# RADIOMETRIC TRACE ANALYSIS OF COBALT* WITH DIETHYLDITHIOCARBAMATE-$^{35}$S, OR $^{203}$Hg

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(Received March 30th, 1961)

## INTRODUCTION AND PRELIMINARY EXPERIMENTS

BODE[1], and BODE AND TUSCHE[2] found the extraction of cobalt with diethyldithiocarbamate (DDC) to be effective up to pH 14. The further behaviour of cobalt was found to be exceptional: the extraction was prevented by cyanide, EDTA or reducing agents, but the cobalt-$(DDC)_3$, once formed, was stable to these reagents. It was also stable to $Pb^{+2}$, $Cu^{+2}$, $Hg^{+2}$ and other ions, but on the other hand exchange between cobalt ions and the DDC-complexes of lead, copper, mercury etc. was negligible.

PŘIBIL[3] used this phenomenon for a selective colorimetric determination of cobalt with DDC: interfering metals were exchanged with mercury(II) which has the highest affinity for DDC and forms a colorless complex.

In our work the technique of PŘIBIL is refined with the help of radio-isotopes: DDC-$^{35}$S is used in method (*A*): the mercury-DDC-$^{35}$S is removed with cyanide and the $^{35}$S in the extract is taken as a measure for the cobalt; $^{203}$Hg is used in method (*B*); the explanation of this technique is given below.

(*A*) In preliminary experiments the co-extraction of DDC-$^{35}$S was studied with a preparation synthesized from $C^{35}S_2$ following GLEU AND SCHWAB[4] and purified by recrystallization from ethyl acetate**. About 0.3% was co-extracted when fresh preparations of DDC-$^{35}$S were used***. This percentage increased on storage of the DDC-$^{35}$S; after 1 month 5% and after 2 months 10% of the $^{35}$S-activity was co-extracted. Evidently radio-decomposition occurred; for example, free sulphur was formed which was especially evident when the sample was recrystallized. No improvement was observed when the DDC-$^{35}$S was kept in a high vacuum over phosphorous pentoxide. The admixture of foreign SH-groups was more successful: a 10-fold excess of cysteinamine (in gequivs.) stabilized the DDC-$^{35}$S. However, this excess of foreign SH-groups decreased the extraction of cobalt appreciably. Extraction of cobalt with a metal-DDC-$^{35}$S complex (prepared from a stabilized DDC-$^{35}$S solution with a large excess of the metal) was therefore applied as an alternative.

The larger amounts of mercury(II) ions and cyanide then required for the back-extractions were a disadvantage. Therefore the excess of the metal-DDC complex over the cobalt had to be kept small. To diminish the consumption of this complex by foreign metals, zinc was chosen for the metal-DDC complex as it is nearest to

* Publication 124° of the Research Institute for Animal Husbandry "Schoonoord".

** Other batches were obtained from the Radiochemical Centre-Amersham, UK.

*** These experiments were all carried out with 500 $\mu$g of DDC-$^{35}$S in order to prevent interference of traces of metal.

cobalt in the sequence of affinities for DDC*. Under these circumstances (a small excess of reagent and a small difference between the affinities of cobalt and zinc for DDC) the process was not 100% effective and isotope dilution with $^{60}Co$ had to be introduced.

(*B*) Method B is founded on the same chemical system, but inactive DDC and labelled mercury(II) are used. Cobalt is extracted with a fixed amount of zinc–DDC in chloroform and the excess of the zinc complex is exchanged with $^{203}Hg^{+2}$ ions. The amount of $^{203}Hg$ in the organic layer (the difference from the blank) is then taken as a measure of the amount of cobalt extracted. Isotope dilution with $^{60}Co$ gives the extracted percentage of the cobalt.

## EXPERIMENTAL [METHOD A]

### *Materials and methods*

*DDC–$^{35}S$.* (*a*) Dissolve 100 mg of DDC–$^{35}S$ dihydrate (3 mC) in 10 ml of 0.01 *N* ammonium tartrate (AnalaR); add 300 mg of cysteinamine hydrochloride (Light & Co.) and adjust the pH to 11 with sodium hydroxide (Merck p.a.). After saturation with pure nitrogen, keep in polyethylene at 4°. (*b*) As above but with 6 g of cysteinamine hydrochloride.

*Zinc–DDC–$^{35}S$.* See below.

*$^{60}Co$.* Dilute (daily) 1 $\mu$g of $^{60}Co$ (10 $\mu$C) with 0.1 *N* nitric acid to 30 ml and use 0.3 ml per experiment (0.01 $\mu$g; 0.1 $\mu$C).

*Ion exchange water*; resistivity 3 to 4 Megohms.

The chloroform, carbon tetrachloride, ammonium tartrate, ammonia (s.g. 0.88) and hydrochloric (s.g. 1.18) and nitric (s.g. 1.42) acids used were "AnalaR".

*Ammonia–ammonium tartrate solutions.* (*a*) 6 *N* and 0.02 *M* respectively, (*b*) 1 *N* and 0.02 *M* respectively.

*Mercury(II) solutions, (X) and (Y).* Each week make fresh dilutions from a stock solution containing 1 mg of mercury(II) per ml of 1 *N* nitric acid: neutralize with sodium hydroxide and adjust with 1% sodium bicarbonate to contain 300 (*X*) and 60 (*Y*) $\mu$g of mercury(II) per ml respectively.

*Cobalt–DDC carrier.* 50 $\mu$g of cobalt as $Co-(DDC)_3$ per ml of chloroform.

*Zinc–DDC solution.* Dissolve 1 mole of zinc sulphate heptahydrate (AnalaR) and 0.14 moles of DDC (Merck p.a.) in 20 ml of 0.01 *M* ammonium tartrate, adjust with sodium hydroxide to pH 11 and extract with 4 successive 20-ml portions of chloroform. After stripping twice with 20 ml, of 0.01 *M* tartrate of pH 8.5, adjust the volume to 100 ml. Keep the chloroform at 4°. It contains about 44 $\mu$g of zinc as $Zn-(DDC)_2$ per ml.

*Potassium cyanide–DDC.* To 100 ml of 1% potassium cyanide (Merck p.a.) in water add 250 mg of DDC dihydrate and purify from traces of lead, thallium and bismuth by extraction with five 10-ml portions of carbon tetrachloride. Store the aqueous layer over tetrachloride in polyethylene and occasionally clean it — from airborne traces of the above metals — by shaking.

The extractions were carried out in polypropylene tubes. When the room temperature increased above 24°, alcohol envelopes were applied (see Fig. 2 of ref. [5]). Shaking was performed with a "Microid Flask Shaker" (Griffin and Tatlock) (see[5]).

The $^{35}S$-activity was measured with an end-window GM-tube (Philips Lamp Works

* Thallium is unsuitable since a trace of its DDC complex, if not exchanged with $Hg^{+2}$, would resist the cyanide treatment.

No. 18506, diameter 3 cm, window 2.5 mg/cm$^2$), combined with a sample changer (Physical Laboratory, University of Utrecht), a scaler (Philips GM 4810-PW 4020) and a printer. The scaler was adjusted to a dead-time of 300 $\mu$sec which resulted in a correction for coincidence loss of 1% per 2000 C/m. The activity of $^{60}$Co (and of $^{203}$Hg, $^{65}$Zn etc.) was measured with a well-type NaI-crystal (Philips PW 4111/w, volume 1 ml) and a scaler (Philips PW 4022–4032–4052).

*Preparation of zinc–DDC–$^{35}$S*

With three successive extractions of a 2-months old preparation of DDC–$^{35}$S, 0.6, 0.3 and 0.3% of the $^{35}$S remained in the final organic layer (after the treatments with mercury(II) and cyanide). Thus about 0.3% of the DDC–$^{35}$S was decomposed into chloroform-extractable products (notwithstanding the cysteinamine). These had to be removed before the preparation of zinc–DDC–$^{35}$S.

For this preparation carbon tetrachloride was preferred to chloroform, as it extracted less DDC–$^{35}$S. It will be shown that chloroform is to be preferred for the reaction with cobalt ions, thus the tetrachloride extract was diluted with chloroform.

DDC–$^{35}$S, (*b*) was used when the preparations were older than three months as it was somewhat better stabilized. The same specific activity was obtained for two samples of zinc–DDC–$^{35}$S made from the two months old DDC–$^{35}$S solutions (*a*) and (*b*) respectively. This indicated that no exchange of $^{35}$SH- and SH-groups had taken place.

*Procedure.* Dilute 0.1 ml of the DDC–$^{35}$S solution with 0.01 *M* ammonium tartrate to 10 ml, adjust with sodium hydroxide to pH 11* and extract with 5 ml of chloroform; add 13 mg of zinc sulphate heptahydrate and extract with 4 successive 3-ml portions of carbon tetrachloride. Shake the combined extracts with 2 ml of 0.01 *M* tartrate of pH 8.5, dilute with chloroform to 28 (*X*) or 140 (*Y*) ml and store in calibrated glass tubes at 4°. The solutions are stable for only three weeks (owing to some radio-decomposition). They contain about 5 (*X*) and 1 (*Y*) $\mu$g of zinc per ml.

*Extraction of cobalt with zinc–DDC–$^{35}$S*

Preliminary experiments were carried out with inactive zinc–DDC and $^{60}$Co. The results for two different concentrations of both reactants, four different compositions of the aqueous layer and two different organic solvents are shown in Table I.

These results show that the extraction from sodium hydroxide solutions is low, especially at small concentrations of cobalt and zinc. Ammonia and chloroform solutions are to be preferred; higher pH values are favourable here. As the zinc complex is not quite stable at pH 10.5, the treatment shown in italics, including a temporary rise of the pH, was chosen.

The foreign ions thallium(I), nickel(II), cadmium(II), zinc(II) and iron(III) reacted only very slowly with zinc–DDC but the ions mercury(I), mercury(II), silver(I), copper(II), thallium(III), bismuth(III) and lead(II) were found to react rapidly. As a large excess of the zinc complex results in a large blank (see below), the amount of the last group of ions in the sample is restricted. In practice a 10-fold excess over the cobalt can be allowed.

A larger excess has to be separated in advance, for instance by: (*1*) Anion-exchange from concentrated hydrochloric acid. With biological samples precipitates may occur.

* At pH 10 to 10.5 precipitation may occur, which slightly hampers the procedure.

(2) Cation-exchange with selective elution. No precipitation is to be feared with biological samples. (3) Extraction with inactive zinc–DDC in chloroform from a sodium hydroxide solution. $\mu$Equiv. amounts of interfering cations are extracted within 1 min with a 3-fold excess of the zinc complex. Only a few percent of the cobalt is lost (Table I); afterwards the aqueous layer has to be acidified to dissolve colloidal cobalt hydroxide and to decompose traces of inactive zinc–DDC, which would decrease the specific activity of the zinc–DDC–$^{35}$S.

TABLE I

PERCENTAGE OF $^{60}$Co EXTRACTED WITH ZINC–DDC IN 2 min[a]

| *Amounts of $^{60}$Co and Zn (as Zn–DDC)* | 2<br>30 | | 0.1<br>1.5 | |
|---|---|---|---|---|
| *Organic layer (2 ml)* / *Aqueous layer (2 ml[b])* | $CCl_4$ | $CHCl_3$ | $CCl_4$ | $CHCl_3$ |
| NaOH pH 8.5 | 40–60 | 20–22 | 1–15 | 0–5 |
| $NH_4OH$ pH 8.5 | 60–80 | 65–85 | 30–50 | 30–50 |
| *$NH_4OH$ (pH 10.5–>) 8.5* | *70–80* | *70–85* | *45–55* | *65–80* |
| $NH_4OH$ pH 10.5 | — | 75–85 | — | 65–80 |

[a] For the further procedure see below.
[b] Containing 0.01 *M* ammonium tartrate

*Removal of excess zinc–DDC–$^{35}$S and other complexes. Blank value*

With regard to the stripping with ionic mercury(II), in the range of 1 to 5 p.p.m. of zinc as zinc–DDC–$^{35}$S, a 20-fold excess of mercury(II) was sufficient to complete the process to more than 95% in a few minutes. Other metal–DDC complexes behaved similarly. With the exception of bismuth, lead and thallium, traces of the DDC complexes which might escape the mercury treatment will be decomposed by cyanide.

The decomposition of mercury–DDC by potassium cyanide was studied with a $^{203}$Hg-labelled complex. Decomposition by 1% potassium cyanide was complete within a few minutes, but 0.32% (st.dev. 0.06) of the aqueous $^{203}$Hg remained suspended in the chloroform*, even with two successive portions of cyanide and intermediate and final centrifugation of the container.

Of course, the same percentage of the aqueous DDC–$^{35}$S (resulting from the decomposition of the mercury–DDC–$^{35}$S by cyanide) also remained suspended in the chloroform. This factor adds $x$ times 0.32% to the blank when $x$ is the excess of the (remaining) zinc–DDC–$^{35}$S over the (extracted) cobalt.

However, in experiments with the $^{35}$S-labelled mercury complex the blank value proved to be larger than 0.32% of the complex. Thus other factors must contribute to this result, *e.g.*: traces of cobalt; traces of decomposition-products of the DDC–$^{35}$S; decomposition of the mercury complex as found below for cobalt–DDC.

"Lead-free" potassium cyanide proved to be favourable for two reasons: traces of

* The greater part of this aqueous layer was removed by filtration, but part of the cobalt–DDC–$^{35}$S was also lost.

lead, bismuth and thallium were removed; the residual inactive DDC in this cyanide exchanged with the DDC–$^{35}$S from the mercury complex.

Two successive treatments with 1% potassium cyanide were more effective than one treatment with 5% potassium cyanide; shaking for 4 min proved to be sufficient.

The mean values of 10 blank experiments with 15 and 3 $\mu$g of mercury as mercury–DDC–$^{35}$S were 1.45% and 3.6% respectively, corresponding to 0.048 (st.dev. 0.005) and 0.024 (st.dev. 0.002) $\mu$g of cobalt.

The stability of cobalt–DDC against the mercury and cyanide treatments was tested in experiments with 0.7 to 0.1 $\mu$g of $^{60}$Co as $^{60}$Co–(DDC–$^{35}$S)$_3$; 2% of both $^{60}$Co and $^{35}$S were lost in the mercury treatment; the results of the cyanide treatment are shown in Table II.

As isotope dilution with $^{60}$Co must in any case be applied in the proposed method, the loss of cobalt as cobalt–DDC in both the mercury and cyanide treatments is not important. However, the loss of cobalt in the ionic form during the cyanide treatment requires a correction. Probably part of the cobalt complex is oxidized to the thiouram compound which in turn forms the monosulfur compound under the influence of the cyanide*.

Evidently no exchange of the DDC–$^{35}$S from the cobalt complex with aqueous DDC takes place, in contrast to the behaviour of the mercury complex.

TABLE II

BACK-EXTRACTION OF $^{60}$Co–(DDC–$^{35}$S)$_3$ WITH CYANIDE[a]

(Experiments in triplicate)

| *Cobalt as $^{60}$Co–(DDC–$^{35}$S)$_3$ (µg)* | *Cobalt back-extracted (%)* | |
|---|---|---|
| | *As $^{60}$Co–(DDC–$^{35}$S)$_3$* | *As $^{60}$Co ions* |
| 0.7 | 2–3 –2[b] | 3–2 –2[b] |
| 0.5 | 6–5 –6[b] | 7–8 –8[b] |
| 0.3 | 5–7[b]–8 | 9–6[b]–8 |
| 0.1 | 9–10–8[b] | 6–7 –8[b] |

[a] 1 ml of $^{60}$Co–(DDC–$^{35}$S)$_3$ in chloroform was shaken during 4 min with two successive 1-ml portions of 1% potassium cyanide in water.
[b] Cyanide–DDC "lead-free" (5 mg DDC), instead of cyanide alone.

### *Measurement of $^{60}$Co and $^{35}$S*

$^{60}$Co was measured with the scintillation detector and the scaler at the discrimination level 200. The background was 60 ± 5 C/m; no correction for $^{35}$S was required. At least 1000 counts were registered so as to give a statistical error of 3%.

$^{35}$S was measured after evaporation on special aluminium dishes (see Fig. 1 of ref. [5]) with the end-window GM-tube. The background was 30 ± 3 C/m; corrections for coincidence loss and $^{60}$Co (C/m Co/2.85 ± 0.02) had to be made. No correction for autoadsorption was required. The error caused by variations in the distribution of the residue was only 6% as compared with 20% for the ordinary flat dishes in which the last traces of chloroform tend to creep to the edges.

* To prevent this decomposition, cobalt–DDC carrier was added; but exchange with the DDC–$^{35}$S of the mercury complex occurred.

## PROCEDURE FOR METHOD A

The procedure is given for a sample containing from 0.1 to 0.5 $\mu$g of cobalt and less than a 10-fold excess of the ions $Hg^{+}$, $Hg^{+2}$, $Ag^{+}$, $Cu^{+2}$, $Tl^{+3}$, $Bi^{+3}$ or $Pb^{+2}$.

Dissolve in nitric and/or hydrochloric acid and evaporate to about 0.5 ml under an infrared heater. Then place in a polypropylene tube (see Fig. 2 of ref.[5]) and add 0.3 ml of $^{60}Co$. Neutralize to phenolphthalein with ammonia–ammonium tartrate solution (*a*) and (*b*), and add 0.1 ml of solution (*b*) in excess (pH 10.5). Readjust the pH to the indicator with 1 *N* hydrochloric acid. Add 1 ml of zinc–DDC–$^{35}S$ solution (*Y*), shake for 4 min at 900 rev./min, centrifuge for 1 min at 1000 rev./min (Ø 25 cm), and remove the aqueous layer. Add 1 ml of mercury(II) solution (*Y*), shake for 2 min, centrifuge as above and again remove the upper layer. Add 1 ml of cyanide–DDC, shake for 4 min, centrifuge as above and remove the upper layer. Repeat this cyanide treatment once. Add 1.5 ml of cobalt–DDC carrier and measure the total volume by suction into a pipet. Measure 1 ml for $^{60}Co$ and two portions of 0.5 ml for $^{35}S$.

*Corrections.* (*a*) When less than 0.5 $\mu$g of cobalt is found in the final organic layer 8% (see Table II) is subtracted from the $^{35}S$-value; (*b*) 0.01 $\mu$g is subtracted from the resulting cobalt value to account for the cobalt added as $^{60}Co$. For amounts of cobalt from 0.5 to 3.0 $\mu$g solutions (*X*) are used instead of solutions (*Y*).

## RESULTS WITH METHOD A

The results are shown in Tables III and IV. Procedure 1 was given above. In procedure 2 treatment (*3*) (see above), was applied in advance to remove excess foreign ions.

## EXPERIMENTAL [METHOD B]

### *Materials and methods (see also method A)*

*Acidic $^{203}Hg$ solution.* 1 mg of $^{203}Hg$ (7 $\mu$C) per ml of 0.5 *N* nitric acid.

*Alkaline $^{203}Hg$ solution(a) and (b).* Neutralize each week 4 ml of the acidic $^{203}Hg$ solution to phenolphthalein using ammonia, add ammonium tartrate to give a concentration of 0.02 *M* and adjust the volume to 40 ml (*a*) or 80 ml (*b*). These solutions contain 100 (*a*) and 50 (*b*) $\mu$g and 1.54 (*a*) and 0.74 (*b*) $\mu$C of $^{203}Hg$ per ml.

*$^{60}Co$ solution.* 0.4 $\mu$g of $^{60}Co$ (4 $\mu$C) per ml of 1 *N* hydrochloric acid.

*Zinc–DDC solution.* See below.

*Mercury–DDC–cobalt–DDC carrier.* 1000 $\mu$g of both metals as the DDC complexes per ml of chloroform.

*Tartrate.* 0.1 *M* ammonium tartrate, adjusted to pH 8.5 with ammonia.

Measurement of $^{203}Hg$ and $^{60}Co$ in one solution was facilitated by an E.K.A.F. single-channel analyser.

### *Preparation of zinc–DDC*

The amount of inactive complex used here has to be accurately known as it determines the amount of $^{203}Hg$ in the organic layer (of the blank). This implies that the volume of the zinc–DDC solution must be corrected for evaporation before use and that decomposition of the complex, either before use or during the extraction of cobalt, must be prevented, or accounted for.

Radio-decomposition is of course not to be feared here, thus the addition of cysteinamine can be omitted. The addition of diethylamine was found to stabilize the complex (compare[5]), but mercury–amine complexes were formed (and extracted into the

TABLE III

DETERMINATION OF 0.1 TO 0.4 μg OF COBALT

| *Procedure* | *Co added* ($\mu g \cdot 10^{-3}$) | *Cu added* (μg) | *Co exchanged* (%) | *$^{35}S$ in the final organic layer (equiv. of 1 μg Co·$10^{-3}$)* | *Idem corrected* | *Co found (corrected)* ($\mu g \cdot 10^{-3}$) | *Co found (mean value)* ($\mu g \cdot 10^{-3}$) | *Standard deviation* (%) | *Error of mean* (%) |
|---|---|---|---|---|---|---|---|---|---|
| 2 | 100 | 6 | 28 | 38 | 35 | 115 | | | |
| 2 | 100 | 6 | 41 | 43 | 39.5 | 86.5 | 103 | 15 | +3 |
| 1 | 100 | 0 | 45 | 62 | 57 | 117 | | | |
| 1 | 100 | 0 | 62 | 68 | 62.5 | 91 | | | |
| 2 | 200 | 6 | 34 | 75 | 68.5 | 192 | | | |
| 2 | 200 | 6 | 36 | 87 | 80 | 212 | | | |
| 2 | 200 | 6 | 56 | 127 | 117 | 198 | 207 | 7.5 | +3.5 |
| 1 | 200 | 0 | 54 | 120 | 110.5 | 195 | | | |
| 1 | 200 | 0 | 55 | 135 | 124 | 215 | | | |
| 1 | 200 | 0 | 64 | 167 | 154 | 231 | | | |
| 2 | 300 | 6 | 18 | 65 | 60 | 323 | | | |
| 2 | 300 | 6 | 26 | 82 | 75.5 | 281 | 301 | 6 | +0.5 |
| 1 | 300 | 0 | 28 | 96 | 88.5 | 306 | | | |
| 1 | 300 | 0 | 49 | 162 | 149 | 294 | | | |
| 2 | 400 | 6 | 22 | 112 | 103 | 457 | | | |
| 2 | 400 | 6 | 31 | 122 | 112 | 353 | 407 | 14 | +2 |
| 1 | 400 | 0 | 35 | 144 | 132.5 | 368 | | | |
| 1 | 400 | 0 | 37 | 185 | 170 | 448 | | | |

TABLE IV

DETERMINATION OF 0.5 TO 3.0 μg OF COBALT

| *Procedure* | *Co added (μg)* | *Cu added (μg)* | *Co exchanged (%)* | *$^{35}S$ in the final organic layer (equiv. of 1 μg Co)* | *Co found (μg)* | *Co found (mean value) (μg)* | *Standard deviation (%)* | *Error of mean (%)* |
|---|---|---|---|---|---|---|---|---|
| 2 | 0.5 | 50 | 47 | 0.24 | 0.50 | | | |
| 2 | 0.5 | 50 | 54 | 0.30 | 0.55 | 0.50 | 7 | 0 |
| 1 | 0.5 | 0 | 38 | 0.19 | 0.49 | | | |
| 1 | 0.5 | 0 | 45 | 0.21 | 0.46 | | | |
| 2 | 1.0 | 50 | 38 | 0.40 | 1.04 | | | |
| 2 | 1.0 | 50 | 46 | 0.42 | 0.91 | 1.01 | 8 | +1 |
| 1 | 1.0 | 0 | 50 | 0.55 | 1.10 | | | |
| 1 | 1.0 | 0 | 45 | 0.44 | 0.97 | | | |
| 2 | 2.0 | 50 | 36 | 0.80 | 2.22 | | | |
| 2 | 2.0 | 50 | 36 | 0.73 | 2.03 | 2.01 | 7.5 | +0.5 |
| 1 | 2.0 | 0 | 40 | 0.77 | 1.93 | | | |
| 1 | 2.0 | 0 | 38 | 0.71 | 1.87 | | | |
| 2 | 3.0 | 50 | 39 | 1.16 | 2.98 | | | |
| 2 | 3.0 | 50 | 36 | 1.10 | 3.05 | 3.06 | 7 | +2 |
| 1 | 3.0 | 0 | 38 | 1.27 | 3.35 | | | |
| 1 | 3.0 | 0 | 47 | 1.34 | 2.85 | | | |

chloroform) on shaking with a $^{203}Hg^{+2}$ solution, thus the amine had to be omitted.

Traces of co-extracted DDC are not detrimental here, as they also react with $^{203}Hg^{+2}$; thus an excess of DDC is permissible.

*Procedure.* Dissolve 300 $\mu$g of zinc as zinc sulphate heptahydrate and 24 mg of DDC dihydrate (a 10-fold excess) in 1 ml of tartrate solution and adjust the pH to 11 with sodium hydroxide. Extract with four successive 5-ml portions of chloroform, strip the collected organic layers with two successive 10-ml portions of tartrate and dilute to 20 ml. Store in a well-stoppered calibrated glass tube at 4°. The solution contains about 15 $\mu$g of zinc as zinc–DDC per ml and is stable for two or three weeks.

*Extraction of cobalt with zinc–DDC*

Cobalt is determined here as the difference between two measurements of $^{203}Hg$. To make this difference as large as possible, the excess of zinc–DDC must be restricted and as much as possible of the zinc must be exchanged with cobalt. In our procedure a 0.5- to 2.0-fold excess is applied (as compared with a 1.3- to 6.5-fold excess with method A). In order to promote the exchange between cobalt and zinc, the procedure is again carried out with an ammonia solution of pH 8.5 which has temporarily been adjusted to pH 10.5 (see Table I); shaking was continued for 10 min.

When the exchange between cobalt and zinc was carried out at pH 10.5 this resulted in a marked fall of the $^{203}Hg$ value, indicating decomposition of the zinc complex at pH 10.5. The same phenomenon was observed when the temperature rose above 22°. An alcohol envelope was again applied to circumvent the latter difficulty.

With the omission of any cobalt, from 2 to 6 % of the zinc was found in the aqueous layer (experiments with $^{65}Zn$–DDC). However, no influence of this effect on the blank (the amount of $^{203}Hg$ in the final organic layer with the omission of cobalt) was observed. Probably traces of copper and other cations from the reagents and the containers were exchanged with the zinc.

*Removal of excess zinc–DDC and foreign DDC complexes with $^{203}Hg^{+2}$ ions*

In contrast to method A, the amount of mercury found in the chloroform after shaking it with an aqueous mercury(II) solution directly contributes to the blank, as here the mercury is labelled. For this reason the excess of $^{203}Hg$ should be minimized. On the other hand one must be sure that the exchange between zinc and mercury is complete: with method A a small residue of zinc–DDC could be supposed to be removed by cyanide but here the amount of $^{203}Hg$ exchanged with the zinc is taken as a measure of the cobalt.

Complete exchange of 2.25 $\mu$g of $^{65}Zn$ (as $^{65}Zn$–DDC in 1 ml of chloroform) with even 50 $\mu$g of $^{203}Hg$ in 1 ml of tartrate (a 7-fold excess) could not be accomplished in the tubes used in method A, within a reasonable time. A small polyethylene pill-case with a somewhat larger diameter (16 mm) and 29 mm high was found to be more favourable. Now the exchange of the $^{65}Zn$ was complete to 96.5% (st.dev. 2.3) within 20 min. A smaller excess of $^{203}Hg$ was not permissible.

The amount of $^{203}Hg$ in the chloroform — the blank — had a standard deviation of 4% or 0.02 $\mu$g. With 7.5 $\mu$g of zinc as Zn–DDC and 100 $\mu$g of $^{203}Hg$ the standard deviation of the blank was again 0.02 $\mu$g of $^{203}Hg$. Experiments with pure chloroform indicated that the greater part of this standard deviation was due to the admixture of a variable amount of ionic $^{203}Hg$ with the chloroform.

Again this blank was lowered by filtration but again losses of the $^{203}Hg$– and $^{60}Co$–DDC complexes occurred (notwithstanding the addition of carrier), when filtration was applied in the determination of cobalt. Desicoted filter papers (KOVÁCS[6]; WEGMANN[7]) were also investigated but proved to be unsuccessful. Thus the values found above had to be accepted.

From 10 to 20% of 0.4 to 0.2 $\mu g$ of cobalt as $^{60}Co$–DDC were exchanged with $^{203}Hg$ within the 20 min required for the exchange of mercury with zinc (2% within 2 min, see method A). This percentage increased with decreasing amounts of cobalt. Especially with the smaller amounts of cobalt this exchange was found to be not quite stoichiometric but extra amounts of $^{203}Hg$ were found in the chloroform. No explanation of this phenomenon could be found.

*Measurement of $^{203}Hg$ and $^{60}Co$*

$^{203}Hg$ and $^{60}Co$ can be measured in the final organic layer with the scintillation detector and the scaler. Discrimination levels of 8 and 200 are then applied respectively. The correction factor $^{60}Co$ at D8/$^{60}Co$ at D200 is about 200%.

With the single-channel analyser better results are obtained. $^{203}Hg$ is measured at the 0.28-MeV maximum with a channel-width of 20 V. $^{60}Co$ is measured above 1 MeV. The correction factor of $^{60}Co$ for the $^{203}Hg$ measurement is 20% (with a more stable detector the channel-width for $^{203}Hg$, and hence the correction factor of $^{60}Co$, can be decreased appreciably). No correction for $^{203}Hg$ need be made in the $^{60}Co$ measurement.

## PROCEDURE FOR METHOD B

The procedure is given for a sample containing from 0.8 to 3.0 $\mu g$ of cobalt and less than a 4-fold excess of foreign cations able to exchange with zinc–DDC (see method A).

Dissolve in nitric and/or hydrochloric acid and evaporate to less than 0.5 ml; add 0.1 ml of $^{60}Co$ and 0.1 ml of tartrate. Neutralize to phenolphthalein with concentrated and/or 1 *N* ammonia. Now add 0.1 ml of 1 *N* ammonia in excess (pH 10.5), readjust the pH with 1 *N* hydrochloric acid to phenolphthalein and transfer the mixture to the pill-case.

Add 1 ml of chloroform and 0.15 ml of the zinc–DDC solution, shake the tube for 10 min (900 rev./min) and centrifuge lightly for 1 min (1000 rev./min; Ø 25 cm). Remove the aqueous layer and rinse by shaking for 1 min with 1 ml of the tartrate. Centrifuge and remove the rinsing liquid. Add 1 ml of the alkaline $^{203}Hg$ solution (*b*). Shake for 20 min, centrifuge and remove the aqueous layer. Finally add 0.2 ml of the mercury–DDC–cobalt–DDC carrier, measure the volume with a pipet and measure the counts of 1 ml in the well-type crystal.

Carry out blank determinations to control the zinc–DDC solution. Measure the $^{203}Hg$- and $^{60}Co$-standards to control the detector. Subtract the $^{203}Hg$-value of the sample (in $\mu$equivs.) from the $^{203}Hg$-value of the blank* to give the amount of cobalt (in $\mu$equivs.) present in the final organic layer. The $^{60}Co$-value for the sample gives the percentage of cobalt in this layer. From the amount of cobalt found, subtract 0.04 $\mu g$ for the cobalt in the $^{60}Co$-solution.

* This value is determined in quadruplicate once a week.

For the determination of 3 to 10 $\mu g$ of cobalt use 0.5 ml of chloroform and 0.5 ml of the zinc–DDC solution. Furthermore use $^{203}Hg$ solution (*a*) instead of (*b*).

## RESULTS WITH METHOD B

The results with 0.4 to 10 $\mu g$ of cobalt are shown in Table V. For the determination of 0.4 $\mu g$ of cobalt only 0.075 ml of the zinc–DDC solution and 0.5 ml of $^{203}Hg$ solution (*b*) were used. The amount of cobalt from the $^{60}Co$ is already included in the values given in the second column.

TABLE V

DETERMINATION OF COBALT WITH ZINC–DDC AND $^{203}Hg$ *2

| *Number of experiments* | *Cobalt added ($\mu g$)* | *Cobalt found ($\mu g$)* | *Standard deviation ($\mu g$ of cobalt)* | *Idem (%)* | *Error of mean (%)* |
|---|---|---|---|---|---|
| 4 | 10 | 10.3 | 0.80 | 8 | + 3 |
| 4 | 5 | 5.0 | 0.35 | 7 | 0 |
| 4 | 2 | 1.95 | 0.15 | 7.5 | — 2.5 |
| 10 | 1.4 | 1.41 | 0.14 | 10 | + 1 |
| 12 | 0.8 | 0.82 | 0.14 | 17.5 | + 2.5 |
| 7 | 0.6 | 0.61 | 0.17 | 28 | + 2 |
| 6 | 0.4 | 0.29 | 0.07 | 24 | —27 |

## DISCUSSION OF METHODS (A) AND (B)

(*A*) The factors influencing the blank were discussed above. In the presence of cobalt a (variable) part of the mercury–DDC–$^{35}S$ complex is replaced by the cobalt complex which does not behave in exactly the same way. Thus the actual blank will probably be (variable and) smaller than the one given above.

The experimental mean of the standard deviation (st.dev.) is 9%. The standard deviation of the measurements of $^{60}Co$ and $^{35}S$ is in total about 7% and a standard deviation of 0.4 to 5% is the result of the variable blank (with 40% exchange of cobalt).

The sensitivity limit can be calculated from the amount of cobalt equivalent to a $^{35}S$-activity of, say, 3 times the background of the GM-tube (0.0045 $\mu g$) and from the percentage of the cobalt which reaches the final $^{35}S$-measurement (7 to 10). This limit (about 0.04 $\mu g$) could be lowered appreciably with a gas-flow or liquid-scintillation counter.

(*B*) Neglecting the experiments with 0.4 $\mu g$ of cobalt, the mean deviation from stoichiometry is 1%. The large deviation from stoichiometry observed with 0.4 $\mu g$ of cobalt is probably due to the extra $^{203}Hg$ in the organic layer observed especially with these smaller amounts. The standard deviation of the blank is evidently one of the factors influencing the accuracy of the method. As cobalt is determined as the difference between two $^{203}Hg$-measurements the (square values of the) standard deviations must be added. Thus the total effect is 3 to 5%.

Another error probably occurs in the determination of the efficiency of the process for the cobalt. When the $^{60}Co$ in all fractions is measured, not more than 90% (st.dev. 5) of the original amount can be found. Probably adsorption effects occur. The error caused in the measurement of the organic $^{60}Co$ thus may be a few percent of

the total $^{60}Co$ or about 6% of the measurement, which accounts for the remaining part of the standard deviation found in Table V.

The main advantages of method *B* over method *A* are (*1*) the omission of a labelled organic reagent, which is liable to radio-decomposition and (*2*) the omission of the cyanide treatment. The main drawbacks are (*1*) the determination of cobalt as a difference of two measurements of $^{203}Hg$ and (*2*) the "exchange" of non-stoichiometric amounts of mercury and cobalt in the smaller concentration ranges, which limits the sensitivity.

Both methods show that determinations of cobalt in the submicrogram range are possible using radiometric techniques. The advantage over activation analysis is that no reactor or other source of nuclear particles is required. Comparison with classical techniques shows our methods to be of equal sensitivity. Undoubtedly further refinement of the procedures presented will be possible with continued research.

ACKNOWLEDGEMENT

The author is indebted to MRS. G. A. OVEZALL-KLAASEN and MISS J. H. F. F. BROEKMAN for valuable technical assistance.

SUMMARY

Two radiometric methods for the determination of submicrogram amounts of cobalt are described. (*A*) Cobalt is extracted from an ammoniacal solution with a zinc-diethyldithiocarbamate-$^{35}S$ solution in chloroform. Excess reagent and interfering metals are removed with mercury(II) and cyanide. The $^{35}S$ in the final organic layer is a measure of the cobalt in this layer. (*B*) Cobalt is extracted from an ammoniacal solution with a fixed amount of zinc-DDC in chloroform. Excess reagent and complexes of foreign metals are removed by exchange with $^{203}Hg^{+2}$ and the $^{203}Hg$ in the chloroform (compared with a blank) acts as a measure of the cobalt. Method *A* is applicable to 0.1 $\mu$g of cobalt and method *B* to 0.8 $\mu$g. As the efficiency of both processes is variable, isotope dilution with $^{60}Co$ is carried out. A 10-fold excess of foreign metals is permitted in method *A* and a 4-fold excess in method *B*; larger amounts are previously removed, *e.g.* by extraction with inactive zinc-DDC from sodium hydroxide media.

RÉSUMÉ

Deux méthodes sont proposées pour le dosage radiométrique de traces de cobalt: (*A*) le cobalt est extrait au moyen de zinc-diéthyldithiocarbamate-$^{35}S$ dans le chloroforme. On mesure $^{35}S$ dans le solvant organique. (*B*) le cobalt est extrait au moyen de zinc-diéthyldithiocarbamate dans le chloroforme. L'excès de réactif est traité par le mercure-203. La mesure de ce dernier permet de déterminer la teneur en cobalt.

ZUSAMMENFASSUNG

Beschreibung von zwei radiochemischen Methoden zur Bestimmung von Spuren von Kobalt: (*A*) Extraktion mit Chloroform in Gegenwart von Zink-Diäthyldithiocarbamat-$^{35}S$. Der Überschuss an Reagenz und störende Metalle werden mit Quecksilber(II) und Cyanid entfernt. Die Messung des $^{35}S$ Gehaltes in der organischen Phase ergibt den Gehalt an Kobalt. Isotopenverdünnung mit $^{60}Co$ ist notwendig. (*B*) Extraktion mit Chloroform in Gegenwart einer gemessenen Menge von Zink-Diäthyldithiocarbamat. Der Überschuss an Reagenz und andere Metalle werden durch Umsetzung mit $^{203}Hg^{+2}$ entfernt. Der Gehalt der Chloroformschicht an $^{203}Hg$ entspricht der Menge an Kobalt.

REFERENCES

1 H. BODE, *Z. anal. Chem.*, 142 (1954) 414; 143 (1954) 182; 144 (1955) 165.
2 H. BODE AND K. J. TUSCHE, *Z. anal. Chem.*, 157 (1957) 414.
3 R. PŘIBIL, *Sborník celostátní pracovní konf. anal. chemiků, 1st Conf., Prague, 1952*, p. 82; *Chem. listy*, 46 (1952) 603; 47 (1953) 842.
4 K. GLEU AND R. SCHWAB, *Angew. Chem.*, 62 (1950) 320.
5 P. C. VAN ERKELENS, *Anal. Chim. Acta*, 26 (1962) 32.
6 E. KOVÁCS, *Naturwiss.*, 44 (1957) 181.
7 K. WEGMANN, *Chemiker-Ztg.*, 83 (1959) 226.