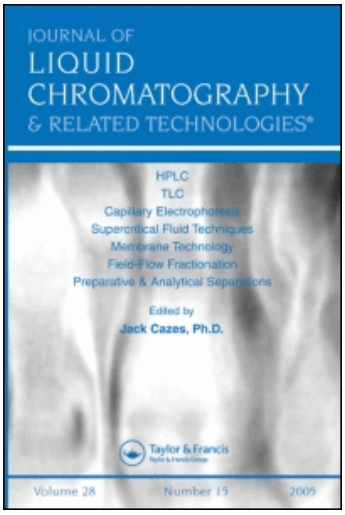

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Steinbrech, Bernd(1987) 'Thin Layer Chromatography and High Pressure Liquid Chromatography of Metal Chelates', Journal of Liquid Chromatography & Related Technologies, 10: 1, 1 — 48

To link to this Article: DOI: 10.1080/01483918708074190

URL: http://dx.doi.org/10.1080/01483918708074190

JOURNAL OF LIQUID CHROMATOGRAPHY, 10(1), 1-48 (1987)

THIN LAYER CHROMATOGRAPHY AND HIGH PRESSURE LIQUID CHROMATOGRAPHY OF METAL CHELATES*

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1. INTRODUCTION

For quantitative trace metal analyses a great number of established methods are available nowadays. Efficient spectroscopic determination methods like atomic absorption spectrometry, plasma emission spectrometry, and X-ray fluorescence analysis are of primary importance in this field, as well as are electrochemical methods like polarography and voltammetry.

Considering the complete analytical procedure, however, preconcentration and separation of the analyt prove frequently to be an indispensible step prior to the actual determination (40, 59, 69, 72). Interferences of the analytical signal due to matrix effects or

* Dedicated to Prof. Dr. K.-H. König on the occasion of his 60 th birthday

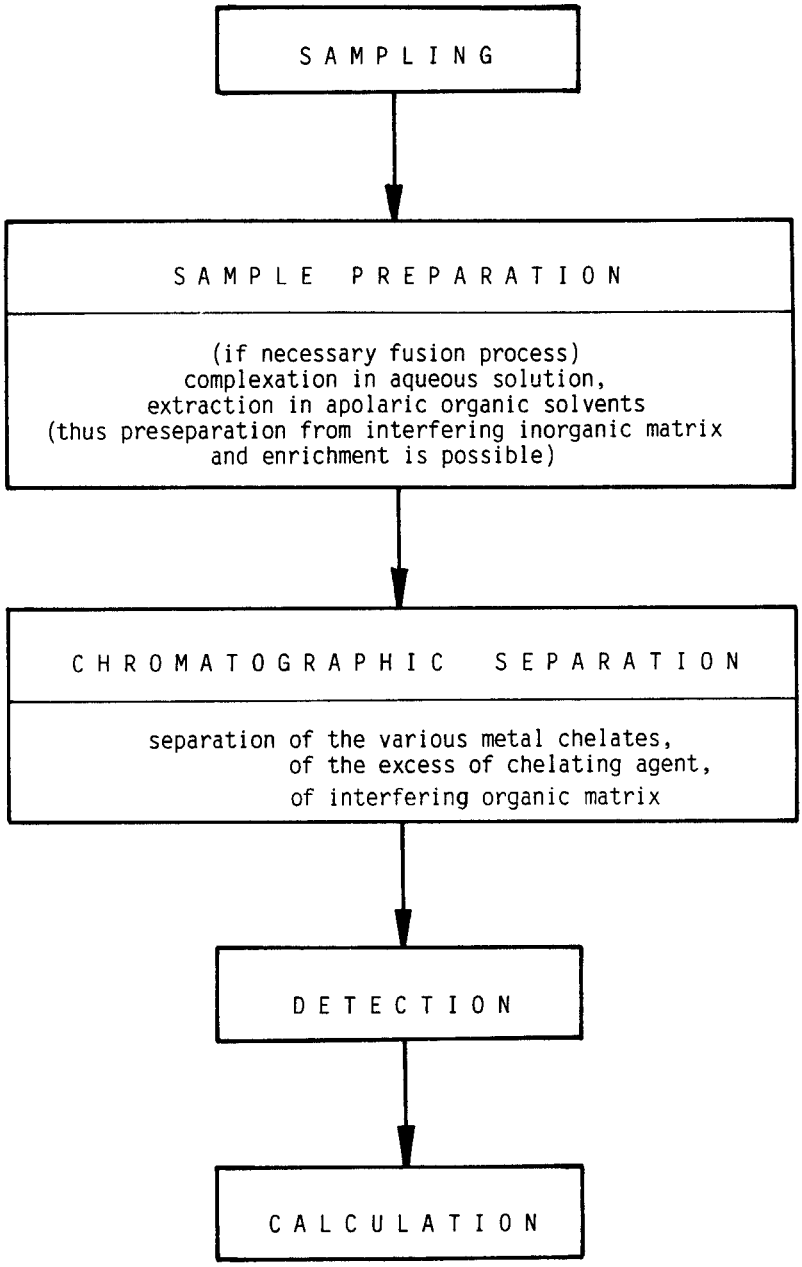

Fig. 1: Trace metal analysis by chromatography of metal chelates (flow chart)

concomitant elements, often met with direct methods, are completely overcome utilizing this approach, in a very generalized way.

Both, separation and enrichment can often combined in a single operation as it is realized by liquid-liquid extraction.

After complexation of metal ions in aqueous solution with organic reagents and separation from interfering inorganic matrix by extraction under enrichment into apolar organic solvents, they can be monitored by spectrophotometric of fluorometric measurements.

Usually, however, organic reagents (e. g. chelating agents) do not react selectively with the element to be determined, but with numerous elements of similar complexchemical properties. Therefore, beside a singel-step separation and extraction, very often more refind separation procedures are necessary. For this task all chromatographic methods are qualified.

All described analytical operations lead to the chromatography of metal chelates as a powerful analytical method for trace metal determination (see Fig. 1).

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

1). Relatively simple sample preparation (complex-formation at appropriate pH-value, if necessary in the presence of masking agents).

2). Elements to be determined can be enriched before determination by extraction of their complexes.

3). Interfering inorganic matrix remain in aqueous phase during extraction.

4). Because of chromatographic separation several elements can be identified and determined quantitatively in a single analytical step (multi-element analysis).

5). Sensitive detection of the metal chelates,e.g. by UV-photometry (high extinction coefficient of the chelates) is possible.

6). Short analysis time, because simple and rapid realization is possible.

Therefore, chromatography of metal chelates is a highly efficient analytical technique, which is able to complement or sometimes even to replace other well known analytical methods of trace metal determination.

2. CHROMATOGRAPHIC TECHNIQUES FOR SEPARATION OF METAL CHELATES

For separation of metal chelates, generally the same chromatographic techniques are used as for the analysis of organic substances. The main field of application is, of course, the liquid chromatography (LC). With LC a great number of inorganic substances and metal complexes with organic chelate-ligands can be separated.

In comparison with LC the possibilities of gas-chromatography (GC) are inferior with the exception of some complexes,like e.g. ß-diketonates and dithiocarbamates, because GC requires substances volatilizing without decomposition. However, metal chelates very often decompose during volatilization.

Detailed overviews of feasibilities of GC of inorganic substances are given for example in (41, 45, 48, 59, 60, 71).

Adsorption chromatography is the most important technique of the various liquid chromatographic separation techniques. Sometimes partition chromatography is also used. In recent years the reversed phase (RP)-chromatography has become more important.

Especially for chromatography of metal chelates, silica gel,and alumina have been used more successfully as stationary phases in TLC and HPLC,because of their good separation qualities. A review of layer-chromatographic procedures up to 1972 is given by BRINKMAN et al (6).

Unfortunately not all elements, forming well defined metal chelates with a given complexing agent, can be used without problems in chromatography. Therefore, a great part of publications in this field report on certain difficulties which generally appear in chromatography of metal chelates. At first decomposition of the chelates before or during elution may appear as well as may incomplete separation of the various complexes or strong tailing. In spite of great efforts it was therefore not possible to achieve a complete chromatographic separation of all elements which form chelates with a given complexing agent. If the excess of ligand can not be separated, more difficulties appear. Moreover, the formation of several components during chromatography of a homogeneous chelate can complicate the determination.

The above mentioned difficulties very often reduce the good qualification of chromatography of metal chelates for trace element analysis. Therefore, only those of the many complexing agents available can be used successfully, which form chelates that can be separated chromatographically and determined analytically. For those complexing agents, metal ions and complexes formed, have to meet certain requirements.

3. RELATIONSHIP BETWEEN COMPLEXCHEMICAL PROPERTIES AND CHROMATOGRAPHY OF METAL CHELATES

Looking at the greatest part of the well known complexing agents in analytical chemistry for photometric, gravimetric and volumetric determination, it is shown, that many of these ligands in spite of their wide range of application do not form metal chelates, which can be chromatographed successfully. Several reasons are responsible for that (29):

- lack of solubility of the formed chelates in apolaric solvents, thus it is not possible to enrich the metal ions from aqueous solution by extraction,

- thus no compact, strongly limited fractions of the chelates in adsorption chromatography on silica gel phases,

- lack of stability of the chelates during contact with the stationary phase,

- decomposition of the chelates during elution,

- strong tailing, no equilibrium-establishment between adsorption and desorption of the chelates on the stationary phase,

- in spite of successful chromatography, not enough differences in chromatographic retention, caused by too voluminous ligands.

A close relationship exists between the chromatographic behaviour and the complexchemical properties of the metal chelates, because these are responsible for type and intensity of interaction with stationary- and mobile phase.

The active positions of silica gel, at that time the most important stationary phase for chromatography of metal chelates, are

the end positioned silanol groups. They form a weak linkage with each neighbouring molecule provided that one of the following interactions is possible (29):

1). dipole - dipole

2). dipole - induced dipole

3). π-complex-linkage to double bondings of the metal chelate

4). hydrogen bridge-bonding between the silanol group as proton donor and the functional groups of the chelate ligand with a free electron pair as proton acceptor

5). polaric electron donor bonding of the silanol anion with the central atom of the complex.

It depends on the individual properties of the chelates, which of the mentioned interactions are responsible for adsorption of the chelate onto the surface of the stationary phase. Most important are dipole momentum, basicity of the coordination sites, metal-specific properties of the central atom like its affinity to oxigen or sulfur, coordination number, steric structure of the chelate including additional ligands like water, solvent molecules or oxigen. It is further important, whether or not the maximum coordination number of the central atom is already realized.

Furthermore, the power of interaction between the effluent and the stationary phase is of definite importance for retention of complexes, because both, effluent and analyte compete for the active sites of the stationary phase. Even a change in the series of the eluotropic activity is possible by solvation and adduct-formation (11).

It is a consequence of the considerable influence of the central atom on the chromatographic behaviour of the metal chelate, that elements, which preferably are coordinated to ligands with oxigen as coordination site (oxigen affinic elements), can be chromatographed less successfully on polaric stationary phases. Moreover, most of these complexes are less soluble in aqueous as well as in apolaric organic solvents.

Even the ß-diketonates, which generally offer good chromatographic properties are suitable for separation of only a few elements, but no alkaline- and earth alkaline elements by adsorption chromatography (20, 49). By the addition of reagents (e.g. ketones) to the mobile phase, sometimes tailing disappears (20). Nevertheless, generally the acetylacetonates are preferably separated by partition chromatography (see below).

Similar chromatographic properties can be obtained by many complexes with N,O-coordination sites. Numerous oxinates are poorly extractable in apolar organic solvents, most of the remaining well extractable oxinates show tailing during chromatography. By addition of strongly polar substances (H_2O, acids) or complexing agents to the mobile phase, tailing can be reduced (42), but then partition effects dominate.

This behaviour can be solved by ligand-derivatisation, but substitution is restricted, since too big substitutens would level out chromatographic retention, or decrease the solubility in organic solvents by "gravimetric effects"(72).

Therefore, low polarity of the metal-ligand bonding is a prerequisite for successful application of a chelate to adsorption chromatography. If the linkage is too polar, tailing or even decomposition of the complex appear, or no elution of the substance takes place. Essential improvement of the chromatographic behaviour can be obtained by substitution of oxigen with sulfur as coordination site.

The metal-sulfur-bonding is more covalent, thus decreasing the enthalpy of adsorption of the complexes on the stationary phases.

Additionally chelates with metal-sulfur-bonding have a more intensive colour, because of their charge-transfer-bonds. This causes a better visual identification of the chelates. In agreement with the substitution of the oxigen-coordination site is a shift in complex formation to the sulfur-affine elements of the PSE (72).

Numerous complexing agents with N-,S-; S-,S-; or O-,S-coordination sites are suitable for chromatography of metal chelates, for example, the dithiocarbamates as well as dithizone and the ß-thioketones.

The following conditions and prerequisites can be deduced from the important properties of the most successful ligands in chromatography, which the resulting metal chelates have to fulfil (29):

1). Formation of stable neutral metal chelates.

2). Control of complex formation by pH-variation or by masking reagents.

3). Formation of well defined chelates with clear stoichiometry.

4). Formation of conjugated, five-membered chelate-rings between metal and ligand, because these possess the highest stability. Four- and six-membered chelate-rings reduce stability or range of application (exception S,S-four-rings of the dithiocarbamates or six-rings with ß-diketone-structure).

5). Chelating agents with N-,O-, S- or Se-atoms as coordination sites possess good complexchemical properties. In addition

these ligands are for analytical applications sufficiently stable under normal circumstances.

6). To receive compact, well resolved elution profiles, the metal chelates have to be well soluble in the mobile phase.

7). The ligand must not be too voluminous, so that the element specific properties of the central atom are maintained and levelling of the chromatographic behaviour of the complexes will be avoided.

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

The last topic indicates the predominate importance of photometric detection after chromatographic separation, though numerous other methods are also in use.

Beside the various methods for visual identification in TLC the chelates could be determined spectrophotometrically by adsorption measurements in transmission or reflectance mode or by fluorescence measurements by means of a TLC-scanner.

Furthermore, radiometric or electrometric measurements or detection by χ-ray fluorescence or emission (see below) are possible.

After removement of the spot from the TLC-plate and elution of the substance from the carrier material,a wide variety of determination methods are available. Because of the less complicated procedure, and therefore increased reproducibility, a direct determination method is advantageous.

In comparison with TLC the sample-elution is "on line" in HPLC analysis, so that no more process steps are necessary to examine

the effluent with various detectors, partly with flow-through detectors.

The most thouroughly investigated complexes in chromatography, which fulfil the mentioned criteria partly or completely, are the dithiocarbamates, dithizonates, complexes with ß-diketone-(thiodiketone)-structure, oxinates, thiooxinates and thionates. They all exhibit more or less excellent chromatographic properties. With these ligands the chromatographic separation of important heavy- and nobel-metals succeed as well as the separation of toxic metals. TLC as well as HPLC can be used for those chromatographic separations, because generally a good transferibility is given for both techniques.

In the following part a review of some important publications with the above mentioned chelating agents on the field of chromatography of metal chelates with both techniques is given. In table 1 the cited TLC- and HPLC-publications are listed.

Further publications regarding TLC or HPLC of metal complexes with partly different ligands are reviewed by (45, 46, 59, 73).

4. CHROMATOGRAPHIC SEPARATIONS AND ANALYTICAL APPLICATIONS

4.1. Dithizonates

During the 60ties, TLC-separation of metals became of general interest. Beside the separation of the metals as ions their separation as chelates was realized.
Dithizone was one of the first ligands being investigated successfully. With dithizone as a typical 'wide range chelating agent' most transition metals were studied.

Table 1: Literature cited in this review

Chelating Agents	Investigated Elements	Technique	References
Dithizone	Pd, Pt, Ag, Au	TLC	19
	Mn, Co, Ni, Cu, Zn, Cd, Hg, Pb	TLC	67, 68
	Co, Ni, Cu, Hg	TLC, HPLC	39
	Co, Ni, Zn, Hg	HPTLC	7
Dithiocarbamates	Cu, Cd, Hg, Pb, Bi	TLC	61
	V, Cr, Mn, Fe, Co, Ni, Pd, Cu, Ag, Zn, Cd, Hg, In, Tl, Sn, Pb, As, Sb, Bi, Te	TLC	2, 25
	Co, Ni, Cu, Zn, As	TLC, HPLC	15
	Co, Ni, Cu, Zn, Pb	HPLC	35, 36
	Cr, Mn, Fe, Co, Mi, Cu, Zn, Cd, Pb	HPLC	38
	Cu	RP-HPTLC	54
	Cr, Co, Ni, Cu, Hg, Pb, Se	RP-HPLC	56, 57
	Co, Ni, Cu, Cd, Hg, Pb	RP-HPLC	5
	Rh, Pd, Pt	RP-HPLC	21
	Co, Ni, Cu, Zn, Cd, Hg, Pb, Bi	RP-HPLC	44
Oxine	Mg, Ca, Cr, Mn, Fe, Co, Ni, Pd, Zn, Al, Pb, Bi	TLC	42
	V, Cr, Mo, W, Co	HPLC	75, 76
	Ru, Rh, Pd, Os, Ir	A-, RP-HPLC	77
	Ru, Rh, Os, Ir, Pt	SFC-HPLC	4
	V, Cr, Co	A-, RP-SEC-HPLC	34
	Pd, Cu, Zn, Al, Ga	SEC-HPLC	62

Thiooxine	Fe, Co, Ni, Ru, Rh, Pd, Os, Ir, Pt, Cu, Zn, Hg, Sn, Pb	TLC	9, 26
	Fe, Co, Ni	HPLC	28
Thiooxine and Derivates	V, Cr, Mo, W, Mn, Re, Fe, Co, Ni, Ru, Rh, Pd, Os, Ir, Pt, Cu, Zn, Hg, Ga, In, Tl, Sn, Pb, As, Sb, Bi, Te		50, 51
		TLC	52, 53
1-Hydroxy-2-pyridine thione (HPT)	Fe, Co, Ni, Ru, Rh, Pd, Os, Ir, Pt	TLC	27
	Rh, Pd, Ir, Pt	TLC, HPLC	28, 33,63
	Ti, V, Cr, Mo, Mn, Fe, Co, Ni, Ru, Rh, Pd, Os, Ir, Pt, Cu, Zn, Hg, Tl, Bi	TLC, HPLC	65
	Sn, Tl, Pb, Bi	HPTLC	10
	Ni in hydrogenated fats	HPLC	30
HPT-Derivates	Ti, V, Cr, Mo, Mn, Fe, Co, Ni, Ru, Rh, PD, Os, Ir, Pt, Cu, Zn, Hg, In, Tl, Pb, Bi, Te	TLC, HPLC	66
ß-Diketones	Be, Zr, Cr, Fe, Co, Ni, Ru, Cu, Zn, Al	HPLC	20
	Ca, Ba, Cr, Mn, Fe, Co, Ni, Cu, Zn	TLC	13
	Cr, Fe, Co	TLC	49
	Be, Cr, Fe, Co, Ni, Ru, Rh, Cu, Al	HPLC	70
	Be, Cr, Co, Ru, Rh, Pd, Pt	RP-HPLC	12
	Be, limit of detection	TLC, HPLC, GC	58
Monothio-ß-diketones	Co, Ni, Rh, Pd, Cu	TLC	43
	Co, Ni, Cu, Zn, Hg, Pb, Cd	TLC	16

(continued)

Table 1 (continued)

Chelating Agents	Investigated Elements	Technique	References
ß-Dithioketones	Mn, Co, Ni, Zn, Rh, Pd, Pt	TLC	17
N,N-Dialkyl-N'-benzoyl-thioureas	Co, Ru, Rh, Pd, Os, Ir, Pt, Cu, Hg	TLC	31
Pyridine-2-aldehyde-2-quinolylhydrazone	Ni, limit of detection	TLC	74
4-(2-Pyridylazo)-resorcinol	Fe, Co, Ni, Cu	RP-HPLC	47
	Cr in presence of V, Mn, Fe, Co, Ni, Cu, Zn, Cd	RP-HPLC	18
1-(2-Pyridylazo)-2-naphthol	Fe, Co, Ni, Cu	TLC	8
1,2-Diketo-bis-thiobenzhydrazones	Zn, Cd, Hg, Pb	TLC	14
Tetrakis (p-tolyl)-porphine	Mg, V, Ni, Pd, Cu, Zn	A-, RP-HPTLC	22, 23
	Ng, V, Ni, Pd, Cu, Zn	RP-HPLC	24

Abbreviations:
A Adsorption
RP Reversed Phase
SEC Size-Exclusion Chromatography
SFC Supercritical Fluid Chromatography
HPTLC High Performance Thin Layer Chromatography

Already 1964 HRANISAVLJEVIC-JAKOVLJEVIC et al. (19) separated Ag (I), Au (III), Pd (II), Pt (II) as dithizonates on silica gel layers with a CH_2Cl_2/ benzene-mixture as mobile phase. The detection of the chelates was carried out visually by identification of the characteristically coloured spots. TEWARI and BHATT (67,68) used dithizone as chelating agents for toxicological analysis and identified Cu in autopsy tissues beside other metal dithizonates by TLC.

LOHMÜLLER, HEIZMANN and BALLSCHMITER (39) transferred TLC-separation of Hg (II), Cu (II), Ni (II), and Co (II)-dithizonates to HPLC. Thereby TLC was used as a pilot method to establish the optimal conditions for HPLC-separation. Using pure benzene as solvent at flow rates of 10 to 20 ml/h the dithizonates were chromatographed on silica gel.

Using the technique of HPLC,BRUNO et al. (7) determined quantitatively Zn (II),Co (II) and Ni (II) in soaps and vitamine B 12 as well as Hg (II) in human urine as dithizonate complexes. As stationary phases they used silica gel-HPTLC-plates, as mobile phase toluene. The development of the chromatograms needed 8 min., followed by densiometric determination using a TLC-scanner. The limit of detection was about 0,5 ng metal.

A survey of other early works on chromatographic separations and behaviour of dithizonates e.g. is given by (6 and 59).

4.2 Dithiocarbamates

However, one of the best investigated complexing agent for the chromatography of metal chelates is dithiocarbamate (DTC, abbreviated for dithiocarbamidic acid). With its two sulfur-coordination-sites it forms very stable chelate-4-rings with a great number of metal ions and so far is also a typical 'wide range chelating agent'.

Already 1965 SENF (61) separated Hg (II), Cu (II), Bi (III), and Pb (II) as diethyl-dithicarbamate (DDTC)- complexes on home made silica gel layers as stationary phase and n-hexane + $CHCl_3$+ diethylamine = 20+2+1 as mobile phase.

BALLSCHMITER systematically investigated the conditions of DDTC-complexes (2, 25, 55, 15).

The TLC-behaviour of 20 different cations which form stable chelates with DDTC was studied in dependence of TLC-material and solvent mixture (2). As an example of the possible seperations, that one of Zn (II), Cd (II) and Hg (II)-DDTC after extraction with $CHCl_3$ and chromatography on silica gel with benzene/cyclohexane (55 + 45) was described in detail.

The effects of ligand-substitution on chromatographic separation of metal dithiocarbamates were also studied (25). With increasing size of the alkyl substituents the chromatographic differences of the chelates were found to decrease, although seperations were still possible. As a typical example the TLC-separation of Fe (III), Co (III), Ni (II), Cu (II), and Hg (II) as diethoxyethyl-DTC on alumina-layers with CCl_4+ CH_3CN_3= 96+4 was demonstrated.

Another paper (15) reported on the HPLC-behaviour of Pb (II), Zn (II), Cd (II), Hg (II), Cu (II), and Co (III) as dialkyl-DTC, 1,2-diketobisthiobenzhydrazones and 1,2- diketobisthiosemicarbazones on silica gel columns. This chromatographic separation was carried out by isocratic and gradient elution, using mixtures of benzene/n-heptane, $CHCl_3$/ n-heptane, benzene/cyclohexane and CCl_4/ CH_3CN_3 and pure benzene. The HPLC-behaviour of the DTC-chelates was identical with their TLC-behaviour, the order of elution was the same.

LISKA, GUIOCHON et al. (35, 36, 38) also reported about HPLC-behaviour of DTC-complexes in detail. For the determination of

Ni-DTC-chelates with different N-alkyl-substitutents (dimethyl to dioctyl-DTC), they used silica gel and chloroform/cyclohexane-mixtures (36). The influence of the alkyl-substitutents on the chromatographic behaviour was studied. The separation of differently substituted Ni (II)-DTC-chelates was complicated by ligand-exchange-reactions forming even completely new species.

Formation and stability of these unsymmetrically N-alkyl-substituted Ni-chelates was verified by two-dimensional TLC (37). Due to the kinetic instability of the Ni-chelates an exchange-equilibrium was found to exist, so that all possible ligand-combinations were formed. The chelates of Cu (II), Co (II), Zn (II), and Pb (II) also showed these ligand-exchange reactions (35). For the separation of Zn (II),Cu (II), Mn (II), Ni (II), Pb (II), Cr (III), Co (II), Cd (II), and Fe (II) mixtures DDTC was used as uniform ligand (38). The separation was carried out on a silica gel column with a mixture of 10 % $CHCl_3$ in cyclohexane as mobile phase. Samples of 5 µl of synthetic mixtures in chloroform ($5{,}6 \times 10^{-7}$ mol chelat/l) were injected.

SCHUNCK and SCHWEDT (54) also reported on the chromatographic behaviour of DTC-chelates in dependence on the chain length of the ligand. They used Cu (II)-DTC-chelates and chose the techniques of RP-chromatography. The examinations were carried out using HPTLC-plates. The R_F-values of the Cu-DTC-chelates were found to be a linear function of the chain length of the chelate,of the water content of the mobile phase,and of the number of the C-atoms in the chain of the stationary, chemically bonded phase. The best eluents for a chemically-bonded octyl phase were octane, isooctane,and a mixture of acetonitrile and water (9:1).

G. SCHWEDT reported in detail about HPLC-separation of metal dithiocarbamates (56). He used also RP-material as stationary phase and methonal/water-mixtures as mobile phase. The DDTC-chelates of

the elements Se, Cr, Ni, Co, Pb, Cu and Hg are suitable for RP-chromatography and with the exception of Pb and Cu were separated with methanol/water = 30/70 in 20 min. on a Li Chrosorb RP 8-column with a flow rate of 2 ml/min..

During the chromatographic process the Cr(III)-complex splitted into two fractions. The first fraction could not be resolved from the Se-peak. The possibilities for quantitative analysis with a UV/VIS-detector with variable wave length adjustment after chromatographic separation were described. Amounts of 100 ng of each metal were determined by peak heights measurements in the absorption maxima of their complexes after extraction as DDTC-chelates and separation by the chromatographic column. Especially the carbamates of Se, Co, Ni and Cr were sensitively determined. The determination of Se was also possible, even in the presence of Cr, since a constant signal ratio was found between the two Cr (III)-fractions (nearly 1:2) at 254 nm, so that the Se-signal could be corrected for the interference by chromium.

Generally in aqueous solutions Cr (III)- ions form hydrato-complexes, which are kinetically very stable, and do not show any ligand exchange reactions.

While chromate(VI)-ions are reduced to Cr (III) already at room temperature, thus forming complexes very easily, the Cr (III)-hydrato-complexes react at higher temperatures only, so that the DDTC-complexes decompose.

Otherwise the ammonium pyrrolidin-dithiocarbamate (APDTC) is less temperature sensitive, so that this complexing agent is better suitable for complexation of Cr (III)-ions. As an example of application SCHWEDT described the quantitative analysis of both Cr (III) and Cr (VI) ions in waste water (57). The determination of both species was demonstrated by HPLC-separation of the diffe-

rent reaction products with APDTC. First the chromate-ions formed complexes at room temperature, which showed spectroscopic differences to the pure Cr (III)-chelate. Besides the Cr (III)-complex also 'disulfide' was formed by oxidation of the ligand and reduction of Cr (VI). After extraction of the reaction products of Cr (VI) with ethylacetate more reagent was added to the sample for reaction of the Cr (III) ions and then heated at $60^{o}C$ for 20 min.. This reaction yielded a defined substance. The combined extracts were set free from the solvent and dissolved in acetonitrile for HPLC-analysis. According to the different reaction products the Cr (VI) and Cr (III) species were separated on a RP 8 column with methanol+ water (70+30) as mobile phase at a flow rate of 1 ml/min.. As little as 0,07 mg Cr (VI)/ l and 0,04 mg Cr (III)/l were detected in waste water applying this technique. Under these chromatographic conditions interfering elements like Fe, Ni, and Cu had greater retention times, so that interferences should be avoided by use of precolumn separation.

In a recent work BOND and WALLACE reported on the HPLC-separation of Pb (II), Cd (II), Hg (II), Co (III), Ni (II), and Cu (II) as DTC-complexes followed by spectrophotometric and electrochemical detection as a procedure for the simultaneous and automated on-line analysis of these elements (5). The complexes were formed in situ in the chromatographic system. Limits of detection in the order of 1 ng/10 µl were achieved by photometric detection. By electrochemical detection, limits for Pb and Cd were improved by a factor of 10 and comparable detection limits were found for the other elements. The linear working range of the calibration curve reached up to 100 ng. The automated HPLC-system operated continuously for periods of several days with either spectrophotometric or electrochemical detection.

MUELLER and LOVETT analysed trace levels of Rh (III) and Pd (II) in aqueous solution after complexation with Na-DDTC and extraction

of the metal complexes into chloroform (44). They used a RP C 18 column and a mixture of CH_3CN/Na-acetate buffer 0,02 M, pH 6,0 (70/30) as mobile phase. Complexation and extraction conditions depended on pH value. The detection limits of Pd (II) and Rh (III) from aqueous solutions were 2,7 ng/ml and 2,8 ng/ml, respectively. Pt-powder, Pt/Rh-alloys and AAS-standards for Pt were analysed.

ICHINOKI and YAMAZAKI also used the RP-chromatography to separate Co (III), Ni (II), Zn (II), Cd (II),Hg (II), Cu (II), and Bi (III) as hexamethylene-DTC-chelates (21). Standard reference materials citrus leaves and rice flour were ashed using nitric acid and perchloric acid. The metals were extracted into chloroform as hexamethylene-DTC-chelates and separated on a RP C 18 column with a mixture of $CH_3OH/H_2O/CHCl_3$/hexamethylene ammonium-hexamethylene-DTC (76/16,5/6/1,5) as mobile phase at a flow rate of 0,8 ml/min.. With the described procedure the simultaneous determination of Ni (II), Cu (II), Zn (II), and PB (II) at ppm-levels was possible within 25 min..

4.3 Oxinates

8-Hydroxy-quinoline(oxine) and 8-hydroxy-quinoline-derivates as well as their metal chelates were investigated very early by TLC. MUCHOVA and JOKL described the chromatographic behaviour of oxinates, dithizonates and DDTC-chelates on silica gel layers (42). They used n-butanol/glacial acetic acid/oxine (100/20/5) saturated with water as mobile phase and chromatographed various standard solutions of oxinate complexes.
Further early-work on this field was reviewed e.g. by BRINKMAN et al. (6) and SCHWEDT (59).

A general problem which appears during adsorption-chromatography of oxine and oxinate-complexes is -as mentioned above- the strong tailing of most of the chelates. ARMIN and JAKOBS reduced tailing

by pretreatment of the TLC-plates with complexing agents as EDTA, and thus improving chromatographic conditions (1).

WENCLAWIAK varied the preparation conditions of the oxine-chelates to improve adsorption-chromatographic separations (75). He used an 'under-water-melt' procedure as technique to prepare the chelates of the elements V, Mo, W, Co, Cr. After elimination of the excess of reagents he separated the complexes by HPLC on a silica gel Si 60 column with THF/$CHCl_3$ as mobile phase. The reproducibility of the separation depended extremely on the age of the sample solution and the elimination of the excess of ligand. With the same procedure V, Mo, W, Co and Cr were determined in steel samples (76).

Recently, metal oxinates were also separated by RP-chromatography. WENCLAWIAK and BICKMANN separated the oxinates of platinum group metals not only on silica gel, but also on RP C 18-columns (77). While the separation of Ru-, Rh- and Ir-oxinate was more successful on a silica gel column with $CHCl_3$/THF (3/2) as mobile phase, the oxinates of Ru, Pd and Os were better separated on a RP C 18 column using CH_3OH/buffer pH 4,6/$CHCl_3$ (57,5/30/12,5). All chelates were formed by an 'under-water-melt' procedure.

These authors also studied platinum metal oxinates, V-, Cu-, Mn- and Fe- acetylacetonates and (Co (II)-, Ni (II)-, Cu (II)- and Zn (II)-DDTC-chelates by supercritical fluid chromatography (4). Silica gel Si 60 and RP C 8 HPLC-columns were used with CO_2 as eluent and methanol/ethanol as modifier. The influence of pressure and temperature on the chromatographic behaviour was investigated in the range of 70 to 140 bar and 40 to 180° C.

LAJUNNEN et al. (34) investigated the HPLC behaviour of Co (II), Cr (III), and V (V) on silica gel and RP as well as on size exclusion columns (LiChrosorb Si 60, 5µm ; LiChrosorb RP 8, 10µm ; Shodex 801/5, 10-15 nm). Mixtures of THF/$CHCl_3$ (60/40), CH_3OH/

$CHCl_3$ (5/95), CH_3CN/H_2O (40/60) and CH_3OH/H_2O (63/37) were used as mobile phases. Linear calibration curves were obtained starting from the lower nanogram level up to the milligram level by peak height and also by peak area calculation. The relative standard deviation was found to range between 0,5 and 5 %.

SHIBUKAWA et al. (62) examined the size exclusion chromatographic behaviour of oxine, Zn (II)-, Ga (III)-, Al (III)-, Cu (II)- and Pd (II)-oxinate on Fractogel PVA 2000 with 1,4-dioxane and 1,2-dichlorothane. The addition of a small amount of chelating ligand to the eluent allowed the metal chelates to be stabilized, so that symmetrical elution peaks resulted. A relationship was established between the distribution coefficient and the molar volume, that allowed conclusions to the effective size of the chelates, as present in different solvents (adduct formation).

4.4 Thiooxinates

The sulfur analoguous of 8-hydroxy-quinoline, the 8-mercapto-quinoline (thiooxine), also forms intensive coloured chelates with a great number of metals beginning with the 5th subgroup to the 6th principal group of the PSE. Mainly BANKOVSKII (3) investigated the properties of this ligand and its analytical application as an agent for photometric trace metal analysis.

Moreover, thiooxine is also a suitable reagent for determination and separation of metals by chromatographic methods (26,28,50). It forms neutral chelates of well defined composition, which are hardly soluble in aqueous solutions, but very well extractable into chloroform. Precipitation and extraction depend on pH-value, so that a preseparation of the metals is feasible.

The TLC-behaviour of the thiooxinate-chelates of the eights, first, and second subgroup of the PSE and of Sn (II) and Pb (II) was ex-

amined on silica gel, silanized silica gel and alumina layers (26). Pure solvents as CH_2Cl_2, $CHCl_3$, benzene and toluene were used as mobile phases. Some of the chelates showed partly strong tailing (Pb (II), Zn (II), Ni (II), Pd (II), Pt (II), Cu (II)). A reduction of tailing is possible by a pretreatment of the layers with 5 % EDTA-solution in analogues to the TLC-separation of the oxine-derivates (1), because contamination of the layers by heavy metals (including Zn and Mn in fluorescenting materials) do not disturb the chromatography of thiooxinates after complexation with EDTA.

Various separations were described. The limits of visual detection were generally found to be between 5 and 20 ng metal absolutely.

If the developed TLC-plates are not monitored by photometrical methods (visual or TLC-scanning in the UV/VIS-region), but by element specific methods like the scanning x-ray spectral analysis, complete separation of the spots is not always necessary. The only precondition is, that chromatographic dispersion complete each other. As an example,this was demonstrated impressively by the thiooxinates of the 8th subgroup (9).

Instead of X-ray fluorescence analysis (XRFA), the proton induced X-ray emission analyses (PIXE) was used, since it is easily capable of performing microanalysis in the scanning mode of trace level elements.

Energy-dispersive X-ray spectrometric analysis suffers from spectral interferences if multielement-samples contain elements of subsequent atomic numbers at concentrations differing by a factor of 10 to 100 (9). These interferences can be overcome by group-separation according to chemical principles. Therefore, a suitable preseparation only needs to consider neighbouring elements.

This succeeds by separation of element-groups of the PSE. Separation of the elements of different periods of the PSE is not required, however, because spectral line of those elements do not interfere each other, so that spectral dispersion allows a simultaneous quantitative determination of those elements.

Chromatographic preseparation of metal chelates on TLC-plates is a powerful means that meets the requirement for group separation. So the simultaneous quantitative determination of the thiooxinates of the 8th subgroup and Hg was carried out by scanning PIXE-analysis of HPTLC-silica gel-layers on aluminium-carrier foil after development with CH_2Cl_2 (9). The length of migration was only 3 cm.

More general, the combination of TLC and PIXE has the advantage to eliminate mutual interfering sample components and matrix effects by separation:

1). Separation and enrichment from interfering inorganic matrix by extraction of the metal chelates into organic phase.

2). Separation of the metal chelates by chromatography.

3). Elimination of interferences from the organic matrix, the ligand and its decomposition products by choosing X-ray emission detection.

4). Measurement of the element specific information by spectral selection and by discrimination (X-ray attention by adsorption filters) of low energy X-rays from the matrix and from TLC-sheets.

By transfering the extracted metal chelates from solution to the solid matrix and by chromatography (separation of groups of the PSE) the performance of PIXE in solid sample analysis can be

utilized without complete chromatographic separation of all metal chelates. Of course, the relatively complicated X-ray emission analysis, especially PIXE-analysis, is not the method of choice for routine analysis in TLC, but it can deliver essential informations for basic research work and method-development in TLC-analysis of metal chelates.

It was found that 14 of the totally 27 thiooxinates extractable by $CHCl_3$ can be chromatographed successfully (compact spots, no tailing, no decomposition, R_F-values<0) (50). This were the chelates of Cr (III), Mo (VI), W (VI), Mn (II), Fe (III), Co (II/III), Ni (II), Rh (III), PD (II), Jr (III), Pt (II), Cu (II), Zn (II), and Hg (II).

The thiooxinates of Ga (III), Sn (II), Sb (III), and Bi (III) were eluted as spots with less sharp boundaries with tailing or they were even not eluted at all (R_F= 0). Re (VII), Ru (III), OS (III), As (III), and Te (IV) decomposed completely, V (IV), In (III), TL (I), and Pb (II) partly. As eluents again pure solvents like $CHCl_3$,CH_2Cl_2 and THF were used.

The separation of the chelates was achieved mainly according to their structure. Moreover,the metal specific properties as are the maximum possible coordination, affinity to oxigen or sulfur, and the atomic weight were responsible for different chromatographic properties of the complexes of identical structure.

A basic study by SCHNEEWEIS and KÖNIG demonstrated the influence of the variation of the basic molecule of the ligand on the chromatographic properties of metal chelates (50).

Quinoline as the basic molecule of oxine and thiooxine is particularly qualified for these studies, because it forms chelate-complexes by substitution in 8-position as well as by inserting the N-oxid-group with additional substitution in 8- or 2- position.

Therfore, these authors carefully compared the adsorption-chromatographic behaviour of the metal chelates of 8-hydroxy-quinoline, 8-mercaptor-quinoline, 8-seleno-quinoline, 8-hydroxy-quinoline-N-oxide, 8-mercapto-quinoline-N-oxide, 2-hydroxy-quinoline-N-oxide, and 2-mercapto-quinoline-N-oxide. Comparative investigations of such a great extent are only tractable by TLC-techniques, because only TLC delivers information about the chromatographic behaviour of all extractable complexes in a sufficiently short time and with low material consumption.

As stationary phases silica gel and silanized silica gel (RP 2), on which the complexes can be eluted with less decomposition and with otherwise rather simular adsorptive properties. For reason of better reproducibility pure solvents were used as eluents.

The studies demonstrated, that in general, those metal chelates were irreversible adsorbed or showed strong tailing during chromatography, that contained O, N or O, O as coordination sites. Chelates with S, N; S,O or S, S as coordination sites were eluted with essentially less problems.

By means of exchange of oxigen to sulfur as coordination site, the metal-ligand-bonding became less polar and consequently the chelates were better suitable for adsorption-chromatographic separations.

The complexes of 8-seleno-quinoline exhibited generally comparably or even better chromatographic behaviour than the thiooxinates, whereas the complexing agent is less stable against oxidation (formation of diselenid), so that it is less suitable for analytical applications.

By introduction of Br in 5-position, the stability of the thiooxinates was improved (51), because the acidity of the mercapto-function increased.

In agreement with the higher stability in aqueous and organic solution the chromatographic behaviour of the 5-Br-thiooxinates was found to be improved as compared to the unsubstituted chelates.

The substitution of methyl-groups in 8-mercapto-quinoline also offered a change in chromatographical properties (52). Substitution in 2- and 4- position yielded the best results. These two derivates as well as 2,4,6-trimethyl-thiooxine completed the range of application of thiooxine.

On the other hand hydrogenation and degradation of the quinoline structure from thiooxine to 1,2,3,4- tetrahydro-thiooxine, N,N-dimethyl-2- mercapto-aniline, and 2-mercapto-aniline deteriorated chromatographic behaviour of the metal chelates, because the power of coordination of the nitrogen atom decreases in the same order (53).

These basic results of SCHNEEWEIS and KÖNIG on the chromatographic behaviour of thiooxine and its coordination sites-, halogen-, methyl- and structure-derivatives supplied essential informations concerning complex-chemical requirements that chelating agents had to comply with, when applied to the chromatography of metal chelates as a method of metal analysis.

4.5 Thionates

1-Hydroxy-2-pyridinethione is another chelating agent, which fulfils all demands as described in section 3 and therefore is extremely suitable for chromatographic determination of metal chelates, (abbreviateded as : HPT or thione, the more stable tautomeric form of 2-mercapto-pyridine-N-oxid). HPT as a cyclic thiohydroxamic acid can be related to the class of hydroxamic acids and their thio analogues. It is a typical 'wide range complexing agent', which forms well defined metal chelates (thionates) with

at least 32 elements including the 4th subgroup up to the metals of the 6th principal group of the PSE (64).

Sulfur and oxigen act as coordination sites. In the complexes stable chelate-5-rings are formed with a conjugated π-system, covering the metal cation and the ligand. The conjugated π-system is additionally delocalized over the pyridine-ring. The charge-transfer-bondings from the central atom to sulfur are responsible for the intensive colour of many complexes. Most of the neutral chelates are insoluble in water, but well extractable into organic solvents as chloroform. According to the type, the valence and coordination number of the central atom the geometry of the complexes is square planar, tetrahedric or octahedric. Complex formation and precipitation depend on pH-value. For the more stable chelates it covers the range from strong acidic solution (6 M hydrochloric acid) to strong alkaline solutions.

Many of the thionates were eluted as uniform fraction without tailing or decomposition (27, 28, 63, 65).

The metals of the 8th subgroup of the PSE were separated by TLC on silica gel and silanized silica gel materials (27). $CHCl_3$/THF- and CH_2Cl_2/THF-mixtures (90+10) were used as mobile phases. On silanized silica gel chloroform and dichlormethane as pure solvents were used. The limits of visual detection were about 0,1 ng to 1 ng complex. Fluorescence quenching of fluorescenting layers at 254 nm improved the detection limits to 15-120 ng complex ($\hat{=}$ 3-40 ng metal).

In a single run 7 of the 9 elements were separated by TLC. These are Fe (III), Co (III), Ni (II), Rh (III), Pd (II), Ir (III),and Pt (II). Most of the results could be transferred to HPLC (28,65).

Because of their economical importance, the platinum metals are of particular interest. Their simultaneous determination without

interference was possible by TLC (33,65) as well as by HPLC (63). With both methods comparably good results were obtained.

An analytical procedure was described (33, 63), which allows quantitative analysis of trace quantities of Rh, Pd, Ir, and Pt. Sample preparation was the same for both techniques, TLC and HPLC. Thereby a preconcentration of the metals up to the factor 50 is possible by extraction into inpolar organic phases as chloroform. An excess of the complexing agent was re-extracted into aqueous phase in alkaline media at pH 12. The chloroform layer was transferred into a suitable syringe and injected into the HPLC-valve or transferred onto the HPTLC-layer.

As stationary phases silica gel HPTLC-plates and as mobile phases $CHCl_3$/THF- and CH_2Cl_2/THF-mixtures 90+10 were used for TLC-studies. The length of run was 6 cm, detection was carried out by reflectance measurements with a TLC-scanner (33).

The resulting detection limits were between 0,1 and 1 ng absolutely. The coefficient of variation of the complete analytical method (formation of the complexes, extraction, chromatography and determination) was between 3 % (Rh) and 16 % (Pd) for solutions containing 10 ppm of the metals.

HPLC - analysis allowed determination of Rh, Pd, Ir, and Pt simultaneously by gradient elution or Rh/Ir and Pd/Pt as separate pairs by isocratic elution (63). The mobile phases were composed of $CHCl_3$/CH_2Cl_2/THF-mixtures at different ratios. As stationary phase a silica gel Si 100 column with 10 μm particle size was used.

By gradient elution Rh, Pd, Ir, and Pt could be determined down to about 30 ng metal absolutely. With isocratic mobile phase the detection limits were 0,5 to 35 ng absolutely.

For standard solutions containing 1 ppm of the analyte, the coefficient of variation of the complete analytical procedure was 3 % (Pd) to 15 % (Ir) using isocratic elution.

In accordance to the excellent chromatographic separation of particular chelates the cross sensitivity between the platinum metals was extremely low. Even a 1000 fold excess of one component did not interfere with the determination of all other components. The calibration curves of the examined complexes were linear over a great range (HPLC: 50 ng-10 ng absolutely, TLC: 10 ng- 170 ng absolutely).

Furthermore, HPT not only forms stable, well defined chelates with the elements of the 8th subgroup, but also with at least 32 maingroup- and transition-metals (64).

A great number of the intensively coloured chelates could be extracted quantitatively into inpolar organic solvents and therefore were suitable for adsorption chromatography. 14 of the 18 thionates extractable into chloroform showed defined chromatographic behaviour with R_F-values, greater than zero (65). These were the chelates of VO (II), Cr (III), MoO_2 (II), Fe (III), Co (III), Ni (II), Rh (III), Pd (II), Ir (III), Pt (II), Cu (II), Zn (II), Hg (II), TL (III), whereas TiO (II), Ru (III), Os (III), and Bi (III) were not eluted (R_F= 0). So numerous separations are possible by TLC as well as HPLC.

Generally those solvents were qualified components for mobile phases, which well dissolved the respective complexes. These were $CHCl_3$, CH_2Cl_2 and THF, as the component with the strongest polarity. Besides the low viscosity and therefore the high fluidity of the mobile phase, the good solubility of the sample was one of the preconditions of TLC without tailing and with sharp limited spots.

Some basic principles, which influence the separation behaviour apply also to the examined thionates as well as to other metal chelates. Generally a separation between square planar MeL_2- and octahedric MeL_3-complexes is possible. The square planar complexes like Ni (II), Pd (II), Pt (II), and Cu (II) showes high R_F-values and so are distinct from octahedric complexes with lower R_F-values. No uniform chromatographic elution behaviour exists for chelates with tetrahedrical structure and also for those complexes which are additionally coordinated to oxigen like $TiOL_2$, $ZrOL_2$, VOL_2, and MoO_2L_2.

Irrespective of the initial oxidation state of the central atom only chelates with unite composition are formed with HPT.

With some elements like Cr (III), Rh (III), Ir (III), and Pt(II), however, kinetically inert cis/trans isomeres are formed with HPT as well as with other unsymmetrical bidental ligands. These can be separated by chromatography . Thereby the more polaric cis isomere (lower elution) has a greater thermodynamic stability than the unpolaric trans isomere as usual for unsymmetrical bidental ligands, which contain sulfur as coordination site, because in square planar and octahedric complexes chelates with cis-geometry possess stronger π-bondings between the S-atom and the corresponding d-orbitales of the central atom.

However, the formation of two different complex species of Cr (III), Rh (III), Ir (III), and Pt (II) do not hinder quantitative determination, if the calculation refers to the more stable and therefore quantitatively larger cis fraction. If this is impossible (due to interfering other chelates or decomposition products of equal retention), so again the sum of the two fractions can be determined along the chromatographic migration length of the HPTLC-plate by element specific detection. With this method the profiles of the metal concentration are neither interfered by

ligand or organic decomposition products nor by other organic products.

So in addition to the more customary UV-reflectance spectrophotometry of TLC-plates the chromatographic properties of metal chelates were studied by PIXE (10). With this technique the quantitative composition of those metal chelates were determined, which led to different separated fractions during chromatographic migration. This enabled a clear identification of optically detected maxima and a quantitative calibration of UV-reflectance measurements refering to the amount of the specific metal.

Since in most cases no complete chromatographic separation is necessary, when utilizing PIXE-detection, further analytical applications result. So e.g. the quantitative analysis of a mixture of the toxical heavymetals Sn (II), TL (III), Pb (II), and Bi (III) was carried out (10). Furthermore, detection limits are usually improved by a factor 3 to 10 using PIXE instead of photometry.

Another analytical application of HPT using HPLC was given by trace analysis of Ni in hydrogenated fats (30). Hydrochloric acid extracts were prepared from hydrogenated coconut oils by standard techniques. In these solutions Ni and Fe were complexed at pH 4-5 with HPT (Na-salt). The resulting chelates were extracted with $CHCl_3$, separated by HPLC and detected photometrically. As mobile phases again gradients with differing compositions of $CHCl_3$ and THF were used, as stationary phase silica gel Si 100. In the analized coconut oil 0,1 ppm Ni were determined. The limit of detection of the described procedure was 0,3 ng Ni, the linear range of the calibration curve reached up to 1 µg Ni.

By derivatisation of thione (e.g. with methyl groups) the number of extractable complexes is still increasing and therefore further elements can be determined by adsorption chromatography

(64, 66). Whereas 18 thionates can be extracted into $CHCl_3$, at least 23 4,6-dimethyl-thionates are extractable quantitatively into $CHCl_3$. These results prove, that it is even possible to optimize chromatographic analysis by special approximation of the analyte-structure to the chromatographic system.

The methylgroup inserted into the basic molecule increases the lipophility of the chelates and so increases the solubility in apolaric organic solvents, without too great changes of structure and dimension of the thionates. So the metal specific properties, which are important for chromatographic separation are not shielded. According to the position of the substituted group at the pyridine-ring and at the polaric metal-oxigen-bonding, the methyl group reduce interaction between complex and stationary phase, however, increases the interaction between complexe and mobile phase. This enable numerous further separations.

The effect of derivatisation of HPT with methyl groups and bromine on the chromatographic behaviour of its metal chelates was studied in detail by means of TLC and HPLC (66). Also separations were worked out using these techniques. So the complete separation of the 6-methyl-thionates of VO (II), Cr (III), MoO_2(II), Co (III), Ni (II), and Cu (II) succeeded.

Basically HPT was found to be an excellent chelating agent for chromatographic separation of metal chelates. This was explained by the following reasons:

1). Advantageous balance of the equilibrium between adsorption and desorption of the thionates at the silica gel phase; affected by the structure of the complexes.

2). Small dimension of the ligand, so metal specific differences of the central atom are not shielded and separations can be worked out very easily.

3). The complexes which show good chromatographic behaviour, are well soluble in the mobile phase.

Altogether the interaction between stationary phase, chelate, and mobile phase is in a balanced relation.

4.6 ß-Diketonates

The substance class of the ß-diketonates is another group of complexing agents, which is very often used for chromatography of metal chelates. A great number of oxigen affinic metals form stable chelat-6-rings with the two oxigen coordination sites of the ligand.

For separation of the ß-diketonates mainly partition chromatography is preferred, because the two polaric metal-oxigen-bondings in the chelate complex show very strong interaction with the stationary phases, commonly used in adsorption chromatography, which often leads to irreversible adsorption or at least to strong tailing.

As early as 1971 HUBER, KRAAK and VEENING separated the acetylacetonates and trifluoroacetyl-acetonates of Be (II), Al (III), Cr (III), Fe (III), Co (II/III), Ni (II), Cu (II), Zn (II), Zr (IV), and Ru (III) by liquid-liquid-chromatography using a home made HPLC-system (20). As stationary phase the aqueous part of the ternary mobile phase, consisting of a mixture of water, 2,2,4-trimethylpentane and ethanol was used. As carrier material the separation column was packed with diatomaceous earth (Kieselguhr, Merck) with a particle size of 5 to 10 and 10 to 20 µm. The addition of a trace of chelating ligand to the phase system suppressed undesirable hydrolysis reactions of the chelates and produced symmetrical and well resolved elution-peaks. Standard so-

lutions with up to 6 acetylacetonates were separated in less than 25 min.

HAWORTH and HUNG studied the TLC-behaviour of acetylacetonates on cellulose layers (13). Diverse binary and ternary solvent mixtures (water, ethanol, acetic acid, acetone etc.) were used. The chromatographic behaviour of the acetylacetonates of Fe (III), Ni (II), Co (II/III), Cu (II), Zn (II), Ca (II), Ba (II), Cr (III), and Mn(III) were examined.

SAITOH and SUZUKI reported about the TLC-behaviour of ß-diketones and their metal chelates on silica gel layers with CCL_4, CH_2Cl_2, toluene, benzene and dieethylether (49). Their studies proved, that with this chromatographic system very often complexing agents as well as many chelates were eluted with strong tailing only.

TOLLINCHE and RISBY described the HPLC-behaviour of metal acetylacetonates and substituted acetylacetonates (70). As stationary phases Al_2O_3, silica gel, polymethane, RP C 8 and RP C 18 columns were used. With silica gel columns and CH_2Cl_2/ CH_3CN (95/5) the best results were obtained, nevertheless in most cases the elution peaks showed no complete baseline resolution.

GURIRA and CARR separated the acetyl-acetonates of Co (II/III), Be (II), Rh (III), Cr (III), Ru (III), Pd (II), and Pt (II) with good resolution on a RP C 18 column with H_2O/CH_3CN (60/40) as mobile phase (12).

SCHWEDT compared the detection limits of Be-acetylacetonate obtained by means of TLC, HPLC, and GC (58). For the TLC normal and silanized silica gel, for HPLC silica gel of 7 µm particle size,and for GC silicone SE-30 as stationary phase were used. Visual detection and remission measurements in TLC, UV-254 nm absorption measurements in HPLC,and measurements with a FID in GC were applied for the evaluation of the calibration curves.

Detection limits of 5 ng Be by TLC, 0,15 ng Be by HPLC and 0,16 ng by GC were obtained.

4.7 Monothio-ß-diketonates

By substitution of one oxigen ligand atom with sulfur as coordination site ß- diketonates change to monothio-ß-diketonates. For this group of unsymmetrically bidental chelate ligands the same statements hold, which already have been discussed when transfering oxinates to thiooxinates. Chelates with metal-sulfur-bondings, which are comparatively less polar than metal-oxigen-bondings, show better chromatographic behaviour on silica gel or alumina materials than their oxo-analogues.

In an interesting study the TLC-separation of Cu (II), Co (III), Ni (II), Pd (II), and Rh (III) as monothio-ß-diketonates was investigated by MÜLLER and ROTHER (43). As an example the separation of the thiothenoyltrifluoroacetonates of Co (III), Ni (II), and Cu (II) as well as Rh (III), and Pd (II) on silica gel with $CHCl_3$ + n-hexane (30 +70) after extraction with chloroform was described. The excellent resolution allowed to extract the separated chelates from the TLC-plate by $CHCl_3$ and to determine them from the resulting solutions photometrically. Detection limits of 10 ng for Co and 50 ng for Ni, Cu, Rh, Pd were obtained with this procedure. The quantitative determination of traces of Co in the presence of Cu and Ni as well as the separation and determination of Rh- and Pd-traces were described.

HONJO and KIBA also studied the chromatographic behaviour of thioanalogues of ß-diketonates. So the TLC-behaviour of Co (II/III), Ni (II), Cu (II), Zn (II), Hg (II), Pb (II), and Cd (II) as 1,1,1-trifluoro-4-(2-thienyl)-4 mercapto-3-buten-2-en complexes (STTA) en silica gel layers were described (16). Pure solvents and binary solvent mixtures were used as mobile phases. Various possibilities for separations were developed.

4.8 ß-Dithioketonates

In the chelate complexes of the ß-dithioketonates both oxigen-ligand atoms are substituted by sulfur.

HONJO and OTAKI studied the qualification of dithioacetylacetone as ligand for chromatography of metal chelates (17). They described the adsorption chromatogrpahic behaviour of Mn (II), Co (II), Ni(II), Zn (II), Rh (III), Pd (II), and Pt (II) on silica gel layers with various organic solvents. Best results were found using CCl_4 and 1,1,1-trichloroethane as eluents. According to the charge-transfer-bondings the examined metal chelates were intensively coloured, which allowed easy identification. However, a disadvantage of the ligand and most of the complexes is the lack of stability. While the dithioacethylacetonates of Co (II), Ni (II), and Pd (II) were stable for at least several days, those of Mn (II), Zn (II), Rh (III), and Pt (II) decomposed gradually within several hours.

4.9 Dialkyl-benzoyl-thiourea chelates

According to their structure the N,N-disubstituted Acylthioureas were also used successfully as ligands for the chromatography of their metal chelates. Complex chemically they possess a great similarity with those monothio-ß-diketonates, already described before. So they form stable, crystalline neutral chelates with numerous metal ions, which are coordinated to both chalkogen atoms (S and O), so that chelate-6-rings result.
TLC-studies proved that silica gel was the best qualified phase for these chelates (31). Thereby resolution depended on the percentage of moisture of the silica gel material. The chelates of the platinum metals showed again excellent chromatographic properties. Their metal ions formed very stable chelates with the thiourea derivates. Moreover, the chelates of Co (II), Hg (II), and Cu (II) showed good chromatographic behaviour.

A great influence upon the chromatographic behaviour was caused by the alkyl substitutens. With increasing chain length of the alkyl-group the R_F-values of the complexes increased strongly with a simultaneous decrease of R_F-values-differences. As mobile phases pure solvents like benzene, toluene and chloroform were used.

For TLC-separations of the chelates the N,N-dimethyl-; N-pyrrolidino-; N,N-diethyl- and N,N-di-n-propyl-N-benzoyl-thioureas were the best qualified ligands. With the ligands mentioned above, platinum metals including Ru (III) and Os (III) were detected sensitively after chromatography on HPTLC-plates by a length of migration of 3 to 4 cm and by UV-reflectance measurements at 260-280 nm (31). The detection limits of the metals were 0,5 ng Pd and 2 ng Pt and Cu.

Nevertheless, the great advantage of these chelating agents in comparison to other sulfur containing ligands are their unusual stability against oxidation. Solutions of dialkyl-benzoylthioureas are totally stable under normal conditions and therefore can be handled without difficulties. Even from nitric acidic solutions the complexes can be precipitated and extracted without oxidation of the ligand. By a very simple synthesis (32) the benzoylthioureas can be derivated with low efforts and so far adapted to different analytical tasks.

Moreover, the dialkyl-benzoyl-thioureas are excellent reagents for pH-selective precipitation and extraction, and therefore are particularly qualified for liquid-liquid extraction of metal ions (32). So it is possible to separate the platinum metals from such important interferring metals like, Cu, Fe or Ni by liquid-liquid extraction and moreover, to separate individual platinum metals and to carry out their fine purification. By extraction high enrichment-factors are possible.

4.10 Some further complexing agents for chromatography of metal chelates

With good success also complexing agents like hydrazones, dithiobenzhydrazones, (pyridylazo)-resorcinol (PAR), (pyridylazo)-naphtol (PAN) and tetraphenylphorphine were used for chromatography of metal chelates.

WEBER and SCHWEDT improved the photometrical detection limit of Ni by pyridine-2-aldehyde-2-quinolylhydrazone (PAC) from 195 ng Ni to 34 ng Ni after TLC-separation of the excess of PAC on silica gel with acetone (74).

ROSTON used 4-(2-pyridylazo) resorcinol (PAR) as complexing agent for liquid-chromatographic determination of Cu (II), Co(II), Ni (II), and Fe (II) (47). The detection was carried out photometrically (fluorescence quenching at 254 nm) and by oxidative thin-layer amperometry. The limit of detection for Co was 0,02 ng by thin-layer amperometry (0,06 ng by UV absorption), and for Cu, Ni, and Fe about 1 ng with both techniques.

For separation of the PAR-chelates a 5 µm RP C 18 column and as mobile phase a mixture of 65 % 0,1 M pH 6,5 $NH_4H_2PO_4/(NH_4)\ HPO_4$-buffer, 35 % distilled CH_3OH was used. Natural water samples were investigated and the amount of Cu, Co, Ni, and Fe determined quantitatively.

HOSHINO and YOTSUYANAGI used a RP C 18 column, too, for analysis of Cr (III) down to the lower nanogram level as PAR-chelate by means of ion-pair RP-HPLC (18). Al (III), V (V), Mn (II), Fe (III), Co (II), Ni (II), Cu (II), Zn (II), and Cd (II) did not interfere up to an excess of a factor 100 to 200. As mobile phase an aqueous methanol (40 %) solution was used containing hexamethyl-1,4-butanediammonium dibromide, Tris-HCl-buffer and EDTA as buffer and ion-pair reagents.

GALIK and VINCOUROVA investigated already 1969 in detail the TLC-determination of Co (III), Cu (II), Ni (II), and Fe (III) as 1-(2-pyridyl-azo)-2-napthol (PAN) (8). The PAN-chelates were extracted into $CHCl_3$ and separated on silica gel layers. The determination was carried out by reflectance photometry with a TLC-scanner. The results were compared with other analytical methods and the experimental variables discussed in detail.

HEIZMANN and BALLSCHMITER used 1,2-diketo-bis-thiobenzhydrazones for extraction and spectrometric determination of metal ions (14). The chelates of Hg (II), Cd (II), Pb (II), and Zn (II) were separated on Al_2O_3-layers with ethylmethylketone.

KOBAYASHI, SAITOH and SUZUKI used tetrakis (p-tolyl) pophine (TTP) as ligand for chromatography of Cu (II), Pd (II), Ni (II), V (IV), Zn (II), and Mg (II) on silica gel-and RP 18- HPTLC-plates (22, 23). However, well resolved separations of TTP-chelates were only received at RP 18 layers with mixtures of acetone/acetonitrile, although the Ni- and Pd-complexes are not completely resolved. On cellulose and silica gel the chelates showed strong tailing and could not be resolved completely.

The results were transferred to HPLC. Thus, the TTP-complexes of Mg (II), VO (II), Ni (II), Cu (II), Zn (II), and Pd (II) and also TTP free acid were separated in about 10 min. on a RP C 18 column with a mixture of acetone and acetonitrile (70/30) at a flow rate of 1 ml/min. (24).

5. CONCLUSIONS

For inorganic analysis chromatographic methods have been used successfully in many cases. In particular, liquid chromatography is of special interest in trace metal analysis.

Considering the complete analytical procedure, LC of neutral metal chelates offers some special advantages. Prior to the chromatographical separation a preseparation from interfering inorganic matrix and enrichment of the analyte is possible by complexation and liquid-liquid extraction, if the solubility of the chelates is low in aqueous solution, but high in organic solvents, such as chloroform.

For chromatographic separation both techniques, TLC (HPTLC) as well as HPLC can be used successfully. Moreover, TLC is a useful pilot-technique to optimize HPLC-conditions. With both techniques, absorption chromatography is the most important separation principle for neutral metal chelates, because it yields sharpest elution profiles with best resolution. For special applications also RP-, partition-, ion-exchange- and gel-chromatography can be used.

To show good adsorption chromatographic behaviour, chelating agents as well as the formed complexes have to fulfil some basic requirements. They must form thermodynamically stable and well defined neutral chelates with clear stoichiometry. The metal chelates have to be well soluble in the mobile phase to avoid tailing. Further the ligand must not be too voluminous, otherwise levelling in chromatographic behaviour of various metal chelates would appear. For sensitive photometric detection the metal chelates should have large molar absorptivity in the UV/VIS-region.

Complexes with excellent chromatographic properties, which fulfil the mentioned criteria, partly or completely are the dithiocarbamates, dithizonates, complexes with ß-diketone/thio-diketone-structure, oxinates, thiooxinates, and complexes of cyclic thiohydroxamic acids, such as 1-hydroxy-2-pyridinethione. Some important publications on chromatography of metal chelates with these complexes are reviewed in this article. Investigations of

chromatographic properties and behaviour of chelating agents and the resulting metal chelates are also described like the analytical separation and determination of many heavy- and nobel metals.

In spite of the numerous important publications, contributing evidence of the great potential usefulness of TLC and HPLC of metal chelates in trace metal analysis, there still remains a certain lack of practicable analytical applications in this field.

Besides the possibilities to monitor the course of reactions, to study chelating equilibria, and separation of geometrical and optical isomers with chromatographic methods, it should be one main task in future to establish chromatographic methods for routine use in trace metal analysis, as it is already done in organic analysis. To transform inorganic metal ions to 'organic' molecules by complexation with organic ligands, nearly all chromatographic possibilities can be used as practised in organic analysis.

On the other hand, a further lack might still remain of very suitable chelating agents, to solve some special analytical problems. If the variation of chromatographical conditions, such as stationary or mobile phase is not successful, the 'organic mask' of the analyte can be optimized. This could be done by variation of the most common and successfully used chelating agents, by derivatisation of a given chelating agent, or by application of a completely new (in chromatography) chelating agent.

Methods development towards this aim is the second main task for chromatography of metal chelates in the future.

6. ACKNOWLEDGEMENT

The author wishes to thank Dr. M. Schuster (J. W. Goethe-Universität) and Dr. R. P. H. Garten (Max-Planck-Institut f. Metallforschung, Laboratorium f. Reinstoffanalytik) for some stimulating discussions during the course of preparation of this manuscript.

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