CHAPTER IV

RESULTS AND DISCUSSION

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

By using the developing solution for TLC plate in developing step, it was necessary to investigate the complex formation conditions. The dissolving ligand together with the mobile phase solvents that moved toward to the metal ion applied on silica gel layer caused the colored complex reaction equilibrium and adsorption equilibrium occurring on the same time. This *in situ* process makes the metal mixture analysis simple by pressing complex formation, separation, and spot visualization in single step. Shape of the complex area, color intensity, and its location on the plate depend on both the complex reaction factors and chromatographic factors: type and amount of metal, type and amount of ligand, type of stationary phase and mobile phase composition.

4.1.1 Study of the Composition of the Mobile Phase in Developing Solution

First, suitable mobile phase compositions for metal complex of each ligand (Group I DMG, Group II DPG, Group III furildioxime, and Group IV nioxime) were investigated and the results expressed in characteristic of complex (spot or band) and calculated R_f values.(Tables 4.1, 4.2, 4.3 and 4.4).

Table 4.1 The characteristic and R_f values of complexes between metal ions and dimethylglyoxime (DMG)(0.05%w/v in various mobile phase systems).

Metal ion 100 ng	Characteristic and (R_f values) of metal-DMG complexes		
	S_1	S_2	S_3
Cr(III)	spot (0.00)	spot (0.00)	spot (0.00)
Mn(II)	spot (0.00)	spot (0.00)	spot (0.00)
Fe(III)	spot (0.00)	spot (0.00)	spot (0.00)
Co(II)	band (0.00-0.22)	band (0.00-0.20)	band (0.00-0.42)
Ni(II)	spot (0.75)	spot (0.89)	spot (0.93)
Cu(II)	band (0.00-0.28)	band (0.00-0.53)	band (0.00-0.44)
Pd(II)	spot (0.63)	spot (0.83)	spot (0.89)
DMG	background (0.51)	background (0.76)	background (0.85)

Abbreviation of mobile phase component systems :-

S_1 $CHCl_3$:EtOH (9:1)

S_2 $CHCl_3$:EtOH (8:2)

S_3 $CHCl_3$:EtOH (5:5)

Table 4.2 The characteristic and R_f values of complexes between metal ions and benzildioxime (DPG) (0.0075%w/v in various mobile phase systems).

Metal ion 100 ng	Characteristic and (R_f values) of metal-DPG complexes				
	S_1	S_2	S_3	S_4	S_5
Cr(III)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)
Mn(II)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)
Fe(III)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)
Co(II)	spot (0.00)	band (0.00-0.71)	band (0.00-0.81)	band (0.00-0.70)	band (0.00-0.90)
Ni(II)	spot (0.00)	spot (0.74)	spot (0.88)	spot (0.89)	spot (0.93)
Cu(II)	spot (0.00)	band (0.00-0.68)	band (0.00-0.79)	band (0.00-0.69)	band (0.00-0.89)
Pd(II)	spot (0.00)	spot (0.76)	spot (0.89)	spot (0.88)	spot (0.93)
DPG	background (0.64)	background (0.66)	background (0.64)	background (0.64)	background (0.64)

Abbreviation of mobile phase component systems :-

S_1 C_6H_{14}:EtOH (8.5:1.5)

S_2 C_6H_{14}:$CHCl_3$:EtOH (5.7:2.8:1.5)

S_3 C_6H_{14}:$CHCl_3$:EtOH (4.25:4.25:1.5)

S_4 C_6H_{14}:$CHCl_3$:EtOH (2.8:5.7:1.5)

S_5 C_6H_{14}:$CHCl_3$:EtOH (3.75:3.75:2.5)

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Table 4.3 The characteristic and R_f values of complexes between metal ions and furildioxime (0.075%w/v in various mobile phase systems).

Metal ion 100 ng	Characteristic and (R_f values) of metal-furildioxime complexes				
	S_1	S_2	S_3	S_4	S_5
Cr(III)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)
Mn(II)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)
Fe(III)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)
Co(II)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)
Ni(II)	band (0.00-0.51)	band (0.00-0.70)	band (0.00-0.70)	band (0.00-0.53)	band (0.00-0.53)
Cu(II)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)
Pd(II)	spot (0.50)	spot (0.74)	spot+tail (0.73)	spot (0.52)	spot (0.51)
Furildioxime	background (0.41)	background (0.36)	background (0.34)	background (0.45)	background (0.46)

Abbreviation of mobile phase component systems :-

S_1 C_6H_{14}:$CHCl_3$:EtOH(1.7:6.8:1.5)

S_2 C_6H_{14}:$CHCl_3$:EtOH (1.2:7.3:1.5)

S_3 C_6H_{14}:$CHCl_3$:EtOH (1.0:7.5:1.5)

S_4 C_6H_{14}:$CHCl_3$:EtOH (1.4:6.4:2.2)

S_5 $CHCl_3$:EtOH (4.3:5.7)

Table 4.4 The characteristic and R_f values of complexes between metal ions and nioxime (0.015%w/v in various mobile phase systems).

Metal ion 100 ng	Characteristic and (R_f values) of metal-nioxime complexes				
	S_1	S_2	S_3	S_4	S_5
Cr(III)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)
Mn(II)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)
Fe(III)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)	spot (0.00)
Co(II)	band (0.00-0.10)	band (0.00-0.22)	band (0.00-0.19)	band (0.00-0.24)	band (0.00-0.15)
Ni(II)	spot (0.66)	spot (0.70)	band (0.00-0.83)	band (0.00-0.88)	band (0.00-0.83)
Cu(II)	band (0.00-0.20)	band (0.00-0.34)	band (0.00-0.56)	band (0.00-0.72)	band (0.00-0.56)
Pd(II)	spot (0.70)	spot (0.75)	band (0.00-0.77)	band (0.00-0.83)	band (0.00-0.75)
Nioxime	background (0.24)	background (0.26)	background (0.19)	background (0.20)	background (0.20)

Abbreviation of mobile phase component systems :-

S_1 C_6H_{14}:$CHCl_3$:EtOH(1.2:7.3:1.5)

S_2 C_6H_{14}:$CHCl_3$:EtOH (1.0:7.5:1.5)

S_3 $CHCl_3$:EtOH (7.7:2.3)

S_4 $CHCl_3$:EtOH (6.8:3.2)

S_5 $CHCl_3$:EtOH (5.7:4.3)

Seven metal ions, namely Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), and Pd(II) were chosen as the tested metals because these metal ions could react with α-dioximes. The N,N donor ligands give very high formation constant and water insoluble complex. The two anionic ligands are interconnected by strong intramolecular hydrogen bonds which thus stabilize the 1:2 metal : ligand chelate.

The presence of the two hydrogen bridges in these complexes makes ring closure and yields planar structure showed in Figure 4.1.

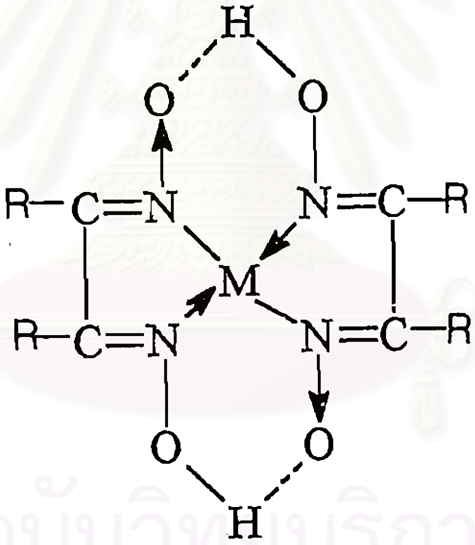

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Figure 4.1 The planar structure of metal-α-dioxime complex.

The consecutive order of the strength of hydrogen bridges was Pd(II) ≈ Ni(II) ≈ Cu(II) > Co(II) > Mn(II) in the complexes. In the case of Pd(II) and Ni(II) complexes, the molecules are interconnected by metal-metal bonds while in the case of the Cu(II) complex, the molecules are interconnected by metal-oxygen bonds. Unlike the metal-complexes mentioned above, the charged Fe(III) and Cr(III) complexes have a distorted octahedral structure with metal-oxygen bonds along Z-axis (8, 34).

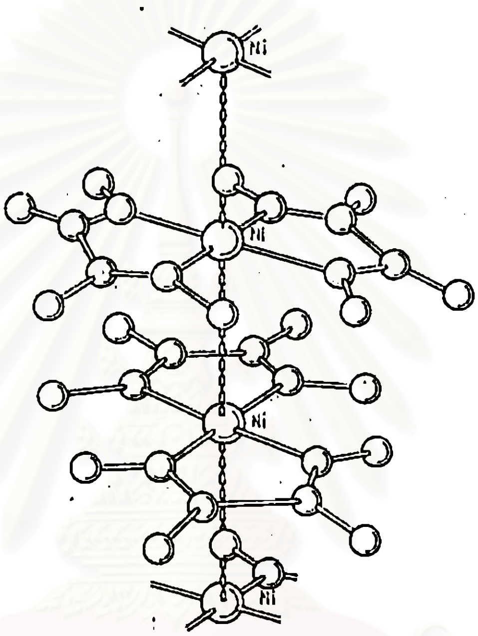

Figure 4.2 Structure model of the nickel dimethylgloxime complex. The nickel-nickel bonds can be seen.

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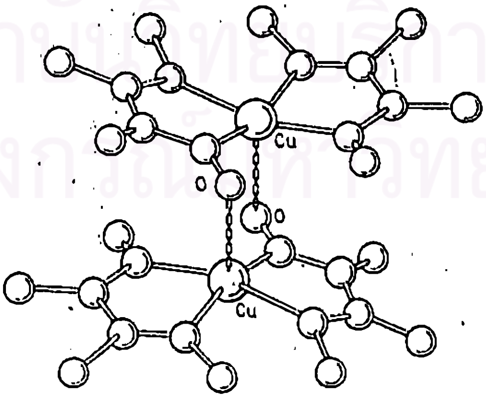

Figure 4.3 Structure model of the copper dimethylglyoxime complex. The Cu-O bonds can be seen.

The obtained chromatograms showed good agreement on the basis of this order. Most complexes of Pd(II), Ni(II), Cu(II), and Co(II) moved from the starting line and in all cases of the experimented dioximes, the R_f values of Pd(II) and Ni(II) complexes were higher than those of Cu(II), Co(II) and Mn(II) complexes. The other complexes of Cr(III), and Fe(III) were seen on the original line ($R_f = 0.00$). These zero R_f value may do so owing to strong adsorption due to charge of the cationic complexes.

On the study of dipolar effect of mobile phase on chromatograms, with the addition of ethanol (10-50%v/v) in aprotic less polar hexane and chloroform, the R_f values of the metal complexes are given in Tables 4.1 to 4.4. Since the planar coordination of metal-nitrogen with strong intramolecular hydrogen bonds leads to the less dipolar structure of Pd(II) and Ni(II) complexes, degree of their migration corresponds to higher distance with hexane and chloroform. Most spots of Pd(II) and Ni(II) complexes are well-defined while tailing or band in lower R_f were observed for those of Cu(II) and Co(II). Adding ethanol to the mobile phase, movement of those complexes on silica gel generally increased.

The mechanism of migration influenced by ethanol in mobile phase could be proposed on three points;

1. The complex could be adsorbed on stationary phase by interaction between silanol group on silica gel surface and loose hydrogen bridge of the complex. However, these weak hydrogen bonds are polar part of the complex molecule and have a good tendency to be solvated by high dielectric ethanol. R_f value of the complex could increase due to increase in higher content of ethanol in mobile phase.

2 When the concentration of ethanol in mobile phase is higher, the silica surface becomes solvated by the polar molecule. This increases eluting power of mobile phase corresponding to higher R_f value.

3. The complexes of Mn(II), Co(II), and Cu(II) which more readily form mixed complexes, including the coordination of ethanol molecule, are more soluble in higher ethanol ratio of mobile phase.

The experimental retention data obtained for metal-dioxime complexes on silica gel TLC plates in various mixed solvent systems showed that the degree of migration of complexes was dependent upon polarity of the eluent used. However further increase in ethanol above the saturated value affected the R_f fairly constant or falling.

In addition, a comparison was also made of migrations of metal complexes on different ligands due to their sizes and steric hindrance. Among the four dioximes used, the R_f values for Pd(II) and Ni(II) complexes with furildioxime were relatively less than the complexes of the other dioximes. Tailing and band were observed for the complexes of Pd(II) and Ni(II) while the R_f values of Cu(II) and Co(II) were almost zero. The exceptionally strong adsorption of these furildioxime complexes on silica gel may have been caused by the loosened hydrogen bridge. The furan oxygen will naturally facilitate hydrogen bridge bond breaking by exerting a pull on the hydrogen atom of the molecule supporting by its very low pKa_1. That such an assumption was verified by the resulting chromatograms where higher concentration of ethanol in developer do not give higher R_f values of the complexes.

The other less polar complexes of DMG, DPG, and nioximes exhibit much higher R_f values because the lipophilic parts of the complexes are preferably dissolved in less polar solvents. As the size of the hydrocarbon substitute in α-dioxime increases, the R_f value of the corresponding metal complexes increases because of the enhanced solubility of the complexes in high content of hexane and/or chloroform. This similar variation of R_f values was also noted when ethanol was added in mobile phases. Their metal complexes R_f values increased with the higher percentage of ethanol. As seen from these data, the smaller DMG and nioxime showed more differences in migration of each metal complexes than those of DPG. The large phenyl rings can reduce such strong binding of the Cu(II) and Co(II) complexes with silica gel.

The feature of this study is a significant difference in R_f values of these metal ion leading to separation possibilities which were realized in practice. It is clear from Tables 4.1-4.4 that Pd(II) and Ni(II) got successfully separated from the other experimental metal ions. Ni(II) and Pd(II) could be separated from Co(II), Cu(II), Mn(II), Cr(III), and Fe(III) by using DMG in $CHCl_3$:EtOH (9:1) system. Similarly the separation of Ni(II) and Pd(II) from those metal ions has been achieved by using DPG in C_6H_{14}:$CHCl_3$:EtOH (5.7:2.8:1.5) developer and nioxime in C_6H_{14}:$CHCl_3$:EtOH (1.0:7.5:1.5) system. However in the case of DPG, the R_f value of Co-DPG and Cu-DPG complexes were close to those value of Ni-DPG and Pd-DPG complexes. Using furildioxime only Pd(II) could be separated from other metal ions except the broad band of Ni(II) in C_6H_{14}:$CHCl_3$:EtOH (1.2:7.3:1.5) system.

4.1.2 Study of the Concentration of the Ligands in Developing Solution

A suitable concentration of ligands in developing solution for metal complex of each ligand (Group I DMG, Group II DPG, Group III furildioxime, and Group IV nioxime) were investigated and the results expressed in characteristic of complex area (spot or band) and calculated R_f values.(Tables 4.5, 4.6, 4.7 and 4.8).

Table 4.5 The characteristic and R_f values of complexes between metal ions and the various concentrations of the dimethylglyoxime (DMG) (%w/v) in the mixed mobile phase of $CHCl_3$:EtOH (9:1).

Metal ion (100 ng)	Characteristic and (R_f values) of metal-DMG complexes		
	0.025%DMG	0.05%DMG	0.1%DMG
Ni(II)	spot+tail (0.76)	spot (0.74)	spot (0.83)
Pd(II)	spot+tail (0.64)	spot (0.59)	spot (0.69)
DMG	background (0.55)	background (0.56)	background (0.64)

Table 4.6 The characteristic and R_f values of complexes between metal ions and the various concentrations of the benzildioxime (DPG) (%w/v) in the mixed mobile phase of C_6H_{14}:$CHCl_3$:EtOH (5.7:2.8:1.5).

Metal ion (100 ng)	Characteristic and (R_f values) of metal-DPG complexes		
	0.0025%DPG	0.005%DPG	0.0075%DPG
Ni(II)	spot+tail (0.71)	spot+tail (0.72)	spot (0.78)
Pd(II)	spot+tail (0.73)	spot+tail (0.73)	spot (0.79)
DPG	background (0.53)	background (0.54)	background (0.56)

Table 4.7 The characteristic and R_f values of complexes between metal ions and the various concentrations of the furildioxime (%w/v) in the mixed mobile phase of C_6H_{14}:$CHCl_3$:EtOH (1.2:7.3:1.5).

Metal ions (100 ng)	Characteristic and (R_f values) of metal-furildioxime complexes		
	0.025% furildioxime	0.05% furildioxime	0.075% furildioxime
Pd(II)	spot+tail (0.70)	spot+tail (0.74)	spot (0.75)
Furildioxime	background (0.31)	background (0.36)	background (0.39)

Table 4.8 The characteristic and R_f values of complexes between metal ions and the various concentrations of the nioxime (%w/v) in the mixed mobile phase of C_6H_{14}:$CHCl_3$:EtOH (1.0:7.5:1.5).

Metal ion (100 ng)	Characteristic and (R_f values) of metal-nioxime complexes				
	0.005% nioxime	0.0075% nioxime	0.01% nioxime	0.015% nioxime	0.02% nioxime
Ni(II)	band (0.41-0.75)	band (0.54-0.78)	spot+tail (0.83)	spot (0.78)	spot (0.73)
Pd(II)	band (0.40-0.65)	band (0.51-0.73)	spot (0.73)	spot (0.74)	spot (0.70)
Nioxime	background (0.26)	background (0.30)	background (0.34)	background (0.34)	background (0.36)

Previous part 4.1.1 dealt with the effect of dipolar component in mobile phase on various metal ions permitted optimal chromatographic for the separation of Pd(II) and Ni(II) by the α-dioximes. The chosen amount of each ligand was not satisfactory for instrumental *in situ* determination, because few diffuse spots and tailing could be observed when the high concentration (100 μg/mL) of metal ion was applied to the TLC plate.

In view of the importance of ligand concentration in the selected mobile phase corresponding to the amount of Pd(II) and Ni(II) applied on the TLC plates, attempts were carried out to obtaine the sufficient amount of ligand in a developing solution to make series of good round spots of their complexes for further quantitative experiments by a densitometer. The lowest recordable limit of the α-dioximes concentration was about 0.05%w/v DMG, 0.0075%w/v DPG, 0.075%w/v furildioxime and 0.015%w/v nioxime in their proper mobile phases for both Pd(II) and Ni(II). These concentrations showed all compact spots of the metal complexes.

The effect of ligand concentration on characteristic of complexes on silica gel can be explained as a metal-ligand formation parameter. Since the α-dioximes are very weak acid (pKa >10), hence significantly non-dissociated in such low dielectric mobile phases. Only the metal complexes with very high formation constant actually achieved on the layer. In our case, basic solvent was not added in the mobile phase for anionic ligand promotion. In essence, insufficiency of the readily anionic ligand causes a tailing or band of the complex. With the higher amount of ligand it is evident from the chromatograms that tailing or band is shortened.

Other concerns associated with the amount of the ligand in developing solution, the R_f values of the diffuse spots are slightly lower than those of dosage. Addition of the ligand over the required concentrations are permitted. However the it offers more developing time because solvent mixture grows tricklingly.

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

A suitable spotted amount of metal ions on TLC plate for metal complex of each ligand (Group I DMG, Group II DPG, Group III furildioxime, and Group IV nioxime) was investigated and the results expressed in characteristic of complexes (spot or band) and calculated R_f values.(Tables 4.9, 4.10, 4.11 and 4.12).

Table 4.9 The characteristic and R_f values of complexes between the various amounts of metal ions (ng/spot) using 0.05 %w/v dimethylglyoxime in the mixed mobile phase of $CHCl_3$:EtOH (9 :1).

Metal ion	Characteristic and (R_f values) of metal-DMG complexes				
	10	50	100	150	200
Ni(II)	spot (0.79)	spot (0.78)	spot (0.80)	spot+tail (0.74)	spot+tail (0.74)
Pd(II)	not detect	not detect	spot (0.70)	spot (0.68)	spot (0.69)

Table 4.10 The characteristic and R_f values of complexes between the various amounts of metal ions (ng/spot) using 0.0075 %w/v benzildioxime in the mixed mobile phase of C_6H_{14}:$CHCl_3$:EtOH (5.7:2.8:1.5).

Metal ion	Characteristic and (R_f values) of metal-DPG complexes			
	10	50	100	500
Ni(II)	spot (0.82)	spot (0.83)	spot (0.75)	spot+tail (0.73)
Pd(II)	not detect	spot (0.80)	spot (0.79)	spot+tail (0.78)

Table 4.11 The characteristic and R_f values of complexes between the various amounts of the metal ions (ng/spot) using 0.075% w/v furildioxime in the mixed mobile phase of C_6H_{14}:$CHCl_3$:EtOH (1.2:7.3:1.5).

Metal ions	Characteristic and (R_f values) of metal-furildioxime complexes			
	10	50	80	100
Pd(II)	spot (0.73)	spot (0.74)	spot (0.74)	spot (0.73)

Table 4.12 The characteristic and R_f values of complexes between the various amounts of the metal ions (ng/spot) using 0.015% w/v nioxime in the mixed mobile phase of C_6H_{14}:$CHCl_3$:EtOH (1.0:7.5:1.5).

Metal ions	Characteristic and (R_f values) of metal-nioxime complexes				
	10	50	80	100	120
Ni(II)	spot (0.73)	spot (0.73)	spot (0.74)	spot (0.73)	spot+tail (0.71)
Pd(II)	ND	spot (0.64)	spot (0.68)	spot (0.69)	spot (0.68)

In this part the acceptable range of the metal amount namely Pd(II) and Ni(II) applied on the TLC plate were examined for further densitometric determination by using the previously selected compositions of dipping solutions. Similar to the part 4.1.2 the amounts of metal should correspond to the chosen amount of the ligand in mobile phase. If the amount of the metal ion is too high the first coming ligand moving together with mobile phase is not enough to react with the entire metal ion that applied on the start line. This requires a sufficient amount of the following ligand. In such cases, bands or diffuse spots could be observed. It was found that all of the selected amount of ligand used in these experiments gave good round and compact spots of their complex area on TLC where the applied metal ion amount was not higher than 100 ng. The certain amounts of Ni(II) that correspond to the actual ligand concentration were determined between 10-100 ng while the amount of Pd(II) were higher due to its better complexing ability with these α-dioximes (29, 35). However, the minimum amount of Pd(II) that could be observed was greater than Ni(II) since the lower color intensity of the Pd(II)-α-dioxime complexes resulted poor visibly observation in locating their area. The chosen conditions of chromatographic systems were summarized in Table 4.13 and the chromatograms of metal-α-dioxime complexes which developed in these conditions were shown in Figures 4.4-4.7.

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Table 4.13 The chosen conditions of chromatographic system.

Metal ion	Mobile phase	Chelating agent in 10.00 mL mobile phase	The amount of metal ion (ng/spot)	Developing time (mins)
Ni(II)	$CHCl_3$:EtOH (9:1)	0.05%w/v DMG	10-100	20
	C_6H_{14}:$CHCl_3$:EtOH (5.7:2.8:1.5)	0.0075%w/v DPG	10-100	15
	C_6H_{14}:$CHCl_3$:EtOH (1.0:7.5:1.5)	0.015%w/v Nioxime	10-100	15
Pd(II)	$CHCl_3$:EtOH (9:1)	0.05%w/v DMG	100-170	20
	C_6H_{14}:$CHCl_3$:EtOH (5.7:2.8:1.5)	0.0075%w/v DPG	20-100	15
	C_6H_{14}:$CHCl_3$:EtOH (1.2:7.3:1.5)	0.075%w/v Furildioxime	10-100	15
	C_6H_{14}:$CHCl_3$:EtOH (1.0:7.5:1.5)	0.015%w/v Nioxime	50-120	15

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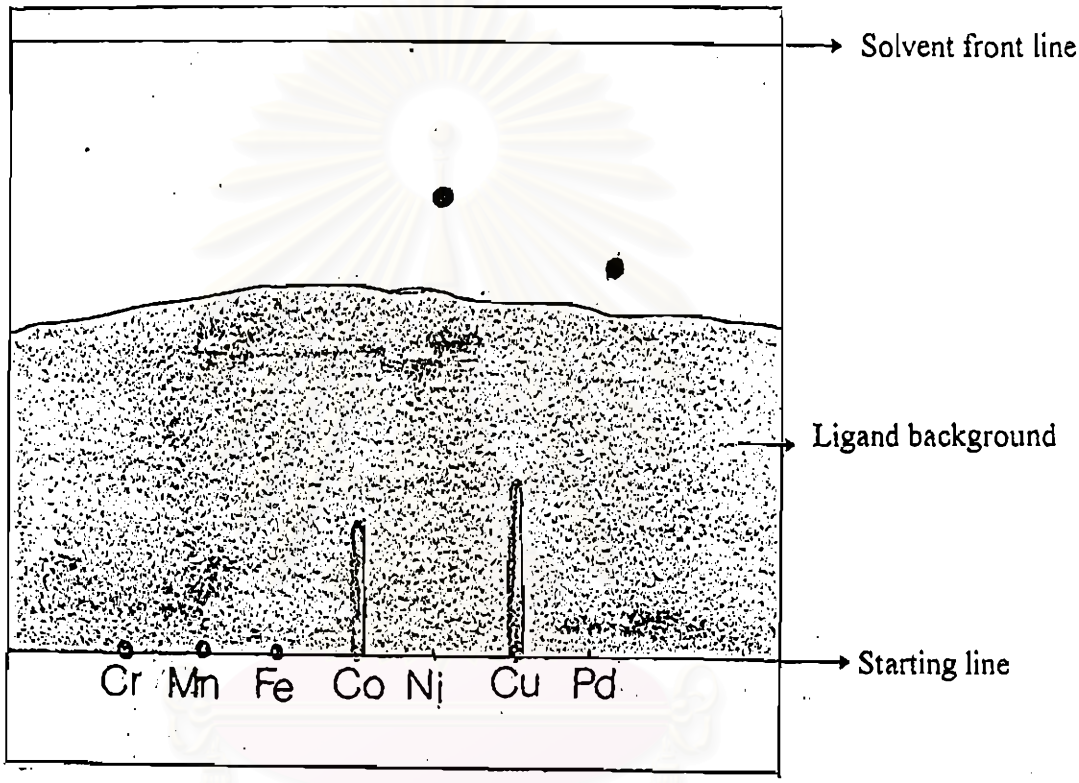

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Figure 4.4 The chromatogram of metal-α-dioxime complexes which developed in $CHCl_3$:EtOH (9:1) and using 0.05%w/v DMG as complexing agent.

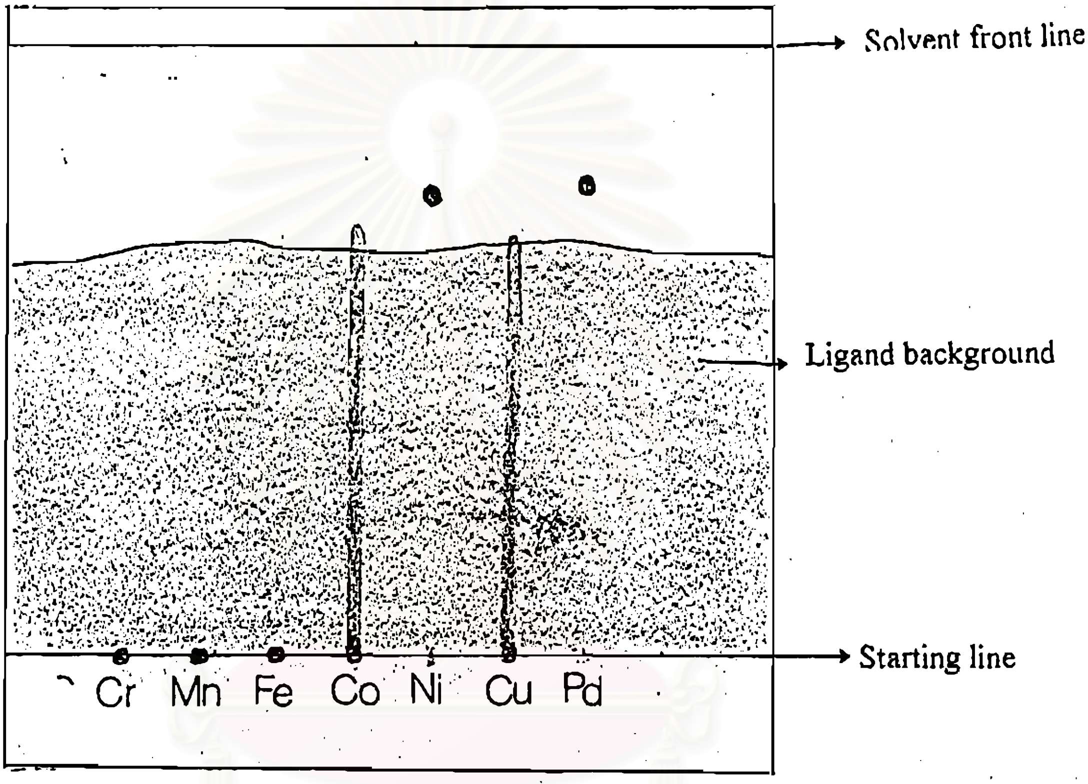

Figure 4.5 The chromatogram of metal-α-dioxime complexes which developed in C_6H_{14}:$CHCl_3$:EtOH (5.7:2.8:1.5) and using 0.0075%w/v DPG as complexing agent.

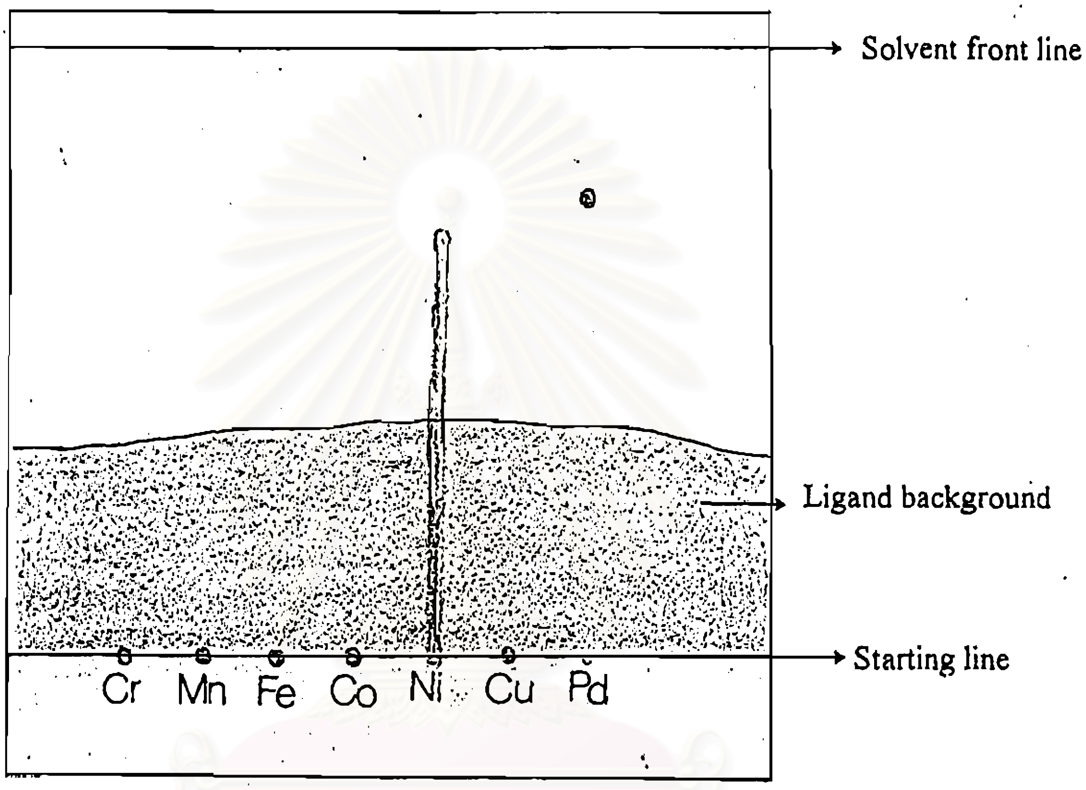

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Figure 4.6 The chromatogram of metal-α-dioxime complexes which developed in C_6H_{14}:$CHCl_3$:EtOH (1.2:7.3:1.5) and using 0.075%w/v furildioxime as complexing agent.

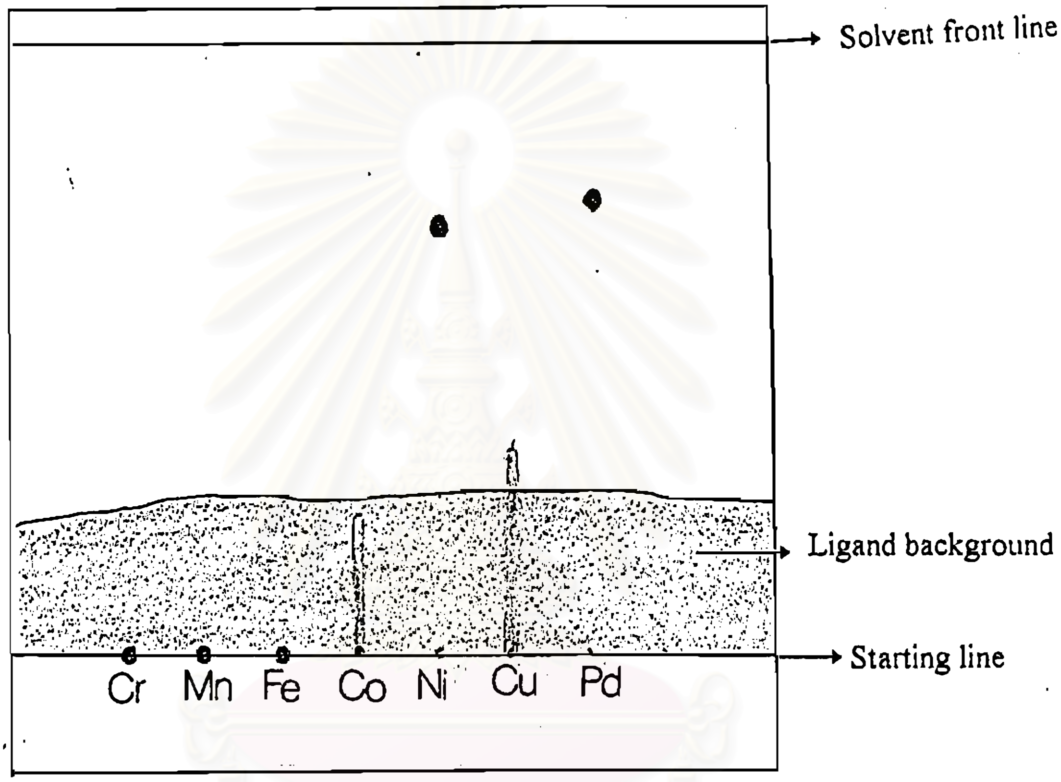

Figure 4.7 The chromatogram of metal-α-dioxime complexes which developed in C_6H_{14}:$CHCl_3$:EtOH (1.0:7.5:1.5) and using 0.015%w/v nioxime as complexing agent.

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4.2. Densitometric Study for Quantification of Ni(II) and Pd(II)

4.2.1 Optimum Absorbed Wavelength of the Complexes on TLC Plate and Densitometric Parameter Optimization

Visually, it was found that the eye could detect these colored complexes and their differences in amount down to 10 ng. This visual inspection of spot location should be carefully watched after the TLC plate dries because the colored spots faded standing. The spot area could be determined more easily when UV light (254 nm) cabinet was used for visualization of the metal complexes on silica gel G F_{254} plate. As instrumental *in situ* determination was used, the lower ligand background fortunately makes the clear observation of the complexes and the silica gel layer background of the spots remained white and fluorescinated.

In the experiments, the peak start and the peak end positions were firstly determined. The importance of these two parameters and a suitable threshold level for integrator subjected to the degree of accurate and precise determination of peak height or peak area. The peak threshold was intentionally selected at high value (10,000) to suppress interference detector noise and the recorder deflection was fully setted at 128 mV. The scanning direction of spot scanning was also chosen. A number of non-Gaussian peak were observed when the TLC plates were scanned in track direction due to some diffuse spots especially Ni-DPG complex. But all Gaussian peaks were obtained after changing to cross direction.

After scanning by a Camag TLC densitometer, the reflectance intensity of the metal complex at various wavelengths were plotted as a spectrum. The wavelengths of the remission minimum were chosen for further determination as the optimum absorbed wavelength. Before employing UV range for this quantitative determination, visible light was attempted, but poor sensitivity of detection. The visible spectra of the red Ni-α-dioxime complexes were quite flat and at about 550-600 nm the signal was not clear enough to obtain a reliable comparison even at high attenuation.

The absorption spectrum of each metal complex at wavelength 200-400 nm was shown in Figure 4.8. All of metal complexes had good absorption in UV range and their spots were located above the ligand background. The optimum absorbed wavelength and densitometric sensitivity of each metal complex were summarized in Table 4.14.

Table 4.14 The optimum absorbed wavelengths and densitometric sensitivities of each metal complexe.

Metal complex	Optimum Absorbed Wavelength (nm)	Densitometric Parameter (Sensitivity)
Ni-DMG	270	160
Pd-DMG	270	160
Ni-DPG	270	165
Pd-DPG	270	170
Pd-furildioxime	280	165
Ni-nioxime	270	165
Pd-nioxime	270	165

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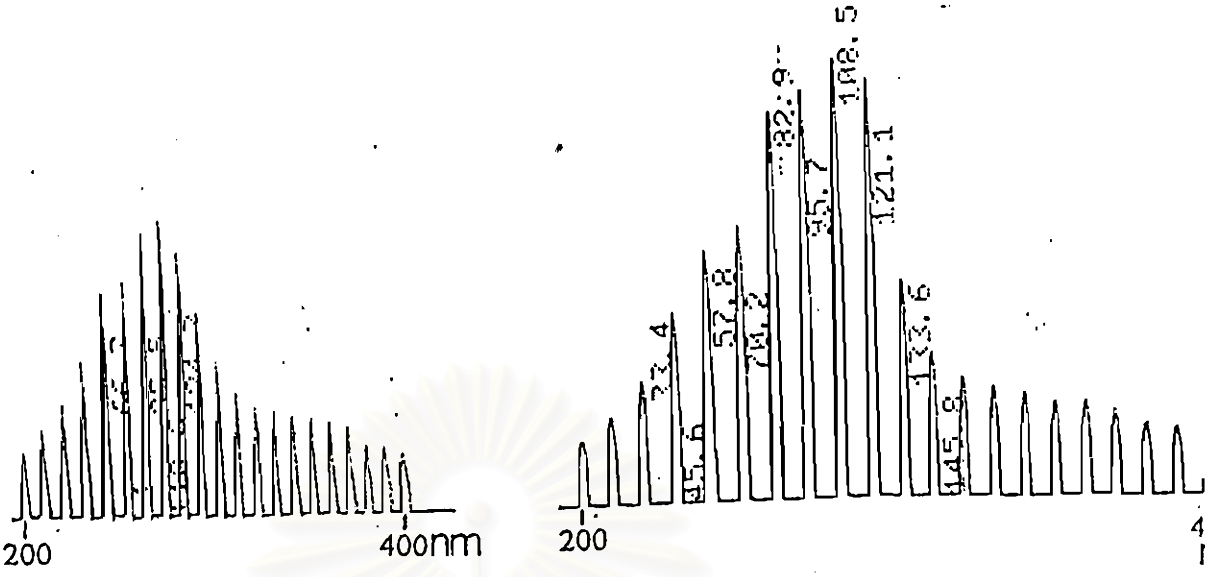
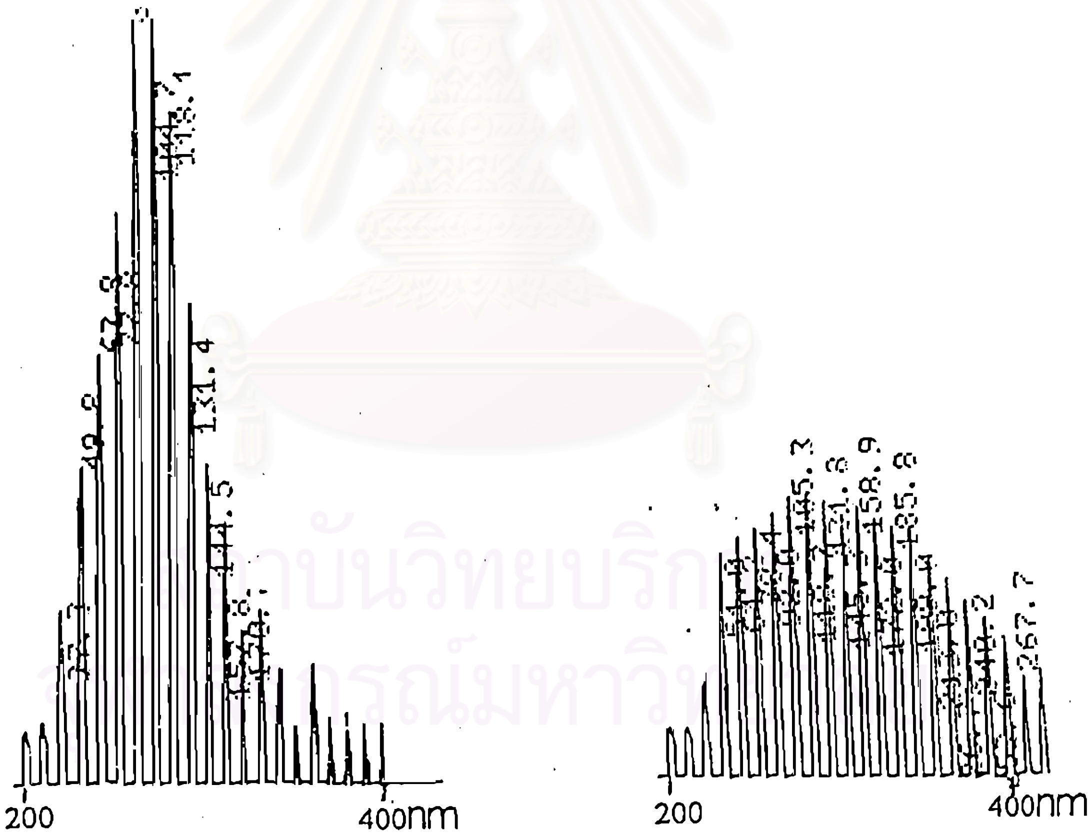


Ni-DMG complex

Pd-DMG complex



Ni-DPG complex

Pd-DPG complex

Figure 4.8 Absorption spectrum of each metal complex at wavelength 200-400 nm.

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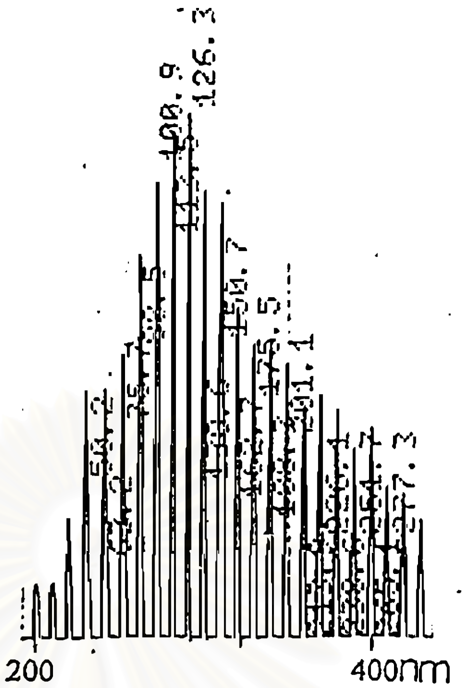
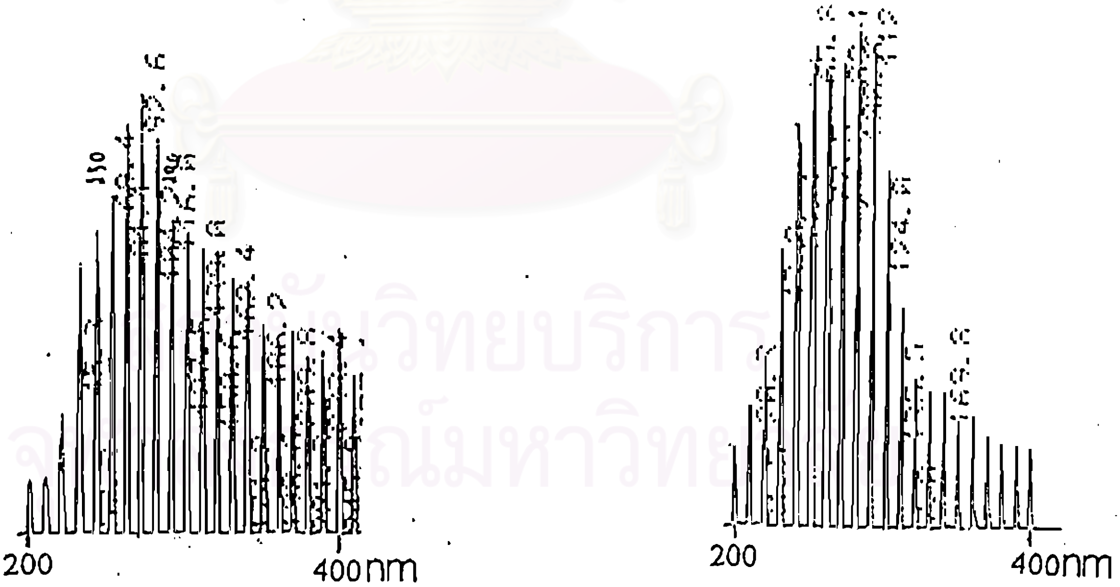


Pd-furildioxime complex



Ni-nioxime complex

Pd-nioxime complex.

Figure 4.8 Absorption spectrum of each metal complex at wavelength 200-400 nm (continued).

4.2.2 The Study of Repeatability of Calibration curves and Linearity of Each Metal-Complex

Since peak height and peak area were related to spot size, the 1 μL fixed volume of the standards having different concentration were applied and the rapid flow of such highly volatile mobile phase made the much slower diffuse rate of the complexes. The definite size and regular shaped spots but varying color intensity were assumed to be obtained.

Absorption of UV light by the standard metal complexes were measured by the scanning densitometer. The diminution of reflected light provided the signal for quantitative measurement. The calibration curves of each metal complexes were plotted between the simultaneous measurement of the light reflection as peak height against the amounts of metal and shown in Figures 4.9 to 4.15.

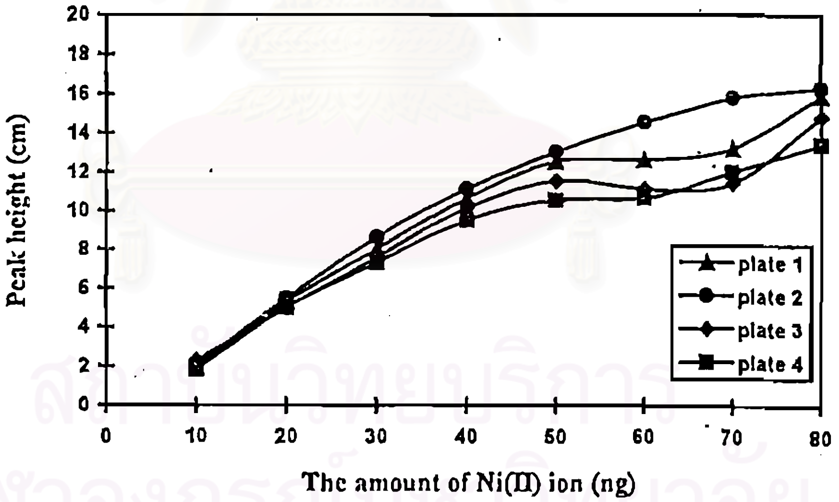


Figure 4.9 Calibration curves : peak height against the amount of Ni(II) (10-80 ng) in Ni-DMG complex.

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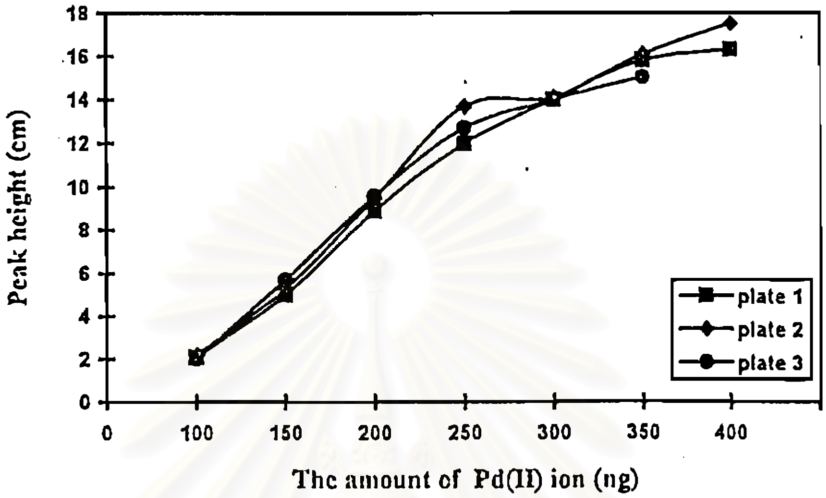

Figure 4.10 Calibration curves : peak height against the amount of Pd(II)(100-400 ng) in Pd-DMG complex.

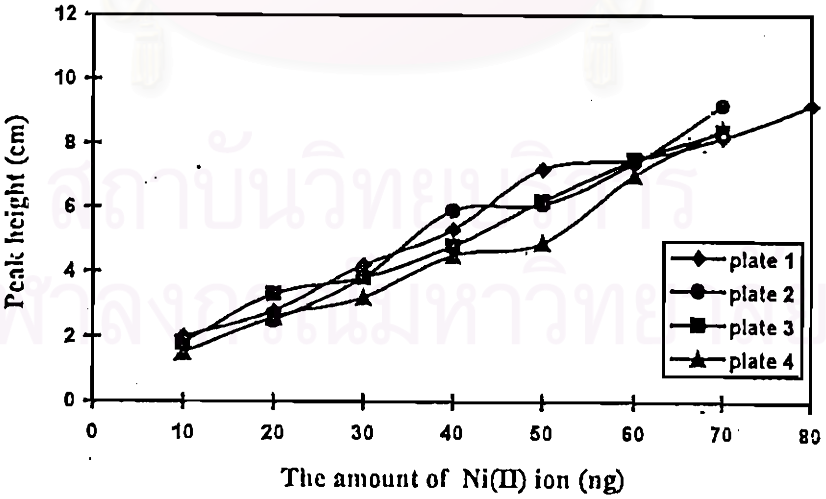

Figure 4.11 Calibration curves : peak height against the amount of Ni(II) (10-70 ng) in Ni-DPG complex.

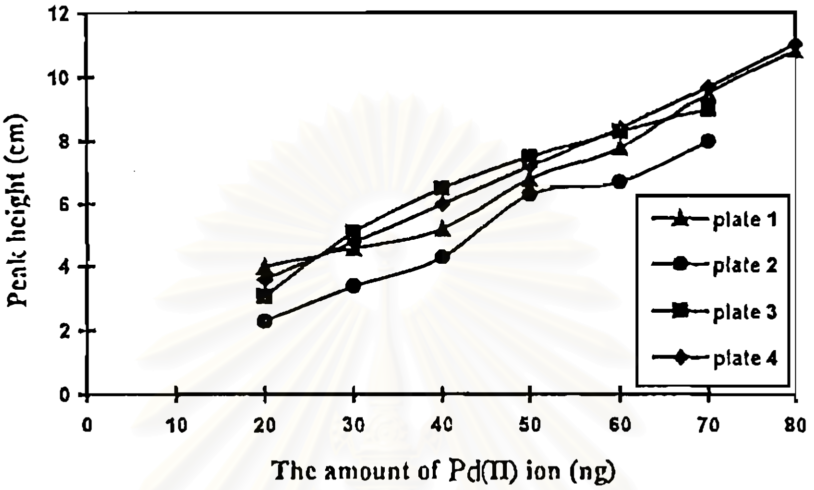

Figure 4.12 Calibration curves : peak height against the amount of Pd(II) (10-80 ng) in Pd-DPG complex.

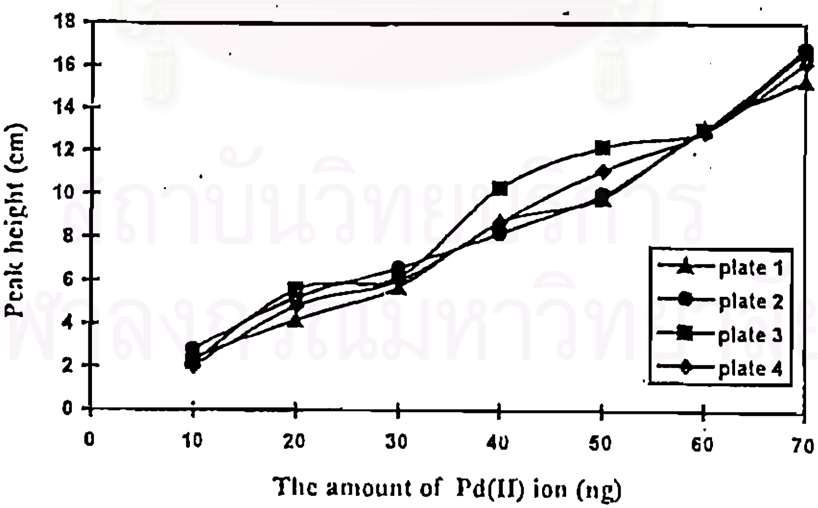

Figure 4.13 Calibration curves : peak height against the amount of Pd(II) (10-70 ng) in Pd-furildioxime complex.

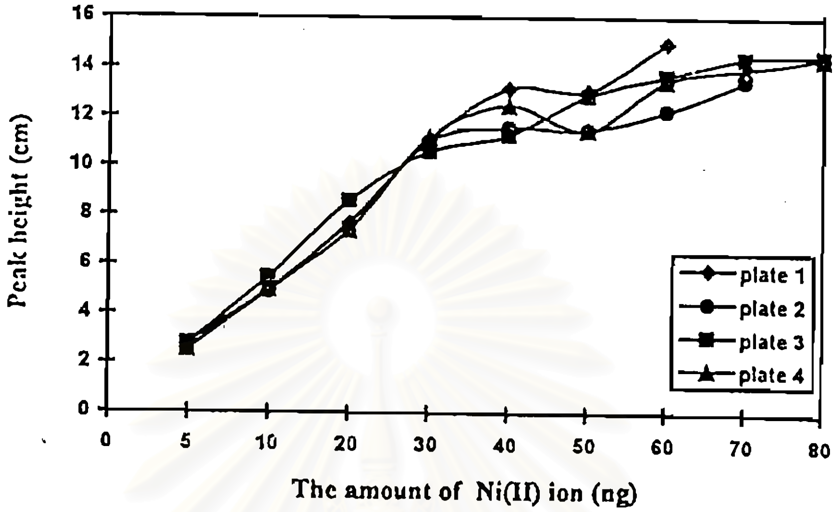

Figure 4.14 Calibration curves : peak height against the amount of Ni(II) (5-80 ng) in Ni-nioxime complex.

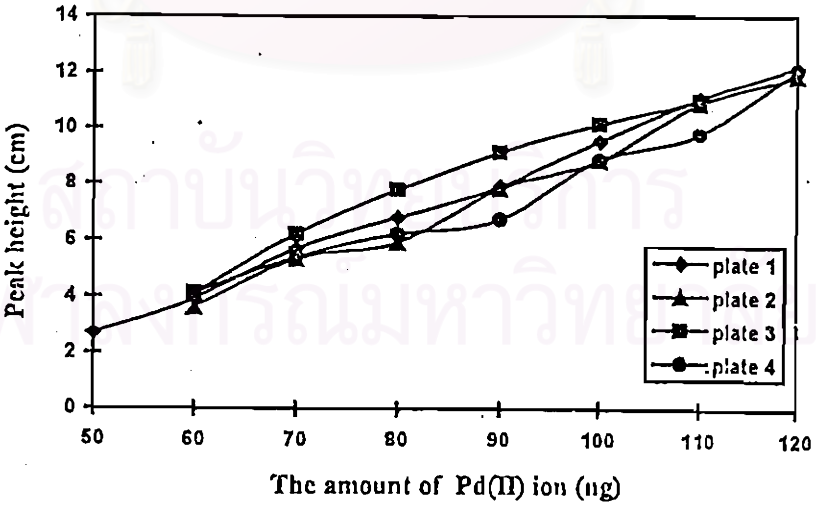

Figure 4.15 Calibration curves : peak height against the amount of Pd(II) (50-170 ng) in Pd-nioxime complex.

Plots obtained by nonlinear regression could be used for quantification, but precision of the measurement would usually be lower than if the relationship was linear. Because of the diffuse reflection of light by the irregular layer of sorbent particles, the propagation of light within the opaque medium is a very complex process. This surface-enhanced light scattering spectrometry could be elaborated by Kubelka-Munk's equation therefore the simple correlation of linearity is possible in a narrow range. The linearity was evaluated from regression analysis of the selected data over the range of the experimental amount of metal. The correlation coefficients were varied from 0.97-0.99. The linear line of the relationship between peak height against the amount of metal (ng) of all metal complexes were show in Figures 4.16-4.22 and the linear range and sensitivity of each metal complex was summarized in Table 4.15. Regression analysis data were summarized in Tables 4.16, 4.18, 4.20, 4.22, 4.24, 4.26 and 4.28.

Table 4.15 The linear range and sensitivity of metal-complexes.

Metal-complexes	The range of linearity (ng/spot)	Sensitivity
Ni-DMG	10-40	0.272
Pd-DMG	100-170	0.062
Ni-DPG	10-80	0.247
Pd-DPG	20-80	0.095
Pd-furildioxime	10-70	0.330
Ni-nioxime	5-25	0.544
Pd-nioxime	50-120	0.139

Since the densitometer was eye scanning instrumental, it was very difficult to establish the certain distance (spot diameter) for every measurements. In practicable locating of the scanning position and direction, the cursory sweep caused the uncertain densitometric peak width so the peak area data were rather fluctuant, while the peak height provided more reproducible reading and also favorable in the case of spots with irregular shape, nonuniform concentration distribution and inhomogenous plate background. However when the amount of metal ions was very low, the error from locating the position of the spots were high so the low accuracy and precision results could be obtained.

Observations of the repeatability of the established range were made in performing the same procedure 4 times and the regression of each chromatographic plates were checked separately. The results from the regression data showed the close-by linearized profiles of the calibration plot and the slope values of each line were quite similar but different in the intercepts. The relative standard deviation of peak height of the same amount of metal on the different plate were shown in Tables 4.17, 4.19, 4.21, 4.23, 4.25, 4.27, and 4.29. The RSD varied from 1.6 to 32% (average value for Ni-DMG 16.1%, Pd-DMG 17.0%, Ni-DPG 10.4%, Pd-DPG 19.9%, Pd-furildioxime 10.1%, Ni-nioxime 11.3% and Pd-nioxime 14.7%). The noticeable errors especially for low concentrations may mainly cause by a little change in the developing conditions of different TLC plates. But it appeared not be necessary to deal with the attributed error by intensely maintained the chromatographic condition for different plate. The inherent advantage that the spot of unknown samples and standards were on the same plate subjected them to the same chromatographic conditions. If the comparison of the different plates was necessary, standardization method could be applicable in the same linear correlation. The systematic errors could be minimized, and accuracy and precision would be favorably obtained.

The enhanced sensitivity of the complex detection by the densitomer were also calculated from the slope of those linear lines in the term of the detector response (R_c). It was defined as the increase in detector output (peak height or peak area) for a nanogram increase in metal ion on the spot. The results of each complex system was summarized in Table 4.15.

As shown in Table 4.15, the analytical methods based UV (270 or 280 nm) absorption for TLC quantification of Ni(II) and Pd(II) could be carried out in satisfactory range and sensitivity. This was improved because the ability of light absorption at the characteristic wavelength of the complex.

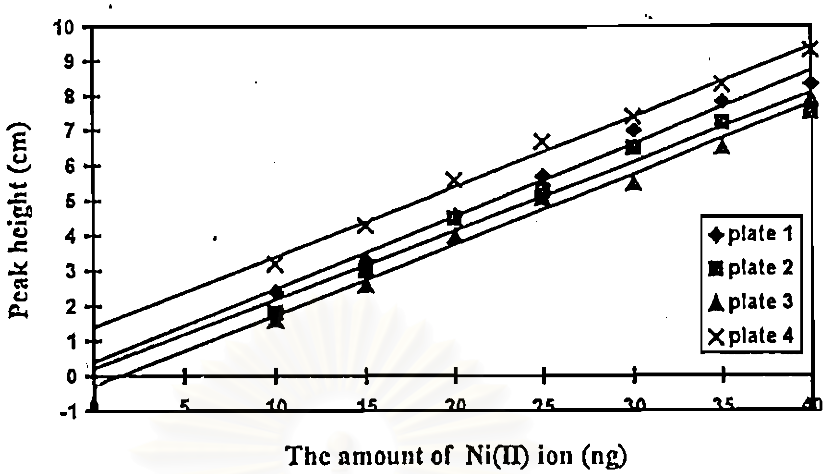

Figure 4.16 Linear calibration curves of Ni-DMG complex (Ni(II) 10-40 ng).

Table 4.16 Regression data of Ni-DMG complex.

Plate No.	Y = Ax	+ B	Correlation coefficient (r)
	A	B	
1	0.207	0.389	0.9937
2	0.196	0.203	0.9852
3	0.201	-0.293	0.9935
4	0.200	1.382	0.9964

Table 4.17 Mean, standard deviation and relative standard deviation values of the various amounts of Ni(II) ion in Ni-DMG complex (10-40 ng) on different plates.

Ni(II) ion (ng)	X±SD	%RSD
10	2.25±0.72	32.00
15	3.30±0.73	22.12
20	4.68±0.67	14.32
25	5.70±0.71	12.45
30	6.60±0.82	12.42
35	7.45±0.77	10.33
40	8.25±0.77	9.33

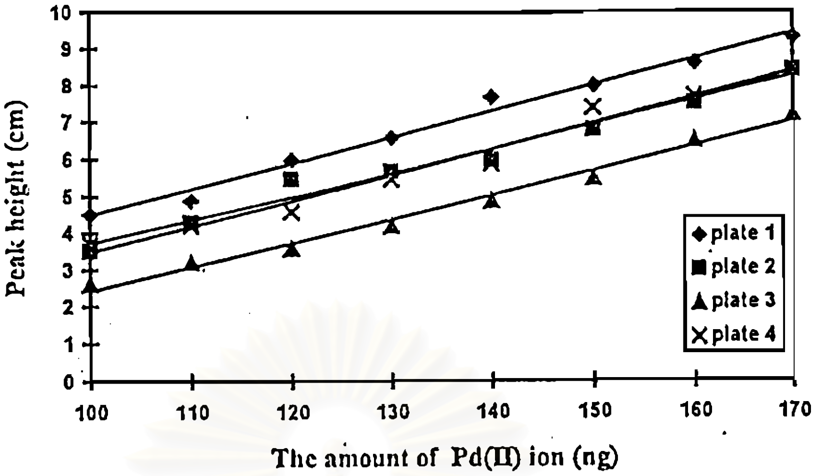

Figure 4.17 Linear calibration curves of Pd-DMG complex (Pd(II) 100-170 ng).

Table 4.18 Regression data of Pd-DMG complex.

Plate No.	Y = Ax	+ B	Correlation coefficient (r)
	A	B	
1	0.070	-2.564	0.9934
2	0.065	-2.796	0.9878
3	0.066	-4.143	0.9940
4	0.070	-3.575	0.9840

Table 4.19 Mean, standard deviation and relative standard deviation values of the various amounts of Pd(II) ion in Pd-DMG complex (100-170 ng) on different plates.

Pd(II) ion(ng)	X±SD	%RSD
100	3.58±0.78	21.80
110	4.15±0.70	16.98
120	4.93±1.06	21.43
130	5.50±0.99	18.00
140	6.13±1.16	18.95
150	6.93±1.07	15.42
160	7.58±0.86	11.37
170	8.08±0.97	12.02

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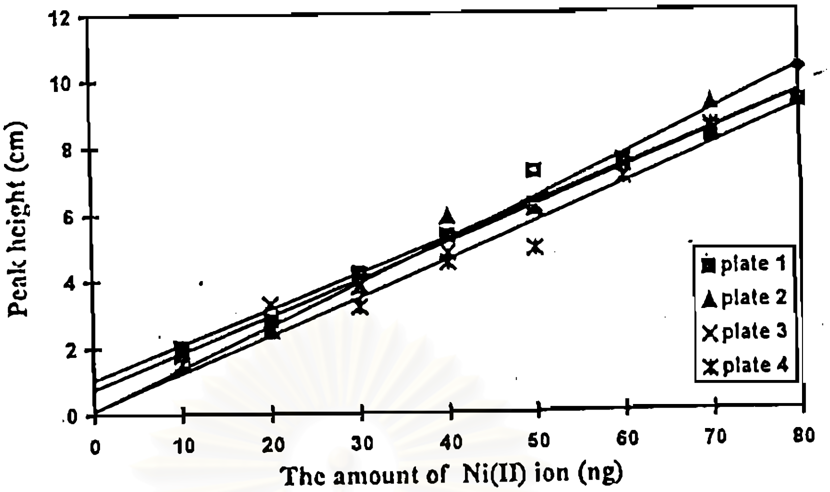

Figure 4.18 Linear calibration curves of Ni-DPG complex (Ni(II) 10-70 ng).

Table 4.20 Regression data of Ni-DPG complex.

Plate No.	Y = Ax + B		Correlation coefficient (r)
	A	B	
1	0.110	0.946	0.9895
2	0.116	0.475	0.9832
3	0.109	0.745	0.9908
4	0.112	0.100	0.9834

Table 4.21 Mean, standard deviation and relative standard deviation values of the various amounts of Ni(II) ion in Ni-DPG complex (10-70 ng) on different plates.

Ni(II) ion (ng)	X±SD	%RSD
10	1.77±0.25	14.22
20	2.80±0.36	12.71
30	3.75±0.41	10.99
40	5.13±0.61	11.95
50	6.10±0.94	15.43
60	7.35±0.24	3.24
70	8.73±0.36	4.12

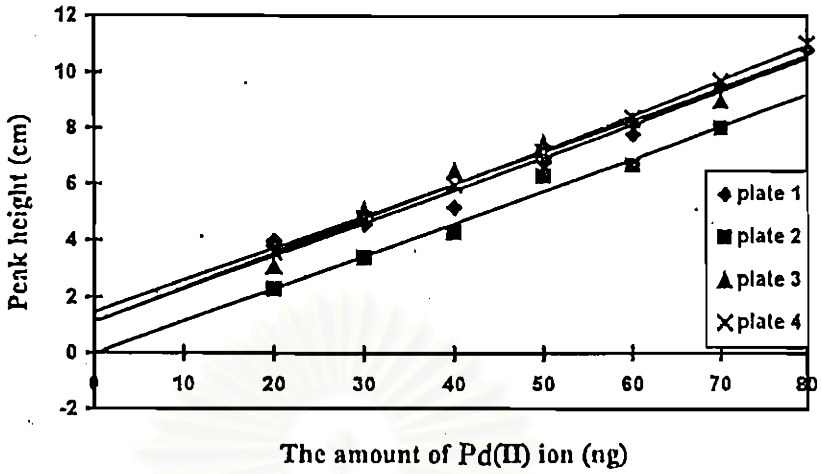

Figure 4.19 Linear calibration curves of Pd-DPG complex (Pd(II) 20-80 ng).

Table 4.22 Regression data of Pd-DPG complex.

Plate No.	Y = Ax	+ B	Correlation coefficient (r)
	A	B	
1	0.117	1.099	0.9881
2	0.112	1.493	0.9766
3	0.120	1.236	0.9994
4	0.115	-0.027	0.9907

Table 4.23 Mean, standard deviation and relative standard deviation values of the various amounts of Pd(II) ion in Pd-DPG complex (20-80 ng) on different plates.

Pd(II) ion (ng)	X±SD	%RSD
20	3.38±0.93	27.51
30	4.70±1.18	25.08
40	6.04±1.50	24.82
50	6.70±1.35	20.08
60	7.78±1.21	15.54
70	8.80±1.30	14.79
80	9.54±1.06	11.16

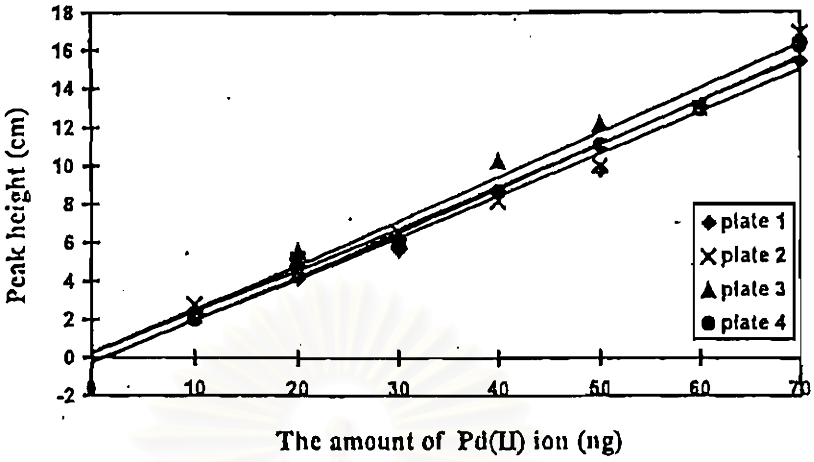

Figure 4.20 Linear calibration curves of Pd-furildioxime complex (Pd(II) 10-70 ng).

Table 4.24 Regression data of Pd-furildioxime complex.

Plate No.	Y = Ax + B		Correlation coefficient (r)
	A	B	
1	0.218	-0.228	0.9941
2	0.229	0.271	0.9876
3	0.218	1.999	0.9837
4	0.228	-0.314	0.9962

Table 4.25 Mean, standard deviation and relative standard deviation values of the various amounts of Pd(II) ion in Pd-furildioxime complex (10-70 ng) on different plates.

Pd(II) ion (ng)	X±SD	%RSD
10	2.55±0.42	16.40
20	4.65±0.67	14.50
30	6.34±0.58	9.17
40	8.27±1.35	16.34
50	10.57±0.96	9.09
60	13.10±0.21	1.60
70	16.43±0.56	3.39

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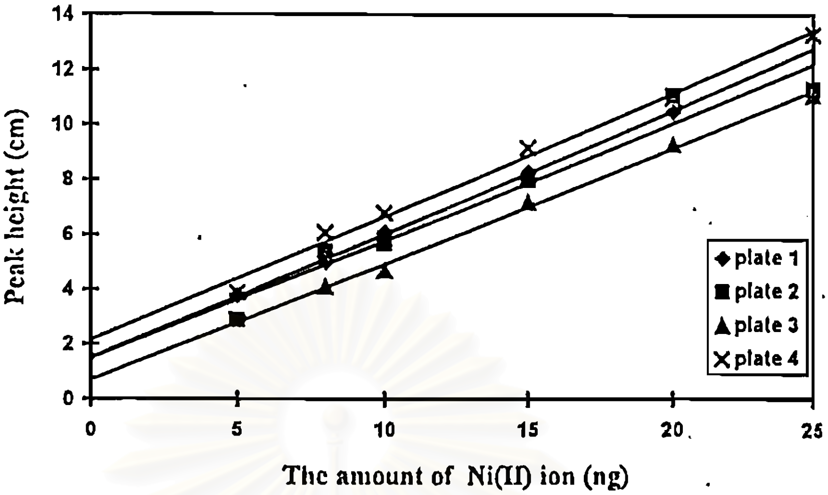

Figure 4.21 Linear calibration curves of Ni-nioxime complex (Ni(II) 5-25 ng).

Table 4.26 Regression data of Ni-nioxime complex.

Plate No.	Y = Ax + B		Correlation coefficient (r)
	A	B	
1	0.433	1.572	0.9937
2	0.509	0.693	0.9912
3	0.423	0.677	0.9982
4	0.450	2.153	0.9956

Table 4.27 Mean, standard deviation and relative standard deviation values of the various amounts of Ni(II) ion in Ni-nioxime complex (5-25 ng) on different plates.

Ni(II) ion (ng)	X±SD	%RSD
5	3.88±0.52	13.44
8	6.04±0.96	15.95
10	6.96±0.83	11.92
15	8.94±1.12	12.52
20	10.90±0.52	4.81
25	11.96±1.06	8.92

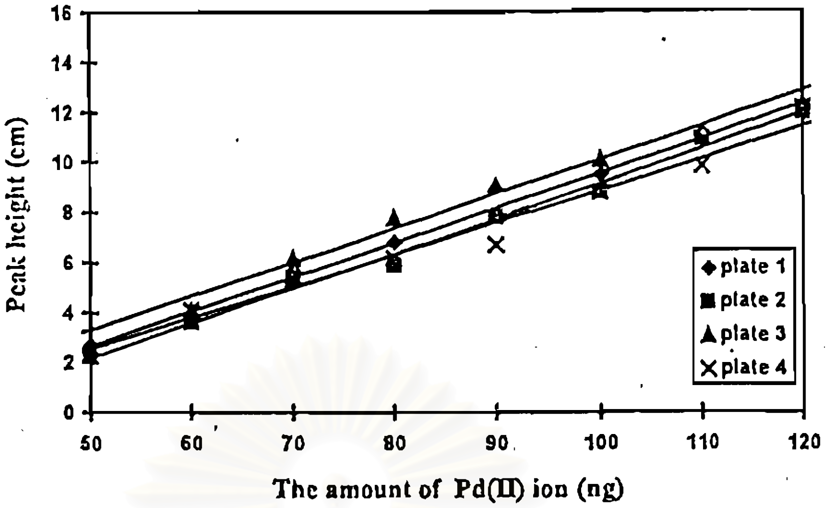

Figure 4.22 Linear calibration curves of Pd-nioxime complex (Pd(II) 50-120 ng).

Table 4.28 Regression data of Pd-nioxime complex.

Plate No.	Y = Ax + B		Correlation coefficient (r)
	A	B	
1	0.136	-4.124	0.9988
2	0.139	-4.973	0.9947
3	0.126	-3.733	0.9879
4	0.136	-3.485	0.9868

Table 4.29 Mean, standard deviation and relative standard deviation values of the various amounts of Pd(II) ion in Pd-nioxime complex (50-120 ng) on different plates.

Pd(II) ion (ng)	X±SD	%RSD
50	3.40±0.74	21.81
60	4.52±0.52	11.32
70	5.64±1.42	25.17
80	7.02±1.28	18.25
90	8.02±0.91	11.40
100	8.92±1.31	14.71
110	9.60±1.12	11.69
120	12.23±0.41	3.36

4.2.3 Repeatability of the Proposed Procedure on the Same TLC Plate

4.2.3.1 Repeatability of the Proposed Procedure on the Same TLC Plate

As mentioned in 4.2.2 , the proposed quantification methods with poor precision (%RSD higher than 10) were likely to be inaccurate because of difficulties in controlling all chromatographic conditions. The precision tended to be better if standards and samples as chromatographed together on the same plate as consistently as possible. The remaining error were mainly caused by samples and standards application mechanism on a TLC plate. In the experiments, no mechanical applicator or any devices were used. The metal ion solutions were manually applied to the TLC plate using a 5 μL Hamilton microsyringe and 1 μL volume was controlled by a plunger. This 1 μL volume of metal ion solution on the layer provided about 2 mm diametered spot and the starting spot dimension depended on the distance between needle tips and the layer surface including speed of injection. However, with smooth delivery of metal solutions by this type of syringe, the precision was reported typically less than ± 1% (6). The higher %RSD obtained was from personal error so the syringe required some practice with care in use for repeatability of sample and standard delivery and application in quantitative work. The misalignment of the spots on the starting line was also possible. The use of the scanning densitometer was also a source of error. Reproducibility of positioning the spot in the center of measuring beam with eye and reproducibility of the optical measurement by the densitometer were included in this obtained precision. The seven parallel spots of the same concentration of metal ion were carried out on the same plate and the data on eleven repeating scans were statistically calculated and given in Tables 4.30-4.36. The obvious better precision was obtained on the same plate experiments. %RSD was in the range 1.12 to 7.20% (average value for Ni-DMG 3.00%, Pd-DMG 4.21%, Ni-DPG 3.65%, Pd-DPG 3.66%, Pd-furildioxime 4.31%, Ni-nioxime 2.93% and Pd-nioxime 3.78%). In general the deviation of peak height was higher at low concentration. These values were regarded as the repeatability of the whole methods. From the error propagation law, the total error (σ_t) was given by the equation

$$\sigma_t^2 = \sigma_v^2 + \sigma_c^2 + \sigma_p^2 + \sigma_m^2 \ldots\ldots.(4.1)$$

where σ_v = repeatability of sample application process

σ_c = repeatability of the chromatographic conditions

σ_p .= repeatability of positioning the spot in the center of the measuring beam

σ_m = repeatability of light measurement by the instrument (6)

As shown from the Tables 4.30-4.36. The σ_m values were in the range 1.04 to 2.00 %. The higher value of the deviation was often observed in low amount of the metal ion based on peak height manual measurement. The value of σ_m of the scanning densitometer changed with respect to the signal to noise ratio, rising as the detection limit was approached. The steady values were obtained for a properly adjusted instrument parameter.

The error of positioning the spot in the measuring beam was not determined directly but evaluated from the equation below.

$$\sigma_p = (\sigma_d^2 - \sigma_m^2)^{1/2} \ldots\ldots..(4.2)$$

where σ_d = repeatability of all determination process using the scanning densitometer. (positioning the spot and light measurement)

The calculated σ_p's were in the range 0.09 to 3.32%. The positioning error was regarded as the personal error discussed in previous 4.2.2. For the manual operating, the use of peak height data could be more precise than those of peak area in different peak width.

The error due to sample application process (σ_v) and the differences in the chromatographic conditions (σ_c) could not determine separately from the experiments. The σ_c caused by migration differences (as a result of edge effects), deviations in adsorbent layer and non-linear solvent front was not effectively eliminated otherwise the predominant σ_v was assumed to be calculated by the equation below by ignoring σ_c.

$$\sigma_v = (\sigma_t^2 - \sigma_p^2 - \sigma_m^2)^{1/2} \text{........(4.3)}$$

The calculated σ_v 's were in the range 2.22 to 3.39 %. In comparison, the largest error would probably be σ_v. It should be mentioned that the errors could be minimized by using modern sample application device, higher quality layer of TLC plate, greater attention in the development process in a special chamber and photodocumentation by high resolution digital camera with automation of the scanning function but these sophisticated devices consumed higher cost of analysis. However, the proposed TLC *in situ* methods gave satisfactory result with less than below 5%RSD, making it a reliable quantitative tool for Ni(II) and Pd(II) analysis.

Table 4.30 Repeatability of the proposed procedures in quantitative determination of Ni(II) by dimethylglyoxime.

Ni(II)ion (ng/spot)	σ_t (%RSD)	σ_d (%RSD)	σ_m (%RSD)
10	2.83	5.55	4.40
15	2.67	3.72	3.98
20	3.69	3.55	1.16
25	4.75	1.30	1.35
30	3.10	3.40	1.00
35	1.58	2.70	1.05
40	2.39	1.77	0.98
Average	3.00	3.14	2.00

Table 4.31 Repeatability of the proposed procedures in quantitative determination of Pd(II) by dimethylglyoxime.

Pd(II) ion (ng/spot)	σ_t (%RSD)	σ_d (%RSD)	σ_m (%RSD)
100	3.55	2.79	2.74
110	6.26	3.45	1.75
120	4.36	2.80	2.44
130	5.07	2.99	1.69
140	3.49	2.29	1.15
150	3.91	3.32	1.79
160	3.56	1.67	1.54
170	3.44	1.99	1.63
Average	4.21	2.66	1.84

Table 4.32 Repeatability of the proposed procedures in quantitative determination of Ni(II) by benzildioxime.

Ni(II) ion (ng/spot)	σ_t (%RSD)	σ_d (%RSD)	σ_m (%RSD)
10	4.38	5.03	2.63
20	4.53	4.96	2.02
30	3.46	4.43	1.96
40	7.20	4.42	1.76
50	3.20	3.54	1.74
60	3.18	3.15	2.32
70	2.12	2.69	1.72
80	1.12	3.03	2.38
Average	3.65	3.91	2.06

Table 4.33 Repeatability of the proposed procedures in quantitative determination of Pd(II) by benzildioxime.

Pd(II) ion (ng/spot)	σ_t (%RSD)	σ_d (%RSD)	σ_m (%RSD)
20	3.12	4.44	3.70
30	3.91	2.14	2.23
40	4.26	3.44	2.63
50	1.82	1.20	2.05
60	4.48	1.57	0.85
70	4.25	1.40	1.32
80	3.82	0.90	0.97
Average	3.66	2.15	1.96

Table 4.34 Repeatability of the proposed procedures in quantitative determination of Pd(II) by furildioxime.

Pd(II) ion (ng/spot)	σ_t (%RSD)	σ_d (%RSD)	σ_m (%RSD)
10	6.67	5.45	2.62
20	4.80	1.95	2.74
30	3.88	2.71	2.23
40	3.51	4.12	2.15
50	3.88	2.41	2.05
60	4.69	3.51	0.89
70	2.77	2.73	1.32
Average	4.31	2.62	2.00

Table 4.35 Repeatability of the proposed procedures in quantitative determination of Ni(II) by nioxime.

Ni(II) ion (ng/spot)	σ_t (%RSD)	σ_d (%RSD)	σ_m (%RSD)
52	1.91	4.82	1.42
8	4.19	4.82	1.74
10	1.86	2.55	1.35
15	2.44	2.04	2.09
20	1.95	0.90	0.50
25	3.14	0.86	0.70
Average	2.93	2.67	1.68

Table 4.36 Repeatability of the proposed procedures in quantitative determination of Pd(II) by nioxime.

Pd(II) ion (ng/spot)	σ_t (%RSD)	σ_d (%RSD)	σ_m (%RSD)
50	3.46	2.41	1.80
60	4.01	2.47	1.55
70	4.00	1.49	1.08
80	5.09	1.71	0.96
90	4.35	1.59	0.87
100	2.49	1.86	0.81
110	3.52	1.11	0.47
120	3.33	0.82	0.52
Average	3.78	1.03	1.04

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4.2.4 Detection Limit

The obtained Gaussian peaks of the complexes signal on the blank (background) signal were maintained after applying the diluted standard metal solution but smaller amplitude signal. The peak to peak noise of a series of background signal were measured and calculated standard deviations. The limit of detection is the mean value of the matrix blank readings ($n \geq 20$) plus 3 standard deviations of the mean, expressed an analyte concentration (35, 36). The calculated detection limits of each Ni(II) and Pd(II)-α-dioxime complexes ($n = 25$) were summarized in Table 4.37.

Table 4.37 The detection limit of all chromatographic systems.

Metal-complexes	Detection limit (ng)
Ni-DMG	3
Pd-DMG	11
Ni-DPG	6
Pd-DPG	6
Pd-furildioxime	3
Ni-nioxime	2
Pd-nioxime	5

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4.3 Study of Ions Interference in the Methods

4.3.1 The Acceptable Amount of Interfering Ions

Since the proposed TLC procedure in Ni(II) or Pd(II) analysis, it should primarily concerned with interference in samples and implied that Cr(III), Mn(II), Fe(III), Co(II), Cu(II), and Zn(II) were expected. Though Ni(II) and Pd(II)-α-dioxime complexes whose relatively large solubility in the organic mobile phases corresponding to the obvious higher R_f leaving other metal ions behind reflected the beneficial effect of the higher complex formation constant on reducing the work required to separate those metal ions before the quantification, the formation of those metal ions with the α-dioxime reagents was probable corequisite. Arising largely of the presence of high concentrations of other metal ions, the direct determination of Ni(II) and Pd (II) might not be carried out satisfactorily. The lowest amount of those metal ions in binary mixture with Ni(II) or Pd(II) that the quantitative treatment of Ni(II) or Pd(II) with accurately tolerate were determined. The results of the Ni(II) complexes were summarized in Tables 4.38-4.40 while the results of Pd(II) complexes were summarized in Table 4.41. As shown from the Table 4.42, the maximum ratio of the quantitatively determined Ni and an expected interfering ions could be estimated on the basis of a certain amount of Ni. It should be pointed out that the measured peak height of the Ni(II) complex signal reduced when the presence of the element was over the limit especially Pd. Hence, one could see that if the first coming ligands were sufficient for the total amount of the both metals the obtained peak height deviation was acceptable. But in the case of insufficient amount of ligands, the competition to form the complexes were occurred and degree of interference depended on complex formation constants and amount of the determined metal ions and the interfere ions. When the amount of the determined Ni was high, the acceptable amount of the interfering metal ions would be low.

Table 4.38 Peak height of Ni-DMG complex without and with interfering ions in various ratios.

Mass ratios (Ni:M) Ni(II) 15 ng	Peak height of Ni-DMG complex without and with interfering ions in various ratios							
	Ni	**Ni:Cr**	**Ni**	**Ni:Mn**	**Ni**	**Ni:Fe**	**Ni**	**Ni:Co**
1:1	-	-	-	-	7.18±0.35	7.18±0.39	-	-
1:5	-	-	-	-	-	-	-	-
1:10	9.88±0.38	9.75±0.47	-	-	8.80±1.13	8.88±0.18	5.88±1.01	5.96±0.72
1:20	8.45±0.37	8.51±0.26	-	-	10.15±0.40	10.25±0.29	10.15±0.40	10.53±0.05
1:30	**7.80±0.11**	**7.04±0.27**	10.05±0.31	10.72±0.15	**12.15±0.53**	**3.56±0.45**	**11.58±0.39**	**5.55±0.27**
1:40	**11.50±0.28**	**2.78±0.41**	**12.46±0.28**	**3.83±0.28**	-	-	-	-
1:50	-	-	-	-	-	-	-	-

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Table 4.38 Peak height of Ni-DMG complex without and with interfering ions in various ratios (continued).

Mass ratios (Ni:M) Ni(II) 15 ng	Peak height of Ni-DMG complex without and with interfering ions in various ratios					
	Ni	**Ni:Cu**	**Ni**	**Ni:Zn**	**Ni**	**Ni:Pd**
1:1	8.48±0.57	8.55±0.39	5.03±0.64	5.18±0.39	10.43±0.59	10.48±0.30
1:5	-	-	-	-	-	-
1:10	6.56±0.55	6.62±0.13	8.45±0.38	8.53±0.26	10.50±1.12	11.73±0.77
1:20	8.45±0.37	8.51±0.26	7.65±0.44	6.83±0.38	**9.03±0.44**	**9.32±0.65**
1:30	**10.58±0.69**	**7.33±0.25**	**9.43±0.15**	**4.68±0.36**	-	-
1:40	-	-	-	-	-	-
1:50	**8.43±0.06**	**5.20±0.62-**	-	-	-	-

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Table 4.39 Peak height of Ni-DPG complex without and with interfering ions in various ratios.

Mass ratios (Ni:M) Ni(II) 60 ng	Peak height of Ni-DPG complex without and with interfering ions in various ratios							
	Ni	**Ni:Cr**	**Ni**	**Ni:Mn**	**Ni**	**Ni:Fe**	**Ni**	**Ni:Co**
1:1	7.20±0.41	7.17±0.32	6.86±0.35	6.78±0.25	5.83±0.67	6.03±0.46	**8.97±0.38**	**6.73±0.76**
1:5	**5.83±0.42**	**4.90±0.41**	**7.36±0.06**	**6.62±0.44**	**7.25±0.40**	**5.98±0.34**	-	-
1:10	**7.85±0.71**	**3.92±0.57**	**10.90±0.38**	**4.23±0.15**	**9.93±0.31**	**3.22±0.30**	-	-
1:20	-	-	-	-	-	-	-	-
1:30	-	-	-	-	-	-	-	-
1:40	-	-	-	-	-	-	-	-
1:50	-	-	-	-	-	-	-	-

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Table 4.39 Peak height of Ni-DPG complex without and with interfering ions in various ratios(continued).

Mass ratios (Ni:M) Ni(II) 60 ng	Peak height of Ni-DPG complex without and with interfering ions in various ratios					
	Ni	**Ni:Cu**	**Ni**	**Ni:Zn**	**Ni**	**Ni:Pd**
1:1	**5.87±0.40**	**4.90±0.40**	6.48±0.71	6.13±0.29	**8.80±0.08**	**9.12±0.32**
1:5	-	-	**9.33±0.22**	**5.85±0.34**	-	-
1:10	-	-	**8.85±0.44**	**5.06±0.38**	-	-
1:20	-	-	-	-	-	-
1:30	-	-	-	-	-	-
1:40	-	-	-	-	-	-
1:50	-	-	-	-	-	-

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Table 4.40 Peak height of Ni-nioxime complex without and with interfering ions in various ratios.

Mass ratios (Ni:M) Ni(II) 15 ng	Peak height of Ni-nioxime complex without and with interfering ions in various ratios							
	Ni	**Ni:Cr**	**Ni**	**Ni:Mn**	**Ni**	**Ni:Fe**	**Ni**	**Ni:Co**
1:1	8.10±0.78	8.20±0.41	10.60±0.53	10.58±0.51	5.72±0.47	5.38±0.33	8.20±0.56	8.50±0.25
1:5	-	-	-	-	7.57±0.57	7.10±0.30	8.00±0.52	8.20±0.24
1:10	-	-	7.20±0.60	7.23±0.38	9.23±0.55	9.08±0.38	**9.93±0.46**	**7.68±0.41**
1:20	8.47±0.47	8.28±0.24	4.90±0.33	4.92±0.28	7.77±0.32	7.78±0.19	-	-
1:30	**7.67±0.72**	**4.40±0.33**	**8.00±0.39**	**7.03±0.45**	**8.40±0.48**	**7.71±0.37**	-	-
1:40	-	-	-	-	-	-	-	-
1:50	-	-	-	-	-	-	-	-

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Table 4.40 Peak height of Ni-nioxime complex without and with interfering ions in various ratios(continued).

Mass ratios (Ni:M) Ni(II) 15 ng	Peak height of Ni-nioxime complex without and with interfering ions in various ratios					
	Ni	**Ni:Cu**	**Ni**	**Ni:Zn**	**Ni**	**Ni:Pd**
1:1	8.60±0.18	8.56±0.19	9.33±0.62	9.24±0.53	8.18±0.45	8.10±0.29
1:5	8.15±0.48	8.13±0.23	7.20±0.60	7.23±0.38	9.23±0.29	9.10±0.16
1:10	7.33±0.43	7.12±0.08	**10.27±0.31**	**8.44±0.34**	8.05±0.66	8.06±0.29
1:20	**9.00±0.40**	**5.47±0.32**	-	-	**9.65±0.49**	**3.10±0.24**
1:30	-	-	-	-	-	-
1:40	-	-	-	-	-	-
1:50	-	-	-	-	-	-

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Table 4.41 Peak height of Pd-dioxime complexes without and with interfering ions.

Pd-dioxime complexes	Mass ratios (Pd:M) Pd(II) 60 ng	Peak height of Pd-dioxime complexes without and with interfering ions							
		Pd	**Pd:Cr**	**Pd**	**Pd:Mn**	**Pd**	**Pd:Fe**	**Pd**	**Pd:Co**
Pd-DMG	1:1	2.45±0.53	3.55±0.55	3.73±0.33	4.02±0.68	3.52±0.47	4.90±0.57	4.05±0.27	5.89±0.14
Pd-DPG	1:1	4.78±0.50	7.45±0.33	4.60±0.36	7.90±0.71	4.55±0.45	7.50±0.17	4.50±0.11	7.88±0.29
Pd-furildioxime	1:1	7.25±0.38	10.83±0.85	7.60±0.47	10.95±0.53	9.17±0.15	11.56±0.39	8.40±0.44	13.96±0.86
Pd-nioxime	1:1	2.55±0.39	3.52±0.44	3.32±1.17	3.98±0.70	2.38±0.42	4.12±0.42	2.73±0.17	4.90±0.14

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Table 4.41 Peak height of Pd-dioxime complexes without and with interfering ions(continued).

Pd-dioxime complexes	Mass ratios (Pd:M) Pd(II) 60 ng	Peak height of Pd-dioxime complexes without and with interfering ions					
		Pd	**Pd:Ni**	**Pd**	**Pd:Cu**	**Pd**	**Pd:Zn**
Pd-DMG	1:1	2.55±0.41	5.55±0.53	4.87±0.19	6.03±0.78	3.34±0.89	6.53±0.44
Pd-DPG	1:1	4.06±0.50	8.65±0.64	4.80±0.41	7.28±0.38	4.00±0.28	6.83±0.45
Pd-furildioxime	1:1	8.41±0.45	11.55±0.46	7.76±0.25	12.80±0.23	8.05±0.68	12.53±0.17
Pd-nioxime	1:1	2.28±0.59	5.65±0.26	2.45±0.17	3.96±0.68	2.25±0.41	3.10±0.22

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Table 4.42 The maximum amount of interfering ions(ng) that could be present together with Ni.

Interfering ion (ng)	The maximum amount of interfering ions (ng) that could be present together with Ni		
	Ni-DMG system (Ni(II) 15 ng)	Ni-DPG system (Ni(II) 50ng)	Ni-nioxime system (Ni(II) 15 ng)
Cr(III)	300	50	300
Mn(II)	300	50	300
Fe(III)	300	50	300
Co(II)	300	*	75
Cu(II)	600	*	150
Zn(II)	150	50	75
Pd(II)	75	*	150

* less than 50 ng

The results of the experiments for the determination of small amount of Ni(II) by means of the α-dioximes (DMG, DPG and nioxime) showed that the co-existing Pd(II), Zn(II), Cu(II) and Co(II) interfered seriously while Mn(II), Fe(III) and Cr(III) moderately interfered. However, it was estimated that Ni could be determined accurately if the total amount of the mentioned element applied on the TLC plate was not higher than 1.0 millimole.

In the case of Pd complexes of the α-dioximes , the results indicated that the presence of Ni(II), Zn(II), Cu(II), Co(II), Mn(II), Fe(III) and Cr(III) was undesirable when the small amounts of Pd(II) were to be determined. Accordingly, the Pd(II) complexes were less sensitive to determine compared with those of Ni(II), the tested amount of Pd(II) was quite high (60 or 90 ng) so the acceptable added amount of the metal could not be determined.

The disadvantage of the presence of these serious interferes should be avoided by applying the method of the Pd(II) determination on a sample that was free from the elements. When necessary, a separation of the interferes should be carried out before the quantitative procedure on Ni(II) or Pd(II). It was not advisable for more steps of sample preparation. The proposed *in situ* TLC quantitative procedure for Ni and Pd were finally adopted by adding a suitable masking reagent in the dipping solution to lower the interferences, simplify and shorten the procedure itself and duration of analysis.

4.3.2 The Use of Triethanolamine as the Masking Agent

4.3.2.1 The Study of the Ability of Triethanolamine in Lowering the Interference

The results in the previous 4.3.1 showed that all of the selected metal ions could interfere the accuracy and precision of the proposed methods for Ni(II) and Pd(II) determination due to their stable complexes with the α-dioximes. The amount of the co-excisting elements with Ni(II) or Pd(II) that the method could tolerate were unsatifactorily low. According to the former literature using these α-dioximes as the gravimetric reagents or solvent-extraction spectrometric reagents for nickel determination, it was suggested that tartrate, citrate, triethanolamine and other polyamines were well applicable for masking of these transition metal ions. Due to its high solubility in the organic mobile phases and its lower R_f background on TLC plates using every selected chromatographic conditions, triethanolamine was chosen and utilized in the experiments, a great excess of the masking ligand was added into the dipping solution together with the α-dioximes and further determinations were carried out similar to those unemployed in 4.3.1. The accuracy and precision in measuring peak height of Ni(II) or Pd(II) complexes given in Tables 4.43 to 4.48 showed some improved results. Triethanolamine was capable of masking Mn(II), Zn(II), and Fe(III) from Ni(II) but not successful in the case of Pd(II) determination.

Table 4.43 Peak height of Ni-DMG complex using the developing solution with and without triethanolamine.

Peak height of Ni-DMG complex			
Developing solution without triethanolamine		Triethanolamine 0.73 M in 10.00 mL developing solution	
Ni (15 ng)	Ni:Cr 1:30	Ni (15 ng)	Ni:Cr 1:30
7.81±0.10	7.04±0.27	7.17±0.23	5.63±0.32
Ni (15 ng)	Ni:Mn 1:30	Ni (15 ng)	Ni:Mn 1:30
10.58±0.69	8.83±0.84	6.80±0.27	4.63±0.21
Ni (15 ng)	Ni:Fe 1:30	Ni (15 ng)	Ni:Fe 1:30
7.87±0.23	6.35±0.68	**5.65±0.38**	**5.27±0.12**
Ni (15 ng)	Ni:Co 1:30	Ni (15 ng)	Ni:Co 1:30
11.58±0.39	5.55±0.27	9.53±0.22	5.36±0.39
Ni (15 ng)	Ni:Cu 1:30	Ni (15 ng)	Ni:Cu 1:30
12.13±0.06	7.33±0.25	6.45±0.45	3.70±0.25
Ni (15 ng)	Ni:Pd 1:10	Ni (15 ng)	Ni:Pd 1:10
10.50±1.12	11.73±0.77	**9.34±0.62**	**9.05±0.30**

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Table 4.44 Peak height of Ni-DPG complex using the developing solution with and without triethanolamine.

Peak height of Ni-DPG complex			
Developing solution without triethanolamine		Triethanolamine 0.73 M in 10.00 mL developing solution	
Ni (50 ng)	Ni:Cr 1:5	Ni (50 ng)	Ni:Cr 1:5
5.83±0.42	4.90±0.40	**7.33±0.90**	**7.43±0.87**
Ni (50 ng)	Ni:Mn 1:5	Ni (50 ng)	Ni:Mn 1:5
7.37±0.06	6.62±0.44	**3.87±0.47**	**3.83±0.40**
Ni (50 ng)	Ni:Fe 1:5	Ni (50 ng)	Ni:Fe 1:5
7.25±0.41	5.98±0.34	**4.50±0.67**	**4.23±0.33**
Ni (50 ng)	Ni:Zn 1:5	Ni (50 ng)	Ni:Zn 1:5
9.33±0.22	5.85±0.34	**4.48±0.73**	**4.53±0.67**

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Table 4.45 Peak height of Ni-nioxime complex using the developing solution with and without triethanolamine.

Peak heignt of Ni-nioxime complex			
Developing solution without triethanolamine		Triethanolamine 0.73 M in 10.00 mL developing solution	
Ni (15 ng)	Ni:Cr 1:30	Ni (15 ng)	Ni:Cr 1:30
7.66±0.72	4.40±0.33	8.15±0.66	7.73±0.26
Ni (15 ng)	Ni:Mn 1:30	Ni (15 ng)	Ni:Mn 1:30
8.00±0.39	7.03±0.45	**10.65±0.24**	**10.60±0.17**
Ni (15 ng)	Ni:Fe 1:30	Ni (15 ng)	Ni:Fe 1:30
8.40±0.48	7.70±0.37	10.98±0.28	9.95±0.44
Ni (15 ng)	Ni:Co 1:10	Ni (15 ng)	Ni:Co 1:10
9.93±0.46	7.68±0.41	6.15±0.21	4.86±0.57
Ni (15 ng)	Ni:Cu 1:20	Ni (15 ng)	Ni:Cu 1:20
9.00±0.40	5.47±0.32	6.03±0.31	3.6±0.14
Ni (15 ng)	Ni:Zn 1:10	Ni (15 ng)	Ni:Zn 1:10
10.27±0.31	8.44±0.34	**8.30±0.62**	**8.44±0.47**
Ni (15 ng)	Ni:Pd 1:20	Ni (15 ng)	Ni:Pd 1:20
9.65±0.49	3.10±0.26	6.93±0.15	5.85±0.21

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Table 4.46 Peak height of Pd-DPG complex using the developing solution with and without triethanolamine.

Peak height of Pd-DPG complex			
Developing solution without triethanolamine		Triethanolamine 0.73 M in 10.00 mL developing solution	
Pd (60 ng)	Pd:Cr 1:1	Pd (60 ng)	Pd:Cr 1:1
4.78±0.49	7.45±0.33	4.16±0.15	5.55±0.07
Pd (60 ng)	Pd:Mn 1:1	Pd (60 ng)	Pd:Mn 1:1
4.60±0.36	7.90±0.71	4.47±0.29	6.63±0.32
Pd (60 ng)	Pd:Fe 1:1	Pd (60 ng)	Pd:Fe 1:1
4.60±0.36	7.50±0.17	4.33±0.25	7.05±0.07
Pd (60 ng)	Pd:Co 1:1	Pd (60 ng)	Pd:Co 1:1
4.50±0.10	7.88±0.29	4.16±0.15	5.50±0.14
Pd (60 ng)	Pd:Ni 1:1	Pd (60 ng)	Pd:Ni 1:1
4.07±0.50	8.65±0.64	4.33±0.25	7.95±0.35
Pd (60 ng)	Pd:Cu 1:1	Pd (60 ng)	Pd:Cu 1:1
4.80±0.14	7.28±0.38	4.16±0.15	5.45±0.07
Pd (60 ng)	Pd:Zn 1:1	Pd (60 ng)	Pd:Zn 1:1
4.00±0.28	6.83±0.45	4.33±0.25	7.50±0.28

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Table 4.47 Peak height of Pd-furildioxime complex using developing solution with and without triethanolamine.

Peak height of Pd-furildioxme complex			
Developing solution without triethanolamine		Triethanolamine 0.73 M in 10.00 mL developing solution	
Pd (60 ng)	Pd:Cr 1:1	Pd (60 ng)	Pd:Cr 1:1
7.25±0.38	10.83±0.85	8.23±0.06	10.83±0.50
Pd (60 ng)	Pd:Mn 1:1	Pd (60 ng)	Pd:Mn 1:1
7.60±0.46	10.93±0.53	8.23±0.06	9.37±0.12
Pd (60 ng)	Pd:Fe 1:1	Pd (60 ng)	Pd:Fe 1:1
9.17±0.15	16.51±0.39	7.00±0.71	16.05±0.35
Pd (60 ng)	Pd:Co 1:1	Pd (60 ng)	Pd:Co 1:1
8.40±0.43	13.96±0.86	9.46±0.57	17.40±0.46
Pd (60 ng)	Pd:Cu 1:1	Pd (60 ng)	Pd:Cu 1:1
4.80±0.14	7.28±0.38	10.26±0.05	14.70±0.14
Pd (60 ng)	Pd:Zn 1:1	Pd (60 ng)	Pd:Zn 1:1
8.05±0.67	12.52±0.17	9.46±0.57	15.27±0.92

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Table 4.48 Peak height of Pd-nioxime complex in developing solution with and without triethanolamine.

Peak height of Pd-nioxime complex			
Developing solution without triethanolamine		Triethanolamine 0.73 M in 10.00 mL developing solution	
Pd (60 ng)	Pd:Cr 1:1	Pd (60 ng)	Pd:Cr 1:1
2.55±0.39	3.52±0.44	2.83±0.35	4.00±0.42
Pd (60 ng)	Pd:Mn 1:1	Pd (60 ng)	Pd:Mn 1:1
3.32±1.17	3.98±0.70	2.27±0.06	2.93±0.76
Pd (60 ng)	Pd:Fe 1:1	Pd (60 ng)	Pd:Fe 1:1
2.38±0.42	4.12±0.42	2.27±0.06	5.27±0.15
Pd (60 ng)	Pd:Co 1:1	Pd (60 ng)	Pd:Co 1:1
2.73±0.17	4.90±0.14	3.10±0.79	6.23±0.40
Pd (60 ng)	Pd:Cu 1:1	Pd (60 ng)	Pd:Cu 1:1
2.45±0.17	3.96±0.68	3.10±0.79	4.90±0.28
Pd (60 ng)	Pd:Zn 1:1	Pd (60 ng)	Pd:Zn 1:1
2.25±0.41	3.10±0.22	3.33±0.57	4.30±0.14

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4.3.2.2 The Study of an Effect of Added Triethanolamine on Ni-α-dioxime Complex Calibration curve

It is evident from the experiments in 4.3.2.1 that as the triethanolamine was added in the developing solutions to mask the interfering metal ions, the recovery of Ni(II) was achieved accurately while those interfering metals were not ensured successful masking in the determination of Pd(II). The data tubulated in Tables 4.43, 4.44 and 4.45 showed that this masking ligand worked well for the recovery of Ni(II) from Fe(III), Mn(II) and Zn(II). However it could be observed that the measured peak height of Ni-α-dioximes complex parallely decreased comparing with those of using the developing solution without triethanolamine. Therefore, it was interesting to examine the effect of the added amount triethanolamine, the established linear calibration curves of various added amounts of the masking agent (0.73-1.10 M) were compared and illustrated in Figures 4.23 and 4.24. The Ni-DMG and Ni-nioxime complex systems were chosen for this study on their linear concentration range. The results obtained were well in agreement with those references using the triethanolamine free developing reagent. Although this did not show deleterious effect but a developing solution in large concentrations of triethanolamine caused partial differences in chromatographic condition due to slower velocity of the viscous mobile phase. This extended the developing time and spot size of Ni-α-dioxime complexes. It was advisable to add triethanolamine 1-2 mL in a 10 mL of mobile phase (0.7-1.5 M).

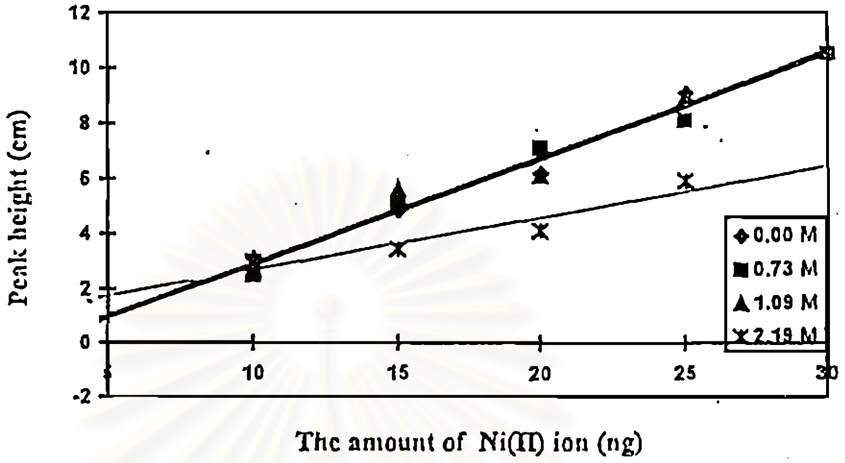

Figure 4.23 Linear calibration curves of Ni-DMG complex (Ni(II) 10-30 ng) when various amounts of triethanolamine was added in the developing solution.

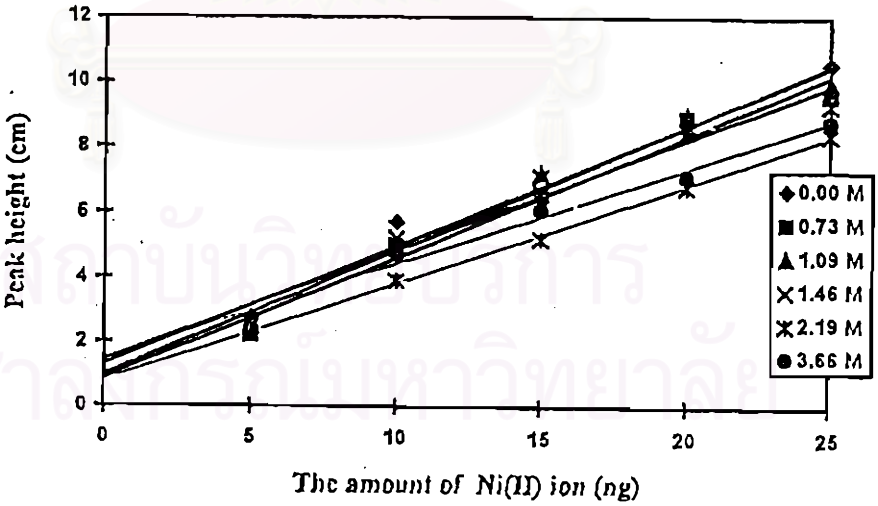

Figure 4.24 Linear calibration curves of Ni-nioxime complex (Ni(II) 5-25 ng) when various amounts of triethanolamine was added in the developing solution.

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4.4 The Quantitative Determination of Nickel Ion in Certified Reference Alloys

To demonstrate the practical utility and reliability chromatographic systems, the procedures of determination of Ni(II) by means of DMG and nioxime were chosen to be applied to a few reference metal alloys of Ni that associated with Cu, Fe, Mn, Zn and Cr. There British chemical standard alloys used as Ni references were

Reference no.1 CUPRO-NICKEL (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%

Reference no.2 Ni-Cr-Mo STEEL (No.219/3) : **nickel 2.52%,** chromium 0.76%,carbon 0.31%, molybdenum 0.6%, silicon 0.20%, sulphur 0.01%, phosphorus 0.019%,copper 0.19%, manganese 0.74%

Reference no.3 3% 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%

It was expected that the proposed methods could be applied to the Ni alloys accurately. In reference no. 1, mass ratio of Ni and Cu was in acceptable value (about 1:2) and in reference no. 2 and no. 3, high amount of Fe(III) should be masked by triethanolamine. The formerly chosen condition of chromatographic systems for both proposed procedures were reported in Table 4.49 and the procedures and accuracy and precision of the methods were also calculated and shown in Tables 4.50 and 4.51 by using DMG and nioxime respectively. The percentage of Ni found was in close agreement with the certified value.

Table 4.49 The chosen conditions of chromatographic systems for nickel determination.

Parameter	**System I**	**System II**
Wavelength	270 nm	270 nm
Mobile phase system	$CHCl_3$:EtOH (9:1)	C_6H_{14}:$CHCl_3$:EtOH (1.0:7.5:1.5)
Complexing agent	0.05%w/v DMG	0.015%w/v nioxime
Masking agent	1 M triethanolamine	1 M triethanolamine
Linearity of calibration curve	10-40 ng	5-25 ng

Table 4.50 The amount of nickel in certified reference alloys when using DMG as complexing agent.

Ref. no.	%Ni in sample	%Ni found	%relative error	%RSD
1	30.85	30.96	0.36	3.57
2	2.52	2.51	0.40	2.26

(n=9)

Table 4.51 The amount of nickel in certified reference alloys when using nioxime as complexing agent.

Ref. no.	%Ni in sample	%Ni found	%relative error	%RSD
1	30.85	30.72	0.42	0.76
2	2.52	2.51	0.40	2.79
3	3.54	3.52	0.56	2.84

(n=9)

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