CHROMATOGRAPHIC AND MASS SPECTROMETRIC STUDIES FOR LANTHANIDES, ACTINIDES AND PLATINUM GROUP ELEMENTS

By

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Enrolment No: CHEM012008040012

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A thesis submitted to the

Board of Studies in Chemical Sciences

In partial fulfillment of requirements

For the Degree of

DOCTOR OF PHILOSOPHY

of

HOMI BHABHA NATIONAL INSTITUTE



September, 2014

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List of Publications arising from the thesis

Journals

- Determination of Palladium by Electrospray Ionization Mass Spectrometry after Preconcentration by Disposable Pipette Extraction
 P.G. Jaison, **Pranaw Kumar**, Vijay M. Telmore and Suresh K. Aggarwal
 Rapid Commun. Mass Spectrom. 26 (2012) 1971-1979.
- Determination of Lanthanides and Yttrium in High Purity Dysprosium by RP- HPLC using α-Hydroxyisobutyric acid as an Eluent

Pranaw Kumar, P.G. Jaison, D. R. M. Rao, V.M. Telmore, A. Sarkar and Suresh K. Aggarwal

J. Liq. Chromatogr. Rel. Technol. 36 (2013)1513–1527.

 Speciation of platinum-benzoylthiourea in gas phase using electrospray ionization mass spectrometry and density functional theory

Pranaw Kumar, P. G. Jaison, Mahesh Sundararajan, Vijay M. Telmore, Swapan K. Ghosh and Suresh K. Aggarwal

Rapid Commun. Mass Spectrom. 27 (2013) 947-954.

 4. Determination of Lanthanides, Thorium, Uranium and Plutonium in Irradiated (Th,Pu)O₂ by Liquid Chromatography using α-HydroxyIsoButyric Acid (α-HIBA)
 Pranaw Kumar, P.G. Jaison, Vijay M. Telmore, Sumana Paul and Suresh K. Aggarwal

International Journal of Analytical Mass Spectrometry and Chromatography **1** (2013) 72-80.

 HPLC Method for Determination of Th, U and Pu in Irradiated (Th,Pu)O₂ using Mandelic Acid as an Eluent **Pranaw Kumar**, Sumana Paul, P.G. Jaison, Vijay M. Telmore, D. Alamelu and Suresh K. Aggarwal

Radiochim Acta; DOI : 10.1515/ract-2013-2242.

Conferences

 Investigations for Trace Determination of Gd, Tb and Ho in Presence of Large Amounts of Dysprosium by HPLC,

Pranaw Kumar, P.G. Jaison, D. R. M. Rao, V.M. Telmore, A. Sarkar and S.K. Aggarwal,

Nuclear and Radiochemistry Symposium (NUCAR) 2011, paper No. B-30, page No.232.

 Electrospray Ionisation Mass Spectrometry (ESI-MS) of Platinum-Benzyolthiourea (Pt- BTU)

Pranaw Kumar, P.G. Jaison, V.M. Telmore and S.K. Aggarwal

ISMAS International Discussion Meet on Elemental Mass Spectrometry in Health and Environmental Sciences (ISMAS-DM-HEAL-2011) during April 14-15, 2011 in New Delhi, CP-5, Pg. 126.

- Determination of Palladium by Electrospray Ionization Mass Spectrometry after Preconcentration by Disposable Pipette Extraction
 P.G. Jaison, Pranaw Kumar, V.M. Telmore and S.K. Aggarwal
 14th ISMAS Symposium cum Workshop on Mass Spectrometry (14th ISMAS-WS
 2011) held in Munnar during November 7 - 11, 2011, CP – 33, p.223.
- Studies on Species of Platinum (II) Complex with Benzyolthiourea in Acetonitrile Solution by Electrospray Ionization Mass Spectrometry
 Pranaw Kumar, P.G. Jaison V.M. Telmore and S.K.Aggarwal

14th ISMAS Symposium cum Workshop on Mass Spectrometry (14th ISMAS-WS 2011) held in Munnar during November 7 - 11, 2011, CP-32, p. 219.

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Dedicated to.....

My Family for unconditional love and support

ACKNOWLEDGEMENTS

At this juncture, I would like to express my sincere gratitude to God, the Almighty for His blessings and number of people whose advice, support, motivation and well wishes have transformed into milestone achievement.

First and foremost, I would like to express my deep sense of gratitude and indebtedness to my guide **Prof. S. K. Aggarwal** for his invaluable guidance, inspirations, persistent encouragement and profound support throughout this work. Deep sense of gratitude to the doctoral committee members, **Prof. K. L. Ramakumar**, **Prof. B. S. Tomar** and **Dr. S.V. Godbole** without their critical comments and suggestion this work would not have taken into the present shape.

I would like to express my sincere gratitude to my immediate superior Sh. P. G. Jaison who has introduced me into this field. His calm demeanor, logical way of guidance, help in the crucial experiments, monitoring the progress of work, teachings about the writing of manuscript helped to bring out the proper shape of this work. I wish to record my heartfelt gratitude and indebtedness to him.

I would like to extent my gratitude to my senior Dr. Arnab sarkar and my lab colleagues Mrs. Sumana Paul and Sh. V. M. Telmore for their wholehearted cooperation, motivation and support for this work. I owe sincere thanks to Dr. D. Alamelu and Sh. A. R. Parab for their encouragement. I am grateful to Dr. S. Kannan and Dr. N. Sivaraman for their guidance and discussion about the results. My special thanks to the all the colleagues of FCD in particular to Dr. Radhika Rao, Dr. J. V. kamat, Sh. Raju Shah, Sh. Sasibhushan, Sh. Jagadish Kumar, Sh. G.K. Prasad, Sh. Vijay Karki, Dr. Manoj Sharma, Sh. S.K. Guin, Mrs. Ruma Gupta, Dr. Satyajit Choudhury, Dr. I.C. Pius and Sh. Rohan Pathak. I would like to sincerely thank to the seniors and colleagues from other division of BARC Partícularly Dr. A. Goswamí, Dr. P.N. Pathak, Dr. R. Acharya, Dr. H. Naík, Sh. S. Jeyakumar, Neelam Kumarí, Dr. Sumít kumar, Sh. Ramamohan Rao for their valuable guídance and support. Special thanks are due to Dr. S. Mahesh for hís help regarding the theoretical work. I would like to thank to my friends Sh. Chandan Kumar, Sh. Sumít Gupta, Sh. Aníl Upreti, Sh. K. Srinivasu and Sh. Sujoy Biswas for their constant support. Special thanks to Sh. R. Shívaram from FCD office for his help and support.

I wish to record my deep sense of gratitude to my graduation teacher **Prof. S.K. Singh** who gave the inspiration for pursuing research in chemistry.

Finally, I owe my heartfelt gratitude to my parents, my brother, my wife and daughter for continuous and unconditional support.

Pranaw

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Synopsis

Chromatography is a physical separation method based on the distribution of components between two immiscible phases i.e. a mobile phase and a stationary phase. Separation of analytes takes place as a result of series of sorption and desorption steps during their flow along the stationary phase. The different chromatographic techniques are classified based on the nature of mobile phase, e.g. if the mobile phase is liquid, then the chromatographic technique is called liquid chromatography (LC). Amongst different LC approaches, high performance liquid chromatography (HPLC) has been one of the most preferred analytical tool for separation, analysis and purification of mixtures of compounds [1, 2]. The online detection system and the miniaturization in particle size of the column have reduced the separation time of many of the complex mixtures from hours to minutes. Hence this technique is quite promising for the separation of lanthanides and actinides [3, 4].

The separation of lanthanides and actinides is important for the industrial, geochemical and nuclear technologies [5-9]. The separation of lanthanides is difficult because of their similar chemical and physical properties. Multi-elemental techniques like neutron activation analysis, X-ray fluorescence, inductively coupled plasma atomic emission spectroscopy and inductively coupled plasma mass spectrometry are used for their determination but are susceptible for spectral interferences when these elements are present in a mixture [7]. Chromatography and solvent extraction are used to separate them individually for minimizing the interferences. In the chromatographic separation method, HPLC involving the use of α -hydroxyisobutyric acid (α -HIBA) as a ligand is the most preferred method for lanthanides separation [10]. Use of ion interaction reagent is advantageous in HPLC separation of lanthanides because it offers high resolution resulting from the faster mass transfer at the surface of the stationary phase [11].

However, co-elution problem of Yttrium with Dysprosium using single eluent is well reported [12, 13]. Use of mixed ligands has been reported for solving this co-elution problem but it requires careful optimization of the composition [13-16]. Present thesis deals with the development of HPLC methods for the separation of trace amounts of lanthanides and yttrium in a given lanthanide. Concerning the measurement of lanthanides, Th, U and Pu, several analytical method are reported varying from classical methods to modern instrumental methods [17, 18]. Among these methods, HPLC is a fast and highly efficient technique and has been applied for the separation of lanthanides and actinides in nuclear fuel samples and geological samples [19 - 23]. HPLC using α -HIBA and mandelic acid are reported for the separation and determination of lanthanides, U and Th using simulated mixtures [24, 25]. In view of the large amount of Th in Th-based fuels and multiple oxidation states of Pu in dissolver solution of irradiated (Th, Pu)O₂ fuel, LC method is attractive since the lanthanides can be separated without interference of Th and Pu. Subsequently, for actinides using α -HIBA the bulk amount of Th can mask the later eluting peak of U. Therefore, the method where U and Pu can be eluted prior to Th is of great interest.

Mass spectrometry (MS) is an analytical tool which provides the data based on the mass-to-charge ratio [26]. Amongst the varieties of ionization techniques, electrospray ionization (ESI) is a soft ionization technique which is capable of providing the information about stoichiometry and about metal and ligand equilibrium [27]. The platinum group of elements which is widely used in varieties of applications is known to make complexes with soft ligands like thiourea (TU) and its derivatives [28]. Although ESI-MS can reveal information regarding the oxidation state and stoichiometric ratio of the elements, the spatial orientation of the metal and the ligands is often predicted through electronic structure methods. It is interesting to study the formation of different species of

platinum with BTU and spatial orientation by density functional theory. Presence of complex matrix can also influence the analyte response in ESI-MS. Hence, it is challenging to determine one element in the complex matrix like simulated high level liquid waste (SHLLW). Disposable pipette extraction using BTU as the complexing agent demonstrates the selective preconcentration of palladium in presence of other interfering elements.

Hence, the objectives of the present thesis were:

- Separation and determination of lanthanides and yttrium in high purity dysprosium
- Separation and determination of lanthanides, Th, U and Pu in irradiated (Th, Pu)O₂ fuel using α -HIBA
- Studies on the chromatographic behavior of Th, U and Pu on reversed phase (RP) using mandelic acid for their determination in irradiated (Th, Pu)O₂ fuel
- Speciation studies of platinum-benzoylthiourea using electrospray ionization mass spectrometry
- Electrospray ionization mass spectrometric studies for the palladium benzoylthiourea complex

The work described in this thesis is divided into six Chapters.

Chapter 1

This Chapter presents an introduction about the different types of chromatography, basic concept of separation, equipment and its applications in various matrices. Amongst the various chromatographic techniques, HPLC developed for various applications in the present work is discussed in detail. Efficient column preparation to minimize the separation time is also discussed. Traditionally used detectors like UV- Visible, conductivity, refractive index and fluorescence etc. are described. Comparison of HPLC with the various other analytical techniques for the present work is also given.

The different varieties of mass spectrometers based on the formation of ions in the source and their separation by using different mass analyzers is described. Isotope dilution thermal ionization mass spectrometry (ID-TIMS) used for the validation of developed chromatographic methods in irradiated fuel is presented. ESI-MS, a soft ionization technique, used for carrying out the speciation studies is described in detail. Finally, the scope of the present work is highlighted.

Chapter 2

This Chapter deals with the studies carried out for the determination of lanthanides viz. yttrium (Y), terbium (Tb), holmium (Ho), erbium(Er), thulium(Tm), ytterbium (Yb) and lutetium (Lu) in high purity dysprosium oxide (Dy_2O_3) matrix. In view of their similar chemical and physical properties, determination of rare-earth elements in bulk of a rare-earth element is a difficult task. The objective of the present work was to enhance the resolution amongst the adjacent lanthanides so that the lanthanides and Y can be separated from Dy. Comparison of two reversed phase (RP) columns viz. C_{18} and C_8 employing α -hydroxyisobutyric acid (α -HIBA) as an eluent is presented. n-Octane sulphonic acid was used as an ion interaction reagent (IIR).

Effect of different chromatographic parameters e.g. IIR, pH and concentration of α -HIBA were studied to arrive at the optimum conditions for the individual separation of lanthanides. C₈ column using n-octane sulphonate as IIR, provided better resolution as compared to C₁₈ based stationary phase. Under the optimized conditions, it was possible to resolve Y from Dy using α -HIBA as a single eluent.



Fig 1. Separation of Ho, Y and Tb from Dy on C_8 RP column where Dy/Tb and Dy/Y amount ratios are 200

Separation and quantification of rare-earth elements was possible in samples with Dy/Tb and Dy/Y amount ratios up to 200 (Fig. 1). Separation and quantification of lanthanides and Y was possible in Dy₂O₃ using α -HIBA as a single eluent with C₈ reversed phase column. The method was validated using the simulated sample containing lanthanides, Y and Dy matrix. Quantification was done using standard addition method. The method was successfully applied for the quantification of lanthanides and Y in high purity Dy₂O₃ sample. Concentrations of Lu, Yb, Tm, Er, Ho, Tb and Y were found to be 74 ppmw, 205 ppmw, 214 ppmw, 225 ppmw, 1207 ppmw, 3325 ppmw and 4463 ppmw, respectively.

Chapter 3

This Chapter discusses the separation and determination of lanthanides (Lns), thorium (Th), uranium (U) and plutonium (Pu) from irradiated (Th, Pu)O₂. The individual separation of Lns, Th, U and Pu is necessary for burn-up measurements in dissolver solution of irradiated fuel. This separation is a challenging task because (i) lanthanides

have similar physical and chemical properties, (ii) presence of complex matrix like irradiated fuel and (iii) the co-existence of multiple oxidation states of Pu. This Chapter presents optimization of procedure for the separation of individual lanthanides and actinides. Since the chromatographic behavior of lanthanides is different than that of tetravalent and hexavalent actinides, two steps separation procedure was developed. In the first step, individual lanthanides were separated on a dynamically modified reversed phase (RP) column using n-octane sulfonic acid as an ion interaction reagent and employing dual gradient (pH and concentration) of α -hydroxyisobutyric acid (HIBA). In order to improve the precision on the determination of Lns, terbium (Tb) was used as an internal standard. The method was validated employing simulated high level liquid waste.



Fig. 2. Direct injection of dissolver solution of irradiated (Th, Pu)O₂ fuel. Column: C_{18} RP (250 mm x 4.6 mm)

Concentrations of lanthanides viz. lanthanum (La) and neodymium (Nd) in the dissolver solution were determined based on their peak areas. In the second step, Th, U

and Pu were separated on a RP column using mobile phase containing HIBA and methanol. Since Pu is prone to co-exist in multiple oxidation states, all the oxidation states of Pu were converted into Pu (IV) using H_2O_2 in 3M HNO₃. The elution pattern was observed as Pu (IV) elution followed by Th and U elution (Fig. 2). The concentrations of Th, U and Pu were determined by standard addition method. These data were in good agreement with the concentration of Th determined by biamperometry and those of U and Pu obtained by isotope dilution thermal ionization mass spectrometry.

Chapter 4

This Chapter deals with the development of HPLC methods for the determination of Th, U and Pu in irradiated (Th, Pu)O₂ fuel using mandelic acid as an eluent. The mechanism of separation of these elements was studied. Th was found to be retained strongly onto the column compared to Pu and U. Retention behavior of U and Pu was greatly influenced by the pH of the medium. At pH < 3.5, Pu was retained strongly compared to U. After pH > 3.5, U was eluting later than Pu. In contrast to HIBA, in case of mandelic acid at pH 3.2, the elution pattern was U elution followed by Pu which eluted prior to Th (Fig.3). The behavior of different species of Th, U and Pu at different pH was studied and it was found that at pH > 3.5, Pu forms stronger anionic complex than in case of U. However, Th was found to exist mainly as hydrophobic species.



Fig. 3. Direct injection of dissolver solution of irradiated (Th, Pu) O_2 fuel. Column: C_{18} RP (250 mm x 4.6 mm)

Chapter 5

This Chapter discusses about the electrospray ionization mass spectrometric (ESI-MS) experiments and density functional theory (DFT) based calculations to shed light on the





Cis



speciation studies of Pt complex with BTU. Various species of Pt complexes in both the

positive and the negative ion modes were studied using ESI-MS having quadrupole-timeof-flight analyzer. Platinum solutions prepared from K_2PtCl_4 with BTU in acetonitrile (ACN) were directly infused into ESI-MS and the formation of different species was monitored in voltage, pH and the positive ion mode. The effects of ESI-MS experimental parameters such as capillary electrolyte on the peak intensity of Pt-BTU complex were studied. DFT calculations were used to characterize the geometric parameters and fragmentation patterns of various Pt complexes in the gas phase. The species observed in ESI-MS were singly charged ions with Na or K as an adduct. In the positive ion mode, solvated species of Pt were observed which were not present in the negative ion mode. Pt containing different species were: PtL₂, PtL₂Cl, PtL₂(ACN), PtL₂Cl(ACN), PtL₃ and PtL_3Cl (where L = BTU), with PtL_3Cl (m/z 771.03) being the most abundant species. For both $Pt(BTU)_2$ and $Pt(BTU)_3$ complexes, the ligand BTU was observed to undergo deprotonation followed by bi-dentate coordination mode. DFT calculations suggested that BTU can coordinate to Pt both in the cis and the trans isomeric forms which are nearly iso-energetic with a slight preference towards trans-isomer (Fig. 4). The preference of trans-BTU binding can be attributed to the exclusive retention of intra-molecular hydrogen bonding which is absent in the cis-form.

Chapter 6

This Chapter presents the studies performed on the species of Pd with BTU. The most abundant peaks of Pd-BTU complex were observed at m/z 464.96 and 502.94 corresponding to $[Pd(BTU)_2]^+$ and $[Pd(BTU)_2Cl]^+$, respectively. Based on the observations, ESI-MS method was investigated for the determination of Pd in simulated HLLW solution. Pd-BTU complex was selectively preconcentrated using disposable pipette extraction (DPX). Extraction parameters as well as ESI-MS parameters such as concentration of BTU, acidity, composition of medium and capillary voltage etc. were optimized based on the major abundant species $[Pd(BTU)_2S]^+$ (Fig. 5). Linearity of the method in synthetic mixtures was observed by mixing known amounts of Pd with a fixed amount of (U+SHLLW) solution so that after dilution, concentration of U in the loading solution is ~ 20 ppm. After introduction of eluted fractions into ESI-MS, no major peaks other than those belonging to Pd were seen in the mass spectrum highlighting the usefulness of the method for the selective extraction of Pd in presence of a host of other metals. The suitability of the optimized method for the uptake of Ru, Rh, Ag and Cd was studied as (i) some of their isotopes interfere with Pd isotopes (ii) they complex with "S' based ligands and (iii) some of these elements are also produced in the nuclear fission. A solution containing 100 ppb each of Ru, Rh, Ag and Cd in 10^{-3} M BTU and 0.1 M HNO₃ was subjected to DPX extraction. No species corresponding to [M(BTU)₂S]⁺, which is used for the determination of Pd, was observed in the mass spectrum. This highlights the selectivity of the method for the determination of Pd in presence of other PGEs.



In summary, the important highlights of the work are as follows:

 RP-HPLC method employing α-HIBA as an eluent was developed for separation of lanthanides and Y from Dy₂O₃ matrix. Using single eluent, separation between Dy and Y was possible with good resolution among all the rare-earths pairs. Studies of pH, concentration of eluent and concentration of IIR were carried out and the method was applied for the separation and determination of different lanthanides and Y in high purity Dy. This method provides the potential use of HPLC for the separation of other lanthanides impurities in Dy₂O₃. Similarly, it can be applied for the determination of lanthanides impurities in bulk of other lanthanides.

- 2. HPLC based method for the separation of Lns, Th, U and Pu in the irradiated fuel was developed. Using the dual gradient of pH and concentration of α-HIBA on modified column, separation and determination of lanthanides from irradiated fuel was carried out. The method was also applied for the determination of Th, U and Pu in the irradiated fuel sample. This method is useful for the determination of burn-up in economical viable way and obviates the need of enriched isotope (spike) which is costly and not easily available.
- 3. RP-HPLC method using mandelic acid was developed for the separation of Th, U and Pu. Elution of U and Pu was carried out prior to Th, which circumvents the interferences in determination of U in Th matrix.
- 4. Electrospray ionization mass spectrometric studies on the formation of different species of platinum with benzoylthiourea in acetonitrile were performed. Effect of experimental conditions on the formation of different Pt-BTU species was studied. It was found that the dominance of the species is dependent on the pH of the solution. Density functional theory based calculations were carried out to find the spatial arrangement of Pt-BTU species. Two different binding modes i.e. mondentate and bidentate coordination of BTU with Pt were identified.
- 5. Different species of Pd with BTU using ESI-MS were studied. BTU as a complexing agent was used for the separation of Pd from the complex matrix like

simulated high level liquid waste using disposable pipette extraction method. In this method, there was no interference from the neighboring elements like Ag, Au, Cd, Rh and Ru which are major interfering species in other analytical techniques.

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Chapter 1

Introduction to

Chromatography and

Mass Spectrometry

1.1 Introduction

Separation and determination of trace elements is important for various fields like environment, toxicology, forensic science and nuclear industries [Heftmann (2004)]. Various analytical techniques are reported for the separation and determination of different elements. Table 1.1 summarizes various separation methods with the principle of separation.

Sl. No.	Method	Basis of Separation
1.	Precipitation and filtration	Difference in solubility of compounds
2.	Distillation	Difference in volatility of compounds
3.	Extraction	Difference in solubility in two immiscible liquids
4.	Ion exchange	Difference in interaction with the ion exchange site
5.	Chromatography	Difference in rate of movement of solute through a stationary phase
6.	Electrophoresis	Under the applied electric field, difference in migration rate of the charged species
7.	Field flow fractionation	Difference in interaction with field or gradient applied perpendicular to transport direction

Table 1.1: Various separation methods [Adapted from Skoog (2004)]

Out of the above mentioned separation processes, chromatography is a powerful and widely applicable method for the separation, identification and determination of individual species from the mixtures [Skoog (2004)]. Term chromatography is defined as a separation process where the sample is distributed in two phases namely stationary and mobile phase [Meyer (2010)]. The chromatographic separation takes place because of repeated sorption/desorption of analyte onto the stationary phase during the movement. Depending upon the nature of mobile phase, chromatography can be classified into three categories namely liquid, gas and supercritical fluid as given in Table 1.2.

1.1.1 Pillars of Chromatography

The development of chromatography began in early 1900. Some of the historical developments are given in Table 1.3. Unger et al. (2010) presented the evolution of liquid chromatography to the current state of development in the field of life sciences as shown in Fig.1.1.



Figure 1.1: *History of the development of chromatography* [Adapted from Unger (2010)].

General Classification	Specific method	Stationary phase	Type of equilibrium
Gas Chromatography	Gas –liquid (GLC)	Liquid adsorbed or bonded to a solid surface	Partition between gas and liquid
	Gas-solid	Solid	Adsorption
	Liquid-liquid or partition	Liquid adsorbed or bonded to a solid surface	Partition between immiscible liquids
	Liquid–solid or adsorption	Solid	Adsorption
Liquid Chromatography	Ion exchange	Ion-exchange resin	Ion exchange
	Size exclusion	Liquid in interstices of a polymeric solid	Partitioning/sieving
	Affinity	Group- specific organic species bonded to a	Partition between surface liquid and
		solid surface	mobile liquid
Supercritical Fluid			Partition between supercritical fluid
Chromatography			and bonded surface

Table 1.2: Different type of column chromatographic methods [Adopted from Skoog (2004)]

Table 1.3: Historical development of High Performance Liquid Chromatography

(HPLC)

Year	Milestone			
1890	David Day at the US Geological Survey demonstrated the separation of			
	petroleum			
Early 1900	Carl Runge, German dye-chemist attempted for crude dye separation			
1903	Invention of chromatography by Russian botanist, Mikhail S. Tswett for			
	the isolation of chlorophyll constituent on chalk column			
1930	First attempt towards reproducible separations: Kuhn and Lederer in			
	Heidelberg, Germany, for the isolation of plant constituents			
1938	Development of thin layer chromatography			
1941	Replacement of liquid mobile phase by gas, concept of gas			
	chromatography			
1941	Concept of HEPT which initiated the development of HPLC			
1942	Development of refractive index detector by A. Tiselius and D. Claesson			
1943	Invention of partition and paper chromatography by A. J. P. Martin			
1950	Separation and isolation of proteins			
1952	Noble prize in chemistry shared by A. J. P. Martin and R. L. M. Synge for			
	the development of partition chromatography			
1956	J. J. vanDeemter, F. J. Zuiderweg & A. Klinkenberg formulated the			
	relation among different types of diffusion & mass transfer phenomena &			
	linear gas flow later known as vanDeemter equation			
1960	Gel-permeation chromatograph by Waters Associates			
1960	Two different groups for development of HPLC, Csaba Horvath in the			
	United States and Josef Huber in Europe			
1960	J. C. Gidding, given dynamics of chromatography a unified theory of			
	separation science			
Late 1960	Introduction of commercial HPLC systems from Waters Associates and			
	DuPont initially in the market			
Late 1970	HPLC system upto pressure 400 bar			
2004	Introduction of UPLC (Ultrahigh performance LC)			

1.1.2 Basic concept related with chromatography

For a better understanding of the chromatographic method, a brief introduction about the basic concepts is presented here.

1.1.2.1 Mechanism of separation

In chromatography, differential migration of the solute is the basis of separation. As shown in Fig. 1.2, when a mixture of two components is allowed to pass through the chromatographic column, one compound moves into the column more rapidly compared to the other one. This differential migration through the column depends upon the distribution of the components between the two phases viz. mobile phase and stationary phase. Therefore, the factors that affect the distribution namely composition of mobile phase, nature of stationary phase and temperature are responsible for the change in differential migration. However, the migration of the components is not identical which is initially narrower but gradually broadens. The different processes responsible for the broadening of the peak namely multiple flow paths, flow distribution, mass transport of the sample between the phases and extra column diffusion will be described briefly [Snyder (1979)].

1.1.2.1.1 Multiple flow paths (Eddy diffusion)

Fig. 1.3 depicts the multiple flow paths of the mobile phase inside the column. During the flow of the mobile phase, there are different flow streams which lead to some molecules traveling at the shortest path length whereas some of them move along with several diversions on the way. As seen in the Figure, for well packed uniformly distributed column, the peak is sharper compared to the poorly packed column. It is approximately independent of the flow rate and depends upon the size and arrangement of the particles.



Figure 1.2: Schematics of chromatographic separation of two components [Adapted from Meyer (2010)].



Figure 1.3: Schematic of multiple flow paths.

1.1.2.1. 2 Flow distribution

Mobile phase flows faster in stream centre than nearer to the particle in a laminar flow of solvent into the stationary phase. As shown in Figure 1.4, the longer arrow represents the higher velocity of the mobile phase. Therefore, the sample molecule in the centre moves to a farther distance compared to the slow moving sample near the particle. This will again lead to broadening of the peak. The broadening because of eddy diffusion and flow distribution can be minimized by packing the column with particles as narrow in size distribution as possible.



Figure 1.4: Schematic of flow distribution.

1.1.2.1.3 Sample molecule diffusion in mobile phase (Longitudinal diffusion)

During the flow of analyte into the stationary phase, there exists a concentration gradient leading to the diffusion of analyte in all directions as shown in Fig. 1.5. This phenomenon becomes more prominent at a lower mobile phase velocity. Since, the time spent on the stationary phase by analyte is inversely proportional to the mobile phase velocity, hence the broadening also inversely depends upon the velocity of the mobile phase. The broadening because of the sample molecule diffusion can be minimized by proper selection of the mobile phase velocity.



Figure 1.5: Schematic of sample molecule diffusion in mobile phase.

1.1.2.1.4 Stagnant mobile phase mass transfer and stationary phase mass transfer

This phenomenon is observed when analytes are trapped into the pores of the column (Fig.1.6). The analyte which penetrates short distance moves out quickly by diffusion and moves down to a certain distance whereas, analyte that penetrated further distance spends more time into the pores and takes longer time to come out. This results into the spreading of the molecules into the stationary phase leading to the band broadening. In both the cases, the broadening of the peak increases with increase in the mobile phase velocity.



Figure 1.6: Schematic of sample molecule diffusion in mobile phase.

More analytically, prevalence of the broadening determines the efficiency of the separation which is described in terms of height equivalent to the theoretical plate (HETP) or (H). Fig. 1.7 depicts the well known van Deemter plot for describing the dependence of the above mentioned factors responsible for peak broadening. Equation (1.1) gives the dependency of H on the mobile phase velocity [van Deemter (1956)].

$$H = A + B/\mu + C\mu$$
(1.1)

where A, B, C and μ are eddy diffusion contribution, longitudinal diffusion contribution, mass transfer contribution and mobile phase velocity, respectively. The H versus μ shows a characteristics shape which depends upon the particle size and type of the column. The graph gives the idea whether a given column performs properly or not. Hence, before the column operation, it is always useful to construct the plot of H versus μ so that proper care could be taken when the performance is not satisfactory.



Mobile Phase Velocity (µ)

Figure 1.7: van Deemter plot for H vs flow rate, where term A represents contribution from multiple flow paths, B represents longitudinal diffusion and C represents contribution from stagnant mobile phase mass transfer and stationary phase mass transfer.

Considering the dependency of H in equation (1.1), H value is smaller for a small particle size column. Fig. 1.8 shows the effect of particle size on HETP. As can be seen, with the decrease of particle size, there is a significant decrease in H value.

H can be determined by using equation (1.2) which is another relation for dependency of H on the number of theoretical plates (N) and column length (L).

H = L/N(1.2)

Equation (1.2) shows an inverse relation between H and N. For an efficient separation, it is desirable to have small value of H and maximum value of N.



Figure 1.8: Effect of particle size on height equivalent to theoretical plate (HETP).

H can be determined experimentally by determining N and knowing the column length. N is dependent on retention time (t_R) and width of the peak (W) as given in equation (1.3)

All the molecules after eluting from the column move to the detector where individual species is represented in term of time spent inside the column, known as retention time. Difference between the retention times of individual components describes the resultant separation. The signal recorded is known as chromatogram. Chromatographic method development requires optimization of separation conditions that provide the acceptable separation for the analytes. Followings is a brief discussion regarding some of the basic parameters involved in chromatography.

Retention time: Time elapsed between sample introduction and maximum of response in the chromatogram of a given solute. Fig. 1.9 shows the retention time of compound 'A'.



Figure 1.9: Measurement of retention time of compound.

As seen in Fig. 1.9, the retention time (t_R) is given by the sum of the time required by mobile phase to pass through the column (t_M) , also known as dead time, and the time of the analyte spent inside the stationary phase (t_s) .

Values of dead time (t_M) and retention time (t_R) depend upon the mobile phase flow velocity, column volume and porosity. Hence, there is a need for a parameter which is independent of these factors.

From the peak of a retained component, qualitative as well as quantitative information can be obtained. Under the identical chromatographic conditions like column dimension, stationary phase and mobile phase composition, mobile phase velocity etc., the retention time of a component is constant. It can be used for identifying the relevant component by comparing the retention times. In chromatogram, as shown in Fig. 1.10, the peak height and peak area are proportional to the concentration of a component injected.

Hence, a calibration plot can be obtained between peak area and concentration and can be used for the determination of concentration of the component in unknown samples.



Figure 1.10: *Representation of a chromatogram indicating peak area and peak height* [Adapted from Meyer (2010)].

Retention factor: It is defined as the ratio of time spent by an analyte into the stationary phase to the time spent into the mobile phase. Mathematically, it can be expressed as

 κ = (amount of analyte in stationary phase) / (amount of analyte in mobile phase)

This can be represented in terms of retention time by following expression

$$\kappa = (t_R - t_o)/t_o$$
 (1.5)

Physical significance of the retention factor is that if κ is less than unity i.e. the degree of separation is inadequate, analyte elutes without retention. On the other hand, higher

values of κ signify the prolonged separation time. The desirable retention factor of analyte in a mixture varies from 1 to 10. Retention factor also allows comparing retention times of a given component obtained using stationary phases of different dimensions.

Separation factor (α): It is measurement of the separation selectivity. If the two components have the value of $\alpha = 1.0$ (i.e. $\alpha = \kappa_A / \kappa_B$), then no separation is possible. Value of α can be altered by selecting the composition of mobile phase and stationary phase.

Resolution: In chromatography, one of the goals is to achieve adequate separation which is defined in terms of resolution. Resolution of the two species given in Fig. 1.11 is defined as the distance between the two bands (denoted as A and B) divided by arithmetic mean of the two peaks width. Mathematically, it is defined by equation (1.6)





Figure 1.11: Representation of measurement of resolution.

where R, t_R and W represent the resolution, retention time and peak width, respectively. When R = 1.0, two bands are reasonably separated. However, for quantitative analysis, it is desirable to obtain the base line separation with a resolution of 1.5. The larger value of resolution is necessary especially for the species of unequally abundance. It is possible to predict the dependency of the various parameters which are generally optimized for obtaining the better resolution in method development in chromatography [Foley (1991)]. The dependency of resolution on various parameters is presented in equation (1.7).

$$R = \frac{1}{4} (\alpha - 1) \sqrt{N} \left(\frac{k}{1 + \bar{k}} \right) \qquad (1.7)$$
(Selectivity) (Efficiency) (Retention factor)

Where α , N and K are separation factor, number of theoretical plates and retention factor, respectively. Term α , which is represented as separation selectivity, changes with the composition of mobile phase and stationary phase. N is represented as separation efficiency which depends upon the column length and particle size whereas K varies with the solvent strength of the mobile phase. For obtaining a quantitative separation, it is desirable to have optimum value of K i.e. $1 \le K \le 10$ and high value of N with α greater than unity.

1.1.3 High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography (HPLC) is a liquid chromatography technique used for the separation and analysis of chemical mixture employing a liquid mobile phase with a stationary phase of very fine particles of diameter less than 10 μ m. To obtain a stable flow rate through smaller particle column, liquid must be pressurized

and hence this technique is also referred to as High Pressure Liquid Chromatography. Due to the wide range of applicability, reasonably good precision and availability of wide range of equipment, column and detectors, it has become one of the most useful analytical techniques [Snyder (2010)]. The mode of separation in HPLC is designed by the type of column being used. Table 1.4 shows different separation modes in HPLC.

HPLC mode	Types of stationary phase and mobile phase applied			
Reversed-phase	Stationary phase is non-polar whereas mobile phase is polar in			
chromatography (RPC)	nature, containing mixture of water and organic solvent. This			
	is the most widely used mode in HPLC especially for water-			
	soluble samples.			
Normal-phase	Stationary phase polar whereas mobile phase is mixture of			
chromatography (NPC)	non- polar organic solvent. Mainly used for water insoluble			
	samples.			
Non-aqueous	Stationary phase non-polar whereas mobile phase is a mixture			
reversed-phase	of organic solvents. Used for hydrophobic water insoluble			
chromatography	samples.			
(NARP)				
Hydrophilic interaction	Stationary phase polar in nature and mobile phase is a mixture			
chromatography	of water and organic solvents. It is useful for highly polar			
(HILIC)	samples.			
Ion-exchange	Stationary phase is a charged group, binds with the opposite			
chromatography (IEC)	charged ions and mobile phase is an aqueous solution of salts			
	with buffer. Useful for separation of ionizable samples.			
Ion-pair chromatography	In RPC, the stationary phase is converted into the charged			
(IPC)	surface employing the ion pair reagent to retain the sample			
	ions of opposite charge.			
Size-exclusion	Separation is based on the molecular weight and useful for the			
chromatography (SEC)	large biomolecules or synthetic polymers.			

 Table 1.4 HPLC Separation modes [Adopted from Snyder (2010)]

The detailed discussion about the individual components of the HPLC system used in the present thesis is given below.

1.2 Instrumentation

1.2.1 High performance liquid chromatography (HPLC)

A schematic diagram of HPLC is shown in Figure 1.12. The important components of HPLC system are pump, sample introduction system, reversed phase column, post-column delivery system, detector and data processor. For radioactive studies, HPLC system excluding the pump and data processor were kept inside the fume hood. Sample introduction system, reversed phase column, post column delivery system and detector were inside the fume hood. A non-returnable valve was placed between the solvent delivery system and the sample injector to prevent contamination due to radioactive solution.



Figure 1.12: Schematic of HPLC system.

1.2.1.1 HPLC pump

In HPLC, the requirement of pump is due to the fact that mobile phase is liquid in nature. The viscosity of the solvent necessitates a pressure driven flow though a small particle packed column. The basic requirements of HPLC pumps are capability to generate high pressure with high flow accuracy and precision at a set flow rate along with ease of operation [Meyer (2010)]. Different types of pumps which can be used are:

- (a) Pneumatic pump
- (b) Hydraulic pump
- (c) Syringe pump
- (d) Reciprocating pump

In the present study, the reciprocating pump was used for HPLC. Figure 1.13 shows the diagram of single headed reciprocating pump. The main component of this pump is a motor, piston, pump seal and check valve. As shown in Figure, the rotating disk or cam guides the piston for the movement in back and forward directions. As a result of this movement, the check valves closes and opens in regular interval. During the refilling stage, the outlet valve remains closed whereas inlet valve is opened. At the time of delivering the solvent, outlet is opened with the closer of inlet valve. Thus the delivery of solvent occurs at regular interval as illustrated in Fig. 1.14. This problem in pump pulsation can be minimized with the use of dual head reciprocating pump working in tandem mode. In this arrangement, the filling of one pump takes place while delivering the solvent by other pump. Further, the pulsation in solvent delivery can be minimized by using a sinusoidal cam as illustrated in Fig. 1.14.



Figure 1.13: Design of a reciprocating pump [Adopted from Meyer (2010)].

The advantages of dual head reciprocating pump is that solvent delivery is continuous without restriction of the size of reservoir. Hence, it is useful for long periods of operation.

For gradient delivery of the solvent, two types of classification are given depending upon the mixing of the solvents namely high pressure solvent programmer and low pressure solvent programmer. If the solvent mixing takes place at high pressure side, then it is classified as high pressure gradient programmer as shown in Fig. 1.15. Solvent passes directly through the pump and is mixed at high pressure followed by transferring into the injector and the column.



Figure 1.14: Flow profile of (a) single head reciprocating pump (b) dual head with circular cam (c) dual head with sinusoidal cam [Adopted from Snyder (1979)].



Figure 1.15: Schematic of high pressure gradient programmer.

In low pressure solvent programmer, the solvents are mixed at low pressure followed by transferring of solvent into the column as shown in Fig. 1.16.



Figure 1.16: Schematic of low pressure gradient programmer.

1.2.1.2 Sample Injector

To obtain a better separation without disturbing the solvent flow, sample injector is one of the essential aspects in HPLC. Different possibilities of sample introduction are available namely syringe and septum injector, with a loop valve and an automated injector system. Among these, the sample injection with loop is the most useful and is widely applied in HPLC injection system. Fig. 1.17 shows the six port injection valve with varying capacity. Sample injector valve has two different positions viz. loading and injection at the time of use. As depicted in Fig 1.17, in loading position, sample is injected in controlled way into the loop with fixed volume. After turning into the clockwise direction (injection position), the filled sample inside the loop is pumped into the column.



Figure 1.17: Six port injector system in loading and injection position.

1.2.1.3 Column

In HPLC, column manufacturing technology has reached a matured state for providing faster separation speed and efficient separation in a reproducible way. Due to the good mechanical strength and high surface area, silica was used widely as the supporting material for stationary phase. Later on, polymeric stationary phase was introduced for wider pH working range compared to the silica based support.

Based upon the particle types, HPLC column can be classified into different categories as discussed below:

Totally porous silica column: This type of column is the most popular in stationary phase due to higher column capacity. In this category, the commonly applied column dimension is of 1.5 to $5 \,\mu m$ size.

Pellicular particle: It is made of the solid surface coated with thin surface layer of stationary phase to provide the faster stationary phase mass transfer. However, this type of column is having low surface area and is limited for small sample sizes.

Superficially porous particle: It is a blend of solid core with porous outer shell. This type of arrangement provides much greater surface area compared to pellicular particle as a result of which larger amount of sample can be injected.

Perfusion particles: This type of column containing large through pores in a connected network. The diameter of the particle is generally higher than 10 μ m and hence it is suitable for the preparative scale separation.

Monolithic stationary phase: During the past few years, monolithic column composed of interconnected porous bed of a single rod is found to be useful for providing the rapid separation. Silica based chemically modified particulate as well as monolithic column have been utilized for the present HPLC studies which will be described briefly here.

Chemically modified silica as stationary phase: Silica as a stationary phase has been used for various niche applications in the field of chromatography. However, silica is having heterogeneous hydroxyl surface containing different silanol groups namely free silanol, germinal silanol and vicinal silanol. As a result of heterogeneity at the surface of silica, the column performance is strongly affected by the acidity of the medium. The severe problem of peak tailing is encountered in case of separation of basic compounds. Also, the presence of metallic impurities hampers the column performance. This reactive nature of the silanol group has been well utilized for chemically molding with varieties of organic groups leading to change in the polarity of the column. The general reaction can be presented as

X-Si
$$(CH_3)_2$$
-R + \equiv Si-OH \implies \equiv Si-O-Si-R+HX

where R = alkyl group, alkoxide etc. and X = halides. Here depending upon R, varieties of HPLC columns have been commercially available.

Monolith column: Monolith consists of an interconnected porous bed of single rod in contrast to particulate column packed with distinct particles [Guiochon (2007),

Snyder(2010)]. After 1990, monolith column has received enormous response from different research groups. The main characteristic of the column is bimodal pore size distribution known as macroporous with diameter of ~ 2μ m and mesoporous with diameter of ~ 10 nm. The retention of the analyte and efficiency of separation depends upon mesopores whereas solvent percolates through the macropores. In case of conventional packed column, the interstitial volume of the column is close to 40% of the total column volume whereas in monolith, this volume is as high as 80%. In monolith, high porosity leads to high permeability as result of which the required pressure at a given flow rate is lower compared to the conventional particle packed column. This provides the opportunity to operate at higher flow rate without loss of column efficiency. Based on the structure material, there are two types of monolith columns viz. silica based and polymer based. The monolith column is having different diameters varying from conventional column type (i.d. of 2 mm to 4.6mm), narrow bore column (i.d. from 10 μ m to 1 mm) and porous layer open tubular column. In the present study, the commercial monolith column manufactured by Merck was utilized.

1.2.1.4 Detector

In chromatography, the separated fraction from column reaches the detector where physical or chemical properties of eluted species can be explored for obtaining the electrical signal. The ideal detector should have high sensitivity, linearity with the change in solute concentration, similar or selective response towards the eluted species, stable towards the change in conditions like temperature and mobile phase composition, stability towards the base line drift and reproducibility etc. In real life, there is no single detector are satisfying all these criteria. The major types of detectors used in liquid chromatography are presented in Table 1.5.

Types of detector	Name of the detector	Working principle		
	Refractive index	Change in refractive index with respect		
Bulk property		to mobile phase is recorded		
detector	Light scattering	Scattered light from evaporated liquid		
		droplets is recorded		
	UV-Visible	Measure absorption of eluted species in		
		UV-Visible range		
	Fluorescence detector	Measure the fluorescence intensity		
		fluorescence compound or fluorescing		
Sample specific		derivative		
detector	Conductivity detector	Suitable mainly for inorganic ions with		
		the change in conductivity		
	Electrochemical	Measure the current for readily		
	detector	oxidizable and reducible species		
	Radioactivity detector	Monitor the emission of light after		
		radioactive decay		
Hyphenated detector	Mass spectrometry	Ions are identified based on m/z values		

Table 1.5: Commonly used detectors in HPLC

The detectors given in Table 1.5 can also be categorized in two groups (a) Concentration sensitive detectors: The signal produced by detector is proportional to the concentration of eluted species. The detectors of this category are UV-Visible, refractive index, fluorescence, radioactivity and mass spectrometry detectors.

(b) Mass sensitive detectors: The signal produced by the detector depends upon the mass flux i.e. number of solute molecules per unit time. The following detectors are of this category: Electrochemical and conductivity detector. In present studies of HPLC, UV-Visible detector was used and hence will be discussed further.

UV-Visible Detector

The UV-Visible detector is the most commonly used detector in HPLC. It is useful for the compounds which absorb light in UV-Visible region. The absorption "A" of a compound by passing light beam through cell is a function of molar absorptivity " ϵ ", molar concentration "C" and the path length 'd'. The relation of A with ϵ cd is known as Lambert-Beer's law and is given by equation

$$A = \log (I_o / I) = \varepsilon cd \dots (1.8)$$

where, I and I_o are intensities of transmitted light and incident light, respectively.

Two different types of configurations of UV-Vis detector are possible namely fixed wavelength and variable wavelength. In fixed wavelength detector, a specific wavelength of the light is generated from the source whereas in the variable wavelength detector, one particular wavelength of light is selected from a broad spectrum source.

In a fixed wavelength detector, different lamp source are available depending upon the types of uses. The different sources are mercury vapor lamp (254nm), zinc lamp (214 nm), cadmium lamp (229 nm). The sensitivity of a fixed wavelength detector is better than that of the variable wavelength detector. On the other hand, variable wavelength detector is versatile, convenience in operation over a wide range of wavelengths that provide maximum selectivity and ability to change wavelength during the run. Now a days, fixed wavelength detector is not widely used as compared to the variable wavelength detector. Further, variable wavelength detectors are broadly classified into two categories (a) Variable wavelength, where use of grating spreads the light into its

components and then a single wavelength of light is allowed to pass through slit, flow cell and photo detector.

(b) Diode array detector (DAD): In this case, the other configuration remains the same as variable wavelength detector. The only difference is that light passes through the flow cell followed by the diffraction grating as shown in Fig. 1.18. This leads to the spreading of light which falls over an array of photo diodes. The number of photodiodes varies with the detectors (512 or 1024 diodes). DAD can be used to collect the data from UV to visible range in a single chromatogram. The deuterium source is used for the generation of light in UV region whereas tungsten lamp is useful in visible region. Simultaneous collection of data at one or more wavelengths is useful to distinguish the two closely eluting peaks having different absorption spectra.



Figure 1.18: Schematics of diode array detector [adopted from Snyder (2010)].

Hyphenated detector

Hyphenated detector in HPLC mainly refers to coupling of liquid chromatography with instruments like mass spectrometer (MS), nuclear magnetic resonance (NMR), infra red (IR) etc. Out of these, mass spectrometry is the most functional detectors at present [Snyder (2010)]. It is useful in providing the structural information or eliminating the ambiguity for the identification of unknown. However, the challenges are mainly due to the differing working conditions of the two techniques. The liquid chromatography operates at atmospheric pressure while MS needs high vacuum during its operation. Hence, it is necessary to develop an interface which can couple these two widely differing analytical instruments. The following section of this Chapter will discuss about the introduction of mass spectrometry and suitable interface like electrospray ionization which has been utilized in the present thesis.

1.3 Mass Spectrometry

Mass spectrometry (MS) is used to determine mass of a molecule or a nuclide by measuring the mass to charge ratio (m/z). The mass spectrometric technologies have achieved maturity over the years and Table 1.6 presents history of development of the MS. Mass spectrometer consist of ion source, mass analyser and detector as shown in Fig. 1.19. Sample is introduced into ion source where the ionization takes place followed by acceleration, collimation and separation of ions based on mass to charge ratio and detection. In order to transfer ions to the detector without collision with the gas molecules, all mass spectrometers operate under high vacuum conditions. Hence, vacuum system is an important part in mass spectrometer for removal of residual gas molecules. The different components of a mass spectrometer are briefly described.

Year	Milestone
1886	Eugen Goldstein discovered positive rays of electricity
1897	Discovery of electron by J. J. Thomson
1898	W. Wien studied the deflection of cathodic rays by electric & magnetic field
1912	Construction of first mass spectrometer by J. J. Thomson
1913	Discovery of ²⁰ Ne and ²² Ne
1918	Development of an electron ionization source with sector shaped magnet with
	direction focusing
1919	Construction of Aston mass spectrometer
1934	First preparative isotope separation
1940	Isolation of ²³⁵ U
1942	Manufacturing of first commercial mass spectrometer
1948	Publication regarding design of time of flight mass spectrometer
1949	Development of mass spectrometer of ion cyclotron resonance type
1952	Theories of quasi equilibrium (QET)
1952	Johnson and Nier developed the double focusing instrument
1953	Development of quadrupole and ion trap analyzer
1956	Coupling of gas chromatograph with mass spectrometer
1958	First commercial mass spectrometer based on L-TOF (linear time of flight)
1966	Discoveries of chemical ionization method
1967	Introduction of collision induced dissociation (CID)
1968	Concept of electrospray by M. Dole
1972	Introduction of reflectron in a TOF instrument
1974	Discovery of atmospheric chemical ionization
1974	Coupling of HPLC with mass spectrometer
1977	Construction of first triple quadrupole mass spectrometer
1980	Houk demonstrated the application of inductively coupled plasma mass
	spectrometer
1983	Development of thermospray
1984	Potential application of electrospray for small molecules by J. Fenn
1987	Description of orthogonal acceleration in TOF
1987	Discoveries of matrix assisted laser desorption ionization (MALDI)
1994	Development of nanoelectrospray
1999	Introduction of Orbitrap mass analyser

Table 1.16: Historical development of mass spectrometers [de Hoffmann (2007)].



Figure 1.19: Schematic of a mass spectrometer [adopted from Becker (2007)].

1.3.1 Ion Sources

Variety of ionization sources are available for mass spectrometers as given in Fig. 1.19. Depending upon the extent of internal energy transferred during ionization, it is classified into two categories (a) extensive fragmentation during ionization e.g. electron ionization, thermal ionization, inductively coupled plasma and glow discharge are of this categories (b) Soft ionization where extent of fragmentation is less, retaining the structure of molecules. Chemical ionization, electrospray, MALDI and fast atom bombardment are categorized as soft ionization techniques. Mass spectrometers are classified according to the type of ionization processes used. Invariably, the ionization process takes place by ionizing neutral gas molecules by different processes such as electron removal, protonation, electron capture, adduct formation or transferring the existing charged ions from the condensed phase to the gas phase. The type of ionization process selected depends upon the application.

1.3.2 Ion Separator

Ion separation is an essential part of mass spectrometer where ions are analyzed based on their difference in mass to charge (m/z) ratios. As the ionization techniques have evolved in the past, several types of mass analyzers have been developed. The analyzers can be classified into two categories (a) separation of ions in time (b) separation of ions in space. Table 1.17 presents the different types of mass analyzers and their working principles. The main characteristics of a mass analyzer are the accuracy, mass range, resolution, analysis speed and transmission of ions. Mass accuracy is the difference between the theoretical m/z and measured m/z value. It is expressed in terms of part per million (ppm). Mass range of a mass analyzer determines the ability to measure ions over the range of m/z. For example, quadrupole can measure ions upto m/z 3000, magnetic sector m/z 10,000 whereas time of flight virtually over unlimited m/z. Resolution is the ability to distinguish the two ions with a small difference in m/z. If Δm is the difference between two masses, then the resolution required to separate the mass m is $R = m/\Delta m$. Analysis speed also known as scan speed measures the rate at which mass analyzer measures a particular mass range expressed in terms of mass unit per second. Transmission of ions by mass analyzer is the ratio of the ions being detected to the ions entering into the analyzer. Since the different mass analyzers are based on different working principles, they have different specifications as summarized in Table 1.17. However, mass analyzers used in present thesis will be discussed briefly.

Table 1.17: Different types of mass analyzers with working principles

Mass analyzer	Mode of separation	Resolution	Mass limit	Accuracy	Ion sampling	Dynamic
		(m /∆ m)				range
Magnetic Sector	Momentum analyzer	10000	20,000	< 10 ppm	Continuous	107
Quadrupole	Stabilities of the trajectories of ions	<400	3000	100 ppm	Continuous	10 ⁵
Time of flight	Difference in the flight time of ions	20000	< 500 000	10 ppm	Pulsed	10 ⁴
Orbitrap	Based on the resonance frequency	50000	50 000	< 5 ppm	Pulsed	10^{4}

1.3.2.1 Magnetic Sector

Under the applied potential, the ions are moving with the kinetic energy given by the following equation:

$$E_k = (mv^2)/2 = qV$$
 (1.9)

where E_k , m, v, q and V are kinetic energy of ions, mass of ions, velocity of ions, charge on ions and potential difference, respectively.

Under the applied magnetic field, the ions are having circular trajectory which is the result of the equilibrium of Lorentz force and centrifugal force balancing each other. Hence these two forces mathematically can be written as

Here, B is the magnetic field, r is the radius of curvature. Thus from equation (1.10), it is clear that for applied magnetic field B, the ions with the same momentum (mv) will be having same circular trajectory with radius r. In other words, the ions with different momentum will follow the different radius of curvature and hence it is acting as a momentum analyzer. However, taking into account kinetic energy and correlating equations 1.9 and 1.10, one obtains

$$m/q = B^2 r^2 / 2V$$
 (1.11)

Thus, from equation 1.11, it can be seen that for a fixed value of magnetic field and acceleration voltage, ions with different m/q ratio will be having different trajectories and those ions can be detected at different positions simultaneously. Alternatively, for a fixed
value of r, the ions with different m/q values will be sequentially detected at the same position by changing the magnetic field B.

1.3.2.2 Quadrupole

Quadrupole mass analyzer consists of four rods in hyperbolic or circular in shape as shown in Figure 1.20. A combination of dc and rf voltages is applied making the opposite rods of quadrupole of the same sign of the potential. The ions inside the quadrupole are influenced by the electric field because of the potential applied on the rods. The potentials are $\Phi = U+V\cos\omega t$ and - $\Phi = -(U+V\cos\omega t)$, where, Φ is the electric potential, U is the dc voltage and Vcos ωt is the rf voltage.



Figure 1.20: Schematics of quadrupole [adopted from Becker (2007)].

As seen from Fig. 1.20, ions with stable trajectory are moving without striking the rods whereas ions with unstable trajectory collide with the rods and are lost. Thus by suitably applying the dc and rf voltages, it acts as a mass filter.

1.3.2.3 Time of Flight

Time of flight is based on principle that ions with different masses are having different velocities under the constant acceleration field. Hence, flight time for the lighter mass is lesser compared to the heavier mass. The relation between applied potential and kinetic energy of the ion can be expressed by the following equation

$$E = \frac{mv^2}{2} = qV = ZeV$$

$$\Rightarrow v = \sqrt{\frac{2\text{ZeV}}{m}}$$

where E, m, v, Ze and V are kinetic energy, mass, velocity, charge and applied potential, respectively. The time required for travelling distance "L" is given by

Thus the difference in time of arrival (Δt) is directly proportional to the mass difference. The resolution can be improved by using the longer flight tube. However, the linear TOF gives the resolution of a few hundred only. In order to improve the resolution, reflectron was introduced. The reflectron consists of parallel ring with retarding potential and is placed behind the field free drift region acting as a mirror. Voltage of reflectron is higher compared to the acceleration voltage to insure the reflection of all the ions with minimization of the spatial distribution. In this way, the TOF with reflectron is very useful for the analysis of large biomolecules or clusters and is helpful for soft ionization source.

1.3.2.4 Hybrid Mass Analyzer

Hybrid analyzer is a combination of different types of analyzer to explore the strength of individual analyzer. The order of combination is abbreviated based on the sequence in which ions travel through the analyzers. The most common combinations are electrostatic with magnetic sector (EB or BE), quadrupole with ion trap (IT), time of flight (TOF), ion cyclotron resonance (ICR) or orbitrap (OT) and TOF with orthogonal acceleration (oa). In TOF analyzer, the most successful combination is oa-TOF with quadrupole analyzer (Q-TOF). This type of combination provides the simplicity of quadrupole with high performance of TOF. This offers high sensitivity and high resolution, allowing the analysis of multiply charged ions and mass range extension with good mass accuracy. The configuration of this type of instrument is QqTOF where first quadrupole Q1, acts as a quadrupole analyser. In second quadrupole q2, the collisions take place followed by analysis in oa-TOF. These combinations are used in two modes viz. MS and MS/MS mode. In MS mode, the quadrupoles, Q1 and q2 are under RF frequency and act as ion guide. In MS/MS mode, the Q1 acts as an ion filter allowing only precursor ions of interest to pass through followed by collisions in q2. The fragmented ions along with remaining parent ions are analyzed in oa-TOF (Chernushevich (2001), de Hoffman(2007)).

1.3.3 Detector

The separated ions in mass analyzer are allowed to generate stable signal in the detector. Since the number of ions exiting from the mass analyzer is very small, special fast ion detection system is required. Depending on the design of instrument and its application, various types of detectors are available. Photographic plate was used as the first detector in mass spectrometry. Detector in mass spectrometry can classified into two

categories (de Hoffman (2007)): (a) Point ion collector which allows to count ions sequentially i.e. one mass at a time. (b) Array collector which allow counting of multiple ions with different m/z values at a time. Another new class of detectors such as charge or inductive detector or cryogenic detector is not utilized frequently for mass spectrometry and are in development stage.

In the beginning, Faraday cup was commonly utilized as a detector in mass spectrometry due to its simple, precise and robust operation. At present, it is only useful for performing a stable isotope ratio measurement. Due to its slow response, it is not appropriate for scanning mode for the unstable ions currents.

Secondary electron multiplier has gained popularity as a detector in mass spectrometry. It consists of 12 to 20 dynodes with good electron emission properties. The incident ion strikes the dynode leading to the removal of electron which is converted into cascade of electrons with the help of other dynodes kept at higher potential and thus current is amplified. Fast response and high amplification provides the usefulness of this detector with the scanning of analyzer. However, this type of detector is not as precise as Faraday cup.

Another commonly used detector in TOF analyzer is microchannel plate. It consists of plate drilled from inside containing parallel cylindrical channels. The semiconductor layer on the surface of the channel performs multiplication of electrons. Due to flat conversion surface with a large area, small path length of secondary electrons inside the channel leads to fast response time suitable for recording the ions having spread in one dimension. Hence, this is commonly used for TOF where the time of arrival of ions is measured precisely. However, microchannel plates are very fragile, expensive and sensitive to air. The studies presented in this thesis were carried out using electrospray ionization mass spectrometry system.

1.3.4 Electrospray Ionization Mass Spectrometry (ESIMS)

Electrospray ionization (ESI) is a process of transforming solution phase ion into the gas phase ions [Cole (2010)]. The process of electrospray was discovered accidently after seeing the painting of car. The electrospray spray based studies started in 1966 where Hines (1966) studied the phenomenon of electrostatic atomization and process of electrostatic spray. Later on, Malcome Dole began with studies on determination of molecular mass of synthetic polymer such as polystyrene. By using the concept of ESI, Dole et al. (1968) were able to analyze macroions polystyrene in kilo-Dalton range. Further, John Fenn utilized ESI for studies on solution of small ions using quadrupole and was able to demonstrate the positive and negative ion formation [Yamashita, (1984a, 1984b). Fenn and co-workers presented the work on the analysis of biomolecules like peptides and proteins by ESI-MS and demonstrated the usefulness of this technique which led to the award of noble prize for the year 2002 [Whitehouse (1985), Wong (1988)]. ESI -MS is very useful for large biomolecules, non-volatile molecules and polymers analyses directly from liquid phase and can be coupled to the separation techniques like high performance liquid chromatography and capillary electrophoresis [Cech (2001), Gelpi (1995), Niessen and Tinke (1995), Slobodnik (1995), Bonvin (2012)].

Figure 1.21 shows schematic of ESI-MS system used in the present works. As seen in Figure, the different parts of ESI-MS are electrospray ion source, ion funnel as ion transfer unit, quadrupole as ion selector and collision cell, TOF as mass analyzer followed by microchannel plate as detector.



Figure 1.21: Schematic of ESI-MS system (Bruker Daltonics).

1.3.4.1. Electrospray ion source

Figure 1.22 presents the schematic of electrospray ionization process. Major steps for the formations of gas phase ions in ESI are (a) formation of charged droplets (b) evaporation of solvent leading to shrinkage of charged ions and (c) subsequent fragmentation of charged species into the gas phase ions.

The solution is infused through the syringe pump at a low flow rate (0.1-10 μ L/min) into the capillary. Nitrogen is used as an auxiliary gas. Under the high voltage (2-4 kV) applied between the capillary and the spray shield, formation of charged droplets takes place. Depending upon the type of analysis of ions, positive or negative potential is applied. Under the high electric field gradient, charged droplets start polarizing from the

liquid surface. The increased surface after the deformation is resisted by surface tension resulting into the formation of "Taylor cone". If the applied electric field is higher than the surface tension, then droplet merges into the fine droplet. These fine droplets are attracted towards the counter electrode. The heated nitrogen gas is flowing opposite to the direction of the droplet extraction, resulting into the solvent evaporation. When the charge on ions exceeds a critical limit known as Rayleigh limit, it leads to the multiple offspring droplet. This process is repeated several times till the ions transform into the gas phase ions. There are two models describing the formation of gas phase ions. (a) Charged residual model (Dole et al. (1968) suggests that the enhancement of charge density after solvent evaporation causes large droplets to undergo fission into smaller and smaller droplets.



Figure 1.22: Schematic of electrospray ionization process (Bruker Daltonics).

(b) Ion evaporation model (Iribarne and Thomson (1976)): After evaporation of solvent, there is a strong coulombic repulsion by which the charged ions overcome the surface tension leading to the emission of ions.

The potential required for the generation of charged droplets should be greater than surface tension of solvent. Table 1.18 shows the approximate voltage required for different solvents with different surface tensions.

 Table 1.18: Required voltages for different solvents with difference in surface

 tensions [Kebarle (2009)]

Col 4	Methanol	Acetonitrile	Dimethyl sulphoxide	Water	
Solvent	(MeOH)	(ACN)	(DMSO)	(H ₂ O)	
Surface tension (N/m)	0.0226	0.030	0.043	0.073	
Voltage (V)	2200	2500	3000	4000	

The generated ions in electrospray have following characteristics [Gaskell (1997)]: (i) The ions and their charges in gas phase reflect the ions in condensed phase, although there are possibilities of ion molecular reactions in the interface. (ii) The transfer of ions from solvent phase to gas phase is not an energetic process. (iii) The disruption of noncovalent interaction between analyte and solvent molecules at the interface takes place.

1.3.4.2. Ion Transmission and Detection

In ESI process, the ions are generated at atmospheric pressure while the mass analysis can only be performed in high vacuum conditions. Hence, necessary steps are required to transfer ions from atmospheric pressure to high vacuum. The differential pumping is employed for this purpose where the mass analyzer is kept at decreasing pressure and ions are transferred using suitable optics. The generated ions are allowed to pass through the capillary from the spray chamber to the ion optics. Capillary is isolating high voltage at the entrance to the low voltages at the ion transfer unit. Ions generated are allowed to pass through the hexapole cartridges which contain the two ion funnels and hexapole. Ion funnels are closely spaced rings with decreasing diameters. Funnels act as ion collector for ions entering into the vacuum system by applying rf and DC voltages. The variation in DC voltage in ion funnel can be used for In Source Collision Induced Dissociation (ISCID) studies. Ion funnels have advantages compared to the skimmer cones in terms of better ion collection and efficient ion transmission [Kelly (2010)]. The repulsive ion optics provides the robustness against the contamination compared to skimmers. Further, hexapole acts as an ion transmitter. The applied rf voltage on hexapole helps in focusing the ions towards the hexapole axis. At the exit of hexapole, gate with ion lenses are able to provide appropriate beam shape for transferring ions into quadrupole. After that, ions travel though the Q-q stage for isolation and fragmentation. The first quadrupole acts as mass filter for allowing ions in a certain mass range to pass. The selected ions undergo fragmentation in collision cell by Collision Induced Dissociation (CID) with neutral collision gas. In MS mode, first quadrupole acts as an ion guide. Due to the presence of neutral gas in collision cell, ions lose energy and gets focused closely at the time of exit. At the end of collision cell, there is a gate lens and transfer lenses. During the collision, the voltages of gate lenses are fixed in such a way that ions are allowed to remain inside and after the collision, the accumulated ions are transferred into the TOF analyzer. The TOF consists of orthogonal acceleration and operates in pulse mode. The ions are stored in pulsing region where they get pushed towards the reflectron and are allowed to travel in field free region. During the orthogonal acceleration, ions will have different velocities and kinetic energies. These energy differences are circumvented by using a reflectron. The ions of same mass but different kinetic energies will penetrate into the different depths resulting in balance in energy. The ions reflected from the reflectron are converted into the electric signal by allowing them to fall into the microchannel plate detector.

ESI –MS being a soft ionization technique enables the identification of species without the fragmentation. However, it does not provide the structural information which can be obtained by using the theoretical tools like density functional calculation. The density function theory is a useful tool for elucidation of structure and possible fragmentation pattern phase [Matsumoto (1999), Conte (2000), Yu (2008), Gresham (2011)].

1.4 Density Functional Theory (DFT) [Neese (2009), Chermette (1998)]

The quantum mechanics began with the historical equation introduced by Austrian Physicist E. Schrödinger. The simplified version of time independent Schrödinger equation can be written as

where H is the Hamiltonian operator, ψ is the wave function and E is the eigen value of the state. Theoretically, Schrödinger equation can be applied to any system. However due to large variables, it is applied with certain approximations for many electron systems. One of the approximations proposed by Max Born and J. R. Oppenheimer states that the nuclei are much heavier in mass compared to the electrons. Hence, nucleus is considered as stationary compared to the motion of electrons. Thus the nuclear kinetic energy terms from Schrödinger equation are omitted and equation remains purely electronic in nature. $H_{ele}\psi_{ele} = E_{ele}\psi_{ele} \quad \dots \quad (1.14)$

Even after applying the approximation, the Schrödinger equation for many electrons is quite complex. Hence Hartree-Fock method was introduced where one varies the functional with respect to form of the orbitals. This method was very successful yet in chemistry rarely used. The term correlation was introduced which is arising from the electron-electron interaction. To overcome problem of multi electrons wave function, density functional theory (DFT) was introduced. Hohenberg and Kohn theorem describes that the density of the system is sufficient to calculate all properties of the system. The energy function can be divided into kinetic energy, potential energy, coulomb repulsion energy and exchange correlation energy. Out of these, the exchange correlation energy of the system is not known and can be calculated by Kohn and Sham (KS) method. The KS equation can be written as

Since the exchange correlation is not known, approximation as accurate as possible is used with the new functional. Exchange correlation energy functional can be categorized into three generations. Local density approximation is the first generation of functional which is proposed by Dirac for exchange. The second generation uses both the density and its gradients. First gradient correlation was introduced by Becke, Perdew and Wang for exchange and Perdew for correlation. The most useful functionals are Becke for exchange, Perdew for correlation and Lee for correlation. These are called generalized gradient approximations. The third generation is hybrid functional which consists of density, gradient and laplacians. It consists of DFT exchange and Hartree-Fock exchange. The most popular hybrid functional for coordination compounds is B3LYP which has been used for the theoretical calculations.

1.5 Scope of studies of separation of lanthanides, actinides and platinum group elements (PGEs)

1.5.1 Lanthanides, Thorium, Uranium and Plutonium

f-block elements in the Periodic Table are derived from the filling of seven 4f and 5f orbitals known as lanthanides and actinides, respectively [Atkins (2010)]. The filling of electrons from f^1 to f^{14} in period 6^{th} are cerium (Ce) to lutetium (Lu) whereas in period 7 are thorium (Th) to lawrencium (Lr). Lanthanum and actinium are considered as f-block elements due to similarity in properties with lanthanides and actinides elements, respectively. As per International Union of Pure and Applied Chemistry (IUPAC), the 4f and 5f elements are called lanthanoids and actinoids, respectively. However, the commonly used names are still lanthanides and actinides where the suffix "-ide" is generally in practice refers to anions. Lanthanoids along with yttrium are known as rare earths. However in the earth's crust, these elements constitute one sixth of the total elements. The chemical properties of lanthanoids are quite different from those of actinoids. In the earth's crust except promethium (Pm), all the lanthaoids are naturally occurring whereas only three actinoids namely Ac, Th and U are found in nature. Lanthanoids are having similar chemical and physical properties. Lanthanoids commonly exist in the +3 oxidation state as given in Table 1.19. The prevalence of trivalent oxidation states in lanthanoids is due to stabilization of 4f orbitals after removal of electron. On the other hand, actinoids' oxidation states show less uniformity. The early members of actinoids from Th to Pu exhibit the variable oxidation states whereas from Am onwards, the trivalent oxidation state is more common. Table 1.20 gives the electronic configuration of actinoids with their stable oxidation states.

Actinoids have remarkable importance for the production of energy in a nuclear reactor. Some of the actinoids like U and Pu have fissile isotopes (²³⁵U, ²³³U, ²³⁹Pu and ²⁴¹Pu) which can undergo fission with thermal neutrons and produce energy whereas the isotopes like ²³²Th and ²³⁸U are used to obtain other fissile radio nuclides (²³³U or ²³⁹Pu) in the nuclear reactor.

Name of the element, atomic		Stable oxidation		
number in parenthesis	Electronic configuration	states		
La (57)	$[Xe] 5d^1 6s^2$	+3		
Ce (58)	$[Xe] 4f^1 5d^1 6s^2$	+3, +4		
Pr (59)	$[Xe] 4f^3 5d^0 6s^2$	+3		
Nd (60)	$[Xe] 4f^4 5d^0 6s^2$	+3		
Pm (61)	$[Xe] 4f^5 5d^0 6s^2$	+3		
Sm (62)	$[Xe] 4f^6 5d^0 6s^2$	+3		
Eu (63)	$[Xe] 4f^7 5d^0 6s^2$	+3, +2		
Gd (64)	$[Xe] 4f^7 5d^1 6s^2$	+3		
Tb (65)	$[Xe] 4f^9 5d^0 6s^2$	+3, +4		
Dy (66)	$[Xe] 4f^{10} 5d^0 6s^2$	+3		
Но (67)	$[Xe] 4f^{11} 5d^0 6s^2$	+3		
Er (68)	$[Xe] 4f^{12} 5d^0 6s^2$	+3		
Tm (69)	$[Xe] 4f^{13} 5d^0 6s^2$	+3		
Yb (70)	$[Xe] 4f^{14} 5d^0 6s^2$	+3, +2		
Lu (71)	$[Xe] 4f^{14} 5d^1 6s^2$	+3		

 Table 1.19: Oxidation states of lanthanoids (most stable are in bold)

Name of the element (atomic		Stable oxidation		
number in parenthesis)	Electronic configuration	states		
Ac (89)	$[\mathbf{Rn}] \mathbf{6d}^1 \mathbf{7s}^2$	+3		
Th (90)	$[\mathbf{Rn}] \mathbf{6d}^2 \mathbf{7s}^2$	+4		
Pa (91)	$[Rn] 5f^2 6d^1 7s^2$	+4, +5		
U (92)	$[Rn] 5f^3 6d^1 7s^2$	+3, +4,+5, +6		
Np (93)	$[Rn] 5f^4 6d^1 7s^2$	+3, +4, +5 , +6, +7		
Pu (94)	$[Rn] 5f^6 7s^2$	+3, +4, +5, +6, +7		
Am (95)	$[Rn] 5f^7 7s^2$	+3, +4, +5, +6		
Cm (96)	$[Rn] 5f^7 6d^1 7s^2$	+3, +4		
Bk (97)	$[Rn] 5f^9 7s^2$	+3, +4		
Cf (98)	$[Rn] 5f^{10} 7s^2$	+3		
Es (99)	$[Rn] 5f^{11} 7s^2$	+3		
Fm (100)	$[Rn] 5f^{12} 7s^2$	+3		
Md (101)	$[Rn] 5f^{13} 7s^2$	+3		
No (102)	$[Rn] 5f^{14} 7s^2$	+3		
Lr (103)	$[Rn] 5f^{14} 6d^{1}7s^{2}$	+3		

Table 1.20: Actinoids and their oxidation states (most stable are in bold)

Similarly, the lanthanoids like La and Nd are employed as the stable fission product monitors for the determination of burn-up.

Burn-up: The term burn- up is defined as the amount of energy extracted from a given amount of fuel during its life in a reactor [Aggarwal (2007)]. It is important for fuel development, performance evaluation, safety of the fuel and validation of the theoretical

codes. Determination of burn-up can be carried out by two methods namely destructive and non-destructive methods. The non-destructive methods are having spectral interferences and hence not accurate [Gysemans (2006)].

In destructive methods, burn-up can be determined by two ways (a) determination of fission product monitor (b) Measuring the change in abundances of heavy element isotopes. By the method of fission product monitoring, certain criteria such as non-volatile, low capture cross section, stable product (non radioactive), not the component of un-irradiated fuels and similar fission yields for different fissile nuclides are necessary to be satisfied. Suitable fission product isotopes such as ¹⁴⁸Nd for ²³⁵U, ²³⁹Pu and ¹³⁹La for ²³³U are commonly used as burn-up monitors. In view of the complex nature of irradiated fuel, it is necessary to separate the burn-up monitor from the dissolver solution of irradiated fuel.

1.5.2 Separation of Lanthanides and Actinides

Separation of actinides was first performed by precipitation method [Nash (1997)]. Due to the non-selectivity leading to co-precipitation of the analyte of interest, it was replaced by solvent extraction method using methyl(isobutyl) ketone (MIBK) and later on tributyl phosphate (TBP) was found to be very efficient for the extraction of tetravalent and hexavalent actinides from trivalent transplutonium elements in a kerosene diluent. Similarly, the fission products like lanthanides resemble the trivalent transplutonium elements and were difficult to separate. Most efficiently, the separation of lanthanides and actinides were achieved by liquid-liquid extraction employing the soft donor ligands [Gorden (2013), Allen (2000)]. The actinides have greater tendency of complexation with soft donor atom as compared to lanthanides. Development of ion exchange resins and processes provided another breakthrough for the separation of

lanthanides and actinides [Nash (1997)]. Earlier, separation was carried out using citrate, lactate, or EDTA as a ligand. But in 1954, α -hydroxyisobytyric acid (α -HIBA) was reported for the separation on cation-exchange based column with the average separation factor of 1.4 for individual lanthanides and actinides [Choppin (1956)]. Later on, this ligand was explored by many researchers for the separation of lanthanides and actinides [Knight (1984), Barkley (1986), Cassidy (1986), Miranda Jr (2002), Raut (2004), Janos (2003), Dixon (2012), Santoyo (2007), Dybczynski (2005)].

Separation of trivalent actinides and lanthanides received another breakthrough with the development of phosphoric, phosphonic and phosphinic acid ester based liquid cation exchangers. Bis(2-ethylhexyl)phosphoric acid (HDEHP) was used for the extraction of trivalent actinides and lanthanides with the average separation factor of 2 [Peppard (1957), Stary (1966), Nash (1997)]. Later on, Weaver et al. (1968) developed a method of separation of lanthanides and trivalent actinides using HDEHP in combination with diethylenetriaminepentaacetic acid (EDTA) with the improved separation factor of 10.

Among the several separation methods for lanthanides and actinides, chromatographic techniques are widely used in different matrices [Nash and Jensen (2001), Robards (1988), Kumar (1994), Lochny (1998), Gouterland (2009), Gunther-Leopold (2005), Rollin (2001), Maheswari (2007), Raju (2007), Barkley (1986), Knight (1984), Cassidy (1986), Raut (2002, 2004), Sivaraman (2002a, 2002b), Deepika (2011), Perna (2002), Joe (2005)]. HPLC along with α -HIBA was utilized for the fast separation of lanthanides and actinides [Verma and Santoyo (2009), Datta (2010, 2011)]. The various complexes of lanthanides using α -HIBA are: [Ln(IBA)]²⁺, [Ln(IBA)₂]⁺, [Ln(IBA)₄]⁻ with the most dominating cationic complexes at pH less than 4. On the contrary, the tetravalent and hexavalent actinides form most dominating

hydrophobic complexes of the type $[MO_2(IBA)]^+$, $[MO_2(IBA)_2]$ and $[MO_2(IBA)_3]^-$ (where M=U and Pu) as well as $[M(IBA)]^{3+}$, $[M(IBA)_2]^{2+}$, $[M(IBA)_3]^+$ and $[M(IBA)_4]$ (where M=Th (IV) and Pu (IV)) [Akhilamaheswari (2007), Raju (2007), Datta (2013)]. The difference in the nature of the complexes of lanthanides and actinides is useful for the separation of lanthanides and actinides by dynamically modifying the hydrophobic reversed phase with ion interaction reagent [Barkley (1986), Elchuk (1991), Cassidy (1990), Fuping (1993)]. Water soluble ion interaction reagent (viz. n-octanesulphonic acid or camphor-10-sulphonic acid) is allowed to pass through the reversed phase column where it provides the charged surface for the interaction of lanthanides [Cassidy (1986), Rao(2005), Raut (2002, 2004)]. The main advantage of this method is the rapid ability to alter the capacity of column and selectivity. Using the dual gradient conditions of pH and concentration of α -HIBA, sequential separation of lanthanides, tetravalent and hexavalent actinides was achieved [Raut (2004), Jaison (2006)].

Under given chromatographic conditions, interaction of tetravalent actinides and hexavalent actinides in presence of α -HIBA on the reversed phase column is hydrophobic in nature. It is, therefore, interesting to use a more hydrophobic ligand having complexation tendency similar to that of α -HIBA. Mandelic acid (phenylhydroxy acetic acid) was found to be the useful in this regard in the present work. The presence of phenyl group is more hydrophobic than the methyl group of α -HIBA as shown in Fig. 1.23. The hydrophobic nature of mandelic acid was exploited by researchers for the chromatographic metals separations [Elchuk (1991), Hao (1996a), Jaison (2009b)]. The studies showed that the presence of phenyl group makes Th-mandelate more hydrophobic than U-mandelate species resulting in the better retention of thorium than uranium [Hao (1996a)]. Hence, it was very useful for determination of uranium at trace levels in presence of bulk of thorium [Jaison (2009b)]. Mandelic acid was also found to be a useful reagent for the preconcentration of uranium at low levels in presence of iron in processed sea water samples [Jaison (2011)].



Figure 1.23: *Structures of Mandelic acid (left) and α-HIBA (right).*

1.5.3 Platinum group of elements (PGEs)

Iridium (Ir), osmium (Os), palladium (Pd), platinum (Pt), rhodium (Rh) and ruthenium (Ru) constitute the platinum group of elements (PGEs). PGEs are having importance due to their applications in catalysts, as anti cancer agent and as automobile catalyzers [IAEA (2005)]. Due to the large applications of PGEs and their limited abundance in earth's crust, nuclear reactors are considered as one of the sources for the production of PGEs namely Pd, Rh and Ru [Bush (1991)]. The amount of the production of these PGEs depends upon the number of reactors, burn-up of fuel and their types [Bush (1991), Kondo (1992)]. PGEs are categorized as the soft metal atoms in the Periodic Table having more tendencies to bind with soft donor ligands. The order of stabilities with the ligands are: S ~ C > I > Br > Cl > N > O [Charlesworth (1981)]. In view of the low matix to analyte ratio and presence of spectral interferences, determination of PGEs poses a serious analytical challenge. Various preconcentration methods are described in the literatures for selective extraction of PGEs [Mladenova (2012)]. Out of the several preconcentration methods, solid phase extraction (SPE) is frequently applied for the preconcentration studies. This method reduces the amount of solvent required. It was successfully utilized for the separation of metal ions [Camel (2003)]. SPE involves partitioning of the analytes between the mobile phase and the solid sorbent phase by passing through a cartridge, a tube, or a disk. The multiple steps involved in SPE are conditioning of the solid surfaces, loading of the sample, washing of the loaded sample followed by elution of the sorbed species. The mechanism of sorption depends upon the type of sorbent. When solid surfaces are non-polar (C_{18} -sorbent), retention of analyte takes place via hydrophobic interaction. Depending upon the type of metals (Hard or soft), the chelating agent is added into the mobile phase for complexation with the metals. For the soft metal like PGEs, sulphur donor ligands are forming better complexes. For elution of the hydrophobic species, nonpolar solvents are preferred which disrupt interaction with the sorbent.

1.6 Scope of work in the Thesis

The main objective of work discussed in the present thesis was to develop HPLC methods for the separation and determination of trace elements in different matrices. The work was also carried out using ESI-MS for the studies of different species formed and the method was also developed for removal of matrices for the determination of trace elements. Thesis contains six Chapters dealing with the use of HPLC and ESI-MS for development of methods for trace impurities determination.

Chapter I: Gives the introduction and brief history about HPLC and ESI-MS techniques. It presents the detailed instrumentation of the two techniques.

Chapter II: Using HPLC, the separation of trace impurities of lanthanides in bulk of dysprosium is presented. The similarities in physical and chemical properties of rare earths poses a challenge for individual separation of rare earths. This is more difficult when one has to determine the trace quantities of one rare earth in presence of bulk of

other rare earth. In this Chapter, α -HIBA was explored as a ligand for the separation and determination of lanthanides and yttrium in high purity dysprosium.

Chapter III: This Chapter deals with the separation of lanthanides, thorium, uranium and plutonium in irradiated (Th,Pu)O₂ fuel. This separation is more difficult in view of the radiation dose associated with the dissolver solution as well as multiple oxidation states of Pu under the chromatographic conditions. In the present approach, separation and determination of individual lanthanides, thorium, uranium and plutonium was achieved keeping Pu in a single oxidation state.

Chapter IV: This Chapter describes the separation of Th, U and Pu using mandelic acid as an eluent. Optimized method was useful for the determination of Th, U and Pu in irradiated (Th,Pu)O₂ dissolver solution.

Chapter V: This Chapter presents the usefulness of ESI-MS as a soft ionization technique for speciation studies of platinum-benzoylthiourea using electrospray ionization mass spectrometry. Various species of platinum with benzoylthiourea were identified and the modes of coordination were given by applying density functional theory.

Chapter VI: This Chapter presents the investigation for the determination of palladium in simulated high level nuclear waste. The removal of matrix was carried out using the loosely bound disposable pipette extraction method. The optimized method was successfully applied for the determination of palladium in complex matrix like simulated high level liquid waste.

Chapter 2

Determination of Lanthanides and Yttrium in

High Purity Dysprosium by

RP- HPLC using α-Hydroxyisobutyric acid as an

Eluent

2.1 Introduction

High purity rare-earths have various applications in different fields viz. optical, electronics, ceramic, nuclear and geochemistry [Rao and Biju, (2000)]. High purity rare earth like dysprosium oxide (Dy_2O_3) finds applications in the nuclear technology. The relatively high neutron absorption cross section of naturally occurring dysprosium (Dy) (Table 2.1) makes it a potential candidate for the reactor control application without the release of gaseous by-product. Dy_2O_3 mixed and pelletized with zirconium oxide is proposed to be used for reactivity control in nuclear reactors [Singh (2008a), Kannan (2010)].

Isotopes of Dy	156	158	160	161	162	163	164
Natural abundance (%)	0.06	0.1	2.34	19	25.5	24.9	28.1
Neutron absorption cross section (σ) in barns	33	43	56	600	194	124	2840

 Table 2.1: Neutron absorption cross section data of Dy isotopes

For the above mentioned applications, stringent specifications are laid down for the chemical purity of Dy₂O₃. In pure Dy₂O₃, trace amounts of other rare-earths like holmium (Ho), terbium (Tb), erbium (Er), thulium (Tm), ytterbium (Yb), lutetium (Lu) and yttrium (Y) etc. are present. The occurrence of other rare earths greatly modifies the property of the material. The specification of Dy₂O₃ for reactor control application is: lighter rare earths (LRE) < 0.1 %, Gd₂O₃ < 0.2 %, Tb₄O₇ < 1 %, Y₂O₃: 1.5 – 5 % [Singh (2008a, 2008b)]. Different analytical techniques are reported for the quantification of rare-earth elements (REE) as impurities in high-purity rare earth materials [Suc (1992), Yoshida (1984), Hwang (1981), Li (1997)]. For quantification of rare earths, mostly destructive methods are employed [Nash and Jensen, (2001)]. The sample is subjected to the digestion process where the selective dissolution is rarely achieved. Two separation approaches are generally pursued: Separation in a group and individual separation of elements. Since the rare-earths are having similar chemical and physical properties, most of the methods suffer from strong interferences [Nash and Jensen (2001), Kumar (1994), Robards (1988)].

Classical methods for the determination of rare earths including gravimetry, complexometry and colourimetry, are typically non-selective and hence are useful for the total rare-earths determinations [Banks and Klingman (1956), Onishi and Banks 1963]. Multi-elemental analysis techniques such as mass spectrometry, neutron activation analysis (NAA), X-ray fluorescence, inductively coupled plasma atomic emission spectroscopy (ICP-AES) etc. are capable of determining individual rare-earths with good sensitivity but are susceptible to spectral interferences when these elements are present in mixtures [Kumar (1994), Biswas (1991), Daskalova (1992)]. Chromatographic techniques and solvent extraction are used to minimize the interferences and thus improve the selectivity of analysis. Chromatography offers the advantage of separating the individual rare-earths in a single separation stage. Different types of chromatographic techniques such as Liquid Chromatography (LC), Gas Chromatography (GC), and Super Critical Fluid Chromatography (SFC) etc. are utilized for the separation of rare-earths [Joe (2005), Cassidy (1988), Nesterenko (1998), Greulich (1986), Wu (1996)]. Among these, liquid chromatography is attractive because it provides the flexibility of selecting suitable stationary phase and mobile phase depending on the elements to be separated.

In liquid chromatography of rare-earths, use of ion interaction chromatography (IIC) is advantageous because it offers high resolution resulting from faster mass transfer at the surface of the ion exchanger [Joe (2005)]. IIC uses bonded micro-particulate alkyl

silica as non-polar stationary phase, aqueous mobile phase containing low concentrations of the ion-pairing reagent and a suitable complexing agent. The separation of rare-earths depends on the differences in the stability constants of the complexes formed by rareearths with the ligand. Different carboxylic acids such as EDTA, glycolic acid, oxalic acid, mandelic acid, lactic acid, α -hydroxy isobutyric acid (α -HIBA), citric acid, tartaric acid etc. have been tested in various laboratories for the separation of lanthanides. For the separation of individual lanthanides, α -HIBA is a popular complexing agent in view of the differences in the stability constants among lanthanide-ligand complexes. Thus, ion interaction chromatography in combination with post-column detection was reported for the determination of lanthanides in complex matrices [Cassidy (1982 and 1986), Barkley (1986), Raut (2002), Jaison (2009a)].

However, for most of the hydroxy carboxylic acids, the separation of Y and Dy is not satisfactory because of the minimal differences in the stability constants of their complexes. The problem of co-elution of Dy and Y has been documented in the literature [Tsakanika (2004), Wu (1999)]. Combination of different ligands like nitrilotriacetic acid, tartaric acid etc. has been used for improving the separation between Y and Dy [Hirokawa (1994 and 1995), Wu (1999), Oguma (1993), Kuroda (1991)]. However, it requires careful optimization of the proportion of the ligands and pH of the solution. This kind of approach was used in the separation becomes a challenging task when the concentration difference between the lanthanide pairs especially Dy-Y and Dy-Tb becomes significant.

Reported work on determination of rare earths in bulk of the rare earth

Various analytical techniques in combination of separation method have been reported. Suc et al. (1992) reported the determination of rare earth impurities in lanthanum oxide by NAA. As La produces high activity, matrix was removed by anion exchange column (Dowex 1 x 8) using mixture of methanol and nitric acid. However, this method involves offline separation which is time consuming and it is difficult to achieve quantitative recoveries. Another limitation of this method is the spectral interferences amongst the different rare earths. Singh et al., (2008b) reported the production of nuclear grade dysprosium oxide using 2-ethylhexylphosphonic acid, mono-2-ethylhexyl ester (EHEHPA) in a solvent extraction process. Mishra et al. (2000) developed solvent extraction process for purification of Dy and Tb from Dy concentrate using 2-ethyl hexyl phosphonic acid mono-2-ethylhexyl ester (PC 88A) in paraffinic kerosene as an extractant whereas Thakur (2000) used PC-88A for separation of Dy and Y from Y concentrate. These methods require careful optimization of experimental conditions like feed acidity, phase ratio, metal concentration in aqueous solution etc. for each application. Zhang et al. (1995) used inductively coupled plasma mass spectrometry (ICP-MS) for the determination of rare earth in high purity europium oxide. However, ICP-MS has the problem of spectral interferences because of formation of polyatomic ions like MO⁺, MOH^+ and multiple charged ions M^{2+} etc. and signal suppression of trace element in presence of bulk of other elements are well known [Takaku (1993)]. Pidreira et al. (2001, 2003 and 2006) resolved this problem by coupling mass spectrometry with the HPLC. The authors reported the trace level determination of rare earths in praseodymium oxide, neodymium oxide and samarium oxide matrices using sector field ICP-MS. However, this method is not cost effective.

Present Studies

This Chapter presents the development of simple HPLC method for the separation and determination of lanthanides, Y in Dy matrix using α -HIBA as an eluent on C₈ reverse phase (RP) column. Experiments were carried out to maximize the resolution for the separation of rare-earths, especially amongst Ho-Dy, Dy-Y and Dy-Tb pairs using α -HIBA as an eluent. Various parameters influencing the resolution are pH, concentration of the eluent (α -HIBA) and concentration of IIR. Conventionally, the above parameters are studied in a one dimensional approach, i.e., optimizing one parameter at a time. However, this approach may not result in the best conditions for the chromatographic separation of rare-earths. In the present work, experiments were performed to study the effect of all the important parameters on resolution simultaneously, to optimize the conditions offering good resolution for the determination of rare-earths based on chromatographic peak.

The salient features of the present Chapter are: (i) separation of individual lanthanides (Lns) and Y using α -HIBA alone as the eluent, (ii) effect of important chromatographic parameters studied simultaneously to arrive at the best conditions for the separation (iii) comparison of C₈ and C₁₈ stationary phases under the IIC conditions and (iv) optimization of separation conditions for holding large amounts of Dy without affecting the resolution so that the method can be applied to real life samples with significant Dy/Ln amount ratios.

2.2 Experimental

2.2.1 Instrumentation

The HPLC system consisted of an L-2130 (Elite, LaChrom) low-pressure quaternary gradient pump and an L-2450 (Elite, LaChrom) diode array detector. C_{18} monolith RP column (100 mm × 4.6 mm, Chromolith, Merck), C_{18} particulate RP column (150 mm × 4.6 mm, 5 µm, Supelcosil) and C_8 particulate RP column (150 mm × 4.6 mm, 5 µm, Merck) were used as the stationary phases. Solutions were injected into the column using a Rheodyne injector (Model 9725i) with a 100 µL loop. The eluted components were monitored after post-column reaction with a metallochromic reagent, which was added with a reciprocating pump (Eldex Laboratories Inc.) into a low dead volumemixing tee (Valco). The signal from the detector was processed by EZChrom software package.

2.2.2 Reagents

All the solutions were prepared using deionised water from Milli-Q system (Millipore) and were filtered through a 0.45 μ m membrane filter (Millipore) prior to use in HPLC. α -HIBA (Lancaster, U.K.) was used as an eluent. Sodium n-octane sulphonate monohydrate (Fluka, Switzerland) was used as the ion interaction reagent (IIR). ICP-standards of all the lanthanides (Inorganic Venture) and Y were used after appropriate dilutions with MilliQ water. HNO₃ and NH₄OH (Merck) were used for adjusting the pH of the mobile phase. Arsenazo (III) (Fluka) was used as the post column metallochromic reagent (PCR).

2.2.3 Procedure

Appropriate quantities of α -HIBA and sodium n-octane sulfonate were dissolved in water and were made to appropriate concentrations. All the solutions were adjusted to the desired pH using high-purity NH₄OH and HNO₃. Mobile phase flow rate of 1.0 mL min⁻¹ was used. The mixture of lanthanides and Y was injected using 100µL loop. The PCR solution containing 1.5×10^{-4} M Arsenazo(III) and 0.01M urea in 0.1M HNO₃ was delivered at a flow rate of 0.3 mL min⁻¹. About 1 g of solid Dy₂O₃ was dissolved in concentrated HNO₃ (~5 mL) by heating on a hotplate. The heating was continued until dissolution was complete. The solution was evaporated to near dryness and was redissolved in 1M HNO₃. Appropriate amount of this stock solution of Dy was taken and was diluted with the mobile phase before injection. The quantification of lanthanides and Y was done by the standard addition method.

2.3 Results and Discussion

Capacity factor or retention factor, k, was determined as $k = (R_1-R_0)/R_0$, where, R_0 and R_1 are the retention times of non-retained peak and that of element retained, respectively.

Resolution between the two adjacent peaks was calculated using the equation: $R = 1.18 \frac{(R_{t_2} - R_{t_1})}{(W_{u(2)1} + W_{u(2)2})};$ where, R_{t_1} and R_{t_2} are the retention times of the two adjacent

peaks 1 and 2, respectively and $W_{1/2}$ denotes the peak width at half maximum. When the resolution between two adjacent peaks was less than 1, the resolution was estimated based on comparison with a standard set of resolution curves [Snyder (1979)].

2.3.1 Study of Chromatographic Parameters

The retention of rare earths (REs) under chromatographic conditions depends on the stability of RE-eluent complex. Therefore, any factor that influences the stability of the RE-eluent complex formed would obviously affect the retention behavior of rareearths in the cation exchange column. Thus, retention of rare-earths on a column will change with the concentration and pH of the ligand. Retention also depends on the concentration of IIR. Thus a study was performed to evaluate the effect of all the three parameters systematically to arrive at the optimum conditions for the separation of rareearths in presence of large amounts of Dy. This strategy was employed simultaneously as it considers the interplay that may exist among the different variables. Preliminary work was carried on a C_{18} monolith column (100 mm x 4.6 mm) using a synthetic mixture containing 1 $\mu g/g$ (w/v) of each of the 4 lanthanides (Gd, Tb, Dy, Ho). Since Tb and Ho are eluting on two sides of Dy, their separation with adequate resolution is critical for determining the rare-earths in Dy matrix based on the peak area. Small C_{18} monolith RP column (100 mm x 4.6 mm) was preferred for optimization studies as this would require less chromatographic run time compared to 150 mm long column. However, for separation of rare-earths in presence of bulk of Dy, longer C_{18} particulate RP column (300 mm x 4.6 mm) was used as it provides large surface area to increase the column capacity. For this study, two different stationary phases i.e. C_{18} and C_8 of the same dimensions were compared.

2.3.2 Effect of IIR

In ion interaction chromatography, the ion-exchange capacity depends on the concentration of the ion interaction reagent in the mobile phase. As the concentration of the IIR increases, there is an increase in the number of active sites created on the stationary phase and this improves the ion exchange capacity and provides better separation. But an increase in the ion exchange capacity may also lead to broader peaks and longer separation times. In the present study, the concentration of IIR was changed from 0.005M to 0.02M at 0.075 M α -HIBA of pH 5.0. Fig. 2.1 shows the change in resolution between the Dy-Tb pair with the increase in concentration of IIR. Tb was chosen since large amount of Dy masks the Tb peak. As expected, the resolution increased steadily with increase in the concentration of IIR. This fairly high pH of α -HIBA was chosen to complete the chromatographic run in a reasonable time and these conditions were confined only to experiments described in Fig. 2.1. For each concentration of IIR, the effects of pH and concentration of α -HIBA were studied in the ranges as described in the following sections.

2.3.3 Effect of pH

In this study, pH of the eluent was changed from 3.0 to 6.0 in increments of 1.0. pH lower than 3.0 was not advisable for the integrity of the column. It was observed that the retention time decreased with increase in pH of α -HIBA. This behavior can be

explained on the basis of increased ionization of α -HIBA (pK_a 3.77) with increase in pH and this enhances its complexation with lanthanide ions.



Figure 2.1: Resolution for Dy and Tb separation as a function of IIR concentration. 0.075 M α-HIBA of pH 5.0 was used as eluent, flow-rate: 1 mL min⁻¹; stationary phase: 100 mm x 4.6 mm, RP column; post-column reagent: 1.5x10⁻⁴ M Arsenazo (III) in 0.01 M urea and 0.1 M HNO₃; flow-rate: 0.3 mL min⁻¹; monitoring wavelength: 653 nm.

Higher pH values led to merging of the peaks whereas lower pH values resulted in prolonged elution time and broadening of peaks. For each pH studied, the effect of concentration of the eluent on the resolution of the above lanthanides was also monitored.

2.3.4 Effect of Concentration of α-HIBA

Effect of concentration of α -HIBA on retention of lanthanides was studied by changing its concentration from 0.075M to 0.15 M. At a given pH, it remains partly

ionized. The positively charged lanthanide-HIBA complex remains in equilibrium between anionic n-octanesulphonate sorbed on to the stationary phase and the dissociated α -HIBA in the mobile phase. With the increase in the concentration of α -HIBA, the fraction of ionized α -HIBA in the mobile phase increases and hence the equilibrium shifts more towards the mobile phase leading to faster elution of lanthanides. Thus retention time of lanthanides decreases with increase in the concentration of α -HIBA leading to degradation of resolution.

2.3.5 Optimization of chromatographic parameters

Effect of pH and concentration of α -HIBA on the resolution of lanthanides was studied simultaneously at different concentrations of IIR in the mobile phase. The plots generated for resolution as a function of pH and α -HIBA concentration at IIR concentrations of 0.005M, 0.01M and 0.02M are given in Figs. 2.2, 2.3 and 2.4, respectively. It is seen that at lower concentrations of α -HIBA and pH of the eluent, the best resolution was obtained and this decreases drastically with increase in pH. An eluent concentration of 0.075M and pH of 3.0 were found to give the best resolution for Dy-Ho and Dy-Tb pairs. However, this condition could not be adopted for the method as some of the lanthanides were eluting as double peaks. Fig. 2.5 shows the elution of 1 µg/g Gd as two peaks separated by ~ 2 minutes. The two peaks are assumed to be due to the different species of Gd formed with the complexing agent, α -HIBA. It may be noted that in the present case, an eluent with relatively low strength was employed (0.02 M α -HIBA at pH 3.0.) and hence splitting of Gd peaks could be seen at an IIR concentration of 0.01 M.



Figure 2.2: Effect of pH and concentration of α -HIBA on resolution of lanthanides at constant concentration of IIR (0.005M), other conditions same as in Fig. 2.1.



Figure 2.3: Effect of pH and concentration of α -HIBA on resolution of lanthanides at constant concentration of IIR (0.01M), other conditions same as in Fig. 2.1.



Figure 2.4: Effect of pH and concentration of α -HIBA on resolution of lanthanides at constant concentration of IIR (0.02M), other conditions same as in Fig. 2.1.



Figure 2.5: Elution of Gd as two separate peaks. Mobile phase: 0.02 M α-HIBA (pH 3.0); 0.01 M sodium n-octane sulphonate; other conditions same as in Fig. 2.1.

The condition was intentionally selected to amplify the separation between the two species of the same lanthanide. In the case where there was peak splitting, the resolution was determined based on the first peak by assuming that the metal ion would be complexed to a greater extent as compared to the latter peak. The same species must be present in greater abundances under conditions favouring complexation with α -HIBA.

Though the different peaks of the same lanthanide ion eluted under the special elution conditions were not characterized, they are supposed to be different species of lanthanide- α -HIBA system. The formation of such double peaks of Ln- α -HIBA species was prominent when complexation of the chelating agent was restricted i.e. under low pH and low α -HIBA concentration conditions. The probability of observing the double peaks increased with the increase in IIR, i.e. when the ion exchange capacity was increased and thereby increasing the chances of separating closely eluting species. It was also observed that when a mixture of lanthanides was injected under such restricted complexation conditions, the lighter lanthanides were found to elute as double peaks whereas the heavier ones would not suffer any peak splitting. This may be explained on the basis of relatively stronger tendency of the heavier lanthanides (charge to radius ratio) to form complexes with α -HIBA as compared to the lighter lanthanides.

For obtaining good separation without any interference, each lanthanide element must elute as a single peak. Use of milder elution conditions caused the excessive broadening of the peaks. Poor complexing ability leads to increase in retention time which in turns causes broadening of the peak (Fig. 2.6). At higher concentration of IIR, it is expected that the retention and hence the separation among the lanthanides would be better. However, when IIR concentration was increased from 0.01 M to 0.02 M, the probability of elution of lanthanides as multiple peaks increased. This necessitated the use of harsh conditions for the elution and resulted in counteracting the improvement in resolution expected at higher concentrations of IIR. Hence 0.01 M of IIR was chosen and isocratic separation of the mixture of 4 lanthanides at 0.075 M of α -HIBA of pH 5.0 was investigated.



Figure 2.6: Width of lanthanide peaks as a function of pH and concentration of α-HIBA. Concentration of IIR in the mobile phase: 0.01M, other conditions same as in Fig. 2.1.

It was found that under these conditions, the four lanthanides were well separated and no peak splitting was observed as shown in Fig. 2.7. However, isocratic condition is not suitable when the lanthanides are in different proportions especially when one of the lanthanides (Dy) is present in bulk amounts. Fig. 2.8 shows the chromatogram obtained using C_{18} particulate RP column (300 mm x 4.6 mm, 5 µm) for a mixture of Ho, Tb, Dy and Gd with Dy/Tb amount ratio of 1,000 employing isocratic conditions.



Figure 2.7: Isocratic elution of a mixture of Gd, Tb, Ho and Dy (1µg/g each). Mobile phase: 0.075 M α-HIBA (pH 5.0); 0.01 M sodium n-octane sulphonate; flow-rate: 1 mLmin⁻¹; temperature 25 ⁰C; stationary phase: 4.6 mm x 300 mm, 5 µm C₁₈ RP column; post-column reagent: 1.5x10⁻⁴ M Arsenazo(III) in 0.01 M urea and 0.1 M HNO₃; flow-rate: 0.3 mL min⁻¹; monitoring wavelength: 653 nm.

Therefore, this condition was not employed for the determination of lanthanides in Dy_2O_3 . Hence a suitable gradient elution condition was explored. From the above studies, pH of the medium was optimized to be 5.0 where the splitting of the peak was not observed. Gradual increase in concentration of the eluent from 0.025M to 0.075M provided a better separation without affecting the resolution. Fig. 2.9 shows the well separated lanthanides peaks using gradient elution on C_{18} particulate RP column where (Dy/Tb) amount ratio was 1,000.


Figure 2.8: Isocratic elution of a mixture of Gd, Tb, Ho (0.1 μ g/g each) and Dy (100 μ g/g). Other conditions same as in Fig. 2.7.

As can be seen from the Fig., there is no adequate separation between Dy and Tb and hence the quantification of the latter cannot be done with a fair degree of confidence based on peak area. The separation was performed under identical gradient conditions employing C_8 particulate RP column. Table 2.2 shows a comparison of the resolution obtained using the two different RP columns. It was found that the resolution between lanthanides was better when C_8 RP column was used with n-octanesulphonate as IIR. This is also supported by the work published previously on the comparison of different IIRs on C_8 and C_{18} stationary phases [Jaison (2009a)].



Figure 2.9: Gradient separation of a mixture of Gd, Tb, Ho (0.1 μ g/g each) and Dy (100 μ g/g) using α -HIBA as an eluent. Stationary phase: 4.6 mm x 300 mm, 5 μ m C₁₈ RP column; Mobile phase: 0.025 to 0.075 M α -HIBA (pH 5.0) in 10 min; other conditions same as in Fig. 2.7.

Table 2.2: Comparison of resolution using two different columns

Sl. No.	Lns pair	Resolution using	g RP Particulate	
		column (300 x 4.6 mm)		
		C_8	C ₁₈	
1.	Ho-Dy	10.4	5.1	
2.	Dy-Tb	10.2	7.3	
3.	Tb-Gd	13.2	9.0	

Present work clearly demonstrates the preference of the C₈ stationary phase for an IIR having an equal number of carbon atoms in its chain (n-Octane suphonate). Hence, the resolution for the separation of lanthanides is influenced by the preference of the stationary phase for the IIR having equal number of carbon atoms in its chain. Hence, C₈ RP column in combination with n-octanesulphonate was used for further studies. Since there is a small difference in the stability constants of Dy and Y with α -HIBA, mixed eluents were reported for the separation of Dy and Y. However, as seen from the Fig. 2.10, under the above optimized conditions, separation between Dy and Y was possible with a resolution of 2.6 using α -HIBA as a single eluent. This was attributed to an increase in the surface area which provided a better differential migration between Dy and Y. Hence C₈ stationary phase along with the optimized gradient conditions was employed for the determination of lanthanides in bulk of Dy.



Figure 2.10: Gradient separation of minor lanthanides (Ho, Y, Dy and Tb) in which Dy/Tb and Dy/Y amount ratios are 200. Stationary phase: 4.6 mm x 300 mm, 5 μm C₈ RP column. Chromatographic conditions same as in Fig. 2.7.

2.3.6 Quantification of Lanthanides and Y

The above optimized parameters were used for the validation of the method by preparing a simulated sample of lanthanides and Y in bulk of Dy. The concentration was determined applying standard addition method using peak areas of different chromatograms. As shown in Table 2.3, there is good agreement between the added concentrations and those determined by HPLC. Subsequently, validated HPLC method was used for the determination of lanthanides and Y in Dy₂O₃ samples by standard addition method. Fig. 2.11 shows the separation of lanthanides (Ho and Tb) and Y in presence of Dy (concentration of 700 μ g/g). However, heavier lanthanides (Lu, Yb, Tm and Er) concentrations in this solution were lower than the quantification limit. Hence, a high concentration of Dy (2300 μ g/g) was used as shown in Fig. 2.12.

Table 2.3: C	oncentrations	of minor	lanthanides an	d Y	in simu	lated	sample	of D	v
									•

Sl. No.	Element	Conc. of	Conc.
		element	determined
		added	(µg/g)
		(µg/g)	
1.	Tb	0.49	0.50 ± 0.02
2.	Но	0.52	0.49 ± 0.01
3.	Lu	0.49	0.49 ± 0.005
4.	Y	0.50	0.49 ± 0.01
5.	Yb	0.50	0.47 ± 0.01
6.	Er	0.50	0.51 ± 0.02
7.	Tm	0.49	0.49 ± 0.04



Figure 2.11: Gradient separation of lanthanides in Dy_2O_3 (Dy concentration 700 $\mu g/g$).

Chromatographic conditions same as in Fig. 2.10.



Figure 2.12: Gradient separation of minor heavy lanthanides in Dy_2O_3 (Dy concentration 2,300 µg/g). Chromatographic conditions same as in Fig. 2.10.

Table 2.4 gives concentrations of lanthanides obtained in Dy_2O_3 sample. Concentrations of lanthanides and Y determined were within an uncertainty of 10 %. However, the results obtained were not compared with those obtained from an independent analytical

method as most of the techniques require pre-separation of the matrix element to take care of the interferences and this is not convenient to achieve particularly when dealing with different lanthanides present at ppm levels in major matrix of another lanthanide.

		Conc. in	Conc. in
S1.	Flement	injected	Solid
No.	Liement	solution	Dy ₂ O ₃
		(µg/g)*	(ppmw)
1.	Tb	0.73 ± 0.04	1207
2.	Но	2.01 ± 0.04	3325
3.	Lu	0.17 ± 0.01	74
4.	Y	2.70 ± 0.2	4463
5.	Yb	0.99 ± 0.07	432
6.	Er	0.13 ± 0.003	59
7.	Tm	0.05 ± 0.01	22

Table 2.4: Concentrations of minor lanthanides and Y in Solid Dy₂O₃

* Employing 3 injections in 3 independent experiments

2.4 Conclusions

A simple and fast RP-HPLC method employing α -HIBA as an eluent was developed for the separation of rare earth impurities in dysprosium matrix. Using α -HIBA alone as the complexing agent, separation between Dy and Y was possible with good resolution among all the rare-earths pairs. This offers simple and fast method for the determination of lanthanides and Y in presence of bulk of Dy. This method obviates the need of mixed ligands, which makes it difficult to optimize the proportion and pH of a combination of eluents. Simultaneous studies of pH, concentration of eluent and concentration of IIR allowed to arrive at the optimum conditions for the separation and determination of different lanthanides and Y in high purity Dy. A C_8 column in combination with n-octane sulphonate as IIR was able to perform the separation and determination of Y and lanthanides in Dy upto an amount ratio ~ 1,000. The optimized method provides rapid and efficient separation of rare earth impurities in Dy for general application using the cost effective traditional HPLC. This approach can be extended for the separation of lanthanides in bulk of other lanthanides.

Chapter 3

Determination of Lanthanides, Thorium,

Uranium and Plutonium in Irradiated (Th, Pu)O₂

by Liquid Chromatography using

a-HydroxyIsoButyric Acid (a-HIBA)

3.1 Introduction

The Indian nuclear program envisages the effective utilization of thorium (Th) as a fertile material for the sustained production of electricity in the country [Ganatayet (2000)]. Unlike the natural uranium (U) based fuel which contains ²³⁵U as the fissile material, thoria based fuel initially requires the addition of fissile materials like ²³³U, ²³⁵U and ²³⁹Pu from outside. (Th, ²³³U)O₂ and (Th, ²³⁹Pu)O₂ mixtures are proposed as fuels for Advanced Heavy Water Reactor (AHWR) to make system self - sustaining in ²³³U [Anantharaman (2008), Sinha (2006)]. In order to assess the performance of (Th, Pu)O₂ MOX fuel, prior to its introduction in commercial reactors, (Th, 4%Pu)O₂ fuel clusters were irradiated in the Pressurized Water Loop (PWL) of CIRUS reactor at BARC. The implementation of mixed oxide based fuel cycle requires development of methodologies for evaluating the performance of the fuel in the reactor. Burn-up is an important parameter for nuclear fuel development, fuel management and fuel performance analysis [Aggarwal (2007), Saha (1977)]. Burn-up is defined as the atom percent fission of heavy element (mass ≥ 225) during its life-time in the reactor. Radiochemical and chemical analysis methods are generally used for burn-up determination of irradiated nuclear fuels [Gysemans (2006)]. The non-destructive methods like Secondary Ionization Mass Spectrometry (SIMS) and γ -spectrometry are used for the investigation of the irradiated fuel material [Portier (2012), Gysemans (2006)]. However the challenge is to solve the isobaric interference of the complex matrix in SIMS and in case of γ -spectrometry, the method is not accurate because it requires the detailed knowledge of fuel power history and interference in counting of burn-up monitoring nuclide from the other fission products [Joe (2006)]. The destructive methods involve dissolution of fuels followed by individual separation and determination of fission products and heavy elements. Isotope dilution - thermal ionization mass spectrometry (ID-TIMS) is an established method for the determination of burn-up [Saha (1977), Koyama (2003), Ramakumar (1980)]. TIMS is a single element analysis technique and it is preferred that the element should be in pure form to achieve the best results. Further, to eliminate isobaric interferences amongst Pu, U and Nd fractions, chemical separation is necessary. In this method, the burn-up monitor element is separated from the heavy element and fission products. The separation of the burn-up monitor element by conventional separation method is time consuming and tedious [Sivaraman (2002b)].

In view of the high radiation dose of irradiated fuel, development of new methodologies for the separation and determination of lanthanides (Lns), Th, U and Pu is essential for burn-up determination of thorium based nuclear fuel [Burcik (1991), Aggarwal (2010)]. Different methods based on solvent extraction, ion exchange, precipitation, liquid chromatography, supercritical fluid extraction etc. have been reported for the separation of Lns, Th, U and Pu in various matrices [Dacheux (1997), Datta (2011b), Jaison (2009), Koyama (2003), Kumari (2012)]. Koyama et al., reported the two steps ion exchange separation for the burn-up measurement by ID-TIMS. In first step, U and Pu were separated from the fission product solution employing anion exchange column at 8 M HNO₃. In second step, separation of Nd was achieved on anion exchange column using mixture of HNO_3 and methanol. Datta et al. (2011b) carried out recovery of actinides from the waste matrices using modified supercritical carbon dioxide. High performance liquid chromatography (HPLC) is a fast and highly efficient technique and has been applied for the separation of lanthanides and actinides in nuclear fuel samples and geological samples [Larsen (1979), Jackson (1994), Rollin (1996), Buchmeiser (2000), Seeber (1999), Datta (2010)]. Due to the fast separation and less amount of the sample handling in HPLC, it minimizes the exposure to highly radioactive samples and is, therefore, attractive for the separation of fission products. HPLC in combination with mass spectrometric techniques like ICP-MS has been reported for the separation of fission products and actinides from the spent fuel [Alonso (1995), Perna (2002), Rollin (1996)].

Reported work on separation of lanthanides from irradiated fuel samples using HPLC

Cassidy et al. studied the separation of lanthanides fission products using a dynamic ion exchange column from different fuel samples like UO_2 and $(Th, U)O_2$ fuels [Cassidy (1986), Knight (1984)]. The dynamic ion exchange separation offers certain advantages over the bound ion exchange separation like faster mass transfer and easy to alter the capacity of the column. However, in presence of bulk of actinides, the individual separation of lanthanides was not reported. Lanthanides were separated in a group by conventional ion exchange method and subsequently injected into HPLC after removal of Th and U. This offline separation requires multiple monitoring elements and there are chances of loss of analytes in different separation stages. The reported method gave good reproducibility among different runs for the same aliquot but results were inconsistent for different aliquots of the same fuel.

Various RP based methods have been reported for the separation of Th, U and Pu using hydroxyl carboxylic acid like HIBA and mandelic acid [Fuping (1993 and 1996), Elchuk (1991)]. Sivaraman et al. carried out extensive studies on the separation of fission products for fast breeder test reactor fuels [Sivaraman (2002a and 2002b), Datta (2013)]. These authors determined the burn-up for the thermal reactor fuel containing UO_2 and fast breeder test reactor fuel (FBTR) containing mixed carbide ($U_{0.3}$, $Pu_{0.7}$)C [Sivaraman (2002b]. Datta et al. (2011a) developed the separation of lanthanides from FBTR dissolver solution on monolith support. They also studied the sorption behavior and rapid separation of U and Th on the bis-2-ethylhexyl succinamic acid coated column. The stability constants of actinide-HIBA complexes under different chromatographic conditions were determined [Datta (2013)]. These researchers also reported the fastest separation of lanthanides in 3.6 min on a short column packed with small particle size (1.8μ m) stationary phase materials [Datta (2010)]. However, (Th, Pu)O₂ is a unique fuel tested for AHWR reactor and liquid chromatographic method for separation of Lns and actinides from this matrix is not reported in the literature. In most of the reported methods, the lanthanides, U and Th are present in comparable amounts. Studies were carried out previously in our laboratory using ion interaction reagent (IIR) on the reversed phase (RP) column for the separation of lanthanides from Th and U [Jaison (2006)]. Under the optimized chromatographic conditions using dual gradient of pH and concentration of the mobile phase, lanthanides were separated from bulk of Th and U from simulated irradiated thoria sample. However, the separation and determination of Lns, Th, U and Pu from irradiated (Th, Pu)O₂ fuel employing HPLC was not attempted so far.

Present studies

This Chapter deals with the individual separation of Lns, Th, U and Pu in an irradiated (Th, Pu)O₂ fuel. Handling of (Th, Pu)O₂ fuel samples is more challenging because of the radiation dose associated with the dissolver solution, presence of large amount of Th which is vulnerable to hydrolysis and multiple oxidation states of Pu under the chromatographic conditions [Datta (2011a)]. Since the chromatographic behavior of lanthanides is different than tetravalent and hexavalent actinides, two different separation procedures were developed. The challenges of the present studies were: (i) individual separation of lanthanide fission products in presence bulk of Th and interference of trivalent actinides in Lns separation, (ii) difficulties in the quantification of Pu because of its multiple oxidation states, and (iii) individual separation of Th(IV), Pu(IV) and U(VI).

3.2 Experimental

3.2.1 Instrumentation

The HPLC system used in this study is mentioned in Section 2.2.1. C_{18} monolith RP column (100 mm × 4.6 mm, Chromolith, Merck) and C_{18} particulate RP column (150 mm × 4.6 mm, 5 µm, Supelcosil) were used as the stationary phases.

3.2.2 Reagents

All solutions were prepared using deionised water from Milli-Q system (Millipore) and were filtered through a 0.45 µm membrane filter (Millipore) prior to using. α -HIBA (Lancaster) was used as an eluent. Sodium n-octane sulphonate monohydrate (Fluka) and tetrabutylammonium iodide (Sigma-Aldrich) were used as the ion interaction reagents (IIR). ICP-standards of lanthanides, U and Th (Inorganic Venture) were used after appropriate dilutions with Milli-Q water and mobile phase. HNO_3 and NH_4OH (Merck) were used for adjusting the pH of the mobile phase. H_2O_2 (Merck) in combination with HNO_3 was used as a redox reagent to bring Pu into the Pu(IV) oxidation state. For ion exchange separation, Dowex 1x8, 200-400 mesh size (Sigma-Aldrich) and Bio-Rad AG 1x2, 200-400 mesh size (Bio-Rad) were used as stationary phases. Arsenazo (III) (Fluka) was used as the post-column metallochromic reagent (PCR). The arsenazo complexes of Lns, Th, U and Pu were monitored at 653 nm. Irradiated (Th, Pu)O₂ sample received from Post – Irradiated Examination Division (PIED) at BARC was used for the method development. NIST-SRM-950a U_3O_8 , K₄Pu(SO₄)₄ and enriched isotope of ¹⁴²Nd were used as spikes for ID-TIMS analyses of U, Pu and Nd, respectively.

3.2.3 Procedure

Appropriate quantities of α -HIBA and sodium n-octane sulfonate were dissolved in water and were made to solutions with concentrations 0.5 M and 0.1 M, respectively. α -HIBA was adjusted to the desired pH using high-purity NH₄OH and HNO₃. Mobile phase flow-rate of 1.0 mL min⁻¹ was used. The PCR solution [1.5 × 10⁻⁴ M Arsenazo(III) and 0.01 M urea in 0.1 M HNO₃] was delivered at a flow rate of 0.3 mL min⁻¹. The lanthanides, Th, U and Pu solutions of appropriate concentrations were prepared after dilution with the mobile phase. Concentration of Pu was determined by biamperometry titration [Nair (1986)]. Dissolution of irradiated fuel sample was done in a facility housed in a shielded glove box. Aliquots from the irradiated fuel dissolver solution were transferred to a shielded fume-hood for further experiments.

For the determination of Lns, about 0.5 g of dissolved sample solution was taken and 2.5 ppm of Tb was added as an internal standard. This solution was directly injected into HPLC through 100 μ L injection port. The concentration of mobile phase containing α -HIBA of pH 6.5 was changed from 0.05 M to 0.15 M in 30 min; whereas the concentration of α -HIBA of pH 3.5 was changed from 0.15 M to 0.3 M in a time interval of 30 to 40 min. A C₁₈ RP column (250 mm x 4.6 mm) was used for the individual separation of Lns. For validation of the method using ID-TIMS, all the aliquots were subjected to necessary chemical treatments to ensure depolymerisation and proper isotopic homogenization. The spiked and unspiked aliquots were used for separation of fission product, thorium, uranium and plutonium by anion exchange column using Dowex 1x8, 200-400 mesh resin in 9 M HCl medium [Ramakumar (1980)]. The effluent containing Th and fission product fraction was collected. Pu and U fractions were sequentially eluted from the column using 0.1 M hydroxylamine hydrochloride in 5M HCl and 0.5 M HNO_3 , respectively. The fraction containing Th and fission products was subjected to a second stage anion-exchange separation using Dowex 1x8, 200-400 mesh resin in 7 M HNO₃ medium. The non-retained fission product fraction was collected and subjected to a third stage separation using Bio-Rad AG 1x2, 200-400 in a mixture containing HNO₃ and MeOH to separate Nd fraction [Ramakumar (1980)].

3.3 Results and Discussion

3.3.1 Separation and Determination of Lns from Irradiated (Th, Pu)O₂

3.3.1.1 Chromatographic Behavior of Lanthanides, Th, U and Pu

In solution, under the chromatographic conditions, lanthanides exist in (III) oxidation state whereas Th and U exist in (IV) and (VI) states, respectively. The situation in the case of Pu is different. In view of the closeness of redox potential values (Fig.3.1), Pu can exist in different oxidation state viz. (III), (IV) and (VI) simultaneously [Coleman (1965)].



Basic Solution

Acidic Solution

$$Pu \xrightarrow{2.42} Pu(OH)_3 \xrightarrow{0.95} Pu(OH)_4 \xrightarrow{-0.76} PuO_2OH \xrightarrow{-0.26} PuO_2(OH)_2$$
$$-0.51$$

Figure 3.1: *Redox potential diagram of Pu in acidic and basic media* [Coleman (1965)].

Retention behavior of Lns and Pu(III) is different from Th(IV), U (VI), Pu(VI) and Pu(IV) when α -HIBA is used as an eluent on RP column [Datta (2013)]. With α -HIBA, Lns and Pu(III) form mainly cationic complexes which are different from those formed by Th, U, Pu(VI) and Pu(IV) [Jaison (2006), Datta (2013)]. Thus Lns-HIBA complexes can be separated on dynamically modified RP column using an IIR which is sorbed unto

the column, converting hydrophobic surface into the charged surface for ion exchange separation of lanthanides [Raut (2004)]. However, Th, U and Pu(IV) get sorbed onto the RP column by hydrophobic interaction, when α -HIBA is used as a complexing agent. In presence of large amounts of IIR, the actinides were found to elute in between the lanthanides which makes lanthanides determination difficult. Hence, at a given concentration of IIR, the surface of RP column remains partly ionic and hydrophobic in nature. The separation of lanthanides from Th, U and Pu was performed using dual gradient (pH and Concentration) of α -HIBA. At higher pH, tetravalent and hexavalent actinides are retained better than lanthanides on dynamically modified reversed phase column leading to the elution and separation of lanthanides prior to actinides. Due to the complex nature of irradiated (Th, Pu)O₂ fuel matrix, two different separation procedures were developed as presented in scheme 3.1. In the first stage, Lns were separated and determined on dynamically modified column whereas the separation and determination of Th, U and Pu was achieved using RP column.

3.3.1.2 Determination of Lanthanides in Irradiated (Th,Pu)O₂ Sample by HPLC

A C_{18} reversed-phase column, dynamically modified with n-octane sulphonate, was used as the stationary phase for separation of lanthanides from irradiated (Th, Pu)O₂ fuel. It was reported that with increase in the concentration of IIR, the retention of Lns increases whereas the retention of Th and U decreases [Raut (2004)]. Th being the matrix element in the present case, the concentration of IIR selected was 5 mM to separate Lns without affecting the Th holding capacity. Effect of pH showed that at lower pH, higher fraction of HIBA remains in the uninonized form, resulting in faster elution of U and Th whereas, at pH \geq 5.0, Th and U showed stronger retention than lanthanides and eluted after Lns. With increase in the concentration of HIBA at pH 4.0, Lns, Th and U showed decrease in retention. Dual gradient (pH and concentration) of HIBA was used for the

separation of Lns, Th, U and Pu. Pu was found to elute as multiple peaks in the chromatographic run due to the presence of its multiple oxidation states. Hence, dissolver solution was evaporated to near dryness and treated with H₂O₂ in 3M HNO₃ to convert all the Pu into Pu(IV), and Pu was re-dissolved in 0.1 M HNO₃ to maintain in single oxidation state. Lns were separated by using the gradient condition. Initially, HIBA of pH 6.5 was used for the separation of lanthanides followed by the elution of actinides with HIBA of pH 3.0. Under these chromatographic conditions, Pu(IV) elutes along with Th and U and hence does not cause any interference to the lanthanide peaks. Henceforth Th, U and Pu are in the oxidation states, Th(IV), UO_2^{2+} and Pu(IV), respectively. The response of the Lns with the post column reagent was found to vary with the mobile phase concentration and pH. Therefore, terbium was used as an internal standard. The advantages of internal standard approach are: (i) calibration plot for a wide concentration range is not required. Single injection of the sample is sufficient, and (ii) matrix effects do not influence the results since the standard is introduced into the sample. Relative response factors (RRF) for the individual Lns pairs were calculated using the simulated samples. For a pair consisting of Tb and La, the RRF can be calculated as

$RRF = C_{Tb}/C_{La} * A_{La}/A_{Tb}$

where, C and A represent concentration and absorbance (peak area), respectively.

The RRFs of the different pairs viz. La/Tb, Pr/Tb, Nd/Tb and Sm/Tb were found to be constant and are presented in Table 3.1.

Validation of methodology developed for lanthanides determination was carried out by employing a simulated high level liquid waste solution. Concentrations of lanthanide

Scheme 3.1: Flow chart for liquid chromatographic separation of Lns, Th, U and Pu

from irradiated (Th, Pu)O₂ fuel



Lanthanide pair	RRF
T //T]	0.04
La/1b	0.94
Ce/Tb	1.03
Pr/Tb	0.77
Nd/Tb	0.92
Sm/Tb	0.89

Table 3.1: Relative response factors (RRF) for the lanthanides pairs

fission products (La, Ce, Pr, Nd and Sm) were determined based on their peak areas using terbium as an internal standard. Fig. 3.2 shows the chromatogram of the separated lanthanides from the irradiated fuel sample. Concentrations of La, Pr, Nd and Sm in the dissolver solution of irradiated (Th, Pu)O₂ sample were determined based on the internal standard approach and were found to be $0.6 \pm 0.05 \ \mu g/g$, $0.4 \pm 0.01 \ \mu g/g$, $1.8 \pm 0.1 \ \mu g/g$ and $0.5 \pm 0.02 \ \mu g/g$, respectively.

3.3.2 Optimization of Chromatographic Conditions for the Separation of Th, U and Pu

U forms $[UO_2(IBA)]^+$, $[UO_2(IBA)_2]$ and $[UO_2(IBA)_3]^-$ whereas Th and Pu form $[M(IBA)]^{3+}$, $[M(IBA)_2]^{2+}$, $[M(IBA)_3]^+$ and $[M(IBA)_4]$ (M = Th or Pu) types of complexes with HIBA [Datta (2013), Raju (2007)]. Dominance of one species over the other depends upon the pH and concentration of HIBA which is responsible for the relative differences in the retention times of Th, U and Pu. Hence the effect of chromatographic conditions such as pH of the mobile phase and concentration of HIBA on the retention of Th, U and Pu was studied.



Retention Time (mins)

Figure 3.2: Direct injection of dissolver solution of irradiated (Th, Pu)O₂ fuel. Conditions: α-HIBA of pH 6.5 changed from 0.05 M – 0.15 M in 30 min; α-HIBA of pH 3.5 changed 0.15 M – 0.3 M from 30 min- 40 min; Column: C₁₈ RP (250 mm x 4.6 mm).

3.3.2.1 pH of Mobile Phase

Fig. 3.3 shows the effect of pH of the mobile phase on the retention behavior of Th, U and Pu. At pH ≤ 2.5 , Pu showed strong retention compared to Th which in turn showed stronger retention than U. This is due to the fact that at lower pH, Pu and Th form predominantly M(IBA)₄ type of species whereas U exists as cationic species. However, at pH ≥ 3.5 , retention of U drastically increases compared to marginal increase in the retention of Th and Pu. This is because at higher pH, Th and Pu undergo hydrolysis and must be forming $[Th(IBA)_4(OH)_n]^{n-}$ and $[Pu(IBA)_4(OH)_n]^{n-}$ (where n = 1 or 2) species, respectively. Being the anionic species, Th and Pu complexes show relatively poor retention on the RP column. In the case of U, the predominant species at higher pH are $[UO_2(IBA)_2]$ and $[UO_2(IBA)_3]^{-}$, which are sufficiently hydrophobic in nature and exhibit strong retention on RP column.



Figure 3.3: Effect of pH of the mobile phase on the retention of Th, U and Pu. Chromatographic conditions: 0.1 M of HIBA and Column: C_{18} RP (100 mm × 4.6 mm, Chromolith).

With further increase in $pH \ge 5.0$, U was not eluted till 60.0 mins whereas the retention times of Th and Pu were 13.3 and 9.9 mins, respectively. Finally, pH 4.3 of the mobile phase was chosen for studying the effect of concentration of HIBA.

3.3.2.2 Effect of concentration of HIBA

Retention behavior of Th, U and Pu was studied as a function of concentration of α -HIBA at pH 4.3. As is seen in Fig. 3.4, the retention of Th, U and Pu decreased with the increase in the concentration of HIBA. The decrease in the retention times of the actinides with increase in concentration of HIBA is attributed to the competition by the undissociated

HIBA molecules for the C_{18} stationary phase. With the increase in concentration of eluent, the number of undissociated HIBA molecules in the mobile phase increases and this results in faster displacement of actinide-HIBA complexes from the stationary phase. However, retention of U decreased drastically indicating the hydrophobic nature of U-HIBA complex. 0.1 M of HIBA was chosen for separation studies.



Figure 3.4: Effect of concentration of α -HIBA in the mobile phase on the retention of Th,

U and *Pu*. Chromatographic conditions: pH of mobile phase, 4.3 and other conditions are same as in Fig.3.3.

3.3.3 Mechanism of Retention of Th, U and Pu

To study the mechanism of retention of Th, U and Pu on the RP column, n-octane sulphonic acid (IIR) was introduced in the mobile phase with the varying concentration. Fig. 3.5 shows the changes in the retention of Th, U and Psu as a function of concentration of n-octanesulphonate (n-OSA). The sorption of n-octanesulphonate on the stationary phase leads to the formation of cation exchange sites. Dominance of cationic

species [M(IBA)]³⁺, [M(IBA)₂]²⁺, [M(IBA)₃]⁺ of Th and Pu would be indicated by an increase in the retention time with increase in the concentration of IIR. As seen from the Figure, the retention times of Th, U and Pu decrease with increase in the concentration of n-octanesulphonate. This shows that the retention of Th, U and Pu on RP column is by hydrophobic mechanism and there is competition from the hydrophobic n-octanesulphonate. The studies on the influence of n-octane sulphonate clearly indicate that the hydrophobic character of U-HIBA is much higher than those of Th-HIBA and Pu-HIBA. The sorption of the IIR molecules onto the stationary phase is also occurring based on the hydrophobic interaction. U-HIBA complex shows a pronounced fall in retention time with the increasing concentration of IIR, owing to its highest hydrophobic character.



Figure 3.5: Effect of n-octane sulphonate on the retention of Th, U and Pu. Chromatographic conditions: Concentration of HIBA, 0.1 M and pH 4.3, Column: C18 RP (100 mm × 4.6 mm, Chromolith).

3.3.4 Effect of composition of MeOH on the retention of Th, U and Pu

Fig. 3.6 shows the retention behavior of Th, U and Pu with change in percentage of MeOH in the mobile phase. The retention of U decreases drastically compared to that of Th and Pu which indicates relatively more hydrophobic nature of U-HIBA complex. The presence of MeOH also resulted in improving the peak shape and shortening the retention time. Since Th and Pu separation was getting affected at higher percentage of MeOH, it was proposed to use MeOH as a gradient for the separation of Th, U and Pu from the actual dissolver sample.



Figure 3.6: Effect of composition of MeOH in mobile phase on the retention of Th, U and Pu. Chromatographic conditions: same as Fig. 3.5.

3.3.5 Separation and Determination of Th, U and Pu from Irradiated (Th,Pu)O₂ by RP-HPLC

The above optimized chromatographic conditions were used for the sequential elution of Pu, Th and U. To improve the resolution between Th and Pu, gradient condition, as given in Table 3.2, was employed. To improve the resolution as well as Th holding capacity, a larger stationary phase column of dimension 250 mm x 4.6 mm was used. Aliquots of irradiated sample were evaporated to near dryness and were taken in the mobile phases containing pH 6.5 of 0.13 M α -HIBA, and were injected through 100 μ L injection port. As shown in Fig. 3.7, lanthanides and other trivalent species are eluted at the solvent front. Th, U and Pu are retained on the column because of the formation of hydrophobic species using α -HIBA as an eluent. Under the optimized conditions, the elution pattern was Pu (IV) followed by Th and U. This is due to the fact that at higher pH for Th and Pu(IV), M(HIBA)₄ type of species are most dominating whereas for U, [UO₂(IBA)₃]⁻ is the major species. However, the lower retention time of Pu and Th compared to U can be explained on the basis of strong hydrolysis nature of Th and Pu(IV) and Pu(IV) in the chromatographic conditions resulting in the formation of [M(IBA)₄(OH)₂]²⁻.

The concentrations of Pu, Th and U were determined by standard addition method and were found to be $27\pm1 \ \mu g/g$, $1.10\pm0.02 \ mg/g$, and $5.3\pm0.3 \ \mu g/g$, respectively, in the fuel dissolver solution.

Time	HIBA	HIBA	%
(min)	(pH=6.5)	(pH=2.0)	МеОН
	(M)	(M)	(v/v)
0.00	0.125	0.005	7
10.00	0.125	0.05	7
20.00	0.125	0.05	30
30.00	0.125	0.05	30

Table 3.2: Gradient condition for separation of Th, U and Pu



Figure 3.7: Direct injection of dissolver solution of irradiated (Th, Pu)O₂ fuel.
Conditions: α-HIBA of pH 6.5, 0.125 M; α-HIBA of pH 2.0 from 0.005 M to
0.05 M in 10.0 min; MeOH 7.0 % till 10 min, then 7% to 30 % till 20 min;
Column: C₁₈ RP (250 mm x 4.6 mm).

3.3.6 Validation of HPLC method

Isotope dilution-thermal ionization mass spectrometric (ID-TIMS) methodology was employed for the concentration determination of U, Pu and Nd in the dissolver fuel samples. Chemical separation of U, Pu and Nd fractions in the dissolver solution was carried out to eliminate the potential isobaric interferences during the mass spectrometric analysis as well as to get good ion yield in TIMS. Nd, U and Pu fractions obtained from unspiked and spiked aliquots were used to determine isotopic composition and concentration by ID-TIMS, respectively. Concentrations of U, Pu and Nd in the sample were found to be $5.3 \pm 0.3 \% \mu g/g$, $26.8 \pm 0.2\% \mu g/g$ and $1.8 \pm 0.2\% \mu g/g$, respectively. Concentration of Th in the dissolver solution, determined biamperometrically using EDTA as a titrant, was found to be $1.03 \pm 0.2\%$ mg/g [Jayachandran (2012)]. The concentrations of Nd, Th, U and Pu obtained were in good agreement with the concentrations determined by HPLC.

3.4 Conclusion

An HPLC method was developed employing α -HIBA as the eluent for the separation of Lns, Th, U and Pu in the irradiated (Th, Pu)O₂ fuel samples. Dynamically modified RP column was used for the separation of Lns employing dual gradient condition. The method was successfully applied for determining the concentrations of Lns using relative response factors (RRF) in dissolver solution. Determination of Th, U and Pu was carried out on RP column. Pu was maintained in single oxidation state using redox treatment with H₂O₂ and HNO₃. A suitable gradient condition of methanol was helpful for the sequential elution of Pu, Th and U. Effect of hydrophobic ion like n-octanesulphonic acid showed that the separation of these actinides is taking place by hydrophobic mechanism. Under the optimized conditions, the concentration of Pu was also determined which was in good agreement with the concentration obtained by ID-

TIMS. The method provides direct measurement of lanthanides fission products for calculation of atom percent fission. The developed method is useful for the determination of burn-up in economical viable way and obviates the need of enriched isotope (spike) in ID-TIMS which is costly and not easily available.

Chapter 4

HPLC Method for Determination of Th, U and

Pu in Irradiated (Th,Pu)O₂ using Mandelic Acid

as an Eluent

4.1 Introduction

In view of the relatively large deposits of thorium (Th) available in the country, India has embarked on the large scale utilization of Th for its long term nuclear power programme [Unak (2000)]. Further, there is a renewed global interest in the thorium fuel cycle in view of its beneficial properties such as (i) intrinsic proliferation-resistance due to the formation of ²³²U, (ii) better conversion ratio in the thermal neutron spectrum compared to that in U-based fuel cycle, and (iii) production of relatively less amounts of minor actinides (Np, Am, Cm) [García (2013), IAEA (2005)].

In addition, thoria fuel offers better operating performance than urania based fuels with respect to thermal conductivity, fission gas release characteristics, dimensional stability at high burn-ups, stable stoichiometry, lower thermal expansion coefficient etc. Thorium-Plutonium (Th–Pu) fuel cycle is useful to burn weapon grade or accumulated reactor grade plutonium [Balakrishnan (2002)]. Several R&D activities are underway on (Th, Pu)O₂ fuels for use in water cooled reactors. Thorium fuel cycle also poses a number of challenges. In the ICP-MS analysis of ²³³U, severe isobaric interference from ²³²ThH⁺ together with peak tailing of the abundant ²³²Th is a problem [Kim (2007), Vais (2004)]. Similarly ²³²U analysis by thermal ionization mass spectrometry is hampered by the isobaric interference of ²³²Th. The most effective strategy to eliminate spectral and non-spectral interferences consists of separating each actinide as an individual fraction. Separation of the actinide elements helps to get rid of complicated matrices and also preconcentrates the analyte(s) [Shi (2013)].

The three step process of separation of Th, U and Pu from spent (Th,Pu)O₂ fuel, though viable, is not easy [IAEA (2005)]. Commonly used separation methods such as column chromatography, solvent extraction etc. are time consuming, susceptible to handling errors, and pose a potential risk of contamination [Guérin (2013), Maxwell (2008)].

Development of new methodologies for the separation and determination of Th, U and Pu is of considerable importance for thorium based nuclear fuel cycle and also in view of the environmental concerns [Jernström (2007), Günther-Leopold (2005), Cozzo (2013), Datta (2011a), Vallet (2013), Dhami (2012)]. The methods offering reduced analysis time, less generation of liquid waste and minimum dose to operator would have an edge in such applications. HPLC methods are reported for the separation and determination of Th and U in different matrices due to their ability to provide rapid and high performance separations [Datta (2013), Jaison (2006)]. HPLC using chelating agents as eluents combines selective complexation, separation and specific detection for the determination of actinide elements [Hao (1996b), Barkley (1992), Fuping (1993)]. For example, the chelating agent α -hydroxy isobutyric acid (HIBA) was reported for the separation of Th, U and Pu [Datta (2013)] and the elution order was found to be Pu, Th and U. In the case of Th based irradiated fuels where U would be present in very small amounts compared to Th, accurate determination based on peak area of U becomes difficult [Meyer (2010), Vidyalakshmi (2004)]. Vidyalakshmi et al., (2004) reported the separation of U from bulk of Th on C_{18} column coated with dihexyl hexanamide where the direct determination of U in presence of large amounts of Th was not possible. Further, with HIBA, the separation between Th and Pu is so close that a longer stationary phase must be employed and the gradient conditions need be carefully optimized to ensure the adequate separation between them. Mandelic acid as an eluent was reported for the separation of Th and U [Jaison (2009b), Elchuk (1991)]. Elchuk et al. (1991) reported the separation of transition metals, lanthanides and actinides using mandelic acid as an eluent. Hydrophobic nature of this reagent was found to be useful for separation of actinides from other elements on reversed phase column. Hao et al. (1996a) studied the retention behavior of Th and U on reversed phase column using mandelic acid and reported the reverse order of elution

between Th and U as compared to that with HIBA. Th was forming neutral complexe and was, therefore, retained strongly compared to uranyl anionic complex. Subsequently, Jaison et al. (2009b) carried out detailed studies on the retention mechanism of Th and U on reversed phase column using mandelic acid. The fast elution of U compared to Th was explored for the determination of traces of U in Th matrix. Though mandelic acid is an aromatic homologue of HIBA, the former gives a very strong retention of actinide elements, especially of Th, under reversed phase (RP) conditions and the elution pattern is different from that observed with HIBA. Hence, it was considered prudent to investigate the use of mandelic acid for the separation of Th, U and Pu in a mixture containing large amounts of Th. Plutonium is known to coexist in aqueous solutions in different oxidation states, Pu(III) to Pu(VI) [Bürger (2007)]. Pu in (IV) oxidation state is expected to follow the chromatographic behavior of Th and it was of interest to investigate the behavior of Pu(III) and Pu(VI) using mandelic acid as the eluent.

Present studies

In this Chapter, HPLC separation of Th, U and Pu employing mandelic acid as an eluent is presented. The method is applicable for the determination of Th, U and Pu in irradiated (Th,Pu)O₂ dissolver solution. Sample treatment was optimised to minimize the loss of Pu and Th during chromatographic determination. Studies are presented here to understand the differences in the chromatographic behavior of Pu(IV) as compared to that of Th(IV) by using ion interaction reagents (IIRs). Chromatographic behavior of different oxidation states of Pu viz. Pu (III), Pu(IV) and Pu(VI) is explored on reversed phase column.

4.2 Experimental

4.2.1 Instrumentation

The HPLC system used in this study is mentioned in Section 2.2.1. C_{18} monolith RP column (100 mm × 4.6 mm, Chromolith, Merck) was used as a stationary phase. The eluted components were monitored after post-column reaction (PCR) with a metallochromic reagent, which was added with a reciprocating pump (Eldex Laboratories Inc.) into a low dead volume-mixing tee (Valco). The signal from the detector was processed by EZChrom software package.

4.2.2 Reagents

All solutions were prepared using deionised water from Milli-Q system (Millipore) and were filtered through a 0.45 µm membrane filter (Millipore) prior to use. Mandelic acid (Merck) was used as an eluent and methanol (Gradient grade, Merck) was used as a mobile phase modifier. HNO₃ and NH₄OH (Suprapure grade, Merck) were used for adjusting pH of the mobile phase. Sodium n-octane sulphonate (Fluka) and tetrabutylammonium bromide (Sigma-Aldrich) were used as the IIRs. ICP-standards of U and Th (Inorganic Venture) were used after suitable dilutions with 0.1M HNO₃ and mobile phase. Pu solution with concentration pre-determined using biamperometric method was used for preparing standard solutions [Nair (1986)]. H₂O₂ (Merck) and HNO₃ were used for the redox treatment of Pu. Hydroxyl amine hydrochloride (Merck) was used for the oxidation of Pu to Pu(VI). Arsenazo (III) (Fluka) was used as the reagent for the post-column reaction. Irradiated (Th,Pu)O₂ sample from Post-Irradiation Examination Division (PIED) of BARC was used for the determination of Th, U and Pu [Anantharaman (2008)].

4.2.3 Procedure

The procedure employed for the sample pre-treatment is similar to that reported in detail in Section 3.2.3. 0.5 M solution of mandelic acid was prepared in water. n-octane sulphonic acid and tetrabutyl ammonium bromide solutions of 0.1 M each were also prepared in water. Dissolution of irradiated fuel sample was carried out in a facility housed in a shielded glove box at the Fuel Reprocessing Division, BARC. Aliquots from the irradiated fuel dissolver solution were transferred to a shielded fume-hood for further experiments. The standard solutions containing Pu as well as the sample solution were treated with concentrated HNO₃ followed by redox treatment with $(H_2O_2 + 3M HNO_3)$ to convert all the Pu into (IV) oxidation state. The treated samples were dissolved in 1 M HNO₃ and the final dilutions were made in 0.5 M mandelic acid of pH 5.0. For studies of chromatographic behavior of Pu(III) and Pu(VI), samples were treated with hydroxyl amine hydrochloride (0.5 M) and $KMnO_4$ (0.1 M) in 0.1 M HNO₃, respectively. Th and U standards were initially diluted with 0.1 M HNO₃ and the final dilution was done with 0.5 M mandelic acid of pH 5.0. The treated sample solution was directly injected into the HPLC column through the 100 μ L injection port. The mobile phase consisted of 0.2 M mandelic acid of pH 3.2 and 25% (v/v) of MeOH. Flow rates of mobile phase and PCR solution were 1 mL/min and 0.3 mL/min, respectively. The eluted species were monitored at 655 nm.

4.3 Results and Discussion

4.3.1 Effect of pH of Mobile Phase

Figure 4.1 shows changes in the retention times of Th (IV), U(VI) and Pu(IV) as a function of pH of mandelic acid. A relatively higher concentration of mandelic acid (0.125 M) and 20% MeOH was used for this study so that Th is eluted out within a reasonable time, especially at higher pH conditions. Throughout the pH range studied,

Th-mandelate is the most hydrophobic species. It is seen that the retention of U follows that of Th and increases sharply with increase in pH of the mobile phase. This is attributed to the increased dissociation of mandelic acid and hence its enhanced complexation with the actinide ions. The competition from the undissociated mandelic acid also weakens at higher pH conditions. It was expected that in solution, Pu(IV) would behave similar to Th(IV). However, the pattern followed by Pu(IV) was anomalous; it showed more retention than U at low pH (< 3.5) whereas at higher pH (> 3.5), it showed lesser retention than U. The previous studies indicated the formation of neutral complex of Th with mandelic acid and the fact that this complex is resistant to hydrolysis in the pH range studied, ensures that Th shows maximum retention on a RP stationary phase [Jaison (2009b)]. The major species responsible for the adsorption of U is anionic in nature which explains its lesser retention compared to Th [Jaison (2009b)]. In the case of Pu(IV), there must be a change in the major species responsible for its adsorption to the stationary phase at low and high pH conditions. The anomalous behavior of Pu(IV) was attributed to its susceptibility to hydrolysis to a greater extent as compared to Th(IV). The ionic radius of Pu(IV) (0.86 Å) is smaller than that of Th (IV) (0.94 Å) leading to increased hydrolysis of Pu(IV) compared to Th(IV) [Greenwood (1997)].

4.3.2 Effect of Concentration of Mandelic Acid

Figure 4.2 shows the effect of concentration of mandelic acid on the retention behavior of Th, U and Pu. This study was carried out at pH 3.2 of the mobile phase containing 20 % MeOH and employing C_{18} stationary phase.



Figure 4.1: Effect of pH of mandelic acid eluent on the retention time of Th, U and Pu(IV). Chromatographic conditions: C_{18} (100 mm X 4.6 mm) monolith column, 0.125 M mandelic acid and 20 % MeOH.

Retention of all the three actinide ions was found to decrease with increase in the concentration of mandelic acid in the mobile phase. This was attributed to the increased displacement of metal-mandelate complexes from the stationary phase by the undissociated mandelic acid molecules. Higher the concentration of mandelic acid, more number of undissociated mandelic acid molecules would be available to compete for the RP stationary phase. Both Th and Pu(IV) showed steep decrease in retention as a function of increasing mandelic acid concentration whereas the retention of U was not significantly affected during the initial concentration range (0.075 M to 0.15 M).


Figure 4.2: Effect of concentration of mandelic acid on the retention of Th, U and Pu(IV). Chromatographic conditions: pH 3.2 of mandelic acid and other conditions same as in Fig. 4.1.

The difference in the trend of retentions is an indicative of varying degree of hydrophobicities of the three actinide-mandelate complexes. The separation factor between Th and Pu decreases at higher concentration of mandelic acid. Thus 0.2 M solution of mandelic acid at pH 3.2, which offers a good degree of separation among Th, U and Pu, was selected for further studies. In order to compare the hydrophobicity of the mandelate complexes of Th, U and Pu, it was considered worthwhile to study the influence of MeOH content in the mobile phase.

4.3.3 Effect of Methanol in Mobile Phase

Figure 4.3 shows the change in the retention times of Th, U and Pu(IV) as a function of methanol content in the mobile phase. This study was carried out with mandelic acid concentration of 0.2 M and at pH 3.2. Introduction of MeOH lowers the overall polarity of the mobile phase.



Figure 4.3: Effect of percentage of methanol in the mobile phase on the retention time of *Th*, *U* and *Pu*(*IV*). Chromatographic conditions: 0.2 *M* mandelic acid of pH 3.2; other conditions same as in Fig.4.1.

The retention times as well as the separation between the actinide ion pairs were found to decrease with the increase in MeOH content. Due to the low pH employed in this study, Pu(IV) showed a retention pattern closely following that of Th. Under the conditions employed, mandelate complexes of both Th and Pu(IV) showed a significant decrease in their retention times indicating greater level of hydrophobicity as compared to U-mandelate. Introduction of MeOH was also found to improve the peak shapes of all the actinides peaks and decrease in the overall separation time. Considering the resolution and the peak shape improvement, 25 % (v/v) of MeOH was selected for further studies. Patterns followed by individual actinide-mandelate complexes suggest that the degree of hydrophobicities of the major species responsible for their retention must be in the order Th > Pu > U. However, this study could not explain the anomalous pattern observed for

Pu(IV) during the pH study. Hence it was decided to investigate the influence of an anionic and a cationic IIR on the retention pattern of these actinide-mandelate complexes.

4.3.4 Effect of Concentration of Ion Interaction Reagent (IIR)

The effect of variation in concentration of ion interaction reagent on the retentions of Th, U and Pu was studied under two different pH conditions as the behavior of Pu(IV) was strongly influenced by the pH of the mobile phase. An anionic IIR viz. n-octane sulphonate and a cationic IIR viz. tetrabutyl ammonium were used to study the retention pattern of the actinide ions.

4.3.5 Effect of n-octane sulphonate

Effect of changes in the concentration of n-octane sulphonate (n-OSA) on the retentions of Th, U and Pu(IV) was studied at pH 3.2 and 4.0. A marked difference in the retention times of the three ions was observed at two different pH conditions and this was attributed to increased complexation of mandelic acid. The concentration of n-OSA was changed from 0 to 0.1 M. During these studies, the mandelic acid concentration was 0.2 M. Under both the pH conditions, the retentions of mandelate complexes of Th, U and Pu(IV) decreased with increase in concentration of n-octane sulphonate indicating the absence of any cationic species responsible for their retention on the stationary phase. At lower pH (3.2), the behavior of Th and Pu(IV) was similar and showed greater hydrophobicity as compared to U. However, pattern observed at pH 4.0 indicated lesser hydrophobicity of Pu(IV) species as compared to that for U and Th. It is seen that there is a possibility of an anionic species controlling the retention of Pu(IV) at higher pH conditions and this can more clearly be seen using a cationic surfactant as the IIR.

4.3.6 Effect of tetrabutyl ammonium bromide

Tables 4.1 and 4.2 show the influence of concentration of the IIR, tetrabutyl ammonium bromide on the retention behavior of Th, U and Pu(IV) at pH 3.2 and 4.0, respectively. Tetrabutyl ammonium being a cationic surfactant, its passage through the mobile phase would cause the formation of positively charged layer on the stationary phase. Hence, the anionic species are expected to show an increased adsorption at higher concentration of this IIR. It is seen that at both the pH, uranyl ion forms negatively charged mandelate complex which is clearly evident from a sharp increase in the retention time. This is more pronounced at higher pH conditions owing to the increased dissociation of mandelic acid leading to increased formation of anionic uranyl mandelate.

Retention of Th showed a decreasing trend in both the cases, though the change is not significant at pH 3.2. Irrespective of the nature of the IIRs used, retention pattern of

Table 4.1: Effect of concentration of tetrabutyl ammonium bromide on the retention time of Th, U and Pu at pH 3.2, C₁₈(50 mm x 4.6 mm) monolith column.

Concentration of tetrabutyl	Retention time	Retention time	Retention time
ammonium bromide (M)	(min)	(min)	(min)
	(Th)	(U)	(Pu)
0	31.4	22.9	28.4
0.0025	26.1	35.6	32.4
0.005	23.9	60.2	34.2
0.01	22.9	not eluted	35.1
0.02	21.1	not eluted	37.7

Table 4.2: Effect of concentration of tetrabutyl ammonium bromide on the retention time of Th, U and Pu at pH 4.0, C₁₈(50 mm x 4.6 mm) monolith column.

Concentration of tetrabutyl	Retention time	Retention time	Retention time
ammonium bromide (M)	(min)	(min)	(min)
	(Th)	(U)	(Pu)
0	112.1	76.6	49.7
0.0025	53.4	101.3	43.6
0.005	44.3	not eluted	49.7
0.01	40.1	not eluted	45.8
0.02	35.4	not eluted	34.9

Th showed decreasing trend with increase in concentration of IIR and this supports that the major species responsible for the retention of Th is neutral in nature. The drastic change observed at pH 4.0 (Table 4.2) is again due to the increased complexation resulting in the formation of more fraction of neutral Th-mandelate.

Retention of Pu(IV) showed different trends under the two pH conditions employed. At pH 3.2, it is expected that Pu(IV) would behave similar to Th. No appreciable change in the retention of Pu(IV) was observed with increase in the concentration of IIR in the mobile phase. At higher pH conditions, Pu(IV) is expected to show an increased retention time in view of the increased hydroxide complexation. However, at higher pH 4.0, the retention of Pu(IV) decreased with increase of IIR concentration.

The difference in the behaviour of Pu(IV) could be due to the higher ionic potential (charge/radius ratio) of Pu(IV) as given in Table 4.3, which might be making the Pumandelate complex susceptible to hydrolysis. It is also probable that the smaller redius of Pu(IV) would cause a restriction for the formation of ML_4 type of complex which is relatively easy in the case of larger Th(IV) ions. This may be due to the fact that at pH 3.2, mandelic acid is less complexing with Pu(IV) and hence there is more probability of complexation by OH⁻ leading to increased formation of hydroxide species. At pH 4.0, the mandelic acid is in more dissociated form leading to increased complexation by ligand thereby reducing the probability of hydroxide complexation. This results in a decreasing trend with increase in tetrabutyl ammonium bromide concentration.

Table 4.3: Charge to radius ratio of Th and Pu [Greenwood (1997)].

Ion	Ionic radius (Å)	Charge/radius ratio
Pu(IV)	0.86	4.651
Th(IV)	0.94	4.255

4.3.7 Separation of Th, U and Pu

Based on the studies of various chromatographic parameters, the separation conditions for Th, U and Pu were optimised. Finally, the method consisted of using 0.2 M mandelic acid of pH 3.2 and 25% (v/v) of MeOH. Figure 4.4 shows a chromatogram obtained by injection of a mixture containing 25 ppm each of Th, U and Pu under the optimised conditions. It is seen that the elution order is U, Pu and Th and the separation is completed in 10 min. It may be noted that the baseline separation was achieved using isocratic elution whereas HIBA required a gradient of both the eluent and MeOH. Figure 4.5 shows the calibration curves obtained for Th, U and Pu using aliquots taken from solutions prepared by the dilution of the synthetic mixture in the concentration range 1 ppm to 50 ppm. Good correlations were obtained between the concentration and the peak area response of all the three actinide elements in the concentration range of 1 ppm to 25 ppm. A saturation trend was observed for Pu(IV) peak area at concentration > 30 ppm.



Figure 4.4: Separation of Th, U and Pu(IV) on RP column. Chromatographic conditions: C₁₈ (100 mm x 4.6mm) monolith column, 0.2 M mandelic acid of pH 3.2 and 25% (v/v) of MeOH.



Figure 4.5: Calibrations curves for Th, U and Pu. Chromatographic condition: same as in Fig. 4.4.

Since U and Pu amounts are only a small fraction of Th present in the dissolved irradiated fuel solution, it is essential that the sample aliquots should be injected at two different concentration levels; concentrated one for Pu and U determination and a diluted sample for Th determination. However, when a concentrated dissolved solution of the irradiated (Th,Pu)O₂ sample was injected into the HPLC column, loss of Pu and Th was observed. Pu loss was found to be more significant than that of Th and U did not show any appreciable loss. This was attributed to different charge/radius ratios which control the tendency of a given ion to undergo hydrolysis during sample drying / evaporation. With an objective to identify the suitable sample medium to minimise the losses, the experiments were carried out with synthetic samples containing Th, U and Pu in proportions similar to those of the actual dissolver solution sample. After the concentrated HNO₃ treatment as well as the redox treatment, the aliquots were dissolved in nitric acid of different concentrations (0.04 M -1.0 M). Table 4.4 presents the data on the peak areas of Th, U and Pu obtained from the aliquots prepared in different HNO₃ concentrations.

Table 4.4: Comparison of recovery of Th, U and Pu at different concentrations	of
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[HNO ₃]	Peak area of		
(M)	U	Pu	Th
0.04	5.01E6 ± 6%	1.05E6 ± 11%	3.16E7 ± 9%
0.07	5.16 E6 ±1%	2.74E6 ± 4%	8.65E7 ± 2%
0.15	5.05E6 ± 4%	2.60E6 ± 2%	8.13E7 ± 2%
0.20	1.14E6 ± 7%	8.0E5 ± 9%	5.62E7 ± 5%

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Table 4.4 shows that an acid strength of 0.07 M is required to keep the actinide ions such as Th and Pu(IV) in solution. At higher acidities (≥ 0.20 M), exclusion of the actinide ions was found to take place at dead-volume and there was a corresponding decrease observed in the peak areas of Th, U and Pu(IV). Hence after the HNO₃ treatment and redox treatment, the samples were diluted with 0.5 M mandelic acid containing 0.15 M HNO₃. Figure 4.6 shows the chromatogram obtained by the direct injection of the treated sample under the optimised chromatographic conditions. It is seen that both U and Pu are well separated and elute before the Th matrix. Another aliquot of the sample was diluted by ~ 100 times in 0.5 M mandelic acid and was injected into HPLC under the same conditions for determination of Th. Chromatogram obtained is shown in Fig. 4.7.



Figure 4.6: Separation of Th, U and Pu(IV) from irradiated (Th,Pu)O₂ sample. Treated sample was directly injected into HPLC under isocratic conditions as in Fig. 4.4.

Th, U and Pu were determined based on the peak areas by employing standard addition method and were found to be 997 \pm 33 µg/g, 5.4 \pm 0.2 µg/g and 27 \pm 4 µg/g, respectively. The data obtained were in agreement with those obtained by isotope dilution-thermal ionisation mass spectrometry.



Figure 4.7: Chromatogram showing the separation of Th by injecting the diluted (Th, Pu)O₂. Other conditions same as in Fig. 4.4.

4.3.8 Chromatographic behavior of different oxidation states of Pu

Pu is known to exist in different oxidation states due to the closeness in their redox potentials. In the present study, Pu was chemically converted into Pu(IV). However, it was of interest to study the retention behavior of different oxidation states of Pu in presence of mandelic acid. Fig. 4.8 shows the different chromatograms of Pu overlaid for different oxidation states viz. (III), (IV) and (VI) under the optimised elution conditions. Pu was reduced into Pu(III) by using hydroxyl amine hydrochloride, injected just before the run. It was found that Pu(III) was poorly retained onto the column and eluted at the dead time. Pu(VI) showed higher retention though this retention was poor as

compared to that of Pu(IV). This is attributed to the different extent of complexation and hence the hydrophobicity follows the order Pu(IV) > Pu(VI) > Pu(III).



Figure 4.8: Overlaid chromatograms showing retention behavior of Pu(III), Pu(IV) and Pu(VI) on RP column. Chromatographic conditions: C₁₈ (50 mm x 4.6mm) monolith column, 0.2 M mandelic acid of pH 3.2 and 25% (v/v) of MeOH.

4.4 Conclusion

This Chapter described the HPLC method developed for the separation of Th, U and Pu using mandelic acid as an eluent. The influence of concentration of mandelic acid, change in pH and MeOH content of the mobile phase was studied to arrive at optimum elution conditions for their separation. The elution of U and Pu prior to Th is useful for samples containing bulk of Th. In view of the adequate separation between U-Pu and Pu-Th pairs, isocratic elution was found to be sufficient to achieve baseline separation among these actinides. Under the optimized chromatographic conditions, Pu was well separated from U and Th. The method was applied for the determination of Th, U and Pu in dissolved solution of irradiated (Th, Pu)O₂ fuel sample after optimizing the sample treatment conditions. Studies were also presented to understand the reasons for the anomalous chromatographic behaviour exhibited by Pu(IV).

Chapter 5

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Speciation Studies of Platinum-Benzoylthiourea

in Acetonitrile using Electrospray Ionization

Mass Spectrometry and Density Functional

Theory

5.1 Introduction

The platinum group of elements (PGEs) are found in environment and in biological samples due to their applications in automobiles and in chemotherapy. Ultra trace amounts of PGEs are also available in environment because of industrial use [Johnson Matthey (2010)]. Platinum (Pt) based compounds like carboplatin and oxaliplatin are used worldwide as anticancer reagents [Ek (2004), Galanski (2005)]. PGEs present in the environment may interact with ligands thus leading to biologically available species. Thus, the knowledge about the concentration and speciation of PGEs is of great interest to evaluate their biological activity and health hazard like allergenic effect. Further, PGEs are important radionuclides whose speciation in various irradiated fuel reprocessing conditions is important to nuclear waste management processes which demand the development of suitable chelating extractants for their recovery [Balcerzak (2011)]. The ultra-trace level determination of PGEs is of great challenge for analyst. Low levels of concentration and presence of matrix necessitate the appropriate sample preparation and selective preconcentration steps. Due to the soft nature of PGEs, the sulfur based donor ligands such as benzoylthiourea (BTU) are known to be strong complexing agents [Dominguez (2002), Pearson (1963)]. Thus, Pt forms strong complexes through the sulfur atom present in BTU and its dialkyl derivatives [Koch (1995, 1993, 1994, 1999), Bourne (1993)] (Fig. 5.1). Koch (1993) reported the reaction of Pt(II) with dibutyl derivatives of benzoylthiourea. In case of Pt salt of chloride and bromide, the species reported were $cis[PtL_2]X_2$ (where X=Cl, Br) whereas in case of iodide, the protonated species $cis[Pt(LH)_2]I_2$ were observed. This group (Koch (1999)) studied the cis-trans equilibrium of N-benzoyl N'-propylthiourea with Pt(II) and Pd(II) in different solvents. They found that the existence of cis or trans form is greatly influenced by the presence of the medium.

In the case of bromo and iodo complexes of Pt(II) and Pd(II), the chelation through Satom was only reported. Bourne and Koch (1993) reported the cis and trans configuration of complex of Pt(II) with N-benzoyl N'-propylthiourea. The crystal and molecular structure of complex revealed the unidentate behavior of the ligand through S-atom and the oxygen of -CO- group involved in intramolecular hydrogen bonding with thioamide proton. These ligands are utilized for the separation by solvent extraction or sorption onto the non-polar stationary phase for selective extraction of PGEs even in the presence of strong interfering matrices [Konig (1985), Vest (1989), Vest (1991), Schuster (1992), Mautjana (2003), Schuster (1996)]. Vest (1989) reported the extraction of PGEs by Nmono and N,N-di substituted benzoylthiourea. Schuster (1992) reported use of N,Ndiethyl N'-benzoylthiourea(DEBT) for selective extraction of platinum metal in ore and rock samples. The formation of neutral complex of Pt with DEBT enables the selective sorption onto a non-polar stationary phase. Further, he reported the application of DEBT in liquid chromatography for separation of PGEs. Mautjana et al. (2003) studied the various hydrophobic N,N-dialkyl N'-acylthiourea for complexation with Pd(II), Pt(II) and Rh(III). The easiness in complex formation with the ligand has been explored for the determination of traces of these metals using reversed phase liquid chromatography. Dominguez et al. (2002) reported 1:2 stiochiometry of Pd (II) complex with DEBT and the different BTU derivatives were used for preparation of ionophores in polymeric membrane electrode. The stability constant of Pd (II) with DEBT was determined and the author demonstrated solvent extraction studies of Pd (II) with DEBT.

In solid state, the coordination behavior of BTU ligand is similar to that of classic β -di-ketone molecule. BTU is found to have intra-molecular hydrogen bonding between the thiourea [-C(S)NH₂] moiety and the oxygen atom of –amidic group resulting in a

planar six membered ring structure (Fig. 5.1). This specific arrangement of BTU results in significant changes in its chemical properties and this leads to preferential complexation



Figure 5.1: *cis and trans isomers of BTU;* ^{*a*} *HOMO=Highest Occupied Molecular Orbital;* ^{*b*} *LUMO=Lowest Unoccupied Molecular Orbital.*

of PGEs for detoxification purposes [Koch (2001), Muhl (1986)]. The strong and selective complexation of BTU with PGEs is due to the increased charge density on the sulfur donor atom resulting from resonance of the ligand [Vest (1989), Koch (2001)]. Thus, the interactions of BTU with Pt are of great interest with geometric parameters frequently studied using X-ray and NMR based techniques.

Electrospray ionization mass spectrometry (ESI-MS) is a soft ionization technique with which the formation, stoichiometry and speciation of complexes involving metals and ligands can be studied [Di Marco (2006)]. Along with the high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) techniques, ESI-MS has been used for the separation and speciation studies of metal complexes [Mautjana (2003), Schuster (1996), Bonvin (2012), Minakata (2006)]. The interactions of Pt complexes with ligands such as sulfur containing peptides [Miao (2005)], 1, 3, 4-tridiazoles and dimethyl sulfoxide [Franski (2005)], phosphaadamantane [Fisher (2000)], amidine [Bertani (2003)], ethylene diamine-N,N'-di-3-propanato ligand [Kaluderovic (2009)], maleonitrile-dithiacrown ether [Drexler (2001)] were investigated using ESI-MS. Miao et al. (2005) studied the interaction of Pt(II) with sulphur containing peptide. They reported the collision induced dissociation for elucidation of structure of the complexes. ESI-MS was also used for studying the gas phase ion reaction and the formation of metal ion adduct for the structure elucidation of complexes [Keith-Roach (2010), Ross (1998)]. Franski et al. (2005) reported varietity of complexes by reaction of 2,5-disubstituted-1,3,4-thiadiazoles with platinum (II) chloride and DMSO. The types of complexes observed are in good correlation with the arrangement of ligand and solvent used indicating ESI-MS spectra represent structurally defined complexes in solution phase. Bertani et al. (2003) reported a series of Pt(II) amidine complexes of cis and trans isomers with Na and/or K adduct. In contrast to trans isomer, cis isomer was found to give different ESI spectra along with adduct peaks.

Although ESI-MS data can reveal valuable information about stoichiometry, oxidation states and fragmentation patterns, the spatial orientation of metal and the coordinated ligands is not available which can be routinely studied using electronic structure methods. In this regard, density functional theory (DFT) can be used in conjunction with ESI-MS to reveal the geometric information. The above mentioned strategy was reported recently to elucidate the structure and possible fragmentation pattern of metal complexes in the gas phase [Matsumoto (1999), Conte (2000), Yu (2008), Gresham (2011), Bryantsev (2008), Groenewold (2006), Crowe (2005)]. Matsumoto et al. (1999) studied the formation of complex of Cu(II) with 2-(5-bromo-2pyridylazo)-5-diethylaminophenol (5-Br-PADAP) in a mixture of methanol and water. Collision induced dissociation experiments were carried out for evaluation of relative binding strength of different species. Crowe et al. (2005) investigated the binding properties and stoichiometries of rare earth with carbamoylmethylphosphine oxide (CMPO) using ESI-quadrupole-ion trap mass spectrometry in the gas phase. Yu et al. (2008) reported the coordination structure of diperoxovanadate with a series of histidine like ligand using ESI-MS and density functional theory calculation. Structure and mode of coordination of experimentally observed species in ESI were theoretically proposed by DFT calculation. Bond energy and enthalpy calculation were reported for the favorable reaction paths for the formation of different species. Gibson and co-workers pioneered the use of DFT methods to understand the hydration sphere of actinglis in the gas phase [Rios (2012)] and also explored the complexation of uranyl nitrate with various amides [Gresham (2011)]. Van Stipdonk and his group have done extensive studies of uranyl complexation studies in different organic solvents. This group reported ESI-MS studies of uranyl with acetone and observed doubly charged uranyl solvated species. Leavitt et al. (2009) studied the ESI-MS observation supported by DFT for the competitive reaction between H₂O and oxygen for acetone and DMSO solvated uranyl (V) dioxocation. Parsons et al. (2006) reported the interesting work of cationic vanadium (IV) oxohydroxo complexes acting as lewis bases using ESI-MS and collision induced dissociation in contrast to common behavior of metal cation as lewis acid. ESI-MS, NMR and ab initio calculation studies were reported by Conte et al. (2000) for structure and exchange reaction of peroxo vanadate with solvent molecules. The relative energy diagrams in gas

phase were constructed for transformation of one species to another.

Present work

Present Chapter explores, the gas phase behavior of BTU with Pt in detail using ESI-MS and quantum chemical calculations. Further, DFT calculations were done to supplement the experimental ESI-MS data through the computation of possible structures and the probable fragmentation pattern.

In this Chapter, DFT calculations are described to supplement the experimental ESI-MS data. Further, extensive ESI-MS experiments were carried out for mono-anions, whereas for DFT, the di-anion (PtCl₄²⁻) was used as initial species which is challenging to model at least from computational viewpoint. The possible species of platinum complexes and their possible low energy pathway fragmentation in the gas phase are proposed. A combined experimental and quantum chemical calculations is found to be useful to understand the interaction between Pt and the commonly used BTU ligand.

5.2 Experimental

5.2.1 Chemicals and Reagents

Platinum solutions were prepared by diluting ICP-MS standard (Merck, Darmstadt, Germany) of 1000 μ g/g concentration and from K₂PtCl₄ (Otto Chemie Pvt. Ltd. Mumbai, India). BTU (Alfa Aesar, Lancashire, UK) was used as a complexing agent. Acetonitrile (LC-MS grade, Fluka, Bengaluru, India) and Milli-Q water (Millipore, Bengaluru, India) were used for dilutions and also as a mobile phase for sample introduction to ESI-MS. Suprapure grade (Merck, Darmstadt, Germany) HNO₃, HClO₄ and NH₄OH were used for pH adjustment.

5.2.2 Instrumentation

Mass spectrometric measurements were performed using a micrOTOF-Q-II (Bruker Daltonik, GmbH, Bremen, Germany) equipped with electrospray ion source and quadrupole-time-of-flight analyzer. Simulations of the stable isotope patterns were made using Compass Isotope Pattern software. Samples were introduced into the ESI using an automatic syringe pump (Nemesys, Cetoni GmbH, Korbussen, Germany) at flow rate of 4 μ L/min. All data were collected and analyzed using Bruker Compass Data Analysis software (supplied by Bruker Daltonics) in MS and MS/MS modes. ESI-MS analyses were performed in the positive ion mode with end plate potential of -3500 V and capillary voltage of -4500 V. In the negative ion mode, end plate potential of +2800 V and capillary voltage of +3800 V were used. High purity argon was used as a collision gas for MS/MS. Nitrogen was used as sheath gas and auxiliary gas. The MS conditions routinely employed were: capillary temperature 180 °C; sheath gas flow rate 4 L/min; and auxiliary gas pressure 0.3 bar.

5.2.3 Procedure

About 1×10^{-5} M solution of K₂PtCl₄ was prepared by dissolving K₂PtCl₄ salt in minimum volume of water, followed by the addition of appropriate amount of acetonitrile (ACN). 0.1M BTU solution was prepared by dissolving appropriate quantity of BTU in ACN. Solutions of Pt metal and ligand were mixed on volume basis to obtain the desired ligand to metal ratios, keeping ionic strength of the solution constant using NaClO₄. pH of the solution was adjusted using HNO₃ and NH₄OH. For pH study, no extra electrolytes were used for maintaining the ionic strength.

5.2.4 Computational Details

In the electronic structure calculations, a 'hybrid' B3LYP density function in conjunction with 6-311++G** basis set for geometry optimizations and energy evaluations was used. For Pt cation, a triple zeta valence polarization basis set (def-TZVP, def is an abbreviation for default as per TURBOMOLE (basis set library)) was

used to describe the valence orbitals, whereas the core electrons (60 electrons) were modeled with def-ECP (effective core potential) pseudo potential. A combination of B3LYP function in conjunction with the Gaussian basis set was used to obtain accurate geometries for transition metal complexes [Uddin (1999)]. All the calculations were carried out using TURBOMOLE 6.0. As the sizes of the chloride anion and sulfur atom are large, we used a large $6-311++G^{**}$ basis set which contains diffuse functions.

5.3 Results and Discussion

5.3.1 ESI-MS study of platinum (II) chloride system

ESI-MS experiments were carried out in the positive as well as in the negative mode, with direct infusion of the solution prepared from K_2PtCl_4 salt in acetonitrile (ACN) maintaining the ionic strength constant. Acetonotrile was used in view of its soft donor nature compared to methanol as Pt-ACN species would allow to monitor the uncomplexed metal ion. Further, the desolvation nature of acetonitrile is not significantly different from that of methanol. Signal assignments of the observed species were confirmed by performing MS/MS and accurate mass measurements after comparing the obtained *m/z* values with the theoretical data. Good correlations among these values were obtained, confirming the identity of the investigated species with agreement of better than 5 ppm. Table 5.1 shows the species obtained by the coordination of Pt with ACN. The species observed in ESI-MS were singly charged ions with Na or K as an adduct. It was found that all the platinum species in the positive ion mode were stabilized by ACN. The species [Pt (CH₃CN)₂Cl₂K]⁺ at *m/z* value of 386.91 was the base peak. Other major peaks were assigned to [Pt(CH₃CN) Cl₃K₂]⁺ and [Pt(CH₃CN)₂Cl₂Na]⁺. This is because of usage of K₂PtCl₄ in the present study. However, lower coordinated Pt species viz. Pt

 $(CH_3CN)Cl_2Na$ (*m/z* 329.91, 7%); Pt(CH_3CN)Cl_2K (*m/z* 345.88, 17%) were also observed in the positive mode.

Species observed	m/z	Relative abundance (%)
[Pt(CH ₃ CN)Cl ₂ Na] ⁺	329.91	7
$[Pt(CH_3CN)Cl_2K]^+$	345.88	17
[Pt(CH ₃ CN) ₂ Cl ₂ Na] ⁺	370.94	36
$[Pt(CH_3CN)_2Cl_2K]^+$	386.91	100
$\left[Pt(CH_{3}CN)Cl_{3}K_{2}\right] ^{+}$	419.82	22

Table 5.1: Species of K_2PtCl_4 in ACN observed in the positive ion mode during ESI

Table 5.2 shows the different chloroplatinate species observed in the negative ion mode. The most abundant Pt species was $[PtCl_3]$ at m/z 300.86. This species is formed by the removal of one Cl⁻ from $[PtCl_4]^{-2}$ under ESI-MS conditions. However, Pt was found to undergo oxidation from +2 to +3 forming $[PtCl_4]^-$ and reduction from +2 to +1 forming $[PtCl_2]^-$. The singly charged, reduced platinum species, $[PtCl_2]^-$ at m/z 265.89 with an abundance of 19% and the oxidized platinum species, $[PtCl_4]^-$ at m/z 335.83 with a relative abundance of 10% were observed.

Species observed	m/z	Relative abundance (%)
[PtCl ₂] ⁻	265.89	19
[PtCl ₃] ⁻	300.86	100
[PtCl ₄] ⁻	335.83	10
[NaPtCl ₄] ⁻	358.82	1
$[KPtCl_4]^-$	374.80	1

Table 5.2: Species of K₂PtCl₄ in ACN observed in the negative ion mode during ESI

5.3.2 ESI-MS study of different platinum and benzoylthiourea species

Platinum solutions prepared from K₂PtCl₄ with BTU in ACN were directly infused into ESI-MS and the formation of different species was monitored in the positive ion mode. Pt containing different species were: PtL₂, PtL₂Cl, PtL₂(ACN), PtL₂Cl(ACN), PtL₃ and PtL₃Cl (where L = BTU), with PtL₃Cl (m/z 771.03) being the most abundant species (Table 5.3 and Fig. 5.2). Table 5.4 shows the MS/MS data of the most abundant peak of Pt. The fragmented products of PtL₃Cl were PtL₃, PtL₂Cl, PtL₂, PtL(thiourea), PtL(SH). The instrumental parameters for fragmentation studies were: collision energy: 20 eV; in source collision induced dissociation energy of 50 eV, isolation width of 5.0 m/z and acquisition factor of 1.0.

Species	m/z
$\left[\operatorname{Pt}(\operatorname{C_8H_8N_2OS})_3\right]^+$	734.06
$[Pt(C_8H_8N_2OS)_3Cl]^+$	771.03
$\left[\operatorname{Pt}(\operatorname{C_8H_8N_2OS})_2\right]^+$	554.02
$\left[Pt(C_8H_8N_2OS)_2Cl \right]^+$	591.00
$\left[Pt(C_8H_8N_2OS)_2Cl(CH_3CN)\right]^+$	632.03
$\left[Pt(C_8H_8N_2OS)_2(CH_3CN)\right]^+$	595.05
$[Pt(C_8H_8N_2OS)_3(CH_3CN)]^+$	775.09

Table 5.3: Pt-BTU species observed in the positive ion mode during ESI



Figure 5.2: Pt-BTU species observed in the ESI-MS (positive ion mode).

The effect of concentration of BTU on the formation of different species was studied by maintaining metal and $HClO_4$ concentrations constant. As can be seen from Fig. 5.3, the intensities of the peaks corresponding to the metal ion decreased whereas those of the metal-ligand peaks increased with the increase in ligand to metal (L/M) ratio. The

Pt(BTU)₃Cl was the most intense peak, which showed a sharp increase in intensity beyond L/M ratio of 2.0.

m/z	Species obtained
734	$\left[\operatorname{Pt}(\operatorname{C_8H_8N_2OS})_3\right]^+$
554	$\left[\operatorname{Pt}(\operatorname{C_8H_8N_2OS})_2\right]^+$
591	$[Pt(C_8H_8N_2OS)_2Cl]^+$
450	$\left[\operatorname{Pt}(\operatorname{C_8H_8N_2OS})(\operatorname{CH_4N_2S})\right]^+$
407	$\left[\operatorname{Pt}\left(\operatorname{C_8H_8N_2OS}\right)(\operatorname{SH})\right]^+$

Table 5.4: Species obtained by MS/MS of [Pt (BTU)₃Cl]⁺



Figure 5.3: Effect of ligand to metal ratio (L/M) on Pt-BTU species (Metal conc. $5x10^{-6}$ M and $HClO_4$ conc. $2x10^{-5}$ M).

5.3.3 Effect of capillary voltage on metal species and complex formation

In ESI-MS, it is necessary to confirm that the mass spectra obtained represent the solution equilibrium. During ionization process, ion adducts can be formed at high applied potential. The study of the effect of capillary voltage was, therefore, undertaken

to establish that the mass spectra obtained are representative of the solution chemistry. As shown in Fig. 5.4, with the decrease of capillary voltage, the pattern of species formation remained the same, indicating that the mass spectra are representative of the solution phase.



Figure 5.4 a: Effect of capillary voltage on metal species formation.



Figure 5.4 b: Effect of capillary voltage on complex formation with BTU.

With the lowering of capillary voltage, the possibility of different reactions during ionization decreases. The effect of capillary voltage on metal ligand species was found to

be similar to that in the metal species. Pt $(BTU)_3Cl (m/z 771.03)$ species was of the highest intensity at all the capillary voltages.

5.3.4 Effect of composition of medium on different Pt-BTU species

To demonstrate the applicability of ESI-MS predictions to the environmental conditions, effect of percentage of H₂O on different species of Pt-BTU was studied and is shown in Fig. 5.5. As can be seen, the intensities of different (Pt-BTU) species decreases with increase in the percentage of water without showing any additional peak. Increase in percentage of water in the mobile phase (ACN+H₂O) leads to increase in the surface tension of the droplets formed and this suppresses the efficiency of the electrospray ionization process. The highest intensity was observed in 100% ACN.



Figure 5.5: *Effect of composition of medium (ACN : H*₂*O) on different Pt-BTU species.*

5.3.5 Effect of pH of medium on ESI-MS of Pt-BTU

Fig. 5.6 shows the effect of pH on intensities of different Pt-BTU species. Since BTU is having dissociable proton on the acidic amido -C (O) NHC(S)-moiety, it was of interest to study the effect of pH. pH of the solution was varied from 3.0 to 7.0 using

HNO₃ and NH₄OH. During this study, the ionic strength was varied from 1×10^{-3} M at pH 3.0 to 1×10^{-4} M at pH 5.0. Fig. 5.6 shows the intensities of different Pt-BTU species with the change of pH. At pH 3.0, Pt(BTU)₃Cl was the most abundant species; at pH 5.0,



Figure 5.6: Effect of pH on different Pt-BTU species.

Pt(BTU)₃ was the most abundant species whereas Pt(BTU)₂(ACN) was the most abundant species at pH 6.0-7.0. At pH 3.0, the Pt-BTU solvated species viz. Pt (BTU)₂(ACN) and Pt(BTU)₃(ACN) were not seen. Higher acidity was found to suppress the ionization of BTU and the protonation of ACN, leading to the formation of ML₃Cl as the most abundant species. With further increase in the pH, the two molecules of BTU showed mono coordination whereas one BTU molecule acts as a bidentate because of dissociation. Further increase in the pH increases the coordination tendency of ACN, which competes with BTU. Thus, Pt (BTU)₂(ACN) becomes the dominating species at pH > 6.0.

5.3.6 Effect of electrolyte on the linearity

The studies were carried out to observe differences, if any, in the formation of different species with the change in the concentration of K_2PtCl_4 . Metal species remained the same with increase in the concentration. However, the poor linearity was observed with the change of concentration indicating suppression effect with increase in the concentration. Since ESI-MS is very sensitive towards the ionic concentration present in the solution, constant ionic strength approach was adopted. To achieve this, HClO₄ of $2x10^{-5}$ M concentration was used because of its non-complexing nature. Under these conditions also, with the increase in Pt concentration, no extra species were observed. Good linearity was observed under constant ionic strength in the concentration range $(1.7x10^{-6}$ M to $1x10^{-5}$ M).

To explore the effect of electrolyte on the formation of various species, $HClO_4$ and $NaClO_4$ were used and data were compared (Table 5.5). $Pt(BTU)_3Cl$ species was of highest abundance in presence of $HClO_4$ whereas $Pt(BTU)_3$ at m/z 734.06 was the most intense in presence of $NaClO_4$. This effect was also seen when HNO_3 was used in place of $HClO_4$.

Electrolyte	<i>m/z</i> of species					
	554.03	591.00	595.05	632.03	734.06	771.03
Nil	1.0±0.1	2.39±0.08	4.4±0.1		15±2	100
NaClO ₄	4.57±0.08	6.58±0.16	16.9±4.7	57.2±3.0	100	40.44±0.20
HClO ₄	5.51±1.2	10.88±1.3		20.7±5.7	33±10	100

Table 5.5: Effect of electrolyte on intensities of different species of Pt-BTU

This can be explained due to suppression of the ionization of BTU into $[BTU-H]^{-}$ in presence of HClO₄ or HNO₃ which leads to the formation of M⁺ peak of Pt (BTU)₃Cl. This observation was also supported by the gas phase proton affinities of BTU and HClO₄ or HNO₃.

5.3.7 Computational results

To corroborate and understand the observed experimental ESI-MS data, DFT calculations were performed for the possible fragmentation patterns of Pt complexes in the gas phase.

5.3.7.1 Gas phase interaction of Pt salt with BTU

Fig. 5.1 shows two different geometric isomers of BTU denoted as trans (S_1 - C_2 - N_3 - C_4 , angle=178°) and cis (S_1 - C_2 - N_3 - C_4 =20°) forms. The trans-form is more stable than cis-form by ~15 kcal mol⁻¹ due to the conserved intramolecular hydrogen bonding. A closer look at the highest occupied molecular orbital (HOMO) of BTU ligand in both the isomers can reveal the binding preference to the Pt centre. The sulfur atom of trans form of BTU ligand can coordinate in a better way to soft Pt centre, due to the availability of sulfur-p non-bonding orbital (86%), as compared to the corresponding cis form. For the cis form, the p-orbital availability is less (76%). Both the lowest unoccupied valence orbitals (LUMO) are of p π -anti bonding type dominantly delocalized on the benzoyl rings [Koch (2001)].

Further, since the gas phase proton affinity (PA) of BTU ligand (in trans conformer) is higher (~9 kcal mol⁻¹) as compared to that of the chloro ligand, the binding of BTU to the soft Pt ion is expected to be stronger in both the anionic and the neutral forms.

$$BTU^{-} + H^{+} \rightarrow BTU \quad (-341.8 \text{ kcal mol}^{-1})$$

$Cl^{-} + H^{+} \rightarrow HCl$ (-332.6 kcal mol⁻¹)

Fig. 5.7 presents the optimized structure of all the species with the possible reaction schemes (scheme 5.1 and 5.2) and the formation energies in gas phase are given in the Tables 5.6 and 5.7.

Scheme 5.1: *Possible reaction for* $PtCl_4^{2-}$ *with BTU in the gas phase.*

$$[Pt(BTU)(BTU^{-})(ACN)]^{1+}$$

$$\begin{array}{c} 9 \\ + ACN \\ [Pt(BTU)(BTU^{-})]^{1+} \\ 5 \\ -HCl \\ \hline \\ -Cl^{-} \end{array} \begin{array}{c} Pt(BTU)_{2}Cl_{3}]^{1-} \\ 1 \end{array} \begin{array}{c} + BTU \\ -Cl^{-} \end{array} \begin{array}{c} Pt(BTU)_{2}Cl_{2}]^{0} \\ -Cl^{-} \end{array} \begin{array}{c} Ft(BTU)_{2}Cl_{3}^{-1+} \\ -HCl \\ \hline \\ -Cl^{-} \end{array} \begin{array}{c} Pt(BTU)_{2}Cl_{3}^{-1+} \\ + BTU \\ 4 \\ + BTU \\ \hline \\ 8 \end{array} \begin{array}{c} Pt(BTU)_{3}Cl_{3}^{-1+} \\ -HCl \\ \hline \\ Pt(BTU)_{3}Cl_{3}^{-1+} \\ -HCl \\ \hline \\ Pt(BTU)_{2}(BTU)]^{1+} \\ \hline \\ 7 \\ + ACN \\ [Pt(BTU)_{2}(BTU)]^{1+} \\ \hline \\ 10 \end{array}$$

Scheme 5.2: *Possible reaction for* $PtCl_4^{2-}$ *with solvent ACN in the gas phase.*

$$\begin{array}{c} (PtCl_4)^{2-} \xrightarrow{+ACN} [Pt(ACN)Cl_3]^{1+} \xrightarrow{+ACN} [Pt(ACN)_2Cl_2]^0 \xrightarrow{+ACN} [Pt(ACN)_3Cl]^{1+} \xrightarrow{+3H_2O} [Pt(ACN)_3(OH)(H_2O)_2]^{1+} \\ 1 & 12 & 13 & -HCl & 14 \end{array}$$

In scheme 5.1, the possible interactions of K_2PtCl_4 (1) with BTU ligand and ACN are shown and their corresponding formation energies are listed in Table 5.6. Displacement of chloro ligand by BTU ligand leads to the formations of species (2). The incoming BTU ligand can interact with Pt ion in two ways. In the first binding mode, the -NH₂ group can form hydrogen bond with the pre-coordinated chloro ligand (denoted as cis-form species (2a), Fig. 5.7), whereas in the second motif, the $-NH_2$ group can interact with chloro ligand, thus the intra-molecular hydrogen bonding is still conserved (denoted as trans species (3b) Fig.5.7).

Reaction	Formation energy
	(kcal mol ⁻¹)
$(1) + BTU \rightarrow (2) + Cl^{-1}$	-63.1
$(2) + BTU \rightarrow (3) + Cl^{-1}$	+12.7
$(3) \rightarrow (4) + Cl^{-}$	+120.6
$(4) \rightarrow (5) + \text{HCl}$	+23.6
$(4) + BTU \rightarrow (6)$	-33.8
$(6) \rightarrow (7) + \text{HCl}$	+18.7
$(4) + \overline{\text{ACN}} \rightarrow (8)$	-23.4
$(5) + \overline{\mathrm{ACN}} \to (9)$	-21.6
$(7) + \overline{\text{ACN}} \rightarrow (10)$	+9.2

Table 5.6: Formation energies of various species in the gas phase

The latter binding motif is slightly more favorable (by $3.9 \text{ kcal mol}^{-1}$) as compared to cis analogue and the formation of species (2) is a favorable process (-63.1 kcal mol⁻¹) (Fig. 5.8).







Figure 5.7: *Optimized structures (Å) of Pt-complexes with BTU and ACN in gas phase.* #colour codes: Pt=Magenta, S=Yellow, O=Red, N=Blue, C=Brown, H=White.

The second BTU molecule can displace another chloride anion from species (2) (Table 5.6) to form species (3) and this is an endothermic process (+12.7 kcal mol⁻¹). The neutral species $[Pt(BTU)_2Cl_2]$ (3) cannot be detected in the mass spectrometer. However, the cationic species $[Pt(BTU)_2Cl]^+$ (4) was identified in these experiments which is derived from the species (3) which has also been identified in the solid state[Koch (2001)]. X-ray crystallographic data are available for cis (3a) and trans (3b) isomers of platinum complex (Fig. 5.7). The calculated geometric parameters of both the isomers are

very close to the experimental data. Here again, the trans-isomer is marginally more stable than cis-isomer due to the apparent stronger hydrogen bonding in trans-isomer compared to the cis-isomer (Fig. 5.7).

 Table 5.7: Formation energies of various species in the gas phase with the solvent

 ACN

Reaction	Formation energy
	(kcal mol ⁻¹)
$(1) + ACN \rightarrow (11) + Cl^{-1}$	-51.9
$(11) + ACN \rightarrow (12) + Cl^{-1}$	-19.0
$(12) + ACN \rightarrow (13) + Cl^{-1}$	+98.0
$(13) + \mathrm{H_2O} \rightarrow (\mathbf{13a})$	-8.5
$(13a) \rightarrow (13b) + \text{HCl}$	+30.8
$(\mathbf{13b}) + \mathrm{H_2O} \rightarrow (\mathbf{13c})$	-9.2
$(13c) + H_2O \rightarrow (14)$	-13.1
$(11) \rightarrow (16) + \mathrm{Cl}^{-1}$	+58.0

The conversion of species (3) to species (4) (Scheme 5.1 and Table 5.6) involving removal of one chloro ligand (Fig. 5.8) is a high energy endothermic process (+120.6 kcal mol⁻¹). Due to the removal of the negatively charged chloro ligand by the neutral BTU ligand, the formation of the mono-cationic species is an endothermic process. In the ESI-MS data, the intensity of this species (4) is lower and hence such species may form transiently only in the gas phase and may undergo further reactions spontaneously. Additionally, the trans and cis isomers of species (4) are nearly iso-energetic (+0.2 kcal mol⁻¹).


Figure 5.8: Computed potential energy surface of Pt with BTU.

Species (4) can further react in three different ways (Scheme 5.1). Removal of hydrogen chloride from (4) and formation of (5) is an endothermic process (+23.6 kcal mol⁻¹). Here again, the intensity of the peak was observed to be poor. The abstraction of the acidic proton from BTU by CI⁻ is indeed the driving force for the formation of this species, whereas the more likely scenario is reaction with another BTU ligand leading to a tetra-coordinated species (6) and this reaction is exothermic (-33.8 kcal mol⁻¹). It is interesting to note that this peak is the most intense in ESI-MS. Here again the trans isomer is marginally favorable over the cis isomer by 3.1 kcal mol⁻¹. This species can be converted to (7), by removal of one hydrogen chloride (endothermic process). In this structure, one BTU ligand is bi-dentate, whereas the other two are coordinated in the mono-dentate binding mode. A similar bi-dentate coordination mode of BTU to Pt has been noted in the past by Circu et al [2009].

Finally, the solvent ACN can react with species (4), (5) and (7) (Scheme 5.1) to form the species (8), (9) and (10), respectively. Except for the conversion of (7) to (10),

the formations of the other two species is exothermic in nature. Hence, the solvent molecules can easily coordinate to platinum complexes in the gas phase.

5.3.7.2 Gas phase interaction of PtCl₄²⁻ with solvent

The possible gas phase interactions of ACN with the platinum salt were also modeled (Fig. 5.9). Unlike the gas phase, the bulk solvent may interact with Pt in different ways as the dielectric constant of ACN is high (ε =35) which will overstabilize the charged species in the gas phase. Similar to the addition of BTU to (1), the addition of one or two ACN molecules to the platinum salt can lead to the replacement of chloro ligand by ACN [species (11) and (12) Scheme 5.2] and the processes are exothermic (-51.9 kcal mol⁻¹ and -19.0 kcal mol⁻¹). It is interesting to note that the formation of species (3) with BTU is slightly unfavorable $(+12.7 \text{ kcal mol}^{-1}, \text{ Table 5.6})$, which signifies that even in the gas phase, the solvent ACN molecules are strongly bound. As far as the geometries are concerned, due to the introduction of asymmetric ACN ligand, the Pt-Cl bond lengths are asymmetric (Fig. 5.7). For species (12), both cis and trans isomers can co-exist since they are close in energy $(-5.5 \text{ kcal mol}^{-1})$. The addition of ACN to (12)leads to a very high energy species (+98.0 kcal mol⁻¹), which can immediately react with water vapor to form the ultimate product (14). The formation of species (14) from species (13) is expected to be a step wise reaction. Initially, one water interacts with a bonded chloride ion through hydrogen bonding which is energetically favorable (-8.5 kcal mol^{-1}). The removal of chloro ligand through the abstraction of proton as hydrogen chloride and the formation of hydroxo complex is somewhat endothermic process $(+30.8 \text{ kcal mol}^{-1})$. However, this species upon addition of two water molecules can interact through hydrogen bonding with the coordinated hydroxo species leading to the ultimate product i.e. species (14).



Figure 5.9: Computed potential energy surface of Pt with solvent ACN.

In the negative mode of ESI-MS, platinum chloro complexes $(PtCl_3^-, PtCl_2^-)$ and $PtCl_4^-)$ were observed. Formation of $PtCl_3^-$ from (1) is an energetically favorable process (~32.4 kcal mol⁻¹), whereas one electron oxidation of (1) is slightly unfavorable (~6.6 kcal mol⁻¹). The removal of the chloride ligand followed by one electron reduction of Pt^{2+} to Pt^+ to form $PtCl_2^-$ is energetically feasible.

5.4. Conclusion

Reactions of BTU ligand and ACN solvent with Pt in the gas phase using ESI-MS experiments were studied. It was found that in the positive mode, the Pt metal ion is stabilized by the solvent ACN molecule which can explicitly bind to Pt centre. The metal ligand equilibrium using BTU as a ligand showed that Pt forms complexes with BTU ligand in various stoichiometric ratios. DFT calculations were carried out to supplement the ESI-MS experimental data and to identify the possible pathways for the formation of various platinum species with BTU and with ACN molecules in the gas phase. Two different binding modes i.e. mondentate and bidentate coordination of BTU with Pt were identified. The calculated geometric parameters for [Pt(BTU)Cl₃]⁻ are in close agreement with the experimental cis and trans isomers of the neutral complex. Many species with different geometric isomers in the gas phase, close in energies, were found to be feasible. The role of hydrogen bonding was not only crucial in the solution phase, but also in the gas phase to stabilize various isomers.

This study provides an opportunity to explore the speciation studies of BTU with other platinum group elements and also with the knowledge of complexation of BTU with PGEs, separation studies of other PGEs like palladium in different matrices can be undertaken.

Chapter 6

Electrospray Ionization Mass Spectrometry

for the Determination of Palladium after

Pre-concentration by

Disposable Pipette Extraction

6.1 Introduction

Platinum group elements (PGEs) find extensive use in high technology applications due to their unique chemical and physical properties. Some of these are vehicle exhaust catalysts, medicine, electronics and chemical industries etc. Wide spread use of PGEs for various applications particularly, in vehicle catalytic converters and tumor treatment is leading to the increased release of these elements into the environment. Studies show that under suitable conditions, certain chemical forms of PGEs emitted from vehicles could become bioavailable [Dubiella (2009), Moldovan (2003)]. Determination of these elements in environmental samples is important to understand their chemical nature and their impact on ecological and biological aspects [Whiteley (2005), Ravindra (2004), Michalke (2010)]. Due to the increase in the usage of Pd in place of Pt in catalytic convertors, a predominance of the former element in the airborne particulate matter is observed over the last decade [Zereini (2012)]. Thus increase in the concentrations of Pd in environmental samples is of concern because among the PGEs, Pd has the highest bio-accessibility and mobility in the environment [Wiseman (2009)].

The process of thermal neutron induced fission of fissile nuclides e.g. ²³⁵U, ²³⁹Pu, leads to the production of a wide range of fission products in varying quantities. Palladium (Pd), Rhodium (Rh) and Ruthenium (Ru) are the only three PGEs formed to significant extent during nuclear fission. The actual amounts of these PGEs produced depends on the type of reactor system as well as on the burn-up of the nuclear fuel [Bush (1991), Kondo (1992)]. With the development of proper recovery and decontamination processes in future, the availability of these metals is likely to increase and thus can serve as an alternative resource to meet the increasing demands of noble metals [Sarkar (2009)]. A reliable analytical procedure for the quantification of the PGEs is, therefore, an important requirement at different stages of irradiated fuel reprocessing. Different

analytical methods have been described for the determination of PGEs in synthetic as well as in actual nuclear waste solutions [Aher (2002), Cavalli (1983)].

Due to the soft ionisation nature and ability to transfer ions from solutions, electrospray ionisation mass spectrometry (ESI-MS) is well suited for the characterisation of metal containing solutions [Di Marco (2006)]. A few studies using ESI-MS are reported for Pd [Bach (2007)]. However, ESI-MS analysis of PGEs in complex matrices, such as geological, nuclear fuel dissolver solutions, environmental samples etc. is a challenging task due to matrix influence. The achievable sensitivity and dynamic range in ESI are greatly influenced by the complexity of the ionisation process and matrix effects [Tang (2004)]. The composition of high level liquid waste (HLLW) solution, generated in nuclear reprocessing, is complex with as many as 30 elements present at different concentrations [Roth (2000)]. This Chapter describes the development of an ESI-MS method for the determination of Pd in simulated HLLW solution. It has been recognized that the purification of the analyte from the sample matrix and optimization of ESI process would lead to increased ionization efficiency. Therefore, removal of matrix elements is important for the reliable quantification of PGEs. The approach of using Pt as an internal standard for the quantification of Pd was reported previously [Minakata (2006)].

Thiourea (TU) and its alkyl or aryl derivatives are commonly employed for the recovery/separation of PGEs. In the present work, benzoylthiourea (BTU) was chosen as a complexing agent as it contains S atom, which would offer selectivity for binding with Pd which is a 'soft' metal [Mautjana (2003)]. In addition, presence of aromatic ring in the complex is expected to facilitate better charging of the analyte during electrospray process and give good ESI-MS response of the analyte. This is in contrast to ICP-MS, where presence of organic ligand and organic eluent is deleterious to

the stability of the plasma. Liquid-liquid extraction method is commonly used for the separation and preconcentration of Pd. However, solvent extraction poses a challenge because of large volume of solvent, low sensitivity, emulsion formation etc. These limitations can be overcome by using solid phase extraction. Disposable pipette extraction (DPX) is a novel form of solid phase extraction technique based on loosely contained sorbent particles in a disposable tip. DPX enables rapid extraction and requires minimal amount of solvent for elution.

Reported work on determination of palladium

Various preconcentration methods and analytical instrumental methods namely ICP-MS, ICP-AES, GFAAS etc. have been reported for Pd determination. The low concentration of Pd and matrix interferences necessitates the development of preconcentration method prior to its determination. Most of the preconcentration methods lead to the analyte losses due to deposition in the tubing. Schuster and Schwarzer (1996) reported a fully automated on-line micro column preconcentration procedure for Pd determination using graphite furnace atomic absorption spectrometry. In this method, selective preconcentration of Pd by N, N'-diethylbenzoylthiourea into the micro column and the optimization of loading and elution condition eliminated the problem of analyte losses and memory effect. Pd complexes were eluted using ethanol with a graphite furnace compatible volume. The effect of presence of other metals like alkali, alkaline earths, Cu, Fe, Co, Ni, Zn, Cd, Pb and Hg was studied. Although ICP-MS has also been utilized for the determination of Pd, however, Pd concentration and high content of matrix interfering elements make the task challenging. To circumvent this problem, different methodologies for preconcentration and separation have been reported. Maldovan et al., (2003) reported preconcentration of Pd on alumina micro column followed by ICP-MS determination. In this method, the interference at the most abundant ^{105}Pd with $(^{40}\text{Ar}^{65}\text{Cu})^{+}$ and $(^{89}\text{Y}^{16}\text{O})^{+}$ formed in the plasma was removed. The authors reported a limit of detection (LOD) of 1 ng/L for Pd determination in urban water sample. Although this method presents the good LOD but interferences from other PGEs were not studied. Jamali et al. (2007) developed functionalized silica for the preconcentration of Pd for ICP-AES. The authors studied the interference with the common coexisting ions. However, poor LOD of 200 ng/L was reported. The optimized method was applied for Pd determination in natural and synthetic water samples. The different solid phase extraction systems were also reported for the preconcentration of Pd. Li et al., (2012) reported the preconcentration and separation of Pd(II) from smelter road dust and waste water using murexide modified halloysite nanotubes prior to detection by ICP-OES. Balarama Krishna (2009) et al., reported the polyaniline loaded mini column for preconcentration of Pd from ground water sample for ICP-MS analysis.

Present Study

This Chapter describes the use of DPX for the pre-concentration of Pd-BTU complex. The conditions for the quantitative recovery of Pd(II) were optimized by systematically studying the extraction parameters such as acid concentration, mobile phase composition, concentration of ligand etc. Tolerance of the method towards the matrix element and possibility of isobaric interference from adjacent elements were also included in the study. In addition, the developed method was applied for the determination of Pd (II) in simulated high level liquid waste (SHLLW) solution. The use of BTU as a complexing agent for the DPX pre-concentration resulted in the minimization of interferences and matrix effects normally associated with ESI-MS. This demonstrated the potential of ESI-MS for the trace level quantitation of Pd from complex matrices.

6.2 Experimental

6.2.1 Chemicals and Reagents

Ru, Rh, Pd, Ag, Cd, Pt and U solutions were prepared by diluting respective ICP-MS standards (Merck) of 1000 ppm concentration. BTU (Alfa Aesar) was used as the complexing agent. Methanol (LC-MS grade, Fluka) and Milli-Q water were used for dilutions and also as the mobile phase for sample introduction to ESI. Suprapure grade HNO₃ and NH₄OH were used for pH adjustment. DPX tips, consisting of C_{18} sorbent particles, from M/s. Gerstel GmbH, were used for the pre-concentration studies.

6.2.2 Instrumentation

Mass spectrometric measurements were performed using micrOTOF-Q-II (Bruker Daltonics, GmbH) mass spectrometer equipped with electrospray ion source and quadruple-time-of-Flight analyzer. Simulations of the stable isotope patterns were made using Compass Isotope Pattern software. Samples were introduced into the ESI using an automatic syringe pump (Nemesys, Cetrol GmbH) at a rate of 4 μ L/min. All data were collected and analyzed using Bruker Compass Data Analysis software in MS and MS/MS modes. ESI-MS analyses were performed in positive ion mode with 3500 V plate potential and 4500 V capillary voltage. High purity argon was used as a collision gas for MS/MS. The following MS conditions were employed: capillary temperature, 180 °C; sheath gas-flow rate, 4L/min; and the auxiliary gas-flow pressure, 0.3 bar.

6.2.3 Procedure

About 0.1 M solution of BTU was prepared in methanol and was mixed with appropriate amounts of Pd to obtain required proportions of Pd:BTU. For carrying out the extraction studies, the sample was drawn into the pipette tip where it gains direct contact with the solid phase. No separate conditioning step was used for the stationary phase as it gets conditioned during the sample loading step itself. Pd-BTU complex, eluted from the

stationary phase using methanol, was mixed with 10^{-4} M BTU and was then directly injected into the ESI-MS system without any further treatment. SHLLW solution obtained from Waste Management Division (WMD), BARC, Mumbai, India was used for preparing the simulated samples. Composition and specifications of the SHLLW used are mentioned elsewhere [Sarkar (2009)]. SHLLW was mixed with U solution to have a uranium concentration of 1000 ppm. Standard solution of Pd was added to (U + SHLLW) mixture and the final dilution was made to 3 M HNO₃ to simulate the HLLW conditions.

6.3 Results and Discussion

6.3.1 Studies on the use of Pt as an Internal Standard for Pd determination

Fig. 6.1 shows a typical mass spectrum obtained by electrospraying a solution containing 10^{-6} M each of Pd(II) and Pt(II) and 10^{-4} M BTU in methanol. The major ions observed are summarized in Table 6.1. Identification of the peaks was done by matching them with the theoretically calculated isotopic pattern of Pd-BTU complexes as well as by



Figure 6.1: ESI-MS spectrum of Pd (II) and Pt (II)-BTU containing 100 ppb of each and $10^{-4}M$ BTU in MeOH.

MS/MS studies. The most abundant peaks of Pd-BTU complexes were observed at m/z 464.96 and 502.94 corresponding to $[Pd(BTU)_2]^+$ and $[Pd(BTU)_2Cl]^+$, respectively. For

Pt-BTU complex, the dominating species were $[Pt(BTU)_3]^+$ and $[Pt(BTU)_3Cl]^+$. It may be noted that the original Pt standard solution was containing about 5% HCl, which is the source for the Cl present in these complexes. Since the ESI response is influenced by the matrix effects, it was initially decided to use an internal standard whose chemical ionization behavior would be similar to that of Pd. Pt was chosen as the internal standard for the quantification of Pd because (a) both the elements belong to the same group in the Periodic Table and (b) Pt is not produced in the nuclear fission.

In order to evaluate the applicability of Pt as an internal standard for the determination of Pd by ESI-MS, the following experiment was carried out. Aliquots containing different concentrations of Pd were prepared in 10^{-4} M BTU. In all the aliquots, a fixed concentration of Pt was added.

Species	m/z.
$[Pd(C_8H_8N_2OS)(CH_4N_2S)]^+$	360.93
$[Pd(C_8H_8N_2OS)_3]^+$	646.99
$[Pd(C_8H_8N_2OS)_2C1]^+$	502.94
$\left[Pd(C_8H_8N_2OS)_2\right]^+$	464.96
$[Pd(C_8H_8N_2OS)_3C1]^+$	682.97
$\left[Pt(C_8H_8N_2OS)_3\right]^+$	734.03
$\left[Pt(C_8H_8N_2OS)_3Cl \right]^+$	771.03
$[Pt(C_8H_8N_2OS)_2]^+$	554.03
$[Pt(C_8H_8N_2OS)_2Cl]^+$	591.03

Table 6.1: Different species of Pd-BTU and Pt-BTU complexes observed in the ESI-MS spectrum

Table 6.2 shows the ESI-MS response of BTU complex species of Pd and Pt from the above aliquots. It is seen that the response of $[Pt(BTU)_3Cl]^+$ was influenced by the amount of Pd in the solution. However, the ESI response of Pd-BTU species show, albeit

not linear, a proportional relationship with the added amount of Pd. The suppression in the ESI response of Pt-BTU complex species on addition of Pd may be due to the difference in the stability constants of Pd(II) and Pt(II) with BTU [Pelaez (2002)]. To the best of our knowledge, the stability constant data of Pt or Pd complex with BTU are not reported. Attempts to determine the stability constant data of Pt-BTU complex in accordance with a reported ESI-MS methodology was not successful [Colette (2002)].

Hence, a different strategy was used for the determination of Pd by separating the matrix elements before introduction into ESI. The effect of different parameters such as concentration of BTU, acidity and composition of the loading medium etc. on the DPX **Table 6.2: ESI response of (Pd-BTU) and (Pt-BTU) complex species with change of Pt/Pd amount ratio**

	ESI Intensity*	% Decrease		
Amount of Pd added (ppb)	[Pt(BTU) ₃ Cl] ⁺ (50ppb)	[Pd(BTU) ₂ Cl] ⁺	in [Pt(BTU) ₃ Cl] ⁺ intensity	
0	16775±557	-	-	
10	10749±457	11613±356	36	
50	5188±11	60048±2565	69	
100	6765±81	102553±4731	60	

Pt/Pd amount ratio

*Average of three ESI-MS runs

extraction of Pd was studied. In all the cases, the elution was carried out using 100% methanol for ensuring maximum recovery for a given volume of the eluent.

6.3.2 Effect of Concentration of BTU

Fig. 6.2 shows the ESI-MS spectrum obtained after DPX extraction of a solution containing 100 ppb of Pd in 10^{-3} M BTU followed by elution with MeOH. It may be noted that after the extraction procedure, the major Pd peak at m/z 496.94 corresponding to $[Pd(BTU)_2S]^+$ was observed in the mass spectrum. The peaks at m/z 464.96 and 502.94, which were the most abundant in the pre-extracted sample, were not seen in this spectrum. In order to follow the transition of the dominating species during the process, the extraction was carried out using Pd loading solution containing different concentrations of BTU.



Figure 6.2: ESI-MS spectrum of Pd (100 ppb) and BTU ($10^{-3}M$) extracted from DPX

The eluate obtained with MeOH was introduced into the ESI-MS system. Fig. 6.3 shows the distribution of Pd peaks with m/z 464.96 and 496.94 as a function of concentration of BTU used in the loading solution.



Figure 6.3: Distribution of species $[Pd(BTU)_2]^+$; m/z 464.96 and $[Pd(BTU)_2S]^+$; m/z 496.94 as a function of concentration of BTU in the loading solution.

It was seen that as the concentration of BTU in the loading solution increases, the abundance of $[Pd(BTU)_2]^+$ decreases and that of $[Pd(BTU)_2S]_+$ increases. The peak corresponding to $[Pd(BTU)_2Cl]^+$ with m/z 502.94 was absent in the mass spectrum obtained by introducing Pd and Pt eluted from the DPX stationary phase for the entire concentration range of BTU studied. This was attributed to the replacement of Cl⁻ by BTU during the extraction stage. $[Pd(BTU)_2S]^+$, m/z = 496.94 being the most stable and abundant species, was used for monitoring the effect of other experimental parameters on the extraction efficiency. As is seen in Fig. 6.3, a concentration of BTU of 10⁻³ M was suitable for the extraction of Pd as the recovery was also found to be the highest at this concentration. Increasing the concentration of BTU in the loading solution enhanced the formation of the hydrophobic Pd(BTU)_2S complex species and hence improved recovery.

The 'S' atom required for the formation of the complex, $Pd(BTU)_2S$, must be generated due to degradation of BTU in acidic conditions. With an objective to verify that the species, $Pd(BTU)_2S$, is formed in solution rather than produced as a result of some gas-phase reactions in ESI, the effect of capillary voltage on the ESI response was studied. Fig. 6.4 shows the change in the ESI response of $[Pd(BTU_2)S]^+$ and $[(BTU)_2]^+$ peaks as a function of capillary voltage. It is seen that the relative intensity of $Pd(BTU)_2S$ species with respect to $(BTU)_2$ is almost unaffected by the changes in the capillary voltage.



Figure 6.4: Effect of capillary voltage on ESI-MS response of $[Pd(BTU)_2S]^+$; m/z = 496.94 and $[(BTU)_2]^+$; m/z = 341.06.

This observation supports the assumption that the species $Pd(BTU)_2S$ must be originally present in the solution phase, provided the dimeric species $(BTU)_2$ is also present in solution phase.

6.3.3 Effect of Concentration of HNO₃

Fig. 6.5 shows the effect of concentration of HNO_3 in the loading solution on the recovery of Pd. Lowering of pH enhances the ion pair formation (charge neutralization) of the Pd(II)-BTU complex which then shows increased preference for the hydrophobic stationary phase, C_{18} . This explains the initial increase in the recovery of Pd as the acidity increases.



Figure 6.5: *ESI-MS intensity of* $[Pd(BTU)_2S]^+$; m/z = 496.94 as a function of concentration of HNO₃ in the loading solution.

Increased dissociation of BTU at higher acidities may also contribute to the availability of S for the complex formation. However, the Pd(II)-BTU complex may not be stable at higher acidities which is indicated by the subsequent decrease in the recovery of Pd. The good recovery achieved at pH 1 is an indicative of the reasonable stability of

the Pd-BTU complex. The preference of Pd to bind to the ligand containing 'S' atom is responsible for the tolerance to moderate acidity and this selectivity can be used as a means for removing the non-soft matrix elements present in the SHLLW. Thus a pH of 1 was chosen for the loading solution.

6.3.4 Effect of Composition of Medium

Effect of proportion of MeOH:H₂O mixture in the loading medium on the recovery of Pd was also studied and the optimum composition was found to be (2:3 v/v) MeOH:H₂O. Because of the hydrophobic nature of the Pd-BTU complex, good retention on the C₁₈ based DPX stationary phase was expected at low MeOH containing loading solution. However, this caused poor wetting of the stationary phase, thereby restricting the analyte mass transfer of the analyte. Higher MeOH content in the loading solution resulted in the stripping of the adsorbed complex from the stationary phase during the loading step itself.

The optimized loading composition consisted of Pd solution prepared in 10^{-3} M BTU and 0.1 M HNO₃ and (2:3 v/v) MeOH:H₂O. A solution of (2:3 v/v) MeOH:H₂O was used for washing the loaded sorbent particles. 100% MeOH in the volume range of 500 – 1000 µL was found to be sufficient to give complete elution of the adsorbed Pd-BTU complex from the DPX stationary phase. The intensities of $[Pd(BTU)_2S]^+$ ion obtained after the pre-concentration of Pd(II) solutions of different dilutions, but maintaining the same amount of Pd(II) were compared. As can be seen from data given in Table 6.3, quantitative recovery of Pd(II) was achieved over a volume range from 1 - 10 mL of the sample solution. Pre-concentration factor, defined as the ratio of the sample volume to elution volume, was found to be independent of the loading solution volume in the volume range studied. Manual operation of the DPX for sample volumes larger than 10 mL was not attempted as the procedure becomes strenuous. However, efficient

extraction for larger sample volumes might be possible with an automated solid phase extraction system.

6.3.5 Figures of Merit of the Method

Linearity of the method was determined by introducing synthetic mixtures into the ESI. The synthetic mixtures were prepared by mixing known amounts of Pd with a fixed amount of (U+SHLLW) solution so that after dilution, concentration of U in the loading solution was ~ 20 ppm.

 Table 6.3: ESI-MS response of [Pd(BTU)₂S]⁺ after DPX extraction of solutions with

 different dilutions

Volume of loading solution	Concentration of Pd in loading solution	Final elution volume	ESI-MS response
(mL)	(ppb)	(mL)	
1	100		80421±1148
2	50	1	82903±610
5	20		83942±1052
10	10		80870±1015

^{*} Average of two extractions

After loading and washing of DPX as per the optimized procedure, the eluted fractions were introduced into ESI-MS. Fig. 6.6 shows the mass spectrum obtained by the DPX extraction of a solution containing 100 ppb of Pd.



Figure 6.6: ESI-MS spectrum of Pd-BTU after DPX extraction of a solution containing Pd (100 ppb), U (20 ppm) and BTU ($10^{-3}M$) in SHLLW.

No major peaks other than those belonging to Pd were seen in the mass spectrum highlighting the efficiency of the method for the selective extraction of Pd in the presence of several other metal ions. It may be noted that the two mass spectra shown in Fig. 6.2 and Fig. 6.6 are quite similar, demonstrating the preferential uptake of Pd by the optimized method. One of the main limitations of ESI-MS is the analyte signal suppression due to the matrix effect. By the use of DPX extraction, linear ESI response was obtained for Pd in the concentration range of 5 ppb to 200 ppb with $r^2 = 0.990$ as shown in Fig. 6.7. The linearity obtained indicated the good pre-concentration ability of the proposed method. The DPX method also helped to circumvent the matrix effect by the selective separation of Pd-BTU complex. Tolerance of the method for the matrix effect of U/Pd amount ratio in the loading solution on the ESI response of Pd after DPX extraction. ESI signal intensity of $[Pd(BTU)_2S]^+$ ion did not changed significantly by the presence of host elements upto U/Pd amount ratio $\sim 7,500$. The relative standard deviation for 50

ppb of Pd extracted from 2 mL of sample solution was found to be 2 %. The limit of detection of the method was estimated using (U+SHLLW) solution spiked with known amounts of Pd. Fig. 6.8 shows the mass spectrum obtained for a 10 mL solution of 0.012 ppb Pd. After extraction, elution was carried out with 1 mL of MeOH and the corresponding $[Pd(BTU)_2S]^+$ peak gave a S/N > 3.



Figure 6.7: *ESI-MS response of* $[Pd(BTU)_2S]^+$ *as a function of concentration of* Pd(II) *in*

The detection limit achieved by the present method may not be at par with that obtained by quadrupole based ICP-MS system due to two factors: (i) owing to the relatively poor ionization efficiency, ESI-MS detection limits are up to 2-3 orders of magnitude poorer than those found using the ICP source [Rosen (2004), Brown (1996)] and (ii) detection limit offered by MS systems employing quadrupole as the analyser, is considered to be better than that of systems using TOF analysers [Pelaez (2002), Pozo (2011)].

SHLLW solution.

Table 6.4: Effect of U/Pd amount ratio in the sample solution on ESI response Pd

Concenti	ation of	U/Pd	ESI-MS		
Pd (ppb)	U (ppm)	amount ratio	response of Pd*		
6	4	764	10732±60		
	18	3,054	10358±485		
	45	7,635	10131±270		
	183	30,542	7506±85		
	764	1,27,257	6630±62		

after DPX extraction

*Average of three extractions



Figure 6.8: ESI-MS spectrum of 0.012 ppb of Pd in SHLLW, by using 10 mL for DPX.

However, the detection limit and the dynamic range obtained by the present method demonstrate the possibility of ESI-MS for the quantification of Pd at trace levels in complex matrices and also shows the potential for direct information on various kinds of Pd species present in the solution. The accuracy of the method was determined by spiking SHLLW solution with known concentration of Pd and determining the concentration based on the calibration curve. A good agreement was obtained between the expected (130 ppb) and determined (125±7 ppb) concentration of Pd.

6.3.5 Interference Studies

The suitability of the optimized method for the uptake of Ru, Rh, Ag and Cd was studied as (i) some of their isotopes interfere with Pd isotopes, (ii) they complex with "S' based ligands, and (iii) some of these elements are also produced in nuclear fission. A solution containing of 100 ppb each of Ru, Rh, Ag and Cd in 10⁻³ M BTU and 0.1 M HNO₃ was subjected to DPX extraction. Fig. 6.9 shows the MS spectrum of the MeOH eluate obtained.



after extraction.

Species of the type $[M(BTU)_2]^+$ were seen in the spectrum e.g. $Ag(BTU)_2$ with m/z: 466.98 and 468.98. It is worth noting that no species corresponding to $[M(BTU)_2S]^+$,

which is used for the determination of Pd, was observed in the spectrum. This confirms the selectivity of the method for the determination of Pd in presence of other PGEs.

6.3.6 Comparison with other methods

The analytical characteristics of DPX method for the ESI-MS determination of Pd were compared with other reported methods and are summarized in Table 6.5. As can be seen, from Table 6.5, the selectivity of the present method is better compared to other preconcentration methods [Moldovan (2003), Balarama Krishna (2009), Liang (2009)]. The use of low pH for pre-concentration eliminates the interference from most of the coexisting ions. Limit of detection of the methods based on ICP-MS and GFAAS is better than that obtained by the proposed method using SHLLW as the blank [Moldovan (2003), Balarama Krishna (2009), Liang (2009)]. In addition, the proposed method gives reasonably good linearity. The time required for the loading step is shorter for most of the reported methods in view of the automation [Li (2012), Jamali (2007), Balarama Krishna (2009), Schuster (1996), Liang (2009)]. Compared to other solid phase extraction methods, DPX does not require a separate conditioning step for the stationary phase and a small volume of the eluent is sufficient.

6.4 Conclusions

This study shows the development of a DPX method for the ESI-MS determination of Pd in SHLLW with good selectivity and sensitivity. The use of BTU and optimization of DPX conditions provided selective and quantitative recovery of Pd. The DPX method is simple, requires minimum eluent volume and takes care of the matrix effect responsible for signal suppression in ESI-MS. Good linearity and sensitivity were obtained for the developed method by virtue of the selectivity and enrichment of the analyte. Further, the selection of $[Pd(BTU)_2S]^+$ ion for determination eliminated the possible isobaric interference from other elements like Ag, Au, Cd, Rh and Ru which are major interfering species in other analytical techniques. The methodology shows a strong potential for Pd determination in HLLW and in view of its selectivity, the method appears promising for its application to other complex samples like environmental, geological etc.

Preconcentration method	Detectio n method	LoD (ngL ⁻¹)	Tolerance	Typical sample volume (mL)	рН	Linea- rity	Analysi s time (min)	Remarks	Referen ce
Microcolumn loaded with Halloysite nanotubes-bound amine	ICP-AES	290	Au, Pt, Ru, Rh, Ir	10	1.0	50 - 400 µg mL ⁻¹	5	Good selectivity; poor LOD	[Li (2012)]
SPE using functionalized mesoporous silica	ICP-AES	200	Ag, Co, Cd, Ni, Zn, Mn, Pb, Al, Cr, Fe, Pt	100	4.0	1–1000 μg L ⁻¹	2	Good selectivity; good linearity; poor LOD	[Jamali (2007)]
Microcolumn loaded with activated alumina	ICP-MS	1	Cu, Y	15 – 30	1.0	0–100 μg L ⁻¹	2 – 15	Good LOD; Tolerance of other noble metals was not investigated	[Moldov an (2003)]
Mini column loaded with polyaniline	ICP-MS	0.4	Na, K, Ca, Mg, Fe, Zn, Cu, Y	25 - 50	6.0- 7.0	5 – 40 ng	> 10	Good LOD; Tolerance of other noble metals was not investigated	[Balara ma Krishna (2009)]
Microcolumn loaded with N,N- diethyl-N'-benzoylthiourea	GFAAS	13	Co, Ni, Cu, Zn, Cd, Pb, Ru, Rh, Os, Ir, Pt, Ag, Au	1-5	<1	0 – 8 ng	5-10	Good selectivity; small sample volume; limited linearity	[Schuste r (1996)]
Dispersive liquid–liquid microextraction using diethyldithiocarbamate	GFAAS	2.4	Cu, Co, Zn, Cd, Ni, Mn, Pb	5	1.0	0.1–5 μg L ⁻¹	> 5	Good LOD; Tolerance of other noble metals was not investigated	[Liang (2009)]
DPX using benzoylthiourea	ESI-MS	12	Ru, Rh, Cd, Ag	1 - 10	1.0	5- 200 μg L ⁻¹	5 – 15	Good linearity; less eluent volume; tolerance to matrix concentration and absence of interference from other PGEs	Present Work

Table 6.5: Comparison of figures of merits of the proposed method with reported methods for Pd determination

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