CHAPTER II

THEORY

THIN LAYER CHROMATOGRAPHY (TLC)

2.1 Introduction (23-26)

Thin layer chromatography is a type of liquid chromatography in which the stationary phase is in the form of a layer on a glass, aluminum, or plastic support. The term "planar chromatography" is often used for both TLC and paper chromatography because each employs a planar stationary phase rather than a column.

The principle of TLC has been known for 100 years . The real break-through of TLC as an analytical method, however, comes later. Egon Stahl (1924-1986), who developed this physico-chemical separation technique more than 40 years ago and made it a standardized method and called it a thin layer chromatography.

For some years it seemed as if TLC was a stagnant, old-fashioned technique, which was displaced by HPLC and almost forgotten. However, the application or development of other adsorbents in addition to the customary silica, the use of new supports and new separation techniques considerably enhance the applicability of TLC. In this respect especially the possibilities of improved quantitative analyses on high performance thin layer plates via photometric methods have caused a substantial recovery of TLC. This technique is used for separation of substances in variety of fields such as chemical, biological, pharmaceutical, and environmental. The reasons for this are many, and include ease of use, speed of separation, high sensitivity, low cost, and the unique ability to analyze multiple samples simultaneously under identical conditions.

Thin layer chromatography is, like all chromatographic techniques, a multistage distribution process. This process involves a suitable adsorbent (the stationary phase), solvents or solvent mixtures (the mobile phase), and the sample molecules. For thin layer chromatography the adsorent is coated as a thin layer onto a suitable support. On this layer the substance mixture is separated by elution with a suitable solvent.

Differential migration is the result of varying degrees of affinity of the mixture components to the stationary and mobile phases. Different separation mechanisms are involved and the predominant forces depend on the exact nature of the two phases and the solutes. The interactions involved in determining chromatographic retention and selectivity include hydrogen bonding, electron-pair donor/electron-pair acceptor, ion-ion, ion-dipole, and van der Waals interactions.

The basis parameter used to describe migration in TLC is the R_f value, where

$$R_f = \frac{\text{distance moved by the solute}}{\text{distance moved by mobile-phase front}} \quad \text{.....(2.1)}$$

R_f values vary from 1 to 0, or from 100 to 0 if multiplied by 100 (hR_f).

The capacity factor, k', is the ratio of the quantities of solute distributed between the mobile and the stationary phases, or the ratio of the respective times the substance depends in the two phases,

$$k' = t_s/t_m = \frac{\text{retention time in stationary phase}}{\text{retention time in mobile phase}} \quad \text{.....(2.2)}$$

The capacity factor and R_f are related by the equation

$$k' = 1-R_f / R_f \quad \text{.....(2.3)}$$

The classical Van Deemter equation and its modifications have been used to describe zone spreading in TLC and HPLC in terms of eddy diffusion, molecular diffusion, and mass transfer. The efficiency of zone in TLC is given by the equation.

$$N = 16 [R_f Z_f / W_b]^2 \quad \text{.....(2.4)}$$

where N = number of theoretical plates

Z_f = distance of solvent migration

W_b = diameter of the zone

An equation for resolution in TLC is

$$R_s = \frac{1}{4} [n / k + 1]^{1/2} [k / k + 1] \left[\frac{k'_1 - 1}{k'_2}\right] \quad \text{.....(2.5)}$$

where k'_1 and k'_2 are the capacity factors constant for spots 1 and 2

k is the mean value of k'_1 and k'_2

High-performance thin layer chromatography (HPTLC) has the highest efficiency for short migration distances, and efficiency eventually is more powerful than for TLC as the migration distance increases and molecular diffusion becomes the limiting factor.

2.2 TLC Procedure (6-7, 26-28)

A basic TLC is carried out as follows. An initial zone of mixture is placed near one end of the stationary phase, a thin layer ; the sample is dried; and the end of the stationary phase with the initial zone is placed into the mobile phase, usually a mixture of solvents, placed inside a closed chamber. The components of the mixture migrate at different rates during movement of the mobile phase through the stationary phase which is termed the development of the chromatogram. When the mobile phase has moved an appropriate distance, the stationary phase is removed. The mobile phase is rapidly dried, and the zones are detected by application of a suitable methods. The entire TLC procedure is summarized in Figure 2.1.

2.2.1 Sample Preparation

Sample preparation procedures for TLC are similar to those for GC and LC . The solution to be analyzed must be sufficiently concentrated so that the analyte can be detected, and pure enough so that it can be separated as discrete, compact spot or zone. Relatively pure samples or their concentrate extracts can often be directly spotted for TLC analysis. If the analyte is present at low levels in a complex sample, solvent extraction, purification, and concentration procedures must precede TLC. Impurities that co-migrate with the analyte, adversely affect its detection must be removed prior to TLC.

TLC Procedure

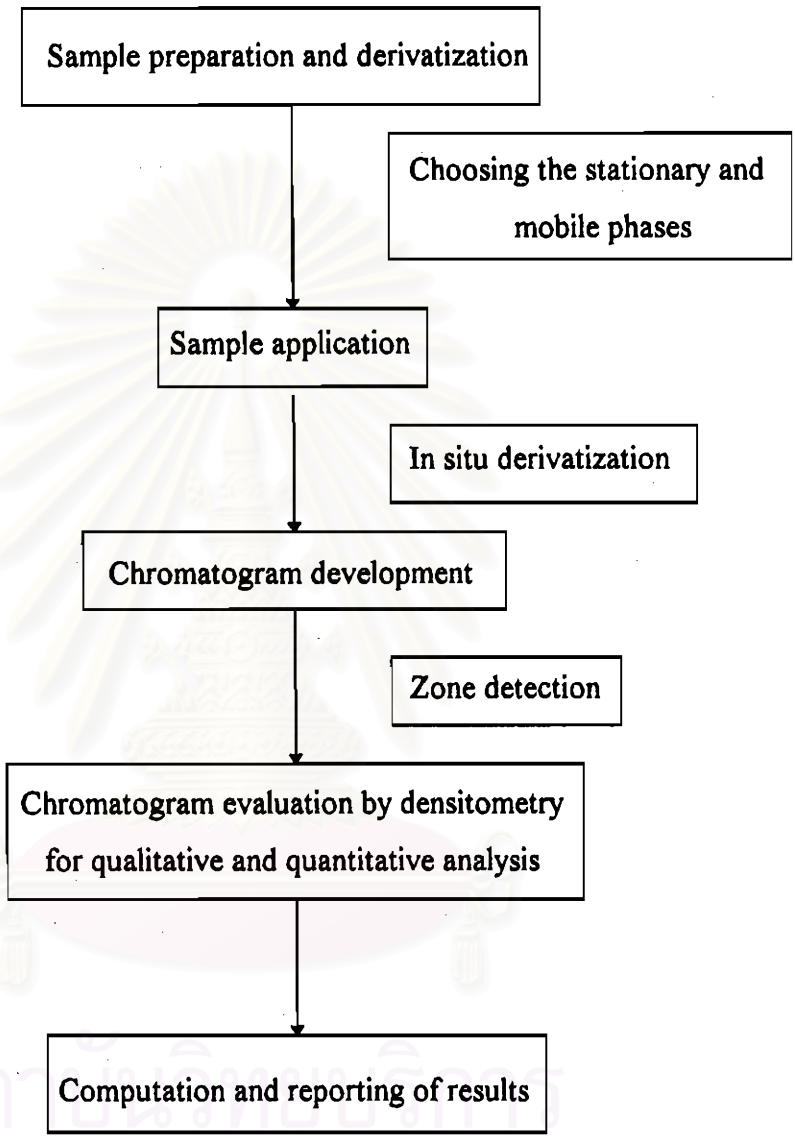

Figure 2.1 The procedure of thin layer chromatography.

2.2.2 Stationary Phase

Thin layer chromatography is a separation method in which uniform thin layers of sorbent or selected media are used as carrier medium. The sorbent is applied to a backing as a coating to obtain a stable of suitable size. The most common support is a glass plate, but other supports such as plastic sheets and aluminum foil are also used. Some examples of adsorbents used for some representative separations by thin-layer chromatography are given in Table 2.1

Table 2.1 Adsorbents for thin-layer chromatography.

Solid	Used to separate
Silica gel	Amino acids, alkaloids, sugars, fatty acids, lipids, essential oils, inorganic anions and cations, steroids, terpenoids
Alumina	Alkaloids, food dyes, phenols, steroids, vitamins, carotenes, amino acids
Kieselguhr	Sugars, oligosaccharides. dibasic acids, steroids
Celite	Steroids, inorganic cations
Cellulose powder	Amino acids, food dyes, alkaloids, nucleotides
Ion-exchange cellulose	Nucleotides, halide ions
Starch	Amino acids
Polyamide powder	Anthocyanins, aromatic acids, antioxidants, flavonoids, proteins
Sephadex	Amino acids, proteins

The choice of the layer and mobile phase is made in relation to the nature of the sample. Normal-or straight-phase adsorption TLC on silica gel with a relatively less polar mobile phase is the most widely used. Lipophilic C_{18}, C_{12}, C_8, and C_2 bonded silica gel phases with a polar aqueous mobile phase are used for reversed-phase TLC. Other commercial precoated layers include alumina, Florisil, polyamide, cellulose, ion exchangers, and chemically bonded phases can function with multimodal mechanisms depending on the composition of the mobile phase. Silica gel may be impregnated with various solvents, buffers, and selective reagents to improve separations. Chiral plates composed of a reversed-phase layer impregnated with copper acetate and a chiral selector, (2S,4R,2'RS)-4-hydroxy-1-(2-hydroxydodecyl) proline, can be used to separate enantiomers through a ligand exchange mechanism. Preparative taper plates have a layer-thickness gradient that reduce spot elongation and overlapping. A recent development is the commercial production of TLC plate in the form of flexible sheets containing about 90% of silica gel or chemically bonded silica gel enmeshed in polytetrafluoroethylene microfibrils with no glass or plastic backing. These particle-loaded membranes are useful where easy sample recovery is important. Layers are routinely cleaned by predevelopment with the mobile phase or dichloromethane-methanol (1:1) prior to sample application, especially for the quantitative and preparative TLC. Two important properties of the adsorbent are its particle size and its homogeneity, because adhesion to the support largely depend on them. The factors to be considered when choosing a sorbent for TLC are

- type of compound to be separated
- visualization technique to be employed
- thickness and stability of the layer desired , and
- mobile phase characteristics

Modern high performance TLC began around 1975 with the introduction of commercially precoated high efficiency plates, which are smaller (10x10 or 10x20 cm); thinner (0.1-0.2 mm), more uniform layer composed of smaller-diameters particles (approximately 5 μm average); and developed over shorter distances (approximately 3-6 cm) compared to classical 20x20 cm TLC plates. Other characteristics of HPTLC compared to TLC include smaller sample volumes (0.1-0.2 μL), starting spot

diameters (1.0-1.5 mm), diameters of separated spots (2-6 mm), and detection limits (0.1-0.5 ng for absorption and 5-10 pg for fluorescence),and a greater number of sample lanes per plate. HPTLC plates are more expensive than TLC plates.

2.2.3 Mobile Phase

The mobile phase in liquid chromatography including TLC is a factor in the selectivity of chromatographic system, exerting a decisive influence on the separation. The success in the technique of TLC depends to a great extent upon selecting the mobile phase that will give the desired separation. The mobile phase is usually a mixture of 2-5 different solvents selected empirically using trial and error guide by prior personal experience and literature reports of similar separations.

Mobile phase for TLC are chosen in relative to the nature of the layer and mixture to be separated. The strength (polarity) of the mobile phase influences the R_f range of the solutes, while the chemical classification of the solvent component determines the interactions and selectivity of the system. These "elutropic series'' are used along with knowledge of solubility (polarity) characteristics of the mixtures to select the chromatographic system to be used. As polarity increases, a solvent becomes stronger (increases R_f values) in normal-phase TLC , while solvents that are strong for RP-TLC are less polar.

2.2.4 Application of Samples and Standards

Sample and standards prepared for TLC are dissolved in appropriate solvent in such concentration that will allow eventual detection of the solute of interest. Typically, 1-5 μL containing 1 ng to 10 μg of solute are applied in the form of spots or narrow bands to TLC plates. The starting zones should be as small as possible for efficient separation.

Samples and standards are applied to the layer as small round spots or streaks using one of a variety of application devices, including glass capillary pipette, microdispensers, or microsyringes. Optimum initial spots size for TLC are typically 3-6

mm. Spots that are too highly concentrated will give poor separation, since the mobile phase solvent tends to flow through the point of least resistance and will travel around the spot. This will result, after development, in separated unsymmetrical spots. Ideally, if the spots are properly applied, the separation zone are symmetrical and compact.

Band application is recommended when sample volumes greater than 50 μL are to be applied, which is often in the case of trace analysis and thin-layer radiochromatography. Bands or streaks of sample are applied manually or automatically or formed automatically by use of plates with a preadsorbent or concentrating area, or formed by a predevelopment on conventional plates. Manual application essentially involves placing a contiguous series of spots from a syringe or micropipette side by side. The band application is also important in preparative TLC.

A sample application is one of the main source of error in quantitative TLC. Therefore a great care must be taken to choose a reliable application device and optimize techniques if accurate and precise analyse are to be realized.

2.2.5 Development

TLC development is the process in which the mobile phase moves across the sorbent layer to effect separation of the sample substances. It may be accomplished a number of ways with several forms of apparatus, ranging from simple to complex. The results of TLC are strongly dependent upon the environmental condition during development, such as small changes in mobile phase composition, temperature, humidity, and the size and type of the chamber and its solvent vapor saturation condition. Development of a chromatogram should never start before the solvent of the applied samples and standards are evaporated completely.

The most frequently used separation technique is ascending TLC in the common trough chamber (standard method, linear development). It is usually applied as a single development. However, the multiple development, with or without change of eluent (step technique) can improve separation results. For the 2-dimensional development only one spot of the sample is applied at one edge of a plate. After chromatography in the first direction, the plate is dried, turned by 90°C and developed

in the second dimension with another eluent. Thus complicated mixtures give two dimensional chromatograms taking advantage of the different separating properties of two eluents.

It is important to pay attention to the atmosphere in the developing chamber. If reproducible migration distances are required, saturation of the chamber atmosphere with eluent vapor is necessary. For this purpose the developing chamber is lined with well absorbing chromatography paper and charged with a correspondingly larger volume of eluent.

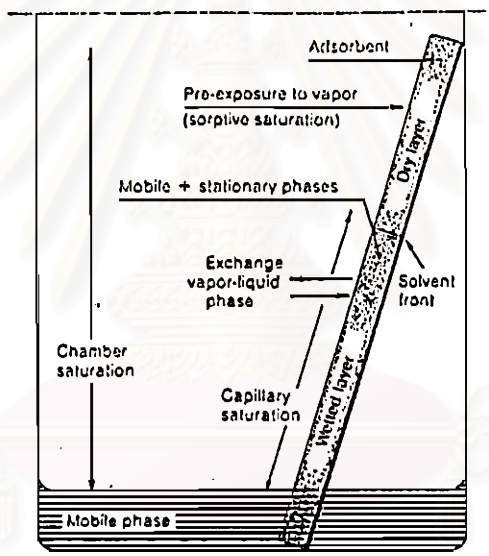

Figure 2.2 Schematic representation of the relationships between development, chamber saturation and pre-loading with solvent vapors of ascending TLC.

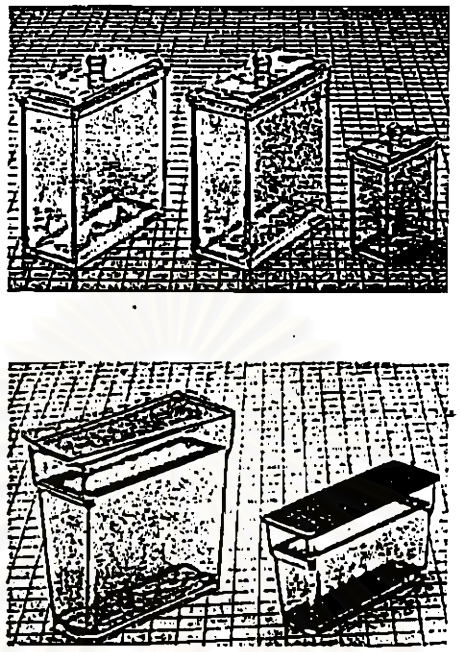

Figure 2.3 Commonly used trough chambers.

A better control of chromatographic conditions, especially of the vapor phase, is achieved in flat chambers or a trough with sandwich configuration. With these techniques the vapor phase is excluded by a covering plate and the eluent supply is supply is linear as seen in the classical TLC.

2.2.6 Zone Detection

Detection of Zones

After development with the mobile phase, the plate is dried in a fumehood and heated to completely evaporate the mobile phase. Separated compounds are detected on the layer by their natural colors, or natural fluorescence, or quenching of fluorescence, or observing the color or the fluorescence of the zone after reacting with an appropriate reagent. Compounds that are naturally colored are viewed directly on the layer in daylight, while compounds with native fluoresce are viewed as bright zone on a dark background under ultraviolet light.

Color, UV absorption, or fluorescence can be induced by spray or dip application of a chromogenic or fluorogenic reagent that reacts chemically with the compound to be detected. The detection reagent solution is usually applied to the layer postchromatography by spraying or dipping , or the reagent may be preimpregnated into the layer prior to spotting and chromatography.

Qualitative Identification of Zones

Qualitative identification is based on characteristic colors produced by a specific detection reagent combined with R_f values. Identification can be aided by using more than one detection reagent, often applied in sequence to a single chromatogram. R_f values of zones from samples should be ideally compared to standards in more than one type of TLC system with different separation mechanisms.

Instead of using chromogenic or fluorogenic reagents to detect sample spots, this treatment can be applied only to standards for their visualization. The corresponding areas on the remainder of the plates are then removed and extracted with an appropriate organic solvent. After filtration and concentration, the extracts can be examined by GC or by visible, or mass spectrometry for identification.

2.2.7 Documentation of TLC Results

Visual Estimation

The simplest method for semiquantitative analysis by TLC is to develop a definite sample aliquot alongside standards containing known weights of analyte. After detection, the weight of analyte in the sample is estimated by visual comparison of the size and intensity of the standard and sample zones. This method has accuracy and reproducibility in the 10-30% range, which is often adequate for the purpose intended. Visual comparison work best if amounts near the detection limit are applied and if the sample is closely bracketed with standard.

Zone Elution

The zone elution method involves drying the layer; location the separated analyte zone; scraping the portion of layer containing the analyte, collecting the sorbent and elution of the analyte from the sorbent; and measurement against standards by an independent microanalytical method such as solution spectrometry ,GC, or electrochemistry.

The zone-elution quantification method is tedious and time-consuming and is likely to be inaccurate because of difficulties in locating the exact zone boundaries, loss of sorbent during scraping and collection, nonreproducible elution from the sorbent, and background interference due to eluted impurities from the sorbent. These errors are minimized if standards and sample are chromatographed, scraped. and eluted together as consistently as possible, and if an equal-size blank area of layer is scraped and eluted in the same way. Prewashing the layer by development with an appropriate solvent will help to minimize the blank value.

Scanning Densitometry

Instrumentation for in situ measurement of TLC chromatograms first appeared in about 1967 and is for the determination of both spot size and location , for a true measure of resolution, and for rapid, accurate quantitation. The relative standard deviation of scanning densitometry can be maintained below 2% , making it a reliable quantitative tool.

Most modern TLC quantitative analyses are performed by in situ measurement of the absorbance or fluorescence of the separated spots using an optical densitometric scanner with a fixed sample light beam in the form of a rectangular slit. The plate is mounted on a moveable stage driven by a stepping motor, which allows each chromatogram to be scanned, usually in the direction of development. Single beam geometry is most often used and produces excellent results when high quality plates and analytical techniques are employed. A schematic diagram of a single beam scanner

arranged for measurement of absorption in the reflectance mode is shown in Figure 2.4.

A halogen or tungsten lamps are used to provide light for the visible region(400-800 nm), deuterium lamps for the UV region(190-400 nm), and a mercury or xenon are source for fluorescence excitation. Filters or monochromators are employed for wavelength selection, and photomultiplier tubes or photodiodes for signal measurement.

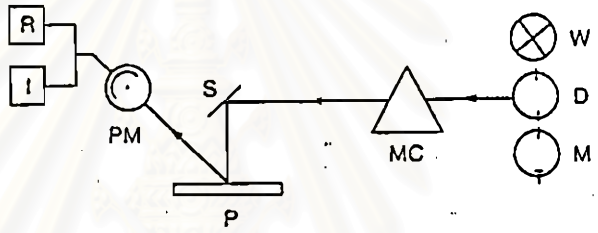

Figure 2.4 Schematic diagram of the optical path of commercially available densitometers for absorption scanning.
R = recorder, I = integrator, PM = photomultiplier detector, P = plate, S = mirror, MC = monochromator, W = tungsten incandescent lamp, D = deuterium lamp, M = mercury lamp.

Light striking the plate surface is both transmitted and diffusely scattered by layer. Light striking a spot on the plate will undergo absorption so that the light, transmitted or reflected, is diminished in intensity at those wavelengths forming the absorption profile of the spot. The measurement of the signal diminution between the light transmitted or reflected by a blank zone of the plate and a zone containing sample provides the mechanism for quantitative measurements by absorption.

$$I_o = I_{ref} + I_{abs} \text{ .....(2.6)}$$

where

I_o = incident light

I_{abs} = absorbed light

I_{ref} = reflected light

Absorbance (reflectance) scanning : The positions of the chromatographically separated substances are generally determined at λ_{max}. As the chromatogram is scanned the voltage differences produced at the detector are plotted as a function of position of measurement to yield an absorption scan (Fig 2.5). Conclusions concerning the amount of substance chromatographed can be drawn from the areas or heights of the peaks.

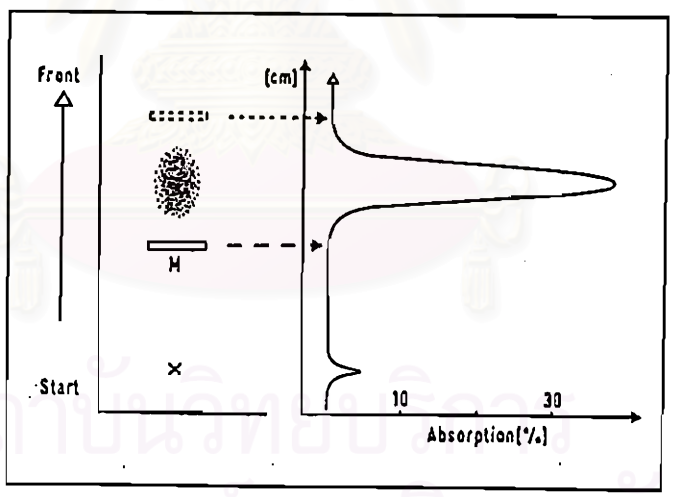

Figure 2.5 Schematic representation of the recording of an absorbance scan - M= measuring.

COMPLEXING AGENTS (23, 29-31)

2.3 α-Dioximes and their chelating properties

Chelating reagents were complexing reagents which formed one or more rings complex compounds with metals. For a chelating reagent to be of photometric value it must contain chromogenic or fluorigenic as well as chelating groups. Most chelating reagents of interest to the analyte are of the type HL, in which H can be replaced by an equivalent of metal. The metal atom is coordinatively bonded to the functional group of basic character to form a ring structure which characterizes a metal chelate. The acidic hydrogen may be present in such groups as

-OH $-NH_2$ -SH -COOH =NOH

Typical coordinating groups are

=O -NH- =S $-NH_2$ =N-

The chelate ring is five- or six-membered; less often it is four-membered, as when both donors are S atoms. Most chelating reagents used in photometry contain one replaceable hydrogen and one coordinating group. The stability of metal chelates varies over a very wide range. It depends on many factors; the nature of the metal and its oxidation state, the nature of the coordinating and acidic groups in the reagent, the stereochemistry of the chelate.

α-Dioxime is the one of chelating reagents that bond metals through N alone or jointly through N and O. Dimethylglyoxime and other 1,2-dioximes having the oxime groups in *cis*-configuration , with the OH groups in *anti* orientation, form chelates with many of the transition metals of the type $M^{II}(HDm)_2$ (H_2Dm = dimethylglyoxime or other α-dioxime). M^{2+} is bonded to N with simultaneous loss of equivalent H^+ from -OH.

The α-dioximes, with dimethylglyoxime as the best known representative, are colorimetric reagents principally for Ni, Pd, and Re. Amphi (γ) *cis* dioximes can form 1:1 metal complexes, whereas the syn (β) isomers usually form no complexes (steric effect ; intramolecular hydrogen bonding).

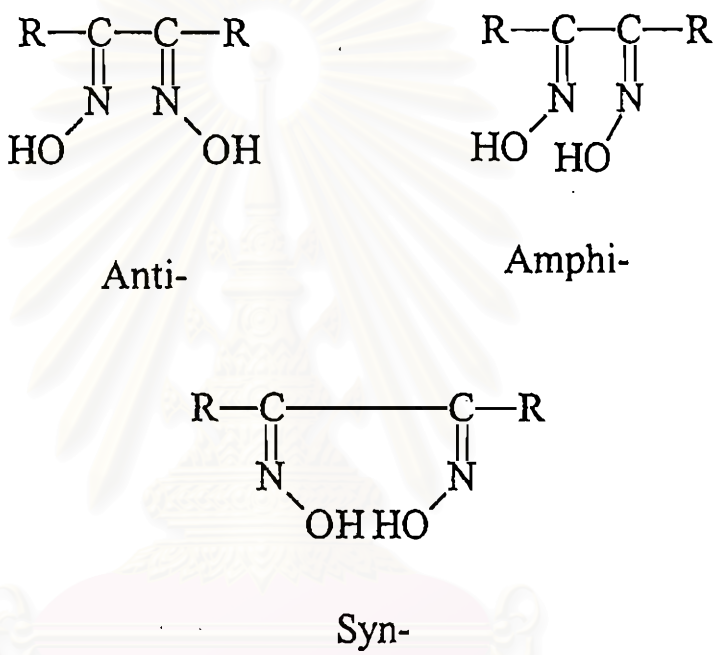

Figure 2.6 Three isomeric forms of α-dioxime.

Although the dioximes are generally thought of as being selective for Ni(II), Pd(II), and Pt(II) in the sense of forming colored slight soluble, and hence extractable, chelates with these metals. Dioximes give slightly soluble chelates also with Co(II), Fe(II and III), and Cu(II), which are extractable into chloroform, carbon tetrachloride, or nitrobenzene. These extracts absorb strongly in the UV or blue region of the spectrum.

2.3.1 Dimethylglyoxime (DMG)

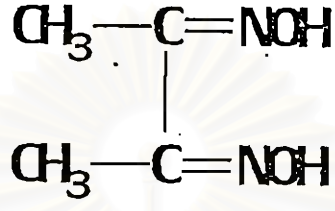

Figure 2.7 The structure of dimethylglyoxime.

Dimethylglyoxime (H_2Dm, biacetyldioxime, Chugaev's reagent) is a white crystalline solid. It melts at 238-240°C with decomposition. Solubility in water is 0.632 g/L. The most important property of dimethylglyoxime (and other α-dioximes) is its ability to form water-insoluble, colored chelates with Ni(II), Pd(II), and Pt(II). Since the dimethylglyoximates of these metals are extractable into immiscible organic solvents, selectivity methods for separation and determination of Ni, Pd, and Pt become available.

Dimethylglyoxime is one of the first and one of the most useful for the detection and determination of inorganic substances. Dimethylglyoxime forms chelates especially with the transition metals. The nickel (II) complex may be represented.

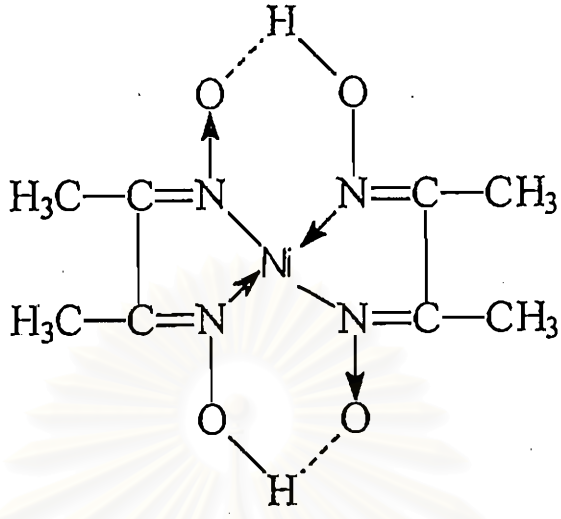

Figure 2.8 The structure of nickel (II) dimethylglyoximate.

The Ni or other metal atoms is bonded to 4N atoms. Formation constants having the following values in water solution (20 or 25°C, μ = 0.1-0.3) are given in Table 2.2.

Table 2.2 Formation constant of metals with DMG.

Metal	log K_1	log K_2	log K_3	logK_1K_2
Co(II)	8.7	9.0	-	17.7
Co(III)	15.0	10.0	7.2	25
Cu(II)	-	-	-	19.2
Fe(II)	-	-	-	7.25
Ni(II)	8.9	8.6	-	17.5
Pd(II)	-	-	-	34.3

Chloroform is generally the best solvent for extraction of the insoluble dimethylglyoximates. From the solubilities of the dimethylglyoximates in chloroform and in water saturated with chloroform, the partition constants are calculated to be

$P_{CHCl3/H2O}$ of $Ni(HDm)_2 = 4.8x10^{-4}/1.2x10^{-6} = 4.0x10^2$

$P_{CHCl3/H2O}$ of $Pd(HDm)_2 = 3.1x10^2$

$P_{CHCl3/H2O}$ of $Cu(HDm)_2 = 0.14$

The extraction of $Ni(HDm)_2$ is primarily important in the separation of nickel but relatively large quantities of nickel have been determined by means of the pale yellow chloroform solution of $Ni(HDm)_2$.

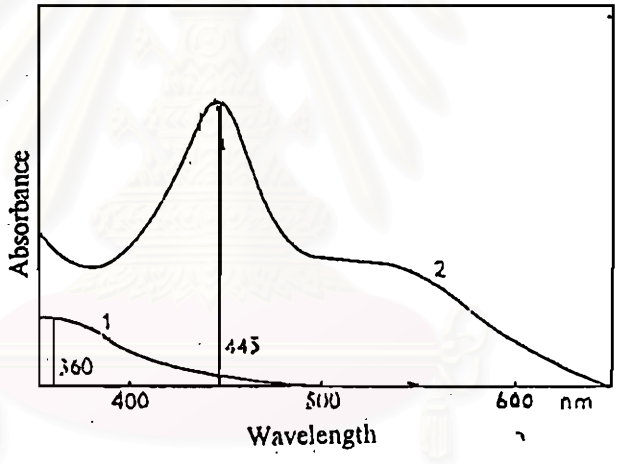

Figure 2.9 Absorption spectra of nickel (II) dimethylglyoxime complex in chloroform (1) and nickel (II) furildioxime complex in chloroform (2).

Curve 1 in Fig. 2.9 represents the absorption spectrum of $Ni(HDm)_2$ in chloroform. The molar absorptivity at λ_{max} = 260 nm is $3.4x10^3$, and at 400 nm is $1.8x10^3$.

This method has been used to determine nickel in niobium, tantalum, and tungsten. The palladium chelate has yellow color in chloroform can be determined in this way, though not with the highest sensitivity in the visible range.

Most ions (e.g. Fe(II), Co(II), and Cu(II)) which form colored, water-soluble complex with H_2Dm interference. However, the complexes of Fe(II), Co(II), and Cu(II) are decomposed by EDTA, and preliminary extraction as $Ni(HDm)_2$ allow nickel to be isolated from large amounts of Co, Cu, Fe, Cr, Al, and Mn. The presence of hydroxylamine ensures the quantitative extraction of nickel and prevents interference from Cu and Mn. In the presence of large quantities of Co(II) and Fe(III) , the use of triethanolamine as masking agent is recommended.

Dimethylglyoxime has been used for the determination of some metal ions (Ni(II), Pd(II), Cu(II)) by the different methods. For example, the determination of nickel in silicate rock by colorimetry. The sensitivity of this method is great enough to permit detection of less than 0.0001% of nickel when a 0.5 g of rock is used. Cu, Mn, Cr, Co, and V in quantities which usually occur in rock do not interfere (32).

2.3.2 α-Furildioxime

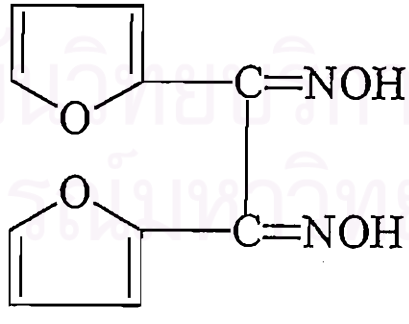

Figure 2.10 The structure of α-furildioxime.

There are three isomers, α, β, and γ of 2-furildioxime. Pure α-furildioxime melts at 192-193 °C, whereas the β and γ compounds melt at 150-152°C and 182-183°C, respectively. The so-called α-furildioxime or 2- furildioxime prepared by the usual procedure is a mixture of α and γ isomers. The α isomer, for which the structural formula is shown above is analytically important.

α-Furildioxime is slightly soluble in water but easily soluble in alcohol and ether. The chloroform solubility is 0.048 M ; $P_{CHCL3/H2O}$ = 0.35. The first acid dissociation constant of α-furildioxime is $(5.5\pm 0.2)x10^{-11}$ at 20°C.

α-Furildioxime reacts with nickel (II) ions similarly to dimethylglyoxime and other α-dioximes, forming a chelate which is sparingly water-soluble, but is extractable into chloroform and similar solvents. The yellow color of the organic extract provides the basis of a spectrophotometric method for determining nickel. The sensitivities of the dimethylglyoxime and the α-furildioxime methods are similar. The absorbance maximum of the nickel α-furildioxime chelate in chloroform is at 435 nm (the molar absorptivity is $2.0x10^4$, absorptivity = 0.34). (see Fig 2.9) The reagent has also been used for the determination of cobalt, palladium, rhenium, and technetium. For example, colorimetric determination of nickel with α-furildioxime. This method can determine nickel in a steel and magnesium alloy. The percentage of nickel in a steel and magnesium alloy is 0.196% and 0.0024% respectively.(33)

2.3.3 1,2-Cyclohexanedionedioxime or Nioxime

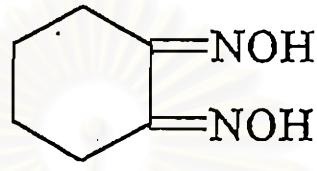

Figure 2.11 The structure of cyclohexanedionedioxime.

Cyclohexanedionedioxime is a white crystalline compound which melts at 187-188°C with decomposition. Unlike dimethylglyoxime, cyclohexanedionedioxime is soluble in water. This is a highly important characteristic, since an aqueous solution of the reagent can be used for the precipitation of nickel without danger of contaminating the nickel compound, as is the case when dimethylglyoxime is used. It has pKa_1 and pKa_2 at 25°C 10.7 and 12.16 respectively.

Cyclohexanedionedioxime gives much the same photometric reactions as dimethylglyoxime, and behaves much like dimethylglyoxime and other α-dioximes in its reactions with Ni, Pd, and other metal ions. It used as 0.01 M solution in EtOH, for extraction-photometric determination of Ni (λ_{max} = 450 nm, ε 18000, $CHCl_3$).

2.3.4 α-Benzildioxime(or α-Diphenylglyoxime)

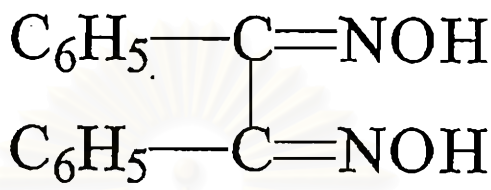

Figure 2.12 The structure of α-benzildioxime

α-Benzildioxime consists of white microcrystalline leaflets, which melts at 235-237°C with decomposition. It is almost insoluble in water, ether, and glacial acetic acid, and is only slightly soluble in alcohol. It dissolves readily in sodium hydroxide solution and in acetone. It has pKa_1 and pKa_2 at 20°C 10.3 and 11.9 respectively.

Like the nickel derivative of other α-orthodioximes , nickel α-benzildioximate consists of two molecules of oxime and one atom of nickel. α-Benzildioxime has been used for extraction photometric determination of Ni(II), Pd(II), and Re(III). Chloroform extraction of the nickel chelate provides markedly greater sensitivity than does that of dimethylglyoximate; Co(II) and Cu(II) are also extracted but their chelate do not absorb in the visible range.