectrum of 50 fold excess DAN. The maximum emission wavelenght of Se(IV)-DAN complex shows variations depending on the medium in which the spectrum is recorded. Therefore in our studies wavelegth of the maximum emission of Se(IV)-DAN complex was determined at each medium (organic solvent and organize media) and fluorescence measurements were carried out at that wavelegth.

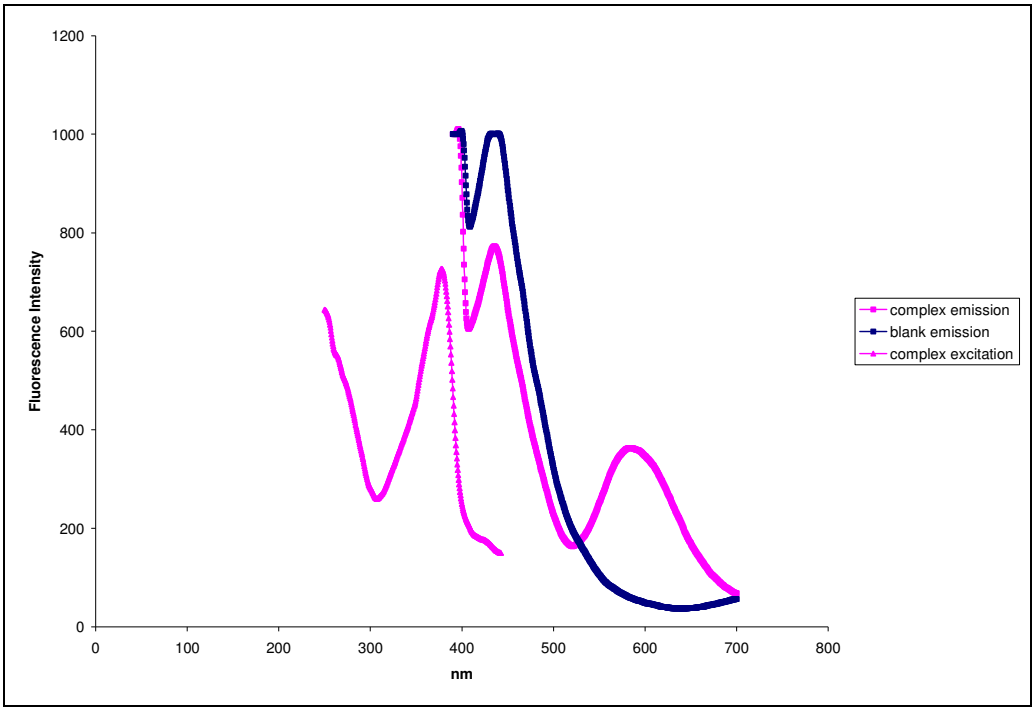


Figure 3.3: Excitation and emission spectra of Se(IV)-DAN complex and blank emission at the excitation wavelength of the complex (λ_{ex} = 379nm).

3.3 Optimization of the Parameters for the Formation of Se-DAN Complex in Cyclohexane

Previous studies [46, 49, 50] indicated that the reaction between Se(IV) and 2,3-diaminonapthalene (DAN) was greatly influenced by pH, temperature, time employed for complex formation and ligand concentration.

The optimizations of the parameters related with the complex formation were carried out by extracting the Se(IV)-DAN complex into cyclohexane phase.

3.3.1 Effect of the pH of the Medium on Se (IV)-DAN Complex Formation

In order to find the optimum pH to yield the maximum fluorescence intensity, solutions containing 0.10 mg/L Se(IV) standards were prepared and their pH valueswere adjusted to 1.0 - 5.0 by the addition of either NH_3 or HCl . Finally 50 µL of 0.10 % DAN were added to each solution prior to the measurement. DAN is not stable in strong acid medium [51], therefore the adjustment of the pH was carried out before the addition of DAN. The final pH values of the solutions were measured and used in Figure 3.4. As can be seen from the Figure 3.4 the most suitable pH range for the complex formation is in between 1.5 and 2. Therefore, pH 1.5 was used throughout this study.

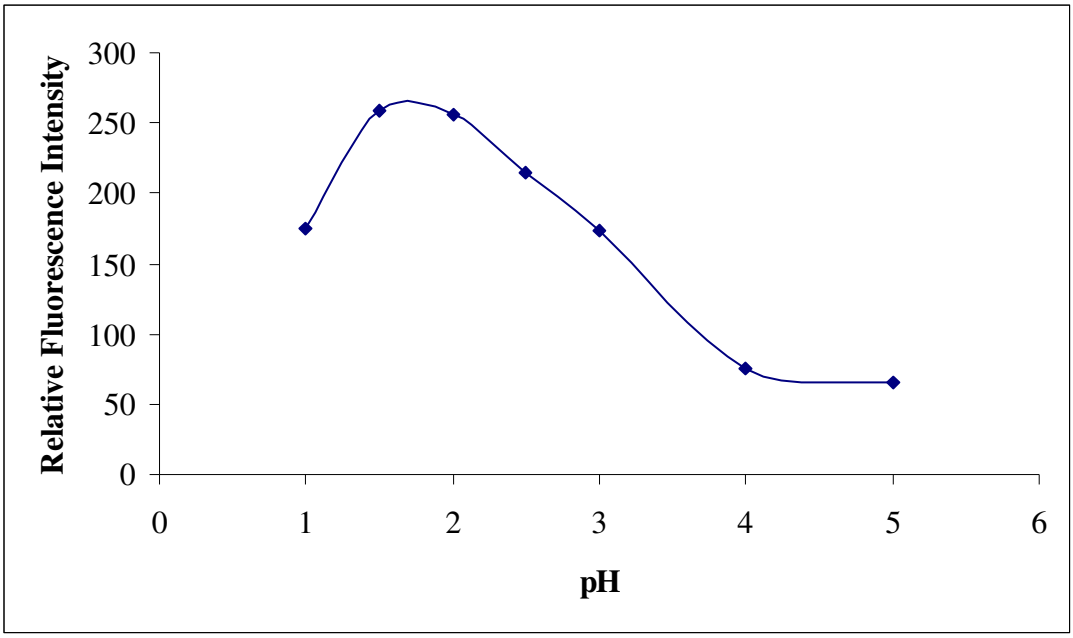


Figure 3.4 Effect of pH on the formation of Se(IV)-DAN complex.

3.3.2 Effect of Reaction Temperature and Reaction Period on Se (IV)-DAN Complex Formation

In order to investigate the duration and the temperature of the complex formation several experiments were performed. One parameter was changed at a time. When reaction period was changed the temperature was kept constant, and when the temperature was changed the reaction time was kept constant. The formed complex was extracted into cyclohexane. Studied reaction time of the complex formations was varied from 10 to 60 minutes and reaction temperature of the complex formation was varied from 50º to 90º C. Results are tabulated in Table 3.1.

Table 3.1: The effect of reaction temperature and time on the fluorescence intensity of the Se(IV)-DAN complex obtained from 0.10 mg/L Selenite standard.

Time (min)	**50ºC**	**60ºC**	**70ºC**	**90ºC**
10	38	45	61	113
20	48	64	98	127
30	76	100	127	**153**
45	103	113	**147**	138
60	101	139	132	123

The rate of complex formation is temperature dependent [46]. It is obvious that the higher the temperature the lower the time required obtaining the best yield. However at high temperature Se(IV)-DAN complex may decompose [52], the rate of the air oxidation of DAN increases and sample loss occurs if $SeCl_4$ has been produced in acidic medium. As expected fluorescence intensity increases when the temperature increases, Table 3.1. Although the maximum fluorescence intensity was observed at 90 ° C with the duration time of 30 min, we decided to use 70 ºC and 45 minutes as the optimum temperature and duration respectively for the reaction. Because it is more practical to work at lower temperature and there is a small difference (% 4) between the fluorescence intensities obtained at the specified reaction conditions.

3.3.3 Effect of Ligand Concentration on Se (IV)-DAN complex formation

In order to find the necessary amount of DAN solution to be used in the experiment, a set of experiments containing different concentration of DAN were performed.

As can be seen from Figure 3.5, the fluorescence intensity increases as the DAN to Se(IV) ratio increases from 20 to 50, becomes steady as the ratio varies from 50 to 500 and decreases slightly afterwards. As reported in the literature [49] when the ratio of DAN to selenium was increased above 50, blank readings were also increased. Besides turbidity was developed during the cloud point extraction in micelle media, therefore DAN to selenium concentration ratio was set to 50 through out this work.

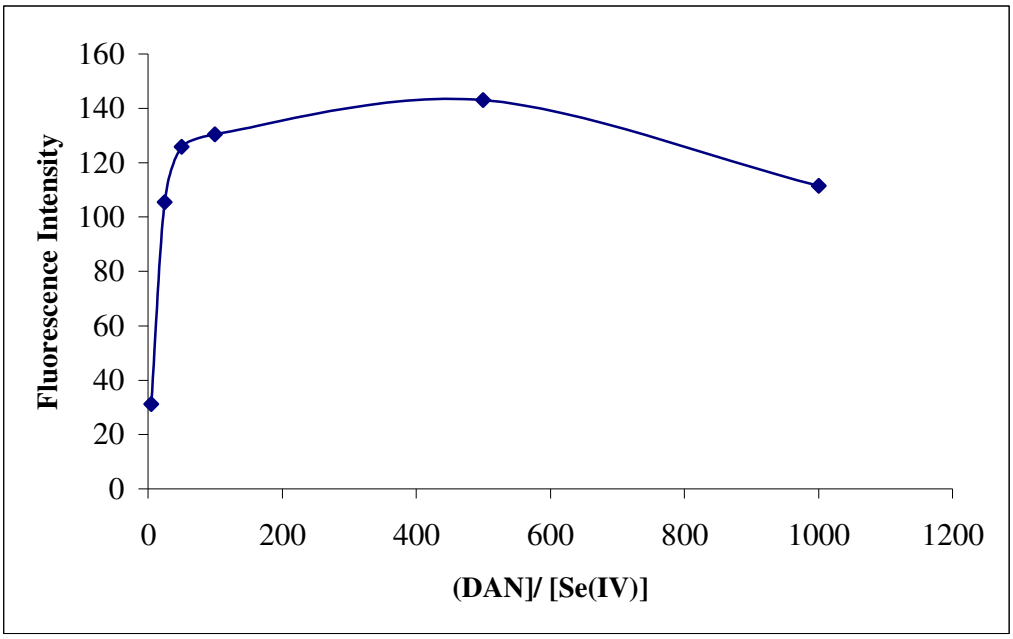


Figure 3.5: Effect of 2, 3-diaminonaphthalene concentration on fluorescence intensity of Se(IV)-DAN complex.

3.4 Optimization of the Parameters for Cloud Point Extraction of Selenite

For inorganic species, the formation of a hydrophobic complex is an essential prerequisite for efficient CPE. Hence the properties of the surfactant system have to be optimized taking into account the variables of complex formation [13]. In our studies DAN and Triton X-114 were selected as the complexing reagent and

surfactant respectively for the CPE. In the following sections surfactant concentration and pH were examined to accomplish a successful CPE.

3.4.1 The Effect of pH on the CPE Efficiency of Selenite

It has been stated that in the case of metal chelates, the optimal pH range for CPE frequently matches the range of the most favorable complex formation [53]. In the previous part (Section 3.3.1), pH 1.5 was found as the optimum pH for the formation of Se-DAN complex in cyclohexane. Similar pH optimization studies were carried out at Triton X- 114 medium in order to find the optimum pH for CPE. As can be seen in Figure 3.6 the optimum pH for complex formation (pH 1.5) also provides the highest extraction efficiency for the CPE. Therefore all CP extractions were carried out at pH 1.5 through out this study.

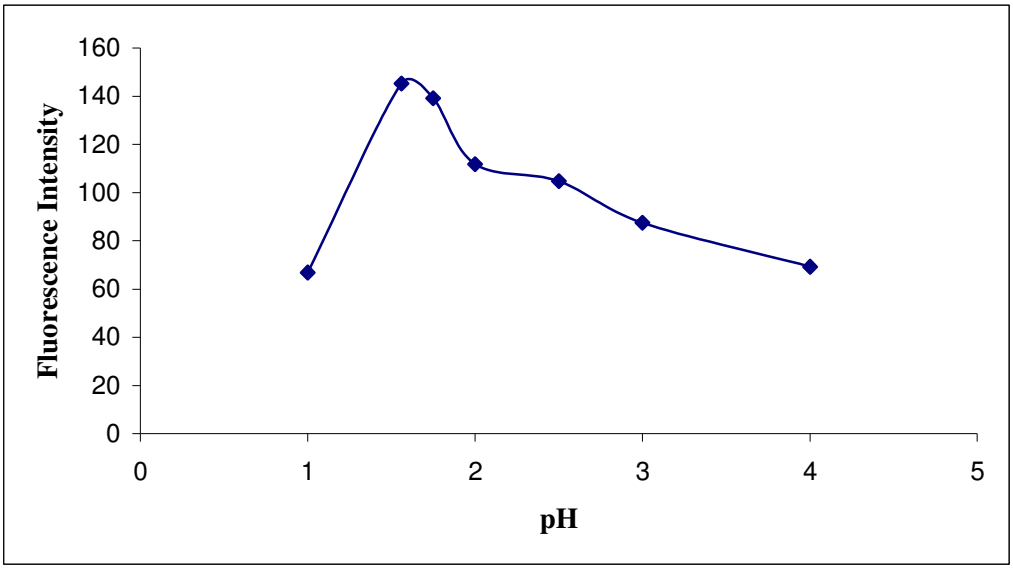


Figure 3.6: Effect of pH on cloud point extraction efficiency

3.4.2 The Effect of TritonX-114 Concentration on the CPE Efficiency of Selenite

The concentration of the surfactant used in CPE is a very critical parameter. Because the volume of the extracted layer and associated with it the preconcentration factor

depend on the nature and the total amount of the surfactant employed. In most extraction experiments given in literature [6, 13], the surfactant concentration is kept in the range of 0.1 to 2.0 % w/v, with a corresponding volume of the extraction layer being between 2 and 10 % of the initial solution volume.

To see the effect of surfactant concentration on the extraction efficiency and the fluorescence intensity of the Se(IV)-DAN complex, 0.15 mg/L Se (IV) standard was reacted with appropriate amount of DAN and CPE was performed with different surfactant concentrations between 0.10 % and 0.50 % Triton X-114. As can be seen in the Figure 3.7, the fluorescence signal reaches a plateau after the usage of 0.25 % (w/v) Triton X-114. However as the surfactant concentration increases, volume of the surfactant rich phase also increases. For example; when we used 0.10 % Triton X-114, the volume of the surfactant rich phase was about 30-40 µL (or 40 mg) whereas the volume of the surfactant rich phase was approximately 100 µL (90 mg) when 0.25 % Triton X-114 was used. Hence, at low surfactant concentrations the preconcentration factor increases. In general, the volume of the surfactant rich phase is between 20-500 µL. In this study 0.25 % surfactant concentration was decided to be used due to the high extraction efficiency and the preconcentration factor obtained at this concentration.

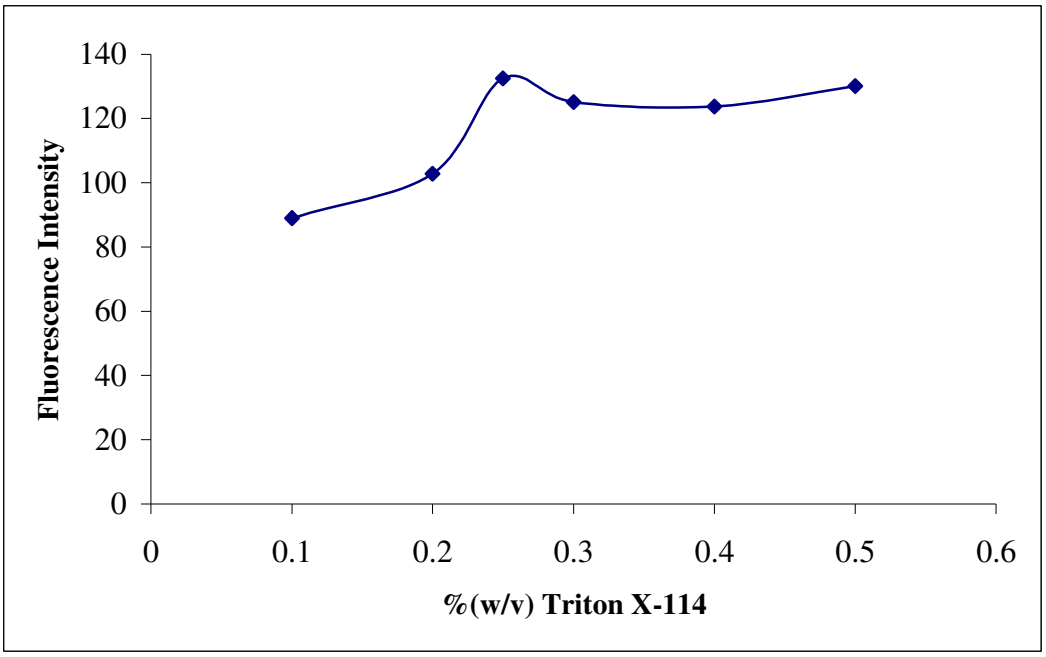


Figure 3.7: Effect of surfactant concentration on the fluorescence intensity of Se(IV)-DAN complex

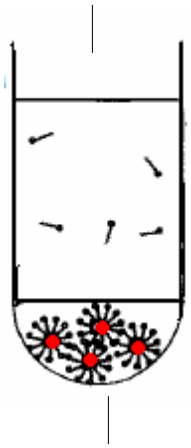
3.5 Recovery Studies for the CPE of Selenite Using HGAAS

The extraction efficiency of CPE method at two different surfactant concentrations were evaluated by using Hydride Generation- Atomic Absorption Spectrometry for the measurement of Se(IV) in the aqueous phase after CPE.

0.50, 0.10, 0.15 and 0.20 mg/L Se (IV) were reacted with appropriate amount ([DAN]/[Se(IV)] =50) of DAN and the formed complex was extracted by CPE with 0.10 % and 0.25 % Triton X-114. After centrifugation, the aqueous phase was transferred to an empty tube by a pipette, HCl was added in order to adjust its concentration to 6 M in the final solution for hydride generation. The experimental conditions used for HG-AAS are given in Section 2.2.2. The surfactant concentration of aqueous phase was expected to be equal to critical micelle concentration of Triton X-114 was 0.020 mM. In the absence of antifoaming agent bubbles were formed

during hydride generation and disturbed the signal measurements. Therefore 150 µL 10 % Antifoam-A was added to each solution.

The selenium concentration in aqueous phase was determined by HG-AAS.



The selenium concentration in the surfactant rich phase was determined by spectrofluorimetric method.

Figure 3.8: The cartoon represantation of phase seperation in cloud point extraction

Table 3.2: The percent recovery of Se(IV) in CPE when 0.25 % Triton X-114 and 0.1 % Triton X-114 aqueous solutions were used as solvent.

Initial Concentration	**Concentration in Aqueous Phase (µg/L)**		**Percentage in Aqueous Phase**		**Percentage in Surfactant Rich Phase**	
	0.10%	**0.20%**	**0.10%**	**0.25%**	**0.10%**	**0.25%**
0.050 mg/L Se (IV)	1.40	1.50	3.0	3.0	97.0	97.0
0.100 mg/L Se (IV)	1.21	1.19	1.0	1.0	99.0	99.0
0.150 mg/L Se (IV)	1.29	1.27	1.0	1.0	99.0	99.0
0.200 mg/L Se (IV)	1.01	1.13	1.0	1.0	99.0	99.0

As shown in the Table 3.2, the efficiency of the CPE is above 97 % at the stated concentrations. The slight decrease in the efficiency at low concentration (0.050 mg/L) of Se (IV) is most likely related to the difficulty in measuring such a low concentration (1.40 μg/L Se (IV) by HGAAS in a CMC of surfactant containing medium.

Besides, as can be seen from the Table 3.2, the extraction efficiencies calculated by using HGAAS measurements for two different surfactant concentrations, 0.10 % and 0.25 % Triton X-114, are the same. This fact is very important in terms of concentration factor. As mentioned in the previous section, the surfactant concentration directly affects the volume of the surfactant rich phase collected in the centrifuge tube and the concentration factor. In our studies the surfactant rich phase was diluted to 1.0 mL for solution phase fluorescence measurements. Therefore the concentration factor was not affected by the concentration of the surfactant. However, if surfactant rich phase is measured without any dilution, higher concentration factor can be obtained by using 0.10 % Triton X-114.

The similarity in the extraction efficiency for the given Triton X-114 percentages seems contradictory to our previous results shown in Figure 3.7. But according to our opinion, the low fluorescence intensity acquired for Se(IV)-DAN complex in the surfactant rich phase of CPE carried out with 0.10 % Triton X-114 was related to the decrease in fluorescence efficiency in dilute micellar media rather than the decrease in CP extraction efficiency.

3.6 Comparision of the Fluorescence Intensities of Se (IV)-DAN in Organic and Aqueous Media

The fluorescence intensities of Se-DAN in organic medium were compared with that in water; in aqueous solution of an anionic detergent and a cavity forming reagent (β-

CD/SDS) and in a concentrated aqueous solution of non ionic detergent Triton X-114 (CPE) at the optimized conditions.

3.6.1 Fluorescence Intensity of Se (IV)-DAN Complex in Cyclohexane

The fluorimetric determination of selenium is usually performed in organic solvents such as cyclohexane, methanol, acetonitrile, 1,4-dioxane [44, 54] due to its extended fluorescence. Among them Cyclohexane is the commonly used one [43]. Figure 3.9 gives the fluorescence spectrum of Se(IV)-DAN complex of 0.20 mg/L selenite in cyclohexane.

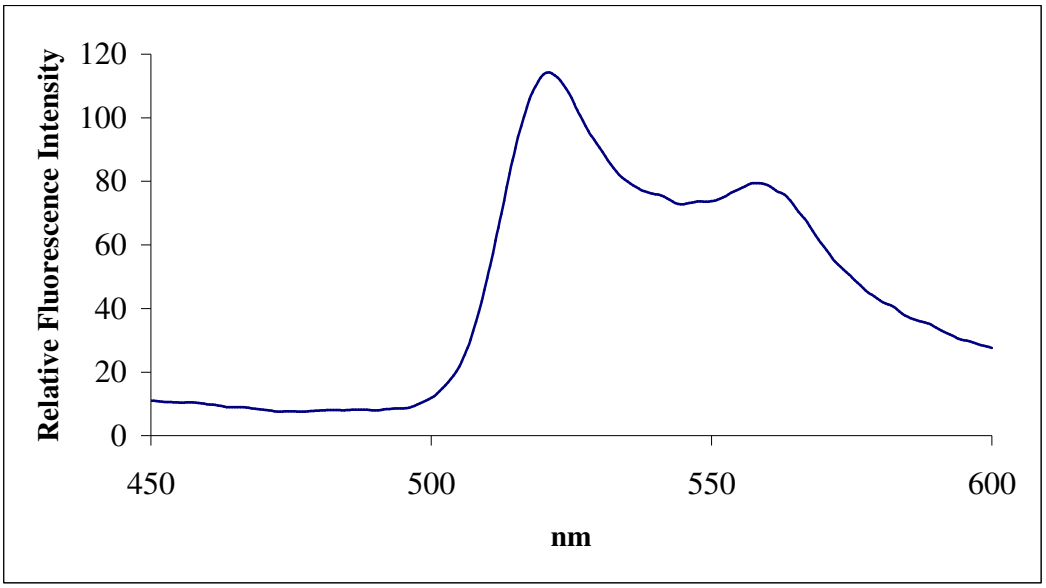


Figure 3.9: Fluorescence emission spectrum of Se(IV)-DAN complex in cyclohexanxane. (λ_{ex}=379 nm)

3.6.2 Fluorescence Intensity of Se (IV)-DAN Complex in Aqueous Media

Se(IV)-DAN complex has very low intensity in aqueous solution. As can be seen in the Figure 3.10, when 2.0 mg/L Se (IV) is prepared in water, it gives only 7.78 units of relative fluorescence intensity at 520 nm.

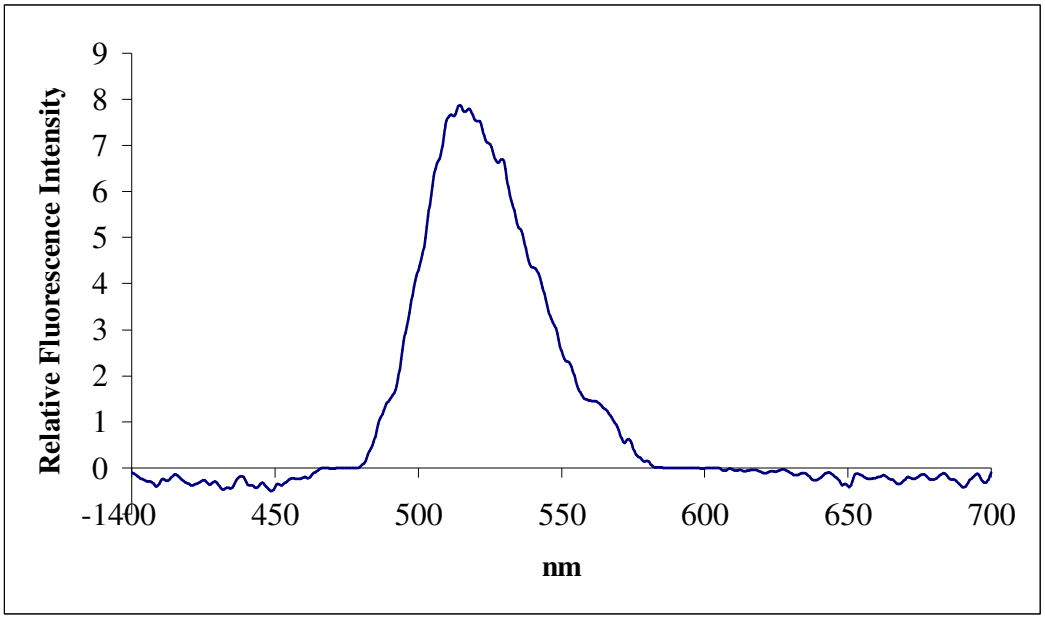


Figure 3.10: Fluorescence emission spectrum of Se(IV)-DAN complex in water. (λ_{ex}=379 nm)

Cavity like structures or surfactant-based organized assemblies, also referred to as "organized" or "ordered" media, may drastically change the spectral characteristics of fluorescent compounds due to the establishment of a special microenvironment for reactions at a molecular level. The properties of the species confined inside these assemblies are fundamentally different from the corresponding species in bulk [55].

Pedro et. al. reported the enhancing effect of addition of β –cyclodextrin (β-CD)-sodium dodecyl sulphate(SDS) mixture to Se(IV)-DAN complex in aqueous medium

[56]. It is claimed that micelles or SDS/ β -CD "aggregates" provide some kind of hydrophobic cavities. When a hydrophobic compound such as Se (IV)-DAN, exists in aqueous solution it can be included into the cavity voluntarily. Hence its chemical environment is changed and the possibilities of quenching are reduced.

The effect of the addition of β-CD / SDS mixture to the fluorescence intensity of aqueous solution of Se-DAN complex was studied. The conditions stated previously [56] were applied without any modification. The molar ratio of SDS/β –CD was kept as 0.6 and the volume of β-CD/SDS added to the 10 mL aqueous solution of Se(IV)-DAN was changed in the range of 2-7 mL. In Figure 3.11 the fluorescence spectra of 2.0 mg/L Se-DAN complex in aqueous medium after the additions of 2, 3, 5 and 7 milliliters of β-CD/SDS solution is given. The highest signal (44.5 relative fluorescence intensity) was obtained when 5.0 mL β-CD/SDS solution was added. Further increase in volume causes the dilution of the complex. As can be seen from the Figure 3.11, when 7.0 mL β-CD/SDS solution was added the fluorescence signal of Se(IV)-DAN complex was decreased.

The enhancement in the fluorescence signal of Se-DAN complex in the presence of β-CD/SDS solution in aqueous medium compared to that of water only medium was about 9 fold.

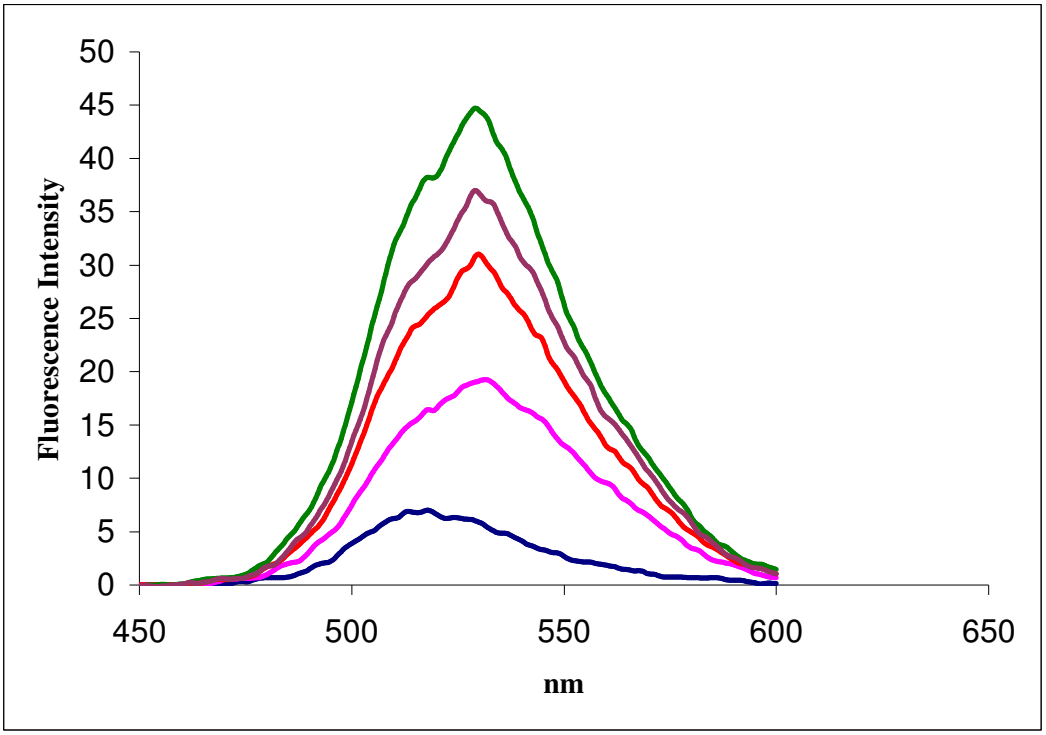


Figure 3.11: Emission signal of — 2 mg/L Se(IV)-DAN complex, — 2 mL SDS- β-CD added to 2 mg/L complex, — 3 ml SDS- β-CD added to 2 mg/L complex, — 5 ml SDS- β-CD added to 2 mg/L complex, — 7 ml SDS- β-CD added to 2 mg/L complex (λ_{ex}= 379 nm, slit= 10 nm)

3.6.3 Fluorescence Intensity of Se (IV)-DAN Complex in Organized Medium (CPE with Triton X-114)

Figure 3.12 depicts the fluorescence spectrum of 2 mg/L selenite obtained after cloud point extraction of 10 mL 0.2 mg/L of selenite to 1 mL of Triton X-114 phase. As can be seen from the figure the 2 mg/L Se(IV) concentration gives 140 units of intensity with respect to its blank.

There is 17.9 fold and 3.1 fold enhancements in the fluorescence signal of Se(IV)-DAN complex in the concentrated Triton X-114 phase of CPE compared to that of the aqueous solution only and β-CD/SDS containing aqueous solution respectively. It is worthy to note that if the starting concentration (0.20 mg/L) is taken into account the total enhancements become 179 and 31 fold. Besides the signal intensity acquired in the surfactant rich phase (0.20 mg/L Se(IV), Relative Fluorescence Intensity (RFI) is 140) is comparable or even better than that of in cyclohexane phase (0.2 mg/L Se(IV), RFI is 120).

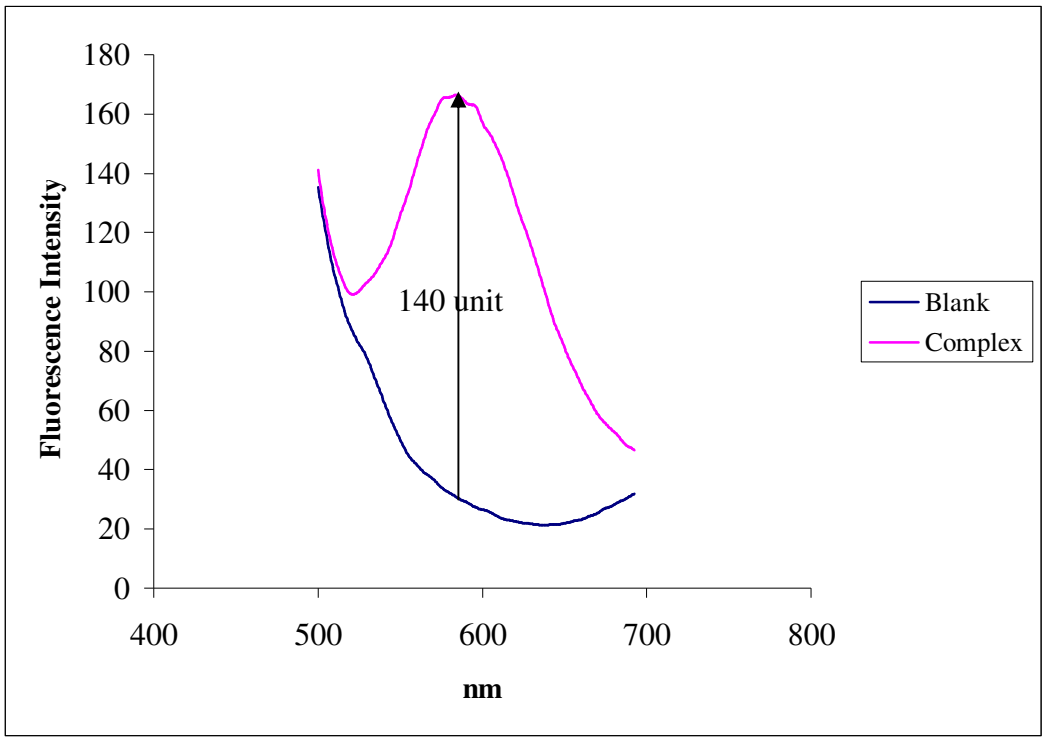


Figure 3.12: Fluorescence spectrum of Se(IV)-DAN obtained after cloud point extraction (λ_{ex}= 379 nm, slit with= 15 nm)

3.7 Analytical Performance Characteristics for CPE and Fluorescence Determination of Selenite

Using the optimized conditions sensitivity and detection limit of selenium determinations by cloud point extraction and fluorescence determination were evaluated. The fluorescence spectra obtained after the cloud point extractions of 0.020 mg/L to 0.70 mg/L selenite as Se(IV)-DAN complex are given in Figure 3.13 and the corresponding calibration line is given in Figure 3.14.

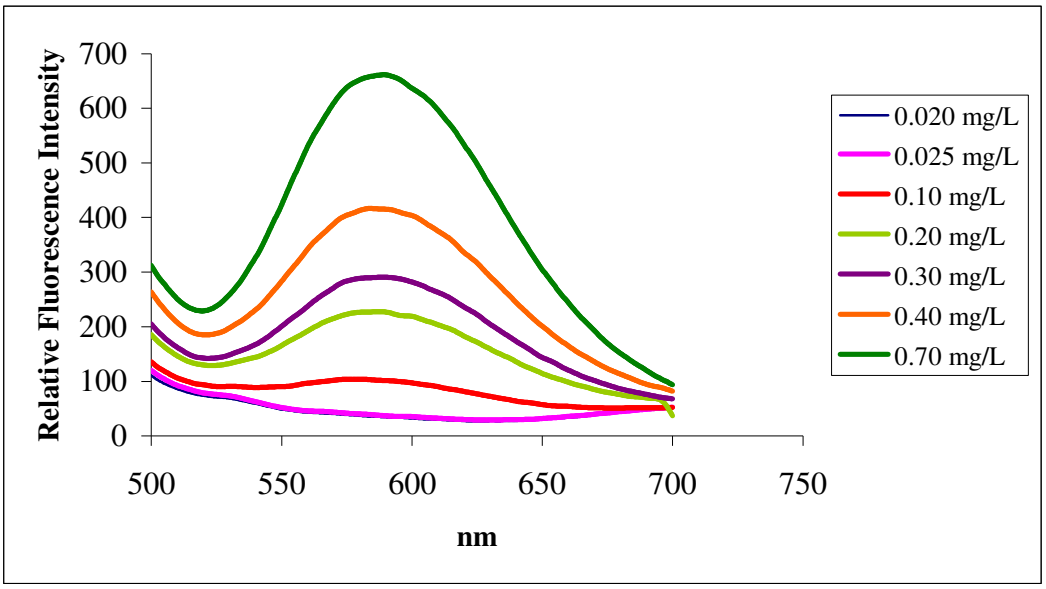


Figure 3.13 Fluorescence spectra obtained after the cloud point extractions of different concentrations.

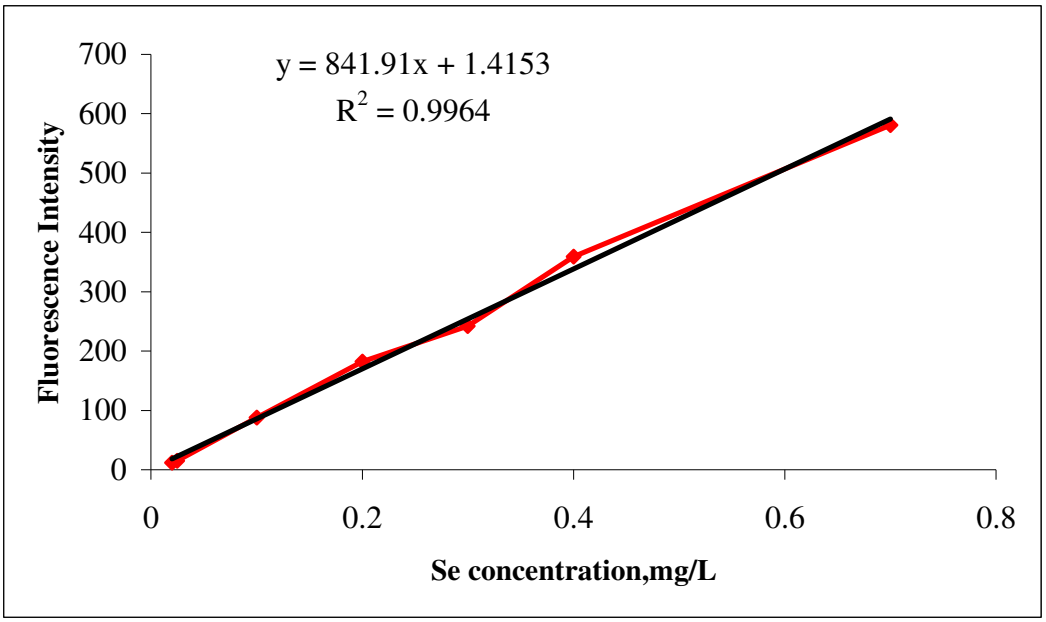


Figure 3.14 Calibration Curve for Fluorimetric Determination of Selenite after cloud point extraction of Se-DAN complex.

As can be seen from the Figure 3.14, the calibration graph was linear up to 0.70 mg/L. The slope sensitivity is 841.9. The detection limit, established as 3*s* /slope where s is the standard deviation of 9 measurements of 0.020 mg/L Se-DAN complex after 10 fold preconcentration was 3.7 µg/L.

In Figure 3.15 is shown the calibration lines obtained with CPE and cyclohexane extraction of selenite standard solutions. The slope sensitivity of CPE method and organic extraction method are 841.9 and 985.5 respectively. As shown quantitatively the sensitivities of CPE and organic extraction methods are comparable when surfactant rich phase of CPE was diluted to 1.0 mL.

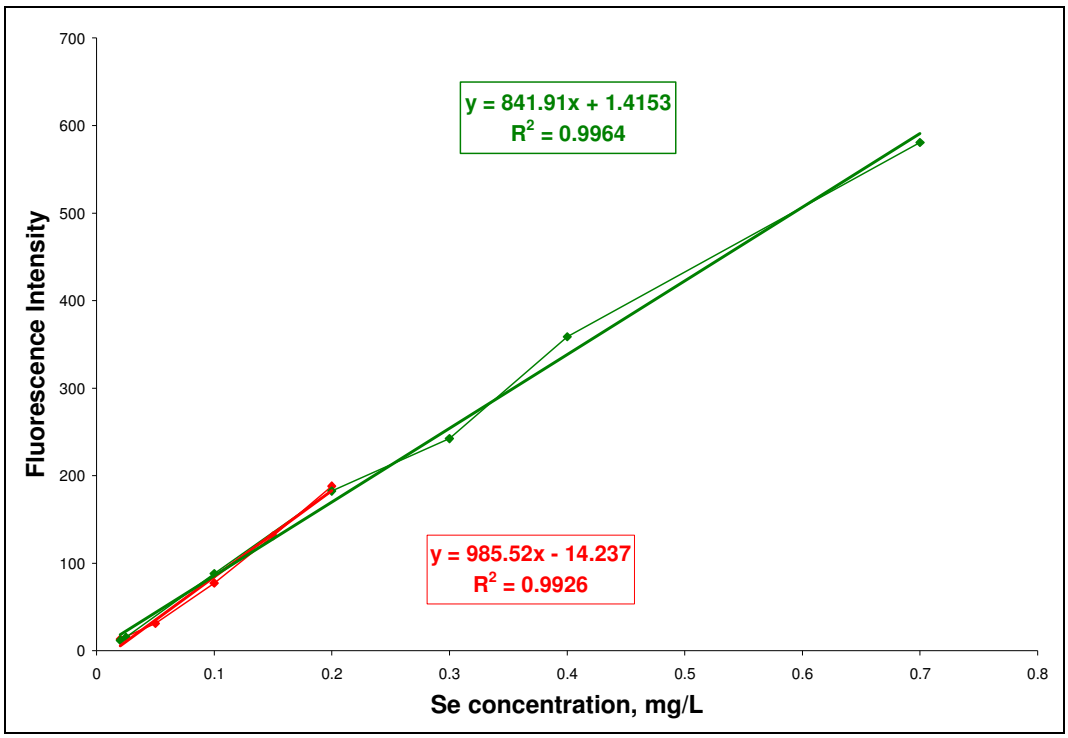


Figure 3.15 Sensitivities of Se(IV)-DAN complex obtained through cyclohexane extraction (—);CPE, the surfactant rich phase was diluted to 1 mL (—)

3.8 Solid Surface Fluorescence Studies

To improve the fluorescence intensity we decided to measure the Se(IV)-DAN complex in surfactant rich phase without any dilution utilizing solid surface fluorescence attachment. A set of CPE studies were performed with various Se(IV) concentrations in the range of 0.010 - 0.35 mg/L. After separation of the phases, 25 µL surfactant rich phase was dropped onto the glass microscope slide and dried at room temperature. The fluorescence emission intensity was measured at 582 nm using the solid surface apparatus. Fluorescence spectra of the standard solutions and the calibration line obtained from this data are shown in Figure 3.16 and Figure 3.17 respectively.

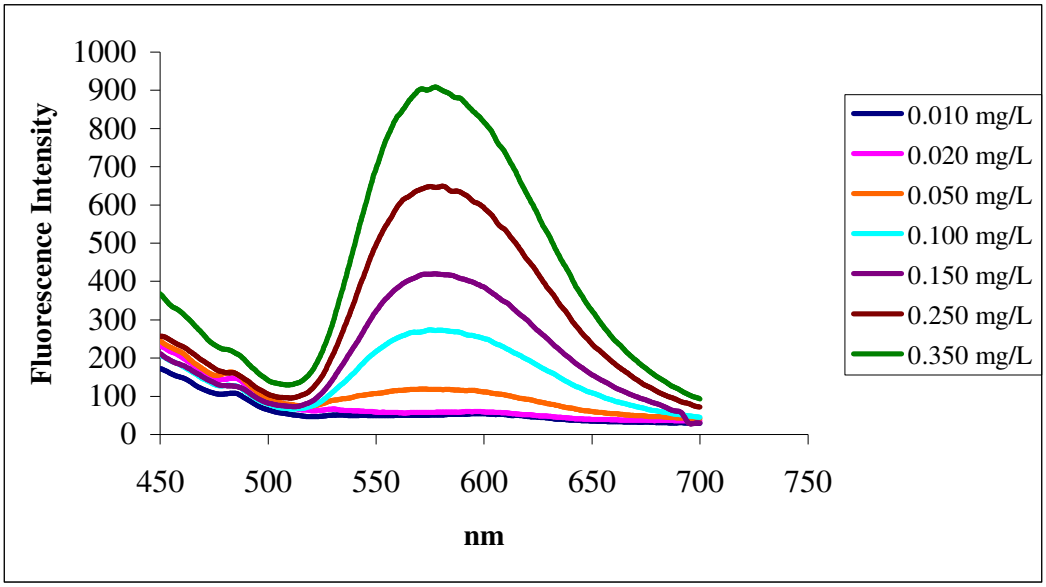


Figure 3.16: Se(IV)-DAN spectra of various selenium concentrations in surfactant rich phase of CPE measured using solid surface fluorescence device.

Alternative surfaces, like various types of filter papers and silica TLC plate were also tried. However a spotlike signal could not be obtained, probably due to the hydrophilicity of the extract solution and the capillary structure of the surfaces. Hence the best performance was obtained with the glass microscope slides.

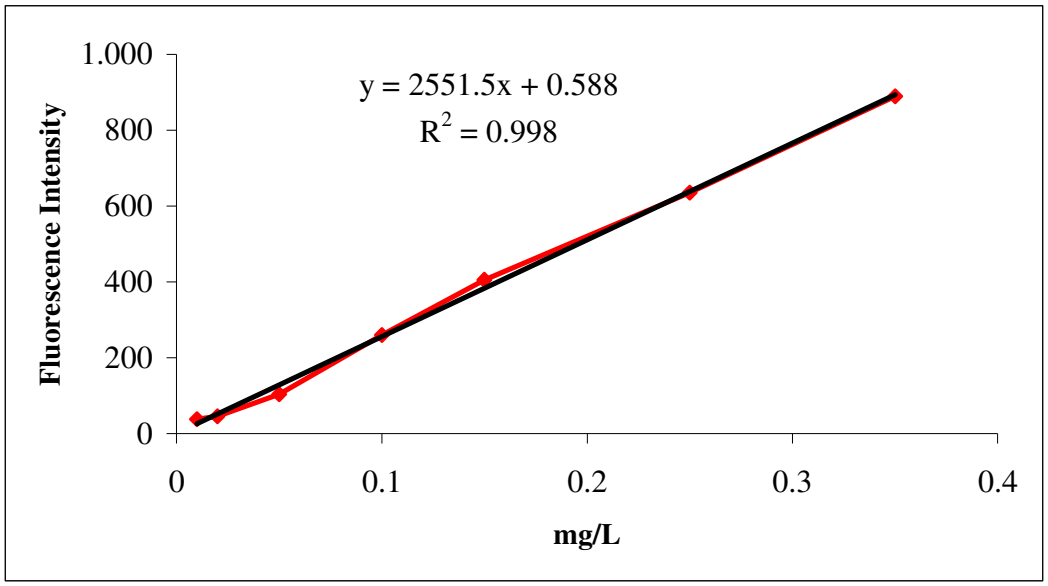


Figure 3.17: Calibration curve of selenite standard solutions preconcentrated in surfactant rich phase of CPE and measured by using solid surface fluorescence device.

As can be seen from the Figure the sensitivity of the CPE method is 2551.5 when concentrated surfactant rich phase is analyzed utilizing solid surface fluorescence attachment.

In Figure 3.18 the comparision of the sensitivities of Se-DAN complex obtained through cyclohexane extraction and CPE is given. Green and Blue lines are corresponding to the diluted (extract was diluted to 1.0 mL) and concentrated forms (measured on glass surface) of the surfactant rich phase of CPE respectively. The slope sensitivity of CPE method with the usage of solid surface measurement (2551.5) is 3 fold higher than that of the organic extraction (985.5) and CPE with solution phase fluorescence measurement (841.9).

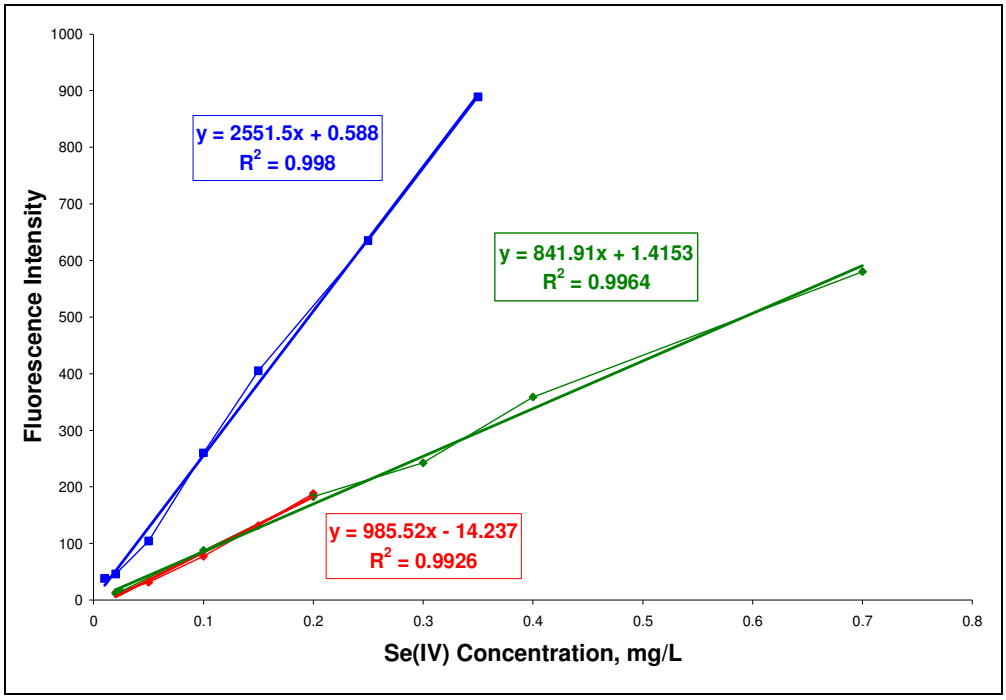


Figure 3.18: Sensitivities of Se(IV)-DAN complex obtained through cyclohexane extraction (—); CPE, the surfactant rich phase was diluted to 1 mL (—) and CPE, the concentrated surfactant rich phase was measured on glass surface (—)

The 3 fold enhancement of the sensitivity of the cloud point extraction method after the direct fluorescence measurement of the concentrated surfactant phase was also reflected on the detection limit of the method. The detection limit given in Table 3.3 (CPE, solution phase fluorescence measurement) was found 1.2 µg/L, which is lower than the other detection limits except the first one given in Table 3.3.

The obtained detection limits (3.7 and 1.2 µg/L) are sufficiently low as to be valuable for detecting selenite in different samples.

3.9. Selectivity of 2,3-diaminonaphthalene(DAN) to Se (IV) in the presence of Se (VI)

Fluorimetric determination of selenium is based upon measuring the fluorescence intensity of the compound (4, 5-benzopiazselenol) formed by the reaction of selenite (selenious acid) with 2, 3-diaminonaphthalene (DAN). It is stated that Se (VI) does not react with 2,3-diaminonaphthalene yielding a Se(IV)-DAN complex. In order to investigate this fact, the same amount of DAN were added to the solutions of 0.1 mg/L Se (IV) standard, 0.10 mg/L Se(VI) and a solution containing both 0.10 mg/L Se (IV) and 0.10 mg/L Se (VI). The emission signals of the surfactant rich phases of these solutions after CPE were shown in the Figure 3.19. As can be seen from the Figure, the presence of the equal amount of Se (VI) in the Se (IV) standard solution does not bring any change in the signal.

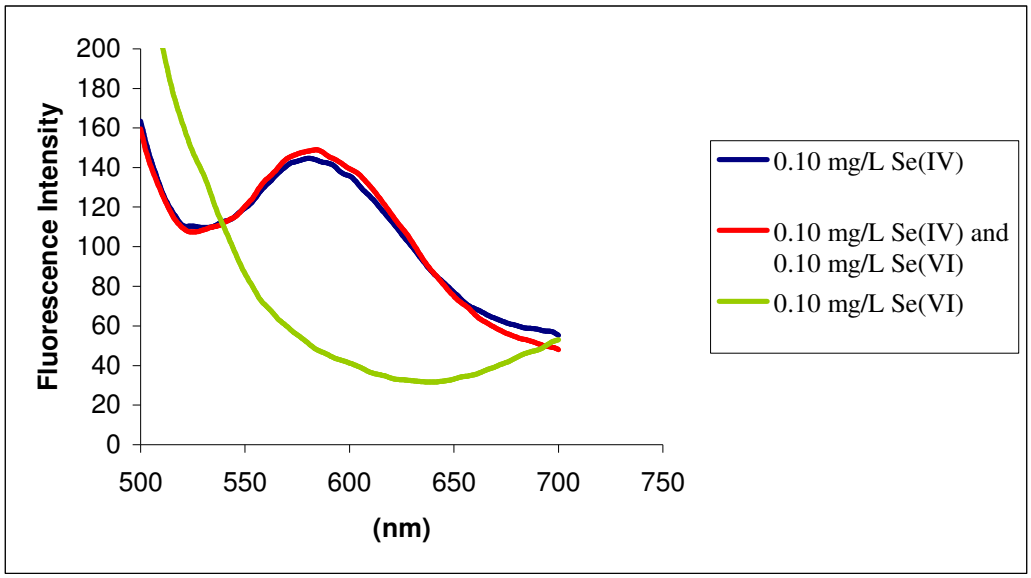


Figure 3.19: Selevtivity of DAN complex formation for Se(IV)

3.10 Interference Studies

Recent studies have been reported that there were no major interferences in the spectrofluorimetric determination of Se(IV) using DAN in the presence of masking agent [52, 57]. It has been reported that [50], Cu^{2+} catalyzes the oxidation of DAN, Sn^{4+} reduces selenium to the elemental state Al^{3+} and Fe^{3+} are potential interfering ions to Se(IV)- DAN complex formation.

To investigate the effect of foreign ion to the complex formation and CPE of Se(IV)-DAN complex at high interferent to analyte concentration ratio, 0.07 mg/L Se was reacted with appropriate amount of DAN in the presence of 1.0 mg/L Cu^{2+}, Sn^{4+}, Al^{3+}, Fe^{3+}. As recommended in the literature [50] and used in the previous section for the analysis of standard reference material, EDTA was decided to be used for removing the interference effect of these ions. Four different EDTA concentrations; 0.0025 M, 0.00375 M, 0.005 M, 0.0075 M were examined.

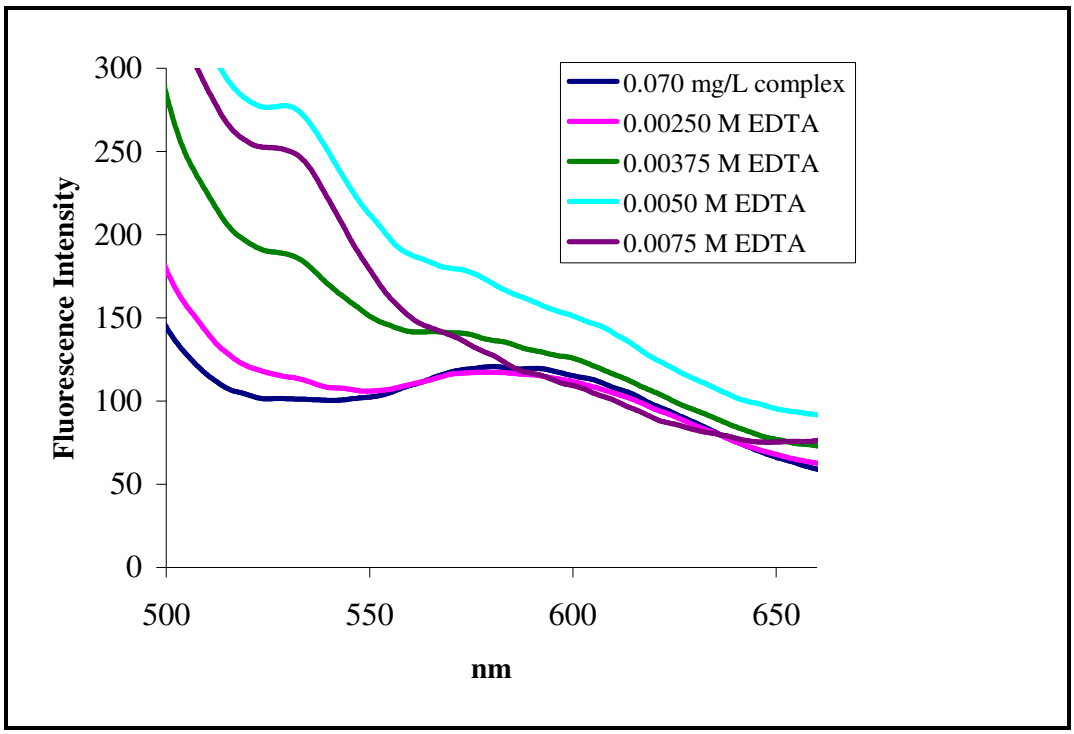


Figure 3.20: Effect of EDTA with different concentration for removing interfering effects; — 0.070 mg/L Se-DAN complex contains added no interference ions, — 0.07 mg/L Se-DAN complex contains interfering ions and 0.0025M EDTA, — 0.070 mg/L Se-DAN complex contains interfering ions and 0.00375M EDTA, — 0.070 mg/L Se-DAN complex contains interfering ions and 0.005M EDTA, — 0.070 mg/L Se-DAN complex contains interfering ions and 0.0025M EDTA.

It was observed that at EDTA concentrations higher than 0.0025 M the solution became cloudy immediately after the addition of EDTA. The pH of our cloud point extraction procedure was 1.5 and it is well known that the solubility of EDTA decreases in acidic medium. Consequently it was concluded that 0.0025 M EDTA was the highest concentration that could be prepared in this medium. Fortunately it was potent enough to remove all the interferences. As can be seen from Figure 3.20 in the presence of 0.0025 M EDTA, the signals of the standard solution alone and the standard solution spiked with the interfering ions are superimposed to each other. As

can also be seen in the Figure 3.20 at high concentrations of EDTA no emission signal was observed (blue, yellow and purple signals) at the Se(IV)-DAN complex emission wavelength (582 nm). This fact probably is related to the removal of selenite from the solution by EDTA precipitated at high acidity before reaching the cloud point of Triton X114.

3.11 Application to the Analysis of Standard Reference Material

In order to demonstrate the accuracy of the proposed method, it was applied to the analysis of standard reference material: EnviroMAT- Waste Water LOW EU-L-1. The concentrations of the other species present in the reference material are tabulated at the appendix. 0.00250 M EDTA was used to prevent any interference effect that may come from the matrix. The surfactant rich phase diluted to 1.0 mL and than fluorescence measurements were obtain.

Table 3.3 Determination of Se(IV) in Standard Reference Material

Sample	***Se concentration (µg/L)***
Se(found)	69 ± 4 (N=5)
Se (informative)	70 ± 3

The result given in Table 3.3 was in good agreement with the certified values which confirmed the accuracy of the fluorometric selenite determination after CPE. Furthermore, the closeness of the result to the reported value was also confirmed that the proposed method for selenite determination was not affected from the other ions present in the waste water sample at their reported concentrations.

3.12 Figures of Merit

The detection limit of CPE method developed was compared with the detection limits of some other fluorimetric selenite determination studies after organic extraction, Table 3.4.

Table 3.4: Detection limits for the fluorometric determination of selenite after organic extraction and detection limit of our method.

Detection Limits (µg/l)	Preconcentration Factor	Determination Method	Ref
0.82	1	Extracted with cyclohexane	[51]
3.6	2	Extracted with cyclohexane	[57]
1.96	3	Extracted with cyclohexane	[54]
3	4	Extracted with cyclohexane	[46]
3.7	**10**	**Surfactant rich phase was diluted to 1 mL**	**This study (1)**
1.2	**10**	**Measured on glass surface**	**This study (2)**

As can be seen from Table 3.4, LOD of our study (3.7 µg/L) is comparable or higher than the others. It is obvious that this C_F and hence LOD can be improved by using small-volume cells and diluting the surfactant-rich phase by small amounts of water or larger volume of initial solution.

In our fluorescence measurements 700 µL sample cell were used. Thus, the surfactant rich phase collected at the bottom of the centrifuge tube (around 100 µL) was diluted to 1 mL. Smaller sample cell can be used, however in case of solution phase fluorescence measurements at least 300-500 µL solution volume is required to fill the

sample cell. Another approach to improve the concentration factor and the detection limit of the method was the CPE of large volume of solution at low selenite concentration. In our studies the size of the centrifuge tubes used was too small for a practical separation of sample volumes larger than 10.0 mL. Therefore our CPE studies were limited to 10 mL volume of the original solution.

3. 13 Future Work

The method is going to be applied for the determination of total selenium in poultry tissue and the obtained results will be compared with that of ICP-MS.

CHAPTER 4

CONCLUSION

It is widely acknowledged that there is a growing need for more environmentally acceptable processes in the chemical procedures. This trend known as 'Green Chemistry' and it mainly concerns about the elimination of waste at source and avoiding the use of toxic and/or hazardous substances. In this respect, analytical procedures carried out at room temperature with small volumes become important.

In this study, a green liquid–liquid extraction method namely, the Cloud Point Extraction (CPE) was used. It is based on the cloud-point phenomenon, a phase separation exhibited by nonionic surfactant aqueous solutions when some conditions, such as pressure or temperature, are modified. The micelle aggregates separate in a small-volume surfactant-rich phase, containing hydrophobic species, which can be extracted and concentrated. Thus the CPE produces 20-500 μL of surfactant as a waste and eliminates the usage of organic solvents commonly employed in conventional extractions.

In this study, the selenium (IV) complex (4,5-benzopiazselenol,DAN-Se) formed with a fluorescing ligand (2,3-diaminonapthalene, DAN) was extracted by Triton X-114. The cloud-point was induced at the optimized conditions: pH 1.5, temperature, 70°C; duration time, 45 min. and [DAN]/[(Se(IV)] = 50. The quantitative measurements were carried out by a spectrofluorometry.

Fluorimetric method is one of the simplest, least expensive and most versatile of all the methods to determine Se(IV) and 2,3-diaminonapthalene is the most widely used reagent due to its lack of toxicity and ready availability. However in aqueous medium Se(IV)-DAN complex has a low fluorescence intensity. Hence the fluorimetric determination of selenium is usually performed in organic solvents. This is the first time that an aqueous solution phase extraction, CPE, coupled with fluorescence detection is exploited for the determination of selenium. The slope sensitivity and detection limit of CPE-fluorescence method proposed in this study were comparable or even better that of the organic extraction-fluorescence method (In Table 3.3) [46, 51, 54, 57].

The sensitivity of the method was further enhanced by using glass surface to measure fluorescence intensity. The detection limit was managed to be reduced to ca. 1.2 µg/L by measuring the surfactant rich phase without any dilution utilizing solid surface fluorescence attachment.

This study offers a safe, sensitive and inexpensive method for the separation and preconcentration of selenite. The accuracy of the method was tested by the analysis of waste water standard reference material (EnviroMAT- Waste Water LOW EU-L-1).

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APENDIX

Table 1: Consensus Values for EnviroMAT – Waste Water LOW EU- L – 1

Parameter	Consensus Value (mg/L)	Confidence Interval (mg/L)
Al	0.15	0.12 - 0.18
As	0.21	0.20 - 0.22
B	0.25	0.24 - 0.26
Ba	0.30	0.29 - 0.31
Be	0.03	0.029 - 0.031
Ca	4.03	3.91 - 4.15
Cd	0.06	0.058 – 0.062
Co	0.20	0.197 - 0.203
Cr	0.15	0.147 - 0.153
Cu	0.26	0.25 - 0.27
Fe	0.11	0.10 - 0.12
K	4.49	4.39 - 4.59
Mg	1.24	1.21 - 1.27
Mn	0.30	0.29 - 0.31
Mo	0.10	0.097 - 0.103
Na	10.4	10.2 - 10.6
Ni	0.20	0.196 - 0.204
P	2.67	2.58 - 2.76
Pb	0.10	0.098 - 0.102
Sb	0.06	0.05 - 0.07
Se	**0.07**	**0.067 - 0.073**
Sr	0.38	0.37 - 0.39
Tl	0.20	0.19 - 0.21
V	0.12	0.116 - 0.124
Zn	0.06	0.057 - 0.063