CHAPTER I

INTRODUCTION

1.1 Problem Definition

Thin layer chromatography is used for the separation and determination of a wide variety of materials ranging from inorganic ions to high molecular weight biological substances. The chief advantages of TLC over the other commonly applied chromatographic methods are convenience, fastness, robustness, and low cost per analysis. It requires minimal sample cleanup, allows high simultaneous sample throughput, offers a wide range of mobile phase and stationary phases and, therefore, gives a high separation selectivity and specific pre- and postchromatographic detection possibilities. It allows an "in-system" calibration and requires minimal quantities of solvents, thus reduces solvent waste.

The poorer separation efficiency and the influence of environmental conditions on the reproducibility of R_f values have , however, been major disadvantage of TLC compared with HPLC and GC. From this disadvantage, TLC is usually used for separation of simple mixtures and for qualitative identification or semiquantitative visual analysis of samples. But this may be compensated for by the use of wider variety of sorbents and solvent systems and by the observation and evaluation of the whole sample track containing the complete chromatogram (1-2).

The separation and determination of inorganic substances by TLC is less applicable than those of organic substances. Especially, metal cations are unfavorable for using this technique. Because of the limit of separation resulted from similar size and chemical properties, therefore, the interested metal cations are interfered by other metal ions. However many research papers showed that some metal cations in the form of metal complexes can be separated from the others and determined by choosing a specific chelating reagent and using optimum stationary phase and solvent systems.

This technique can be used for the separation and determination of the metal cations which give good results in terms of sensitivity, accuracy, and reproducibility (3-5).

The determination of interested metals after their separation on thin layer plates is an important step which affects the accuracy and the reproducibility of the TLC method. There are many approaches to accomplish these ultimate goal. Scraping the interested metal together with the sorbent off the thin layer plate, extracting with suitable solvents and analyzing with appropriated methods such as AAS spectrometry have been carried out. The disadvantage of this approach is the error due to the scraping causing the lost of analytes leading to the uncertainty of analytical results. Additionally , in some cases, the amount of extracted metals is not sufficient for the analytical determination. The comparison of the intensity of spots of analytes and standards is therefore introduced. However, this method gives the accuracy and the reproducibility in the range of 10-30% and is only applicable to the concentration range of 1-10 micrograms. Until the densitometer has been introduced in the determination of interest metals on thin layer plates based on the measurement of the analystes absorbance relative to those of standards. This method seems to give better accuracy and reproducibility and to improve the detection limit. For this reason, this approach is currently widely used in TLC analytical method (6).

In order to apply the method to wider range of sample, the development of the selectivity and efficiency of the method is essential. The efficiency of separation and the sensitivity and selectivity of detection may be accomplished by pre-or postchromatographic derivatization of sample analytes. For the quantitative TLC, this is usually performed by the use of spraying specific reagents to analytes on TLC plates or dipping TLC plates into solution containing specific reagents. In some cases, the use of dipping solution gives better result (7).

Those above-mentioned procedures are usually carried out after TLC development which are obviously time-consumed. For this reason, it is advisable in this study to develop a TLC procedure based on a combined separation and derivatization with the aims of improving the selectivity and speed of the analytical method for the determination of some transition metals without any sacrifice on the accuracy and reproducibility. An organic reagent that gives a specific and spectrometrically sensitive

complex compounds with the interesting metal ion is suggested to be added in the suitable mobile phase solvent for developing process in TLC. This may caused the colored complex reaction equilibrium and adsorption equilibrium occurring on the same time on an adsorbent layer. This *in situ* process makes the metal mixture analysis simple by pressing the selective reaction, separation and spot visualization in single step. The obtained TLC plate is readily further investigated in the metal quantity by a densitometer. The selectivity is improved again by adding a masking agent in the mobile phase.

The interested metals used in this study are first row transition metals since these metals are usually present in various industrial /and environmental samples. The proposed TLC procedure is based on the complexation reaction between the metal ions especially Ni and Pd with dioximes as chelating agents in the presence of triethanolamine as masking agent. This procedure is therefore proposed to be the first TLC method to be used for quantification of the metal analysis.

1.2 Literature Reviews

α-Dioximes as Complexing Agents

α-Dioximes are today considered as one of reagents giving higher selectivity. Dimethylglyoxime (DMG) is the first reagent which discovered by Tschugaeff in 1905. DMG, as well as other α-dioximes, forms insoluble compounds with relatively few elements. Among these are nickel(II), palladium(II), platinum(II), iron(II) and bismuth(III). With many other ions, water soluble complexes are formed (8).

Kolthoff and Langer (9) used dimethylglyoxime to determine nickel by amperometric titration using dropping mercury electrode as indicator electrode. This method is especially useful for the titration of very dilute nickel solution giving good results in a concentration range between 0.01 and 0.0001 M. Other metal ions such as cobalt(II), copper(II) or iron (II) may interfere in the titration. Warren (10) described a spectrophotometric method using dimethylglyoxime as the reagent for the determination of palladium in titanium-base alloys. None of elements in common with

titanium alloys interfere. This method is rapid, simple, reproducible and can determine 0.1-1.0% of palladium in titanium-base alloys.

α-Benzildioxime was introduced and used to the direct *in situ* measurement of nickel (II) by using spectrophotometric method after preconcentration (11). The method is simple, sensitive and selective and the results are, for all practical purpose, independent of sample volume for the same amount of nickel.

Dimethylglyoxime is rather expensive while α-benzildioxime is probably easily prepared. However it is water insoluble and tends to crystallize. This problem may be overcome by introducing the proper substituent into the benzene ring. α-Furildioxime was therefore investigated in 1925 (12). α-Furildioxime was used to determine rhodium spectrometrically(13). The method is free from interference by most ions.

One of water soluble α-dioximes is 1,2-cyclohexanedionedioxime (nioxime). This reagent was first prepared and studied by Wallach in 1924 (14).

α-Dioximes in TLC

Netz and Funk (15) determined nickel and cobalt cations by using HPTLC cellulose F_{254} as stationary phase and ethanol-water-nitric acid (65%) (70+18+12) as mobile phase. After the development the chromatogram was dried for 5 minutes in the stream of cold air, immersed in the dipping solution (1%w/v dimethylglyoxime in ethanol) for 2 seconds, dried for 2 minutes in a stream of cold air and exposed to ammonia vapor for minutes. Nickel cations (hR_f 35-40) appeared as red and cobalt cations (hR_f 40-45) as yellow chromatogram zones on a colorless background. Their detection limits were approximately 20 ng substance per chromatogram zone. Wattanacheep and Wongkachataewon (16) studied the separation and determination of Ni(II), Cu(II) and Co(II) by chelating with 1-(2-pyridylazo)-2-naphthol or dimethylglyoxime on silica gel TLC plates. The chromatograms were developed in EtOH-CHCl$_3$ with the chelating agent as the mobile phase. The light absorption of these chelates were measured by spectrodensitometer. It was found that this method could be used for quantitative analyses of Ni(II) and Cu(II) in the range of 0.02-0.09 μg with relative deviation of 4-14%.

Method of Detection

Two basic methods used for detection analytes in quantitative TLC are direct methods, in which the separated spots are determined *in situ* on the plate; and indirect methods, in which quantitative measurements are carried out after elution of the spots on the chromatoplate (7).

Indirect methods

Sharma, Misra and Gupta (17) determined some toxic metal ions and studied the factor of R_f values by using normal phase, reversed phase and chelation thin layer chromatography. Pb(II) ion was separated from binary mixtures and synthetic alloys, containing Cu(II), Sr(IV), Bi(III), Sb(II), Cd(II), Ni(II) and Hg(II) on DMSO, TBA, TBP and EDTA impregnated silica gel G layers, which DMSO-HNO₃ and DMSO-HCl systems were used as the mobile phase. Pb(II) ion was scratched from the plates and eluted with 1:1:3 $H_2SO_4:HNO_3:H_2O$. Pb (II) ion was determined spectrophotometrically using dithizone in carbontetrachloride. The detection limit is 10 μg with relative error 5%. Similarly, Gupta, Ali , Khurana and Dhagarra (18) studied the separation of eight component mixture of transition metal ions (Cr(III), Mn(II), Fe(II), Co(II), Ni(II), Cu(II), Zn(II) and Ca(II)) on plain and impregnated silica gel layers. The solvent systems used were pyridine-benzene-acetic acid-water (6:5:8:4, v/v) on 1,10-phenanthroline(1%), *n*-butanol-benzene- formic acid (5:10:9, v/v) on DMG (1%) and pyridine-benzene-acetic acid-water (5:5:4:1, v/v) on EDTA (2%). β-naphthol was used as locating reagent. These metal ions were separated and extracted from silica gel G in double distilled water. The concentration of each metal ion was determined by atomic absorption spectrophotometer. The percentage recovery of these metal ions in quantitative estimation by atomic absorption spectrophotometer ranged between 65.2 and 89.9.

Direct methods

In situ densitometry offers a simple way of quantifying directly on the plate. A definition of direct densitometry might be : resolving the compounds to be separated on the chromatoplate and measuring the optical density of the separated spots directly on the plate. The amounts of compounds are determined by comparing them to a standard curve from reference materials chromatographed simultaneously under the same conditions.

Soljic and Jurlina (19) determined platinum and rhodium in Pt-Rh catalysts by *in situ* TLC. Pt and Rh were separated on microcrystalline cellulose with solvent system of methylisobutyl ketone-conc. HCl (40+60, v/v) and KI in ethanol as detecting reagent. The R_f values for Pt and Rh were 0.76 and 0.44 respectively. Pt and Rh on developed plate were determined by TLC scanner at maximum absorbance of 480 nm for Rh and 510 nm for Pt. The linearity range were 0.06-1.5 µg/cm. The limit of detection were approximately 0.06 µg/cm with relative error 0.25-1.00% for Rh and 0.18-0.57% for Pt. The simultaneous determination of light rare earths in monazite sand by densitometery on thin layer chromatograms using diisopropyl ether-diethyl ether-bis(2-ethylhexyl)phosphate-nitric acid (8:8:0.4:0.07) as eluent and 1% carboxymethyl cellulose as stationary phase. The R_f values or lanthanum, cerium, praseodymium, neodymium, samarium and yttrium were 0.13, 0.39, 0.55, 0.69, 0.90 and 0.98, respectively, showing good separation possibilities. The densitometric calibration graphs were linear in the range 0.015-0.06 µg of individual of rare earth metals. The limits of detection for lanthanum, cerium, praseodymium, neodymium and samarium were in the range 9-12 ng (20). Similarly, Maneerattanachatchai and Sirisoksukon (21) studied the development of TLC method for the separation and determination of metal ions by observing their colored chelates on silica gel TLC plates developed in ethanol-chloroform with the complexing agent 1-(2-pyridylazo)-2-naphthol or 8-hydroxyquinoline as mobile phase. The optimal conditions for separation for Ni(II), Co(II), Cu(II), Pd(II), Al(III) and Fe(III) were investigated. Results from this study showed that the quantitative analysis was feasible and recommended. The

standard graphs and the sensitivity for cobalt determination methods were studied and the detection limit in the range of 1.0-5.0 ng of Co(II) with 10% relative error.

Masking Agent

The masking agents were recommended to prevent interfering ions. Masking is a process in which a substance, without physical separation of it or of its reaction products, is so transformed that certain of its reactions are prevented (22). Many reports showed satisfactory results when masking agents were used. The presence of hydroxylamine ensures the quantitative extraction of nickel and prevents interference from Cu(II) and Mn(II). In the presence of large quantities of Co(II) and Fe(III) , the use of triethanolamine as masking agent is recommended (23). Other polyamines are also well applicable for keeping in solution or masking of transition metal ions, while the alkaline earth metals remain free under such circumstances (8).

1.3 Frame Work

The proposed method is used for separation and determination samples that containing some transition metal cations (Cr(III), Mn(II), Fe(III), Co(II), Cu(II), Ni(II), Zn(II) and Pd(II)). Silica gel G was used as stationary phase. The solvent systems used were ethanol, chloroform, and hexane in different ratios. The four α-dioxime compounds namely dimethylglyoxime, α-benzildioxime, α-furildioxime and cyclohexanedionedioxime were chosen as complexing agents. Because they have a very good tendency to form chelates with transition metal ions immediately. The separation of their metal chelates was expected by the difference of their complex stabilities in adsorption on silica gel surface. Ni(II) and Pd(II) ions could be suggested to be separated from other metal ions and were determined by a densitometer. Some masking agents were further used in diminishing interfering ions if necessary. The complexing reagent was added into the mobile phase to form colored complexes. This separated complexes were determined by densitometer. The separation efficiency depends on type of stationary phase, the composition of solvent systems and the

environmental condition during development. Therefore, in this study ,the quantitative analysis of metal cations by TLC were developed, the type and composition of the mobile phase, zone detection, quantities determination and masking agents to prevent interfering ions were studied.

1.4 The Purpose of the Study

1.To study optimal conditions, namely, ratios of solvent systems used in the mobile phases, concentration of ligands, suitable range of concentration of metal ions and masking agent for a rapid and convenient quantitative analysis of metal cations by using TLC.

2. To study of accuracy, reproducibility, sensitivity and detection limit of the proposed methods.

3. To develop a TLC method for the determination of Ni(II) ion or Pd(II) ion in certified reference alloys.