SEPARATION AND QUANTITATION OF SOME PLATINUM GROUP METALS BY RP-HPLC

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Approval of the Graduate School of Natural and Applied Sciences.

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This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

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ABSTRACT

SEPARATION AND QUANTITATION OF SOME PLATINUM GROUP METALS BY RP-HPLC

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In this study, a reversed-phase high performance liquid chromatography (RP-HPLC) method has been developed to separate and determine Pt and Pd after formation of their chelates with N,N-diethyl-N'-benzoylthiourea (DEBT). With the aim of reducing the number of steps in treating the samples, the method developed does not require the elimination of excess chelating reagent before the analysis of metal chelates. The different physical and chemical parameters affecting separation were examined in details. The whole analysis was completed on a C_{18} column in 16 min at 280 nm, with the mobile phase of acetonitrile-methanol-water (80:10:10, v:v:v) containing 0.20 mol l^{-1} pH 5.0 acetate buffer at a flow rate of 0.8 ml min^{-1}. Detection limits of the method, based on 3s, were found as 14.2 μg l^{-1} for Pd and 0.77 mg l^{-1} for Pt using a 20-μl sample loop.

Reproducibility of the method for ten repeated measurements was found as 2.36 % for 0.60 mg l^{-1} Pd and 2.58 % for 10.0 mg l^{-1} Pt as % RSD. The proposed method is a rapid, simple and highly selective method for the simultaneous determination of Pt and Pd by HPLC without the need for any interference elimination process.

Keywords: Reversed-phase high performance liquid chromatography (RP-HPLC); platinum group metals (PGMs); N,N-diethyl-N'-benzoylthiourea (DEBT); Platinum; Palladium; Metal Chelates

ÖZ

TERS FAZ-YÜKSEK PERFORMANSLI SIVI KROMATOGRAFİ İLE BAZI PLATİN GRUBU METALLERİN AYRILMASI VE TAYİNİ

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Bu çalışmada, platin ve palladyum'un, N,N-dietil-N'-benzoiltiyoüre (DEBT) ile şelatları oluşturulduktan sonra ayrılmaları ve tayin edilmeleri için ters-faz yüksek performanslı sıvı kromatografisi (RP-HPLC) yöntemi geliştirilmiştir. Örnek hazırlama aşamasında gereken basamakları azaltmak amacıyla geliştirilen bu yöntemde, metal şelatların tayininden önce, ortamdaki şelat yapıcı reaktifin aşırısının uzaklaştırılması gerekmemektedir. Ayırma işlemine etki eden farklı fiziksel ve kimyasal parametreler detaylı olarak incelenmiştir. Tüm analiz, C_{18} kolonu ile 280 nm de, 0.20 mol l^{-1} derişimindeki pH 5.0 asetat tamponu içeren asetonitril-metanol-su (80:10:10, v :v :v) karışımında ve 0.8 ml dk^{-1} akış hızındaki akıcı faz kullanılarak 16 dakikada tamamlanmıştır. Bu yöntemle, 3s e karşı gelen gözlenebilme sınırları (DL), 20 µl-lik örnekleme hacmi kullanarak, Pd için

14.2 μg l^{-1} ve Pt için 0.77 mg l^{-1} olarak bulunmuştur. Yöntemin tekrarlanabilirliği, 10 kez tekrarlanan ölçümle, 0.60 mg l^{-1} Pd için % 2.36 ve 10.0 mg l^{-1} Pt için % 2.58 BSS (RSD) olarak bulunmuştur. Önerilen yöntem, Pt ve Pd un eş zamanlı tayini için hızlı, basit, çok seçici ve girişimi gidermek için proses gerektirmeyen bir HPLC metodu olarak önerilmektedir.

Anahtar Sözcükler: Ters-Faz Yüksek Performans Sıvı Kromatografisi (RP-HPLC); Platin Grubu Metalleri (PGM); N,N-dietil-N'-benzoiltıyoüre (DEBT); Platin; Palladyum; Metal Şelatlar

TO MY GREAT PARENTS,

AHMED & FAYZA

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This thesis is dedicated to my family, who deserve an award for their patience, understanding and prayers for me during my studies in Turkey. I am also grateful to my relatives and friends both in Palestine and Turkey, who provided me with moral and spiritual support.

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INTRODUCTION

1.1 Platinum Group Metals (PGMs)

The 4d and 5d metals in groups 8 through 10 in the periodic table are referred to as the platinum group metals because they occur together in platinum-bearing ores (Shriver D. F., Atkins P. W., 1994). These metals are platinum (Pt), palladium (Pd), rhodium (Rh), iridium (Ir), ruthenium (Ru) and osmium (Os). Platinum group metals (PGMs) together with gold (Au) are known as the precious metals. This term reflects their economic value as well as their rare occurrences in nature (Barefoot R. R., Van Loon J. C., 1999). The prices of the individual platinum metals vary widely because they are recovered together but their consumption is not proportional to their abundance. Rhodium is by far the most expensive metal in this group because it is widely used in industrial catalytic processes and in automotive catalytic converters. For example, rhodium is about 40 times more costly than the catalytically less useful metal, palladium, even though they occur in similar abundance (Shriver D. F., Atkins P. W., 1994). The PGMs have very low natural background concentrations in most rocks, sediments and soils of the order of 1-2 ng g^{-1} or less (Vos E. De et al, 2002).

Platinum, palladium and rhodium are mined in a limited number of countries, the most important being South Africa, the former USSR, the USA and Canada. These countries report huge reserves, but with different mine ratios of Pt, Pd and Rh (Table 1.1) (Ertl G. et al., 1999).

Table 1.1. Relative Importance of Some Countries in the Supply of Pt, Pd and Rh in 1997

Country	Pt (%)	Pd (%)	Rh (%)
South Africa	75.0	29.9	83.3
Russia	15.2	55.8	12.6
USA and Canada	5.8	10.4	4.1
Others	4.0	3.9	0

Over the past few decades, PGMs have become widely used in the automotive, chemical, electronics, glass, medical/biomedical and petroleum industries, in jewellery and in coins and bars used in investment. Another field of application is dental restorative alloys (Vos E. De et al., 2002; Philippeit G. et al., 2001).

1.2 Usage of PGMs in the Automotive Industry

Spark-ignition (gasoline) and compression-ignition (diesel) engines are a major source of urban air pollution. Carbon monoxide (CO) unburned or partially burnt hydrocarbons (HC) and nitrogen oxides (NOx) are the main contaminants present in engine exhaust fumes. Due to increasing ecological awareness of the need for the preservation of acceptable environmental conditions, catalytic exhaust after-treatment

technologies for gasoline- and diesel-powered vehicles have been developed. These technologies were classified according to engine type, which determines the catalyst operating conditions (Moldovan M. et al., 2002).

The early days of catalytic converter research and development targeted the use of base metal catalysts. Numerous publications described the results obtained with catalysts that contained, for example, the oxides of Cu, Cr, Fe, Co and Ni. Despite this effort, no real breakthrough was achieved, i.e., they were not efficient in the conversion of engine contaminants to harmless compounds (Ertl G. et al., 1999).

1.2.1 Catalytic Converters

Catalytic converters containing PGMs were first introduced in the United States in the 1970s and then in Europe in the 1980s (Moldovan M. et al., 2002). The precious metals are known to have a much higher intrinsic activity, i.e., the activity per gram component, for the simultaneous conversion of CO, HC and NOx than base metal catalysts. Furthermore, it is easier to obtain and to keep precious metals in a very finely divided state to increase their surface areas. Precious metal-based catalysts are also much more resistant to sulfur poisoning at temperatures below 750 K than base metal catalysts (Ertl G. et al., 1999).

1.2.2 Three-Way Catalyst

The most widespread car catalyst employed nowadays is the ‘three-way catalyst’ (Figure 1.1), so-called because it can reduce the three main

contaminants, namely CO, HC and NOx, to below the legislated level (Palacios M. et al., 2000). It consists of a honeycomb type monolithic support made of a high melting point ceramic material, namely cordierite ($2MgO.2Al_2O_3.5SiO_2$). The cordierite is coated with a highly porous wash coat (a highly porous alumina coating on the substrate, which provides a high surface area for the catalytic reactions) that consists of approximately 90% γ-Al_2O_3 where PGMs are finely dispersed in a metallic form (Palacios M. A. et al., 2000).

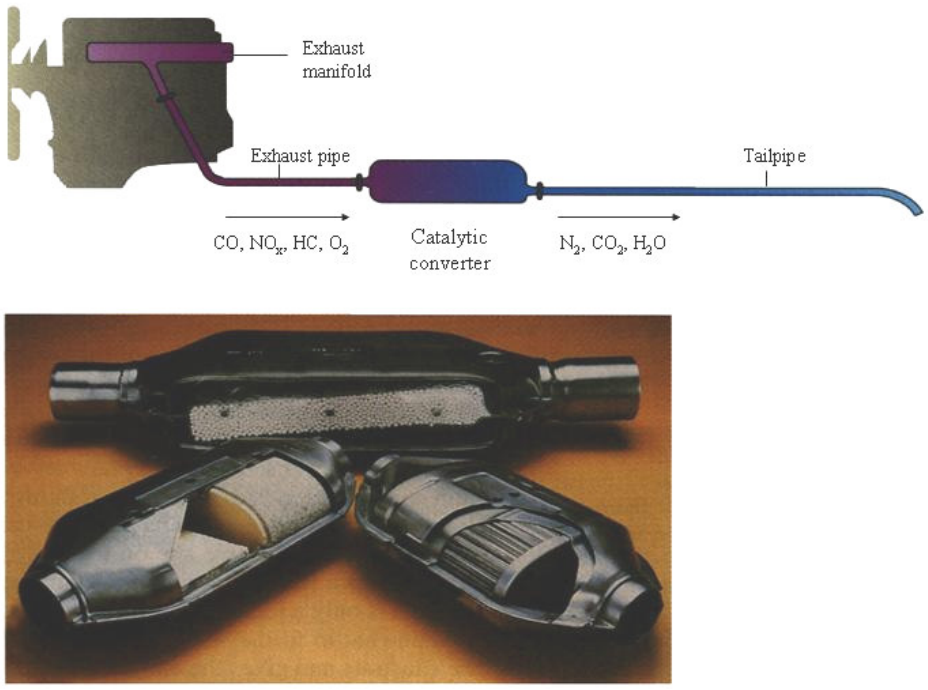


Figure 1.1. Autocatalytic Converter. Top: Exhaust gases from the automobile engine pass to the exhaust manifold, then to the catalytic converter, where pollutants are converted. Bottom: Cross-sectional views of some autocatalytic converters, showing the packing material with catalysts on its surface (Ebbing D. D., 1996).

The precious metals are generally introduced in the catalyst by wet chemical methods such as incipient wetness impregnation, typically using

aqueous solutions of the three precious metal salts, followed by a drying step to remove the water, and then by a calcination step to decompose the precious metal salts. Sometimes, a reduction step is applied to convert the precious metal oxides into the metallic state.

The precious metals currently used in three-way catalysts are palladium, platinum and rhodium. In the past, also ruthenium and iridium have been tested, but because of the volatility and/or the toxicity of these metals or their oxides, neither has found practical application (Ertl G. et al., 1999).

There is a wide range of possible combinations and concentrations of Pt, Pd, and Rh that can be used to achieve the different performance features required by car manufacturers. In the various Pd-Rh, Pt-Rh, Pt-only or Pt-Pd-Rh catalysts, the percentage of PGMs with respect to the bulk material is < 0.1 wt. %. Other elements present in the catalyst, such as Ce, Zr and rare earths are used as components of the wash coat to increase defined properties such as catalyst PGM impregnation, oxygen storage capability and chemical inertness (Palacios M. A. et al., 2000).

The gasoline engine, which is the most popular today, is supported by the use of three-way catalysts. CO and HC are oxidized to CO_2 and H_2O, while at the same time NOx are reduced to N_2. Pt and Pd are involved in the oxidation of HC and CO, while Rh is used in the reduction of NOx.

The diesel engine, which is the second most popular, is supported by the use of oxidation catalysts, which were put on the market in 1991 for

diesel passenger cars. A diesel oxidation catalyst converts a large part of the hydrocarbon constituents of the soluble organic fraction, as well as gaseous HC, CO and odor-creating compounds to CO_2 and H_2O.

In a typical diesel oxidation catalyst, Pt or Pd is used. Similar to the three-way catalyst, the diesel oxidation catalyst consists of a ceramic monolith on which a special wash coat is deposited, which contains the precious metal components. In both types, a key factor affecting the performance of the catalyst system is the type, quantity and distribution of PGMs on the wash coat layer (Moldovan M. et al., 2002).

1.3 Release of PGMs into the Environment

Since the introduction of catalytic converters in the United States in the 1970's and in Europe in the 1980's, there has been a clear link between their use and the increasing concentration of PGMs in the environment. Under real driving conditions, thermal and chemical mechanisms led to a partial release of PGMs into the environment through car exhaust fumes.

Although other sources of PGMs are possible in highly populated cities, of these, the metallurgical, pharmaceutical and petroleum industries etc., none of them can be established as comparable in importance to catalyst contamination (Moldovan M. et al., 2002). Those sources are 1 to 6 orders of magnitude lower (Dongarra G. et al., 2003).

On the basis of the documented evidence produced to date, it is generally accepted that the loss of catalyst performance during on-road lifetime, and so is the release of PGMs in the environment from

automobile exhaust catalytic converters, can be attributed to a number of mechanisms namely mechanical stresses, hot-temperature chemical reactions with oil fumes, fouling and thermal processes which all lead to deterioration of the catalytic film (Caroli S. et al., 2001; Palacios M. A. et al., 2000; Dongarra G. et al., 2003).

From the environmental point of view, the mobility of PGMs on/from the catalyst surface is the most important for the increase of their environmental background level and can be mainly attributed to thermal sintering, evaporation and mechanical or thermal erosion.

Sintering occurs when small crystallites migrate towards larger crystallites or when larger ones capture single atoms emitted from small crystallites. During this migration, some particles or atoms can leave the catalyst surface. Evaporation of the precious metals results in a loss of catalytic active surface. Although PGMs are quite thermally stable (boiling points between 3000 and 4000 °C), losses of some PGMs were observed above 900 °C and under oxidizing conditions. This temperature and oxidizing conditions can be easily reached on the surface during automobile operation (Palacios M. A. et al., 2000).

It is believed that PGMs are emitted from catalytic converters in particulate form, mainly in the (0) oxidation state or as oxides. Nanocrystalline PGMs particles are attached/coated on μm-sized aluminum oxide particles or bound to other carriers. According to some studies, only 1-10 % of the total platinum emissions are water-soluble compounds (Moldovan M. et al., 2002; Dongarra G. et al., 2003; Moldovan M., Rauch S. et al., 2001).

No definite data is yet available about the emission factors from converter-equipped vehicles, because the PGM emissions vary according to catalyst age and car speed (Dongarra G. et al., 2003). Nevertheless, the total amount of PGMs released into the environment by catalysts can be directly evaluated by determining their content in car exhaust fumes or indirectly by quantifying the anthropogenic PGMs in environmental materials such as soil, road dust, airborne particles, sludge, water, plant, etc., and modeling these data together with traffic statistics. Direct determination requires an effective sampling procedure where representative amounts of the released PGMs are collected. Table 1.2 shows the results obtained by authors following either strategy. Comparison of the data for Pt, which is the element most frequently analyzed, reveals an evident discrepancy with indirect determinations giving generally higher values (Palacios M. et al., 2000; Palacios M. A. et al., 2000). This indicates that other sources of Pt such as its usage in polymerization of olefins, refining of petroleum, ammonium oxidation, electronic, glass and jewellery manufacturing and hospital effluents containing Pt drugs are also important sources for the emission of Pt into the environment. However, little data is available about the contribution of these sources.

Table 1.2. PGMs Released into the Environment by Car Catalysts (Palacios M.A., et al., 2000)

Direct determinations from gasoline cars exhaust fumes	
Type of catalyst	PGM emission
Pellet catalyst	1.2 μg km^{-1} of Pt when running at 48 km h^{-1} 1.9 μg km^{-1} of Pt when running at 96 km h^{-1}
Three-way catalyst	2-60 ng km^{-1} of Pt
Three-way Pt-Rh catalyst	120 ng m^{-3} of Pt at 140 km h^{-1} 0.3 ng m^{-3} of Pd at 140 km h^{-1} 20 ng m^{-3} of Rh at 140 km h^{-1}
Three-way catalyst	67 ng m^{-3} of Pt under idling conditions
Three-way catalyst	Pt, Pd and Rh concentrations at blank filter level under constant sampling volume following EC-Directive 70/220
Three-way catalyst	7-72 ng km^{-1} of Pt
Indirect determinations of environmental Pt by data modeling	
Sample	PGM emission
Soil, sludge, water, etc.	270 ng km^{-1} of Pt
Rainwater discharges along roadsides	14 ng m^{-2} of Pt of daily emission
Soil, plants, sewage ashes	2-10 μg km^{-1} of Pt

The variability among various study estimates and the difference between engine test benches and real driving conditions make an accurate assessment of the emission rates quite difficult. The range depends on the operating conditions and the age of the converter as well. It was observed that the highest content of particulate PGMs are released by fresh catalysts, while the emissions are significantly reduced in aged converters (Dongarra G. et al., 2003).

Because PGMs are not atmophile elements, once released to the air they 'travel' short distances due to their masses and are deposited along bordering traffic routes. Therefore, they may accumulate in neighboring soil, water and vegetation in a process called bioaccumulation (Dongarra G. et al., 2003). They deposit either directly or through runoff and hence they enter the food cycle. The behavior of PGMs in soils and other environmental matrices has already been studied by many authors (Cinti D. et al., 2002).

1.3.1 Effects of PGMs

Anthropogenic emissions of PGMs can accumulate in freshwater and estuarine sediments and their effect on aquatic life will depend on their biological availability. Platinum concentration in river water and pore water is at nanograms per liter level. But it was found that sediments with high organic content accumulate anthropogenic platinum and increase its concentration. Similar conditions can be expected for palladium and rhodium. The toxicity of Pt to aquatic life has been studied for several freshwater invertebrates. However, the effects of Pd and Rh on aquatic life are not well known yet (Moldovan M., Rauch S. et al., 2001).

A number of previous studies reveal significantly elevated concentrations of PGMs in road dust and in the vicinity of highways, directly linked to the use of catalytic converters in automobiles. Uptake by and effects on organisms living in and plants growing on these contaminated soils are of some concern. The soluble fraction of the emitted PGMs represents the immediately available amount for all kinds of organisms; however, further transformation reactions such as oxidation and complexation, can increase the bioavailability.

Attempts have been made to establish a general guideline value for PGMs in soil, but important information regarding bioavailability, toxicity, etc., is not yet available, and therefore, a model for these processes could not be established. Further investigations on the solubility and transformation (oxidation, complexation) of Pd, Pt and Rh under environmental conditions are required (Gomez B. et al., 2002).

Although the introduction of catalytic converters resulted in the lead pollution level decreasing in the air, this device is not totally 'clean'. In fact, the release of PGMs into the environment has caused a new environmental risk.

PGMs come into contact with man both directly, through inhalation of dust, and indirectly through the food chain. Palladium is currently the element arousing most concern for man, as it is considered to be a powerful allergen. Although inert in metallic form, Pt salts of less than 10μm are easily inhaled and cause hypersensitivity and susceptibility to asthma. It was found that more than 90% of the bioavailable Pt is bound to high molecular weight compounds as proteins and that finely dispersed Pt may be significantly dissolved in the presence of adenosine triphosphate (ATP). In soil, PGMs can be dissolved by humic substances and fixed in metal-organic complexes (Dongarra G. et al., 2003).

The long-held belief that PGMs are generally harmless stems from their chemical inertness. On the other hand, their role as sensitizers in the etiology of allergenic pathologies such as: asthma, conjunctivitis; dermatitis; rhinitis; and urticaria has been ascertained (Caroli S. et al., 2001).

Even though the data available today predicts PGM concentration in ambient air to be at least two orders of magnitude below the guidance range of 15-150 ng m^{-3}, indicating no obvious health effects, there are still a number of aspects related to PGMs and catalysts that justify further research. First, continual monitoring of changes in PGM levels in air and road dust is warranted, to make sure that there is no dramatic increase from today's levels. In fact, in the last 15 years, the PGM concentration in air has increased by more than two orders of magnitude in heavy traffic areas. In the future, there will be an increased number of catalyst-equipped vehicles, and new types of catalysts might also come into use. Secondly, more detailed information on the chemical composition of the PGM-containing substances or complexes leaving the catalyst surface, as well as the size distribution of PGM-containing particles released during driving, will facilitate a more in-depth human risk assessment (Gomez B. et al., 2002; Artelt S. et al., 1999; Merget R. et al., 2001).

1.4 Methods Used for the Determination of PGMs in Soils

In recent years the development of analytical methods for the determination of PGMs in various matrices has attracted considerable interest (Pyrzynska K., 1998). In fact, this group of elements is one of the most difficult groups to be accurately determined at low concentration levels (Andreia Mesquita da Silva M. et al., 2001). In spite of the increase in the concentrations of these metals in the environment caused by the introduction of catalytic converters, their concentration is still low. Therefore, specific and sensitive analytical techniques are required to achieve accurate results. Care in sample preparations in order to avoid

losses of analytes is equally important (Motelica-Heino M. et al., 2001; Barefoot R. R., 1999).

Schramel and coworkers (Schramel P. et al., 1995) used Q-ICP-MS for Pt in road side dust after digesting it with aqua regia or a mixture of acids (HNO_3+$HClO_4$+HF). This technique was also used by Parent and coworkers (Parent M. et al., 1996) to analyze Pt in street dust and soil making use of 4-bis(carboxymethyl)dithiocarbamate complex and an XAD-4 resin for preconcentration of the metal. In 1997, Lustig and coworkers (Lustig S. et al., 1997) determined Pt by ICP-AES after separating and preconcentrating it by HPLC. Müller and Heumann (Müller M., Heumann K. G., 2000) used Q-ICP-MS for the determination of Pt and Pd in road dust and surface soil after preconcentrating them with anion exchange chromatography. Garcia and coworkers (Garcia R. et al., 2001) analyzed road dust for Pt using Q-ICP-MS after preconcentrating it on a modified silica capillary with a strong cation exchanger. A GFAAS method that followed column preconcentration with N,N-diethyl-N'-benzoylthiourea (DEBT) was suggested by Boch and coworkers (Boch K. et al., 2002). Cation exchange chromatography, which was followed by Q-ICP-MS, was suggested by Higney and coworkers (Higney E. et al., 2002) for analysis of Pt in road dust. The use of ICP-AES, combined with ion exchange chromatography after digestion with aqua regia, for the determination of Pt and Pd was suggested by Kovacheva and Djingova (Kovacheva P. and Djingova R., 2002). Road dust was digested in microwave oven by aqua regia and hydrofluoric acid by Gomez and coworkers (Gomez M. B. et al., 2003) who then preconcentrated Pt and Pd using Te coprecipitation and $SnCl_2$, and determined their amounts by Q-ICP-MS. Limbeck and coworkers (Limbeck A. et al., 2003) used DEBT as a complexing agent on a C_{18} micro column for Pd which was then

determined by GFAAS. Table 1.3 summarizes the various methods used in the determination of Pt and Pd in soil and dust.

Table 1.3. Methods Used in the Determination of Pt and Pd

Matrix	Digestion / dissolution method	Preconcentration / separation	Detection	LOD (ng l^{-1}) Unless stated somewhere else		Reference
				Pd	Pt	
Road side dust (tunnel)	Aqua regia or HNO_3+$HClO_4$+HF	-	Q-ICP-MS	-	10	Scramel P. et al., 1995
Street dust/soil	MW with HNO_3+$HClO_4$+HF	Preconcentration on 4-bis(carboxymethy)dithiocarbamate complex on XAD-4	Q-ICP-MS	-	40	Parent M. et al., 1996
Soil	Aqua regia + HF with MW digestion	HPLC	ICP-AES	-	200	Lustig S. et al., 1997
Road dust (tunnel) surface soil (road soil)	Aqua regia	Anion exchange chromatography	Q-ICP-MS	0.075 (ng g^{-1})	0.15	Müller M., Heumann K. G., 2000
Road dust	MW with aqua regia + HF	Modified silica capillary (with strong cation exchanger)	Q-ICP-MS	-	8.8	Garcia R. et al., 2001
Road dust	NiS fire assay	-	ICP-AES	-	200	Sures B. et al., 2001

Table 1.3 (Continued)

Matrix	Digestion / dissolution method	Preconcentration / separation	Detection	LOD (ng l^{-1}) Unless stated somewhere else		Reference
				Pd	Pt	
Road dust	H_3BO_3 + H_2O_2 and HNO_3 in MW oven (+HF)	DEBT complex on C_{18}	GFAAS	18	-	Boch K. et al., 2002
Soil	Aqua regia in MW	-	Q-ICP-MS	0.2 (ng g^{-1})	0.6	Cinti D. et al., 2002
Road dust	Aqua regia	Cation exchange chromatography	Q-ICP-MS	-	0.1	Higney E. et al., 2002
Road dust	Aqua regia	Ion exchange on Dowex 1-X10	ICP-AES	15 (ng g^{-1})	15	Kovacheva P. And Djingova R., 2002
Road dust	Aqua regia + HF in MW	Te Coprecipitation by $SnCl_2$	Q-ICP-MS	0.6 (pg m^{-3})	0.3	Gomez M. B. et al., 2003
Road dust	HNO_3 + HF + $HClO_4$	FI with DEBT as a complexing agent on a C_{18} microcolumn	GFAAS	23	-	Limbeck A. et al., 2003

1.5 High Performance Liquid Chromatography (HPLC)

HPLC is the most widely used of all of the analytical separation techniques (Skoog D. A. and James J. L., 1992). The reasons behind this popularity are its sensitivity, its ready adaptability to accurate quantitative determinations, its suitability for separating nonvolatile species or thermally fragile ones, and above all, its widespread applicability to substances that are of prime interest to industry, to many fields of science, and to the public.

In analytical HPLC, the focus is to obtain information about the sample compounds. The information that can be obtained includes identification, quantification and resolution of a compound.

With respect to separation principle, liquid chromatography can be divided into four basic types: partition chromatography; adsorption chromatography; ion exchange chromatography; and size exclusion chromatography.

Partition chromatography has become the most widely used of all of the four types of liquid chromatography procedures. Within this type, two different kinds are distinguishable based on the relative polarities of the mobile and stationary phases. Early work in liquid chromatography was based upon highly polar stationary phases such as water supported on silica or alumina particles; a relatively nonpolar solvent such as hexane then served as the mobile phase. This type of partition chromatography is now known as normal-phase chromatography (NP). In reversed-phase chromatography (RP), the stationary phase is nonpolar, often a

hydrocarbon, and the mobile phase is relatively polar (such as water, methanol, or acetonitrile). In normal-phase chromatography, the least polar component of the sample is eluted first because in a relative sense, it is the most soluble in the mobile phase; increasing the polarity of the mobile phase has the effect of decreasing the elution time. In contrast, in the reversed-phase method, the most polar component of the sample appears first, and increasing the mobile phase polarity increases the elution time.

Bonded-phase packings are classified as reversed-phase when the bonded coating is nonpolar in character and as normal-phase when the coating contains polar functional groups. Most commonly, the R group of the siloxane in these coatings of the reversed-phase is a C_8 chain or a C_{18} chain. The mechanism by which these surfaces retain solute molecules is at present not entirely clear.

As expected, longer chains produce packings that are more retentive. In addition, longer chain lengths permit the use of larger samples.

In most applications of RP chromatography, elution is carried out with a highly polar mobile phase such as aqueous solution containing various concentrations of such solvents as methanol (MeOH), acetonitrile (AcN), or tetrahydrofuran (THF).

1.5.1 Method Development in RP-HPLC

Method development tends to be more complex in liquid chromatography than in gas chromatography because in a liquid mobile phase, the sample components interact with both the mobile phase and the stationary phase.

Successful chromatography with interactive mobile phases requires a proper balance of intermolecular forces among the three active participants in the separation assay- the solute, the mobile phase, and the stationary phase. These forces are described quantitatively in terms of the relative polarity of the three participants.

Often in choosing a column for a partition chromatographic separation, the polarity of the stationary phase is matched roughly with that of the analytes; a mobile phase of a considerably different polarity is then used for elution.

Improving the resolution of a chromatographic column is based upon varying one of the three parameters (N, k', and α) (Table 1.4).

Table 1.4. Important Relationships in HPLC

Name	**Equation**
Number of theoretical plates	$N = 16\ (t_R/W)^2$
Retention factor	$k' = (t_R - t_M) / t_M$
Selectivity factor	$\alpha = [(t_R)_B - t_M] / [(t_R)_A - t_M]$
Resolution	$R_s = 2\ [(t_R)_B - (t_R)_A] / [W_A + W_B]$

Where,

t_R : retention time, time between injection of a sample and appearance of a solute peak at the detector.

t_M : dead time, time required for an unretained species to pass through a column.

$(t_R)_A$: retention time of species A.

$(t_R)_B$: retention time of species B.

W_A : peak width at its base (in units of time) for species A.

W_B : peak width at its base (in units of time) for species B.

The retention factor (k') is experimentally the most easily manipulated of the three because of the strong dependence of this constant upon the composition of the mobile phase. For optimal performance, k' should be in the ideal range of 1 to 10; for complex mixtures, however, this range must often be expanded to perhaps 20 in order to provide time for peaks of all of the components to appear.

Sometimes, adjustment of k' alone does not suffice to produce individual peaks with no overlap. If resolution is very poor (below 0.5), variation in selectivity factor (α) must be resorted to keeping k' within a reasonable range. This can be achieved by choosing a different stationary phase or by changing the mobile phase identity.

1.6 HPLC of Metal Chelates

In quantitative trace metal analysis, the analytical signal is affected by interferences because of matrix effects or contaminant elements in direct methods.

Both separation and enrichment can be possible in a single operation step simultaneously by liquid-liquid extraction for trace metal analysis. After complexation of metal ions in aqueous solution with organic reagents and separation from interfering inorganic matrix by extraction into nonpolar organic solvents, they can be monitored by spectrophotometric measurements (Figure 1.2).

Generally, the following advantages can be pronounced in the chromatography of metal chelates for trace metal analysis:

1. Relatively simple sample preparation (complex formation at appropriate pH values),
2. Elements to be determined can be enriched before determination by extraction of their complexes,
3. The interfering matrix remains in the aqueous phase during extraction,
4. Because of chromatographic separation, several elements can be identified and determined in a single step (multi-element analysis),
5. Sensitive detection of the metal chelates using UV-spectrophotometry is possible making use of the high extinction coefficients the chelates have,
6. Short analysis time, simple and rapid procedure.

Therefore, chromatography of metal chelates is a highly efficient analytical technique and it is a good alternative to other well known analytical methods of trace metal determination.

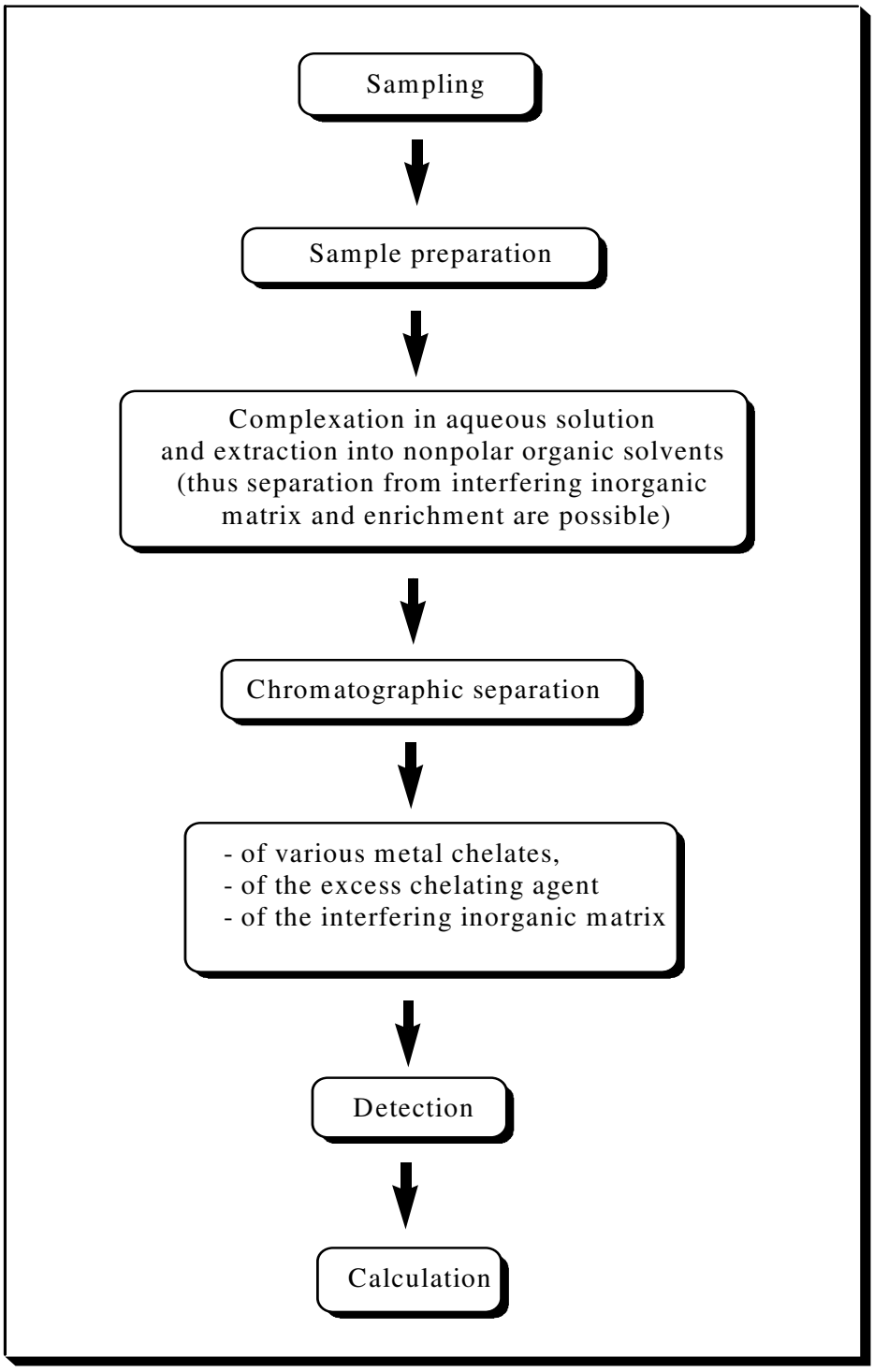


Figure 1.2. Trace Metal Analysis by the Chromatography of Metal Chelates

Unfortunately, not all elements can be used without problems in chromatography. Certain difficulties, like decomposition of the chelates before or during elution may appear, as well as incomplete separation of the various complexes or strong tailing in chromatograms may appear. These difficulties often reduce the good qualification of the chromatography of metal chelates for trace metal analysis. Therefore, only certain complexing agents, which form chelates that can be separated chromatographically and determined analytically, can be used successfully (Merdivan M., 1994).

The well-known complexing agents in analytical chemistry for photometric, gravimetric and volumetric determinations do not form metal chelates which can be chromatographed successfully. There are several possible reasons:

1. Weak solubility of the formed chelates in nonpolar solvents. Thus, it is not possible to enrich the metal ions from aqueous solutions by extraction,

2. Lack of stability of the chelates during contact with the stationary phase,

3. Decomposition of the chelates during elution,
4. Strong tailing and/or fronting due to slow equilibrium of adsorption and desorption of the chelates on the stationary phase,
5. In spite of successful chromatographic behavior for individual analytes, not enough differences in chromatographic retention for the mixture.

A close relationship exists between the chromatographic behavior and the complex chemical properties of the metal chelates because these are responsible for type and intensity of interaction with stationary and mobile phases. The silanol groups (-SiOH), of the stationary phase (Figure 1.3) form weak linkages with one of the following interactions (Merdivan M., 1994; Snyder L. R., Kirkland J. J., 1974):

1. Dipole-dipole,
2. Dipole-induced dipole,
3. π-complex-linkage to double bondings of the metal chelate,
4. Hydrogen bonding between the silanol group (as a proton donor) and the functional groups of the ligand with a free electron pair (as a proton acceptor),
5. Polar electron donor bonding of the silanol anion (-SiO^-) with the central atom of the complex

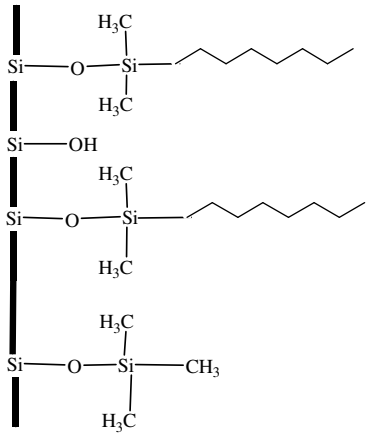


Figure 1.3. Silanol Groups of the Stationary Phase

The interaction between the chelates and the surface of the stationary phase depends on the properties of the chelates. These are dipole moment, basicity of the coordination sites, metal-specific properties of the central atom - like its affinity to oxygen or sulphur -, coordination number and steric structure of the chelate.

The power of interaction between the mobile phase and the stationary phase is very important for retention of complexes, because the mobile phase and the stationary phase compete for the analyte.

Numerous complexing agents with N-,S- ; S-,S- ; and S-,O- coordination sites are suitable for chromatography of metal chelates. However, whatever the complexing agent used is, metal chelates must have the following conditions and prerequisites (Merdivan M., 1994):

1. Formation of stable neutral metal chelates,
2. Control of complex formation by pH variation,
3. Formation of well defined chelates with clear stoichiometry,
4. Five-membered chelate rings between the metal and the ligand possess the highest stability, but four- and six-membered chelate-rings reduce stability,
5. Chelating agents with N-, O-, S- and Se- atoms as coordination sites have good properties and chemical properties for complex formation. In addition, these ligands are stable under normal circumstances for analytical application,
6. The metal chelates must have high stability in the mobile phase,
7. The ligand must not be too large,

8. The chelating conjugated π-system should cover the complete ligand for sensitive photometric detection (high extinction coefficients).

Some of the investigated chelating agents in HPLC are: 2-(2-thiazolylazo)-5-diethylaminophenol, dimethylglyoxal bis(4-phenyl-3-thiosemicarbazone), 8-hydroxyquinoline, N,N-dialkyl-N'-acylthiourea and N,N,diethyl-N'-benzoylthiourea (DEBT). They are summarized in Table 1.5. The studies using these chelating agents are given in more detail in Section 1.8.

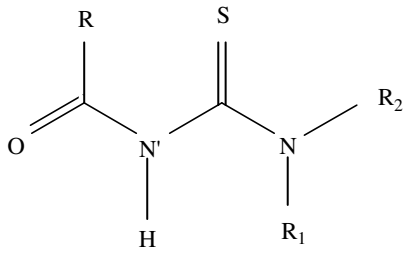
N-pyrolidino-; N,N-diethyl-; N,N-dimethyl- and N,N-di-n-propyl-N'-benzoylthioureas are the best-qualified ligands for chromatographical applications (Merdivan M., 1994). The great advantage of these chelating agents in comparison to other sulphur-containing ligands is their unusual stability against oxidation (Merdivan M., 1994; Koch K. R., 2001; Philippeit G. et al., 2001). Solutions of dialkyl–benzoylthioureas are totally stable under normal conditions and therefore can be handled without difficulties. Besides, the dialkyl-benzoylthioureas are excellent reagents for pH-selective precipitation and extraction, and therefore are qualified for liquid-liquid extraction of metal ions. Within these, N,N,diethyl-N'-benzoylthiourea (DEBT) is one of the widely used chelating agents. So it is possible to separate the platinum group metals from highly complex matrices like soil by liquid-liquid extraction forming their metal chelates and moreover to separate the individual platinum metals chromatographically.

Table 1.5. Investigated Chelating Agents in HPLC

Chelating Agent	**Elements Determined**	**Reference**
Dimethylglyoxal bis(4-phenyl-3-thiosemicarbazone)	Pt(II), Pd(II)	Hoshi S. et al., 1997
2-(2-thiazolylazo)-5-diethylaminophenol	Pt(II), Ir(IV), Ru(III), Rh(III), Os(IV)	Wang H. et al., 1999
8-hydroxyquinoline	Pt(II), Pd(II), Rh(III), Ir(IV)	Sanchez J. M. et al., 2000
N,N-dialkyl-N'-acylthiourea	Pt(II), Pd(II), Rh(III)	Koch K. R., 2001
N,N,diethyl-N'-benzoylthiourea (DEBT)	Pd(II)	Philippeit G. et al., 2001

1.7 DEBT and Its Metal Chelates

N,N-diethyl-N'-benzoylthiourea (DEBT) is one of a class of deceptively simple ligands based on the N,N-alkyl-N'-aroylthiourea motif below:

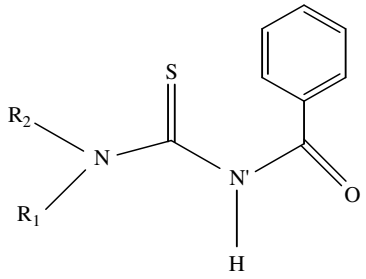


R = aryl or alkyl
R_1R_2 = alkyl; N,N-dialkyl-N'-aroylthiourea **HL**
R_1 = alkyl, R_2 = H; N-alkyl-N'-aroylthiourea **H_2L**

The co-ordination chemistry of the ligands H_2L and HL is much more varied than that of simple thioureas. Moreover, the physiochemical properties of their metal complexes are more favorable, resulting in a number of interesting potential, technical and analytical applications. The co-ordination chemistry and potential applications of such ligands have only been explored to some extent in the last three decades.

Amongst the attractive features of these ligands is their facile synthesis from readily available and inexpensive starting materials, giving in the case of the N'-benzoylthiourea derivatives, usually high yields of pure product in a two-step synthesis. In general, ligands of type HL, derived from benzoyl chloride are stable, relatively hydrophobic substances, with one dissociable proton on the weakly acidic amido –C(O)NHC(S)- moiety. N,N-dialkyl substituted HL ligand assumes a

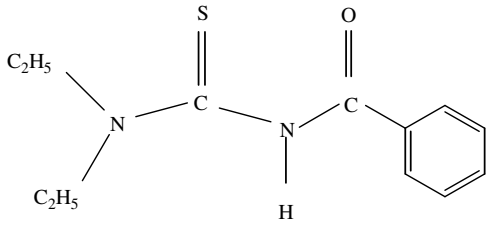
twisted conformation in the solid state, with the sulphur and oxygen atoms pointing approximately in opposite directions as shown schematically below:



The use of N,N-diethyl-N'-benzoylthiourea (DEBT) for the chromatographic separation of PGMs is based on a study by Melek Merdivan (Merdivan M., 1994). She pointed out that DEBT is a very good reagent for selective extraction for the PGMs due to its high extraction values with these metals. It was mentioned that the metal chelates of DEBT have very high complexation stability and that there was no problem of dissociation of these chelates in aqueous and organic solvents. Schuster and Schwarzer (Schuster M. and Schwarzer M., 1996) described that the ligand provides an extraordinary chemical resistance against oxidation and hydrolysis and a striking selectivity for PGMs.

In the literature, the selectivity of DEBT for PGMs was shown in numerous studies (Philippeit G et al., 2001; Schuster M. and Schwarzer M., 1996; Limbeck A. et al., 2003; Tilch J. et al., 2000). The essential part of all studies was that thioureas, in general, act as selective complexing agents for the enrichment of PGMs even from strongly interfering matrices.

Considering the facts mentioned above about the high stability of these metal chelates, the high extraction efficiency in organic solvents, and their high resistance and selectivity towards these metals rendered DEBT an optimal chelating agent for this analytical procedure.



N,N-diethyl-N'-benzoylthiourea
(DEBT)

1.8 Related Studies Using HPLC for PGMs

In 1997, Hoshi S. and coworkers (Hoshi S. et al., 1997) used reversed phase HPLC to separate and determine Pt and Pd in a commercially available dental alloy and a platinum-palladium wire. Pt (II) and Pd (II) were preconcentrated from aqueous solutions by complexing them with the ligand dimethylglyoxal bis(4-phenyl-3-thiosemicarbazone), abbreviated as DMBS. This ligand was coated on an amberlite XAD-7 resin and DMBS-XAD-7 was added to a solution containing Pd and Pt. This solution was then filtered through a polyethylene column and then Pt and Pd were eluted from the resin as their DMBS chelates with 1 ml N,N-dimethylformamide (DMF). DMF was used as the solvent because dithiosemicarbazones have the disadvantage of low solubility in common solvents. A 10 μL volume of the eluted solution was injected into the

reversed phase HPLC system. The column used was an ERC-ODS 1282 (250 x 6 mm I.D.). The peaks of the metal dithiosemicarbazone chelates were not separated by methanol-water or acetonitrile-water mobile phases. Resolution was achieved by using a 65:35 (v/v) acetone: water mobile phase. A UV-VIS detector was used; the most suitable detection wavelength for DMBS chelates was 425 nm. Using a flow rate of 1.0 ml min^{-1}, these authors could get a full separation of Pt and Pd chelates in 24 min under these chromatographic conditions.

In 1999, Wang H. and coworkers (Wang H. et al., 1999) could separate Pt and Rh by RP-HPLC after complexation with 2-(2-thiazolylazo)-5-diethylaminophenol (TADAP). Acetate buffer (pH = 5.0) and TADAP solution were transferred into an aqueous solution containing Pt and Rh. The mixture was heated at 80 °C for 1 h, after which methanol was added immediately. After having cooled to room temperature, this solution was diluted with water. A 20 µl aliquot of this solution was injected into the column (an ODS column, 5 µm, 150 mm x 4.6 mm I.D.) for analysis. Pt and Rh complexes were eluted at a flow rate of 1.0 ml min^{-1} and were detected at 574 nm with a UV-VIS detector. Acetonitrile-water was tested as a mobile phase. However, better resolution could be achieved using a 69.5:30.5 (v/v) methanol: 0.01 mol l^{-1} acetate buffer (pH = 5.0). Pt and Rh complexes could be separated in approximately 17 min with detection limits of 0.39 ng ml^{-1} for Pt and 0.29 ng ml^{-1} for Rh.

In 2000, Mautjana A. N. and Koch K. R. (Koch K. R., 2001) developed another reversed phase HPLC-, chelate formation-based method for separating and quantifying Pt, Pd and Rh. A hydrophilic N,N-dialkyl-

N'-acylthiourea ligand, which was soluble in acetonitrile, was mixed with a sample containing traces of Pt (II), Pd (II) and Rh (III) in dilute HCl. This homogeneous solution was then heated at 70-80 °C for 10 min, followed by a salt-induced (NaCl) phase separation into an acetonitrile-rich phase containing the PGMs complexes and a water-rich phase, which was discarded. A 20 µL of the acetonitrile-rich phase was then directly injected into a LUNA C_{18} HPLC column (5 µm, 150 mm x 4.6 mm). A mobile phase, having a composition of 90:10 (v/v) acetonitrile: 0.1 mol l^{-1} sodium acetate buffer (pH = 6.0), was applied at a flow rate of 1.0 ml min^{-1}. These authors could separate the three metal chelates in 15 min with detection limits of 2.0 mg l^{-1}, 0.1 mg l^{-1} and 3.0 mg l^{-1} for Pt, Pd and Rh, respectively, at optimized photometric detection.

In 2000, Sanchez J. M. and coworkers (Sanchez J. M. et al., 2000) used normal phase HPLC to separate and quantify Pt, Pd and Rh. To achieve this goal, they tried different HPLC methodologies i.e., Reversed phase (RP), Non-aqueous reversed phase (NARP) and Normal phase (NP) HPLC after the formation of PGMs 8-hydroxyquinolate chelates. The RP method only permitted the quantitative separation of Rh and Pd. The NARP method allowed the effective separation of the three elements tested, but a high detection limit for Pt and peak width did not favor its application. NP-HPLC with a cyano column (Nucleosil 100 CN, 150 mm x 4.6 mm, 5 µm), used for the first time for this purpose, could effectively separate these PGMs in less than 15 min. A gradient elution of hexane-chloroform gave the best results. Under the chromatographic conditions of 20 µl injection, a flow rate of 1.0 ml min^{-1}, detection limits of 1.0 ng Pt, 0.3 ng Pd and 0.3 ng Rh were obtained with a UV-VIS detector.

In 2001, Philippeit G. and coworkers (Philippeit G. et al., 2001) used HPLC with UV detection to determine traces of palladium in human urine. The organic matrix of urine was completely destroyed using UV photolysis and then Pd was enriched by solid-phase extraction (SPE). A reversed phase C_{18} material was filled into the SPE cartridges and then a solution of the ligand N,N-diethyl-N'-benzoylthiourea (DEBT) was passed through the column. Sample solutions containing Pd were then loaded onto the column and $Pd(DEBT)_2$ complex was eluted with ethanol. To separate the complex from the free ligand, 200 µl of the ethanolic elute were injected into the HPLC system. A reversed phase column (Lichrosper 100 RP 18-e, 5µm, 250 x 4 mm I.D.) was used. The UV detection was performed at 274 nm. The flow rate of the mobile phase (98:2 methanol: water (v:v) was set to 1.0 ml min^{-1}. Under these conditions, DEBT eluted after 2.63 min and $Pd(DEBT)_2$ followed at 7.24 min. As these authors could not find an adequate certified standard reference material for Pd in urine, they spiked urine samples for calibration standards. Taking into consideration a threefold signal-to-noise ratio, they could reach a detection limit of 10 ng Pd l^{-1} using this method.

1.9 Objective of This Work

The most important aim of this study was to develop a Reversed Phase method combined with the usage of a highly selective reagent, DEBT, for enrichment and removal of some PGMs from a strongly interfering matrix, soil. To achieve this goal we aimed at:

1. Synthesis of pure chelating agent (DEBT) and verification of its formation using UV-absorption and FT-IR analysis,

2. Synthesis of pure metal chelates with DEBT for Pt and Pd, and verification of their formation using UV-absorption and FT-IR analysis,

3. Examining the different separation parameters that affect PGM separation,

4. Designing a systematic approach toward the separation of Pt-DEBT and Pd-DEBT chelates,

5. Usage of salt-induced phase separation for extraction and preconcentration of Pt and Pd as DEBT chelates from soil, after verification of its high extraction efficiency using ICP-OES,

6. Separation of interferences that are present in soil and affect the analysis of PGMs using the systematic approach designed.

CHAPTER 2

EXPERIMENTAL

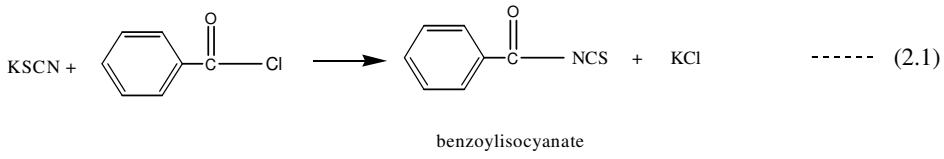
2.1 Synthesis of DEBT and Metal Chelates

2.1.1 Synthesis of DEBT

The synthesis of N,N-diethyl-N'-benzoylthiourea (DEBT) as a chelating agent for the separation and determination of platinum group metals was performed by following the procedure given in the literature (M. Merdivan, 1994).

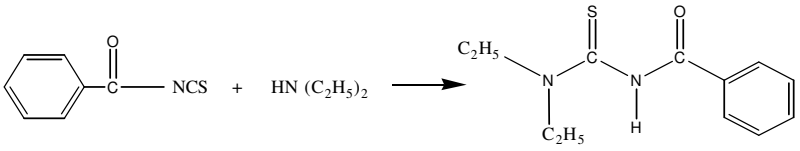
0.10 mol of potassium thiocyanate (Fisher) was dissolved in 100.0 ml anhydrous acetone (Riedel-deHaën) and heated in a reflux condenser. 0.10 mol (approximately 11.60 ml) of benzoyl chloride (Merck) was added drop by drop to the mixture after heating was stopped. The reaction mixture was stirred about 30 min at room temperature. Then, the precipitate of potassium chloride was removed by filtration and the orange colored filtrate was reacted with 0.10 mol (approximately 10.50 ml) of diethylamine (Merck). The reaction mixture was crystallized in 1.0 mol l^{-1} HCl solution that had been previously cooled with ice. Then, the supernatant solution was removed by filtration and the residue was recrystallized with ethanol (absolute, Baker). Synthesis of DEBT in two

steps is shown with chemical equations below [equation (2.1) and equation (2.2)].



(2.1)

benzoylisocyanate



(2.2)

N,N-diethyl-N'-benzoylthiourea
(DEBT)

2.1.2 Synthesis of Metal Chelates

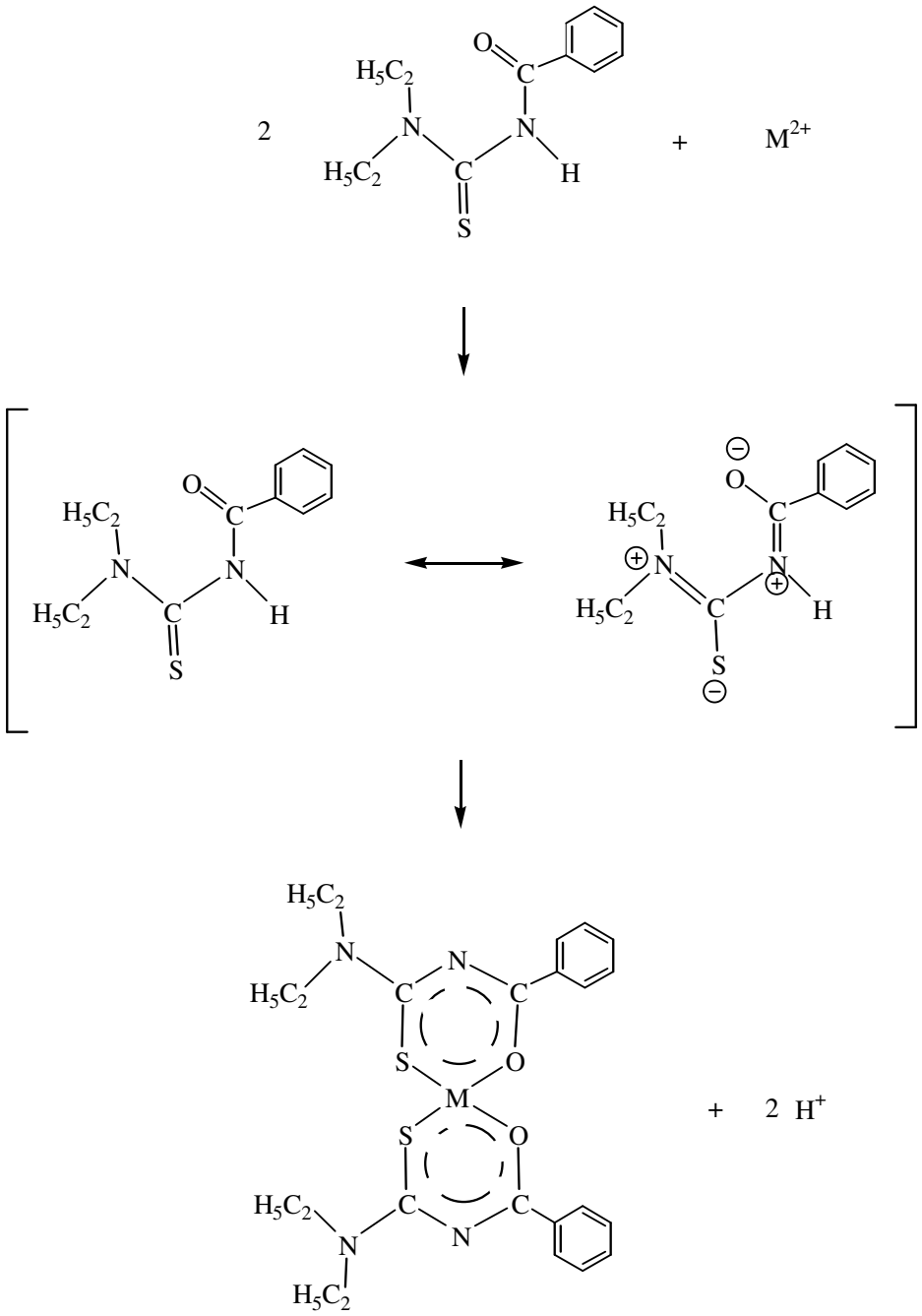
Metal ion solutions of Pt (II) and Pd (II) were prepared from Platinum (II) chloride (73 % Pt, Acros) and Palladium (II) chloride (59 % Pd, Acros) in 1.0 mol l^{-1} HCl. A 0.10 mol l^{-1} DEBT solution, which was insoluble in water, was prepared in ethyl alcohol and was added to the 0.010 mol l^{-1} aqueous metal solution in stoichiometric ratio (ligand slightly in excess) by stirring vigorously at the suitable pH values as shown in Table 2.1. Formation of the metal chelates can be expressed in general with equation (2.3). Also, open formulas of the metal chelates are given below (Figure 2.1).

Table 2.1. Experimental Parameters for the Preparation of Chelates

Metal	pH	Color	Remarks
Pt (II)	1-7	Yellow	Complexation at 40 °C
Pd (II)	1-7	Orange	Room temperature

$$M^{n+} + nHL \longrightarrow ML_n + nH^+ \quad \text{------} \quad (2.3)$$

The ratio of metal ion solution to ligand solution, in volume, was 4:1; this results in a solution where metal ion concentration is 0.008 mol l^{-1} and ligand concentration is 0.020 mol l^{-1}, this molar ratio is in excess for the ligand. Heating to 40 ºC was required for Pt complex, whereas, Pd complex formed at room temperature (see Table 2.1). The precipitated metal complexes were filtered. To obtain very pure metal complexes, they can be recrystallized twice by ethanol. Crystals of the complexes were kept for drying in a vacuum desiccator that contained P_2O_5.



M = Pt or Pd

Figure 2.1. Formation of a Neutral Complex between DEBT and Pt (II), Pd (II) (Philippeit G. et al., 2001)

2.2 Characterization of DEBT and Metal Chelates

To verify the formation of the chelating agent and the metal complexes, UV-VIS and IR analysis were carried out.

2.2.1 UV-Absorption Analysis

A sample of DEBT was examined using a double beam UV-visible absorption spectrophotometer (SHIMADZU UV-160, uv-vis) in the range 200-400 nm with 10 mm quartz cells. DEBT was dissolved in ethanol which was used as blank in the reference cell.

Samples of Pd and Pt complexes were examined using the same UV-VIS spectrophotometer in the same range. Each sample of the complexes was dissolved in chloroform and the spectrum was obtained against chloroform.

2.2.2 Infrared Analysis (IR)

A FT-IR instrument (Unicam, Mattson 1000) was used in the region between 4000-400 cm^{-1} for characterization of DEBT and the metal complexes. 1.0 mg of finely ground sample was intimately mixed with 150.0 mg of dried potassium bromide powder by using an agate mortar. The mixture was then pressed and the pellets were investigated by FT-IR.

2.3 Optimization of HPLC for Separation

2.3.1 Instrumentation

A CECIL 1100 HPLC (England) with a SHODEX RSpak DS-613 column (5 µm, 150 x 6 mm i.d., Phenomenex), ACE 3 C_{18} column (3 µm, 150 x 4.6 mm i.d, ACT) and a CE 1220 UV-VIS detector (England) was used for HPLC analysis (Figure 2.2).

For convenient results, this instrument had to be computerized, which required a specially designed software. This software (Forlab Version 1.0) was updated many times to meet our needs. The final version had the following properties:

1. It converted the detector's analog signal into a digital signal that could be easily processed,

2. It obtained an average of the continuous data with a specified range,

3. It continuously plotted 'Absorbance vs. Real Time' chromatogram of the assay,

4. It enabled the transfer of these chromatograms to other programs such as MS PowerPoint or MS Word, and the extraction of their data to mathematical programs such as MS Excel.

Figure 2.2. The View of CECIL 1100 HPLC and CE 1220 UV-VIS Detector.

2.3.2 Procedure for Optimization

Stock solutions of (100.0 mg l^{-1}) of Pt (II) and Pd (II) were prepared in 1.0 mol l^{-1} hydrochloric acid from platinum (II) chloride (73% Pt, Acros) and palladium (II) chloride (59% Pd, Acros), respectively. A 2.50 ml of each stock solution was transferred into a 10.0 ml flask and the solution was diluted to the mark with 1.0 mol l^{-1} hydrochloric acid. This solution contained 25.0 mg l^{-1} of each metal. A stoichiometric amount of DEBT crystals was dissolved in 4.0 ml acetonitrile and was added to the metal solution, the ligand being slightly in excess. The mixture was transferred into a distillation flask and was heated to 40 °C and stirred vigorously for 30 min in a water bath. The homogeneous solution obtained

was cooled to room temperature, followed by a salt-induced (2.20 mol 1^{-1} NaCl) phase separation of the sample phase into an acetonitrile-rich phase containing the PGM complexes and the extra DEBT, and an aqueous phase which was discarded. A 20 µl of the acetonitrile phase was then directly injected into the RP-HPLC system after being filtered under vacuum through a 0.45 µm filter (Millipore, Bedford, USA). An initial set of experimental conditions was selected (Table 2.2) and a chromatogram was obtained under these conditions.

Table 2.2. Initial Experimental Conditions for Separation

Data Collection	150 ms
λ	280 nm
Flow Rate	1.5 ml min^{-1}
pH	6.0
Acetate Buffer Concentration	0.10 mol 1^{-1}
Mobile Phase	90:10 (v:v) acetonitrile: buffer

2.3.3 Optimization of Flow Rate

The initial experimental conditions shown in Table 2.2 were kept constant while the mobile phase flow rate was decreased gradually from 1.5 ml min^{-1} to 0.8 ml min^{-1}. In each flow rate examined, a sample of the final metal solution obtained in section 2.3.2 was injected into the HPLC system and a chromatogram was obtained.

2.3.4 Optimization of Mobile Phase

2.3.4.1 Mobile Phase: Acetonitrile-Water

Different compositions of the mobile phase acetonitrile-acetate buffer were prepared and filtered to remove impurities and to degas them from dissolved air. Mobile phases were decreased in the organic content starting at a composition of 90:10 (v:v) acetonitrile : buffer and ending at a composition of 75:25 (v:v) acetonitrile: buffer. Samples of the metal solutions were injected into the system under the following conditions (Table 2.3).

Table 2.3. Experimental Conditions for Optimization of Mobile Phase

Data Collection	150 ms
λ	280 nm
Flow Rate	0.8 ml min^{-1}
pH	6.0
Acetate Buffer Concentration	0.10 mol 1^{-1}

2.3.4.2 Mobile phase: Methanol-Water

Different compositions of the mobile phase methanol-acetate buffer were prepared and filtered. Compositions were studied over the range 90:10 (v:v) methanol : buffer to 65:35 (v:v) methanol : buffer. Samples of the metal solutions were injected into the HPLC system under the conditions stated in Table 2.3.

2.3.4.3 Mobile Phase: Methanol-Acetonitrile-Water

A mobile phase of the composition 10:80:10 (v:v) methanol : acetonitrile : acetate buffer was prepared and filtered. Samples of the metal solutions were injected into the HPLC system under the following experimental conditions (Table 2.4).

Table 2.4. Experimental Conditions for Separation with Methanol-Acetonitrile-Water

Data Collection	150ms
λ	280 nm
Flow Rate	0.8 ml min^{-1}
pH	5.0
Acetate Buffer Concentration	0.10 mol 1^{-1}

2.3.5 Optimization of pH

pH was studied over the range of pH 4.0 - pH 6.0. It was adjusted to the required value using a 0.10 mol 1^{-1} acetate buffer. An appropriate amount of sodium acetate was dissolved in de-ionized water (Millipore, Elix, Elga System); the solution was transferred into a 250.0 ml volumetric flask and was diluted to near the mark (approximately 245.0 ml). This solution was transferred into a beaker and was magnetically stirred. The pH value required was achieved by dropwise adding of 1.0 mol 1^{-1} acetic acid. The solution was then transferred into the 250.0 ml flask again and diluted to the mark with de-ionized water. This resulted in 0.10 mol 1^{-1} acetate buffered solutions of pH between 4.0 and 6.0. An ORION, 420 A model pH meter was used for all pH adjustments.

A mobile phase composition of 90:10 (v:v) acetonitrile : acetate buffer was used to study pH effect on separation. Mobile phases of this composition having different pH values were prepared, filtered and applied in the HPLC assays. A sample of the metal solutions was injected in each case under the following conditions (Table 2.5).

Table 2.5. Experimental Conditions for Optimization of pH

Data Collection	150 ms
λ	280 nm
Flow Rate	0.8 ml min^{-1}
Mobile Phase	90:10 (v:v) acetonitrile : buffer
Acetate Buffer Concentration	0.10 mol 1^{-1}

2.3.6 Optimization of Buffer Concentration

The effect of acetate buffer concentration on separation was investigated throughout the range 0.10 - 0.50 mol 1^{-1}. Buffer solutions in varying amounts of sodium acetate were prepared by following the procedure described in Section 2.3.5. A sample of the metal solutions was injected in each case under the following conditions (Table 2.6).

Table 2.6. Experimental Conditions for Optimization of Buffer Concentration.

Data Collection	150 ms
λ	280 nm
Flow Rate	0.8 ml min^{-1}
pH	5.0
Mobile Phase	75:25 (v:v) acetonitrile : buffer

2.4 Determination of Pt and Pd by ICP-OES

Stock solutions of (60.0 mg 1^{-1}) of Pt (II) and (10.0 mg 1^{-1}) Pd (II) were prepared separately in 1.0 mol 1^{-1} hydrochloric acid from platinum (II) and palladium (II) AAS standards, respectively. A stoichiometric amount of DEBT crystals was dissolved in 4.0 ml acetonitrile and was added to 10.0 mL of the Pt stock solution, the ligand being slightly in excess. The mixture was transferred into a distillation flask and was heated to 40 °C while stirring vigorously for 30 min in a water bath. The homogeneous solution obtained was cooled to room temperature, followed by a salt-induced (2.00 mol 1^{-1} NaCl) phase separation of the sample phase into an acetonitrile-rich phase containing the PGM complexes and the extra DEBT, and an aqueous phase which was analyzed for Pt remaining in the aqueous solution by ICP-OES. This procedure was also used for Pd but Pd complex formed instantaneously at room temperature.

The same procedure was repeated to check the effect of NaCl concentration on the extraction efficiency of metal chelates from the aqueous phase by increasing salt concentration from 2.00 mol 1^{-1} to 2.20 mol 1^{-1} NaCl.

For determinations of Pt and Pd concentrations that remained in the aqueous phase, a Leeman DRE ICP-OES instrument was used. The instrument employs a photomultiplier tube (PMT) as a detector. An axial plasma torch was used. Burgener 2002 Meinhardt type nebulizer was used in sample introduction system of ICP-OES. The operating parameters of the instrument are given in Table 2.7.

Table 2.7. ICP-OES Instrumental Parameters Used for the Determination of Pt and Pd.

Power	1.4 kw
Coolant Flow	16 LPM
Auxiliary Flow Rate	0.5 LPM 20 mA
Nebulizer Pressure	35 PSI
Pump Rate	1.0 ml min^{-1}

2.5 Analysis of Soil

2.5.1 Digestion of Soil

The soil material is dried at 40°C and ashed at 450°C for 3h. Then Microwave-assisted digestion of this soil was performed according to a procedure by Boch k. and coworkers (Boch K. et al., 2002) with some modifications. A microwave sample preparation system (MILESTONE MICROWAVE Laboratory Systems, ETHOS PLUS Microwave Labstation equipped with LabTerminal 800 Controller) was used in this study. This system is pressure and temperature controlled, so that reaching maximum pressure or temperature the microwave energy input is restricted automatically.

The vessels of the microwave system were charged with 500 mg of ashed soil, different volumes of 1000 mg l^{-1} Pt AAS standard solution, different volumes of 1000 mg l^{-1} Pd AAS standard solution, 5 ml of HNO_3 (65 wt. %) and 2 ml of H_2O_2 (31 wt. %). After 10 min, when the first

vigorous reaction has taken place, the pressure vessels were closed and the first microwave step was performed (Table 2.8). When opening the vessels after they have cooled down, nitrous gases escaped and a precipitation of silicate was observed.

For the second step, 3 ml of hydrofluoric acid (40 wt. %) was added. The vessels were closed and heated according to step 2. After they have cooled down, they were opened again and 2 g of H_3BO_3 and 30 ml of H_2O were added for the third reaction step. In this step, no further gaseous reagent products were formed – only masking of fluoride took place. The microwave energy programs used for the three-step digestion are summarized in Table 2.8.

The digested solutions were then transferred into 100-ml glass flasks, filled up to volume with 1.0 mol l^{-1} HCl, and complexation procedure with DEBT was followed. Metal complexes were determined by the RP-HPLC method proposed.

Table 2.8. Energy Programs and Reagents for the Microwave-Assisted Digestion Procedure

Procedure number	**Energy (W)**	**Time (min)**	**Temperature (ºC)**
First step: 5 ml HNO_3, 2 ml H_2O_2			
1	500	10	150
2	500	5	180
3	500	15	180
Cooling	0	10	Room Temperature (RT)
Second step: 3 ml HF			
1	500	10	150
2	500	5	180
3	500	15	180
Cooling	0	10	RT
Third step: 2 g H_3BO_3, 30 ml H_2O			
1	500	5	150
2	500	5	180
3	500	5	180
Cooling	0	10	RT

CHAPTER 3

RESULTS AND DISCUSSIONS

3.1 Characterization of DEBT and Metal Chelates

3.1.1 UV-Absorption Analysis

UV –absorption bands of DEBT are similar to those given in the literature (Figure 3.1)

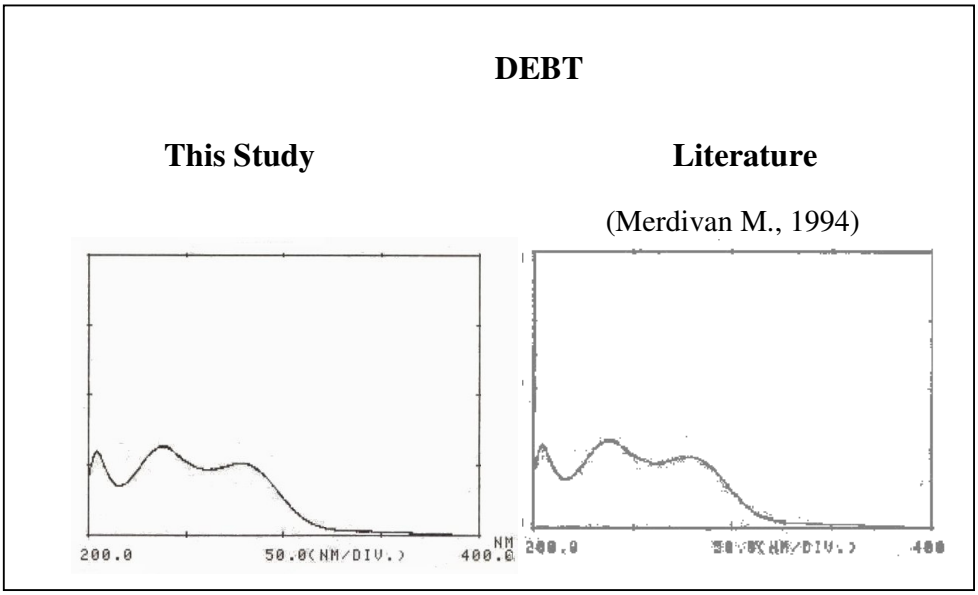


Figure 3.1. UV-Absorption Spectra of DEBT

DEBT has a very weak absorption band above 300 nm. However, it has two strong but broad absorption bands at 240 nm and 280 nm.

Absorption spectra of the metal complexes are given in Figure 3.2. No absorption band was observed in the visible region for any of the compounds. It can also be observed that the spectra of the complexes are similar regarding the absorption wavelengths although absorptivities are different. The absorption maxima of the chelating agent remain either unchanged or suffer slight shifts in the complexes. This is because the spectra of the complexes are attributed mainly to the ligand.

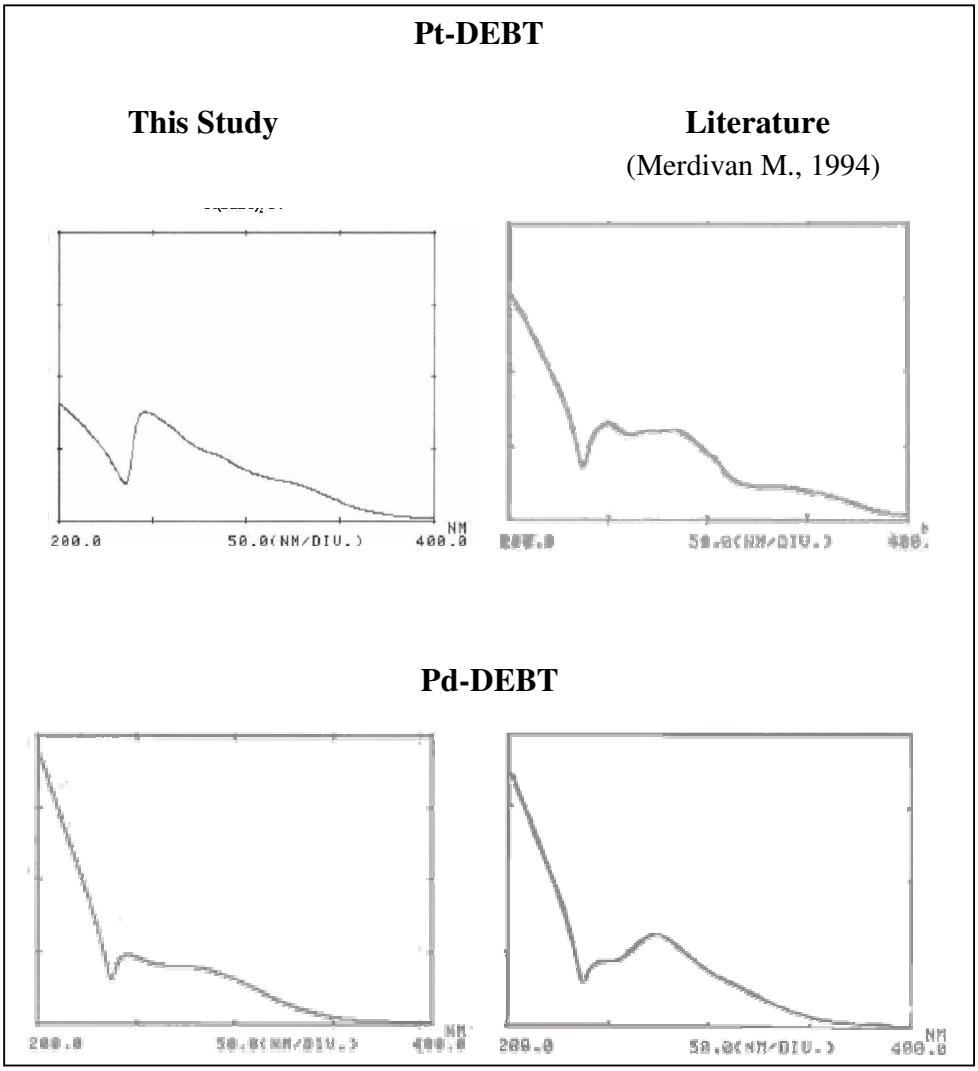


Figure 3.2. UV-Absorption Spectra of Metal Complexes

3.1.2 Infrared Analysis (IR)

FTIR spectrum of DEBT given in the literature and that of the synthesized reagent in this study resemble each other as shown in Figure 3.3. The characteristic absorption bands for N-H, C-H and amide I (C=O), amide II and amide III at 3276, 3066-2936, 1656, 1538 and 1309 cm^{-1}

respectively appearing in both of the spectra support the formation of DEBT.

The IR spectral bands corresponding to C=N, C-H, N-H and C=O bands of the chelating agent and complexes are given in Table 3.1 and the spectra of the chelates are given in Figure 3.4 and Figure 3.5.

Table 3.1. IR Spectral Bands of DEBT and Metal Chelates

	DEBT	**$Pt(DEBT)_2$**	**$Pd(DEBT)_2$**
ν N-H	3276	-	-
ν C=O	1656	-	-
ν CH_3	2877, 2794	2872, 2972	2869, 2979
ν CH_2	2936	2930	2929
ν C-H	3020, 3066	3066, 3090	3065, 3086
ν C=N	-	1588	1587

The complexes have similar IR spectra, which indicate that they have similar structures and there is no significant dependence on the central metal ion. The characteristic IR bands of $-N(CH_2CH_3)_2$ group, appearing at 2877 and 2974 cm^{-1} (ν CH_3) and 2936 cm^{-1} (ν CH_2) in the spectrum of the ligand, remain almost unchanged in the spectra of the complexes. In the complexes of DEBT, $-N(CH_2CH_3)_2$ group and aromatic ring displayed slight shifts on complexation. The small change in the $-N(CH_2CH_3)_2$ band indicates that this group does not take part in coordination. In the aromatic ring upon the formation of the metal-ligand bond the C-H vibration is shifted to higher frequencies.

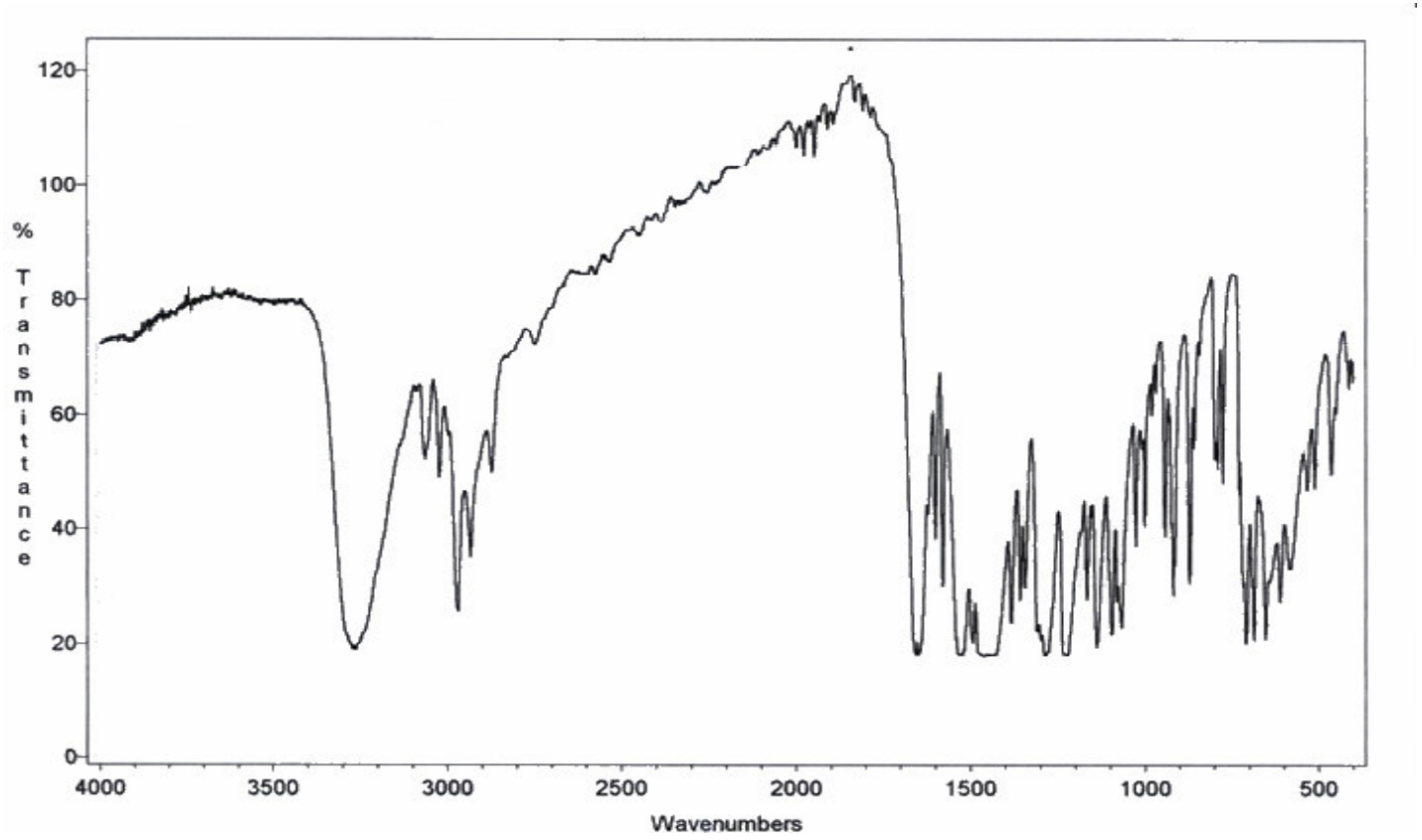


Figure 3.3a. Infrared Spectra of DEBT. (This Study)

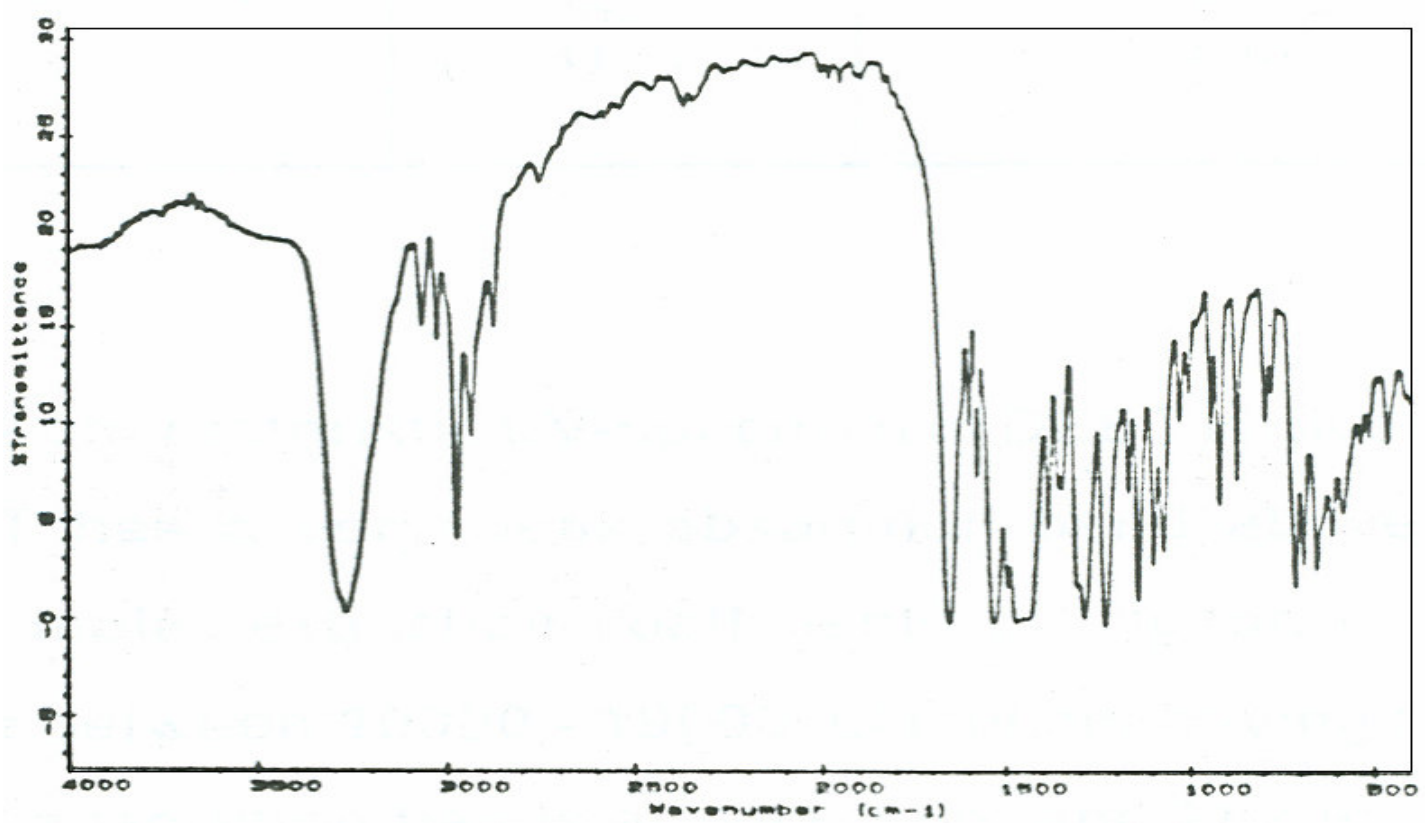


Figure 3.3b. Infrared Spectra of DEBT. (Literature)

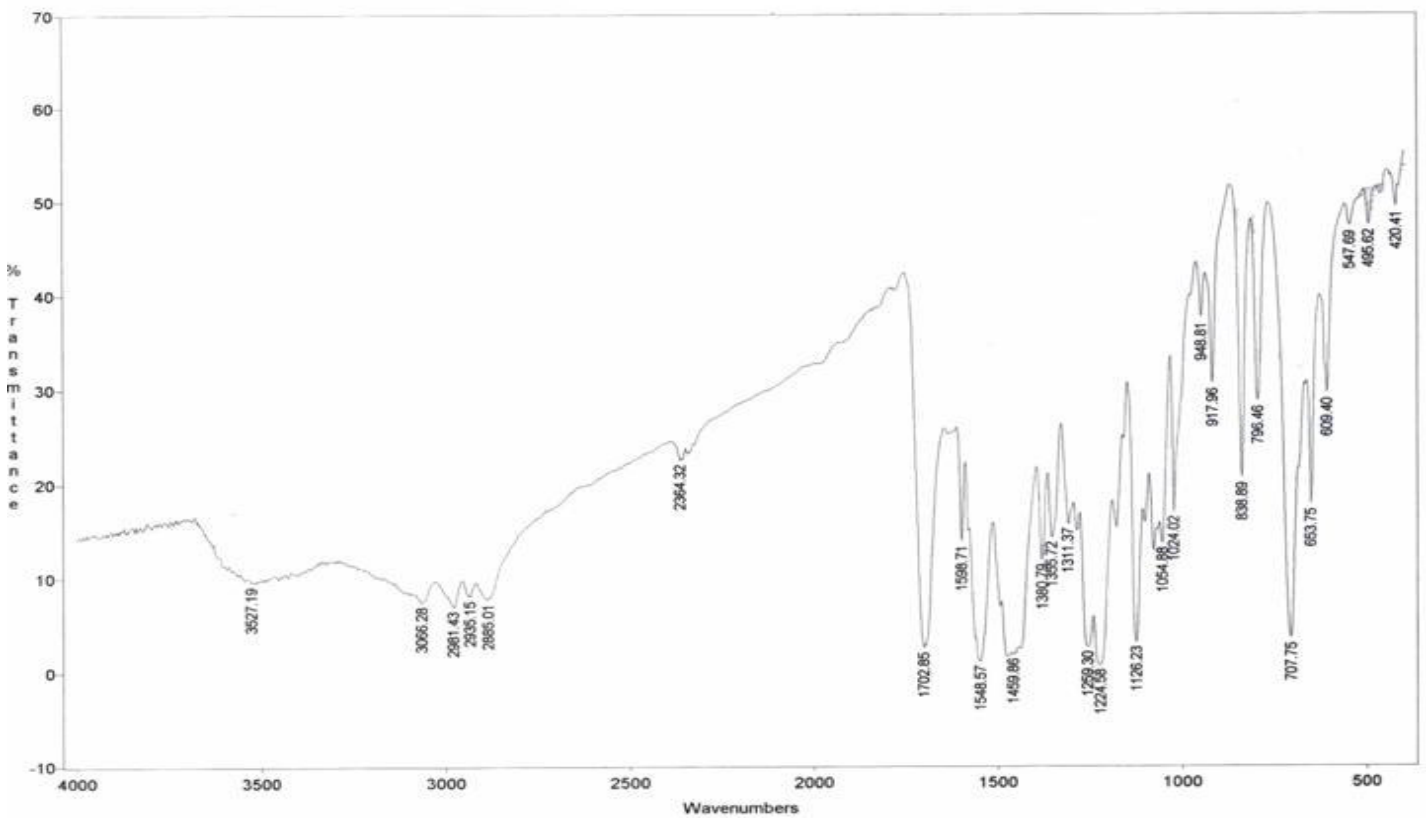


Figure 3.4a. Infrared Spectra of $Pt(DEBT)_2$. (This Study)

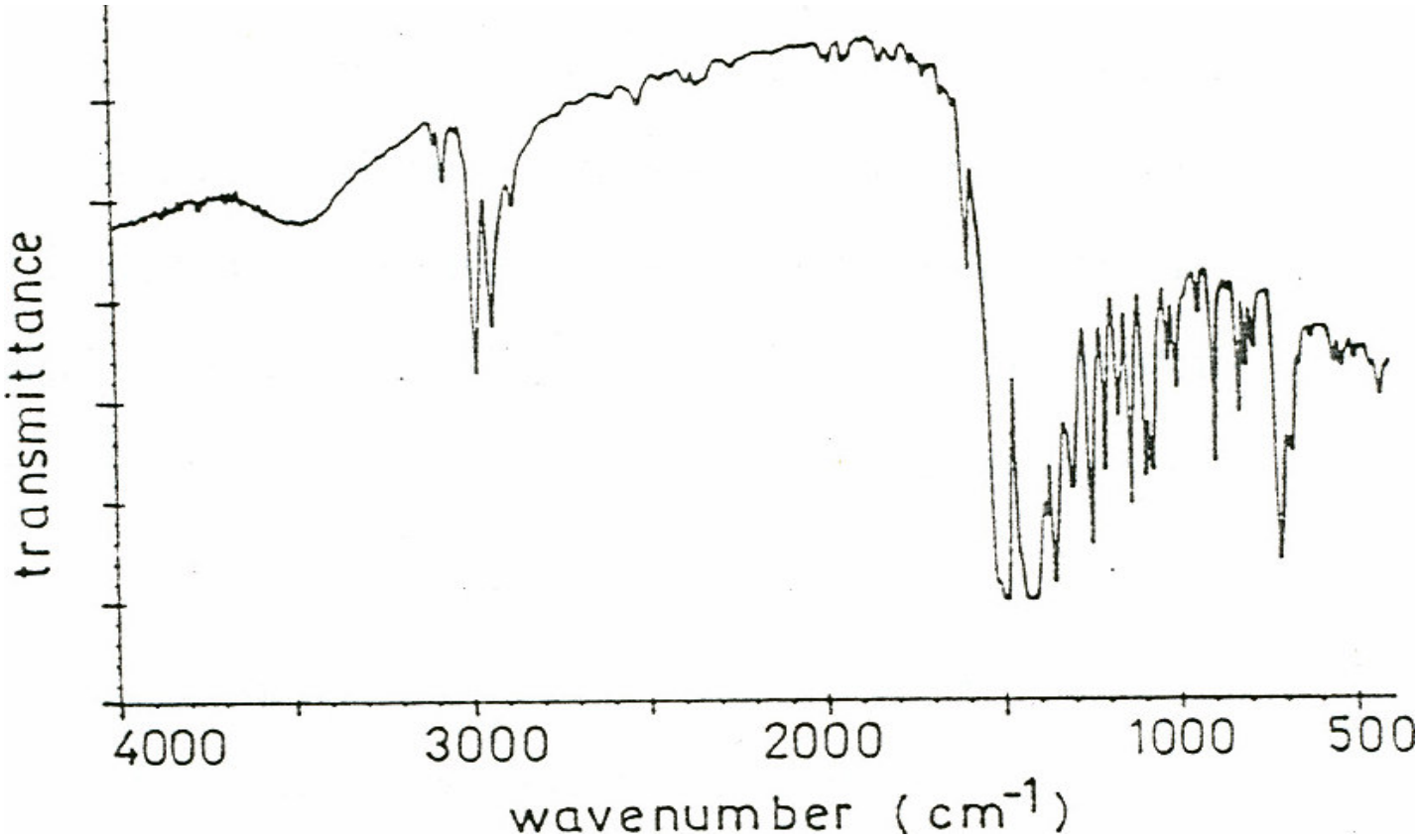


Figure 3.4b. Infrared Spectra of $Pt(DEBT)_2$. (Literature)

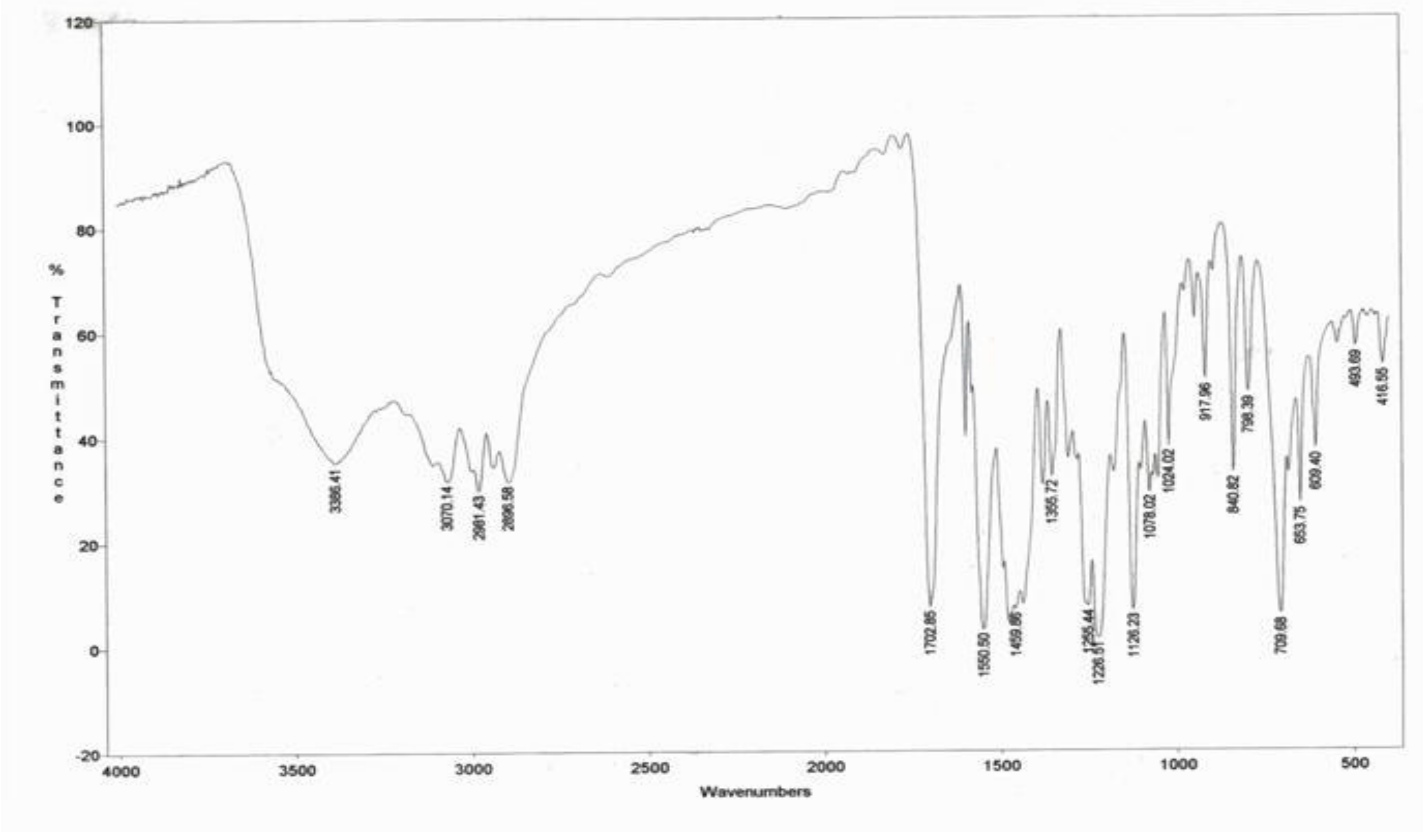


Figure 3.5a. Infrared Spectra of $Pd(DEBT)_2$. (This Study)

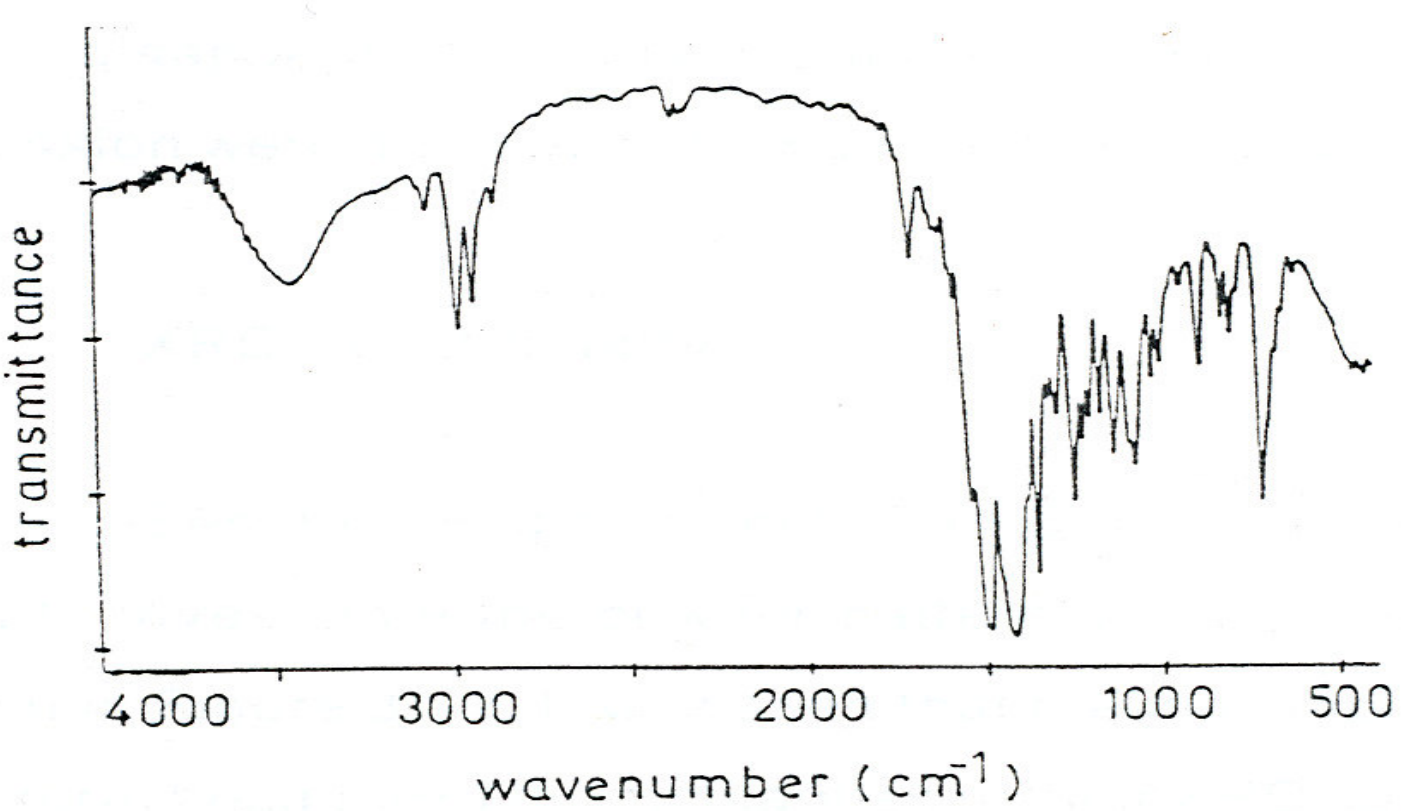


Figure 3.5b. Infrared Spectra of $Pd(DEBT)_2$. (Literature)

The positions of the amide I, amide II and III bands of DEBT (1656, 1533 and 1309 cm^{-1}), arising from the carbonyl of the benzamide moiety, disappeared in the complexes. The vibration band of the secondary amide at 3276 cm^{-1} also disappeared in the complexes. Similar discussion is also valid for IR spectrum of Pd complex.

3.2 Optimization of HPLC for Separation

Having synthesized the chelating agent DEBT and its metal chelates with Pd and Pt, and after verification of their formation using UV-VIS and IR spectrometry, they were ready for application with HPLC for later quantification of the two metals in environmentally polluted soils. However, the HPLC system had to be optimized for separation first, which was not an easy work due to two reasons.

First, stoichiometric formation of the metal complexes required that the chelating agent be in an excess amount. Extra DEBT then gave a high peak in the chromatogram due to its high extinction coefficient. This peak interfered with metal complexes' peaks in some cases and therefore it had to be separated from the other peaks for accurate results. Spectral elimination of DEBT's peak was not possible because DEBT highly absorbed at the most suitable wavelengths of the metal complexes.

Second, Pt and Pd complexes are indeed chemically very similar and hence they are closely migrating species. Both metals form square planar-structured complexes with DEBT as shown in Figure 2.1, with the only difference being the central metal in the core of the complex.

Therefore, a successful use of HPLC for solving this problem depended on the choice of the right combination of operating conditions: the type of column packing, column dimensions, particle size, flow rate of the mobile phase, the mobile phase composition and identity, pH of the mobile phase, concentration of the buffer used for adjusting the pH, and temperature of the column. This choice in turn required a basic understanding of the various factors that control HPLC separations.

A strategy or an approach to the design of this HPLC assay can be broken down into the following six steps:

1. Selecting an HPLC methodology,
2. Selecting an HPLC column,
3. Selecting initial experimental conditions,
4. Carrying out an initial separation,
5. Evaluating the initial chromatogram and determining what change in resolution is required,
6. Establishing conditions required for the necessary final resolution.

Equation (3.1) is a fundamental relationship in Liquid Chromatography, which allows a chromatographer to control resolution (R_s) by varying k'_{av}, N and α, where k'_{av} is the average of retention factors of a critical pair, N is the number of theoretical plates (efficiency) and α is the selectivity factor. (See table 1.4). The three terms (i) - (iii) of the equation are essentially independent, so that one term can be optimized first then another. k'_{av} [term (i)] is varied by changing solvent strength, the ability of the mobile phase to provide large or small k'_{av} values. Separation

$$R_s = \frac{1}{4} \left[k'_{av}/(1+k'_{av})\right] \quad N^{1/2} \quad (\alpha - 1)/\alpha \qquad \text{------- (3.1)}$$

(i) (ii) (iii)

efficiency as measured by N [term (ii)] is varied by changing column length or mobile phase flow rate. Separation selectivity as measured by α [term (iii)] is varied by changing the identity of the mobile and/or stationary phases.

Terms (i) – (iii) can each be varied to improve resolution. At this point let's assume that we are at step 4 of our strategy and we carried out an initial separation. Now, if we find that R_s is poor and k'_{av} for the initial separation is small, k'_{av} should first be increased into the optimum range $1 \leq k'_{av} \leq 10$. No other change in separations will give as large an increase in R_s for as little effort.

When k'_{av} is already within the optimum range of values, and resolution is still marginal, the best solution is usually an increase in N. Normally this means an increase in separation time. However, the necessary change in experimental conditions is easily predicted, and little effort will be spent in achieving the required increase in N and R_s.

If k'_{av} is in the optimum range but with a very small resolution between the two bands, here the necessary increase in N will probably require a very long separation time, and it might even be impossible to achieve (e.g. when $\alpha = 1$). In this case, what is needed is an increase in α.

An increase in the separation factor α results in a displacement of one band center, relative to the other, and an increase in R_s. The time of

separation and the heights of the two bands are not much changed for moderate changes in α. However, predicting the right conditions for the necessary change in α is seldom a straightforward procedure, and it often involves much effort. Thus, an increase in α can provide the shortest possible separation times, but the effort required to discover the right experimental conditions may represent a greater investment than we care to make. So, a change in α may well be preferable when a large number of such separations is involved. Adding to this the fact that as N is increased and so is the analysis time, band heights rapidly decrease, which is not favorable for later quantitative analysis. Figure 3.6 summarizes a systematic approach toward separation of the ligand (DEBT) and the metal complexes.

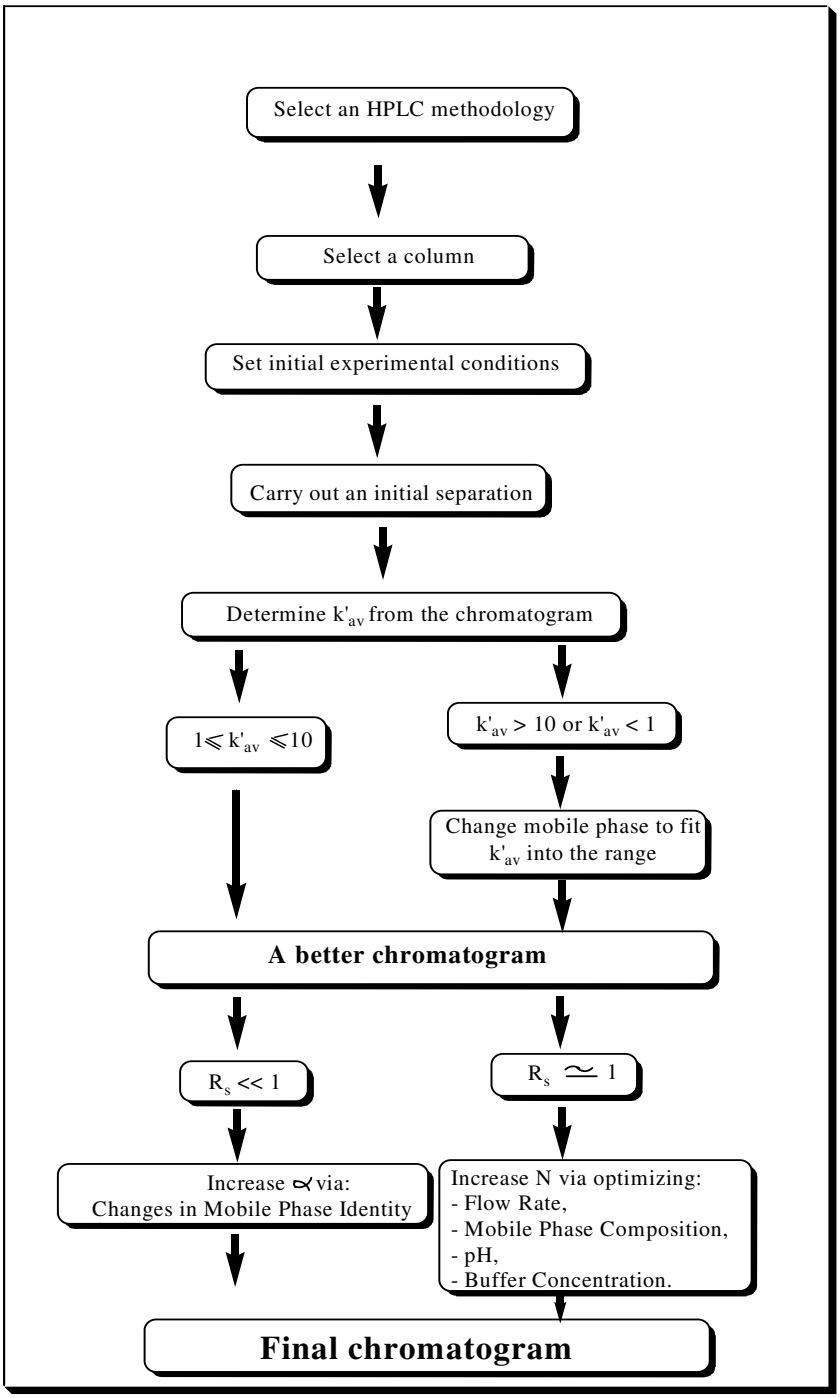


Figure 3.6. Design of an HPLC System for DEBT and Metal Complexes (Pt and Pd)

3.2.1 Selection of HPLC Methodology

Despite the demonstrated application of N,N-dialkyl,N'-benzoylthiourea in normal-phase (NP)-TLC separation of a series of transition metal ions, including Pt (II), Pd (II), Ir (III), Os (III), Rh (III) and Ru (III), it was found by Koch (Koch K. R., 2001) that the metal complexes derived from N'-benzoylthioureas show some disadvantages. Foremost amongst these is the relatively poor solubility of the N'-benzoylthiourea ligands and their corresponding metal complexes. This necessitated either the quantitative precipitation of uncharged metal complexes from the aqueous phase or a solvent extraction process using water-immiscible solvents, an approach advocated by Schuster et al. (Koch K. R., 2001) and successfully applied by Merdivan M. (Merdivan M., 1994).

As has been shown by these authors, silica gel (SG)-based NP-TLC and high performance-TLC gave good separations of the resultant N'-benzoylthiourea complexes of PGMs with careful sample preparation. Moreover, the separated complexes can be successfully quantified at ultra-trace levels by means of a TLC scanner. Attempts to transfer this methodology to conventional normal-phase high-performance liquid chromatography (NP-HPLC) with photometric detection failed, however (Koch K. R., 2001). This was due to ‘‘on-column’’ complex decomposition and irreversible retention of metal complexes, the latter probably due to the inherent acidity of the SG column packing material. Furthermore, the notorious sensitivity of the NP-HPLC with SG-based packing materials to traces of water is a considerable disadvantage resulting in poor control of retention behavior for these complexes, rendering NP-HPLC impractical for separation of these complexes. This

gave us the impression that reversed-phase (RP-HPLC) is promising toward the separation of PGM complexes.

If the systematic approach we have designed for separation of the metal chelates and the excess ligand (Figure 3.6) is investigated, it can be seen that after selecting a suitable RP column, the system is ready for a first trial. Setting the initial experimental conditions shown in Table 2.2, a chromatogram was obtained (Figure 3.7).

From the chromatogram, it can be seen that two peaks are present. This shows that two compounds 'traveled' at the same rate and therefore their peaks overlapped. To have an idea about the relative migration rates, solutions of the three compounds were injected individually; it was found from the chromatograms (not shown here) that DEBT and Pd-DEBT migrated at the same rate whereas Pt-DEBT migrated at a lower rate. This resulted in tailing of the first peak of the chromatogram given in Figure 3.7. It can be seen from the chromatogram that $R_{s1,2}$ is less than 1, therefore peaks 1 and 2 were considered to be the critical pair of the chromatogram.

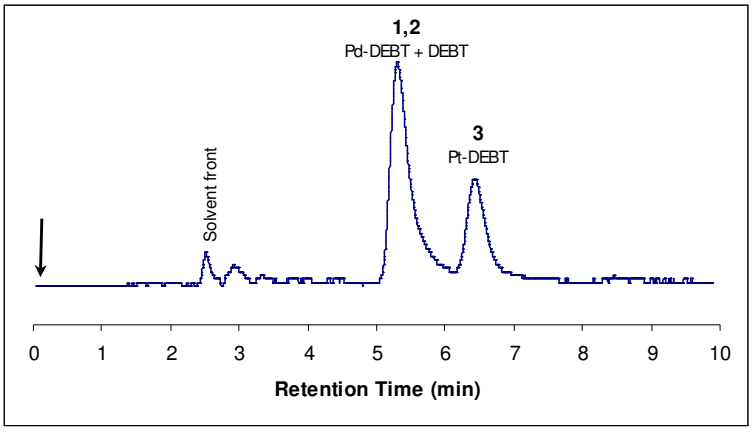


Figure 3.7. Initial Chromatogram for Evaluation of Separation

The next step is to evaluate the chromatogram in quantitative terms. The average k' of peaks 1 and 2 was calculated as 1.555, a value that falls within the optimum range of k'_{av}. At this point, estimation of resolution between peaks 1 and 2 was important. Our approach to estimating R_s was based on comparison with a standard set of resolution curves given by Snyder (Snyder L. R. and Kirkland J. J., 1974) (Figure 3.8).

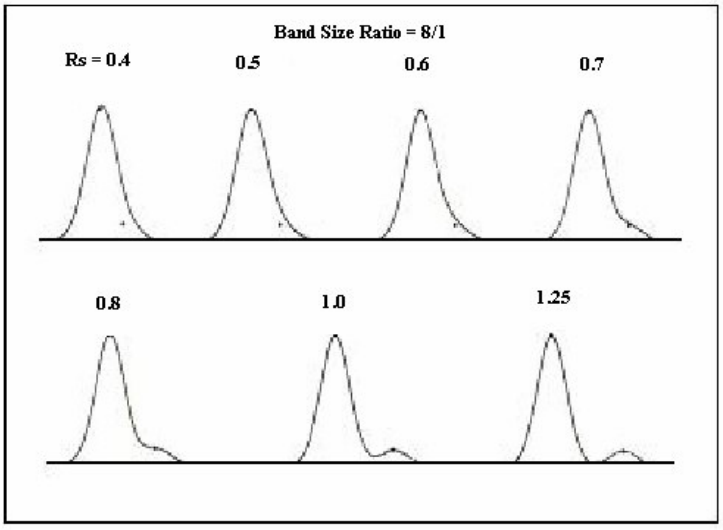


Figure 3.8. Standard Resolution Curves for a Band-Size Ratio of 8/1 and R_s Values of 0.4 – 1.25 (Snyder L. R. et al., 1974)

These resolution curves were provided by Snyder as a quick and convenient tool to estimating resolution. They are a set of hypothetical chromatograms arranged according the relative peak heights (band-size ratio) of the two peaks that are considered critical in a chromatogram. Figure 3.8 is just one of this set that was chosen because the relative heights of the peaks corresponding to DEBT and Pd-DEBT in the initial chromatogram (Figure 3.7) were thought to be 8/1.

In the figure, a good match can be seen with the peak having R_s = 0.4. Therefore, R_s between 1 and 2 was estimated as 0.4. Since this value was not much less than 1 (e.g. 0), two ways to increasing R_s were possible in this case (see Figure 3.6). First, R_s can be increased via increasing efficiency (N). This in turn can be done by decreasing the flow rate of the mobile phase, which can sometimes be a short way. Second, R_s can be increased with increase in selectivity (α) through changes in mobile phase identity, which is always a long way that involves more trials and errors.

3.2.2 Effect of Flow Rate

In order to separate peaks 1 and 2 in the initial chromatogram (Figure 3.7), flow rate of the mobile phase was gradually decreased from 1.5 to 0.6 ml min^{-1} as described in section 2.3.3. Figure 3.9 shows the chromatograms obtained at each value.

It can be seen from the chromatograms below that decreasing flow rate had a little effect on resolution although the total time required increased about three fold. The standard resolution curves in Figure 3.8 show a good match between the curve of $R_s = 0.7$ and the first peak (1,2) at 0.5 ml min^{-1} in Figure 3.9 with respect to the extent of tailing. This indicates a change in resolution from 0.4 to 0.7. It can be reported here therefore that changing flow rate failed in resolving Pd-DEBT and the excess DEBT peaks in spite of increasing efficiency (Figure 3.10).

Resolution changes directly with the square root of the change in column plate number. Since plate number changes inversely with flow rate, resolution will change inversely with the square root of the ratio of flow rates (F).

$$R_{s2} = R_{s1} \times (F_1/F_2)^{0.5} \qquad \text{---------- (3.1)}$$

Where:

R_{s1} = Resolution at a flow rate 1 (F_1)

R_{s2} = Resolution at a flow rate 2 (F_2)

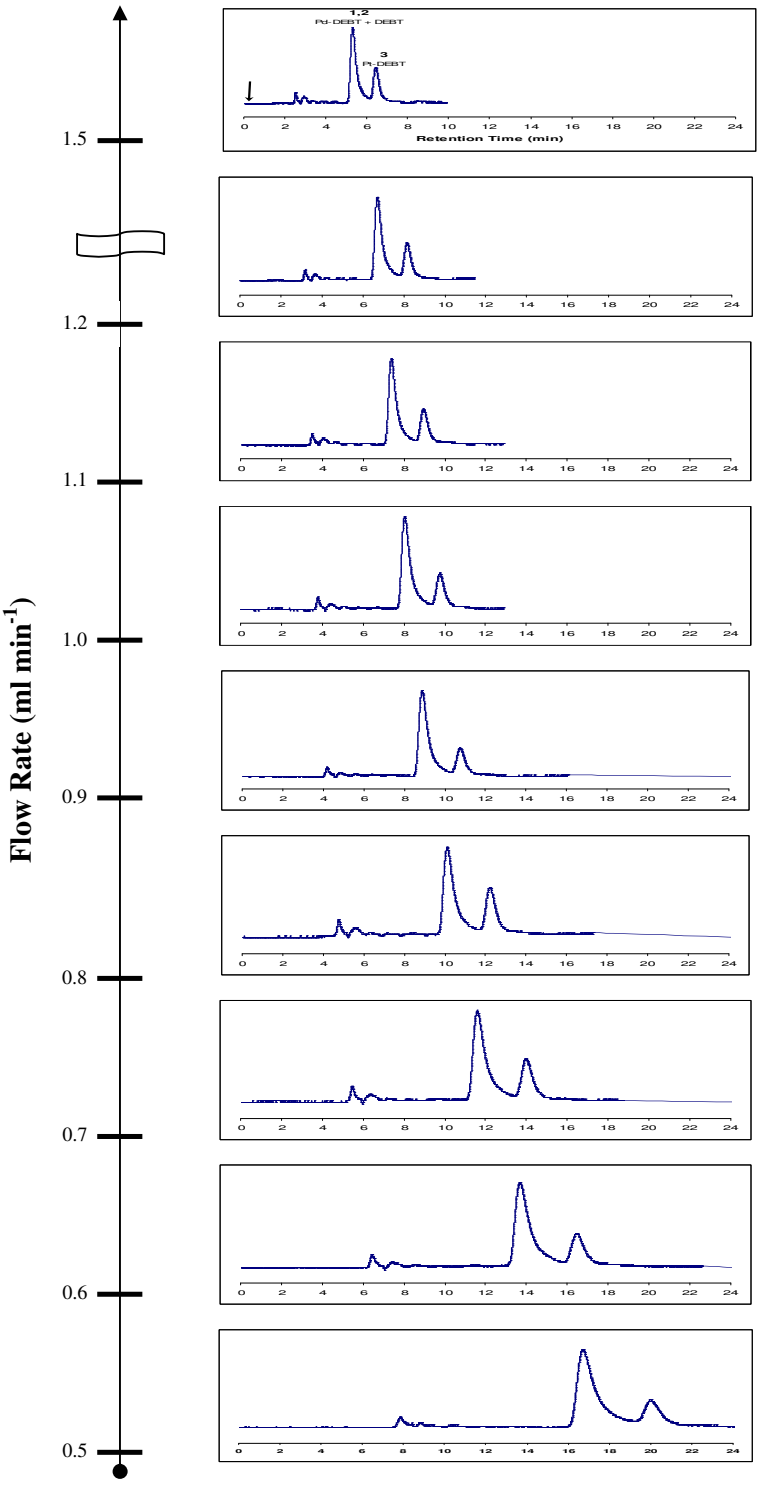


Figure 3.9. Effect of Flow Rate on Separation

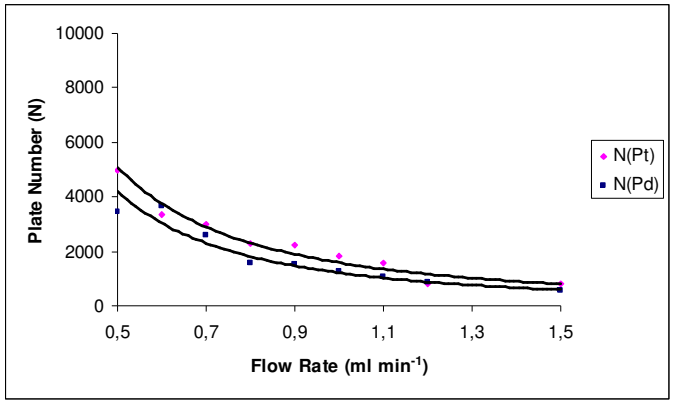


Figure 3.10. Effect of Flow Rate on Plate Number (Efficiency)

Having a resolution of 0.4 at a flow rate of 1.5 ml min^{-1}, enables us to calculate the resolution between peaks 1 and 2 at a flow rate of 0.5 ml min^{-1} making use of equation (3.1). It is found that $R_{s(0.5)} = 0.69$, which is in a good agreement with the value (0.7) obtained directly from the standard resolution curves (Figure 3.8).

In similar fashion, how the analysis time (T) and back pressure (P) will change with flow rate can be predicted [equation (3.2) and equation (3.3)].

$$T_2 = T_1 \times F_1/F_2 \qquad \text{---------- (3.2)}$$

Where:

T_1 = Analysis time at a flow rate 1(F_1)

T_2 = Analysis time at a flow rate 2 (F_2)

From the chromatograms obtained (Figure 3.9), it can be seen that the three components were eluted in 7.0 min at a flow rate of 1.5 ml min^{-1} and in 21.0 min at a flow rate of 0.5 ml min^{-1}. Using equation (3.2) for

$T_{0.5}$ gives the same value of 21.0 min. It is obvious here that although decreasing the flow rate results in better resolution, it increases the retention time by the same factor.

Back pressure through the column is directly proportional to the flow rate (equation 3.3).

$$P_2 = P_1 \times F_2/F_1 \qquad \text{---------- (3.3)}$$

Where:

P_1 = Back pressure at a flow rate 1 (F_1)

P_2 = Back pressure at a flow rate 2 (F_2)

This means that low flow rates are favorable for low back pressures because high back pressures decrease life time of the column.

Considering the different parameters affected by flow rate: efficiency (plate number) (N), resolution (R_s), analysis time (T) and back pressure (P), the optimum flow rate for separation of Pd-DEBT, Pt-DEBT and the extra DEBT is 0.8 ml min^{-1}.

3.2.3 Effect of Mobile Phase Composition

The mobile phase must be chosen according to its chromatographic properties: it must interact with a suitable stationary phase to separate a mixture as fast and as efficiently as possible. As a general rule, a range of solvents is potentially able to solve any particular problem, so selection must be based on different criteria:

1. Viscosity: a low-viscosity solvent produces a lower back pressure than a solvent with higher viscosity for a specific flow-rate. It also allows faster chromatography as mass transfer takes place faster,

2. UV transparency: if a UV detector is used, the mobile phase must be completely transparent at the required wavelength,

3. Purity: this criterion has a different meaning depending on the intended use, e.g. absence of compounds that would interfere with the chosen mode of detection,

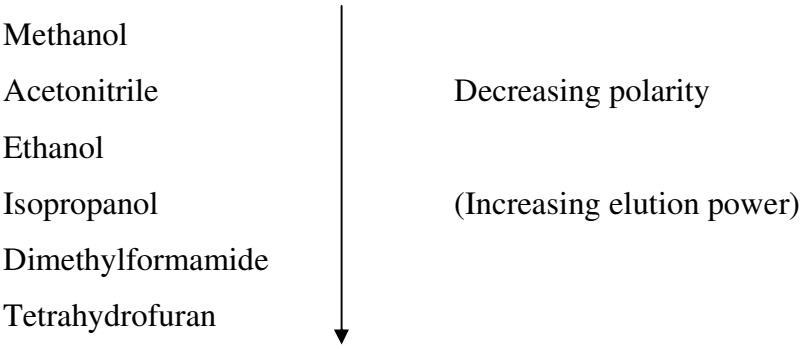
4. Inert with respect to sample compounds: the mobile phase must not react at all with the sample mixture

5. Toxicity: here the onus is on each individual laboratory to avoid toxic products as far as possible,

6. Price: solvent consumption in HPLC is relatively high. Therefore solvents with high purity but moderate prices are preferred.

As a general rule, the mobile phase should not be detector-active, i.e. it should not absorb in the same region used for the analytes. Otherwise, it is very possible that unwanted baseline effects and extra peaks will show up in the chromatogram.

The mobile phase in reversed-phase chromatography consists of mixtures of water or aqueous buffer solutions with various water-miscible solvents, e.g.



The polarities of solvents are given in Snyder polarity index in Appendix A.

The mixtures of water with organic solvents often have a markedly higher viscosity than the pure compounds. For the three most frequently used solvents, all compositions of methanol – water, have higher viscosity than tetrahydrofuran – water which have higher viscosity than acetonitrile – water. Since the back pressure through the column is proportional to the viscosity, compositions of acetonitrile – water seem to be the best of the three.

Water is often described as the strongest elution medium for chromatography, but in fact this is only completely true for adsorption

processes. Water may interact with the active centers in silica and alumina, so that adsorption of sample molecules becomes highly restricted and they are rapidly eluted as a result. Exactly the opposite applies in reversed-phase systems: water cannot wet the non-polar hydrophobic alkyl groups and does not interact with them in anyway. Hence, it is the weakest mobile phase of all and gives the slowest sample elution rate. The greater the amount of water in the eluent, the longer is the retention time. The chromatograms depicted in Figure 3.11 demonstrate this point.

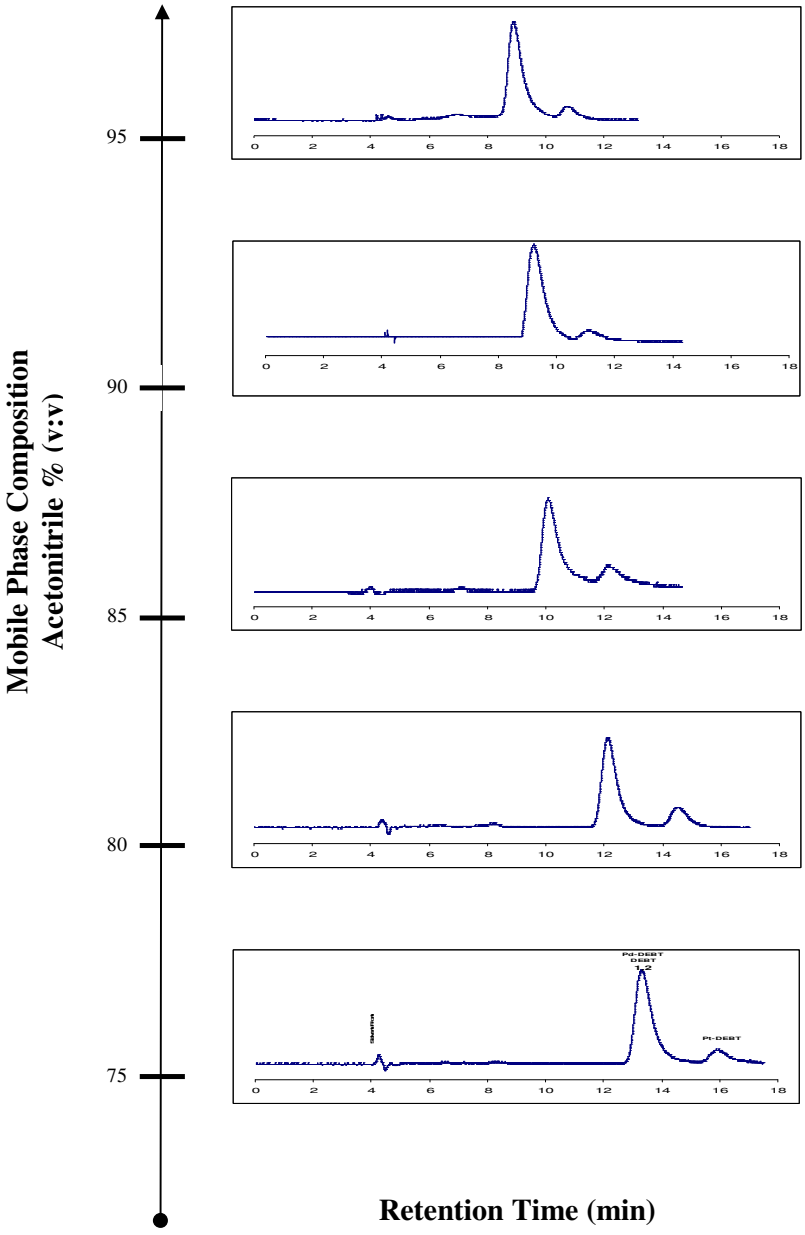


Figure 3.11. Effect of Mobile Phase Composition on Separation

3.2.4 Effect of pH

The effect of pH on separation of Pt and Pd complexes was studied through the pH range of 4.0 to 6.0. This range was chosen because at pH greater than 7.0 silica dissolves, and at pH lower than 3.0 cleavege of the silanol groups occurs. Changes in pH (as shown in Figure 3.12) did not affect the resolution or the elution time of the chelates. However, peak areas were affected, with the peak areas being maximum at pH 5.0. The appearance of extra peak at pH 4.0 was not clearly understood but it is thought to be a complex of a different oxidation state of Pt, i.e. Pt (IV), which may be verified by different analysis. From these results, pH 5.0 was considered optimum for later studies through this study.

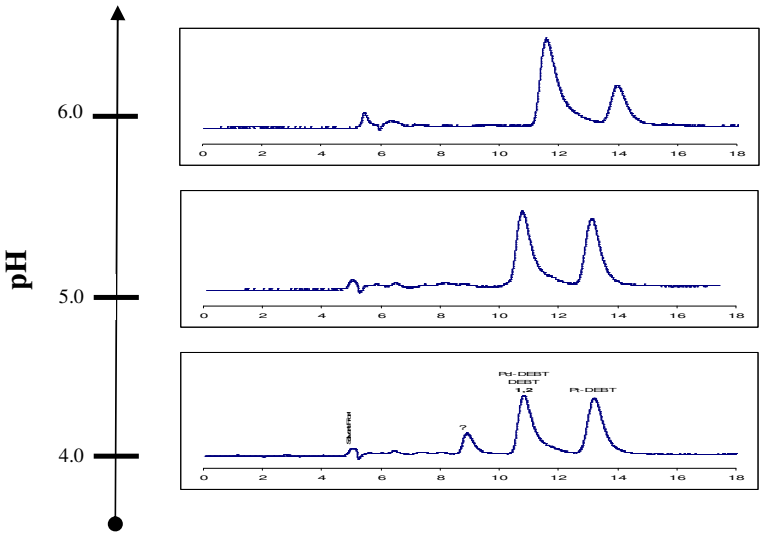


Figure 3.12. Effect of pH on Separation

3.2.5 Effect of Buffer Concentration

Buffer is used in RP-HPLC to adjust the pH of the aqueous part of the mobile phase and to get reproducible retention times and a stable baseline. The range was chosen as 0.10 – 0.50 mol l^{-1} because at higher values, buffers may foul the column by plugging its frits and packing, and at lower values it may lose its functions.

It can be seen from the chromatograms (Figure 3.13) that decreasing buffer concentration increased resolution and elution time slightly. The concentration of 0.20 mol l^{-1} was set optimum.

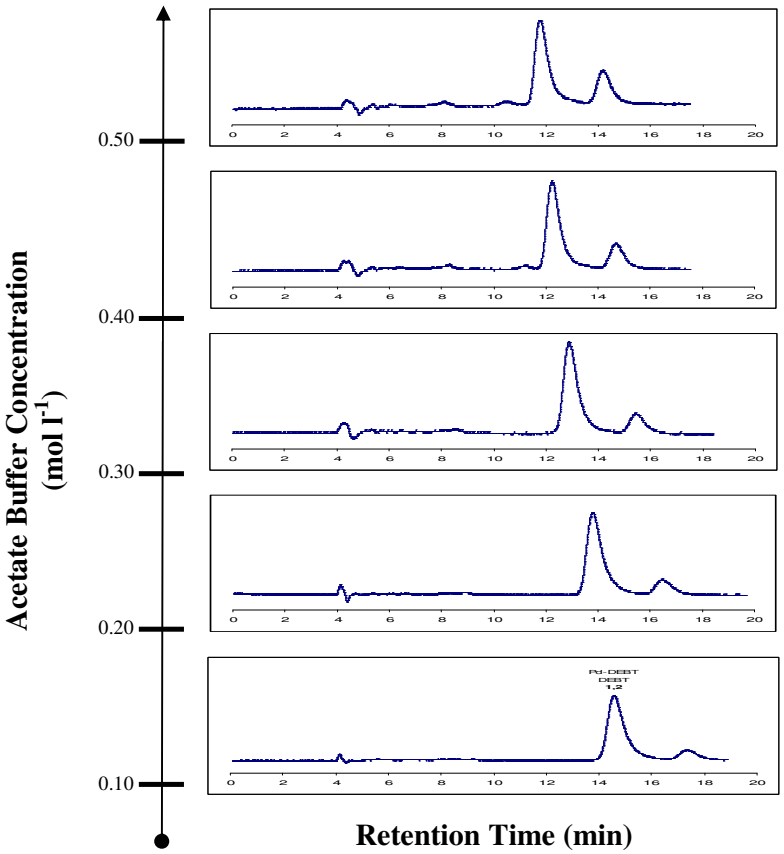


Figure 3.13. Effect of Buffer Concentration on Separation

3.2.6 Effect of Mobile Phase Identity

The most powerful means to influence the separation is by changing the selectivity properties of the phase system. This can be done by the use of another method (e.g. normal vs. reversed phase), the use of another stationary phase (e.g. octadecyl vs. phenyl silica) or the use of another mobile phase. In the letter case, it will be best to choose solvents with large differences in their selectivity properties.

As shown in the chromatograms in Figure 3.11, the mobile phase of AcN: H_2O (75:25, v:v) was accepted to be optimum. Calculating the polarity of this composition at this step is necessary. This can be done using the list of solvent polarities (shown in Appendix A) which was developed by Snyder (Snyder L. R. and Kirkland J. J., 1974) and equation (3.4) below.

$$P'_{AB} = \phi_A P'_A + \phi_B P'_B \qquad \ldots\ldots\ldots\ldots (3.4)$$

Where P'_A and P'_B are polarity indexes of the solvents and ϕ_A and ϕ_B are the volume fractions of each.

Accordingly a mobile phase of AcN: H_2O with a composition of (75:25, v:v) has a polarity of 6.90. Using the same list, it was possible to construct the ‘‘Mobile Phase Composition vs. Polarity’’ curves using different solvent combinations (Figure 3.14 through Figure 3.16). For example, Figure 3.14 is plotted using equation (3.4) at different compositions of methanol to water, acetonitrile to water and tetrahydrofuran to water.

These curves are very useful in calculating the different compositions that correspond to a desired polarity. For example, some possible compositions having a polarity of 6.90 can be found as:

1. 75 % AcN, 25 % H_2O (from Figure 3.14)

2. 87.5 % MeOH, 12.5 % H_2O (from Figure 3.14)

3. 43.8 % THF, 56.2 % H_2O (from Figure 3.14)

4. 45 % AcN, 35 % MeOH, 20 % H_2O (from Figure 3.15)

5. 65 % AcN, 5 % THF, 30 % H_2O (from Figure 3.16)

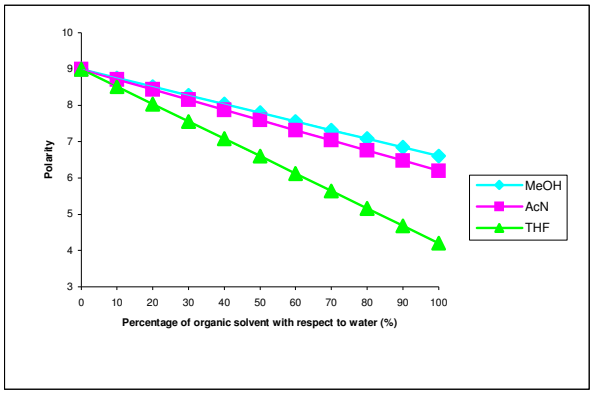


Figure 3.14. Variation of Polarity as a function of Mobile Phase Composition (Binary Solvent)

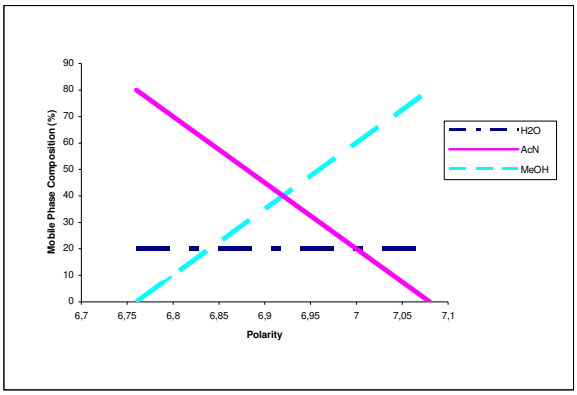


Figure 3.15. Variation of Polarity as A function of Mobile Phase Composition (AcN-MeOH- H_2O)

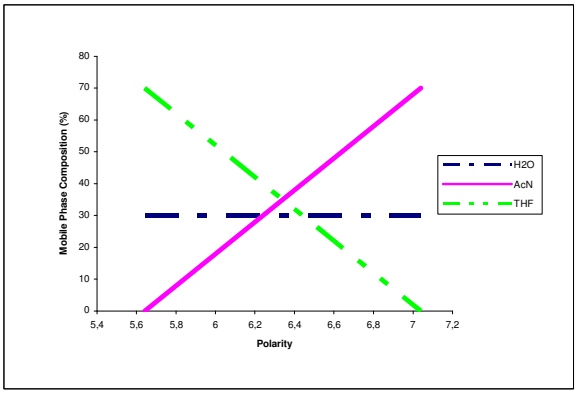


Figure 3.16 Variation of Polarity as A function of Mobile Phase Composition (AcN-THF- H_2O)

As it can be seen from these examples and figures, many other possibilities may also exist, e.g. changing the percentage of water besides the other two in the tertiary compositions. That is the reason why optimization of selectivity is the hardest part of the optimization considering all the other parameters being investigated. It should be noted here that these calculations give only approximate results and even do not give closer-to-optimum results in some cases. However, using them enabled us to minimize the trials and errors.

In the literature, a four-solvent optimization system is frequently used for adjustment of α in RP chromatography (Meyer V., 1998). Three compatible solvents are used in combination with water. In the case of AcN, MeOH, and THF, polarities can also be used to construct the so-called solvent triangle (Figure 3.17)

From this triangle, it can be seen that after finding an optimum polarity of AcN/H_2O, it is easier to start optimization of α using another 2-solvent system of the corners of the triangle, e.g. MeOH/H_2O. If poor resolution is still obtained, the use of a 3-solvent system remains a possibility. Finally, the 4-solvent system of MeOH/AcN/THF/H_2O, which would make the optimization more complex, should be tried.

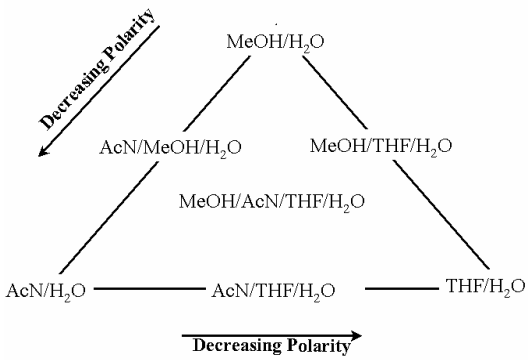


Figure 3.17. Solvent Triangle for RP-HPLC

In this study, this approach was used. First, we started with AcN-H_2O and then tried MeOH-H_2O but no resolution was obtained. Finally, we decided to use the tertiary mobile phase of AcN-MeOH-H_2O, which resulted in a very good resolution of the three sample components as shown in the chromatogram below (Figure 3.18).

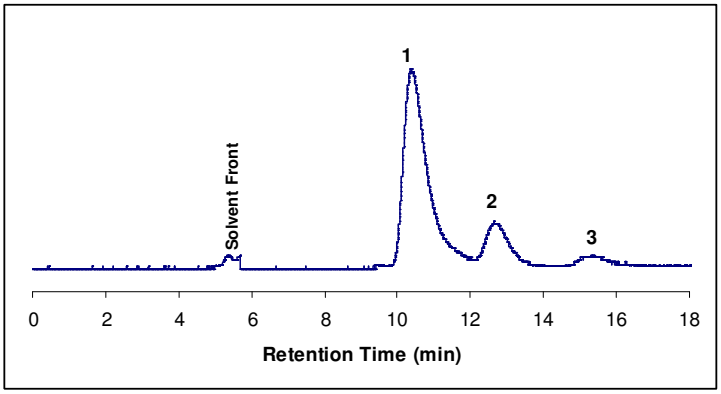


Figure 3.18. Final Chromatogram: Separation under the Optimized Parameters

Conditions: mobile phase, AcN: MeOH: Buffer (0.20 mol l^{-1}, pH 5.0 Acetate) (80:10:10, v:v:v); Flow Rate = 0.8 ml min^{-1}. Column: SHODEX RSpak DS-613 (5 µm, 150 x 6 mm i.d., Phenomenex)

Peaks: 1 = DEBT; 2 = $Pd(DEBT)_2$ (5.0 mg l^{-1}); 3 = $Pt(DEBT)_2$ (10.0 mg l^{-1})

3.3 Calibration Curves and Figures of Merit

The performance of the method was evaluated by plotting calibration curves for Pt and Pd [Figure (3.19) and Figure (3.20)]. Under the previously optimized conditions, chelates of the two metals in different concentrations were injected into the HPLC system as described in section 2.3.2.

A detection limit of 0.77 mg l^{-1} (based on 3s) was obtained for Pt and the relative standard deviation obtained by analyzing ten independent replicates of a solution was 2.58 % for a platinum concentration of 10.0 mg l^{-1}. The analytical response was found to be linear throughout the range 3.0 – 70.0 mg $l^{-1}$of Pt.

A detection limit of 14.2 µg l^{-1} (based on 3s) was obtained for Pd and the relative standard deviation obtained by analyzing ten independent replicates of a solution was 2.36 % for a palladium concentration of 0.60 mg l^{-1}. The analytical response was found to be linear throughout the range 0.60 – 10.0 mg $l^{-1}$of Pd. The results obtained are summarized in Table 3.2.

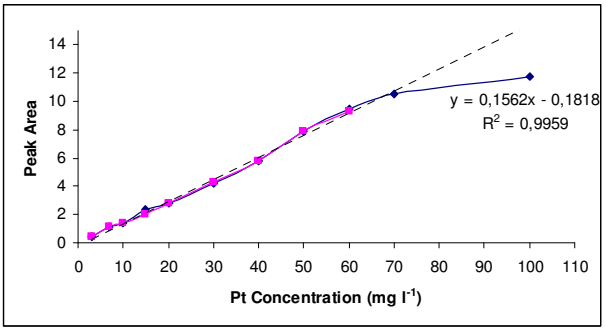


Figure 3.19. Calibration Curve Obtained Using Different Concentrations of Pt

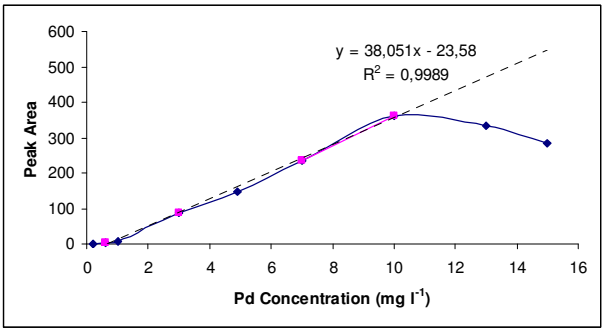


Figure 3.20. Calibration Curve Obtained Using Different Concentrations of Pd

Table 3.2. Figures of Merit of the Method for Pt and Pd under the Optimized Experimental Conditions

Element	Detection Limit	Limit of Quantitation ($mg\ l^{-1}$)	Working Range ($mg\ l^{-1}$)	RSD (%)
Pt	0.77 $mg\ l^{-1}$	3.0	3.0 – 70.0	2.58
Pd	14.2 $\mu g\ l^{-1}$	0.20	0.60 – 10.0	2.36

Limit of quantitation was set as the lowest concentration at which measurements could be made. This was taken to be approximately equal to ten times the average of noise signals of the base line in the chromatograms.

3.4 Determination of Pt and Pd by ICP-OES

ICP-OES was used to check for the extent of removal of Pt and Pd from the aqueous phase after applying the salt-induced phase separation of acetonitrile and water as described in section 2.4. In the literature, phase separation was achieved using 2.00 mol l^{-1} NaCl (Koch K. R., 2001). However, we have found that this concentration of NaCl was not enough for full recoveries of the metals from the aqueous phase as shown below. Therefore, salt concentration was increased to 2.20 mol l^{-1}. However, during analysis of the aqueous phase with ICP-OES, we have found that this concentration of NaCl resulted in clogging of the nebulizer.

In order to overcome this problem, two ways can be followed. First, the concentration of NaCl can be decreased from 2.20 mol l^{-1} to 2.00

mol l^{-1} during the preparation procedure of metal complexes. A NaCl concentration of 2.00 mol l^{-1} was observed to be the minimum concentration that can be applied in the salt-induced phase separation process to obtain two separate phases. Second, the aqueous samples that contained 2.20 mol l^{-1} NaCl and to be analyzed with ICP-OES must be diluted with de-ionized water (1:1, v:v). Both ways were followed and the results obtained are discussed below.

Standard solutions of Pt and Pd in the range of concentrations, supplied as 1000 mg l^{-1} by Aldrich, were analyzed and calibration curves were plotted (Figure 3.21 and Figure 3.22). The effect of high concentration of NaCl on the calibration data was checked via standard addition at one point i.e., 5.00 mg l^{-1} Pd and 5.00 mg l^{-1} Pt. It was found that NaCl had no effect on the determinations at the two salt concentrations studied.

Using these calibration curves, the concentrations of the metal ions that remained in the aqueous solutions that contained 2.00 mol l^{-1} NaCl and the other aqueous solutions that contained 2.20 mol l^{-1} NaCl were determined.

Duplicate samples were prepared for two different salt concentrations and for each sample three measurements were performed. The average of six measurements with its precision in terms of 3s is given as the metal ion concentration in the sample.

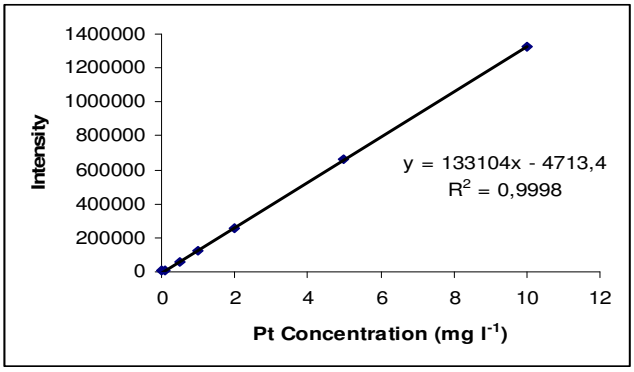


Figure 3.21. Calibration Curve Obtained for Pt by ICP-OES

Initial Pt concentration before salt-induced separation = 60.0 mg l^{-1}

Pt concentration in the sample at 2.00 mol l^{-1} NaCl = 6.24 ± 0.22 mg l^{-1} (3s)

Extraction Efficiency at 2.00 mol l^{-1} NaCl = 89.6 %

Pt concentration in the sample at 2.20 mol l^{-1} NaCl = below LOQ

Extraction Efficiency at 2.20 mol l^{-1} NaCl ≈100.0 %

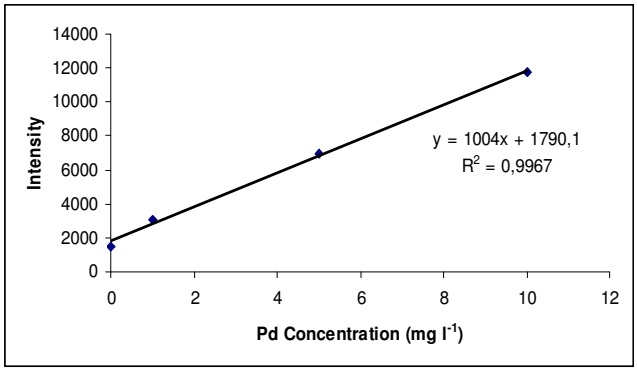


Figure 3.22. Calibration Curve Obtained for Pd by ICP-OES

Initial Pd concentration before salt-induced separation = 10.0 mg l^{-1}

Pd concentration in the sample at 2.00 mol l^{-1} NaCl = 1.66 ± 0.06 mg l^{-1} (3s)

Extraction Efficiency at 2.00 mol l^{-1} NaCl = 83.4 %

Pd concentration in the sample at 2.20 mol l^{-1} NaCl = below LOQ
Extraction Efficiency at 2.20 mol l^{-1} NaCl ≈ 100.0 %

The extraction efficiencies calculated as 89.6 % for Pt and 83.4 % for Pd showed that incomplete recoveries of the metals were obtained by this salt-induced phase separation process when 2.00 mol l^{-1} NaCl was used.

By increasing NaCl concentration to 2.20 mol l^{-1} and analyzing the samples after dilution, metal concentrations were found below the limit of quantitation, which indicates recovery values close to 100% for both metals.

Comparing the results obtained with ICP-OES with both salt concentrations, it can be seen that the minimum concentration of NaCl should be at least 2.20 mol l^{-1} in order to obtain a full phase separation of the aqueous and organic phases and a full extraction of Pt- and Pd-DEBT complexes.

3.5 Interference and Peak Characterization

In soil matrices, many contaminating elements are present. The main constituents were found to be Ca, Fe and Zn, and the trace elements with the highest contents were found to be Cu, Mn and Co (Boch. et al., 2002). In the literature, almost 20 metals were studied with DEBT, including these six metals. It was found that Ca did not form chelates with DEBT. Among the others, Fe (III) and Cu (II) were the only contaminants

that were precipitated together with PGMs during complexation below pH 3. The other remaining metals could only be precipitated at higher pH values (near neutral region) (Merdivan M., 1994).

In this part of the study, the optimized parameters found earlier had to be extended to include the separation of Fe- and Cu-DEBT complexes before analysis of soil. As can be seen from the chromatograms below, Fe-DEBT complex interfered with the peak of the extra DEBT and that of Cu overlapped with Pd-DEBT (Figure 3.23).

Here, separation of Cu-DEBT from Pd-DEBT was important. To achieve this goal, the systematic approach set in section 3.2, was also followed. Since k'_{av} of this critical pair was about 5, a value that is considered optimum in most cases, changing the selectivity factor (α) was thought to be the most promising parameter for their separation. By so doing, a resolution of about 1 was obtained by using a mobile phase of AcN-THF-H_2O (44: 26: 30, v:v:v) (step 2 in Appendix B). Decreasing the polarity of this mobile phase gave better resolution. But, it seemed that the dependence of resolution on polarity was critical here since it is a 3-solvent system. Poor resolution was obtained at high and low polarities and therefore a medium polarity value was required to achieve the final resolution. The final chromatogram obtained under these new conditions is shown below in Figure 3.24.

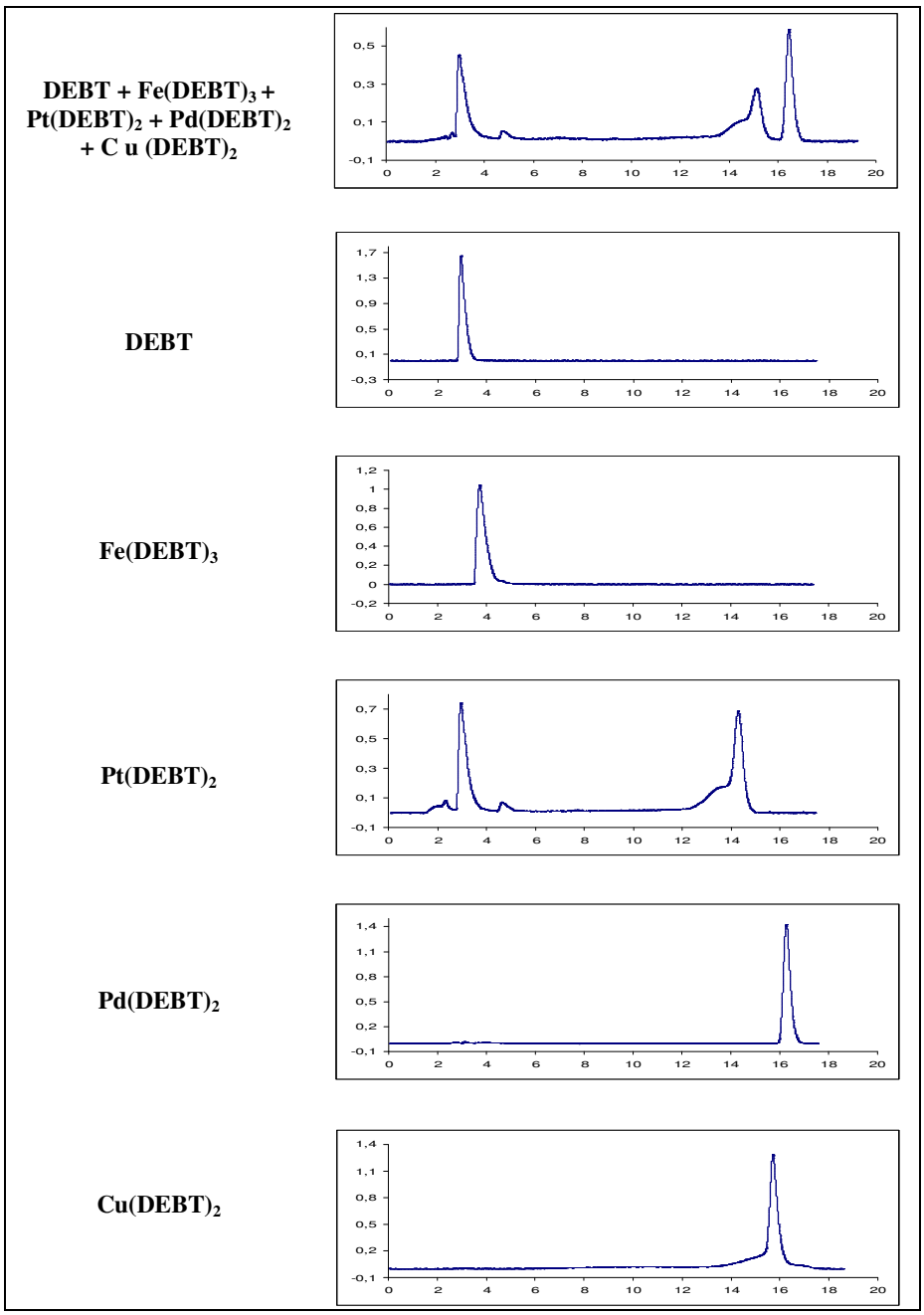


Figure 3.23. Interferences and Peak Characterization

Conditions: mobile phase, AcN: MeOH: H_2O (80:10:10), Flow Rate, 0.8 ml min^{-1}, pH: 5.0, Buffer: 0.20 mol l^{-1} Acetate Buffer. Column: ACE 3 C_{18} (3 µm, 150 x 4.6 mm i.d, ACT)

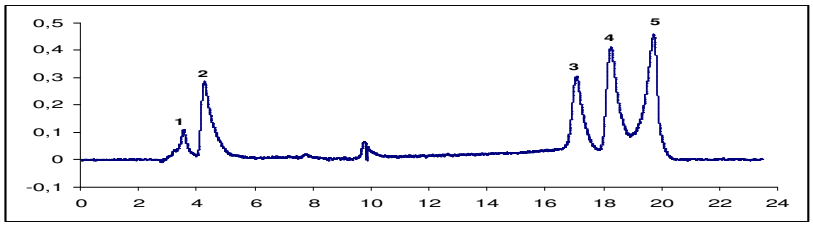


Figure 3.24. Final Chromatogram: Separation of PGMs and Interfering Metals

Conditions: mobile phase, AcN: THF: H_2O (38:32:30), Flow Rate, 0.8 ml min^{-1}, pH: 5.0, Buffer: 0.10 mol l^{-1} Acetate Buffer. Column: ACE 3 C_{18} (3 µm, 150 x 4.6 mm i.d, ACT)
Peaks: 1 = DEBT; 2 = $Fe(DEBT)_3$; 3 = $Pt(DEBT)_2$; 4 = $Cu(DEBT)_2$; 5 = $Pd(DEBT)_2$

3.6 Analysis of Soil

3.6.1 Digestion of Soil

PGMs, especially palladium, bind strongly to silicate residues even in acidic solutions. Therefore, soil had to be digested completely. This required the application of oxidizing acids as well as hydrofluoric acid.

To facilitate sample preparation, a pressure and temperature controlled microwave digestion system for the simultaneous treatment of samples was used. The power programs which were used are listed in Table 2.8.

In the first digestion step, mainly organic components of the mixture were oxidized by a mixture of nitric acid and hydrogen peroxide. Nitrogen oxides formed during that reaction were partly re-oxidized to nitric acid by hydrogen peroxide, and the pressure increase, which usually

occurs during the formation of nitrogen oxides, was therefore reduced. Addition of hydrogen peroxide thus led to higher temperatures of the treated solutions and to a more complete digestion.

In the second digestion step, an excess of hydrofluoric acid was applied to guarantee the complete dissolution of the silicate residue. In this step, temperature was held at 180 °C for 20 min to ensure complete mineralization of remaining organic components. In comparison with digestion step 1, lower amounts of gaseous reaction products were formed and therefore, the resulting pressure increased gradually with the rise in temperature.

The most important effect of the addition of boric acid in step three was the masking of fluoride ions as tetrafluoroborate. To avoid the stepwise hydrolysis of tetrafluoroborates to hydroxofluoroborates, and with it the re-formation of hydrofluoric acid, a two-fold stoichiometric excess of boric acid was required. Another positive effect of boric acid was the dissolution of sparingly soluble fluorides.

Due to the masking of hydrofluoric acid as tetrafluoroborate, all digestion solutions could be transferred directly to glass vessels. This also prevented the solution from attacking the silicon matrix of the RP-HPLC column used.

3.6.2 Determination of Pt and Pd in Soil

In order to check the accuracy of the proposed method, the analyses of soil samples spiked with different amounts of Pt and Pd individually and together were performed.

Figure 3.25 is a typical chromatogram obtained by injecting a digested soil sample that had been previously spiked with 5.00 mg l^{-1} Pt and 5.00 mg l^{-1} Pd. Investigating the chromatogram, some notes can be drawn. First, it can be seen that retention times of metal chelates were not affected and that they eluted with the same order found earlier in this study under the same chromatographic conditions when the mixture of metal chelates was injected. Second, a comparison of peaks 2 and 3 in the chromatogram reveals that the detector is more sensitive to Pd complex than to Pt complex under these conditions. Third, the chromatogram obtained upon injection of soil extracts that had been spiked with one of the metals alone resulted in two peaks only, one for DEBT and the other for metal-complex, but three peaks for the mixture as given in Figure 3.25. Finally, no peaks for the interfering metals Cu and Fe were observed in the chromatogram which indicates that the analyzed soil samples did not contain either of these two metals or their concentrations were below the detection limit of the proposed method.

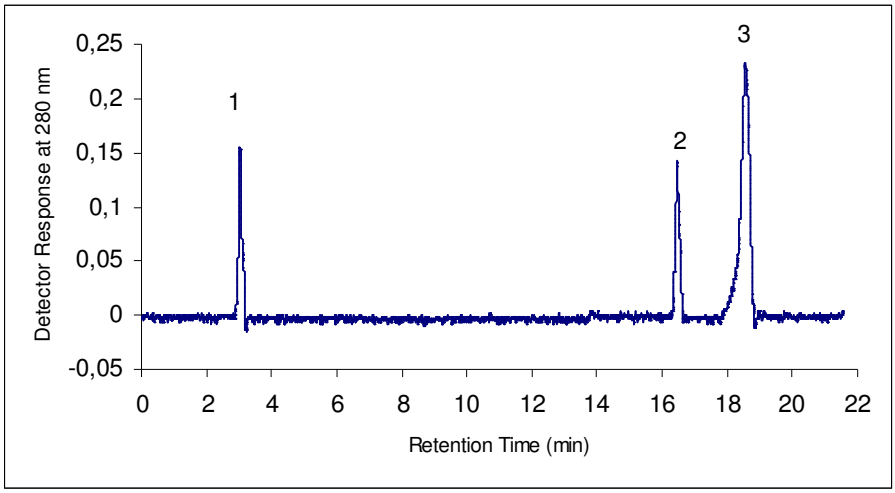


Figure 3.25. Separation of PGMs in a Spiked Soil Sample

Conditions: mobile phase, AcN: THF: H_2O (38:32:30), Flow Rate, 0.8 ml min^{-1}, pH: 5.0, Buffer: 0.10 mol l^{-1} Acetate Buffer. Column: ACE 3 C_{18} (3 µm, 150 x 4.6 mm i.d, ACT)
Peaks: 1 = DEBT; 2 = $Pt(DEBT)_2$ (5.00 mg l^{-1}); 3 = $Pd(DEBT)_2$ (5.00 mg l^{-1})

Table 3.3. Determination of Pd and Pt in Spiked Soil Samples

Spiked Value		**Experimental Results**	
Pd (mg l^{-1})	Pt (mg l^{-1})	Pd (mg l^{-1})	Pt (mg l^{-1})
5.00	**20.00**	4.93, 4.75, 4.68, 4.86 **Av. 4.81 ± 0.33***	21.70, 19.16, 20.59, 20.28 **Av. 20.43 ± 3.13***
2.00	**5.00**	1.97, 1.96, 2.00, 1.95 **Av. 1.97 ± 0.06***	4.98, 4.35, 4.78, 5.24 **Av. 4.84 ± 1.14***

* Taken as 3s.

The analysis results are given in Table 3.3. The experimental results given for the two metals are the averages of four determinations obtained by spiking the soil samples with two different amounts of Pd and Pt. The precision of the method is given as three times standard deviation. It can be seen that precision of Pd results are better than those of Pt.

The accuracy of the method is found to be better for the lower concentration of Pd; namely, a relative error of 1.5 % is found for 2.0 mg l^{-1} and 3.8 % for 5.0 mg l^{-1}. The opposite is observed for Pt; a relative error of 2.2 % is found for 20.0 mg l^{-1} and 3.2 % for 5.0 mg l^{-1} spiking. For both of the metals, relative errors less than 4.0 % were found. It can be seen that the measured concentrations are in good agreement with the spiked values for both metals.

3.7 Future Work

In the future, three objectives can be suggested:

1. During the optimization of pH, it was observed that an extra peak always appeared in the chromatograms obtained by injecting Pt-DEBT samples at low pH values. This peak was thought to belong to a new chelate with a different oxidation state of Pt, i.e., Pt (IV). Further studies can therefore be done to verify this. This method may then be used for speciation of Pt.

2. Although this method provided better detection limits compared to other studies using HPLC for Pt and Pd, these detection limits are still high regarding the low concentrations of these metals in environmental samples. Therefore, a combination of a preconcentration procedure, more efficient than the salt-induced phase separation used with this method, would provide an inexpensive, interference-free alternative.

3. At the early stages of this study, we aimed to separate Pt, Pd and Rh (the three pollutants emitted from autocatalytic converters). However, we faced two difficulties. First, formation of Rh-DEBT chelate was hard. Second, after formation of its chelate, when applied to HPLC, it gave more than one peak at the optimum conditions for separation of Pt and Pd. Therefore, further studies can be suggested to separate the three metals making use of the systematic approach used for Pt and Pd.

CHAPTER 4

CONCLUSION

The most important aim of this study was to provide a Reversed Phase-HPLC method for separation and quantitation of some PGMs from a strongly interfering matrix, soil, making use of a highly selective chelating agent, DEBT.

DEBT was a very good reagent for the selective extraction of PGMs investigated (Pt and Pd) due to its high extraction values with these metals. The ligand provided an extraordinary chemical resistance against oxidation and hydrolysis and a striking selectivity toward these PGMs. The metal chelates of DEBT had very high complexation stability and there was no problem of dissociation of these chelates in aqueous solutions or organic solvents.

In order to characterize and confirm the formation of DEBT and PGM chelates, UV-absorption and FT-IR analyses were carried out. In UV-absorption analyses, absorption bands of DEBT and its chelates were similar to those given in the literature. IR spectra obtained were also very similar to their literature counterparts. In addition to that, IR spectra of Pt and Pd chelates resembled each other indicating that they have similar structures.

The successful separation and quantitation of the PGMs investigated in this study using RP-HPLC demonstrated the superiority of RP-HPLC over NP-HPLC for this kind of chelates. It had been shown that NP-HPLC failed to separate N'-benzoylthiourea complexes of PGMs. This was due to ‘‘on-column’’ complex decomposition and irreversible retention of metal complexes which was due to the inherent acidity of the silica gel column packing material.

A comparison of the proposed method with other methods using RP-HPLC combined with DEBT and other chelating agents for the determination of Pt and Pt is given in Table 4.1. This method appears to be the most sensitive of all for Pd and more sensitive than most of the others for Pt in spite of applying an easier preconcentration method.

In contrast to the other HPLC methods proposed so far for separation of PGMs, this method provided a systematic approach (design) that can be easily followed aiming at minimizing the number of experiments involved. In this design, suitable HPLC methodology and column were first selected. Then, initial experimental conditions were set for carrying an initial separation. From the chromatogram obtained, the average retention factor (k'_{av}) of the critical pair was determined. It was found as 1.555 which fit in the optimum range of 1 to 10. Therefore, changing the mobile phase was not required at that point. Using Snyder’s standard resolution curves for a band-size ratio of 8/1, resolution between the peaks of Pd-DEBT and Pt-DEBT was estimated as 0.4. Increasing the number of theoretical plates (N) is straightforward and can be easily predicted through changes in mobile phase flow rate, mobile phase composition, pH of the aqueous part of the mobile phase, and buffer concentration. Therefore, this path was followed first.

Table 4.1. Comparison of This Study with Other RP-HPLC Methods

Matrix	**Digestion / dissolution method**	**Preconcentration / separation**	**Chelating agent**	**LOD (mg l^{-1})**		**Analysis Time (min)**	**Reference**
				Pd	**Pt**		
Dental alloy	Aqua regia	Complexation on XAD-7 resin	Dimethylglyoxal bis(4-phenyl-3- thiosemicarbazone)	3.25	0.5	24	Hoshi S. et al., 1997
-	-	-	2-(2-thiazolylazo)-5-diethylaminophenol	0.39	-	23	Wang H. et al., 1999
-	-	Salt-induced (NaCl) phase separation	N,N-dialkyl-N'-acylthiourea	2.0	0.1	15	Koch K. R., 2001
Human urine	UV photolysis	Complexation on C_{18} microcolumn	DEBT	-	0.5	7	Philippeit G. et al., 2001
Soil	MW with HNO_3 + H_2O_2 + HF + H_3BO_3	Salt-induced (NaCl) phase separation	DEBT	0.77	0.014	16	This Study

Moreover, by so doing, the optimum values for the different parameters could be found.

Since increasing N did not solve the problem, changing the selectivity factor (α) was necessary. This was achieved by resorting to other combinations of solvents such as methanol-water and acetonitrile-methanol-water. A composition of AcN-MeOH-H_2O (80:10:10, v:v:v) resulted in resolving DEBT, Pt-DEBT and Pd-DEBT completely in 16 minutes.

ICP-OES was used to check for the extent of removal of Pt and Pd from the aqueous phase after applying the salt-induced phase separation of acetonitrile and water. In the literature, phase separation was achieved using 2.00 mol l^{-1} NaCl. However, we have found that this concentration of NaCl was not enough for full recoveries of the metals from the aqueous phase (89.6 % recovery for Pt 83.4 % for Pd). Therefore, salt concentration was increased to 2.20 mol l^{-1}, which resulted in full recoveries of the two metals.

The total amount of PGMs released into the environment by autocatalytic converters can be directly evaluated by determining their content in car exhaust fumes or indirectly by analyzing environmental materials such as soil, road dust etc. In this study, we have chosen to analyze soil for PGMs. However, we were faced with two major difficulties.

First, PGMs, especially Pd, bind strongly to silicate residues even in acidic solutions. Therefore, soil had to be digested completely. This

required the application of oxidizing acids and hydrofluoric acid in a pressure and temperature controlled microwave digestion system. At the end, masking of hydrofluoric acid as tetrafluoroborate was necessary so that the digested solutions could be transferred to glass vessels and could be analyzed by the RP-HPLC method without attacking the silicon matrix of the column.

Second, soil matrices contained six contaminating elements; Ca, Fe and Zn were the main constituents, whereas, Cu, Mn and Co were the trace elements with the highest contents. Accurate determination of Pt and Pd, therefore, required the elimination of these metals. Ca did not form chelates with DEBT. Among the others, Fe (III) and Cu (II) were the only contaminants that were precipitated together with PGMs during complexation below pH 3. To separate these two interfering metals from Pt and Pd, the systematic approach set earlier was also followed. It was found that changing the selectivity factor via changing the mobile phase identity was the most pronounced parameter affecting resolution of the four metal chelates. The final resolution was obtained using a mobile phase composition of AcN-THF-H_2O (44:26:30, v:v:v).

Although this method provided better detection limits compared to other studies using RP-HPLC for Pt and Pd, these detection limits are still high regarding the low concentrations of these metals in environmental samples. Therefore, a combination of a preconcentration procedure, more efficient than the salt-induced phase separation used with this method, would provide an inexpensive, interference-free, sensitive, simple and highly selective method for the simultaneous determination of Pt and Pd by HPLC.

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APPENDIX A

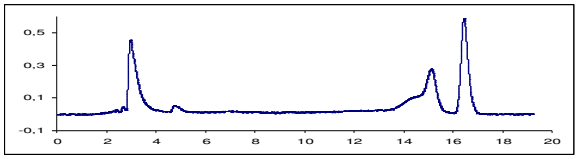
Table A.1. Snyder Solvent Polarity Index

Solvent	**Polarity index** (Snyder L. R., 1974)
Acetic acid	6.2
Acetone	5.4
Acetonitrile	**6.2**
Benzene	3.0
Carbon tetrachloride	1.7
Chloroform	3.4-4.4
Cyclohexane	0
Dichloromethane	3.4
Dimethyl sulfoxide	6.5
Ethanol	5.2
Ethyl acetate	4.3
Ethylene dichloride	3.7
i-octane	0.4
i-propyl ether	2.2
Methanol	**6.6**
Methoxyethanol, 2-	5.7
Methyl acetate	4.4
Methyl ethyl ketone (MEK)	4.5
Methylene chloride	3.4
n-decane	0.3
n-hexane	0
Propanol, 2-	4.3
Pyridine	5.3
Tetrahydrofuran	**4.2**
Toluene	2.3
Water	**9.0**

APPENDIX B

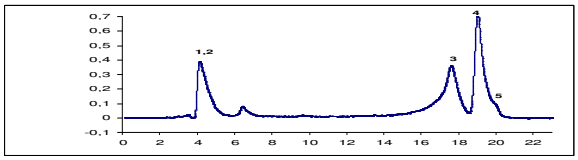
Figure B.1. Some Steps Toward Separation of Interferences

1



P = 6.52. MP: **AcN: MeOH: H_2O** (80:10:10), FR: 0.8 ml min^{-1}, pH: 5.0, Buffer: 0.2 mol l^{-1} Acetate Buffer.

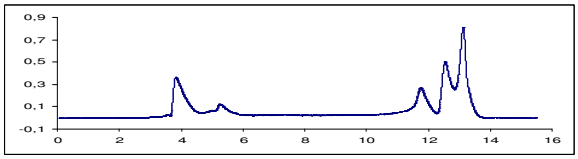
2



P = 6.52 MP: **AcN: THF: H_2O** (44:26:30), FR: 0.8 ml min^{-1}, pH: 5.0, Buffer: 0.1 mol l^{-1} Acetate Buffer.

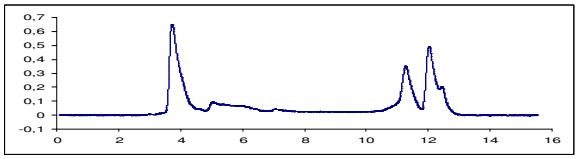
1: DEBT
2: $Fe(DEBT)_3$
3: $Pt(DEBT)_2$
4: $Cu(DEBT)_2$
5: $Pd(DEBT)_2$

3



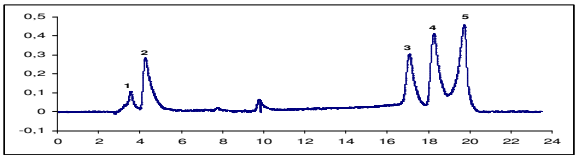
P = 6.36 MP: AcN: THF: H_2O (60:20:20), FR: 0.8 ml min^{-1}, pH: 5.0, Buffer: 0.1 mol l^{-1} Acetate Buffer.

4



P = 6.32 MP: AcN: THF: H_2O (70:15:15), FR: 0.8 ml min^{-1}, pH: 5.0, Buffer: 0.1 mol l^{-1} Acetate Buffer.

5



P = 6.40 MP: AcN: THF: H_2O (38:32:30), FR: 0.8 ml min^{-1}, pH: 5.0, Buffer: 0.1 mol l^{-1} Acetate Buffer.