RADIOMETRIC TRACE ANALYSIS OF LEAD

STELLINGEN

I.

Er bestaat in West-Europa en Amerika weinig waardering voor de radio-uitwisselings analyse. Deze geringe waardering mist elke reëele grond.

W. W. Meinke — Anal. Chem. *30* 686, (1958).
E. Broda en T. Schönfeld — „Radiochemische Methoden der Mikrochemie" pagina 130 ev. — (2eband van: „Handbuch der Mikrochemischen Methoden" — Springer — Wenen — 1955).

II.

Voor de kwantitatieve papierchromatografie van microgram-hoeveelheden kationen kunnen gemerkte (anorganische) anionen met vrucht gebruikt worden.

Dit proefschrift — Hoofdstuk 4.

III.

De bewering van Broda en Schönfeld, dat anionen niet merkbaar aan chromatografeerpapier geadsorbeerd worden, is in zijn algemeenheid onjuist.

E. Broda en T. Schönfeld — „Radiochemische Methoden der Mikrochemie" pagina 59 — (2e band van: „Handbuch der Mikrochemischen Methoden" — Springer — Wenen — 1955).
Dit proefschrift — Hoofdstuk 2 en appendix 4.

IV.

De radiochemische zelfontleding van (metaal-) dithiocarbaminaten berust in hoofdzaak op secundaire effecten. Fysische stabilisatie is aantrekkelijker dan chemische.

Dit proefschrift — Hoofdstuk 2 en 6.

E. Broda en T. Schönfeld — „Radiochemische Methoden der Mikrochemie" pagina 85 — (2e band van: „Handbuch der Mikrochemischen Methoden" — Springen — Wenen 1955).

Proefschrift P. C. van Erkelens — 23 mei 1960.

V.

Oosting heeft een uitgesproken voorkeur voor de extractie, boven bijvoorbeeld de (papier)chromatografie en de ionen-wisseling, als algemene scheidingsmethodiek voor sporenelementen. Deze voorkeur is onvoldoende gemotiveerd.

M. Oosting — Mededeling 58-1 van het Analytisch Instituut T.N.O. — pagina 11 ev.

VI.

Het is onbegrijpelijk hoe Lacourt c.s. de door hen gemelde resultaten bij de kwantitatieve papierchromatografie van [sub-]microgram hoeveelheden Fe, Al en Cu hebben kunnen verkrijgen op ongewassen papier.

A. Lacourt c.s. — Mikrochim. Acta *1951* 312, *1954* 630, *1956* 700, *1957* 278.
idem — Analyst *77* 943 (1952).
E. en M. Lederer — „Chromatography" — Pagina 80 — (Elsevier - 1953).
J. N. Balston en B. E. Talbot — „A guide to filterpaper- and cellulosepowder-chromatography" — Pagina 19 ev. (Reeve-Angel-London-1952).

VII.

De door Hamm toegepaste *droge* verassing van biologisch materiaal is om meer dan één reden misplaatst.

R. Hamm — Mikrochim. Acta *1956* 268 (1956).

VIII.

Bij het opstellen van een balans van chemische elementen in de landbouw worden de nutriënten aanwezig in regen en sneeuw vaak ten onrechte verwaarloosd.

G. Ingham — J. Agr. Sci. *40* 55 (1950).
A. I. Virtanen — Angew. chem. *65* 2 en 10 (1953).
C. Richard en A. Vialard-Goudou — C. R. *237* 1548 (1953).
S. Muto — J. Chem. Soc. Japan — Pure Chem. Sect. *73* 446 (1952) *74* 99, 420 (1953).
S. Samson — Landbouwgids 1954 — pagina 281 ev.

IX.

Het percentage geladen terugstoten, gevonden door Yosim en Davies bij (n, γ) reacties aan ^{197}Au, geeft de mogelijkheid waarden af te leiden voor de spin en de pariteit van de eerste aangeslagen toestand van ^{198}Au.

S. Yosim en T. H. Davies — J. Phys. Chem. *56* 599 (1952).

X.

De discussie over stellingen, gelegen buiten het vakgebied van de promovendus, neemt tijdens de meeste promoties een onevenredig groot gedeelte van de tijd in beslag.

XI.

De vrees van het grote publiek voor „radio-actieve straling" is onevenredig groot in vergelijking met overeenkomstige gevoelens ten opzichte van andere moderne bronnen van gevaar.

RADIOMETRIC TRACE ANALYSIS OF LEAD

ACADEMISCH PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN
DOCTOR IN DE WIS- EN NATUURKUNDE
AAN DE RIJKS-UNIVERSITEIT TE UTRECHT
OP GEZAG VAN DE RECTOR-MAGNIFICUS
Dr J. F. NUBOER, HOOGLERAAR IN DE
FACULTEIT DER GENEESKUNDE VOLGENS
BESLUIT VAN DE SENAAT DER UNIVERSI-
TEIT IN HET OPENBAAR TE VERDEDIGEN
23 MEI 1960 DES NAMIDDAGS
4 UUR PRECIES

DOOR

PIETER CORNELIS VAN ERKELENS
GEBOREN TE AMSTERDAM

1960
DRUKKERIJ H. J. SMITS - OUDEGRACHT 231 - UTRECHT

Promotor:
Prof. Dr. Ir. J. SMITTENBERG

Aan mijn vrouw

VOORWOORD

Bij het verschijnen van dit proefschrift, ruim 14 jaar na mijn doctoraal examen aan de Universiteit van Utrecht, zijn helaas nog slechts enkele van mijn toenmalige leermeesters in leven. Hen breng ik mijn oprechte dank voor hun bijdrage tot mijn wetenschappelijke vorming, de overledenen gedenk ik hierbij met eerbied.

Hooggeleerde *Smittenberg,* ik dank U in het bijzonder, waar U zich ondanks drukke werkzaamheden heeft willen verdiepen in dit voor U nieuwe onderwerp. Uw waardevolle suggesties, bij de keuze van een deel mijner experimentele resultaten voor de bewerking tot een proefschrift en bij het uitwerken van mijn manuscript, zijn mij tot grote steun geweest.

Zeergeleerde *de Ligny,* ook U betuig ik gaarne mijn dank voor het kritisch doorlezen van mijn manuscript.

Het is verheugend, dat een gedeelte van mijn werk op het gebied der radiometrische analyse, verricht onder auspiciën van de Landbouworganisatie T.N.O. — later de Nationale Raad voor Landbouwkundig Onderzoek — in het Instituut voor Veeteeltkundig Onderzoek „Schoonoord", hier gepresenteerd kan worden in de vorm van een proefschrift.

Allen die hiertoe hebben bijgedragen breng ik mijn hartelijk dank; met name wil ik hier noemen: *Mevr. C. J. H. Stutterheim-Kanis, Mevr. W. Cliné-Theil* en *Mej. J. H. F. F. Broekman* die elk een groot deel van de experimenten hebben uitgevoerd.

122*nd publication of the Research Institute for Animal Husbandry „Schoonoord"*

RADIOMETRIC TRACE-ANALYSIS OF LEAD

Contents

INTRODUCTION

In the last two or three decades the important role, played by the so-called *minor constituents* [1]) in various phenomena, is becoming increasingly evident. In widely different fields of endeavour extremely small concentrations of many trace elements [1]) — sometimes as low as 10^{-6} percent — were found to exert profound influences which, in the dimension of their effect, could be compared with the actions of vitamines and hormones on the living organism.

Minute amounts of foreign metals proved to be decisive for instance for the properties of *semiconductors* as silicium, germanium, etc. The development of the transistor, which has become such a powerfull tool in electronics, would have been impossible without a thorough knowledge of the influence of foreing ions on the crystal-structure of these materials.

Other aspects, which are again of direct economical importance in the technical field, are for instance the effect trace elements have on (1) *corrosion* — where both the minor constituents in the agent and in the corroded material are of interest — on (2) the *cracking of crude oil* — where traces of certain metals present in only a few parts per billion can be detrimental for the catalyst — and on (3) the properties of the newly developed *materials in the field of nuclear energy* as: zirconium, beryllium, aluminium, uranium, etc.

In the *biological field* the role of trace elements in vital processes like *blood formation, bonegrowth,* general *metabolism,* and the function of the *brain,* is hardly less striking. Although practical use of trace elements is made here on a large scale already — especially in *mineral supplements* for animal feeding — the working-mechanism of these constituents is as yet badly understood. This is in contradiction to most of their actions in the technical field.

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[1]) The words "minor constituents" or "trace elements" are used in the general sense of. an element occurring in a very low concentration in an organic or inorganic matrix but having an important effect on [some of] its properties.

Apart from this growing interest in the *action* of the minor constituents, interest in the *occurrence* (and isolation!) of precious and rare elements in geological formations, seawater, industrial air, etc., has also promoted the attention paid to trace-analytical techniques.

The difference between trace-analysis and micro-analysis must be stressed here: in the latter, which is dealing with samples from 0.1 to 10 mgrams, the analytical methods are often similar to macro-techniques, but in the former new analytical tools are to be developed as the *concentrations* of the unknown elements are uncommonly low. Sometimes small samples are involved in trace-analysis as well, for instance when the sample-materials are precious or rare, or when ashing of larger quantities is difficult.

Especially in the latter cases the requirements for trace-analysis are rather severe. The analytical technique should allow the simultaneous determination of (sub-)microgram quantities of many elements, in a large amount of organic and inorganic materials, with a standard-deviation of say 10 to 20 percent. To eliminate statistical fluctuations, the method should be quick and inexpensive in order to allow several repetitions to be made within a reasonable period of time. In analysing biological or precious samples one gram of dry material — and sometimes less — should suffice.

To accommodate trace-analysis to these requirements many special techniques have been devised. None of them is universally applicable however and each has his specific advantages and disadvantages which vary greatly with the individual sample. As there is no general solution to the problem — which is in the meantime growing steadily by new discoveries of roles played by the minor constituents — a constant interest exists in new trace-analytical techniques.

New tools in this field were offered by the development of nuclear energy. In our investigation the application of (1) radioactive reagents and (2) radio-exhange, to the trace analysis of lead, is reported [2]).

In chapter 1 the principles underlying methods (1) and (2) are described, and a comparison is made with other techniques using

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[2]) "Radiometric analysis" is the expression commonly used to cover methods (1), (2) and (3).

the "nuclear tool", as there are: (3) radiotracer-analysis, (4) isotope dilution, and (5) activation-analysis [3]). A survey of the literature on (1) and (2) is given.

In chapter 2 the purpose of the present work is formulated and preliminary experiments are described. Out of the chemical systems investigated in these experiments, precipitation of Pb with $^{32}PO_4^{3-}$ (chapters 3 and 4) and exchange between the Pb- and ^{204}Tl--complexes of diethyldithiocarbamate (chapters 5 and 6) are chosen as being the most promising.

[3]) The application of scattering- and absorption-effects of nuclear particles in analysis is not included.

CHAPTER 1.

THE USE OF NUCLEAR ENERGY IN ANALYSIS

The 5 methods mentioned above are compared in table 1; this comparison is made in the first place to review which of them may offer a solution to the present problem — the trace analysis of lead — and secondly to clear up the differences between the respective methods.

1.1. *Analysis with radioactive reagents* (R.R.) consists of a reaction of the (total amount of the) substance to be determined (xM) with a radioactive reagent (*R), subsequent separation between excess *R and the reactionproduct (xM*R), and determination of the radioactivity of the latter.

Organic molecules like pyridine, quinoline, etc. may be added and included in M*R. Both M^+ and $*R^-$ may be replaced by more complex ions.

Either xM*R or the excess of *R has to be removed completely from the original system. For this separation use can be made of precipitation, paperchromatography, extraction, ion-exchange, etc. The separation should not interfere with the stability of one of the compounds M, *R or M*R.

Further requirements are: (1) a fixed stoechiometry [M/*R] and (2) accomplishment of the reaction for a fixed and known percentage (preferably 100) in a reasonably short time (The latter requirement can be forestalled by isotope dilution with *M).

A disadvantage of R.R. analysis is that most radioactive reagents have to be synthesized by the investigator himself. R.E. analysis does not suffer from this drawback.

1.2. The name *radioexchange analysis* (R.E.) is somewhat misleading. It is of course impossible to exchange radioactivity from one atom to another. What is meant is: replacement of a non-radioactive atom by a radioactive one.

Table 1.
Comparison of analytical methods using nuclear energy as a tool.

Number and symbol	1 R.R.	2 R.E.	3 R.T.	4 I.D.	5 A.A.
Name of the analytical method	Analysis with radioactive reagents	Radio-exchange analysis	Radio-tracer analysis	Isotope-dilution	(Neutron-) activation analysis
Scheme of the procedure	$xM^+ + {}^*R^- \rightarrow xM^*R$	$xM_1^+ + {}^*M_2A \leftrightarrows xM_1A + x^*M_2^+$	$[xM^+ + y^*M^+] + R^- \rightarrow [xMR + y^*MR]$ while $y \ll x$	$[xM + y^*M] \cdots > Q[xM + y^*M]$ while $Q < 1$ and $y \ll x$	$xM + \text{Neutr.} \rightarrow \psi x^*M$ $[\psi x^*M + zM] \cdots > Q[\psi x^*M + zM]$ while $z \gg x$
Procedure; Addition to the sample of:	Radioactivity and mass. (in 1 compound)	Radioactivity and mass. (in 1 compound)	Radioactivity and mass. (in 2 different compounds)	Radioactivity	(Neutrons and) mass
Separation required between	xM^*R and excess $^*R^-$	$x^*M_2^+$ and excess $^*M_2A$	$[xMR + y^*MR]$ and $[xM^+ + y^*M^+]$	$Q[xM + y^*M]$ and the rest of the system	$Q[\psi x^*M + zM]$ and the rest of the system
Measurement of mass	None	None	$(x + y).R^-$	Q.x.M	QzM
Measurement of radioactivity	x^*MR or excess $^*R^-$	$x^*M_2^+$ or excess $^*M_2A$	Only the *change* of the radioactivity in one phase of the system is observed.	y^*M and Qy^*M [or $(1-Q)y^*M$]	$Q\psi x^*M$
Limit of sensitivity	Micrograms or milli-micrograms [1]	Micrograms or milli-micrograms [1]	Milligrams or micrograms [2]	Milligrams, micrograms or millimicrograms [2]	Millimicrograms (for lead: micrograms)

[1] Depending on the specific activity of the reagent (or the metal) available.
[2] Depending on the measurement of mass.

R.E. analysis can for instance consist in the exchange of the total amount of the element to be determined (xM_1) with another (labelled) element or group ($*M_2$) and subsequent isolation of the exchanged amount: $x*M_2$. The reaction may start at either side (see table 1) and complex ions may be involved instead of the simple ones symbolised here.

$$MC_1 + *MC_2 \leftrightarrows *MC_1 + MC_2$$

Other exchange reactions are for instance:

$$M_1^+ + *M_2 \leftrightarrows M_1 + *M_2^+$$

in which two ions or complexes *) containing the same metal, but in two different chemical forms, are exchanged; and

in which a cation of M_1 is exchanged with an atom of another [of even of the same] element (M_2). In the case that $M_1 = M_2$ the name "isotope exchange" is used.
As realisation of the reactionscheme given in column 2 of table 1 seemed to be the most attractive, these alternative reactions were not examined in the present work.

An advantage of R.E.- over R.R.-analysis is the larger choice of labelled compounds: simple ions or ionic complexes of ordinary elements can be used. Radioisotopes of many of these elements are readily available with a high specific activity. As these isotopes emit both β- *and* γ-radiations, measurement is facilitated, as compared with the measurement of the β-emitters ^{32}P, ^{35}S, ^{14}C and ^{3}H, which are the labelled atoms commonly used in the radioactive reagents.

1.3. In *radiotracer- of radioindicator-analysis* (R.T.) the substance to be determined (M) is labelled with an amount (y) of *M which is small compared to the amount (x) of M in the sample. M and *M are then gradually removed from the sample — for instance by titration — by means of an inactive reagent (R). The end-point is indicated by the total removement of the radioactivity from the solution. [1]) The method is similar to other tracer applications of radioisotopes.

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*) Compounds which have been proved to be *un*suitable for the Szilard-Chalmers process could for instance be used.

[1]) *Foreign* metals may be used as a label as well: Duncan et. al. (1957; 1958) titrated 7 μgrams of zinc and 10 μgrams of cobalt in *one* procedure, using ^{60}Co as the indicator, with a carbontetrachloride solution of Dithizon. The end-point of the zinc-titration was indicated by the start of the decrease

Measurement of the required amount of inactive reagent is necessary; therefore the sentitivity of radiotracer analysis is in principal restricted, *independent* of the radioactive tool. Thus it was excluded from our investigations, although remarkable results were obtained with this method for other purposes [2].

1.4. In *isotope dilution* (I.D.) — as in R.T.-analysis — an unknown amount (x) of substance M is labelled with an amount (y) of *M which is small compared with x. The radioactivity of y*M is a_1.

In contradistinction with R.T.-analysis only a fraction Q of M (+ *M) is separated from the original system and in this part the amount [= Q (xM + y*M) $\approx$ QxM] of M is determined, as a rule by classical methods [3].

At the same time the radioactivity of this portion is measuared: a_2. The ratio a_2/a_1 provides the factor Q and hence xM.

It is clear that isotope dilution is merely a makeshift: it is easier to separate *part* of substance M free from interferences than to separate the complete amount of it. Thus the method is not specifically usefull in trace analysis: its usefullness depens on the analytical method applied to determine QxM [4].

1.5. *Activation analsis* (A.A.) is in a sense the reverse of isotope dilution. Here a fraction φ of the unknown substance (xM) is made radioactive, usually by placing it during an appropriate time in the flux of thermal neutrons from a reactor.

To this activated sample, containing an amount φx of substance *M, a known amount (z) of inactive M is added which is large compared with x. Part of (φx*M + zM) is then reparated and is measured with classical methods: Q (φx*M + zM) $\approx$ QzM. From this measurement the factor Q is known. At the same time the radioactivity of this separated part is measured: a. The activity

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of the radioactivity in the aqueous layer and the end-point of the cobalt determination by its total disappearance.

[2]) See for instance note [1] on pag. 3.

[3]) In chapter 6, isotope dilution is used in combination with R.E. analysis.

[4]) Zimakov et. al. (1958) demonstrated, that isotope dilution can be maae *independent* from other analytical methods by repeating the dilution-process with equal aliquots of the sample and different amounts of *M, provided that *equal* amounts of (M + *M) can be seperated from solutions of different concentrations. The authors determined 0.1 p.p.m. of Sb in lead.

of the original activated sample is then: $a/Q = a_0$ [5]). Either by a theoretical formule or — better — by comparison with standards the factor φ is determined and a_0 is related to the original amount x of M.

In contradistinction to isotope dilution, activation analysis can be very usefull for trace analysis, independent of the additional classical method. For the purpose of this work — the determination of (sub-) microgram amounts of lead — activation analysis is not very suitable however, as the sensivity of the method for lead is low: according to Meinke (1957), with a flux of 10^{11} neutrons per cm^2 per sec. an activation time of 30 days is required to determine 10 ppm of lead in an aqueous solution.

From table 1 and from the foregoing paragraphs, it is evident that out of these five methods, the numbers 1 and 2 [R.R. and R.E. analysis respectively] offer the best possibilities for our purpose.

1.6. *Survey of the literature on R.R.- and R.E.-analysis.*

Ehrenberg discovered *R.R. analysis* (1925) and devised an imposing number of ingeneous applications, using naturally occurring radioisotopes [see also Broda and Schönfeld (1955) page 141].

Since 1925 however, not more than fifty to sixty original publications appeared in the inorganic field [6]). As far as is known to the present author, there are only a few examples of radio-exchange analysis among these publications. More than fifty percent of the authors are Russians or Japanese, the French being the most productive in Europe.

On the first sight, this poor development of a promising method is somewhat astonishing, especially as the availibility of man-made radioisotopes should have promoted the method. Meinke (1958), citing Catch (1956; 1957), tried to find an explanation for this phenomenon (which is especially predominant in his own country) in: "... exaggerated estimation of the danger and cost ..., ... ignorance of the possibilities, and perhaps a measure of conservatism".

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[5]) Sometimes a_0 is measured directly, when γ- of β-spectrometry can be used to distinguish the radiations of *M from those of other activated elements.
[6]) P. Daudel (1958) reviewed the applications of activation analysis, R.R.- and R.E.-analysis in the organic field.

Consideration in detail of the literature, which is reviewed by Broda and Schönfeld (1955); Alimarin (1955; 1958) and Meinke (1958), reveals the following: *R.R. analysis with inorganic reagents* — generally very simple ones as for instance the ions $^{32}PO_4^{3-}$; $^{131}J^-$; $^{110}Ag^+$ and $^{210}Pb^{2+}$ — was used almost exclusively. Occasionally larger molecules, as for example complexes containing ^{60}Co, have found application.

Some extraction procedures were described by Korenmann et. al. (1957) but in general *separation by precipitation in bulk solution* was used. Langer (1941) introduced a titration method which became comparatively populair. Microtitrations and microcentrifugations [Moureu et. al. (1944)], or precipitations in filter paper [Peixoto Cabral and Götte (1955) and van Erkelens (1953)] were used bij some authors to obtain a higher sensitivity, while others added carriers, either of foreign substances or of the inactive form of the precipitate, for the same purpose. Sue (1941; 1946) could lower the limit of sensitivity from 100 to 5 micrograms of magnesium by adding an amount of inactive magnesiumphosphate to the solution, prior to the addition of phosphate-^{32}P, exchange being small within the time and at the temperature of the experiment.

In conclusion: it is remarkable that the literature on R.R. analysis is mainly restricted to inorganic reagents applied in bulk solution.

The scarce application of organic radioactive reagents is obviously in the first place due to difficulties encountered with their synthesis. As Broda and Schönfeld (1955, page 138) state: "... die Markierung des Komplexbildners (its) meist schwierig ...". Alimarin's more optimistic exceptation (1955): "... there is no doubt, that in the nearest future wider use will be given to organic precipitating agents with labelled atoms", did not come true.

Another reason is certainly to be found in the rapid decomposition which many organic (metal-)reagents undergo as a consequence of their own radiation.

As indicated already in 1.2., these considerations advocate the application of radioexchange analysis where radioactive reagents are not required and a wide choice of radioisotopes — of the metals themselves — is availlable.

Just as R.R. analysis, the *R.E. method* was discovered in Germany — by Peixoto Cabral and Götte (1955) — and was mainly applied by Russian and Japanese authors. Traces of metals were analysed by Troitskii (1958), who determined 100 μgrams of lead, cerium, or lanthanum by exchange with $^{45}CaCO_3$ and by Suzuki (1959), who analysed 0.1 ppm of silver by exchange with ^{110}Ag-dithizonate in carbontetrachloride. Anions were determined by the discoverers, who used the exchange of fluoride with zirconium-phosphate-^{32}P for the analysis of the former and by Zubrzycki et. al. (1959), who analysed phosphates by reacting them with silverions and exchanging them subsequently with ^{131}J.

Exchange reactions with *inactive* molecules were frequently applied in analytical chemistry — a.o. bij: B. L. Clarke (1937; 1938), Martin (1952), M. Fujimoto (1956; 1957), Kato (1957) and R. E. D. Clark et. al. (1959) —. In many instances it seems to be possible to refine these methods with radioactive tools.

In the meantime inorganic radioactive reagents remain to be attractive, while organic reagents are of interest only when they are easily synthesized and when their radiodecomposition can be limited.

As to the separation-techniques used in the literature, no obvious reasons can be given for the limitation to precipitation in bulk solution. It will be shown in chapters 5 and 6 that extraction-procedures are very well suited to R.E. analysis. The application of extraction in R.R. analysis is described elsewhere

CHAPTER 2.

INTRODUCTION TO THE EXPERIMENTS.

2.1. *Purpose of the present work.*

As we were primarely concerned with animal materials, the elements Fe, Cu, Zn, Mn, Mo, Pb and Co were of special interest. The amounts of Fe, Cu and Zn present in these materials are mostly sufficient for comparatively simple classical methods and thus only for Mn, Mo, Pb and Co radiometric methods are worth investigating. For molybdenum no sensitive radiometric method could be developed so far; results with cobalt and manganese will be published elsewhere; in this thesis the determination of traces of *lead* with R.R.- and R.E.-analysis is described.

The *physiological role of lead* as a trace element will not be discussed here in detail. It is not one of the most important trace elements, but on the other hand it surely has a cumulative toxicity for men and for the higher animals and plants (Orchard sprays!).

The high concentration of lead in the outer enamel layers of teeth, as compared with the inner layers — and with the distribution of for instance Sn and Cu which are equally available to the teeth from the ingested food — was a reason for Brudevold et. al. (1955) to suppose a physiological role of lead in teeth.
In general it is considered as an accidental or toxic trace element however.

The mechanism of this toxis action is as yet not understood; one of the suggestions is a reaction with vital phosphates, the other, from Dunlop (1939), a depression of the availability of copper.

Most animal materials — including blood, liver, urine and faeces — have a lead-content between 0.1 and 5 p.p.m. (dry) with a mean value of 3 p.p.m. The leadcontent of muscle is about 0.01 p.p.m. however. The amount of the sample which is available for analysis may be limited to a few grams or to part of a gram; moreover, the ashing of amounts of biological material larger than 1

gram (dry) is always to be avoided. Thus a *method which allows the determination of 0.1 to 1.0 μgrams of lead is desirable.* [For the determination of lead in muscle, an even more sensitive method is required.] Although this conclusion is mainly based upon the lead-content of material from the cow, it also holds for human material and for plants.

In inorganic materials still smaller concentrations of lead may be of interest, but the size of inorganic samples is seldom restricted to a gram.

In animal material lead is to be determined in a mixture with (1) other trace-elements (2) cations like Na^+, K^+, Ca^{2+}, Mg^{2+} and (3) anions like SO^{2-} and PO_4^{3-}; so either specific reactions of the lead-ion or separations or both wille be required. Our investigations into the *separation* of several trace elements, in which ion-exhange, paperchromatography and extraction are used, will be published elsewhere. For the present purpose, the following remarks may suffice.

(1) It is not necessary to separate hundred percent of the lead: isotope dilution can often be used to indicate the percentage which has reached the final step.

(2) Separation of lead from other cations and from anions can often be combined with separation of the former from excess reagent (see chapters 3 and 4) or from the exchanging radioactive metal (see chapters 5 and 6).

2.2. *Radionuclides and general techniques.*

The radionuclides applied are shown in table 2 with their characteristic half-life and mode of deacay. Their radioactive decay-products and the techniques applied for their measurement are also indicated.

Measurement A was carried out with an end-window GM-tube (Philips Lamp Works — Eindhoven. No. 18506; diameter 3 cm, window 2.5 mgrams per cm^2, zero-effect surrounded by 3 cm's of lead: 30 ± 3 c/m) and a scaler (Philips GM 4810 — PW 4020). This equipment was either combined with a sample-changer and a printer or with a ratemeter (Philips PW 4041) and a recorder (Nieaf — Utrecht). Sample-changer and scanner were build in one

Table 2.
Radionuclides applied.

Nuclide	Half-life [1] in hours (H) days (D) or years (Y)	Mode of Decay Radiation—(Max) Energy in Mev [1]	Radioactive decay-products	Measurement [2]
^{14}C	5568 Y	β^- —— 0.155		A
^{32}P	14.3 D	β^- —— 1.701		AB
^{35}S	87.6 D	β^- —— 0.168		A
^{54}Mn	310 D	γ —— 0.845 and K-capture		C
^{59}Fe	45.9 D	β^- —— 0.257–0.455 γ —— 1.100–1.295		C
^{60}Co	5.29 Y	β^- —— 0.311 γ —— 1.172–1.332		C
^{65}Zn	248 D	β^+ —— 0.323 γ —— 1.118 and K -capture		C
^{89}Sr	54 D	β^- —— 1.482		BC [3]
^{203}Hg	46.0 D	β^- —— 0.206 γ —— 0.280		C
^{204}Tl	3.1 Y	β^- —— 0.770 and K-capture		BC [3]
^{210}Pb (Ra-D)	22 Y	β^- —— 0.018–0.056 γ —— 0.047 and below	^{210}Bi (=Ra–E) β^- —— 1.17 half life 5 days ^{210}Po (=Ra–F) α —— 5.3 γ —— 0.786	BC [4]
^{212}Pb (ThB)	10.6 H	β^- —— 0.350–0.580 γ —— 0.115–0.176–0.238 –0.249–0.299	half lives ^{212}Bi — 60.5 min ^{208}Tl — 3 min ^{212}Po — 0.3 μsec together: numerous β's and γ's	not applied

[1] (Mean values) from: Radiological Health Handbook – S. Kinsman et al. – R. Taft, San. Eng. Center-Cincinnati – Ohio – U.S.A. – 1954.
[2] For the meaning of A, B, and C, see text.
[3] The "bremsstrahlung" is measured (C).
[4] With purified ^{210}Pb (see chapter 6) only measurement C is possible.

apparatus by the Physical Laboratory of the University of Utrecht. The scaler was regulated on a dead-time of 300 μseconds which resulted in a dead-time correction of 1 % per 2000 c/m.

Measurement B was carried out with the same scaler and a GM--tube for liquid samples (20th Century Electronics - M6; zero--effect surrounded by 2 cm's of lead: 22 ± 2 c/m) which was desicoted between the electrodes to prevent leakage.

Measurement C was carried out with a well-type scintillation-detector (Philips PW 4111/W; NaJ-crystal: Ø 7 cm, height 6 cm, hole Ø 1.1 cm and 3.4 cm deep) and a scaler (Philips PW 4022 — 4032 — 4052). In most measurements this scaler was combined with a single-channel analyzer (E.K.A.F. — The Hague, type 30301 — 20102). The zero-effects are given in the following chapters.

The usual *precautions for radioactive* work on a μcurie-level were taken: pipetting was done with a bulb; tables were covered with adsorbing paper and part of the work was carried out in polyethylene troughs — 60x40x3 cm —. Gloves were only worn in making the dilutions.

The usual *precautions for trace-analysis* were taken as well: glass- and polyethylene-ware was cleaned with a mixture of sulfuric- and chromic-acid, rinsed in 3% hydrofluoric-acid and kept for at least one night under 2n. nitric-acid; pipets were desicoted and lead bricks — used for the screening of radioactive radiation — were carefully painted to prevent pollution.

2.3. *Preliminary investigations on R.R.-analysis.*

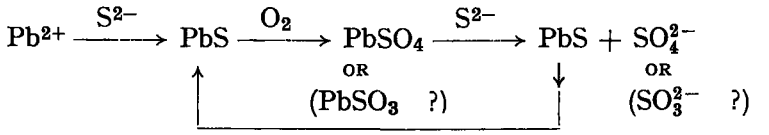
As radioactive reagents were investigated: Hydrogen-sulfide-^{35}S, Meraptobenzthiazol-^{35}S, Mercaptonaphthiazol-^{35}S, Acetylacetone-^{14}C, Thiocyanate-^{35}S, Diethyldithiocarbamate-^{35}S, Phosphate-^{32}P and Sulfate-^{35}S.

2.3.1. *Hydrogen-sulfide-^{35}S.*

This method consisted of a paperchromatographic separation of the metal-ions and a subsequent reaction with $H_2{}^{35}S$ in a reducing atmosphere.

As was described already in a short publication (van Erkelens — 1953) the dried and neutralised papergram was treated with $H_2{}^{35}S$ — in fact $(NH_4)_2{}^{35}S$ — by putting it in a bell-jar which had a volume of about 2 liters and contained two beakers, one with concentrated ammonia and the other with 10 mgrams of $Zn^{35}S$. Pure nitrogen (99.99 percent) was led in while the jar was surrounded by a second one — containing also pure nitrogen — to diminish the effect of leakage. The inner jar was then evacuated and again filled with pure nitrogen 4 times. After the final evacuation, sulfuric-acid which had been boiled out under nitrogen, was added to the $Zn^{35}S$ through a series of stopcocks which prevented any air to enter the bell-jar. After 10 minutes the surplus of $(NH_4)_2{}^{35}S$ was swept out of the reaction vessel.

Notwithstanding alle these precautions, taken to prevent the oxidations of the culfides, this oxidation was not excluded completely and gave rise to a reaction-chain which caused false and irreproducible results:



Further favourable effects were obtained by (1) the addition of hydrazine to the atmosphere in the bell-jar and by (2) the exclusion of light, but here again oxidation disturbed the results, at least when amounts of lead below 10 μgrams [2]) were concerned.

This high oxidation-rate cannot be understood from the oxygen content of the nitrogen. Only 3 μgrams of oxygen are present in 2 liters of nitrogen at a pressure of 7 mm. These 3 μgrams are sufficient for the oxidation of not more than 40 μgrams of lead, while 20 to 40 spots of 1 cm^2, each containing 5 to 20 μgrams of lead, were handled simultaneously. Probably traces of oxygen, adsorbed on the wal of the vessel and/or on the paper, are responsible for the observed effect. Hydrazine was added to the paper, but appeared to prevent the reaction of the metals with the S^{2-}-ion. A mixture of H_3PO_2 and HJ as described by Archer (1956) will reduce sulfates to sulfides. This is not applicable in our case however, as reaction of the metals with the J^-- and PO_2^{3-}-ions is to be expected.

2.3.2. *Mercaptobenzthiazol-^{35}S and Mercaptonaphthiazol-^{35}S.*

According to Spacu (1935, 1936) and Kuras (1939), these reagents precipitate the ions of Cu, Pb, Bi, Hg, Tl, Co, Ni, Zn, Mn and Ag. Oxidation — and chainreactions — of the metalcomplexes is not to be feared here; the synthesis of the radioactive reagent is a new complication however. Following J a c o b s e n et. al. (1891) the two reagents were prepared from elemental ^{35}S, which is commercially available, and inactive phenyl- or naphtylisothiocynate respectively.

63 mgrams of elemental ^{35}S, with a specific activity of 10 mc/mmole, were heated with 344 mgrams of naphtylisothiocyanate during 4.5 hours at 250°C in

[2]) The limit of sensivity set by the measurement of ^{35}S, was about 20 mμgrams of lead for our preparation, which had a specific activity of 10 mc. per millimole.

a closed Carius tube with an inner diameter of 1 cm and 10 cm's long. This tube stood in a sand-bath which in turn was placed in a hood and surrounded by a wire cage.

After cooling, the tube was opened in the hood by means of a flame and of the pressure of its contents. After standing for 30 minutes, 1.5 ml 8n.NaOH and an equal volume of alcohol were added and mixed with the dark mass in the tube; the mixture was then left standing for 5 hours.

The glass vessel was centrifuged at 3000 r.p.m. and the precipitate washed two times with 3 ml ethanol and two times with 3 ml water. To the combined supernatant and washing-liquids 2 ml of 6n.HCl as added and the mixture was left standing overnight.

The crude mercapto-naphthiazole was then dissolved in 6 ml of a 10 percent Na_2CO_3-solution, boiled with active coal for 30 minutes, filtered through glasswool, precipitated again with 6n.HCl, and left standing overnight.

It was now centrifuged and recrystallised from hot alcohol by the addition of water. This method gave better results than recrystallisation from 50 percent acetic acid. The yield was 20—25 percent, which is somewhat low in comparison with the figures given by Jacobsen. The melting point was 238°C (178°C for the phenyl-compound) which is in fair agreement with Jacobsen (1891) and Spacu (1936).

The reagents were tested with Cu and Pb, with which they produced — in ammoniacal solution — a green and a white precipitate respectively. According to Spacu this leadcompound has the composition Pb-R-OH, in which R is the reagent; in neutral solution the yellow compound PbR_2 is precipitated. The latter contains variable amounts of additional reagent however and is thus unsuitable for our purpose. Further tests showed the reagents to be soluble in many organic solvents, a.o. in alcohol, and in aqueous ammonia, but not in water. The metal-complexes were soluble in alcohol, but not in water, ammonia or acetic-acid.

Experiments were carried out as follows:

An alcoholic or acetonic solution, containing about a 4-fold excess of the reagent, was sprayed on a — previously neutralised — papergram of Cu, Pb, etc.

A suitable solvent, either organic or aqueous, was used to separate the excess reagent from the metal-complexes.

Unfortunately, traces of the reagent could not be washed out of the paper thus producing a variable blank which made the method unsuitable for our purpose.

Alternatively the reagent was applied with a small paintbrush to part A of the papergram (figure 1) and chromatographed immediately in direction II with an organic solvent as for instance petroleum-ether (b.p. 80—100°C), butanone, isopropyl alcohol, or ethylacetate. A typical result is shown in figure 2: the greater part of the reagent

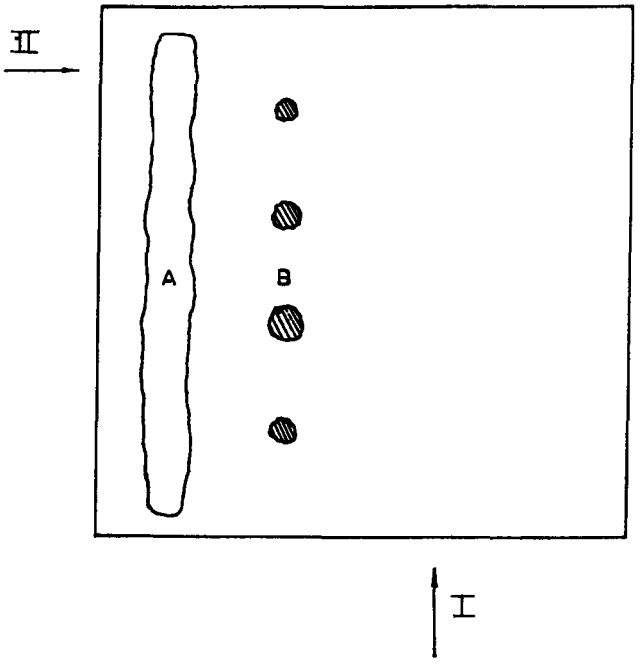


FIGURE 1:
Application of a radio-active reagent (A) beside a papergram (B).
I: direction in which the papergram was developed.
II: direction in which A is moved over B.

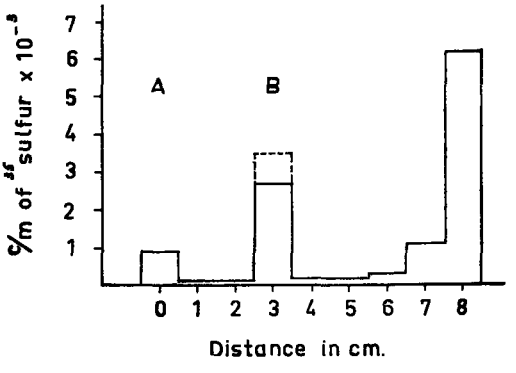


FIGURE 2:
Typical result of the reaction of mercaptobenthiazol-^{35}S moving in petroleum-ether (80—100) over a spot containing 20 μgrams of lead.
A and B correspond with A and B in figure 1.
.....: Expected value.

is found on Rf 1.0, about 10 percent is left at Rf 0.0 (A in figure 1), and only a small amount is found to be adsorbed to the rest of the paper. The reaction-product is found where the metal was to be expected. The totale activity is insufficient — and variable — however, indicating an incomplete reaction.

Solvents containing water and ammonia were also tested, as a better reaction could be expected here. These solvents did not carry the reagent directly in the front however. As a consequence the metal-ions were partly washed out before they were able to react with the mercaptothiazoles.

With organic solvents and a large excess of the reagent a more complete reaction than shown in figure 2 was observed. The variations in the background — caused by the adsorption of this large excess reagent to the paper — were too high however. Above, the reagents undergo radiochemical decomposition, probably through a reaction between their SH-groups and the free radicals formed by the radiochemical decomposition of traces of water. Even when kept under vacuum over P_2O_5, this decomposition spoiled the reagent within a few months.

2.3.3. *Acetylacetone-*^{14}C.

Our first task was again to prepare the reagent in a radioactive form.

Following Sixt et. al. (1949) acetylacetone-^{14}C was prepared from acetone-^{14}C — which is commercially available — ethylacetate and sodium-methylate. The procedure of Sixt proved to be superior to the method of Adkins et. al. (1955).

With the samples of sodium-methylate which were available to us (Th. Schuchard AG — München) satisfactory results could not be obtained. Following an indication of Pluyger[3]) sodium-methylate had to be prepared immediately before the synthesis of acetylacetone from 1 mole of sodium and 3 moles of methanol. The latter was prepared free of water by the procedure of Lund and Bjerrum (1931). Acetone and ester were dried by the methods given by Adkins (1955).

The procedure was carried out with 1 mcurie acetone-^{14}C — diluted with inactive acetone to 3.5 ml —. The yield was 33 percent. The product had a boiling point of 136—138°C; it was diluted to 2 ml with inactive acetylacetone.[4])

Extraction of lead with this reagent would be rather dangerous

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[3]) The authors thanks are due to Dr C. W. Pluyger of the Institute for Organic Chemistry — T.N.O. — Utrecht.

[4]) Merck, p.a.

and uneconomical. Paperchromatotografic separation seemed to be best policy. Solvents containing much acetylacetone-^{14}C again could not be used for reasons of economy. On the other hand *some* acetylacetone-^{14}C had to be present in the solvent, otherwise the Rf-values changed into the values for the metal-ions, which indicated that the complexes were broken down [5]).

Of a solution containing per ml: 1 mgram each of Fe, Cu, Zn, Mn, Pb, Co and Mo, and 200 mgrams of the acetylacetone-^{14}C or about a 10-fold-excess, 0.01 ml was placed on the paper and chromatographed with the solvents: C_2H_5acetate[8]-HCl[0.7]-CH_3OH[1.3] or: Dioxane[4]-4nHCl[1]. A separation: Zn—Mn—Pb+Cu—Fe—Co was obtained; most of the excess acetylacetone-^{14}C was found in the solvent front, but part of it — an equivalent of 1 to 5 μgrams of lead per cm^2 — was retained in the paper and could not be removed, neither by ordinary evaporation nor by a stream of warm air. Elution of the metal-acetylacetonates and evaporation of the excess acetylacetone would offer a solution to this problem, this method would be rather cumbersome however.

2.3.4. *Thiocyanate-*^{35}S.

This reagent is commercially available: Oxidation does not seem to be a serious danger. In general, separation of the reagent from metalthiocyanates by (1) evaporation of HCNS from an organic extract, (2) precipitation of the salts, or (3) chromatography, seems to be feasible.

(1) Extraction of lead with CNS^--ions is not possible. Above it would be uneconomical as a large excess reagent would be required.

(2) Precipitation of lead with $Cr(CNS)_6^{3-}$ is possible but as yet not worked out.

(3) Paperchromatographic separations of the thiocyanates of some heavy metals were carried out by Beck (1953), who separated Fe^{3+}-, Cu^{2+}- and Co^{2+}-thiocyanates, and by Martin (1951), who separated the lead salt from the Fe^{3+}-, Ni^{2+}-, Co^{2+}-, Cu^{2+}-, Cd^{2+}-, Hg^{2+}-, As^{3+}-, Ag^+-, Sb^{3+}-, Cr^{3+}- and Bi^{3+}-thiocyanates.

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[5]) This result is not in accordance with the experiments of Berg et. al. (1955).

The solvent of Martin — butanol saturated with 2n. HCNS — was used in our experiments. Again the reagent adhered to the paper in amounts comparable with the amount of CNS^--ion chemically bound in the leadsalt however. Esterification of the OH-groups of the paper with methyljodide, carried out to prevent this adsorption, was found to be unsuccesfull.

2.3.5. *Diethyldithiocarbamate-^{35}S (DDS-^{35}S).*

This reagent was synthesized [6]) following G l e u and S c h w a b (1950); the solvent was evaporated by a stream of nitrogen and the whole procedure was carried out in an efficient hood. Three different R.R.-methods were used:

(1) The first method consisted of a reaction of the metals with excess DDC-^{35}S in filterpaper, followed by a chromatografic separation of the metal-DDC-^{35}S complexes from each other and from the excess reagent. A large variety of solvents and papers [W h a t m a n 1; S c h l e i c h e r and S c h ü l l (SS) 2043; SS-2043-acetylated (by S.S.); SS-Glasspaper [7]); SS 1001- and 1002-PVC papers, called "Rhovyl-Selecta"; and papers acetylated or butyrated by Z i j p (1955) and M i c h e e l [8]) (1954)] was tried but only very small differences in the Rf-values were observed.

Separations proved to be possible with Al_2O_3-coated papers, but with many experimental difficulties (see appendix 1).

(2) Alternatively the application of DDC-^{35}S to a papergram of metal-ions, followed by separation of the excess reagent, was studied. The papergram [9]) was dried and neutralised in the vapour of concentrated ammonia. The reagent was applied in the form of a long spot as shown in figure 1 [2.3.2.] or as a spray over the chromatogram [9]).

As DDC is only soluble in solvents containing an appreciable

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[6]) Other preparations were received from the Radiochemical Centre—Amersham (UK).

[7]) So called "glasfaserpapier". The authors thanks are due to Dr. A. G r ü n e — Schleicher and Schüll Company; Dassel - Germany — for these specimens.

[8]) The authors thanks are due to Dr. Z i j p and to Dr. M i c h e e l for these specimens.

[9]) Details of the separation of lead by paperchromatography and or the technique for the application of the reagent, are given in chapter 4.

amount of water, the first variant suffered from the same disadvantage as described before: the metalspots dissolved in the first amount of solvent before the reagent could reach them.

In the second variant the reagent again adhered to the paper even when solvents were used like: CH_3OH^2-0.01 n. NH_4OH^8 in which DDC is soluble to a very large extent. Consequently, paper was rejected as a substratum for DDC-^{35}S.

(3) When metal-DDC complexes are extracted with organic solvents the greater part of the reagent will remain in the aqueous layer; Pb, Bi and Tl are selectively extracted from a KCN-solution at a pH above 11.

In our experiments the (radiochemical-) instability of the reagent was again a drawback, but stabilisation could be obtained by adding a large excess of inactive SH-groups in the form of cysteïn or cysteïnamine. This prevented the extraction of metal-DDC-^{35}S complexes to a large extent however. Thus an organic solution of a metal-DDC-^{35}S complex — prepared with a large excess of this metal over both DDC-^{35}S and foreign SH-groups — was applied as a reagent for the extraction of lead. The excess of this complex was backextracted with KCN.

A similar method was worked out for cobalt and will be published elsewhere. The smallest amount which could be analysed in this way was 0.1 μgram of cobalt, equivalent with 0.5 μgrams of lead. As better results were obtained with the same chemical-system in R.E.-analysis — using inactive DDC and a radioactive metal — this determination was not worked out for lead.

2.3.6. *Phosphate-^{32}P.*

The experiments with this reagent are described in detail in chapters 3 and 4. The only way to use this reagent is obviously by precipitation, here carried out in filterpaper to obtain a higher sensitivity. Oxidation of the reaction-product or radiochemical decomposition of the reagent are not be feared. *The adsorption of the reagent to the paper is much smaller than with the reagents described before.*

Other ions can be analysed by this reagent as well. This universality is of course also a drawback as interferences from foreign metals are to be expected. In the chapters 3 and 4 two different methods are given to circumfere this interference.

2.3.7. *Sulfate-^{35}S.*

With this reagent again no exidation or radiochemical decomposition is to be feared. The adsorption of the reagent to the paper is again moderate. Interferences from other metals are restricted to Ba and Sr and larger amounts of Ca. For the determination of lead this is of course an advantage, but on the other hand the reagent does not satisfy our premise to be applicable to other cations.

Ethanol^{1}-H_2O^{1} can be used for separation of excess agent. The sensitivity is about a factor of 4 smaller then with phosphate-^{32}P owing to the higher solubility of the precipitate. In contradistinction to ^{32}P the radiation of ^{35}S is partly adsorbed by the paper and thus inhomogenities in the latter add to the error made.

2.3.8. *Other reagents for lead.*

Ishimori et. al. (1957) used a ^{204}Tl-compound for the determination of lead by R.R. analysis in bulk solution. With very fine capillary tubes they were able to precipitates "less than 78 μgrams of lead".

Not applied so far but obviously usefull are the reagents: arsenite, selenide, pyrophosphate, triphenyl methylarsonium-iodide, chromate — used in the inactive form for instance by Guthrie and Nance (1951) — and diethyldithiophosphate used in the inactive form by Busev et. al. (1958[a, b]). With the last reagent R.R. *and* R.E. analyses should be possible.

2.3.9. *Conclusion.*

From the radioactive reagents for lead considered here, phosphate-^{32}P is obviously the most promising; this method will be worked out in chapters 3 and 4.

Sulfate-^{35}S is more selective but less sensitive. All other reagents tried, suffer either from oxidation or from adsorption on the paper. Procedures avoiding the paper as a substrate are not sensitive enough, because of the large volume of the system.

Paperchromatography of metal-DDC-^{35}S complexes, with solvents which leave the excess reagent at Rf 0.0, is attractive but could not be realised in our experiments although a great variety of papers and solvents was tried. This conclusion is in accordance with the results obtained with other radioactive reagents bij Fink and Fink (1949) already.

2.4. *Preliminary investigations on R.E. analysis.*

2.4.1. *Cation-exchange columns treated with* ^{89}Sr *or* ^{65}Zn.

When a cation-exchange column is saturated with a radioactive cation and treated with a solution of another cation — which has a higher affinity to the exchange-material — an equivalent amount of the radioactive cation will in principle be released to the eluate. Samuelson (1952) gave some sequences of affinity. The selectivity of the exchange-material is larger when it has a higher percentage of cross-linking.

In our experiments, Dowex 50 × 8 — 50 to 100 mesh — was used in small columns 6 cm long and 0.27 square cm in diameter, furnished with a G-2 fritted glassfilter at the lower end. These columns can take up about 1 μequivalent of cations, while about 0.2 μequivalents are to be expected in the ash of 1 gram of dry cow liver for instance. The columns were saturated with (1) $^{89}Sr^{2+}$ or (2) $^{65}Zn^{2+}$ by repeated sorption of the 0.1 molair solutions of these ions in 0.0001 n HCl.

(1) Strontium-89 with a specific activity of 0.05 mc/mmole was used. Elution with 1 μequivalent of HCl (10 ml 0.0001 n) gave rise to 0.2—0.8 μequivalents of ^{89}Sr in the eluate. This means that 20—80 percent of the HCl are exhanged! As the differences between successive washings with 10 ml portions of HCl were large, ^{89}Sr-columns can not be used in this way for the analysis of trace elements.

(2) Better results were obtained with columns containing ^{65}Zn with a specific activity of 0.1 mc/mmole: 10 ml of 0.01 n HCl (100 μequivalent) produced only 0.4 — 8.3 μequivalents of ^{65}Zn, and 10 mls' of 0.001 n HCl (10 μequivalents) produced only 0.0 — 0.25 μeq.'s of ^{65}Zn in the eluate.

When μequivalent amounts of the cations Mg^{2+}, Ca^{2+}, Zn^{2+} and Cu^{2+} (in 0.001 n HCl) were percolated through the columns, they gave rise to appreciable amounts of ^{65}Zn in the eluate. The figures for two columns filled with the same ^{65}Zn differed by a factor of 2 however. In all cases the amounts of ^{65}Zn released from the column, were below the equivalent of the ions added on the top of it. Aging effects were also observed.

One of the reasons for these unsatisfactory results may be the slowness of the exchange-reaction; longer columns should be used. Another confusing factor might be the "elution" of small parts of

the columnmaterial. No gross contamination by whole grains is to be feared as the diameter of our grains is larger than the holes in the filter, but it is a well known fact, that trace amounts of the exchange material are often to be found in the eluate [11]). As the figures found in our experiments are all too low however, this factor is certainly not predominant.

2.4.2. *Anion-Exchange columns treated with S^{2-}- and $^{65}Zn^{2+}$-ions.*

D. L. Clarke et. al. (1937; 1938) used papers in which ZnS was precipitated, for the determination of traces of Cu and other metals. The color-change of the paper was observed. Similar exchange-phenomena were observed by Delfino Lema (1956) between freshly prepared MnS and the ions Fe^{2+}, Fe^{3+}, Zn^{2+}, Ni^{2+} and Co^{2+}, and by Kodera and Onishi (1953) who used Ag^{+}-ions to detect the reaction-product of H_2S with several metal-ions in a papergram.

Of course, the method of Clarke et. al. is not sensitive enough for the determination of μgram-amounts. When ^{65}ZnS is used, the radioactivity in the eluate can be taken as a measure and the sensitivity is enhanced appreciably. Oxidation is to be feared, as (1) oxidation of ^{65}ZnS gives $^{65}ZnSO_4$ and hence free ^{65}Zn-ions, and (2) oxidation of CuS produces new copper-ions able to exchange with ^{65}ZnS.

With a view to this oxidation, paper was avoided in our experiments (see 2.3.1.) and an ionexchangecolumn was used, filled with Dowex 1 × 10 — 50 to 100 mesh. A solution containing a tenfold excess Na_2S was left in contact with the column-material overnight and excess sulfide was eluted with water untill the reaction of the eluate with copper was negative. The column was then emptied in a pyrex beaker containing a tenfold excess of zinc in the form of a $^{65}ZnSO_4$-solution. After standing overnight and repeated decantations with water, the column was filled again and rinsed with 10 ml of slightly alcaline 0.01 molair tartrate: practically no ^{65}Zn was eluted. The column contained now 1 μequivalent of ^{65}ZnS with a specific activity of 0.02 mc/mmole.

Experiments were then carried out with Cu and Pb in slightly alcaline 0.01 molair tartrate: 0.5 μequivalents of copper eluted only

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[11]) See 3.2.4.

0.05 to 0.1 μequivalents of ^{65}Zn, 5.0 μequivalents of copper eluted only 0.25 to 0.5 μequivalents of ^{65}Zn; with lead similar results were obtained.

These *low results* are an indication that oxidation of the sulfides is improbable. The loss of ^{65}ZnS, either in the form of a colloidal solution or adsorbed to small parts of the Dowex material, is evidently not to be feared.

On the other hand an excess sulfide in the columns, which could be an explanation for the low results, is very improbable with regard to the method of preparation. The *formation of colloïdal hydroxydes* — in spite of the tartrate — *or a slow exchange-reaction are more probable reasons for the low results.*

2.4.3. *Exchange between metal-ions and metal-D.D.C. complexes:*

$$M_1^+ + M_2\,(D.D.C.) \rightleftarrows M_1\,(D.D.C.) + M_2^+$$

This method was chosen for the determination of lead, and is described in detail in chapters 5 and 6. There are two variants, symbolised by both directions of the reaction given above: either lead-ions are exchanged with the radioactive metal-component of a metal-DDC complex (chapter 6), or the lead in the lead-DDC complex is exchanged with a radioactive metal-ion (chapter 5). The first variant is the most simple one. The second allows the separation of lead by selective extraction with DDC, prior to the exchange. This selectivity can also be introduced in the method of chapter 6 by the addition of KCN to the exchanging system, as only the ions Pb^{2+}; Bi^{3+}; Tl^{3+} and Tl^+ are [extracted and] exchanged from a KCN-solution at a high pH. The order of decreasing affinity to DDC is Tl^{3+}; Bi^{3+}; Pb^{2+}; Tl^+. Consequently Tl^+ labeled with ^{204}Tl is used in chapter 6 and labeled Tl^{3+} in chapter 5.

This exchange-reaction offers a solution to the analysis of other trace-elements as well [12]). The advantage of DDC is, that it is universal to a large extent, and on the other hand easily transformed into a selective reagent. As a sequence of affinities between metals and DDC exists, this can be accomplished not only by (1) regulation of the pH and (2) addition of masking agents but also by (3)

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[12]) In contradistinction to the phosphate-method of chapter 4, *simultaneous* determination in the same — part of the — sample is impossible or is at least difficult to achieve.

the choice of the metal with which the exchange is going to take place.

2.4.4. *Other exchange-reactions of lead.*

The only R.E.-analysis of lead known in the literature is the method of Troitskii [1958] who exchanged lead-ions with $^{45}Ca^{2+}$-ions from the surface of $^{45}CaCO_3$ particles and obtained a sensitivity of 100 μgrams of lead.

The method of Suzuki [1959], who exchanged silver-ions with ^{110}Ag-dithizonate in carbontetrachloride, came to our attention after our own experiments were finished. A sensitivity of 0.1 μgram of Ag is reached by the author.

Other systems, not used in R.E.-analysis so far, but obviously adaptable to it with adequate separation-techniques, were mentioned already in chapter 1. Further exemples which seem to be especially suited for lead are the observed exchanges between:

- (1) Pb(II)-acetate and Pb(IV)-acetate – Hevesy (1920)
- (2) Th^{4+}-ions and Pb(II)-versenate – Flaschka et. al. (1957)
- (3) Pb^{2+}-ions and $CdSnJ_4$ – Feigl (1947)
- (4) Acetylacetonates – Martell and Calvin (1953)
- (5) Diethyldithiophosphates – Busev et. al. [1958 a and b]

2.4.5. *Conclusion.*

Exchange-reactions carried out on cation- and anion-echange columns were found to be unsuitable for R.E.-analysis. Probably the differences in the affinities of the respective ions to the column-material, and hence the reaction rates, were too low. Other anion-exchange columns than the one examined here — for instance columns loaded with chlorides — may be investigated however.

The exchange between a metal-ion in water and a metal-DDC complex in an organic solvent proved to be a suitable basis for R.E.-analysis and will be worked out for lead in chapters 5 and 6.

In addition, exchange-reactions with dithizonates and diethyldithiophosphates are considered to be of great value in R.E.-analysis.

CHAPTER 3.

DETERMINATION OF LEAD WITH PHOSPHATE-^{32}P, WITHOUT PRECEDING SEPARATIONS.

3.1. *Introduction.*

As indicated in chapter 2, the purpose of our investigations is to find a radiometric method for the determination of lead down to 0.01 or 0.001 μequivalents. This method should be applicable to a large variety of materials and preferably to the determination of other metals as well. The preliminary experiments on R.R. analysis described in chapter 2 led us to the choice of precipitation with phosphate-^{32}P in filter paper.

This reagent was used already by Sue (1941; 1946), Langer (1941), Möller et.al. (1948), Barcia Goyanes (1951), Tananaev (1955) and Mevel et.al. (1956). These authors could not obtain a sensitivity of 0.01 μequivalents however. Filter paper was not applied. It is an attractive medium for the reaction however, notwithstanding the paperblank which is to be expected (chapter 2). As the isotope ^{32}P emits hard β-rays (2.2 table 2), measurement of phosphate-^{32}P in filter paper does not involve any difficulty. Interference of other metals has to be prevented: either a preceding separation is necessary (chapter 4) or the problem has to be solved by a suitable choice of the pH and/or the addition of complexing agents.

3.2. *Experimental.*

3.2.1. *Radioactive chemicals.*

Phosphate-^{32}P. A batch of 0.5 mc.carrier-free ^{32}P as Na_2HPO_4 in water was diluted and carrier was added to prepare the following solutions:
(A) 40 μc. and 27 mgrams PO_4 in 100 ml. 1n.HNO_3.
(B) 400 μc. and 270 mgrams PO_4 in 100 mil. 1n.HNO_3.
^{210}Lead. A batch of 1 mc.^{210}Pb — Radium-D in equilibrium with its decay products — as $Pb(NO_3)_2$ in 2.5 n.HNO_3 was diluted and carrier was added to prepare a solution containing 4 bc. and 400 bgrams of lead per ml. 1n.HNO_3. The lead was measured — on the filterpaper — by means of the 1.17 MeV

β-particle emitted by its daughter: ^{210}Bi. A check was made after 5 days to control whether equilibrium betwee Pb and Bi was established.
$^{65}Zinc$. A batch of 0.5 mc. ^{65}Zn as $ZnCl_2$ in 1n.HCl was diluted and carrier was added to make a solution containing 100 μc. and 5 mgrams of zinc per ml. 1n.HNO_3.
Other solutions of radioactive metals were prepared in a similar way. All solutions were kept in polyethylene bottles, and their radioactivity was measured periodically, to control losses by adsorption to the wall.

3.2.2. *Other chemicals.*

Ion exchange water was used which had a resistivity of more than 3 to 4 Megohms. All solutions were kept in polyethylene bottles.
Hydrochloric acid — Analar — sp.gr. 1.18; *Nitric acid* — Analar — sp.gr. 1.42; *Hydrogene peroxyde* — Merck p.a. — 30%; *Ammonia* — Analar — sp.gr. 0.88; *Methanol* — Analar; *Ethanol* — Merck p.a.; *n-Propanol* — Merck p.a.
Oxine-solution — 5 grams of 8-Hydroxy-quinoline (Analar), were dissolved in 100 ml. of ethanol.
Alcoholic-Buffer-solutions (1) — *Sodium acetate 3 aq.* was added to 0.05n.HCl untill a pH of 4.4 was reaced. This solution was about 0.1n. in acetate. A mixture of 3 parts n-propanol, 3 parts ethanol and 4 parts of the buffer was prepared.
(2) — To 4 parts of a *borax-buffer* — 0.025 molair in borax and adjusted to pH 8.0 with HCl — 3 parts of ethanol and 3 parts of n-propanol were added and the mixture was readjusted to pH 8.0.

3.2.3. *Apparatus etc.*

The counting equipment was described in 2.2 as were the general precautions taken in all experimental work.
pH-values were measured with a Beckman model- G instrument, which was calibrated on aqueous standards. Ordinary *chromatografic sprayers* were used for the oxine-solution. The *papers* were either Whatman 1 or Schleicher and Schüll 2043 b.

3.2.4. *Pretreatment of the sample.*

The sample — or its ash — was dissolved in a minimum of nitric-acid or hydrochloric-acid. The amounts of these acids are to be kept small, as even the Analar-reagents contain some lead. Sulfuric- or phosphoric-acids are to be avoided completely, as they interfere with the reaction between lead and phosphate-^{32}P. Larger amounts of foreign metals interfere; up to a 10-fold excess (in gram-equivalents) of metals like Fe, Zn, Mn, Cu, etc., up to a 20-fold excess of Ca (Sr or Ba) and up to a 50-fold excess of Mg are allowed.

Excess phosphate-^{32}P was added to the solution; 0.25 ml of solution (B) was required per μequivalent of lead — a 13-fold excess — and in addition 0.05 ml of the same solution per μequivalent of foreign cations.

The transport of milliliter amounts of aqueous solutions to the paper is not practicle. Some methods have been devised, for instance bij Phillips (1956) and by French and Gibson (1957), but for a routine-method they are too cumbersome. Thus, the solution usually has to be *concentrated by evaporation.*

This was carried out in a polyethylene- or teflon-dish. As is shown in figure 3, the dish was held in a somewhat slanting position, to force the last drops to collect, thus facilitating the estimation of their volume. The dishes were placed under an infrared heater and supported by a hot plate; the latter was operated carefully however, to avoid splashing of the liquid.

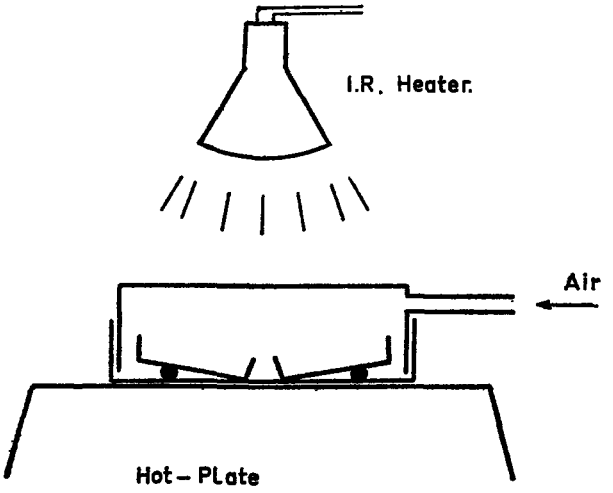

FIGURE 3:

Evaporation of the sample-solution in "teflon"-dishes placed in pyrex-glass Petri-dishes.
¼ of the real dimension.

When the sample contained less than 10 μgrams of lead, the dishes were placed between two pyrex-glass Petri-dishes through wich filtered air was led (see figure 3 and R. E. Thiers et. al. (1955)). Thus, pollution with airborne *traces of lead was avoided; above evaporation was promoted, especially* when the air was warmed up by sending it through a heating-spiral. When a dish run dry, the contents was taken up in 0.1 or 1n. hydrochloric acid; precipitates were also brought into solution with HCl. The final volume should be about 0.05 to 0.1 ml, the estimation of which is a matter of exercise.
Sometimes a small organic rest appeared in the residue. Small amounts of ionexchange material may occur for instance in the eluates of ionexchange columns (Raleigh (1958); Kitchener (1958)), traces of charred material may result from the ashing-procedure, etc. As these organic molesules

are inclined to adsorb appreciable amounts of lead, they had to be ashed completely. A mixture of equal volumes of concentrated nitric-acid and concentrated hydrogene-peroxyde proved to be suitable for this purpose, provided it was administered in small drops to avoid splashing caused by the sudden formation of a large volume of oxygen. The total volume of the mixture has to be kept small, as nitric acid contains some lead. The completion of this ashing is easily observed in the white teflon dishes.

3.2.5. *Transport to the paper and purification of the latter.*

It is not absolutely necessary to *produce a small spot* on the paper; as a somewhat lower blank and a quicker separation of the reagent would be the result, it was tried to solve this wellknown problem of paperchromatography (see Appendix 2). No simple solution was found however. Therefore *a larger spot was accepted and dipping of the paper into the solution was applied,* as being the most convenient method. It does not need any special arrangement and does not involve much time (in fact these spots could easily be produced with a pipet as well; this would involve the use of an additional instrument however).

The paper was cut into — or bought as — strips of 2.5 or 3.0 x 30 cm. and folded at 6 cm. from one end. The dish was held in its slanting position and the fold was dipped into the few drops of liquid assembled in the lower part, untill the spot was about 5 cm^2. This spot was dried and the paper was dipped again untill the total solution had been taken up. Mostly 2 dippings were sufficient. Finally the paper was dried and neutralised in ammonia-vapour (the pH remains 4 to 5).
The efficiency of the method was tested with ^{210}Pb; 0.01 ml. of the solution of 3.2.1 was added to the sample and inactive phosphate was used instead of the reagent; 97 to 99 percent of the lead was found on the paper as compared with the direct administration.

From the literature, and from our preliminary experiments with other radioactive reagents, it was clear that appreciable amounts of several *metals are contained in chromatographic paper.* Although the greater part of these metals certainly consists of Fe, Ca, Al, Mg and Cu, the use of a purified paper in the procedure given above proved to be of advantage in our experiments with amounts of lead smaller than 8 μgrams. Investigations on this purification are described in appendix 3. The procedure is given in 3.4.

3.2.6. *Separation from foreign cations and excess reagent.*

The masking-effect of *several complexing agents* on interfering cations was examined, *in combination with a pH-range of 3 to 10;*

the complexing agent was either sprayed on the paper or dissolved in the liquid used for the separation.

In principle water is suitable for the latter. *A pH of 4.4 was optimal for the separation from other cations* however (see table 3). Unfortunately about 0.3 ± 0.5 μgrams of lead were coeluted with an aqueous buffer of pH 4.4; at lower pH-values this phenomenon was still more pronounced.

Ebel et.al. (1951) used *alcoholic solvents* for the paperchromatographic separation of inorganic phosphorcompounds; a R_f value of 0.45 was found for instance for PO_4^{3-}-ions with the mixture: n-propanol[3]-ethanol[3]-water[4]. As it was to be expected that the solubility of lead-phosphate in an alcoholic mixture would be low compared with water, this solvent was used, with the buffer inserted for the aqueous part. Tests with 1 and 4 μgrams of lead — labelled with ^{210}Pb — revealed that only 0.04 to 0.09 μgrams were eluted now. A small part of the reagent was found in the original spot (see 3.5.1.) and in the intermediate part of the paper, but this blank was not larger than with purely aqueous solvents.

The *separation from [some] foreign cations* is shown in table 3. The percentages remaining on R_f 0.0 as the phosphates are given for alcoholic acetate-buffers of pH 4.4 to 6.4 and for an alcoholic borax-buffer of pH 8.0. In combination with this alcoholic buffers in some experiments a spray with 8-Hydroxyquinoline was used (see below).

Table 3

Percentages of several metals remaining at R_f 0.0 — as the phosphate-^{32}P — after separation of the reagent.

Buffers and Sprays / Ions	Borax [1]		Sodium-acetate [2]					
	pH 8.0	pH 8.0 oxine spray	pH 6.4	pH 6.0	pH 5.5	pH 5.0	pH 4.4	pH 4.4 oxine spray
Ca	100	100	23	23	3.3	2.0	0.7	<0.6
Mg	100	100	1.8	1.6	0.9	0.6	0.2	<0.2
Pb	100	100	100	100	100	100	100	100
Zn	100	<1	100	90	75	4	1.2	<1
Mn	100	<1	30	27	27	27	25	<1

[1] and [2] See descriptions in 3.2.2.

N.B. The values of 100 percent are approximately, and used here for simplicity.

It is clear that the effect of the spray on the phosphates of the heavy metals is more pronounced at higher pH values, but also that for Ca and Mag combination of the spray with a separation at pH 4.4 is superior to all other treatments. The latter procedure was chosen for general work. Application of the spray *before* the neutralisation with ammonia — in order to prevent the precipitation of foreign phosphates in advance — dit not have any additional effect. In fact it could be shown that the pH is only 4 to 5 after the „neutralisation", so precipitation was already largely prevented.

As compared with the pH-ranges given by Kolthoff and Sandell (1952) for the reaction with 8-Hydroxyquinoline in purely aqueous solutions (Ca > 9.2; Mg > 8.2; Pb > 8,4; Zn > 4.5; Mn > 5.9) the marked influence of the Oxine-spray on the reaction between phosphate and manganese at pH 4.4 is surprising (at least when the pH-values and the affinities in our alcoholic solutions are comparable with the values in water).

In our procedure the spray was applied to both sides of the paper. About 80 cm^2 can be sprayed with 1 ml. of the solution and 650 µgrams of the reagent are delivered per cm^2. This is equivalent to 150 µgrams of a 2-valent metal of atomic weight 50 and sufficient for the masking of 50 µgrams of the latter. Larger amount can be

Table 4

Influence of 50 µgrams of zinc on the determination of 4 µgrams of lead, in 5 cm^2 spots on acid-washed paper. Equivalents for 1 µgram of lead found [3] on R_f 0.0.

	1	2	3	4	5	6
	Procedure / Sample	Borax [4] pH 8.0; and Oxine-spray	Sodium-acetate [4] pH 5.5	pH 5.0	pH 4.4	pH 4.4 and Oxine-spray
1	4 µgrams of lead [1]	4.2	3.8	4.1	4.2	4.0
2	50 µgrams of zinc [2]	1.3	113.1	6.8	1.7	1.5
3	4 µgrams of lead [2]; 50 µgrams of zinc	5.2	113.9	10.3	5.7	5.4
4	Row 3 minus row 2: lead calculated	3.9	0.8	3.5	4.0	3.9

[1] Reagent: 27 µgrams of $^{32}PO_4^{3-}$.
[2] Reagent: 135 µgrams of $^{32}PO_4^{3-}$.
[3] The blank value is subtracted already (see 3.4.1).
[4] See 3.2.2 for the composition of these buffers.

complexed with more oxine but the uncertainty in the blank goes up in proportion.

The effectiveness of this treatment for zinc is shown for pH 8.0 in the second column of table 4. In the fifth and sixth columns it is shown that the oxine-treatment is practically superflous for the same element at pH 4.4.

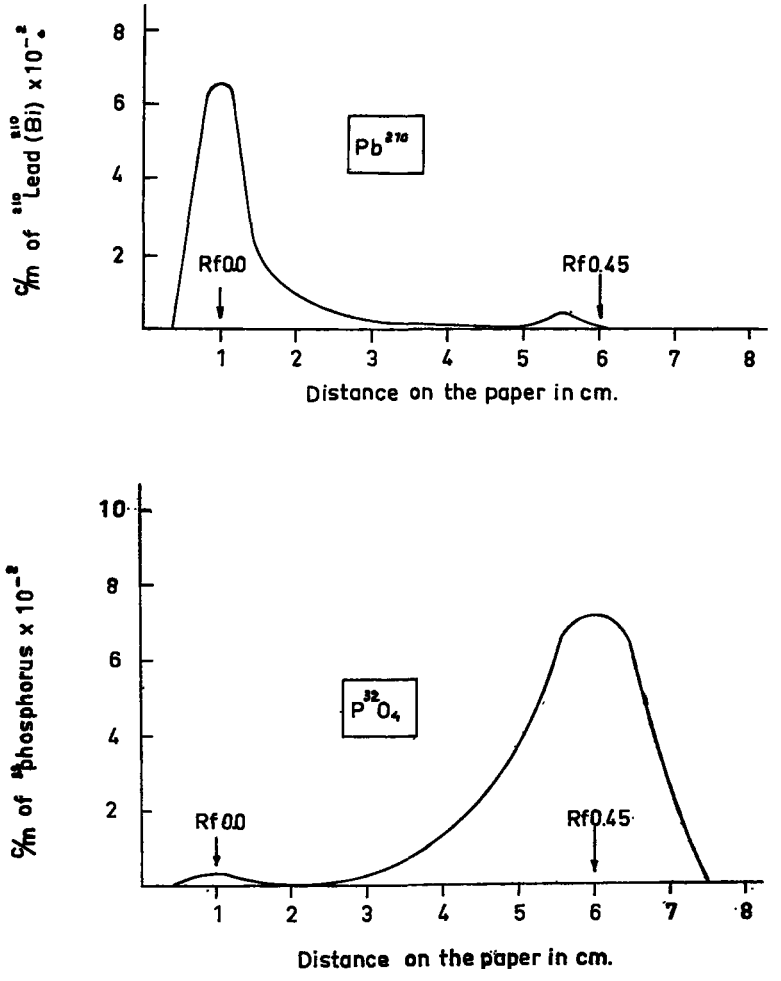

FIGURE 4:

Distribution of the ^{210}Pb- (^{210}Bi-) and ^{32}P-activities over the paper, after the precipitation of lead with $^{32}PO_4^{3-}$ (in the presence of excess zinc) and the elution of excess phosphate- ^{32}P with an alcoholic acetate-buffer op pH 4.4.

From the calculations of row 4 it is evident that a much higher excess of foreign metals is allowed than was indicated in 3.2.4., provided the amount of these metals it at least approximately known

To obtain the results of table 4 a higher excess reagent had to be added in the presence of 50 μgrams of zinc (see footnotes). Thus part of this metal is present as the phosphate. At pH 4.4 this phosphate is rather soluble and the complex-formation with oxine is small. At pH 8.0 the phosphate is insoluble (see table 3) but here the oxinate has a higher stability.

The *separation is visualised* — for the pH 4.4. buffer with the oxine spray — in figure 4^{a-b}, the isotopes ^{210}Pb, and ^{32}P were used respectively. Lead was found for the greater part on R_f 0.0; phosphate on R_f 0.45. Results with Fe (III) and with 2-valent Fe, Cu, Co, Mn, Ca and Mg were comparable to zinc. *Interference from* 2-valent UO_2, 3-valent *Cr, Bi and Ti,* and 4-valent *Zr, Th and Ce was found to be not prevented by this method however.*

3.2.7. *Interfering anions.*

Anions precipitating with lead at pH 4.4 may interfere. These are for instance: chromate, iodate, sulfate, fluoride, pyrophosphate and inactive phosphate itself. Three μgrams of the latter gave a 10 percent deviation when 0.1 ml. of phosphate-solution (A) containing 27 μgrams of phosphate-^{32}P were used as the reagent. The influence of pyrophosphate was comparable. Chromate was rapidly reduced on the paper to the Cr^{3+}-ion, which interfered. Results with iodate were variable; some iodine was formed on the paper — probably after a partly reduction to iodide — but lead-iodate was also precipitated. The influence of 1 μequivalent of sulfate was about equal to 0.2 μequivalents of phosphate. Fluoride *enlarged* the amount of phosphate-^{32}P by a factor of 2 when an amount equivalent to the amount of lead was added, more fluoride had no further influence. Probably a complex $Pb_xF_y\ (PO_4)_{2x}$ is formed.

There is no other remedy to these interferences as a carefull separation of the anions. Ion-exchange or paperchromatography (chapter 4) offer adequate solutions to this problem.

Only in exceptional cases the anion-contents of the reagents are a serious problem. When the nitric acid-peroxyde mixture is not required, less interfering anions than an equivalent of 1.3 μgrams of phosphate are introduced by the reagents; the error is thus smaller than 5 percent for the lowest range of lead, where 27 μgrams of phosphate are used as the reagent. When destruction of

organic residues with HNO_3-H_2O_2 is required (in 3.2.4.) however, an amount of interfering anions up to an equivalent of 10 μgrams of phosphate is introduced per ml. of the acid mixture. Thus, with the smaller amounts of lead, either more phosphate-reagent has to be used, which enhances the blank, or the addition of the HNO_3-H_2O_2 mixture has to be restricted to a few tenths of a ml. Alternatively a purer nitric-acid, which is the main source of the anions, has to be prepared, for instance by isothermic destillation following Irving and Cox (1958).

3.2.8. *Interference or complexing agents.*

Complexing agents — as for instance ethylenediaminetetra-acetic-acid, acetylacetone and diethyl-dithiocarbamate — may interfere with the formation of the lead-phosphate. When these, or other interfering complexing agents, are present in the original solution of the sample — or are introduced by some separation-technique — they should be removed previous to the transport of the sample-solution to the paper. For the three reagents mentioned above, the nitric-acid-peroxyde mixture, bromine water and mere acid evaporation are sufficient respectively.

3.3. *Measurement of the radioactivity of the lead-phosphate-^{32}P.*

After the separation of lead-phosphate from excess reagent and interfering metals, the paper is dried and cut off at R_f 0.30. The part which contains the leadphosphate is folded lenghtwise and sticked to a thick aluminium strip which is passed through the scanner. Thus folded the paper is narrower than the window by a factor of 2 and the geometrical error made by differences in the distribution of the activity is minimized. Less than 1 percent of the radiation from the underlaying paperhalf is absorbed. The total error due to both effects is smaller than 3 percent.

The slitwidth and the scanningspeed are regulated in accordance with the activity on the paper; usually a slit of 5 mm. and a paper-speed of 2 to 32 cm. per hour are sufficient to obtain a statistical error which is smaller than 3 percent. The total of all errors is about 4 and 3 percent for the smaller and larger amounts of lead respectively.

The resulting curve is either scissored out and weighed, or planimetered. After evaluation of the halflife correction, the amount of lead is found by comparison with standards.

With preparations of phosphate-^{32}P older than 1 or 2 months (2 to 4 half-lives) spots containing only a few μgrams of lead are

cut out directly, folded once or twice, sticked in a flat aluminium dish and passed through the sample changer. While in the scanning-procedure paperblanks are found automatically, here extra dishes containing these blank parts are to be inserted, preferably between the smallest samples.

3.4. *Procedure.*

The procedure is *given for a sample containing* at least 1 μgram of lead and — per μequivalent of this metal — not more than the following amounts of foreign substances — also in μequivalents —: heavy metals 10; calcium 20; magnesium 50; phosphate 1.5; pyrophosphate 1.5; sulfate 7.5; chromate 0.1; iodate 0.1 and fluoride 0.1. [1])

This sample is *solved* in HCl or/and HNO_3. *Phosphate-^{32}P is added:* 0.25 ml's of solution (B) (3.2.1.) are required per μequivalent of lead. The mixture is *evaporated* in a teflon-dish (figure 3) to 0.05—0.10 ml. The dishes A of figure 3 are only used for amounts of lead below 10 μgrams. When a dish runs dry or a precipitate occurs, 0.1—1.0 n.HCl is used to reestablish the solution. Small organic residues are carrefully ashed with HNO_3[1]—H_2O_2[1].

Strips of paper — 2.5 or 3.0 x 30 cm — are hung in a chromatografic jar and *rinsed* during 24 hours with methanol[9]-HCl[1] in the descending way. The eluate is removed and the jar is ventilated a few hours. Then the strips are run 24 hours with methanol[9]-NH_4OH[1], again ventilated, finally run 24 hours with methanol[95]-H_2O[5] and allowed to dry in the atmosfere of the last solvent. [2])

These strips are folded at 6 cm from one end and *dipped* into the sample-solution until the spot is about 5 cm_2. They are *dried* while hanging on a glass-frame in a clean atmosfere and *dipped* again until the total solution is taken up. After a final *drying* (during 60 minutes) they are *neutralised* in the vapor of concentrated

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[1]) When a larger — but approximately known — amount of foreign metals is present in the sample, an additional amount of phosphate-^{32}P is added: 0.05 ml of solution (B) per μequivalent. A "blank" is dertermined by an experiment with a simulated sample containing no lead. In this way up to a 50-, 100-, and 250-fold excess of heavy metals, Ca and Mg respectively — but in total not more than 2 μequivalents can be handled.

[2]) The second solvent reestablishes the pH and the third one the watercontent of the paper. Several paperchromatografic separations with these papers proved to give essentially the same results as with untreated paper.
For amounts of lead larger than 8 μgrams non-washed papers may be used.

ammonia during 30 minutes (pH 4 to 5) and *ventilated* for 15 minutes. Each spot is then sprayed with about 0.1 ml. of the *oxine solution* (3.2.2.) and dried.

The glass-frame is put in a glass-jar and the *spots are chromatographed* directly in the ascending way with the alcoholic buffer-solution of pH 4.4. (3.2.2.).

After 16 hours the papers are taken out of the jar and dried.

The remaining lead-phosphate is then *measured* as described in 3.3.

3.5. *Results.*

3.5.1. *Blank value.*

Some blanks, found with different sizes of the spot and different amount of phosphate-^{32}P, are shown in table 5.

Table 5.

Blank values in μgrams of lead
(Mean values and standard-deviations of 8 experiments each).

Size of the spot and amount of $^{32}PO_4^{3-}$† / Papers	1 cm²	5 cm²		
	0.01	0.01	0.05	0.15
Acid-washed	0.3 ± 0.04	1.3 ± 0.08	2.1 ± 0.14	—
Non-washed	—	—	3.0 ± 0.9	4.6 ± 0.9

† In ml's of solution (B) of 3.2.1.

This table shows that the blank goes up with (1) the surface of the spot and (2) the amount of the reagent, for both factors not in equal ratio to their increase however. Non-washed papers cannot be used below 8 μgrams of lead. A still higher blank is found when (3) the nitric-acid peroxyde mixture is used to wet-ash organic residues: an equivalents of 0.5 μgrams of lead has to be added per ml. of the mixture (usually only 0.1 to 0.5 ml. of the latter is required). Another reason for a higher blank is furnished (4) by the presence of appreciable amounts of foreign metals in the sample (see 3.2.6.).

3.5.2. *Table and Graph for 1 to 16 μgrams of lead.*

Table 6 and curve 1 of figure 5 represent the results for 1 to 16 μgrams of lead. Acid-washed papers were used throughout these experiments; the results for 8, 12 and 16 μgrams of lead obtained with ordinarily paper were comparable however, only the standard-deviations were higher: 13, 10 and 9 percent respectively.

For the range of 1 to 4 μgrams of lead 0.1 ml. of solution (A) was used as the reagent and for the higher range 0.05 ml. of solution (B). As both solutions were 2-months — or 4 half-lives — old, the lower range had to be measured with the sample-changer to obtain a 4 percent error.

Table 6

Determination of 1 to 16 μgrams of lead with phosphate-^{32}P.

1	2	3	4	5	6	7	8	9
Nr.	Lead added in μgrams	Phosphate-^{32}P in c/m; corrected for the blank	Mean of column 3	Standard deviation in c/m	Lead •• found in μgrams	Standard deviation in μgrams	Error in percent	Standard deviation in %
1	1	133						
2	1	174	155	21	1.04	0.15	+ 4	15
3	1	168						
4	1	144						
5	2	271						
6	2	337	300	32	2.07	0.22	+ 3[5]	11
7	2	316						
8	2	277						
9	4	591						
10	4	539	600	45	4.20	0.31	+ 5	8
11	4	634						
12	4	636						
13	8	1102						
14	8	1254	1190	80	8.3	0.6	+ 4	7
15	8	1267						
16	8	1137						
17	12	1844						
18	12	1764	1740	93	12.2	0.7	+ 2	6
19	12	1620						
20	12	1732						
21	16	2151						
22	16	2419	2293	113	16.0 ••	0.8	0	5
23	16	2266						
24	16	2338						

•• Calculated from the specific activity of the phosphate-^{32}P and with a P/Pb ratio of 1.10 (see 3.6.1).

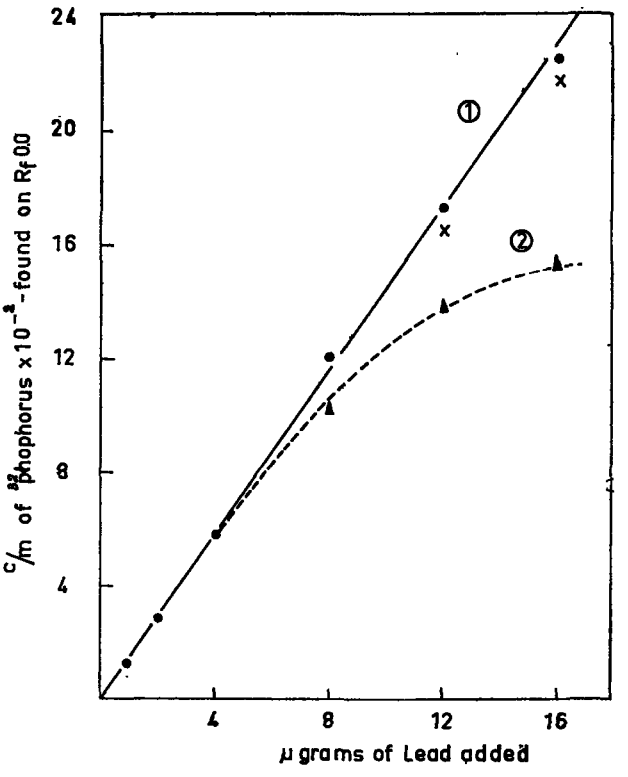

FIGURE 5:

Determination of 1 to 16 µgrams of lead with $^{32}PO_4^{3-}$. Mean values of 4 experiments are given.
Curve 1: See procedure in 3.4.
Curve 2: 50µgrams of zinc and 0.05 ml. of phosphate-B added.
X : 50 µgrams of zinc and 0.15 ml. of phosphate-B added.

3.5.3. *Interferences.*

Curve 2 of figure 5 represents the values obtained for (acid-washed paper and) 4 to 16 µgrams of lead, when 50 µgrams of zinc were added without any rise in the amount of the reagent (see 3.5.2.), but with the extra blank of table 4 — 1.5 µgrams of lead — substracted.

This small excess reagent is evidently just sufficient for 4 µgrams of lead (compare table 4) but insufficient for larger amounts. With a 3-fold increase of the reagent — to become a 50-fold excess over the lead and a 4.5-fold over both ions together — the values found

in 3.5.2. for 12 and 16 μgrams are again approached: see the crossindicated points in figure 5.

3.6. *Discussion.*

3.6.1. *Chemical form of the precipitate.*

The ratio P/Pb can be calculated from the specific activity of the phosphate. As a mean value of 10 experiments with 16 μgrams of lead, this ratio was found to be 1.10 ± 0.06 (St.D.). With smaller amounts of lead similar results were obtained. The mean ratio P/Pb for all experiments of table 6 was evidently 1.125.

As the blank is substracted already, it is likely that a lead-phosphate containing equal amounts of lead and phosphate — probably $PbHPO_4$ [4]) — is precipitated and that an extra 10 or 12.5 percent phosphate is adsorbed on or occluded in this precipitate.

Comparison with table 6 shows that: (1) for 4 to 16 μgrams of lead the standard-deviation is mainly due to this factor, but (2) for 1 to 2 μgrams of lead one or more other factors must contribute to the total standard-deviation.

Subsequent repetition of the separation, was found to be favourable; the mean ratio found in the 10 experiments with 16 μgrams of lead was lowered to 1.06 ± 0.035 for instance.

3.6.2. *Blank value.*

The blank value of 5 cm^2 spots on acid-washed paper — 1.3 μgrams of lead — has a standard-deviation of 0.08 and thus contributes only to the variation in the determinations of 1 and 2 μgrams of lead. When non-washed paper is used the blank has a standard-deviation of 0.9 μgrams and contributes to the standard-deviation of even 16 μgrams of lead (see table 6).

It can be shown that the greater part of the blank of acid-washed paper is due to the adsorption of $^{32}PO_4^{3-}$-ions to the paper (see appendix 4).

Subsequent repetition of the separation of excess phosphate-^{32}P was found to be favourable for a smaller and more constant blank. Alternatively, papers made from polyethylene fibers, or papers in

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[4]) Hubicki et. al. (1950) found the solubility-product of $PbHPO_4$ to be minimal at pH 4.5; the addition of alcohol decreased the solubility. $Pb_3(PO_4)_2$ was only precipitated at pH-values above 6.

which the OH-groups are changed into OCH_3-groups could be used, when amounts of lead smaller than 1 μgram are to be determined.

3.6.3. *Loss of lead.*

A negligable contribution to the standard-deviation is made by the variations in the amount of lead lost in the separation process (3.2.6.). When an aqueous buffer had been used this influence would have contributed even to the standard-deviation of the highest amounts of lead determined. From the figures of 3.2.6. it is probable that the solubility of lead-phosphate at pH 4.5 is diminished by a factor of 5 through the addition of alcohols up to 60 volume percent.

In 3.6.1. it was shown that the standard-deviation of the ratio P/Pb is diminished by repetition of the separation 3.2.6. Experiments with 1 μgram of lead — labelled with ^{210}Pb — indicated a loss of 0.05 to 0.12 μgrams of lead through this repetition. Consequently its influence is not favourable for the determination of 1 μgram of lead.

3.6.4. *Total standard-deviation and sensitivity-limit.*

As the error made by the measurement of the radio-activity is below 4 percent, it can be stated that the greater part of the standard-deviation is due to variations in the factor P/Pb. Only for the determination of 1 and 2 μgrams of lead — and also for higher amounts and non-washed paper — the variation of the blank is important.

3.6.5. *Foreign Ions.*

The interference of foreign *anions* precipitating with lead at pH 4.4 is the main drawback of the method. When these ions are present they should be removed in advance, for instance by the technique of chapter 4.

Interference from the *cations* present in biological ashes is prevented to a large extent by the choice of the pH and the application of the oxine-spray. For biological materials containing much calcium the technique of chapter 4 is to be preferred however.

Interference from Zr^{4+}, Th^{4+}, Ce^{4+}, Cr^{3+}, Bi^{3+}, Ti^{3+} and UO_2^{2+}could not be prevented in this way. If the figures for purely

aqueous solutions given by Kolthoff and Sandell (1952) may be applied it can be concluded that Zr^{4+}, Th^{4+}, Bi^{3+}, Ti^{3+} and UO_2^{2+}-oxinates are formed at pH 4.4. Our results show that the complex-strengh of the phosphates is higher however. For samples containing appreciable amounts of these ions either the technique of chapter 4 or other masking agents should be applied.

It is clear that the phosphates of the „masked" cations have formed to a considerable extent. Apart from the small amount retained at R_f 0.0, considerably larger amounts must exist in solution, otherwise the excess reagent need not be enhanced in 3.5.3. On the other hand, as the excess reagent need not be enlarged to a 13-fold over the lead and the zinc together, a third portion of the foreign metal is evidently complexed by the oxine sprayed on the paper.

3.7. *Conclusion:* with the restrictions indicated above the method has proved to be successfull for the analysis of 0.0.1 μequivalent of lead. This is about a factor of 40 below the smalles amount of any metal ever determined with phosphate-^{32}P and is evidently due to the application of filter-paper as a medium for the reaction.

CHAPTER 4

QUANTITATIVE PAPERCHROMATOGRAPHY OF LEAD WITH PHOSPHATE-^{32}P

4.1. *Introduction*

The interference of foreign ions in the determination of lead with phosphate-^{32}P can also be prevented by the separation of lead previous to the application of the radioactive reagent. For this separation paperchromatography will be used here. [1])

As the oxine spray (3.4) can be omitted, the method has the distinct advantage that other elements in the sample can be analysed with the same or with other reagents in one procedure. [2])

4.2. *Experimental*

Only those details are described, which differ from chapter 3. Procedures are indicated for two cases: (1) in which only lead is of interest, and (2) in which other ions are of interest as well. Only case (1) is worked out in detail however.

4.2.1. *Materials*

Borax-oxalate-buffer: 60 ml. of ethanol, 60 ml. of n-propanol, 20 ml. of water, 40 ml. of 0.05 m. borax and 20 ml. of 0.05 m. oxalic acid were mixed and adjusted to pH 8.0 with the borax-solution. (The tartrate- and citrate-buffers of 4.2.5. were prepared in an analogous way.)
Phosphate-^{32}P — (a) and (b) —: A 1 percent Na_2HPO_4 solution in water, containing 10 μcuries $^{32}PO_4^{3-}$ per ml., was used for the range of 4 to 16 μgrams Pb (a) and was diluted four times for 1 to 4 μgrams Pb (b).
Ordinary dryboxes were used for the radioactive spray. The counting equipment has been described in 2.2.

4.2.2. *Pretreatment of the sample*

The technique of 3.4. was used; the addition of the radioactive

[1]) The *ion-exchange* separation of lead from the trace elements Fe, Cu, Zn, Mn, Mo and Co, from other metals like Na, K, Ca, Mag, etc. and from anions like SO_4^{2-}, PO_4^{3-} etc. will be described elsewhere.
[2]) Preliminary experiments indicated that submicrogram amounts of for instance Fe, Zn, and Mn, can be determined with phosphate-^{32}P.

reagent was omitted however. The sample should contain at least 1 μgram of lead. Foreign cations and anions are allowed in amounts up to a few hundred micrograms. Of the cations Ca^{2+}, Sr^{2+} and Ba^{2+} not more than a 250-, 70- and 20-fold excess (in μequivalents) respectively should be present however.

4.2.3. *Transport to the paper*

(1) When only lead was to be determined the sample was placed on a strip of paper (3.4). For less than 8 micrograms of lead acid-washed strips were used (3.4.). The neutralisation of the spot and the oxine-spray were omitted and the ions were separated as described in the next paragraph.

(2) When other cations were to be determined as well and the corresponding spots were separated less than 4 cm., a sheet of paper — 20 x 20 cm. — was used instead of the paperstrips. For amounts of lead smaller than 8 μgrams these sheets were acid-washed. The dipping-technique was somewhat more difficult here; satisfactory results were obtained however, when the paper was folded twice (figure 6) and the tip was immersed in the intersection of the two folds. When the solution was placed on the paper with a pipet, a more rectangular spot was formed, (figure 7) which had some advantage in connection with the subsequent chromatografic separation. The spot was not neutralised or sprayed; it was chromatographed in direction I as described in the next paragraph.

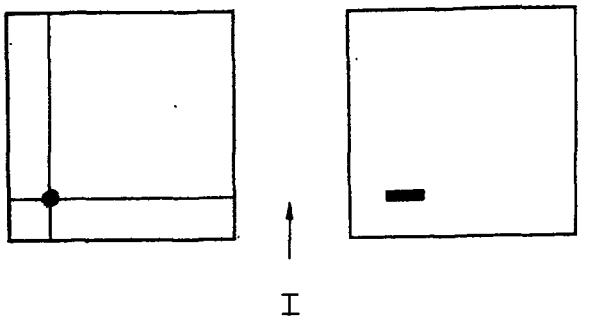

FIGURE 6 AND 7

Application of an aqueons solution to a papersheet. For the procedure: see text.

(3 When other cations were to be determined as well but the corresponding spots were separated 4 cm. or more, the paper*strips* were applied.

4.2.4. *Paperchromatographic separation of lead*

(1) When lead is the only element to be determined, the only requirement for the solvent used for the development of the paper-

gram is that it should separate lead from interfering cations and anions (see 3.2.7.). The choice of this solvent depends on the composition of the sample. Directions are given here for the ash of 50 miligrams of dry cow liver, which contains at least 1 μgram of lead.

For this sample the alcoholic solvents used by Lederer (1951) Walker and Lederer (1951), Burstall et.al. (1950) and Hunt et.al. (1954) were found to be the most convenient. These authors were able to separate Pb^{2+} from: Fe^{3+}, Zn^{2+}, Cu^{2+}, Mn^{2+}, Co^{2+}, Tl^{3+}, Tl^{+}, Hg^{2+}, Zr^{4+}, Ag^{+}, Al^{3+}, Mg^{2+}, Ni^{2+}, Sn^{2+}, Bi^{3+}, Cd^{2+} and MoO_4^{2-}, but not from Ca^{2+}, Sr^{2+}, Ba^{2+}, Ce^{3+}, Th^{4+}, the rare-earths and Hg^{+}.

In our own experiments the cations Fe^{3+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Tl^{3+}, Tl^{+}, Hg^{+}, Hg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Al^{3+} and Mg^{2+} were tested, as well as the anions PO_4^{3-}, MoO_4^{2-} and SO_4^{2-}. Paperstrips with these ions, in amounts ranging from 1 to 200 μgrams, were chromatographed in the *ascending way with the solvent ethanol*[9] *— 5 n HCl*[1] (see 4.4.). [3])

The radioisotopes ^{59}Fe, ^{65}Zn, ^{54}Mn, ^{80}Co, ^{204}Tl, ^{203}Hg, ^{89}Sr, ^{32}P, ^{35}S and ^{210}Pb were used to detect the respective ions. The ions Cu^{2+}, Ca^{2+}, Ba^{2+}, Mg^{2+}, Al^{3+} and MoO_4^{2-} were detected with the tests described by Pollard and McOmie (1953).

The main part of the lead was found at R_f 0.15 [with 1 to 3 μgrams of lead the size of the spot was about 1 cm^2] but 0.09 ± 0.03 μgrams of lead were eluted and found at higher R_f-values.

Ca^{2+}, Sr^{2+}, Ba^{2+}, Hg^{+} and Tl^{+} could not be separated from lead. The last two ions can easily be oxidised before the chromatografic separation and then do not interfere anymore; thus *only the ions Ca +, Sr + and Ba + interfere seriously. For biological material only calcium is of much importance.*

(2) If one is interested in other cations as well, the separation should be carried out (on the papersheet of 4.2.3.) by another solvent, as the alcoholic solvent used above will in general not produce mutual separations of these other cations. The choice of the solvent depends on the ions of interest. The separation of the trace-elements Fe, Cu, Zn, Mn, Pb, Mo and Co by paperchromatography will be described elsewhere.

With most solvents, the *earthalcali-ions* are separated from lead and need not be considered anymore. On the other hand, with none of these solvents the separation of lead from large amounts of other *heavy metals* is as good as with the alcoholic-HCl used in case 1.

(3) When other ions are of interest, but the distance between their different spots is larger than 4 cm., paperstrips can be used.

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3) Methanol[95]-HCl[5] is another satisfactory solvent.

4.2.5. *Reaction with phosphate-^{32}P. Separation from excess reagent and from earthalcali-phosphates*

(1) After the separation of Pb^{2+} from anions and from (most) other cations, the paper was dried, neutralised in ammonia-vapour (3.4.) and *sprayed* — on both sides — *with one of the phosphate-^{32}P solutions* of 4.2.1., the choice depending on the amount of lead to be expected.

Spraying was carried out in a drybox or in an efficient hood; it did not involve any danger to the operator. The walls of the drybox or the hood were covered with absorbing paper, which was removed daily or twice a week. The paper could not be sprayed too heavily, otherwise the solvent dripped off and the spots spread out. (It is also possible to supply the reagent with a pipet or a small paint brush; these methods give somenwhat less satisfactory results however.)

After the strip had been dried from the spraying, *the separating solvent* (see below) *was allowed to ascend in the same direction* in which the papergram was developed. (When there was interest to detect some of the cations between R_f 0.3 and R_f 1.0, the strip was cut off at R_f 0.3 and the separating solvent was applied in the *descending* way and allowed to drip off at the point R_f 0.3.)

The solvent used in chapter 3 for the separation of excess reagent — an alcoholic HCl-acetate buffer of pH 4.4. — could be used here with advantage only when the amounts of earthalcali-ions were not too large.

As was shown already in chapter 3 table 2, about 0.7 percent of the calcium was retained as the phosphate in the original spot. Results with barium and strontium were similar. When an oxinespray was inserted, less than 0.6 percent of Ca, Sr or Ba were found in the original spot after the separation with this buffer.

In many samples the excess of Ca, Sr or Ba over Pb is too large for this buffer however; in biomaterials much calcium is to be expected. For these samples another solvent was used, consisting of an alcoholic borax-oxalate buffer of pH 8.0. (Citrate or tartrate instead of oxalate were not effective; probably insoluble earthalcali-oxalates were formed.)

About 10 percent (St. dev. 1.5 %) of the leadphosphate was also transformed into the oxalate. Results with Sr and Ba were slightly inferior to those with Ca. In table 7 the percentages of the three earthalcali-metals found as the phosphates in the original spot and the ultimate amounts permitted bside 1 μgram of lead — for 10 % error — are given.

Table 7

Interference of Ca, Sr and Ba.
*Percentages found — as the phosphates — in the original spot (%) and maximum permissable amounts beside 1 µgram of lead * (M.A.) for three different treatments.*

Solvent and Treatment / Element	Sodium-acetate buffer, pH 4.4		Sodium-acetate buffer, pH 4.4 with Oxine-spray		Borax-oxalate buffer, pH 8.0	
	%	M.A.	%	M.A. †	% †	M.A. †
Ca	0.7	3	< 0.6	5	0.04	50
Sr	0.7	6	< 0.6	10	0.13	30
Ba	0.7	9	< 0.6	15	0.44	15

* For 10 percent deviation. When the amounts of Ca, Sr and Ba were approximately *known*, a larger excess was permitted (see note in 4.4).
† Approximate values.

(2) When more metals were to be determined *and* the successive spots were separated less than 4 cm., sheets of paper were used for the papergram (see 4.2.2.). After drying and neutralisation of the papergram on the 20 x 20 cm.-sheet, the hatched part (figure 8) was sprayed wits the phosphat-^{32}P, the rest being masked with a screen. In this case the buffer — used for the separation of excess reagent — had a direction (II) perpendicular to that (I) of the solvent used for the paperchromatography. After drying, the hatched part was cut out and measured.

The composition of the buffer depended on the chromatogram and on the ions of interest. When for instance Pb, Mg, Zn and Mn were to be determined, the borax-oxalate buffer of 4.2.1. was used; when Pb, Ca and Mg were to be analysed, an oxine-spray was inserted and the borax buffer of 3.2.2. was applied. This general buffer was indeed suitable for many metals — when the oxine-spray was omitted — as was shown in 3.2.6. (column 1 of table 3).

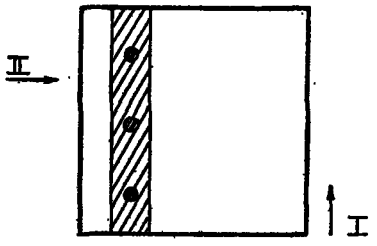

FIGURE 8:

Spraying of a papergram with phosphate-^{32}P. Separation of the excess phosphate-^{32}P by elution in direction II perpendicular to the direction (I) in which the papergram was developed.

(3) When the spots on the paperstrip were separated more than 4 cm., the paper was cut into pieces containing the different metals. To the successive pieces of the strip, the technique described above was applied; different solvents were used for the separate parts. As the pieces of paper were rather short for a satisfactory separation of metal-phosphates and reagent, the descending technique was used and the solvent was allowed to drip off.

4.3. *Measurement of the radioactivity of the lead-phosphate-^{32}P*

In case (1) the procedure was essentially the same as in 3.4. In case (2) the hatched part of the paper (figure 8) was cut out, folded lengthwise when more than 1.5 cm. wide and sticked together and to the aluminium strip to be scanned.

4.4. *Procedure (for case 1).*

The procedure is described for a sample containing from 1 to 4 μgrams of lead and — per μequivalent of lead — not more than the following amounts of foreign metals — in μequivalents —: 250 Ca, 70 Sr, 20 Ba [6]), 0.1 Hg (I), 0.1 Tl (I) [7], 0.1 Ce (III) and 0.1 Th (IV) [8]). In total less than 1 mgram of inorganic material should be present.

This sample is dissolved in HCl or/and HNO_3 and evaporated to 0.05—0.1 ml. (3.4.).

Strips of paper — 2.5 or 3.0 x 30 cm. — are acid-washed (3.4.), folded at 6 cm. from one end and dipped in the solution untill the spot is about 5 cm^2. [9]) The strips are dried while hanging on a glass-frame in a clean atmosphere and dipped again untill the total solution is taken up.

The strips are then dried for 60 minutes, conditioned in the vapour of ethanol[9]-5 n.HCl[1] for 16 hours and chromatographed with this solvent in the ascending way during 4 hours (about 20 cm.).

The strips are again dried for 60 minutes, neutralised in the vapour of concentrated ammonia for 30 minutes and ventilated during 15 minutes.

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[6]) With up to 4 times larger — but approximately *known* — amounts of Ca, Sr and Ba a "blank", determined with a simulated sample containing no lead, can be substracted. Alternatively another solvent should be sought for chromatography.

[7]) The interference of Hg (I) and Tl (I) is easily prevented by oxidation to Hg (II) and Tl (III).

[8]) With larger amounts of Ce (III) and Th (IV) another solvent should be sought for chromatography.

[9]) With amounts of lead larger than 8 μgrams non-washed papers can be used.

The region of R_f 0.1 to 0.2 is now sprayed with the $Na_2H^{32}PO_4$-solution (b) (4.2.1.) in a dry-box the inside of which is covered with absorbing paper. Spraying is performed on both sides and to thorough dampness; about 1 ml. is required per 80 cm^2. [10])

The strips are again dried and the borax-oxalate buffer (4.2.1.) is allowed to ascend in the direction of the chromatogram.

Finally the strips are dried and the region of R_f 0.1 to 0.2 is measured (3.3.).

4.5. *Results*

4.5.1. *Blank value and interferences*

The blank amounts to 0.51 μgrams of lead (St.dev. 0.06) per cm^2 of acid-washed paper. The influences of (1) the size of the spot, (2) the amount of the phosphate-^{23}P, (3) the HNO_3—H_2O_2 mixture, etc. are the same as in chapter 3. Hundred micrograms of the ions Fe^{3+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, MoO_4^{2-}, Co^{2+}, Al^{3+}, Hg^{2+}, Tl^{3+} and Mg^{2+} have no influence on the blank. The same amounts of Ca^{2+}, Sr^{2+} and Ba^{2+} produces a blank corresponding to 0.2, 0.3 and 0.7 μgrams of lead respectively (St. dev.s 0.05, 0.08 and 0.17).

4.5.2. *Examples of papergrams*

In table 8 some examples are given of the determination of 1 μgram of lead. The amount of lead is calculated from the ^{32}P-value found — in a spot of 1 cm^2 — in the absence of foreign ions and hence by omission of the paperchromatografic separation. Thus a correction for the loss of 0.09 μgrams of lead in this separation (4.2.4.) had to be made. On the other hand only the *variations* found in the loss of lead-phosphate (in 4.2.5.) had an influence while a correction for the 10 percent loss itself was not required.

Results with larger amounts of lead showed smaller standard-deviations — comparable with chapter 3 table 6 — while a larger excess of foreign ions was allowed. As the latter tend to disturb the papergram by overloading, these results are not very interesting: an aliquot of the sample containing at least 1 μgram of lead should be taken.

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[10]) Solution (a) is used for 4 to 16 μgrams of lead.

Table 8

Determination of lead — with procedure 4.4. — in samples containing 1 µgram of this element and additional foreign ions.

Experiment	Foreign ions in µgrams	Substracted blank (in µgrams) of Pb) *	Lead found (in µgrams) Triplicate	Mean value	St. Dev. in percent	Mean value (corrected)**
1	Ca 100 Sr 20 Ba 20	0.91	0.90–1.10–1.10	1.03	11.5	1.12
2	Fe 300 Cu 100 Zn 200	0.51	0.99–0.80–0.88	0.89	9.5	0.98
3	Na 150 Mn 1 K 400 Fe 15 Ca 15 Cu 5 † Mg 10 Zn 10 Mo 1 Co 0.1	0.54	0.93–0.85–1.07	0.95	11.0	1.04
4	As in the preceding experiment † Above: PO_4 30 SO_4 20	0.54	0.98–0.80–0.94	0.91	9.5	1.00
5	PO_4 100 SO_4 100	0.51	0.95–0.99–0.83	0.92	8.5	1.01

† Cations (and in experiment 4: 2 percent of the anions) in the ash of 50 milligrams of dry cow liver, which contains in general more than 2 µgrams of lead.

* From the figures of 4.5.1 an extra blank, equivalent to 0.40 ± 0.06 (St. Dev.) µgrams of lead, can be calculated for experiment 1, and an extra of 0.03 µgrams of lead for experiments 3 and 4.

** For the loss of 0.09 µgrams of lead in the paperchromatographic separation of 4.2.4.

4.6. *Discussion*

Comparison with chapter 3 shows the advantages of the method described here.

(1) To a certain extent procedure 3.4. can be made selective for other ions [11]), but combination with paperchromatography (4.4.) certainly makes the method more universal.

(2) In the analysis of lead, interference from other cations and from anions is prevented to a much larger extent.

As to the latter: more than 98 percent of the anions occurring for instance in a sample of dry cow liver, can easily be separated from lead with a cation-exchange column. Table 8 shows, that the

[11]) A mixture of complexing agents for the selective determination of manganese was developed for instance.

remaining amounts of these anions — and even 3- to 5-times larger amounts — do not have any influence on the determination of 1 μgram of lead. Larger amounts of anions tend to overload the paper.

As to the foreign cations: with the paper and the chromatografic separation applied here, most cations are allowed in amount up to 100 μgrams — or more — beside 1 μgram of lead. [12])

This means a 400-fold excess in μequivalents as compared with a 10-fold excess in chapter 3.

The *masking effect of the oxalate-buffer* is obviously due to precipitation of the earthalcali-oxalates. When the phosphate-^{32}P is sprayed on the "neutralised" papergram, lead-phosphate will precipitate but the pH of 4 to 5 (3.4.) prevents precipitations of the earthalcali-phosphates to a large extent (chapter 3 table 4). Separation of excess reagent with the borax-oxalate buffer of pH 8.0 will cause precipitation of Ca, Sr and Ba, partly as the phosphates but for the greater part as the oxalates. As the solubility-products of the latter are smaller — for Ca by a factor of 2500 — and as the concentration of the oxalate-ion increases rapidly whereas the concentration of the phosphate-ion decreases during the separation-process, the greater part of the earthalcali-phosphates will finally be transformed into the oxalates. Owing to the change in the concentrations of the two anions, part of the lead-phosphate will also be transformed into the oxalate. The solubility-products of both lead-salts are comparable however; above, the process — which will undoubtedly be further modified by the paper and by the partly alcoholic medium — starts with totally *precipitated* lead-ions.

In contradiction to chapter 3 *the experimental standard-deviations can be totally understood by the factors known to contribute to them:* (1) 0.015 μgrams are due to variations in the transformation of lead-phosphate described above, (2) 0.03 μgrams are the consequence of variations in the amount of lead lost in the paperchromatografic separation, (3) 0.06 μgrams are due to the variable paperblank, (4) 0.04 μgrams are due to the error made in the measurement of ^{32}P and (5) 0.06 μgrams are caused by the adsorption of the reagent on — or occlusion in — the precipitate (see 3.6.1.). In experiment 1 a factor (6) of 0.06 μgrams due to

[12]) With thicker papers — f.i. what man nr. 3 m.m. — larger samples can be handled.

variations in the Ca-, Sr-, and Ba-blanks has to be added to this list.

The factors (3), (4) and (5) were also valid in chapter 3, factor (3) even to a somewhat larger extent owing to the larger spot. The other factors are new contributions, of which only factor (6) is of considerable importance.

4.7. *Conclusion:* combination with paperchromatography makes the determination of lead with phosphate-^{32}P less sentitive to interferences; in addition the method becomes applicable to many other cations as well.

CHAPTER 5.

DETERMINATION OF LEAD WITH DIETHYLDITHIO-CARBAMATE AND $^{204}Tl^{3+}$-IONS

5.1. *Introduction.*

In chapter 2 it was pointed out, that the system: M^{+}_{1} + $^{*}M_2$-D.D.C. $\rightleftarrows$ $^{*}M^{+}_{2}$ + M_1-D.D.C. is of general applicability in R.E.-analysis, but on the other hand can be made selective by a proper choice of pH and masking-agents. *A sequence of affinities of the different ions to D.D.C. exists, which makes another selective factor available.*

Experiments on this sequence (Hg^{2+}, Ag^{+}, Cu^{2+}, Tl^{3+}, Ni^{2+}, Bi^{3+}, Pb^{2+}, Co^{3+}, Cd^{2+}, Tl^{+}, Zn^{2+}, Sb^{3+}, Fe^{3+}, Mn^{3+}) and on the rates of exchange were carried out by Wickbold (1956), Bode (1957) and Eckert (1957), *colourreactions* being the base of their quantitative conclusions. Sedivec and Vasak (1951; 1952; 1957), Pribil et.al. (1952a; 1952b; 1953), Martens and Githens (1952), Kovarik and Vins (1955) and Deszö and Fülöp (1959) used the same principles for the quantitative determination of metals. Sedivec and Vasak (1952) showed that lead can be determined by the exchange of Pb-$(D.D.C.)_2$ with copper-ions and a subsequent measurement of the *colour* of the Cu-$(D.D.C.)_2$ complex. This methode is not selective for lead however, and in addition has a restricted sensitivity.

Tracer techniques have only scarcely been used in this system. (Johnson and Hall (1948) studied the exchange of labelled nickel-ions with some nickel-dialkyldithiocarbamates.) *Radioisotopes enable the estimation of the exchanged quantities of metals to much lower concentrations* than is possible with a spectrophotometric method. In *addition, colourless complexes can be meausured as well.* Tracers can be used in several ways. In this work the exchange of a radioactive metal-ion with the D.D.C.-complex of lead and the exchange of lead-ions with the D.D.C.-complex of a radioactive metal will be studied (in this chapter and in chapter 6 respectively).

To make the method selective, a high alcalinity and masking with KCN are applied.

5.2. *Preliminary experiments with $^{203}Hg^{2+}$-ions.*

As Hg^{2+} has the highest affinity to D.D.C., this ion was chosen for our first experiments. Pb-(D.D.C.)$_2$ was extracted with CCl_4 from a KCN solution and exchange with $^{203}Hg^{2+}$-ions was accomplished by shaking 1 ml. of the organic extract with 1 ml. of an aqueous solution of ^{203}Hg(II)-nitrate containing 0.01 m. ammonium-tartrate and adjusted to pH 8.5 with ammonia.

In general a rapid exchange was observed. Traces of cyanide — retained in the organic layer from the extraction of Pb (D.D.C.)$_2$ — interfered with the process however. Two successive washings with alcaline-solutions *and* masking of the last traces of cyanide with Fe^{3+}were required for a satisfactory exchange. [1]) In tables 9 and 10 the influence of the cyanide on the exchange-procedure is shown. Hg^{2+}-ions were in excess in table 9 and Pb.(D.D.C.)$_2$ was in excess in table 10 (this complex was prepared here with the omission of KCN).

Table 9.

*Influence of CN$^-$-ions on the exchange *) of 40 μgrams of ^{203}Hg (II) with 20 μgrams of Pb as Pb.(D.D.C.)$_2$.*

CN$^-$-ion added. In μgrams.	0	5	10	20	40
^{203}Hg exchanged. In μgrams.	20	19	13	5	0
Idem. In the presence of 100 μgrams Fe^{3+}	20	20	20	20	18

*) Shaken to equilibruim (3 minutes).

Table 10.

*Influence of CN$^-$-ions on the exchange *) of 20 μgrams of ^{203}Hg (II) with 30 μgrams of Pb as Pb-(D.D.C.)$_2$.*

CN$^-$-ion added. In μgrams.	0	2	5	10	20
^{203}Hg exchanged. In μgrams.	20	18	2	1	0
Idem. In the presence of 100 μgrams Fe^{3+}	20	20	20	19	17

*) Shaken to equilibrium (6 minutes).

[1]) CN$^-$ was estimated with CuS-paper (Feigl: 1947). Instead of Fe^{3+} formaldehyde could be used (Feigl and Goldstein; 1952).

Table 9 shows, that 5 μgrams of the CN^--ion are just about sufficient to eliminate the excess mercury of 20 μgrams; obviously $Hg(CN)_2$ is formed. On the other hand 10 and even 20 μgrams of the CN^--ion are *not* sufficient to prevent all exchange: the stabilities of $Hg(CN)_2$ and $Hg(D.D.C.)_2$ are evidently comparable.

In table 10 these results are confirmed; owing to the fact that lead is in excess over mercury here, the limit of 5 μgrams (of the CN^--ion) is more striking. Even traces of CN^- as small as 2 μgrams have an influence on the exchanged amount. Table 9 and 10 show in their last rows that Fe^{3+}-ions can be used to mask the cyanide. About a 7-fold excess — in μequivalents — over the CN^--ion is required when Hg^{2+} is in excess.

With the precautions mentioned above satisfactory determinations of lead were achieved, as is shown in figure 9. The procedure is rather lengthy however. To avoid this, thallic-ions labelled with ^{204}Tl were introduced instead of $^{203}Hg^{2+}$. The formation of the $Tl(D.D.C.)_3$ complex is *not* prevented by CN^--ions. Bismuth could have been used for the same purpose, but a convenient radioisotope was no available [2]).

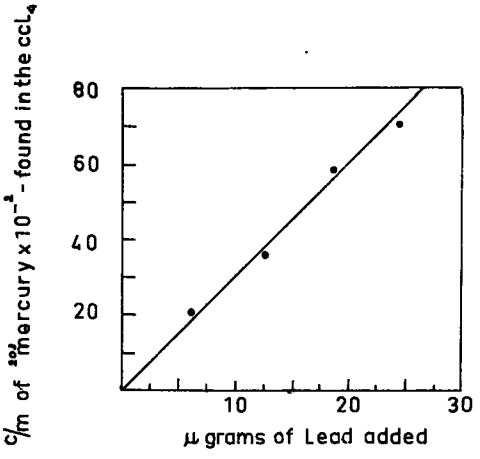

FIGURE 9:

Determination of 6 to 25 μgrams of lead with $^{203}Hg^{2+}$: exchange of $^{203}Hg^{2+}$-ions with Pb, after the extraction of the latter as $Pb(D.D.C.)_2$ in CCl_4.

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2) A further advantage of $^{204}Tl^{3+}$ over $^{203}Hg^{2+}$ is shown in 5.3.4.

5.3. *Development of the exchange-procedure with $^{204}Tl^{3+}$-ions.*

5.3.1. *Radioactive chemicals.*

^{204}Tl — (a) — A batch of ^{204}Tl, containing 1 m.curie and 2.4 milligrams of Tl as Tl_2So_4 in H_2O, was diluted with 1n.HNO_3 to 10 ml. and boiled 30 minutes with 0.5 ml. of bromine-water to produce Tl^{3+}. After cooling, the solution was diluted with 1 n.HNO_3 to 100 ml. and standardised iodometrically following S a n d e l l (1950) — page 561. (Traces of nitrites had to be removed carefully!)
^{204}Tl — (b) — From solution (a) 8 ml's were neutralised with alcali and diluted to about 25 ml.; KCN and $NaHCO_3$ were added to 0.12 percent each, the pH was adjusted to 13 and the volume to 30 ml. This solution contained 6.1 μgrams of $^{204}Tl^{3+}$ per ml. It had to be prepared daily fresh in order to avoid adsorption or formation of radiocolloids (which both tend to diminish the Tl-content).
^{210}Pb — see chapter 3.
D.D.C.-^{35}S — 100 mgrams of D.D.C-^{35}S — with a specific activity of 7 mc. per mmole [3]) were dissolved in 10 ml. 0.01 n.sodiumtartrate and the pH was adjusted to 11 with NaOH. To diminish the radiochemical decomposition (see 2.3.5.) 300 mgrams of β-mercapto-ethylamine-HCl were added. The solution was saturated with nitrogen and stored in a polyethylene bottle in the refrigerator.

5.3.2. *Other Chemicals.*

Of the reagents used, only the KCN (M e r c k p.a.) contained appreciable amounts of lead (10 p.p.m.) and had to be purified:
KCN-DDC — (a) — To 100 ml. of a 10 percent KCN-solution in H_2O, 0.2 moles of NaOH and 20 mgrams of D.D.C. (both: M e r c k p.a.) were added. The mixture was purified by extraction with 5 successive 10 ml. portions of CCl_4 and stored in a polyethylene bottle in the refrigerator. It contained about 200 μgrams of D.D.C. per ml.; some CCl_4 was added to clean it — by occasional shaking — from airborne traces of lead.
KCN-DDC — (b) — To 100 ml. of a 1 percent KCN-solution in H_2O, 0.01 moles of ammonium-tartrate (Analar) and 0.2 mgrams of D.D.C. were added. The solution was extracted and stored as solution (a). It contained about 1.8 μgrams of D.D.C. per ml. and had a pH of about 10.5.
KCN-$NaHCO_3$ —(a) — An aqueous solution 0.1 percent in both (M e r c k p.a.) reagents and with a pH of 10.
KCN-$NaHCO_3$ — (b) — The same solution adjusted to pH 13.
Tartrate — 0.1 molair ammonium-tartrate (Analar).
Acid Tl-carrier — 2 mgrams $Tl(NO_3)_3$ per ml. 2n.HNO_3.
Tl-(D.D.C.)$_3$carrier — 100 mgrams $Tl(NO_3)_3$ were dissolved in 10 ml. 0.01 NaOH, 1500 mgrams D.D.C. were added and the Tl[D.D.C.]$_3$ complex was extracted two times with 10 ml. portions of carbontetrachloride. The organic volume was adjusted to 100 ml.
Hydrochloric-acid (Sp.gr. 1.18) *Nitric-acid* (Sp.gr. 1.42) and *Carbontetrachloride* were of Analar-quality.

5.3.3. *Apparatus and general precautions.*

The counting-equipment was described in 2.2.
The procedure was carried out in a small pill-case (figure 10) covered with

[3]) From: the Radiochemical Centre — Amersham U.K. —

a very efficient lid and consisting of clear polyethylene. Shaking was performed — in a vertical position — with a "Microid Flask Shaker" (from "Griffin and Tatlock") which made a vertical movement of about 0.5 cm. The arm of the shaker was connected with a simple device for measuring its velocity. A ball-bearing was added to the spindle of the drivingmotor to give a more constant velocity for a given position of the dial. To prevent spillage of a radioactive solution in case of an accident, the shaker was placed in a shallow trough (2.2.).

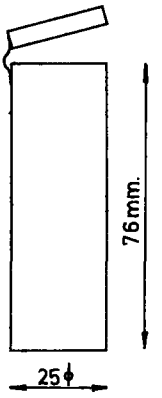

FIGURE 10:
Polyethylene pill-case for discontinuous extractions.

5.3.4. *Scheme of the method.*

$Pb(D.D.C.)_2$ was extracted with CCl_4 from an alcaline solution (of pH 14), containing: (1) an excess D.D.C., (2) KCN to obtain selectivity and (3) tartrate to prevent the precipitation of hydroxydes and carbonates.

In the extract, containing all the lead as $Pb(D.D.C.)_2$, lead was exchanged with ^{204}Tl by shaking with an alcaline solution of $^{204}Tl^{3+}$-ions. After an alcaline rinsing to remove each trace of the aqueous layer, the ^{204}Tl-activity of the CCl_4-layer was measured; this was taken as a measure for the amounts of lead originally present in the sample.

Part of the D.D.C. was coextracted with the $Pb(D.D.C.)_2$ and would also react with the ^{204}Tl during the exchange-procedure. As a large excess of D.D.C. was required for the complete extraction of traces of lead, this coextraction of D.D.C. would result in a considerable blank. Thus backextractions of D.D.C. (carried out with KCN) were inserted.

The total scheme then became:

1) — Aqueous Pb + D.D.C. (+ KCN, tartrate, pH 14)
2) — Extraction with CCl_4 (5.3.5) Remove aqueous layer
3) — First backextraction of D.D.C. with alcaline KCN (5.3.6) Remove aqueous layer
4) — Second backextraction of D.D.C. with alcaline KCN (5.3.6) Remove aqueous layer
5) — Exchange of lead with alcaline $^{204}Tl^{3+}$-ions (5.3.7.) Remove aqueous layer
6) — Removal of traces of $^{204}Tl^{3+}$-ions by rinsing (5.3.7.) Remove aqueous layer
7) — Measurement of ^{204}Tl in the CCl_4 (5.4.)

The procedure is described in detail in 5.5.

5.3.5. *Extraction of lead and co-extraction of D.D.C.*

The radio-isotope ^{210}Pb [= radium-D in equilibrium with its decay products] was used to study the extraction (back-extraction and exchange) of lead. [Separation of radium-D from radium-E and -F was not achieved here (see chapter 6). The mixture was measured at the 47 KEV-maximum of ^{210}Pb however. Above, measurements were checked after one half-life of ^{210}Bi (= 5 days; see 2.2.)].

Three ml's of an alcaline solution [pH 14], containing 0.1 to 3.0 μgrams of lead [$+^{210}Pb$], 5 % KCN, 0.01 m. tartrate and 300 μgrams of D.D.C., were shaken with organic solvents. With CCl_4 as the organic solvent, shaking at 800 to 1000 periods per minute proved to be optimal: unsufficient extraction was obtained [within 5 minutes] at lower velocities and emulsions occurred at higher ones. Afterwards the pill-case was centrifuged lightly to force all drops of liquid to go down and to speed up the separation of the two layers.

In fact chloroform was slightly more efficient for the extraction, but co-extraction of D.D.C. was also enhanced (see note on page 60). As this D.D.C. reacts with $^{204}Tl^{3+}$ during the exchange, the result is a larger blank.

A large volume of CCl_4 — as compared with the aqueous volume — was applied in order to extract the lead in one procedure. So only one pill-case was required and pipetting of the organic layer was

avoided. This large volume of CCl_4 resulted of course in a higher D.D.C.-blank too. Comparison of the extraction of lead and D.D.C. with either large volumes of CCl_4 or small volumes of CCl_3H showed the former method to be the most satisfactory however.

When the aqueous solution was shaken during 5 minutes with 8 ml's of CCl_4, 96 percent of the lead was extracted with a standard-deviation of 3 percent. [4]) Smaller amounts of D.D.C. resulted in an incomplete extraction, especially of the smaller amounts of lead.

With the exception of Bi and Tl, *other metals are not coextracted* in appreciable amounts. [Traces of metals which might be co-extracted are backextracted by KCN together with the D.D.C. (5.3.6.). This is an additional advantage of the use of ^{204}Tl instead of ^{203}Hg.]

Bismuth and thallium are easily separated from lead, for instance by the anion-exchange procedure described in chapter 6. For small amounts of both metals a "blank" can be determined by carrying out the extraction with D.D.C. — with part of the sample — in the presence of KCN and *E.D.T.A.*: lead is not extracted in contradistinction to bismuth and thallium (Kinnunen and Wennerstrand — 1957; 1959).

The extraction of lead was influenced by the presence of Fe^{3+}-, Cu^{2+}-, $P_2O_7^{2-}$- and PO_4^{3-}-ions. Up to 50, 50 100 and 10 μgrams were allowed respectively beside 0.1 μgram of lead. No explanation of the behaviour of Fe^{3+} and Cu^{2+} could be found; the ions Hg^{2+}, Ag^+ and Zn^{2+} did *not* interfere. When larger amounts of the anions were present citrate had to be added to the acid sample-solution as described by Sandell (1950) for the extraction of lead with dithizon. With a 5 % citrate solution 200 μgrams of phosphate are allowed beside 0.1 μgram of lead for instance.

Part of the D.D.C. was co-extracted. In the literature only very scarce and approximate values are found for the extraction of D.D.C. at higher pH-values. Bode (1954), using the colourreaction with excess aqueous copper as the detection-method, stated that the extraction is negligible above pH 8.5, provided the extract is dried by filtration.

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[4]) With other, less efficient, shakers a mixture of 6 ml's CCl_4 and 2 ml's $CHCl_3$ was to be preferred.

In our experiments, D.D.C.-^{35}S was used. As this compound contained small amounts of decomposition-products — some of which were extracted better than the D.D.C.-^{35}S itself — the solution (5.3.1.) was extracted three or four times. The extracted amount of D.D.C.-^{35}S was then determined by pipetting 0.5 ml. of the *last* organic layer on an aluminium dish, drying and measuring (2.2.). The form of the dish promoted a homogeneous distribution and thus minimized the error due to a variable selfabsorption (figure 11) [5].

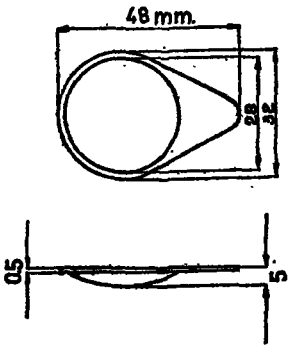

FIGURE 11:
Aluminium tray for counting D.D.C.-^{35}S under an end-window GM-tube.

From 0.2 to 0.4 percent of the D.D.C.-^{35}S were found in the CCl_4-layer, depending on the velocity of the shaker: with a more energetic shaking the percentage went up. Centrifugation of the pill-case up to 2000 r.p.m. (Ø 25 cm.) was unsuccesfull in lowering this blank. [6]) Filtration decreased the "extracted" percentage to about 0.02 but was found to remove also appreciable amounts of lead when applied to a Pb-$(D.D.C.)_2$solution [7]). Thus the CCl_4-layer contains a D.D.C.-surplus of 0.2 to 0.4 percent of the applied amount [corresponding with an equivalent of 0.3 to 0.6 μgrams of lead).

A *second D.D.C.-surplus* in the "CCl_4-layer" results from the

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[5]) With the ordinary dishes — which have a rectangular profile — the last drops of the solvent and thus the precipitate are collected in the outer regions.
[6]) Higher velocities are dangerous with regard to the construction of the pill-
[7]) Filtration through hydrophobised paper, as recommended by K o v á c s (1957) and W e g m a n n (1959), was found to be equally unsatisfactory.

insufficient removal of the aqueous layer. Provided this D.D.C — 1 to 2 percent — is totally extracted with $^{204}Tl^{3+}$ in the exchange-procedure, this second D.D.C.-surplus is equivalent with 1.5 to 3.0 μgrams of lead. From these figures it is clear that backextraction of the D.D.C. [and rinsing of the organic layer] is required.

5.3.6. *Backextraction of D.D.C.*

As was to be expected from the figures of B o d e et.al. (1954), strongly alcaline solutions were the most effective here. Appreciable amounts of lead were also backextracted however. A solution of pH 10.5 — containing 1 percent KCN to prevent the interference of foreign metals — was found to be optimal: 90 to 95 percent of the D.D.C. an only 1 to 6 percent of the lead were backextracted. As again 1 to 2 percent of the aqueous layer could not be removed, the remaining D.D.C.-blank can be calculated to be:

$$\frac{1 \text{ to } 2}{100}[(1.5 \text{ to } 3.0) + (0.27 \text{ to } 0.57)] + \frac{5 \text{ to } 10}{100}(0.3 \text{ to } 0.6) = 0.082 \pm 0.049 \text{ μgrams of lead.}$$

(not removed) (backextracted-1 to 2% not removed) (not backextracted)

Thus a *second backextraction is required for amounts of lead below 0.5 μgrams.* In our procedure (5.5.) a KCN-D.D.C. solution — resulting from the purification of KCN with D.D.C. — was used instead of KCN alone. It can be calculated that the D.D.C.-blanks for 1 and 2 backextractions are enlarged to 115 ± 60 and 36 ± 14 m μgrams of lead respectively by this additional D.D.C.

It can also be calculated that the whole process is efficient for 87 to 98 and 81 to 97 percent of the lead with 1 and 2 backextractions respectively. In practive the figures 94 (±3) and 89 (±7) were found.

5.3.7. *Exchange with* $^{204}Tl^{3+}$.

In some preliminary experiments the optimal values for the pH, the shaking-time and the excess thallium were evaluated.

Figure 12 shows the influence of the pH; 6.1 μgrams of $^{204}Tl^{3+}$ were shaken during 5 minutes with 3 μgrams of lead as $Pb(D.D.C.)_2$. Evidently the whole range from pH 12.5 to 14 could be used, but pH 13 is optimal.

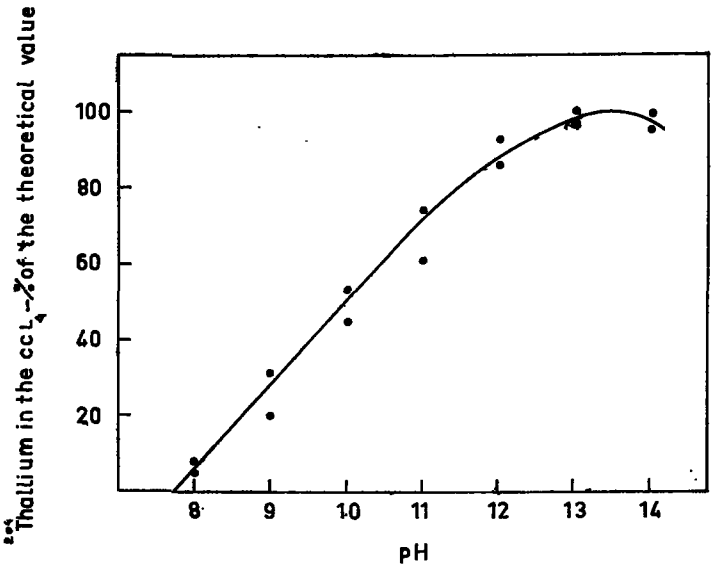

FIGURE 12:

Influence of the pH on the rate of excange between 6.1 μgrams of (aqueous) ^{204}Tl (III and 3 μgrams of Pb as Pb(D.D.C.)$_2$ in CCl_4. Percentage of exchange within 5 minutes.

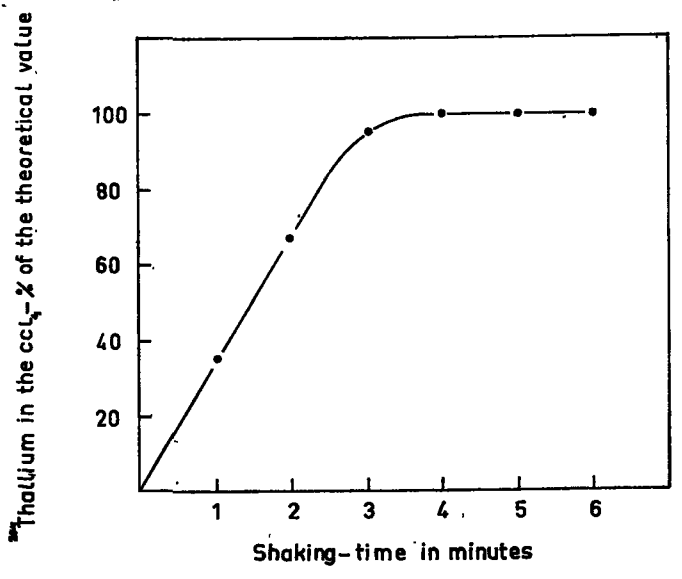

FIGURE 13:

Influence of the shaking-time on the exchange of 6.1 μgrams of (aqueous) ^{204}Tl (III) and 3 μgrams of Pb as Pb(D.D.C.)$_2$ in CCl_4. Percentage of exchange with an aqueous layer of pH 13.

In figure 13 the influcnce of the shaking-time is shown for pH 13 and for the same amounts of thallium and lead as used above. Although for smaller amounts of lead the situation was somewhat less favourable than shown in the figure, five minutes were always sufficient for total exchange.

With a 3.5-fold excess of $^{204}Tl^{3+}$, pH 13 and a shaking-time of 5 minutes *the exchange-procedure was effective for 96 (± 2) percent* for the whole range of lead examined (0.1 to 3.0 μgrams). Thus the total procedure was effective for 90 (±5) and 85 (±9) percent for the two ranges of lead respectively.

For amounts of lead below 0.1 μgrams the efficiency of the process soon dropped to 75 percent with a standard-deviation larger than 15 percent.

To prevent all interferences from traces of Hg, Ag and Cu in the reagents etc., 0.1 percent KCN was added to the alcaline $^{204}Tl^{3+}$ solution. In most cases this additional KCN will be superflous as the small amounts of KCN retained with the CCl_4 (see 5.1.) are efficient enough in this respect. This was shown for copper — the most probable element to interfere in this way — by shaking 40 μgrams of the latter with 10 μgrams of $^{204}Tl^{3+}$ as the D.D.C.-complex: 100 μgrams of KCN were sufficient to prevent the exchange totally, whereas 20 and 40 μgrams prevented the exchange (of ^{204}Tl) for 90 and 95 percent respectively

After the separation of excess $^{204}Tl^{3+}$ *from the organic layer, the latter has to be washed* in order to remove (1) the usual small rest of the aqueous layer — 1 to 2 percent — and (2) part of the ^{204}Tl "dissolved" in the CCl_4.

As to the former: after washing this factor still results in a *blank* equivalent with 2.2 (± 1.4) and 0.5 (± 0.2) mμgrams of lead for the two amounts of ^{204}Tl applied in 5.5.

As to the latter: experiments with ionic ^{204}Tl and pure CCl_4 indicated a *blank* of 0.1 to 0.3 percent (after the washing) [8]). This blank decreased through filtration (see 5.3.5.) but again part of the metal-D.D.C.-complex [$Tl(D.D.C.)_3$ here] was adsorbed by the filterpaper. Factor (2) attributes a Tl-blank of 18 (± 9) and 3.6 (± 1.8 mμgrams of lead for the two amounts of ^{204}Tl applied in 5.5. respectively. This is still small compared with the D.D.C.-blank.

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[8]) For chloroform a figure of 0.7 to 0.8 percent was found.

5.4. *Measurement of the radioactivity of* ^{204}Tl (see also 2.2.).

^{204}Tl can be measured in two ways; with the M6-GM-tube or with the scintillation-detector (see 2.2.). The sensitivity of both detectors is about equal, the GM-tube has a lower — and more stable — zero-value however and was therefore used to measure the small activities. The well-type crystal has the advantage that separate glass- or plastic-tubes can be used for the samples and thus the detector need not be cleaned between measurements. With both methods 10^4 counts were registered resulting in a 1 percent statistical error.

Direct measurement of the CCl_4 (8 ml.) is of course to be preferred with the GM-tube (10 ml. capacity). Sometimes losses by adsorption occur notwithstanding the addition of Tl-(D.D.C.)$_3$ carrier. For this reason a thorough rinsing of the plastic pillcase with the carrier can not be omitted.

Although the organic layer could be measured in the well-type crystal as well — partly or after evaporation to 1 ml. — backextraction with a small volume of the acid Tl-carrier is to be preferred here. (Since a few percent of the excess $^{204}Tl^{3+}$ are adsorbed to the wall of the plastic pill-case during the exchange of Tl and Pb this backextraction has to be carried out in a second container.) Less than 0.5 percent of the $^{204}Tl^{3+}$, present as Tl-(D.D.C.)$_3$ in the organic solution, is not backextracted.

5.5. *Procedure.*

The procedure is given for a sample containing from 0.1 to 0.5 μgrams of lead, less than a 0.1-fold amount of bismuth and thallium, less than 10 and 100 μgrams of phosphate and pyrophosphate respectively [9]), less than 50 μgrams of Fe or Cu and less than 1000 μgrams of other heavy metals, including Zn, Hg and Ag.

This sample is dissolved in HNO_3 or/and HCl, evaporated when necessary to about 0.5 ml. (see 3.4.) and transported to the tube of figure 10; 0.3 ml. of the tartrate solution (5.3.2.) are added and the mixture is neutralised with concentrated ammonia on phenolphtaleïn. The volume should now be about 1.5 ml. Of the KCN-D.D.C. solution (a) (5.3.2.) 1.5 ml. are added and Pb-(D.D.C.)$_2$

[9]) Beside 0.5 to 3.0 μgrams of lead 50 and 100 μgrams of these anions are allowed respectively. With larger amounts citrate has to be added (see 5.3.5.).

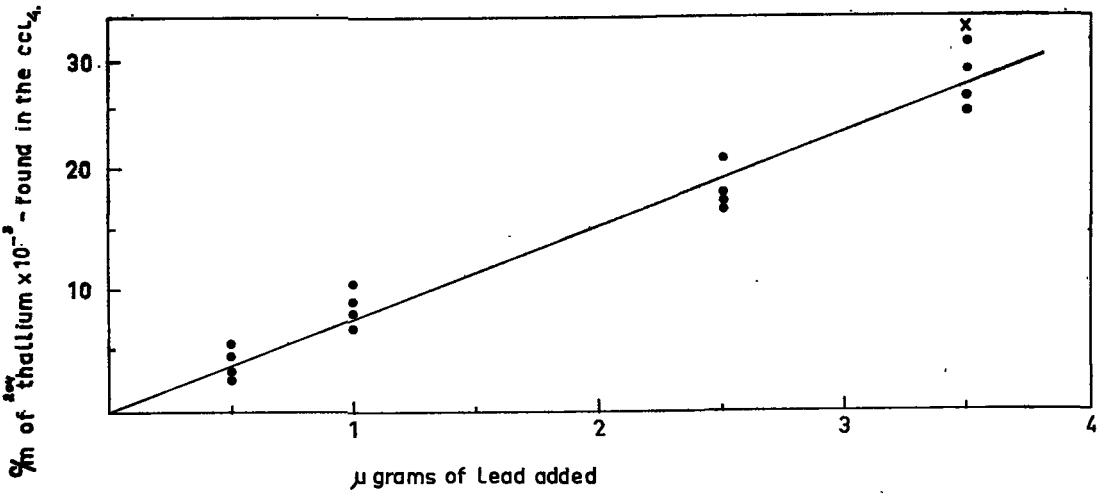

FIGURE 14:

Determination of 0.5 to 3 µgrams of lead with 6.1 µgrams of $^{204}Tl^{3+}$: exchange of Tl^{3+}-ions with Pb, after the extraction of the latter as Pb (D.D.C.)$_2$ in CCl_4. X: Theoretical value for 100% efficiency.

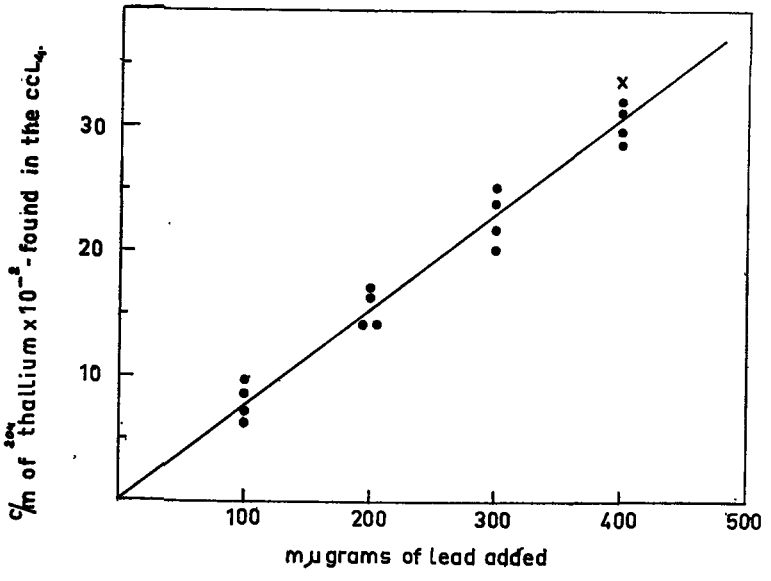

FIGURE 15:

Determination of 0.1 to 0.4 µgrams of lead with 1.2 µgrams of $^{204}Tl^{3+}$: exchange of Tl^{3+}-ions with Pb, after the extraction of the latter as Pb (D.D.C.)$_2$ in CCl_4. X: Theoretical value for 100% efficiency.

is extracted with 8 ml. CCl_4 by shaking during 5 minutes with a frequency of 800 to 1000 per minute in the "Microïd Flask Shaker".

The pill-case is centrifuged during 1 minute at 1000 r.p.m. (Ø 25 cm.), the aqueous layer is pipetted off and D.D.C. is backextracted by shaking 2 minutes (see above) with 2 ml. of KCN-D.D.C.-solution (b) (5.3.2.). This is repeated once more [10]. Again the pill-case is centrifuged and the upper layer is pipetted off.

Exchange with $^{204}Tl^{3+}$ is accomplished by shaking 5 minutes with 0.2 ml. of $^{204}Tl^{3+}$-solution (b) (5.3.1.) and 0.8 ml. of the KCN-$NaHCO_3$-solution (b) (5.3.2.) [11]. After centrifugation the water layer is taken off and the organic layer is shaken 1 minute with 2 ml. of KCN-$NaHCO_3$-solution (a) (5.3.2.), centrifuged and separated.

A half ml. of the Tl-$(D.D.C.)_3$ carrier is added, the mixture is pipetted in a calibrated glass-tube and the pill-case is rinsed by shaking two times during 10 minutes with 0.5 ml. of the Tl-$(D.D.C.)_3$ carrier. The collected solutions are adjusted with CCl_4 to 10 ml., pipetted in the GM-tube and measured (2.2). [12].

5.6. *Results.*

In the figures 14 and 15 and in table 11 the results for the two ranges of lead are shown. To facilitate comparison, all values are corrected for measurement of the CCl_4 with the GM-tube [13].

For the range of 0.1 to 0.5 μgrams of lead the blank was found to be equivalent with 30 m μgrams of lead with a standard-deviation of 6 m μgrams; for the range of 0.5 to 3.0 μgrams of lead the blank was 115 (± 45) m μgrams. Amounts of Fe, Cu and Zn up to 1000 μgrams had no influence on this blank.

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[10]) When more than 0.5 μgrams of lead are present one backextraction is sufficient.

[11]) When 0.5 to 3.0 μgrams of lead are present, 1 ml. of $^{204}Tl^{3+}$ —(b) is used, and no KCN-$NaHCO_3$.

[12]) With 0.5 to 3.0 μgrams of lead the organic layer is pipetted in a second pill-case, the first one is rinsed 2 times during 10 minutes with 2 ml. of the Tl-$(D.D.C.)_3$ carrier and the collected solutions are shaken 4 minutes with 0.8 ml. of the acid Tl-carrier. After centrifugation the aqueous layer is pipetted in a measuring-tube, the pill-case is rinsed with 0.3 ml. of the acid carrier and the collected aqueous solutions are measured with the well-type crystal.

[13]) See 5.4.; part this correction is due to the different detector, another part to the different solvent which is measured.

Table 11

Determination of 0.1 to 3.0 μgrams of lead with D.D.C. and $^{204}Tl^{3+}$

Number of experiment	Lead added (in mμgrams)	c/m ^{204}Tl in CCl_4 (minus blank) [1]	Mean of column 3 with stand.-deviation	Lead exchanged, [2] and stand.-deviation (in mμgrams)	Lead corrected [3] (in mμgrams)	Error (in percent)	Standard-deviation (in percent) (from column 4)
1	100	653	770; 85	89; 10	104	+ 4	11
2	100	858					
3	100	807					
4	100	731					
5	200	1411	1540; 160	177; 18	208	+ 4	10
6	200	1658					
7	200	1382					
8	200	1714					
9	300	2190	2240; 250	258; 29	303	+ 1	11
10	300	2513					
11	300	1878					
12	300	2337					
13	400	2918	2980; 80	343; 10	402	+ 0.5	3
14	400	3064					
15	400	3029					
16	400	2891					
17	500	3648	3900; 500	450; 55	500	0	13
18	500	3451					
19	500	4411					
20	500	4168					
21	1000	7437	7950; 550	915; 65	1010	+ 1	7
22	1000	7978					
23	1000	8701					
24	1000	7713					
25	2000	14212	14800; 800	1700; 90	1890	— 5.5	5
26	2000	14701					
27	2000	15887					
28	2000	14424					
29	3000	22617	23000; 1700	2650; 200	2930	— 2	7
30	3000	23348					
31	3000	24921					
32	3000	21289					

[1] 260 and 1000 c/m for 0.1 to 0.4 and 0.5 to 3.0 μgrams of lead respectively.
[2] Calculated from column 4 ; 1 mμgram of ^{204}Tl in CCl_4 produces 8.7 c/m in the GM-tube.
[3] As 85 and 90 percent (5.3.7) of the lead are exchanged in the two ranges respectively.

5.7. *Discussion.*

5.7.1. *Stoechiometry of the exchange.*

As column 5 in table 11 is calculated under the assumption of the exchange of equal amounts of both metals — in gramequivalents — the figures of column 7 show that this assumption is right with a mean error of + 0.5 percent.

5.7.2. *Blank value.*

The D.D.C.-blank (5.3.6.) and the thallium-blank (5.3.7.) are responsible for a large part of the blank value. For the lower range of lead [less than] 36 m μgrams are due to the former and about 3.6 m μgrams to the latter factor; for the higher range these figures are 115 and 18 m μgrams respectively.

From the experimental blanks it can be concluded that the D.D.C.-blank is smaller than indicated above: evidently the reaction between aqueous D.D.C. and $^{204}Tl^{3+}$ — and/or the extraction of the reaction-product — during the exchange of $^{204}Tl^{3+}$ with Pb-(D.D.C.)$_2$, is incomplete. This is the more clear since other contributions to the blank are probably due to (1) small amounts of lead introduced by and not removed from the KCN-D.D.C.-solutions (a) and (b) in the procedures of (5.3.2.) and to (2) small amounts of lead contained in the other reagents or in the plastic tubes.

For the determination of still smaller amounts of lead the KCN should be purified without the use of D.D.C. (M a y n e s and M c B r y d e 1957), or otherwise a purer batch of KCN (Analar) should be applied [see "Analytical methods committee — 1959].

5.7.3. *Standard-deviation and limit of sensitivity.*

The standard-deviation is due to the following factors:

(1) Only a small contribution is made by the *counting-error* (5.4.).

(2) Variations in the blank-value are especially important in the determinations of 100, 500 and 1000 mμgrams of lead. This factor is of increasing weight with amounts of lead below 100 mμgrams. With the application of extra purified reagents this influence could be considerably decreased however.

(3) Variations in the efficiency of the process make the main contribution to the standard-deviation and are the main limitation to the sensitivity of the method; additional tools to reach a higher sensitivity are:
   (a) Evaporation of the CCl_4, prior to the exchange (and handled with care (see 3.2.2.)),
   (b) Higher amounts of $^{204}Tl^{3+}$, and
   (c) Prolonged shaking-times.

Alternatively to (a), (b) and (c) isotope dilution with ^{210}Pb could be introduced.

The limit set by the specific activity of the ^{204}Tl is not completely

reached in our experiments; a preparation with a higher specific activity should be used for amounts of lead smaller than 10 m μgrams. It can be calculated that theoretically the limit of sensitivity is about 1 $\mu\mu$gram.

5.8. *Conclusion.*

With the method described here 0.1 μgrams of lead can be determined with a standard-deviation of 11 %. The technique is rather complicated however, owing to the co-extraction of D.D.C.

A simpler — and more sensitive — technique is decribed in chapter 6.

CHAPTER 6.

DETERMINATION OF LEAD WITH ^{204}Tl-(D.D.C.)$_1$.

6.1. *Introduction.*

The exchange of Pb^{2+} [in water] with ^{204}Tl-(D.D.C.)$_1$ [in an organic solvent] can be applied in the analysis of lead: the amount of ^{204}Tl found in the aqueous layer (after substraction of the blank) is taken as a measure for the (exchanged) amount of lead. The method is in principle simpler than the one described in the foregoing chapter. In practice, the rate of exchange appears to be very low however. Total exchange is only achieved with long shaking-times and a large excess of ^{204}Tl-(D.D.C.)$_1$, both circumstances producing a high blank and therefore a higher inaccuracy of the method. The necessity of a total exchange can be avoided however by the introduction of isotope dilution with radiolead. The percentage exchanged can be found from the distribution of the radiolead over the two phases. Of course, separate measurements of both radioistotopes [^{204}Tl and ^{210}Pb] are required.

6.2. *Experimental.*

6.2.1. *Radioactive chemicals.*

^{204}Tl — A batch of ^{204}Tl, containing 1 mcurie and about 3.4 mgrams of Tl as Tl_2SO_4 in less than 1 ml. H_2O, was diluted with 0.2 n.HCl to 10 ml. and 22 mgrams of Tl as Tl_2SO_4 were added. This solution contained about 2540 μgrams and 100 μcuries of ^{204}Tl per ml.
^{210}Pb — A batch of ^{210}Pb (radium-D in equilibrium with its decay-products) containing 0.11 m.curies and about 12 μgrams of Pb as $Pb(NO_3)_2$ in 0.3 ml. 2 n.HNO_3, was diluted with 9 n. HCl to 10 ml. This solution contained about 1.2 μgrams of lead and 11 μcuries of ^{210}Pb per ml.

6.2.2. *Other chemicals.*

KCN — tartrate (a): An aqueous solution containing: 1 % KCN (Analar), 0.02 m. ammonium-tartrate (Analar) and (2X + 0.1) n.NaOH (Merck p.a.). For the value of X see below.
KCN — tartrate (b): Idem but: 0.5 % KCN and 0.01 m.ammoniumtartrate; adjusted (with X gramequivalents of NaOH per liter) to pH 11.5.
Acid Bi PbTl-carrier: 100 μgrams of each metal [as the nitrate] per ml. 1n.HCl.

BiPbTl-D.D.C.-carrier: 100 μgrams of each metal as the D.D.C. complex per ml. chloroform.
D.D.C.-solution: A fresh solution of 10 mgrams D.D.C. in 10 ml. H_2O.
Chloroform — (Analar) — *Ammonia* — (Analar) — Specific gravity 0.88. *Hydrochloric acid* — (Analar) — Specific gravity 1.18. *Diethylamine* — (May and Baker — Residue on volatilization less than 0.05 %). *Bromine water* — Made from bromine (Merck p.a.).

6.2.3. *Apparatus and general precautions.*

The equipment and the precautions were in general the same as described in chapter 5.

6.2.4. *Preparation of ^{204}Tl-(D.D.C.)$_1$.*

The stock-solution of the "reagent" ^{204}Tl-(D.D.C.)$_1$ was prepared in *chloroform* as this solvent showed the smallest backextraction of ^{204}Tl during the exchange (i.e. the smallest blank; see 6.2.5.1). The drawback of the relatively high co-extraction of D.D.C. with chloroform (compare 5.2.4.) was forestalled by using excess $^{204}Tl^+$ for the preparation of the complex, instead of excess D.D.C.

Again KCN was applied to obtain selectivity. Diethylamine was added to the chloroform-extract for reasons of stabilisation (see 6.2.5). A 3 weeks old solution, prepared as indicated below, showed a 2 percent increase of its radio-activity when shaken with ionic-$^{204}Tl^+$ of the same specific activity, which indicated that after 3 weeks about 2 percent of the Tl-[D.D.C.]$_1$ in the chloroform was decomposed to D.D.C. (or to other molecules able to extract thallium).

The *procedure* for the preparation of the Tl-[D.D.C.]$_1$-solution was as follows: 1 ml. of the ^{204}Tl-solution (a) is pipetted in a glass- or polyethylene-tube and neutralised with 2 n.ammonia on phenolphtaleïn; 1.1 ml. of the KCN-tartrate solution (a) and 2.4 ml. of the D.D.C.-solution are added and ^{204}Tl-(D.D.C.)$_1$ is extracted by shaking 3 minutes at 900 periods per minute with 5 ml. chloroform. The tube is centrifuged lightly (1000 r.p.m.; 25 cm Ø) and the aqueous layer is pipetted in a second tube in which the extraction is repeated. The assembled organic layers are stripped 3 times with 2 ml. of the KCN-tartrate-solution — (b), 1 mmole of diethylamine is added to the chloroform-solution and the volume is made up to 20 ml. When stored in a refrigerator, the solution is stable (enough) for 3 weeks (see 6.2.5.7). It contains about 4.0 μcuries and 100 μgrams of thallium as ^{204}Tl-(D.D.C.)$_1$ per ml.

6.2.5. *Factors influencing the stability of the* $^{204}Tl[D.D.C.]_1$-*solution.*

The instability of the $^{204}Tl[D.D.C.]_1$-reagent is a drawback; it brings part of the ^{204}Tl back in the waterlayer during the exchange with Pb^{2+} and thus produces a (variable) blank. The same is of course effected by interfering cations. The influence of several factors on this blank was investigated:

(1) *Organic solvent.* A few experiments were carried out with carbontetrachloride and amylalcohol. As indicated above already, backextractions from these solvents were higher than from chloroform however.

(2) *pH of the [Pb-containing] sample-solution.* A pH-value of 11.5 proved to be optimal; the blank, and especially its standard-deviation, went up at higher as well as at lower pH-values.

(3) *Concentration of KCN.* In order to prevent the exchange of (most) foreign cations with ^{204}Tl-(D.D.C.)$_1$, KCN had to be added to the sample-solution. For amounts of heavy metals larger than 2 μequivalents, 0.5 % KCN was required; for smaller amounts 0.1 % KCN was sufficient. Further details

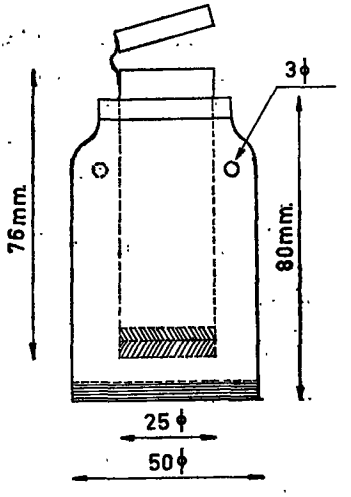

FIGURE 16:
Polyethylene pill-case with an alcohol-envelope.

are given in 6.5. As the influence of the KCN-concentration on the stability of the ^{204}Tl-(D.D.C.)$_1$ was not significant in the range from 0.1 to 0.5 % KCN, the latter solution was chosen for all experiments.

(4) *Temperature.* When the room-temperature went up to 25°C (or above) higher blanks were observed; 10 to 15 percent of 100 μgrams of thallium as ^{204}Tl-(D.D.C.)$_1$ were backextracted for instance at 30°C with the procedure described below, instead of the usual 2.4 percent backextracted at 20°C. Evidently the decomposition of the reagent is enhanced at the higher temperature. Thus on hot days an alcohol-envelope was used which prevented the temperature from going up above 20°C (figure 16).

(5) The influence of *the concentration of the* ^{204}Tl*-(D.D.C.)*$_1$ *in the* chloroform is given in table 12. The solution of 6.2.4. (diluted with chloroform) was shaken during 6 minutes with 2 ml.'s of the KCN-tartrate solution (b), at a velocity of 1400 periods per minute. The further procedure is described under 6.4. (This procedure was repeated once; the values given below are the blanks after the *second* backextraction (see 6.2.5.7.)).

Table 12.

*Backextraction of ^{204}Tl from ^{204}Tl-(D.D.C.)$_1$ in $CHCl_3$ * (Influence of the concentration)*

μgrams of ^{204}Tl as ^{204}Tl-(D.D.C.)$_1$	25	20	10	5	2.5
μgrams of ^{204}Tl backextracted	1.6	1.6	1.5	0.9	0.6
Percentage of ^{204}Tl backextracted *	7.1	9.2	20.0	25.7	40
Number of experiments	7	9	6	10	5
Standard deviation of ^{204}Tl backextracted (in μgrams)	0.9	0.6	0.5	0.3	0.3
Idem; in percentage of the ^{204}Tl in ^{204}Tl-(D.D.C.)$_1$	4.0	3.4	6.7	8.5	20

* With the tubes of figure 16, 2 ml-volumes of both layers, a shaking time of 6 minutes and a velocity of 1400 periods per minute.

* Of the ^{204}Tl still present as ^{204}Tl-(D.D.C.)$_1$ after the first blank.

Neither a constant *amount* nor a constant *percentage* of the ^{204}Tl is backextracted! Evidently a higher concentration of ^{204}Tl-(D.D.C.)$_1$ is favourable for a smaller and more constant blank (in percentage). Thus a small volume of the organic

layer was tobe preferred. The following experiments were all carried out in the polypropylene tubes of figure 17; with 1 ml-volumes of both layers.

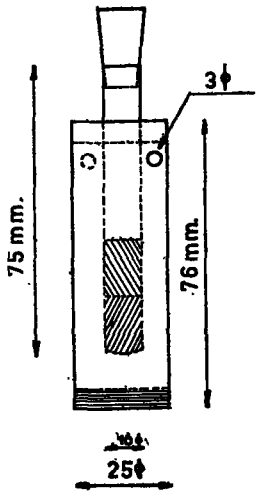

FIGURE 17:
Polypropylene tube with an alcohol-envelope.

Table 13.

*Backextraction of ^{204}Tl from ^{204}Tl-(D.D.C.)$_1$ in $CHCl_3$ * (Influence of the shaking-time and -velocity)*

Shaking-procedure	3 minutes 900 periods p.m.					6 minutes 900 periods p.m.			6 minutes 1400 periods p.m.		
µgrams of ^{204}Tl as ^{204}Tl-(D.D.C.)$_1$	25	12.5	6	1.5	0.5	6	1.5	0.5	6	1.5	0.5
µgrams of ^{204}Tl back-extracted	0.70	0.38	*0.292*	0.120	0.055	0.332	*0.160*	*0.073*	0.611	0.352	0.149
Percentage of ^{204}Tl backextracted *	2.9	3.2	5.3	9.3	13.7	6.3	13.3	19.2	12.2	37.1	49.8
Number of experiments	4	6	7	10	14	6	14	10	4	4	4
Standard-deviation of ^{204}Tl backextracted (in µgrams)	0.09	0.04	0.020	0.006	0.002	0.021	0.006	0.002	0.176	0.066	0.052
Idem; in percentage of the ^{204}Tl in ^{204}Tl-(D.D.C.)$_1$	0.38	0.34	0.36	0.46	0.50	0.39	0.50	0.53	3.5	6.8	17.3

* With the tubes of figure 17 and 1 ml. volumes of both layers.
* Of the ^{204}Tl still present as ^{204}Tl-(D.D.C.)$_1$ *after* the first blank.
N.B. The italicized figures are applied in Practice (6.4).

(6) The influences of *the time and the velocity of the shaking* are shown in table 13 (in addition the influence of the concentration is shown once more. Again the second blank is given — see 6.2.5.7).
Table 13 shows that a shorter shaking-time and a lower -velocity are both favourable. Comparison with table 12 shows that the use of smaller tubes and volumes contributes to the lowering of the blank. The smallest blanks of table 13 are a factor of 5 below the corresponding values of table 12.

The variation found in all these blanks might be due to a variation in the polypropylene tubes. These tubes are manufactured on metalmoulds containing small amounts of lead. Although they are purified thoroughly (2.2.) small differences in their lead-content might excist. The *smaller* standard-deviations (in μgrams of ^{204}Tl) of the blanks obtained with *smaller* amounts of ^{204}Tl-(D.D.C.)$_1$ are an indication that this factor is not of much importance however.

Apart from the variations shown in table 12 and 13, distinct differences between several fresh solutions were observed, a phenomenon for which as yet no explanation has been found.

(7) *Effect of aging of the* ^{204}Tl*-(D.D.C.)*$_1$*-solution.* Older preparations required a preliminary cleaning [by and alcaline back-extraction]: the amounts of thallium backextracted in the second and third procedures were (about equal and) appreciably lower than in the first one. For preparations not older than 2 or 3 weeks this second or third blank was comparable with the first one of the freshly prepared solution. Evidently decomposition had taken place.
[a] An appreciable part of the decomposition of ^{204}Tl-(D.D.C.)$_1$ was prevented already through the *addition of diethylamine* [in 6.2.4] to the fresh preparation. The first blank of a 2 weeks old solution, containing 100 μgrams of ^{204}Tl as ^{204}Tl-(D.D.C.)$_1$ per ml., decreased from 4 to 1.5 percent through the addition of 1 mmole of the amine per 20 ml. of the solution, whereas 0.7 percent was the value of the fresh solution (and of the second blank). The stabilisor was not stripped from the chloroform by KCN-tartrate.
[b] No influences of reducing agents or a nitrogen-atmosphere were observed, thus *oxidational* decomposition is improbable.
[c] To test whether *radio-decomposition* occurred, excess Ag-

-(D.D.C.)$_1$ was added [1]). No influence was observed when 50 μgrams of Ag as Ag-(D.D.C.)$_1$ per ml were added to a solution of 20 mμcuries and 2 μgrams of ^{204}Tl as ^{204}Tl-(D.D.C.)$_1$. With 2000 μgrams of Ag per ml. added to 6000 mμcuries and 20 μgrams of ^{204}Tl, the blank of a 2 days old solution was only $^2/_3$ of the Ag-free preparation. Thus a distinct radio-decomposition was observed at the higher specific activities. [2])

(8) *Adsorption of ^{204}Tl-ions to the walls.* Apart from the ionic thallium found in the aqueous solution, 0.45 $\pm$ 0.14 percent (standard-deviation) of the thallium-ions were found to be adsorbed to the wall of the polypropylene tube [3]). These adsorbed thallium-ions are part of the blank or, in an experiment with a sample containing lead, part of the thallium to be evaluated as being exchanged with lead-ions. No influence of the presence of lead-ions on the adsorbed amount of ^{204}Tl was observed and thus this adsorption could be neglected.

6.2.6. *Exchange with Pb^{2+}-ions.*

The influence of the organic solvent, the pH [4]), the concentration of the KCN and the age of the ^{204}Tl-(D.D.C.)$_1$ on the rate of exchange, proved to be small.

Higher temperatures, shaking-velocities and concentrations of both components were all favourable for a high rate of exchange. No use of the first factor could be made however with regard to the higher blank (6.2.5.4.).

A chloroform-solution of ^{204}Tl-(D.D.C.)$_1$, a temperature of 20 $\pm$ 2°C and aqueous solutions of pH 11.5, containing 0.5 percent KCN, were chosen for our first exchange-experiments. With 2 ml. of both layers, the tubes of figure 16 and a velocity of 1400 periods per minute, exchange between 5 μgrams of lead and 25 μgrams of

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[1]) Ag [D.D.C.]$_1$ is supposed to be decomposed in the same way as Tl-[D.D.C.]$_1$ by the radiationeffect.

[2]) These blanks were obtained with KCN-free aqueous layers in order not to decompose the Ag-complex.

[3]) Apart from the adsorption of ^{204}Tl-ions, adsorption of ^{204}Tl-(D.D.C.)$_1$ itself was observed. This ^{204}Tl-(D.D.C.)$_1$ had to be removed, previous to the determination of the adsorbed ^{204}Tl+, by prolonged shaking with the Bi, Pb, Tl-D.D.C. carrier.

[4]) Between pH 8.5 and 13.5. This is in contradistinction with the exchange of $^{204}Tl^{3+}$ with Pb-(D.D.C.)$_2$ (5.2.6.).

^{204}Tl as ^{204}Tl-(D.D.C.)$_1$ proved to have taken place for about 90 percent within 6 minutes. The standard-deviation of the amount of aqueous ^{204}Tl was 20 percent (or 2μgrams), about half of which was evidently due to variations in the blank (see table 12). The variation in the exchanged percentage of lead was of course another source or error.

Less than 80 percent of amounts of lead smaller than 3 μgrams were exchanged with the same 2.5-fold excess of ^{204}Tl-(D.D.C.)$_1$. A 10- to 25-fold excess was required here for 90 percent exchange within 6 minutes. Owing to the larger blank, the standard-deviations of the latter determinations went up to 40 or 50 percent however. Consequently a large excess of thallium is to be avoided. As total exchange of the smaller amounts of lead becomes impossible then, isotope dilution with radio-lead has to be introduced.

6.2.7. *Isotope dilution with radiolead.*

The radio-isotopes of lead and thallium are to be measured in the presence of eachother. The only suitable and available radio-isotopes are ^{204}Tl, ^{212}Pb and ^{210}Pb (see 2.2.).

^{212}Pb (Th.B.) can be separated from a thorium-source but *not* from its decay-products. Measurement of ^{204}Tl in the presence of the mixture Th-B-C-C'-C'' is very difficult.

^{210}Pb can be separated from a radium-source *and also* (owing to the larger half-life of its first daughter, as compared with Th-C) from its decay-products. Measurement of ^{204}Tl and ^{210}Pb in the (presence of eachother is f.i. possible with the M6 GM-tube (^{204}Tl) and the single-channel analyzer (^{210}Pb).

^{210}Pb was separated from its decay-products by anion-exchange in concentrated HCl. Results with this technique were reviewed a.o. by Kraus and Nelson (1954). Special applications to mixtures containing lead, bismuth, thallium and polonium were published by Ishimori (1955) and Gorsuch (1959).

As lead was the only element to be separated here, their technique could be simplified: Tl^{3+}, Bi^{3+} and Po^{4+} were adsorbed from concentrated HCl by Dowex 1 X 10, whereas Pb^{2+} was found in the eluate. A batch-procedure was unsatisfactory: only 92 and 95 percent of the ^{210}Bi were removed from 9 n. and 6 n. HCl respectively. Thus a column had to be used (figure 18). It was filled with Dowex 1 X 10 — 100 to 200 mesh, by the addition of this material as a slurry in 9n. or 6 n. HCl and washed with 10 ml. of the same acid solution. The mixture of the isotopes was then dissolved in HCl of the same concentration and added from the funnel to the column at a rate of about 0.25 ml. per minute. The eluate was tested on ^{210}Bi with the M6 GM-tube and on ^{210}Pb and ^{210}Po

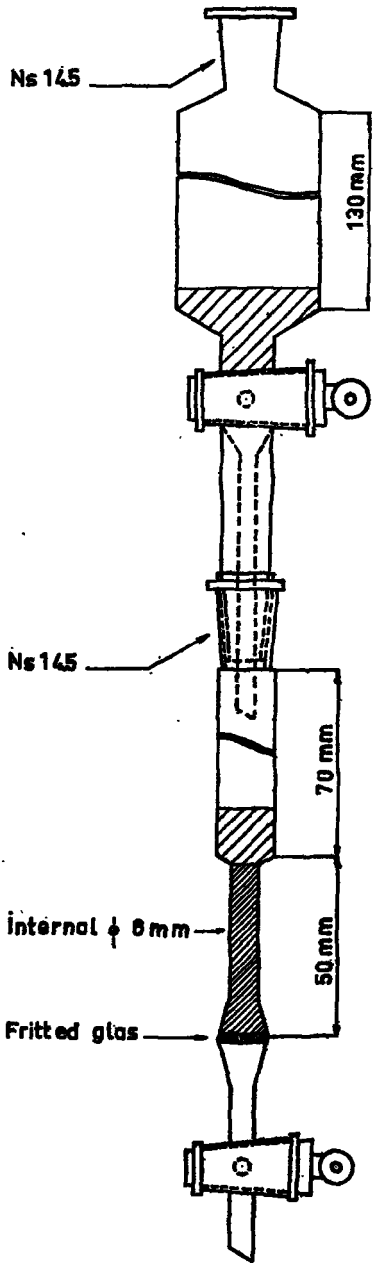

FIGURE 18:

Ion-exchange column for the separation of ^{210}Pb from its decay-products.

with the single-channel analyzer (2.2.). As the first ml's from an old column contained some $^{210}Bi^{3+}$ and $^{210}Po^{4+}$, they were always returned to the latter.

More than 99.8 percent of the ^{210}Bi was removed from 6 n. HCl but 25 to 30 percent of the ^{210}Pb were also retained in the column. For 9 n. HCl these figures were 99 and 2 percent respectively. The influence of carriers was negligable. With 9 n. HCl ^{210}Po was found for 10 percent in the eluate; this isotope does not influence the measurements of both ^{210}Pb and ^{204}Tl to a large extent, however.

The "GM-percentage" $\left(\frac{\text{c/m M6-tube} = {}^{210}Bi}{\text{c/m 47 Kev.Maximum} = {}^{210}Pb} \times 100\right)$ of a fresh eluate was about 4 with our detectors and increased with 2 units per hour owing to the formation of new ^{210}Bi. Another increase of the GM-percentage of the aqueous layer took place during the exchange-procedure between Pb and Tl-[D.D.C.]$_1$. An increase of 1.8 $\pm$ 1.0 units was found as a mean of 10 experiments with different amounts of lead and inactive Tl-(D.D.C.)$_1$. The value of 1.8 has of course only a practical meaning, it is obviously due to the difference between the rates of exchange of Bi and Pb with Tl-(D.D.C.)$_1$.

Evidently the carrier-free bismuth exchanged with Tl-[D.D.C.]$_1$ at a lower rate than the lead, which is not in accordance with their places in the sequence of affinities and is probably due to the smaller concentration of the Bi. (An uncertainty of 1 unit was introduced in the "GM-percentage" by this "rise-through-exchange"; the total standard-deviation of the "GM-percentage" was in practice about 1.6 units.)

It could be shown through exchange-experiments with excess lead instead of excess Tl-(D.D.C.)$_1$, that no rise of the "GM-percentage" exists when only 15 percent of the lead is exchanged. Obviously about 15 percent of the bismuth-isotope are exchanged in all experiments.

6.3. *Measurement of the radioactivity of ^{204}Tl and ^{210}Pb* (see also 2.2.).

Measurement of ^{204}Tl in the aqueous layer gives — after substruction of the blank — the amount of thallium which is exchanged with lead. The GM-tube was preferred for this measurement as the correction-factor for ^{210}Pb ("GM-percentage") was more favourable than with our scintillation-detector (see also 5.3.).

Direct measurement of the exchanged percentage of lead in the organic layer had to be avoided as this layer contained as a rule a large excess of ^{204}Tl. Thus the aqueous ^{210}Pb was measured. As about 2 percent of the lead are adsorbed to the wall during the exchange-procedure, the percentage of aqueous ^{210}Pb is substracted from 98 to find the exchanged percentage.

As indicated above, the measurement of ^{204}Tl had to be corrected with the help of the "GM-percentage" of ^{210}Pb; on the other hand the measurement of ^{210}Pb had to be corrected for ^{204}Tl (this "channel-percentage" was usually about 5 to 7).

With all measurements at least 1000 counts were registred. Including the errors in the correction-factors the total error was about 5 percent.

6.4. *Procedure.*

The procedure is given for a sample containing from 0.1 to 0.6 μgrams of lead, less than a 0.1-fold quantity of bismuth and thallium, a 6.000-, 8.000- and 28.000-fold amount of Fe, Cu [or Ag, or Hg] and Zn [or Mn etc.] respectively, and less than a 15.000-fold excess of PO_4^{3-} [or $P_2O_7^{2-}$] (all in gramequivalents; see also 6.5.).

Daily standardisations of ^{204}Tl and ^{210}Pb. The ^{204}Tl-solution (a) is standardised and the "channel-percentage" is determined. The ^{210}Pb-solution is purified (6.2.7.) and standardised. It is then neutralised with concentrated ammonia on bromocresol-purple (pH 1.0). The volume is corrected to a 2-fold of the original ^{210}Pb-solution. The "GM-percentage" is determined during the measurement of the samples [1.8 is added to this value when the exchanged percentage of the lead is larger than 20].

Weekly determination of the $Tl[D.D.C.]_1$-blank. The ^{204}Tl-$(D.D.C.)_1$-solution (a) (6.2.4.) is checked on losses by adsorption to the wall, an aliquot containing 1.5 μgrams of ^{204}Tl is brought into a tube (figure 17) and the volume is adjusted with chloroform to 1 ml. One ml. of KCN-tartrate (b) is added, the tube is shaken during 6 minutes at 900 p.p.m. and centrifuged during 1 minute (1000 r.p.m.; Ø 25 cm).

The aqueous layer is pipetted off and the organic layer is rinsed with 1 ml. of KCN-tartrate (b). The whole process is then repeated; this second time the assembled aqueous layers are diluted to 10 ml. with the acid BiTlPb-carrier and measured in the GM-tube.

Determination of 0.1 to 0.6 μgrams of lead. The sample is dissolved in HNO_3 and/or HCl and evaporated (3.4.) to about 0.2 ml.; then 0.02 ml. of the neutralised ^{210}Pb-solution are added, the mixture is neutralised with concentrated ammonia on bromocresol-purple and the volume is adjusted to 0.5 ml.

The Tl-$[D.D.C.]_1$ blank is now carried out as described above, but, instead of the third 1 ml.-portion of KCN-tartrate (b), 0.5 ml. of KCN-tartrate (a) is added. Then the neutralised *sample* is

pipetted in this KCN-solution and the further procedure is carried out as given above. To the assembled aqueous layers only 1 ml. of the acid carrier is added however; ^{210}Pb is measured in 1 ml. of the mixture. The remaining volume is measured by suction in a pipet, diluted with the carrier to 10 ml. and the radioactivity of ^{204}Tl is measured in the GM-tube.

The calculation is carried out as indicated in 6.3. The ^{204}Tl-value gives the *amount* of lead exchanged, the ^{210}Pb-value gives the *percentage* of lead exchanged. The amount of lead found in this way is corrected for the lead in the ^{210}Pb [0.012 μgrams].

For the *determination of 0.6 to 3.0 μgrams of lead* the same

Table 14

Determination of 0.05 to 0.6 μgrams of lead with ^{204}Tl-(D.D.C.)$_1$

1	2	3	4	5	6	7	8	9	10	11
Nr. of experiment	Lead added -in mμ-grams*	^{204}Tl used as ^{204}Tl-(DDC) -in mμ-grams	Lead exchanged -in %	^{204}Tl exchanged-in mμ-grams*	Lead exchanged-in mμgr. (from column 5)	Lead found -in mμ-grams (from columns 4 and 6)	Mean value of column 7	Standard deviation-in mμ-grams	Error of mean -in %	Standard deviation -in %
1	600	1500	43	476	245	570				
2	,,	,,	42	496	255	608	593	46	— 1	8
3	,,	,,	40	506	260	650				
4	,,	,,	48[5]	514	264	545				
5	300	,,	26	176	90	346				
6	,,	,,	27	153	78	289	322	24	+ 7	7[5]
7	,,	,,	30	190	97	323				
8	,,	,,	38	245	125	328				
9	100	,,	26	56	28[5]	110				
10	,,	,,	45	85	43	95[5]	96[5]	9[5]	— 3[5]	9[5]
11	,,	,,	45	80	40[5]	90				
12	,,	,,	29	51	26	90				
13	150	500	18	50	25[5]	142				
14	,,	,,	20	58	29[5]	148	142	10	— 5[5]	7[5]
15	,,	,,	21	62	31[5]	150				
16	,,	,,	24	60	30[5]	127				
17	100	,,	20	44	22[5]	112				
18	,,	,,	24	53	27	112	112	9[5]	+ 6	9
19	,,	,,	28	53	27	96[5]				
20	,,	,,	30	55	28	93[5]				
21	50	,,	18	19	9[5]	53				
22	,,	,,	22	24	12	54[5]	51	6	+ 2	12
23	,,	,,	26	27	14	54				
24	,,	,,	24	20	10	41[5]				

* Including the lead in the ^{210}Pb.
[5] After substraction of the blank.

amount of ^{210}Pb but instead of 1.5 μgrams of ^{204}Tl as Tl-(D.D.C.)$_1$ now 6 μgrams are used. A shaking-time of 3 minutes is sufficient here.

For *0.05 to 0.15 μgrams of lead* only 0.005 ml. of the neutralised ^{210}Pb-solution are added and only 0.5 μgrams of ^{204}Tl are used.

6.5. *Results.*

The values of the *blanks* are italicized in table 13.

Some *results with standard amounts of lead* are given in table 14 and figures 19 and 20. In the table the standard amounts of lead

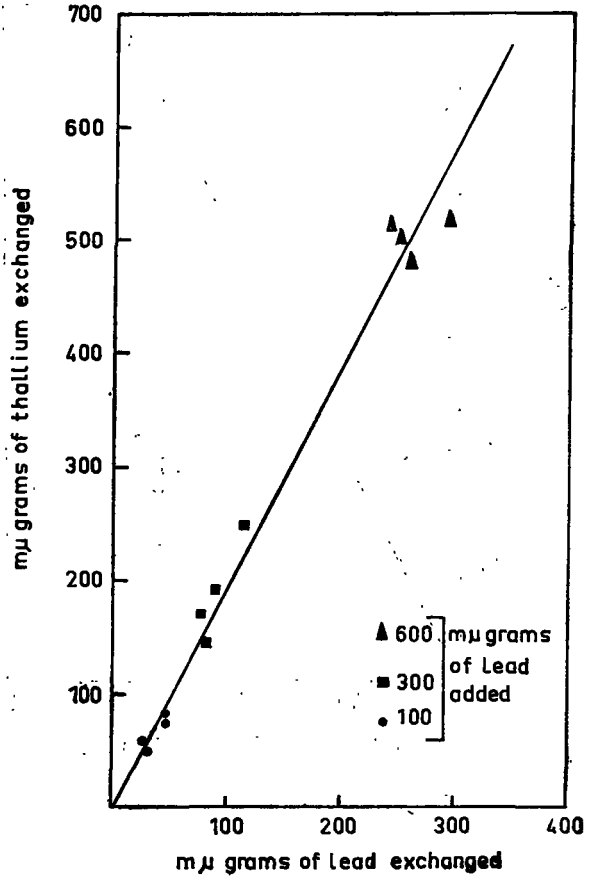

FIGURE 19:
Determination of 100, 300 and 600 mμgrams of lead with 1.5 μgrams of ^{204}Tl (I) as ^{204}Tl-(D.D.C.)$_1$: mμgrams of lead and thallium exchanged, and theoretical curve.

added are compared with the values calculated from the exchanged amounts of thallium and the exchanged percentages of lead. In the figures the quotients of the exchanged amounts of thallium and lead are compared with the theoretical curve.

As to the interference of foreing ions: thallium and bismuth can be removed by anionexchange from 9 n. HCl. [5]) This was shown

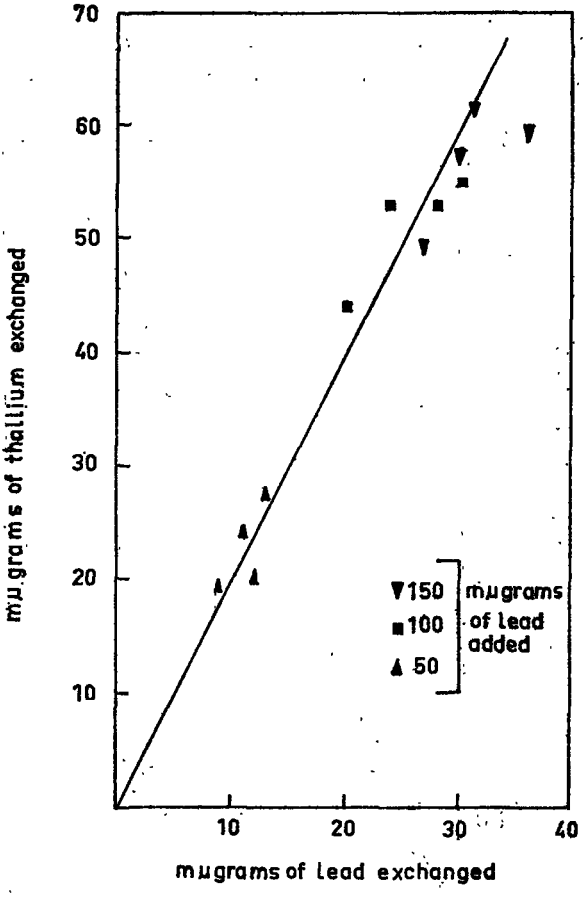

FIGURE 20:

Determination of 50, 100 and 150 mμgrams of lead with 0.5 μgrams of ^{204}Tl (I) curve.

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[5]) Evaluation of the effect of Bi and Tl by a „blank" with E.D.T.A. [see as ^{204}Tl-(D.D.C.)$_1$: mμgrams of lead and thallium exchanged, and theoretical chapter 5] is impossible here.

for bismuth in 6.2.7. already. The adsorption of thallous-ions to the resin is small (K r a u s and N e l s o n – 1954); oxidation – with a trace of bromine — to the thallic form is required; only 1 to 2 percent of μgram amounts of $^{204}Tl^{3+}$ were found in the eluate of the column.

The interference of al other ions is moderate: a 6.000-, 8.000-, 28.000- and 15.000-fold excess of the ions Fe^{3+}, Cu^{2+}, Zn^{2+} and PO_4^{3-} respectively resulted in less than a 6 percent error; Hg^{2+} and Ag^{+} behaved like Cu^{2+}; Mn^{2+} was equivalent to Zn^{2+} and pyrophosphate to phosphate.

6.6. *Discussion.*

6.6.1. *Stoechiometry of the exchange.*

From column 10 of table 14 it is clear that equal amounts of both metals are exchanged. The mean values of lead found have an error of about 1 percent.

Factors influencing the ratio Pb/Tl in a negative way are:

(1) The lead-content of the KCN (less than 10 mμgrams of lead).
(2) The admixture of part of the chloroform layer with the aqueous solution (about 0.3 percent).
(3) The backextraction of part of the Pb-$(D.D.C.)_2$ formed, during the exchange-procedure (probably less than 2 percent [6]).

Factors influencing the ratio Pb/Tl in a positive way are:

(4) The admixture of part of the aqueous layer with the chloroform (about 0.7 percent).
(5) The extraction of lead-ions with D.D.C. resulting from the Tl-blank; or with the free D.D.C. present in the older preparations of the Tl-$(D.D.C.)_1$ (probably less than 6 percent [7]).
(6) The extraction of thallium-ions with the aqueous D.D.C. (in excess of the same effect with the blank).
(7) For a small excess of Tl-$(D.D.C.)_1$ over Pb: the decrease in the actual blank as a result of the exchange of part of the Tl-$(D.D.C.)_1$ with lead-ions.

The effect of factor (7) is illustrated by a duplicate experiment with 0.7 μgrams of lead: 0.290 and 0.320 μgrams of lead were exchanged

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[6]) As found by experiments with ^{210}Pb-$(D.D.C.)_2$ in the absence of ^{204}Tl-$(D.D.C.)_1$, $^{210}Pb^{2+}$-ions and $^{204}Tl^{+}$-ions.

[7]) As found in some experiments with $^{210}Pb^{2+}$-ions and small amounts of D.D.C., in the absence of ^{204}Tl-$(D.D.C.)_1$

whereas only 0.460 and 0.440 μgrams of thallium [excluding the blank] were found in the aqueous layer respectively. Evidently the amount of Tl as Tl-[D.D.C.]$_1$ [1.5 μgrams minus the first blank] was too small here.

6.6.2. *Blank value. Radio-decomposition.*

The factors influencing the blank were discussed already in 6.2.5. The radiochemical decomposition was the only factor which could not be controlled.

Comparison of the specific activities of the two ^{204}Tl-(D.D.C.)$_1$ solutions used in 6.2.5.7. (10 and 330 mμcuries per μgram of Tl respectively) with the solution used in procedure 6.4. (40 mμc/μgram) shows that the radio-decomposition of the latter can be supposed to be low. A preparation of ^{204}Tl-(D.D.C.)$_1$ with a specific activity of 330 mμc/μgram, proved to be stable for only a few days. [8])

6.6.3. *Standard-deviation and limit of sensitivity.*

The standard-deviation is due to:

(1) The error made in the measurement of ^{204}Tl and ^{210}Pb, including the variations in the correction-factors; in total approximately 5 percent.

(2) The error made through variations in the blank-value; a variable contribution (depending on the excess Tl-(D.D.C.)$_1$ and the exchanged percentage of the lead) of about 2 to 10 percent.

Comparison with the experimental values shows that other contributions are probably made by variations in the factors of 6.6.1. With decreasing amounts of lead and ^{204}Tl-(D.D.C.)$_1$ the positive factors (1) and (3) of 6.6.1. and the negative factor (7) will probably limit the sensitivity of the method together with

(a) the increasing standard-deviation of the blank (in percentage)

(b) the higher excess of Tl-(D.D.C.)$_1$ required for a reasonable exchange of the lead and

[8]) To diminish the radiodecomposition, a "diluting-substance" is required. This should either be (1) a substance containing SH-groups [and in addition: soluble in $CHCl_3$ *and not* extracting lead], or (2) a substance able to convert the nuclear radiation into vibrational- or/and rotational-energy [f.i. benzol], or finally (3) a porous substance or finely divided powder, insoluble in $CHCl_3$, able to absorb the β-radiation of ^{204}Tl.

Preliminary experiments, with a system combining (2) and (3), were carried out. A distinct enhancement of the stability was observed.

(c) the increasing influence of either the counting-error or the radiochemical decomposition (6.6.2.).

The error made by uncertainties in the amount of lead present in the ^{210}Pb is very small. Theoretically the specific activity of the latter can be increased with a factor of 100.

Comparison with chapter 5 shows that, without a considerable increase in the standard-deviation, the sensitivity is doubled.

A further advantage of the method described here is the simpler procedure: about 40 minutes (30 working-minutes) are required for a duplicate as compared with 90 minutes (50 working-minutes) in chapter 5.

An advantage of the method of chapter 5 is that no alcohol-envelopes are required when the temperature goes up: the stability of the Pb-$(D.D.C.)_2$ is larger than that of the Tl-$(D.D.C.)_1$; in addition the excess $^{204}Tl^{3+}$ will probably react with traces of D.D.C. resulting from the decomposition of the lead-complex.

6.7. *Conclusion.*

The exchange of aqueous lead-ions with the thallium from a ^{204}Tl-$[D.D.C.]_1$-solution in chloroform is suitable for the analysis of 0.05 μgrams of lead — or 0.5×10^{-9} gramequivalents — with a standard-deviation of 12 percent.

Addition of KCN and NaOH [pH14] to the sample-solution makes the technique highly selective for lead. The method is simple and inexpensive; it can be applied to other cations by a proper choice of (1) the D.D.C.-complex, (2) the pH and (3) masking agents.

APPENDIX 1

Experiments on the chromatografic separation of metal-D.D.C. complexes on Al_2O_3-papers.

According to Al Mahdi and Wilson (1951; 1953), separations of metal-D.D.C. complexes are possible on a column of aluminiumoxide. For our purpose, where D.D.C.-^{35}S had to be used, these columns were not pratical however, as the soft β-radiation of ^{35}S would not penetrate either column or glasswall. Selective elution was considered to be too elaborate for a routine method of analysis.

Separation on paper was tried as an alternative. *Aluminium-oxide-papers* were prepared by the methode of Flood (1940; 1949). As Al Mahdi and Wilson indicated already, and was confirmed by our own experiments, heating of the Al_2O_3 to 250°C (for 3 hours) is essential for the separation. The papers were dried by heating them in a high vacuum over P_2O_5 to 125°C (which is about the highest temperature the papers will stand). After this treatment, the papers were kept in a vacuum-desiccator over silicagel. On further drying (under decomposition of the structure) they proved to contain still 10—20 percent of water, calculated on the amount of Al_2O_3. According to Prettre et.al. (1953) this percentage is still too high and should be lowered to 6 or 9 percent by heating in a high vacuum to about 200°C. As a consequence, the separations carried out with these papers were unsatisfactory. Other papers containing Al_2O_3 were prepared by successive dippings in (1) a solution of anhydric $AlCl_3$ in absolute alcohol and (2) an alcoholic solution of ammoniagas. The results with these papers were even worse however; obviously the oxide toke up moisture from the atmosphere.

Glasspapers (Schleicher and Schüll Company) were *covered with aluminium-oxide* — following again the procedure of Flood — heated in vacuum to 250°C for 3 hours and kept in a desiccator over silicagel. Results with these papers were in accordance with the experiments of Al Mahdi and Wilson. The papers soon became brittle on keeping however, which made the experiments a nuisance.

Further experiments were carried out with *papers sprayed with a 2 percent suspension of Al_2O_3* — previously heated on 250°C for 3 hours — in absolute alcohol. The papers were dried at 90°C in a vacuum before and after the spraying. Results with these papers were reasonable, but the oxide did not adhere very good to the paper, which was especially dangerous with a view to the radioactive substances to be chromatographed.

In conclusion: The experimental difficulties, encountered in the realisation of the chromatografic separation of metal-D.D.C. complexes on Al_2O_3-papers, could not be solved.

APPENDIX 2

Application of larger volumes of (acid) aqueous solutions to a paper.

Administration with a pipet or an automatic micropipet ("Agla") was found to be inconvenient with regard to the time required to produce a small spot. A buret or a pipet with a fine capillary-end which needed no supervision (Merz - 1957), was more convenient in this respect. The procedure remained to be rather lengthy however; a föhn could be used to accelerate it but an infrared heater decomposed the paper slightly. With the föhn, choking-up of the fine capillary had to be watched.

The ring-dike of Ultée (1952) and the rectangular dikes of Wiegand and Schrank (1956) were found to be adequate for neutral solutions; 0.05 ml. per minute could be applied; with acid solutions some leakage of the dikes was observed however. The funnel-and-pin described by Urbach (1949) was found to be practical with organic-but not with aqueous-solutions. Above, all methods mentioned up to now have the disadvantage of a special arrangement to keep the paper in a horizontal position over longer periods of time.

An extra paper, either folded to a zigzag (van Erkelens - 1953) or left flat (von Euler - 1952), which can be dipped into the evaporation-dish and then has to be fixed in a fold of the chromatographic paper, does not have the latter disadvantage. It implicates a special set up in the chromatographic separation however.

In conclusion: No satisfactory procedure could be found for the application of larger volumes of (acid) aqueous solutione to a chromatografic paper.

APPENDIX 3

Metal-contents and purification of the paper.

Balston and Talbot (1952) claim an *ash-content* for the acid-washed Whatman papers of 0.008 percent — or 6 μgram ash per cm^2 — whereas the commonly used Whatman paper Nr. 1 is said to contain 0.07 percent ash. The Company "Machery and Nagel" (Düren, Germany) claims an ash-content of 0.005 percent in its best paper (No. 640 d.d.) and additionally gives the *composition* of the ash: 40% Fe_2O_3; 18% Al_2O_3; 25% CaO; 8% MgO and 5% SiO_2. Phosphate is also contained in filterpapers but lead is mentioned very seldom.

Pollard and McOmie (1953) do mention the presence of *lead* in filterpaper in their review on the methods of purification. They remark that the otherwise acceptable procedure in which the paper is washed with 8-hydroxyquinoline in ethanol is not very succesfull when lead is to be removed. As the pH of a paper wetted with water or ethanol is about 4.0 to 4.6, this can easily be understood from the pH-range (8.4—12.3) given by Kolthoff and Sandell (1952) for the reaction between lead and oxine. From their values it can be predicted, that *calcium and magnesium* will also not be removed with this procedure.

In general, two groups of cleaning-procedures are to be distinguished: the one, using complexing agents, is a.o. propagated by Pollard and McOmie (1951), the other, using acids in organic or aqueous solution, is recommended a.o. by Osborn (1949) and Hanes et.al. (1949). Most agree, that chromatografic rinsing is best policy.

Two different purity-tests were used in our experiments. In a first series of tests hydrogensulfide-^{35}S was used. Although the oxidation of the sulfides as described in 2.3.1. made the quantitative evaluation difficult, dependable limits could be found by comparison with spots containing known amounts of heavy metals.

Strips of paper were rinsed chromatografically in the descending way with 2 or 3 successive solvents, each rinsing lasting one night. The solvents were allowed to drip off. The papers were then dried in the chromatographic jar, neutralised and treated with $H_2{}^{35}S$ (see 2.3.1.).

Another series of papers was tested with phosphate-^{32}P. The cleaning-procedure was carried out as described above, most of the rinsings lasted only a few hours however. Spots of phosphate-^{32}P of 1 cm^2 were then placed on the paper and the excess was eluted with the borax-buffer of 3.2.2., so as to leave all metalphosphates including Ca- and Mg-phosphate, in the original spot (see table 3).

The results of both series are shown in table 15, and are given in equivalents of a two-valent metal with an atomic weight of 50 ($^{50}Me^{2+}$).

Table 15.

Metal-contents of washed Whatman Nr. 1 papers.

Nr. of experiment	Treatment	Residue in μgrams $^{50}Me^{2+}$ per cm^2		
		Tested with • $H_2{}^{35}S$; rinsings lasting one night	Tested with • $^{32}PO_4{}^{3-}$	
			Rinsings lasting one night	Rinsings lasting 3 hours
1	(A) 1% Oxine in 60% v/v Ethanol (B) Methanol	0.05	—	6.1
2	(A) 1% Benzoylacetone in Ethanol (B) Methanol	0.05	—	8.2
3	(A) 2n.Acetic acid (B) 1n.Ammonia (C) Water	0.04	—	0.4
4	(A) 1n.Nitric acid (B) 1n.Ammonia (C) Water	—	—	0.8
5	(A) 1n.Hydrochloric acid (B) 1n.Ammonia (C) Water	—	—	0.3
6	(A) 0.1n.Hydrochloric acid (B) 1n.Ammonia (C) Water	—	—	7.1
7	(A) 90% v/v Methanol 10% v/v conc. HCl (B) 90% v/v Methanol 10% v/v conc. Ammonia (C) 95% v/v Methanol 5% v/v Water	0.03	0.20	0.25
8	(A) 90% v/v Methanol 10% v/v conc. HCl (B) Ammonia vapor	—	—	0.4

• The figures given here are upper limits with a standard-deviation of 40 to 60 percent.

* Of these values 0.05 μgrams are due to the adsorption of phosphate-^{32}P to the paper (see 3.5.1).

The difference between to two tests is a (rough) measure for the sum of the amounts of residual calcium and magnesium. It is evident that a large amount of these ions is present in the oxine- and benzoylacetone-washed papers and consequently in the untreated paper as well. The total metal-content in the latter was found to be about 8.0 μgrams $^{50}Me^{22}+$ per cm^2 in this batch of paper, which is a factor of 2 larger than the figure given by the manufacturer. In another batch 5.2 μgrams were found. These results indicate that all metals in the paper have reacted completely with the phosphate (except Na, K etc. of course).

Hydrochloric-, nitric- or acetic-acid, either in water or in methanol (and 1 normal), are the only reagents which have removed calcium and magnesium to a considerable extent. For experiment 7 it can be calculated, that 0.25 — 0.03 — 0.05 = 0.17 and 0.20 — 0.05 = 0.12 μgrams of $^{50}Me^{2}+$ are equivalent with the sum of the Ca- and Mg-contents per square cm., for the two washing-methods (24 and 3 hours) respectively. According to the manufacturers 0.07 and 0.10 μgrams of Ca and 0.025 and 0.035 μgrams of Mg are present per cm^2 in the acid-washed papers of Machery and Nagel and of Whatman respectively

(when the composition of the ash given by the first manufcturer is accepted). The sum is equivalent to about 0.14 and 0.20 μgrams of $^{50}Me^{2}+$ and in good agreement with our results.

As an organic liquid has the advantage to dry up more quickly, *treatment 7 (methanol^{9}-HCl^{1}) was chosen for the purification.* The papers lost the last traces of acid very slowly; thus neutralisation was accomplished with (methanol-) ammonia, which is practically free of trace-metals and consequently does not spoil the result of the first rinsing. The ammonium-salt was removed with methanol^{95}-water^{5}. The composition of the last liquid was such as to reëstablish the water-content of the paper.

APPENDIX 4

Blank value of acid-washed paper, found with phosphate-^{32}P.

The equivalent of 0.3 μgrams of lead, found for the blank value of 1.0 cm^2-spots on acid-washed paper, is the total result of at least four influences.

(1) Less than 0.1 μgram originates from the lead-content of the reagents (with the exclusion of the nitric-acid-peroxyde mixture).

(2) Very small parts are due to the calcium-, magnesium- and (3) heavy-metal-content of the acid-washed paper.

As to the former metals: the sum of the Ca- and Mg-contents is calculated from table 15 to be equivalent to 0.17 μgrams $^{50}Me^{2+}$ per cm^2.[1]) According to table 3 less than 0.6 and 0.2 percent of these ions react with the phosphate respectively, thus their effect is always smaller than the equivalent of 0.004 μgrams of lead.

As to the H_2S-metals: the test of table 15 shows, that only 0.03 μgrams of $^{50}Me^{2+}$ are present per cm^2. As less than 1 percent of these heavy metals are retained at R_f 0.0 as their phosphates (table 3) this part of the blank is smaller than 0.0012 μgrams of lead, provided the heavy-metals of the paper behave normally in relation to phosphate. As certainly less than 10 percent of the heavy metals or less than the equivalent of 0.003 μgrams $^{50}Me^{2+}$ consist of lead (Zr, Th..), the whole contribution of the metal-content of the acid-washed paper to the blank is smaller than the equivalent of 0.004 + 0.0012 + 0.012 μgrams of lead per square cm.

Consequently the greater part of the blank, about 0.2 μgrams of lead or the equivalent of 0.05 μgrams of $^{50}Me^{2+}$ per cm^2, is due to the retention of part of the phosphate-^{32}P by the paper.[2])

Probably a reaction with the OH-groups takes place. According to Schönfeld et.al. (1950, 1951, 1952) and to Husemann and Weber (1942) chromatografic papers contain about 50 μequivalents of OH-groups per cm^2, which is a large excess over the remainder of our blank.

In fact, a reaction of the phosphate-^{32}P reagent with an excess of OH-groups does not explain the relatively large *variations* of the blank. When these variations are due to inhomogenities in the paper however, these inhomogenities must be smaller than 1 cm^2, as can be concluded from the blank-values in 3.4.1.

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[1]) Or better: 0.12 μgrams, when corrected for the adsorption of phosphate-^{32}P, which is found as a result of the present considerations however!

[2]) This value was substracted in table 15 under the assumption that this adsorption is also active at pH 8.0, where it is difficult to distinguish from the reactions of the metals (contained in the paper) with the phosphate reagent.

SUMMARY AND CONCLUSIONS.

The *purpose* of this work was: to test the applicability of some techniques, which use the tool of nuclear radiation, in trace-analysis and in the determination of traces of lead in particular.

In *chapter 1 a comparison was made* of (1) analysis with radioactive reagents (R.R.), (2) radio-exchange analysis (R.E.) (3) radio-tracer-analysis (R.T.), (4) isotope-dilution (I.D.) and (5) (neutron-)activation analysis (A.A.).

The last method is not very sensitive for lead: with a flux of 10^{13} neutrons per cm^2 per second an activation-time of 30 days is required for the determination of 0.1 μgrams of lead. Methods (3) and (4) (nearly) always require an additional classical chemical determination, which restricts the sensitivity in advance.

Methods (1) and (2) are in principle suitable for our purpose however. R.E. analysis (2) has some advantages over R.R. analysis (1): a radioactive synthesis is not required, radiochemical decomposition is to be feared less and the number of possibilities for the choice of the radio-isotope is enlarged. A survey of the literature showed that the use of both methods (1) and (2) (but especially of R.E. analysis) is still rather scarce.

In *chapter 2* some preliminary investigations were described on the use of several chemical systems in R.R. and R.E. analysis.

Most *R.R.methods* were carried out *in filterpaper* in order to enhance the concentrations of both components and hence the rate of the reaction. Filterpaper proved to have several drawbacks however.

The finely divided metalsulfides for instance, formed with $H_2{}^{35}S$ on a papergram of heavy metals, showed an abnormal sensitivity to traces of oxygen. A chain-reaction of the oxidation-product with new $H_2{}^{35}S$-molecules was the result. This made the method useless, at least for amounts of lead below 10 μgrams. Several other reagents showed a high adsorption to the paper, thus producing a high — and variable — blank.

At last two reagents were found which showed only a moderate adsorption: phosphate-^{32}P and sulfate-^{35}S. These reagents have the additional advantages of being commercially available and insensitive to radio-decomposition. Separation of the excess reagent was possible with the help of alcoholic buffers. *Phosphate-^{32}P was chosen* for our further work as it is more universal and in addition better measurable in the paper.

Cationexchangers loaded with $^{89}Sr^{2+}$ or $^{65}Zn^{2+}$, *and anion-exchangers loaded with* ^{65}ZnS, were applied in some preliminary experiments on the use of ionexchange-materials in *R.E.-analysis*, but were all found to be unsuitable.

With the exclusion of many other systems only the reaction: $M_1^+ + M_2$ (Diethyldithiocarbamate) $\rightleftarrows M_2^+ + M_1$ (D.D.C.) was investigated. Separation of ions and metal-complexes is possible here by extraction with an organic solvent. The direction *and* the rate of the reaction depend on the affinities of M_1 and M_2 to D.D.C. The exchange is made specific for lead, bismuth and thallium through the addition of KCN and NaOH up to pH 14. As Pb(II) stands between Tl(III) and Tl(I) in the sequence of affinities to D.D.C., ^{204}Tl can be used in two different R.E.-methods for lead.

The *R.R.-analysis with phosphate-^{32}P in filterpaper* was worked out in chapter 3 and 4. Selectivity for lead was obtained in *chapter 3* by the choice of the pH (4.4) and the addition of a masking-agent (8-hydroxyquinoline).

This method is simple but the possibilities are restricted: only a 20-fold excess of Mg(II), Ca(II), Sr(II) and Ba(II) and a 10-fold excess of most heavy metals is allowed, whereas traces of the metals: Cr (III), Bi(III), Ti(III), Ce (III and IV), Zr(IV), Th (IV) and U(VI) interfere already. In addition, the anions precipitating with lead at pH 4.4, as for instance: F^-, $P_2O_7^{2-}$, CrO_4^{2-}, SO_4^{2-} en PO_4^{3-} interfere in μgram quantities already.

The blank, which is mainly the result of the adsorption of part of the phosphate on the paper, restricts the sensitivity to 1 μgram of lead. The standard-deviation [about 15 % for 1 μgram of lead] is the result of this variable blank but especially of the adsorption of some phosphate-^{32}P to — or occlusion in — the precipitate. The composition of the latter probably is $PbHPO_4$.

The method is only applicable to other metals with a proper choice of the pH and of masking-agents.

In *chapter 4* selectivity for lead was obtained by a paperchromatografic separation previous to the application of the phosphate-^{32}P. The earthalcali-metals had to be masked with oxalate.

This method is slightly more elaborate but has the advantage that much larger amounts of foreign ions are allowed. Only Ba(II), Ce(III and IV) and Th(IV) interfere (with the paperchromatografic separation chosen here).

The same factors as mentioned above influence the blank and the standard-deviation [about 11 % for 1 μgram of lead]. Application to other metals is easily accomplished.

The *system chosen for R.E.-analysis* was worked out in chapter 5 and 6. In *chapter 5* excess D.D.C., KCN and tartrate were added to the sample and lead was extracted at pH 14 with CCl_4. It was then exchanged with ^{204}Tl(III) by shaking the extract with an alcaline solution containing an excess $^{204}Tl^{3+}$-ions. The amount of ^{204}Tl in the CCl_4 was taken as a measure for the lead.

As a large excess of both D.D.C. and ^{204}Tl is required for a reasonable efficiency of the process (about 90 %), traces of both reagents are retained in the CCl_4, which results in a troublesome blank (D.D.C. reacts with excess ionic ^{204}Tl). To confine this blank to reasonable proportions the procedure has to be lengthened with two backextractions of D.D.C. The standard-deviation (1.1 % for 0.1 μgram of lead) is the result of the variable blank and of the variable efficiency of the process.

The limit set by the specific activity of the ^{204}Tl is not reached; smaller amounts of lead can probably de determined with the help of an additional isotope dilution with ^{210}Pb.

In *chapter 6* tartrate, KCN and NaOH were added to the sample-solution and lead was exchanged directly with ^{204}Tl-$(D.D.C.)_1$ which was kept in a stock-solution in $CHCl_3$. The rate of this reaction is still smaller than the one used above; in order to determine the output of the exchange reaction isotope dilution with ^{210}Pb is required for microgram amounts of lead already. The amount of ^{204}Tl found in the aqueous layer (after substraction of the blank) is but a measure for the amount of lead which is exchanged.

A blank results from the spontaneous decomposition of the ^{204}Tl-$(D.D.C.)_1$. As complete exchange is not required here, the excess ^{204}Tl-$(D.D.C.)_1$ and thus the blank can be kept relatively small. The determination of 0.05 μgrams of lead proves to be possi-

ble with a standard-deviation of 12 percent. The sensitivity is restricted by: (1) variations in the blank, (2) variations in the measurement of ^{204}Tl and ^{210}Pb and (3) the difficult interpretation of the blank: the conversion of part of the $Tl(D.D.C.)_1$ into $Pb(D.D.C.)_2$ will diminish the blank in an unknown way. The limit set by the specific-activity of our ^{204}Tl is not quite reached.

Only bismuth and thallium interfere with the determinations of chapter 5 and 6. These interferences can easily be prevented however, or accounted for by the addition of E.D.T.A.

The R.E.-analysis used here for lead is in principle applicable to other trace elements as well. Apart of the choice of the pH and masking agents, the sequence of affinities to D.D.C. can be applied for this purpose. The method of chapter 6 is in general to be preferred.

Comparison of our results with the literature shows:

(1) our R.R.-analysis of lead (in paper) is about 10 times as sensitive as the R.R.-analysis of Langer (1941) and Ishimori (1957), both carried out in solution.

(2) our R.R.-analysis of lead has the same sensitivity as the spectrophotometric method with dithizon [Mc.Cord and Zemp (1955), Gage (1955), Addink (1956) and „Analytical methods committee" (1959)].

(3) the sensitivity of our R.E.-analysis of lead (in solution) is comparable with the R.E.-analysis of Suzuki (1959) — also carried out in solution — but 10 times as sensitive as the method of Troitskii (1958) who applied the exchange on a solid-liquid interface to analysis.

(4) our R.E.-analysis of lead is 3 to 4 times as sensitive as the spectrographic method [Heggen and Strock (1953), Addink (1956), Pfeilsticker (1956) and Mitchell 1957)]; its sensitivity is matched only by the massspectrometric technique (Urech — 1958). The sensitivity of the polarografic method is between that of spectrophotometry and spectrography (Mayer and Schweda — 1956).

As only simple tools are required for the methods described here, it may be claimed that both R.R.- and R.E.-analysis are usefull methods for the trace analysis of lead.

In addition it has been made probable that the principles applied here do have a more general applicability in trace analysis.

SAMENVATTING EN CONCLUSIES.

Het *doel* van dit proefschrift was: na te gaan in hoeverre enkele methodieken, die gebruik maken van kernstraling, een bijdrage kunnen leveren tot de sporen-analyse in het algemeen en de bepaling van sporen lood in het bijzonder.

In *hoofdstuk 1 werden de volgende technieken vergeleken:* (1) analyse met radioactieve reagentia (R.R.), (2) radiouitwisselings-analyse (R.E.), (3) radio-tracer-analyse (R.T.), (4) isotopenverdunning (I.D.) en (5) (neutronen-)activeringsanalyse (A.A.).

De laatstegenoemde methode bleek voor lood niet erg gevoelig te zijn: met een flux van 10^{13} neutronen per cm^2 per seconde is een activerings-tijd van 30 dagen vereist om 0.1 μgram lood te bepalen. Voor de technieken (3) en (4) is (bijna) altijd een additionele, klassiek chemische bepaling noodzakelijk die de gevoeligheid reeds van te voren beperkt.

Methode (1) en (2) zijn in principe bruikbaar voor het gestelde doel. Methode (2) bezit verschillende voordelen boven methode (1): een radioactieve synthese is niet vereist, radiochemische ontleding behoeft minder te worden gevreesd en het aantal mogelijkheden voor de keuze van het radio-isotoop is belangrijk groter.

Een overzicht van de literatuur toonde aan, dat het gebruik van beide technieken (maar vooral van de R.E.-analyse) nog opvallend schaars is.

In *hoofdstuk 2* werd de bruikbaarheid van verschillende chemische systemen voor de R.R.- en R.E.-analyse oriënterend experimenteel onderzocht.

Praktisch alle *R.R. analyses* werden uitgevoerd in *filtreerpapier* teneinde de concentraties der componenten, en daardoor de reactiesnelheden, te vergroten. Helaas bleken aan het gebruik van filtreerpapier enkele bezwaren te kleven. Zo trad in het geval van een reactie der metalen met $H_2{}^{35}S$ een onverwacht snelle *oxidatie* der sulfides op. Deze leidde tot de vorming van sulfaten, dus tot een „kettingreactie", waardoor de methode onbruikbaar werd (althans voor hoeveelheden lood kleiner dan 10 μgram). Verschillende andere

reagentia bleken sterk door het papier te worden *geadsorbeerd*, waardoor een hoge- en variabele-blanco ontstond.

Ten slotte werden evenwel twee reagentia gevonden waarbij de adsorptie redelijk laag is: fosfaat-^{32}P en sulfaat-^{35}S. Deze reagentia hebben bovendien het voordeel commercieel verkrijgbaar te zijn en geen radiochemische ontleding te vertonen. Afscheiding van de overmaat reagens bleek mogelijk met behulp van alcoholische buffers. Onze keuze voor een nader uit te werken R.R.-methode viel op het fosfaat-^{32}P vanwege (1) de betere meetbaarheid van ^{32}P in papier en (2) de bruikbaarheid van fosfaat voor de precipitatie van ook andere sporenelementen.

Wat de *R.E.-analyse* betreft bleek uit de experimenten van hoofdstuk 2, dat uitwisselingsreactie aan *kationwisselaars* beladen met $^{89}Sr^{2+}$ en $^{65}Zn^{2+}$, evenals reacties aan *anionwisselaars* beladen met ^{65}ZnS, niet bruikbaar waren voor het gestelde doel.

Met terzijde laten van een groot aantal andere mogelijkheden werd hier nog slechts onderzocht: *de uitwisseling tussen een metaalion (M^{+}_1) en de metallische component (M_2) van een metaaldiaethyldithiocarbaminaat (M_2-D.D.C.)*, waarbij de kwantitatieve scheiding van ionen en metaal-D.D.C.-complexen gemakkelijk door extractie met een organisch oplosmiddel tot stand kon worden gebracht. De reactiesnelheden zijn hierbij vooral afhankelijk van de plaats van M_1 en M_2 in de affiniteits-reeks tot D.D.C. Door toevoegen van loog (pH 14) en KCN aan de waterphase wordt de uitwisseling specifiek voor Pb, Tl en Bi. Daar het ion Pb^{2+} in de affiniteitsreeks tot D.D.C. tussen Tl^{3+} en Tl^{+} in staat, zijn met het isotoop ^{204}Tl twee verschillende loodbepalingen mogelijk.

De R.R. analyse met fosfaat-^{32}P in fitreerpapier werd uitgewerkt in de hoofdstukken 3 en 4. In *hoofdstuk 3* werd selectiviteit voor lood verkregen door de keuze van de pH (4.4) en door het toevoegen van een maskeringsmiddel (8-hydroxy quinoline).

Deze methode is eenvoudig maar de mogelijkheden zijn beperkt: slechts een 20-voudige overmaat van de metalen Mg(II), Ca(II), Sr (II) en Ba(II) en een 10-voudige overmaat van de meeste andere metalen is toegestaan (in gramaequivalenten) terwijl zelfs sporen van de metalen: Cr(III), Bi(III), Ti(III), Ce(III en IV), Zr(IV), Th(IV) en U(VI) onoverkomelijke moeilijkheden opleveren. Bovendien storen anionen die met lood bij pH 4.4 precipiteren, zoals:

F^-, $P_2O_7^{2-}$, CrO_4^{2-}, SO_4^{2-} en PO_4^{3-}, reeds in zeer geringe hoeveelheden.

De blanco blijkt vooral te worden veroorzaakt door de adsorptie van een deel van het reagens aan het papier. Dit beperkt de gevoeligheid tot 1 μgram lood.

De standaard-deviatie [circa 15 % voor 1 μgram lood] wordt veroorzaakt door de variabele blanco maar vooral door de adsorptie of occlusie van een deel van het reagens aan (in) het lood-fosfaat, waarvan de samenstelling $PbHPO_4$ blijkt te zijn.

De methode is slechts toepasbaar op andere metalen bij een goed aangepaste keuze van pH en maskeringsmiddel(en).

In *hoofdstuk 4* werd selectiviteit verkregen door de meeste storende metalen vóór de toevoeging van het reagens papierchromatografisch van het lood te scheiden. De storingen van de in het chromatogram niet ruimtelijk gescheiden aardalkali-metalen werden opgeheven door maskering met oxalaat.

Deze techniek is wat ingewikkelder maar heeft het voordeel dat veel grotere hoeveelheden van vreemde ionen toelaatbaar zijn. Slechts de metalen Ba(II), Ce(III en IV) en Th(IV) storen bij de gekozen papierchromatografische scheiding nog in belangrijke mate.

De blanco-waarde, de standaard-deviatie [circa 11 % voor 1 μgram lood) en de gevoeligheid worden door dezelfde factoren beheerst als boven beschreven. Toepassing van de techniek op andere metalen is eenvoudig.

Het voor de *R.E.-analyse gekozen systeem* werd verder uitgewerkt in de hoofdstukken 5 en 6. In *hoofdstuk 5* werd het lood, na toevoegen van overmaat D.D.C., KCN en tartraat, bij pH 14 met CCl_4 geëxtraheerd, waarna het werd uitgewisseld met $^{204}Tl(III)$ door schudden met een alcalische $^{204}Tl(III)$ oplossing. De hoeveelheid ^{204}Tl in de CCl_4 vormde een maatstaf voor het lood.

Doordat een grote overmaat van D.D.C. zowel als van $^{204}Tl^{3+}$ is vereist om het proces althans voor ongeveer 90 procent te laten verlopen, komen sporen van deze beide reagentia in de CCl_4 laag terecht, wat een storende blanco tengevolge heeft. (vóór D.D.C.: na reactie met ionisch ^{204}Tl). Het inperken van deze blanco vereist enkele extra moeite. De variatie in de blanco *en* de variabele efficientie van het proces bepalen de standaard-deviatie (11 % voor 0.1 μgram lood).

De grens gesteld door de specifieke activiteit van het ^{204}Tl is hiermee niet bereikt; geringere hoeveelheden lood zijn wellicht te bepalen door een additionele isotopen verdunning met ^{210}Pb.

In *hoofdstuk 6* werd het lood, na toevoegen van tartraat, loog en KCN, direct uitgewisseld met een (in voorraad gehouden) oplossing van $^{204}Tl(D.D.C.)_1$ in chloroform. Deze reactie verloopt echter *nog* trager dan de boven beschrevene, zodat het — ter bepaling van het uitwisselingsrendement — reeds voor microgram-hoeveelheden lood nodig is isotopen verdunning met ^{210}Pb toe te passen. De hoeveelheid ^{204}Tl in de waterlaag vormt immers (na aftrekken van de blanco) slechts een maatstaf voor de *uitgewisselde* hoeveelheid lood.

Door de spontane ontleding van $^{204}Tl(D.D.C.)_1$ ontstaat een blanco. Aangezien volledige uitwisseling hier niet vereist is, kan de overmaat $^{204}Tl(D.D.C.)_1$, en dus de blanco, relatief klein worden gehouden. Daardoor blijkt hier de bepaling van 0.05 μgram lood met een standaard-deviatie van 12 procent nog mogelijk. De gevoeligheid wordt beperkt door de samenwerking van in hoofdzaak 3 factoren: (1) variaties in de $^{204}Tl(D.D.C.)_1$-blanco, (2) fouten bij de meting van ^{204}Tl en ^{210}Pb, en de onderlinge correctie factoren, en (3) onzekerheid over de interpretatie van de ^{204}Tl-$(D.D.C.)_1$-blanco: deze daalt namelijk indien een gedeelte van het thallium-complex in $Pb(D.D.C.)_2$ is omgezet. De grens gesteld door de specifieke activiteit van ons ^{204}Tl is dichter benaderd, maar nog niet geheel bereikt.

Slechts bismuth en thallium storen de bepalingsmethoden van hoofdstuk 5 en 6 in belangrijke mate. Deze storingen zijn bijvoorbeeld te verdisconteren door de toevoeging van E.D.T.A. Ook is afscheiding van het lood door anionwisseling aantrekkelijk.

De hier voor lood uitgewerkte R.E.-analyses zijn door een aangepast gebruik van (1) de pH, (2) maskeringsmiddelen en (3) de affiniteitsreeks tot D.D.C., ook toepasbaar op andere metalen.

De in hoofdstuk 6 beschreven modificatie verdient daarbij waarschijnlijk altijd de voorkeur.

Vergelijking van onze resultaten met de literatuur leert:

(1) onze R.R.-anlyses van lood (in papier) zijn meer dan 10 maal zo gevoelig als de R.R.-analyses van L a n g e r (1941) en I s h i m o r i (1957), welke beiden in oplossing werden uitgevoerd.

(2) onze R.R.-analyses van lood bezitten ongeveer dezelfde ge-

voeligheid als de spectrofotometrische bepaling van lood met dithizon [Mc.Cord en Zemp (1955), Gage (1955), Addink (1956) en „Analytical methods committee" (1959)].
(3) onze R.E.-analyses van lood (in oplossing) zijn wat hun gevoeligheid betreft vergelijkbaar met de R.E.-analyses van Suzuki (1959), die eveneens in oplossing werden uitgevoerd, maar meer dan 10 maal zo gevoelig als de methode van Troitskii (1958),
(4) onze R.E.-analyses van lood zijn 3 tot 4 maal zo gevoelig als de spectografische methode [Heggen en Strock (1953), Addink (1956), Pfeilsticker (1956) en Mitchell (1957)] en worden in dit opzicht slechts geëvenaard door de massa-spectrografische techniek (Urech — 1958). De polarografische analyse ligt qua gevoeligheid tussen de spectrofotometrische en de spectrografische in (Mayer en Schweda — 1956).

Aangezien voor de hier beschreven methoden slechts eenvoudige hulpmiddelen vereist zijn, terwijl men van de meetapparatuur voor radioactieve straling in de naaste toekomst een grote verbreiding mag verwachten, is hiermee aangetoond dat zowel R.R.- als R.E.-analyse een bijdrage van betekenis kunnen leveren voor de sporenanalyse van lood.

Verder is aannemelijk gemaakt, dat de hier toegepaste principes een meer algemene bruikbaarheid bezitten.

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