

CHAPTER III

EXPERIMENTAL

3.1 Apparatus

A Camag TLC Scanner II, Switzerland.

A Camag SP 4290 Integrator, Switzerland.

A Camag UV-CABINET II, Switzerland.

A Camag Twin Trough Chamber for 10x10 cm plates, with stainless steel lid

Microsyringe 5.0 μ L (needle has 90° bevel), Hamilton Company, USA.

TLC Aluminium sheets, 20x20 cm Silica gel G 60 F₂₅₄, E.Merck, Darmstadt, Germany.

Graduated pipettes 1.00, 5.00, and 10.00 mL

Volumetric flasks 5.00, 10.00, 25.00, 50.00, and 100.00 mL

Beakers 10, 25, 50, 100, 250, and 500 mL

Plastic microtubes, 1.7 mL, AxyGen Inc., USA.

All glasswares were washed in detergent, rinsed with deionized water and then soaked in dilute HNO₃ overnight.

For the plastic tubes, they were washed in detergent, rinsed with deionized water, soaked in 0.01 M EDTA overnight, soaked in 0.1 M HNO₃ overnight, and finally rinsed with deionized water before use.

3.2 Chemicals

3.2.1 Stock Standard Solutions of Metal Ions

Seven transition metal salt solutions namely chromium (III) nitrate, manganese (II) nitrate, iron (III) nitrate, cobalt (II) nitrate, nickel (II) nitrate, copper (II) nitrate, and palladium (II) nitrate were used. Each stock standard solutions containing 1.000 ± 0.002 g/L ($1000 \mu\text{g/mL}$) of metal ions was prepared by dissolving in 0.5 mol/L HNO_3 .

3.2.2 Organic Solvents

Analytical grade (A.R.) of absolute ethanol, chloroform, and hexane from J.T. Baker Chemical Company, Deventer, Holland were used.

3.2.3 Reagents

Nitric acid and hydrochloric acid, analytical grade, from E.Merck, Darmstadt, Germany.

Dimethylglyoxime (DMG), puriss.p.a, from E.Merck

Diphenylglyoxime (DPG), puriss.p.a, from Fluka

Furildioxime, puriss.p.a, from Sigma

Cyclohexanedionedioxime (nioxime), puriss.p.a; from Fluka

Triethanolamine, purum from Fluka

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จุฬาลงกรณ์มหาวิทยาลัย

3.2.4 Certified Reference Alloys

British chemical standard

- CUPRO-NICKEL alloy no.180/1 : copper 67.36%, nickel 30.85%, iron 0.82%, manganese 0.81%, carbon 0.025%, sulphur 0.010%, silicon < 0.01%, zinc 0.05%, lead < 0.01%, cobalt 0.005%, arsenic 0.006%, antimony < 0.005%, oxygen < 0.005%, and bismuth < 0.0005%

- Ni-Cr-Mo STEEL no.219/3 : nickel 2.50%, chromium 0.76%, carbon 0.31%, molybdenum 0.60%, silicon 0.20%, sulphur 0.01%, phosphorus 0.019%, copper 0.19%, manganese 0.74%

- NICKEL STEEL no. 222/1: nickel 3.54%, carbon 0.32%, silicon 0.22%, sulphur 0.009%, phosphorus 0.020%, manganese 0.62%, chromium 0.05%, molybdenum 0.029%, copper 0.14% and 0.037%

3.3 Preparation of the Standard Metal Solutions

The working standard solutions of each metal ion (Cr(III), Mn(II) Fe(III), Co(II), Ni(II), Cu(II), and Pd(II)) were prepared from 1000 µg/mL stock standard solution of metal ions. These solutions were suitably diluted to give concentration of each metal ions in the range 5-500 µg/mL with deionized water.

3.4 Preparation of the Ethanolic Ligand Solutions

Among the three solvents used as mobile phase; ethanol, chloroform, and hexane; ethanol gave good solubility for all ligands used. Ethanolic ligand solutions were prepared at high concentration as possible.

4.1 Dimethylglyoxime 1.0%w/v in ethanol

The 1.0%w/v of dimethylglyoxime in ethanol was prepared by dissolving 1.0 g and diluting it to the mark with ethanol in a 100.00 mL volumetric flask.

4.2 Benzildioxime 0.025%w/v in ethanol

The 0.025% w/v of benzildioxime in ethanol was prepared by dissolving

0.025 g and diluting it to the mark with ethanol in a 100.00 mL volumetric flask.

4.3 Furildioxime 0.5% w/v in ethanol

The 0.5% w/v of furildioxime in ethanol was prepared by dissolving 0.5 g and diluting it to the mark with ethanol in a 100.00 mL volumetric flask.

4.4 Nioxime 0.1% w/v in ethanol

The 0.1% w/v of nioxime in ethanol was prepared by dissolving 0.1 g and diluting it to the mark with ethanol in a 100.00 mL volumetric flask.

3.5 Preparation of Developing Solutions

The developing solutions were composed of mixed solvent of ethanol, and chloroform, or ethanol, chloroform and hexane in various ratio as mobile phases with various amount of the ligand added.

3.6 Preparation of TLC Plates

The stationary phase was commercial precoated silica gel G 60 F₂₅₄ which was cut into 10x10 cm or 5x10 cm and marked the starting line above the lower edge of the plate about 1.5 cm and the solvent front line about 8.0 cm from the starting line.

3.7 Preliminary Study of the Formation of Complexes between Metal Ions and Ligands on TLC Plates and the Factors of R_f Values.

To study the formation of complexes between the metal ions (Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), and Pd(II)) and the four ligands (dimethylglyoxime, benzildioxime, furildioxime, and nioxime) on precoated silica gel G TLC plates, 1.0 µL of each metal standard solutions were applied as a series of spots to TLC plates of 10x10 cm size at the starting line by using a 5.0 µL microsyringe. The spots were dried with hot air, and the plates were developed in twin trough chamber containing developing solution. This plate was developed by using one-dimensional ascending technique. Before developing, the chamber that contained the developing solution was

covered with its lid for about 5 minutes so that the chamber would be presaturated with the mobile phase vapor. The ligand moved to the spot together with the mobile phase and reacted with the spotted metal. The colored complexes were formed and moved on the plate under the distribution equilibrium between two phases.

It was convenient to classify these experiments into four groups regarding the ligand used (Group I DMG, Group II DPG, Group III furildioxime, and Group IV nioxime). The compositions of the developing solution (mobile phase solution and ligand concentration) and the amount of spotted metal were varied to obtain good round spots of the complex and the difference in distance on TLC plate. The characteristic and the R_f values of the observed spots were compared. The optimum condition for separation and quantification of metal cations was then selected.

3.7.1 Study of the Composition of the Mobile Phase

To study the effect of the composition of the mobile phase to the formation of complexes between metal ions and ligands and their locations on TLC plate, the procedure for study of the composition of the mobile phase was as follows:

1. The seven standard metal solutions prepared for spot application were 100 $\mu\text{g/mL}$ of Cr(III), 100 $\mu\text{g/mL}$ of Mn(II), 100 $\mu\text{g/mL}$ of Fe(III), 100 $\mu\text{g/mL}$ of Co(II), 100 $\mu\text{g/mL}$ of Ni(II), 100 $\mu\text{g/mL}$ of Cu(II), and 100 $\mu\text{g/mL}$ of Pd(II).
2. 1.0 μL of each standard solution (100 ng) was applied as a series of spots on 10x10 cm TLC plates at the starting line by using a 5.0 μL microsyringe.
3. The spots were dried with hot air and the plate was developed in the twin trough chamber (12.1x10.8x8.3 cm) containing 10.00 mL of developing solution at certain concentration of the ligands.
4. The mobile phase was allowed to migrate up to 8.0 cm from the starting line.
5. After development, the plate was dried with hot air and the spots were examined in the UV-CARBINET II.
6. The characteristic of spots were observed and the distance of spots were measured to calculate R_f values.

7. The other experiments were carried out in the same manner by varying developing solution (different ligands and different composition of mixed solvent mobile phase) as

Group I DMG ; 0.05%w/v DMG in various mobile phase when the compositions of mobile phase were $\text{CHCl}_3:\text{EtOH}$ (9:1), $\text{CHCl}_3:\text{EtOH}$ (8:2), and $\text{CHCl}_3:\text{EtOH}$ (5:5).

Group II DPG ; 0.0075%w/v DPG in various mobile phase when the compositions of mobile phase were $\text{C}_6\text{H}_{14}:\text{EtOH}$ (8.5:1.5), $\text{C}_6\text{H}_{14}:\text{CHCl}_3:\text{EtOH}$ (5.7:2.8:1.5), $\text{C}_6\text{H}_{14}:\text{CHCl}_3:\text{EtOH}$ (4.25:4.25:1.5), $\text{C}_6\text{H}_{14}:\text{CHCl}_3:\text{EtOH}$ (2.8:5.7:1.5), and $\text{C}_6\text{H}_{14}:\text{CHCl}_3:\text{EtOH}$ (3.75:3.75:2.5).

Group III Furildioxime ; 0.075%w/v furildioxime in various mobile phase when the compositions of mobile phase were $\text{C}_6\text{H}_{14}:\text{CHCl}_3:\text{EtOH}$ (1.7:6.8:1.5), $\text{C}_6\text{H}_{14}:\text{CHCl}_3:\text{EtOH}$ (1.2:7.3:1.5), $\text{C}_6\text{H}_{14}:\text{CHCl}_3:\text{EtOH}$ (1.0:7.5:1.5), $\text{C}_6\text{H}_{14}:\text{CHCl}_3:\text{EtOH}$ (1.4:6.4:2.2), and $\text{CHCl}_3:\text{EtOH}$ (4.3:5.7).

Group IV Nioxime ; 0.015%w/v nioxime in various mobile phase when the compositions of mobile phase were $\text{C}_6\text{H}_{14}:\text{CHCl}_3:\text{EtOH}$ (1.2:7.3:1.5), $\text{C}_6\text{H}_{14}:\text{CHCl}_3:\text{EtOH}$ (1.0:7.5:1.5), $\text{CHCl}_3:\text{EtOH}$ (7.7:2.3), $\text{CHCl}_3:\text{EtOH}$ (6.8:3.2), and $\text{CHCl}_3:\text{EtOH}$ (5.7:4.3).

8. The solvent systems that gave good results were chosen for further experiments.

9. The results were shown in the Tables 4.1, 4.2, 4.3 and 4.4.

3.7.2 Study of the Concentration of the Ligands in Developing Solution

To study the effect of the amount of ligand in developing solution, the procedure for study of the optimum concentration of ligand was as follows:

1. The two standard metal solutions prepared for spot application were 100 $\mu\text{g}/\text{mL}$ of Ni(II), and 100 $\mu\text{g}/\text{mL}$ of Pd(II).
2. 1.0 μL of each standard solution (100 ng) was applied as two spots side by side on 5x10 cm TLC plate at the starting line by using a 5.0 μL microsyringe.
3. The spots were dried with hot air and the plate was developed in the twin trough chamber (12.1x10.8x8.3 cm) containing 10.00 mL of the developing solution that composed of the mobile phase (that chosen from 3.7.1).

4. The mobile phase was allowed to migrate up to 8.0 cm from the starting line.
5. After development, the plate was dried with hot air and the spots were examined in the UV-CARBINET II.
6. The characteristic of spots were observed and the distance of spots were measured to calculate R_f values.
7. The other experiments were carried out in the same manner by varying developing solution (different ligands and different ligand concentrations) as

Group I DMG; when the concentrations were 0.025%, 0.05%, and 0.1%w/v DMG in 9:1 CHCl_3 :EtOH.

Group II DPG; when the concentrations were 0.0025%, 0.005%, and 0.0075%w/v DPG in 5.7:2.8:1.5 C_6H_{14} : CHCl_3 :EtOH.

Group III Furildioxime; when the concentrations were 0.025%,0.05%, and 0.075%w/v furildioxime in 1.2:7.3:1.5 C_6H_{14} : CHCl_3 :EtOH.

Group IV Nioxime; when the concentrations were 0.005%, 0.0075%, 0.01%, 0.015%, and 0.02%w/v nioxime in 1.0:7.5:1.5 C_6H_{14} : CHCl_3 :EtOH.

8. The concentration of the ligand in developing solution that gave good results were chosen for further experiments.
9. The results were shown in the Tables 4.5,4.6,4.7 and 4.8.

3.7.3 Study of the Spotted Amount of Metal Ions on TLC Plate

To study the effect of the amounts of Ni(II) and Pd(II) on the TLC plate, the procedure for study of the optimum range of metal amount was as follows:

1. The two series of various concentrations of standard metal solutions prepared for spot application were a) 10, 50, 80, 100, 120, 150, 200 and 500 $\mu\text{g}/\text{mL}$ of Ni(II) and b) 10, 50, 80, 100, 120, 150, 200 and 500 $\mu\text{g}/\text{mL}$ of Pd(II).
2. 1.0 μL of each standard metal solution (10-500 ng) was applied as series of spot on 10x10 cm TLC plate at the starting line by using a 5.0 μL microsyringe.
3. The spots were dried with hot air and the plate was developed in the twin trough chamber (12.1x10.8x8.3 cm) containing 10.00 mL of the developing solution (that chosen from 3.7.2).

4. The mobile phase was allowed to migrate up to 8.0 cm from the starting line.
5. After development, the plate was dried with hot air and the spots were examined in the UV-CARBINET II.
6. The characteristic of spots were observed and the distance of spots were measured to calculate R_f values.
7. The other experiments were carried out in the same manner by varying metal and the amount of metal that spotted on TLC plate as

Group I DMG; on the first plate, amount of Ni(II) were 10, 50, 100, 150, and 200 ng/spot, on the second plate, amount of Pd(II) were 10, 50, 100, and 200 ng/spot, and developing solution as 0.05%w/v DMG in 1:9 CHCl_3 :EtOH.

Group II DPG; on the first plate, amount of Ni(II) were 10, 50, 100, and 500 ng/spot, on the second plate, amount of Pd(II) were 10, 50, 100, and 500 ng/spot, and developing solution as 0.0075%w/v DPG in 5.7:2.8:1.5 C_6H_{14} : CHCl_3 :EtOH.

Group III Furildioxime; on the first plate, amount of Pd(II) were 10, 50, 80, and 100 ng/spot, and developing solution as 0.075%w/v furildioxime in 1.2:7.3:1.5 C_6H_{14} : CHCl_3 :EtOH.

Group IV Nioxime; on the first plate amount of Ni(II) were 10, 50, 80, 100 and 120 ng/spot, on the second plate, amount of Pd(II) were 10, 50, 80, 100, and 120 ng/spot, and developing solution as 0.015%w/v nioxime in 1.0:7.5:1.5 C_6H_{14} : CHCl_3 :EtOH.

8. The range of metal amount on TLC plate that gave good results were chosen for further quantification experiments.

9. The results were shown in the Tables 4.9, 4.10, 4.11 and 4.12.

3.8 Densitometric Study for Quantification of Ni(II) and Pd(II)

In the previous experiments (Part 3.7.1-3.7.3), seven chromatographic conditions showed possibility for the quantitative determination of Ni(II) and Pd(II) by using the α -dioximes as complexing agent. The colored complexes that formed on the plates were located by visual observation (with or without 254 nm light). The characteristic of these complex zones were reported and the distance of spots were measured to calculate R_f values. These optimum conditions of mobile phase, ligand concentration and metal amount were shown in Table 3.1. For quantitative analysis, light absorption of the spot was measured as a function of metal amount by a densitometer. Some important densitometric parameters were optimized. The calibration curve, the linearity, the repeatability, the sensitivity and the detection limit of the method in each system were investigated.

Table 3.1 The optimum conditions for quantitative analysis of Ni(II) and Pd(II).

Metal complex	Mobile phase	Concentration of Ligand (%w/v)	Amount of Metal (ng/spot)
Ni-DMG	CHCl ₃ :EtOH (9:1).	0.05	10-100
Pd-DMG	CHCl ₃ :EtOH (9:1).	0.05	100-200
Ni-DPG	C ₆ H ₁₄ :CHCl ₃ :EtOH (5.7:2.8:1.5)	0.0075	10-100
Pd-DPG	C ₆ H ₁₄ :CHCl ₃ :EtOH (5.7:2.8:1.5)	0.0075	20-100
Pd-furildioxime	C ₆ H ₁₄ :CHCl ₃ :EtOH (1.2:7.3:1.5)	0.075	10-100
Ni-nioxime	C ₆ H ₁₄ :CHCl ₃ :EtOH (1.0:7.5:1.5)	0.015	10-100
Pd-nioxime	C ₆ H ₁₄ :CHCl ₃ :EtOH (1.0:7.5:1.5)	0.015	50-120

3.8.1 The Procedure for the Determination of the Optimum Absorbed Wavelength of Complexes on TLC Plates and the Densitometric Parameter Optimization

1. The two standard metal solutions prepared for spot application were 100 $\mu\text{g/mL}$ of Ni(II) and 100 $\mu\text{g/mL}$ of Pd(II).
2. 1.0 μL of each standard solutions containing 100 ng was applied as two spots side by side on 5x10 cm on TLC plate by using a 5.0 μL microsyringe.
3. The spots were dried with hot air and the plate was developed in the twin trough chamber containing a suitable developing solution (see Table 3.1).
4. The mobile phase was allowed to migrate up to 8.0 cm from the starting line.
5. After development, the plate was dried with hot air and the spots were examined by using a Camag UV-CARBINET II.
6. Spot of the colored complex on the developed plate was scanned comparing with background area to obtain the absorption spectrum of the complex (200-400 nm) by densitometer.
7. The light absorption of the spots was measured again at their chosen wavelength. The sensitivity of the densitometer was adjusted by increasing value until photomultiplier showed input signal over range.
8. The optimum sensitivity values was chosen for further experiment.
9. The results were shown in the Table 4.14.

Table 3.2 The densitometric condition setted for spectrum determination.

TLC Scanning densitometric parameter	TLC Scanning densitometric condition
Wavelength range (source)	200-400 nm (D ₂ lamp)
Wavelength increment	10 nm
Scanning speed	1.0 mm/sec
Slit width	2 nm
Sensitivity	100-220
Chart speed	2 cm/min
Attenuation	128 mV (maximum value of integrator)

3.8.2 Procedures for the Study of Repeatability of Calibration curves and Linearity of each Metal-Complex

1. Series of different concentration of standard solutions of Ni(II) or Pd(II) preparation for spot application were in the range that reported in Table 3.1.
2. 1.0 μL of each concentration was applied as a series of spots on 10x10 cm TLC plates at the starting line by using a 5.0 μL microsyringe (10 to 200 ng).
3. The spots were dried with hot air and the plate was developed in the twin trough chamber containing a suitable developing solution (see Table 3.1).
- 4 The mobile phase was allowed to migrate up to 8.0 cm from the starting line.
5. After development, the plate was dried with hot air and the spots were examined by using a Camag UV-CARBINET II.
6. The light absorption of spots were measured as a function of metal amount (ng) by a densitometer at their suitable wavelength and sensitivity.
7. From the densitogram obtained, height and integration area of peaks were measured and plotted against the amount of metal (ng) to give a calibration curves and straight part of the curve was brought forth a linear range.
8. The data were statistically calculated and a regression obtained according to the least squares.
9. The repeated experiments were carried out on new plates to observe the distribution zones of calibration lines (about 4-5 times repeatedly).
10. Repeatabilities of line were reported as relative standard deviation ($n=4$) and sensitivities as peak height or peak area per ng.
11. The results were shown in the Tables 4.15-4.29 and the Figures 4.9-4.22.

3.8.3 The Procedure for Study of Repeatability of the Proposed Procedure on the Same TLC Plate

- 1 Series of different concentration of Ni(II) or Pd(II) in the linear range (that chosen from Table 4.15) were prepared for spot application.
2. 1.0 μL of a certain concentration were applied as a series of same amount metal spots on 10x10 cm TLC plate at the starting line by using a 5.0 μL microsyringe.
3. The spots were dried with hot air and the plate was developed in the twin trough chamber containing a suitable developing solution (see Table 3.1).
4. The mobile phase was allowed to migrate up to 8.0 cm from the starting line.
5. After development, the plate was dried with hot air and the spots were examined by using a Camag UV-CARBINET II.
6. The light absorption of spots were measured as a function of metal amount (ng) by a densitometer at their suitable wavelength and sensitivity.
7. From the densitogram obtained, height of peaks were measured; mean, standard deviation and relative standard deviation of each amount of metal were calculated.
8. For other concentrations of each metal, experiments were carried out in the same manner.
9. Repeatabilities were reported as relative standard deviation. This error was regarded as the whole method error.
10. A spot on the obtained chromatographic plate was scanned again seven times by resetting the scanning position.
11. The seven peak heights were measured and relative standard deviation of each amount of metals were calculated. This error was regarded as the densitometric step error.
12. A spot on the obtained chromatographic plate was scanned eleven times by fixing the scanning position.
13. The eleven peak heights were measured and relative standard deviation of each amount of metals were calculated. This error was regarded as the instrumental error.
14. The results were shown in Tables 4.30-4.36.

3.8.4 The Procedure for the Determination of Detection Limit

- 1 Series of different concentration of standard Ni(II) or Pd(II) solutions in the range 1-100 $\mu\text{g/mL}$ were prepared for spot application.
- 2 1.0 μL of each concentrations was applied as a series of spots on 10x10 cm TLC plate at the starting line by using a 5.0 μL microsyringe.
3. The spots were dried with hot air and the plate was developed in the twin trough chamber containing a suitable developing solution (see Table 3.1).
4. The mobile phase was allowed to migrate up to 8.0 cm from the starting line.
5. After development, the plate was dried with hot air and the spots were examined by using a Camag UV-CARBINET II.
- 6 The light absorption of spots were measured as a function of metal amount (ng) by a densitometer at their suitable wavelength and sensitivity.
7. From the densitogram obtained, height of the background signal and the analytical signal were measured.
8. The method was repeated with successive lowering of the amount of metal ion until spots could no longer be detected.
9. The detection limit of the methods were calculated and the results were shown in the Table 4.37.

3.9 Study of the Ions Interference in the Methods

The effect of the other metal ions such as Cr(III), Mn(II), Fe(II), Co(II), Cu(II), and Zn(II) on the formation of Ni-complexes and Pd-complexes were studied. Though these metals give large different R_f values from Ni or Pd on the plate by the chromatographic conditions and had less complex formation constant, large amount of these competitive ions may cause ligand deficiency and affect the position and/or amount of Ni or Pd complex on TLC plate. This can be studied using binary mixture of Ni or Pd with the expected interfering ions at various ratios (1 to 50 folds).

3.9.1 The Procedure for Study of the Interfering Ions

1. Series of binary metal mixture solutions containing a certain concentration of Ni(II) (or Pd(II)) with various concentration of each metal ion (Cr(III), Mn(II), Fe(II), Co(II), Cu(II), or Zn(II)) were prepared for spot application solutions. The mass ratio of Ni(II) (or Pd(II)) and the other metal ion were 1:1, 1:5, 1:10, 1:20, 1:30, 1:40, and 1:50 respectively.
2. 1.0 μL of each binary mixture solutions were applied as a series of spots on 10x10 cm TLC plate at the starting line by a using 5.0 μL microsyringe and single Ni(II)(or Pd(II)) solution was also spotted side by side to with the mixture.
3. The spots were dried with hot air and the plate was developed in the twin trough chamber containing a suitable developing solution (see Table 3.1).
4. The mobile phase was allowed to migrate up to 8.0 cm from the starting line.
5. After development, the plate was dried with hot air and the spots were examined by using a Camag UV-CARBINET II.
6. The characteristic of metal complex spots were observed and the distance of spots were measured to calculate R_f values.
7. The light absorption of Ni-complex (or Pd-complex) spots were measured as a function of Ni (or Pd) amount by a densitometer.
8. The results were shown in the Tables 4.38-4.42.

3.9.2 The Study of the use of Triethanolamine as the Masking Agent

Among the frequent masking agents used to eliminate these interference, triethanolamine was chosen because of its good solubility in ethanol and chloroform. A suitable amount of triethanolamine added in developing solution was studied including its effect on the calibration of Ni-dioxime or Pd-dioxime complex.

3.9.2.1 The Procedure for the Study of the Ability of Triethanolamine in Lowering the Interference

1. Series of binary metal mixture solutions containing a certain concentration of Ni(II) (or Pd(II)) with various concentration of each metal ion (Cr(III), Mn(II), Fe(III), Co(II), Cu(II), or Zn(II)) were prepared for spot application. The ratio of Ni(II) (or Pd(II)) and the other metal ion were 1:1, 1:5, 1:10, 1:20, 1:30, 1:40 and 1:50 respectively.
2. 1.0 μL of each binary mixture solutions were applied as a series of spots on 10x10 cm TLC plate at the starting line by using a 5.0 μL microsyringe and single Ni(II) (or Pd(II)) solution was also spotted side by side to the mixture.
3. The spots were dried with hot air and the plate was developed in the twin trough chamber containing a suitable developing solution (see Table 3.1) and 1.00 mL of triethanolamine was added into 10.00 mL of this solution.
4. The mobile phase was allowed to migrate up to 8.0 cm from the starting line.
5. After development, the plate was dried with hot air and the spots were examined by using a Camag UV-CARBINET II.
6. The characteristic of metal complex spots were observed and the distance of spots were measured to calculate R_f values.
7. The light absorption of Ni-complex (or Pd-complex)) spots were measured as a function of Ni(II) (or Pd(II)) amount by a densitometer.
8. The results were shown in the Tables 4.43-4.48.

3.9.2.2 The Procedure for the Study of an Effect of Added Triethanolamine on Ni- α -dioxime Complex Calibration curve

1. Series of different standard Ni(II) solutions prepared for spot application were in the range of 5 to 40 $\mu\text{g/mL}$.
2. 1.0 μL of each concentration was applied as a series of spots on 10x10 cm TLC plate at the starting line by using a 5.0 μL microsyringe (5 to 25 ng for Ni-nioxime complex and 10 to 40 ng for Ni-DMG complex).

3. The spots were dried with hot air and the plate was developed in the twin trough chamber containing a suitable developing solution (see Table 3.1), and 1.00 mL of triethanolamine was added into 10.00 mL of this solution.
4. The mobile phase was allowed to migrate up to 8.0 cm from the starting line.
5. After development, the plate was dried with hot air and the spots were examined by using a Camag UV-CARBINET II.
6. The light absorption of spots were measured as a function of metal amount (ng) by a densitometer at their suitable wavelength and sensitivity.
7. From the densitogram obtained, height of peaks were measured and plotted against the amount of metal ion (ng) to give a calibration curves and straight part of the curve was brought forth a linear range.
8. The experiments were carried out again by changing amount of triethanolamine in developing solution (1.00, 1.50, 2.00, 3.00, 5.00 and 7.00 mL).
9. The slopes and interceptions of these calibration curves were compared.
10. The results were shown in the Figures 4.23 and 4.24.

3.10 The Quantitative Determination of Nickel in Certified Reference Alloys

The Procedure for Quantitative Determination of Nickel Ion in Certified Reference Alloys

1. The sample solutions were prepared by dissolving an accurate amounts of powdered certified reference alloys in 37%v/v hydrochloric acid and conc. nitric acid. This solutions were subsequently diluted to give a sufficient amount of analytes.
2. Series of various concentration of standard Ni(II) solution prepared for spot application were 5 to 25 $\mu\text{g/mL}$ for Ni-nioxime complex and 10 to 40 $\mu\text{g/mL}$ for Ni-DMG complex.
3. 1.0 μL of each standard solutions of Ni(II) and sample solutions were applied as a series of spot on 10x10 cm TLC at the starting line by using a 5.0 μL microsyringe.

4. The spots were dried with hot air and the plates were developed in the twin trough chamber containing a suitable developing solution (Table 3.1) and 1.50 mL of triethanolamine was added into this solution.
5. The mobile phase was allowed to migrate up to 8.0 cm from the starting line in all case.
6. After development, the plates were dried with hot air and the spots were examined by using a Camag UV-CARBINET II.
7. The light absorption of spots were measured as a function of Ni(II) amount by a densitometer.
8. From the densitogram obtained, peak height were measured and plotted against the various amount of metal ion.
9. The data were statistically calculated and regression lines were obtained according to the least squares.
10. The amounts of Ni(II) in samples were determined from this line and the accuracy of the method was calculated.
11. The results were shown in the Tables 4.50 and 4.51.