

RADIOMETRIC TRACE ANALYSIS\*  
QUANTITATIVE PAPER CHROMATOGRAPHY OF LEAD WITH  
PHOSPHATE-<sup>32</sup>P

P. C. VAN ERKELENS

*Institute for Animal Husbandry "Schoonoord", Utrecht (The Netherlands)*

(Received March 30th, 1961)

GENERAL INTRODUCTION

The name "radiometric analysis" is often used for the measurement of the amount of a nucleide by means of its radio-activity, but in the opinion of the present author, this term should be reserved for analytical methods in which induced or added radio-activity is applied. Of these methods isotope dilution and activation-analysis are specially important, the latter requiring a strong source of nuclear particles, the former being in fact merely a make-shift, the sensitivity of the analysis depending on an *addition* technique.

The term radiometric analysis is often reserved for three other types of analysis. The first (1) and most widely applied, uses the radio-active tool merely as a *tracer* to denote the end-point or the starting point of a reaction; as with isotope dilution the sensitivity is in principle restricted\*\*. Both other techniques, (2) analysis with radio-active reagents and (3) analysis by exchange-reactions involving radio-active isotopes, do not suffer from this disadvantage. A survey of the literature<sup>1</sup> revealed that, notwithstanding this, both methods (2) and (3), but especially method (3), are less popular than method (1).

Preliminary experiments<sup>1</sup> led to the choice of (a) phosphate-<sup>32</sup>P for the quantitative paper chromatography of traces of metals — the application to lead is described below — and (b) the exchange reactions of the system: metal ion in water–metal–diethyldithiocarbamate complex in chloroform for the determination of traces of metals in solution. Applications of the latter system to lead and cobalt will be described in later papers.

QUANTITATIVE PAPER CHROMATOGRAPHY OF LEAD WITH PHOSPHATE-<sup>32</sup>P

*Introduction*

Phosphate-<sup>32</sup>P has been used in the analysis of metals by SUE<sup>2</sup>, LANGER<sup>3</sup>, MOELLER AND SCHWEITZER<sup>4</sup>, BARCIA GOYANES<sup>5</sup>, TANANAEV<sup>6</sup> and MEVEL<sup>7</sup>. Filter paper was not used by these authors. It was chosen as a medium for our experiments because a

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

\*\* Method (1) is mostly carried out as a titration with a radio-active indicator and a non-radio-active reagent. It can be made more sensitive by using a radio-active reagent, but this converts method (1) to a special form of method (2).

higher sensitivity could be expected. Interferences from other metals could have been diminished by a proper choice of pH and masking agents, but a much better selectivity was obtained by the development of a paper chromatogram to yield a separation of the relevant elements.

Phosphate- $^{32}\text{P}$  is a rather general reagent for metals. Thus procedures are indicated here for two cases: (1) in which only lead is of interest, and (2) in which other ions are also of interest. Only case (1) is worked out in detail.

### *Experimental*

#### *Materials*

*Phosphate- $^{32}\text{P}$  solutions:* (a) 1%  $\text{Na}_2\text{H}^{32}\text{PO}_4$  in water; 10  $\mu\text{C}/\text{ml}$ ; (b) Solution (a) 4 times diluted.

*$^{210}\text{Pb}$  solution:* 400  $\mu\text{g}$  of  $^{210}\text{Pb}$  (Ra-D in equilibrium with its decay products) and 4  $\mu\text{C}$  per ml of 1 *N* nitric acid.

*Other solutions of radio-active metals* were prepared in a similar way. All were kept in polyethylene vessels. The radio-activity was measured periodically, to control losses by adsorption to the wall.

*Ion exchange water* was used which had a resistivity of more than 3 to 4 Megohms.

Hydrochloric acid ( $d = 1.18$ ), nitric acid ( $d = 1.42$ ), ammonia ( $d = 0.88$ ) and methanol were "AnalaR"; ethanol, *n*-propanol and hydrogen peroxide (30%) were "Merck p.a."

*Borax-oxalate buffer:* Mix 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 and adjust to pH 8.0 with the borax solution.

As the isotope  $^{32}\text{P}$  emits hard  $\beta$ -rays, measurement in filter paper does not involve any difficulty. *The counting equipment* consisted of an end-window GM-tube (Philips 18506), a scanner-sample changer (Phys. Lab. Univ. of Utrecht) and a scaler-ratemeter (Philips). The usual precautions for radioactive tracer work *and* for trace analysis were taken. Lead-210 was measured by the  $\beta$ -radiation of its daughter  $^{210}\text{Bi}$ . A check was made after 5 days ( $= \tau_{1/2}$  of  $^{210}\text{Bi}$ ).

*pH-values* were measured with a Beckman model-G instrument, calibrated on aqueous standards. Ordinary chromatographic sprayers and dry boxes were used for spraying the phosphate- $^{32}\text{P}$  solution. The papers were either Whatman no. 1 or Schleicher and Schüll 2043 b.

#### *Pretreatment of the sample*

The sample — or its ash — is dissolved in a minimum volume of nitric or hydrochloric acid. The amounts of these acids must be kept small, as even the AnalaR reagents contain some lead. Complexing agents should be absent; they can be destroyed for instance by ashing with 1:1 nitric acid-hydrogen peroxide.

The sample should contain at least 1  $\mu\text{g}$  of lead. Foreign cations and anions are allowed in amounts up to a few hundred micrograms. Larger amounts tend to overload the paper.

#### *Application to the paper*

The application of milliliter amounts of aqueous solutions to the paper is not practicable. Some methods have been devised, for instance by PHILLIPS<sup>8</sup> and by

FRENCH AND GIBSON<sup>9</sup>, but for a routine method they are too cumbersome. Thus the solution usually has to be concentrated by evaporation.

The method of THIERS *et al.*<sup>10</sup> proved to be preferable. The final volume should be about 0.05 to 0.1 ml, the estimation of which is a matter of practise. If a dish becomes dry or precipitation occurs, dilute hydrochloric acid should be added; organic residues are ashed with 1:1 nitric acid–hydrogen peroxide. It is not absolutely necessary to produce a small spot on the paper although a somewhat better chromatogram would result. A larger spot was accepted and the paper was dipped into the solution, this being the most convenient method. The efficiency of the method was tested with <sup>210</sup>Pb: 97 to 99% of the lead was found on the paper (as compared with direct administration).

From the literature, and from our preliminary experiments with other radio-active reagents, it was clear that appreciable amounts of several metals are contained in chromatographic paper. Although the greater part of these metals consists of Fe, Ca, Al, Mg and Cu, the use of a purified paper proved to be of advantage in our experiments with amounts of lead smaller than 3  $\mu$ g. Investigations on the method of purification are described elsewhere<sup>1</sup>. The method of choice is given below.

Cut strips of paper (2.5 or 3.0  $\times$  30 cm) and hang them in a chromatographic jar; rinse for 24 h with 9:1 methanol–concentrated hydrochloric acid in the descending way; remove the eluate and ventilate the jar for a few hours. Then run for 24 h with 9:1 methanol–ammonia, ventilate and run for 24 h with 95:5 methanol–water. Allow to dry in the atmosphere of the last solvent.

The second solvent re-establishes the pH and the third one the water content of the paper. Several paper chromatographic separations with these papers proved to give essentially the same results as with untreated paper.

(1) When only lead is to be determined, the sample is placed on a strip of paper as indicated above and the ions are separated as described in the next paragraph.

(2) When other cations are to be determined as well and the corresponding spots are separated by less than 4 cm, a sheet of paper — 20  $\times$  20 cm — is used instead of the paper strip. The dipping technique is somewhat more difficult here; satisfactory results are obtained however, when the paper is folded twice (Fig. 1) and the tip is immersed in the evaporation dish. A more or less irregular spot forms around the intersection of the two folds. When the solution is placed on the paper with a pipet, a more rectangular spot can be formed (Fig. 2), which has some advantage in connection with the subsequent chromatographic separation.

(3) When other cations are to be determined as well, but the corresponding spots are separated by 4 cm or more, a paper strip is used.

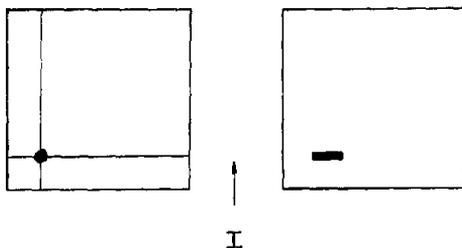


Fig. 1. Application of the sample by dipping.

Fig. 2. Application of the sample with a pipet.

### *Paper chromatographic 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 chromatogram is that it should separate lead from interfering cations and anions. The choice of this solvent depends on the composition of the sample. Directions are given here for the ash of 50 mg of dry cow liver, which contains at least 1  $\mu\text{g}$  of lead.

For this sample the alcoholic solvents used by LEDERER<sup>11</sup>, WALKER AND LEDERER<sup>12</sup>, BURSTALL *et al.*<sup>13</sup> and HUNT<sup>14</sup>, were found to be the most convenient. These authors were able to separate  $\text{Pb}^{+2}$  from  $\text{Fe}^{+3}$ ,  $\text{Zn}^{+2}$ ,  $\text{Cu}^{+2}$ ,  $\text{Mn}^{+2}$ ,  $\text{Co}^{+2}$ ,  $\text{Tl}^{+3}$ ,  $\text{Tl}^{+}$ ,  $\text{Hg}^{+2}$ ,  $\text{Zr}^{+4}$ ,  $\text{Ag}^{+}$ ,  $\text{Al}^{+3}$ ,  $\text{Mg}^{+2}$ ,  $\text{Ni}^{+2}$ ,  $\text{Sn}^{+2}$ ,  $\text{Bi}^{+3}$ ,  $\text{Cd}^{+2}$  and  $\text{MoO}_4^{-2}$ , but not from  $\text{Ca}^{+2}$ ,  $\text{Sr}^{+2}$ ,  $\text{Ba}^{+2}$ ,  $\text{Ce}^{+3}$ ,  $\text{Th}^{+4}$ , the rare earths and  $\text{Hg}^{+}$ .

In our own experiments the cations  $\text{Fe}^{+3}$ ,  $\text{Cu}^{+2}$ ,  $\text{Zn}^{+2}$ ,  $\text{Mn}^{+2}$ ,  $\text{Co}^{+2}$ ,  $\text{Tl}^{+3}$ ,  $\text{Tl}^{+}$ ,  $\text{Hg}^{+}$ ,  $\text{Hg}^{+2}$ ,  $\text{Ca}^{+2}$ ,  $\text{Sr}^{+2}$ ,  $\text{Ba}^{+2}$ ,  $\text{Al}^{+3}$  and  $\text{Mg}^{+2}$  were tested, as well as the anions  $\text{PO}_4^{-3}$ ,  $\text{MoO}_4^{-2}$  and  $\text{SO}_4^{-2}$ . Paper strips with these ions, in amounts ranging from 1 to 200  $\mu\text{g}$ , were chromatographed in the ascending way with the solvent 9:1 ethanol-5 N hydrochloric acid.

The radioisotopes  $^{59}\text{Fe}$ ,  $^{65}\text{Zn}$ ,  $^{54}\text{Mn}$ ,  $^{60}\text{Co}$ ,  $^{204}\text{Tl}$ ,  $^{203}\text{Hg}$ ,  $^{45}\text{Ca}$ ,  $^{89}\text{Sr}$ ,  $^{99}\text{Mo}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$  and  $^{210}\text{Pb}$  were used to detect the respective ions. The ions  $\text{Cu}^{+2}$ ,  $\text{Ba}^{+2}$ ,  $\text{Mg}^{+2}$  and  $\text{Al}^{+3}$  were detected by the tests described by POLLARD AND McOMIE<sup>15</sup>.

The main part of the lead was found at  $R_F$  0.15 (with 1 to 3  $\mu\text{g}$  of lead the size of the spot was about 1  $\text{cm}^2$ ) but  $0.09 \pm 0.03$   $\mu\text{g}$  of lead were eluted and found at higher  $R_F$  values.

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

(2) If the determination of other cations is also required, the separation should be carried out (on the paper sheet) with 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.

With most solvents, the alkaline earth ions are separated from lead and need not be considered further. On the other hand, with no other solvent tested was the separation of lead from large amounts of other heavy metals as good as with the alcoholic hydrochloric acid used in case (1).

### *Reaction with phosphate- $^{32}\text{P}$*

(1) After the paper chromatographic separation of  $\text{Pb}^{+2}$ , the paper was dried and neutralized in ammonia vapor. By this treatment the pH was increased to about 4.5. The paper was then sprayed (on both sides) with one of the phosphate- $^{32}\text{P}$  solutions, the choice depending on the amount of lead expected.

Spraying was carried out in a dry box or in an efficient hood; it did not involve any danger to the operator when the walls were covered with absorbing paper. The paper must not be sprayed too heavily, otherwise the solvent drips off and the spots spread. It is also possible to apply the reagent with a pipet or a small paint brush, but these methods give somewhat less satisfactory results.

(2) When other metals were to be determined (and the successive spots were

separated by less than 4 cm), after drying and neutralization, the developed part only (Fig. 3) was sprayed with the phosphate- $^{32}\text{P}$ , the rest being masked with a screen.

(3) When the spots were separated by more than 4 cm, the paper was cut into pieces containing the different spots. To these pieces technique (1) was applied.

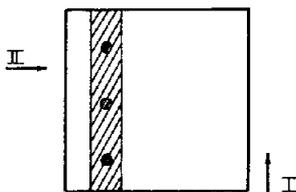


Fig. 3. Spraying of a paper sheet with phosphate- $^{32}\text{P}$ .

#### *Separation from excess $^{32}\text{PO}_4^{-3}$ and alkaline earth metals*

(1) 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 chromatogram was developed. If some of the cations lying between  $R_F$  0.3 and  $R_F$  1.0 had to be detected, the strip was cut off at  $R_F$  0.3; the separating solvent was then applied in the descending way and allowed to drip off at the point  $R_F$  0.3.

The solvent for the elution of excess phosphate- $^{32}\text{P}$  was chosen so as to elute the alkaline earth metals (not separated from lead above) as well. A borax-oxalate buffer was found to be suitable\*; alcohol was added to diminish the solubility of the lead phosphate (see EBEL AND VOLMAR<sup>16</sup>). With this solvent only 0.04, 0.13 and 0.44% of the original calcium, strontium and barium respectively remained in the spot as the phosphate; only small amounts of lead (10%, St. dev. 1.5) were eluted, probably as the oxalate.

Another suitable solvent was found to be an alcoholic acetate buffer of pH 4.5: 0.7% of the calcium, strontium or barium were retained and only 6% of the smaller amounts of lead was eluted.

(2) In this case the elution is carried out perpendicularly to the chromatographic development (directions II and I in Fig. 3).

The composition of the eluting solvent depends on the chromatogram and the ions of interest. For example, borax-oxalate can be used for magnesium, zinc and manganese; borax alone is suitable for calcium, magnesium and many other metals (for calcium and magnesium alone a spray with 8-hydroxyquinoline is inserted).

(3) Here, different solvents can be used for the successive pieces of the chromatogram. The descending technique is used and the solvent is allowed to drip off.

#### *Measurement of the radioactivity of the lead phosphate- $^{32}\text{P}$*

After the separation of lead phosphate from excess of reagent and interfering metals, the paper is dried and cut off at  $R_F$  0.30. The part which contains the lead phosphate is folded lengthwise and fixed to a thick aluminium strip which is passed through the scanner.

\* Citrate and tartrate are not effective. Probably insoluble oxalates are formed.

Thus folded the paper is narrower than the window by a factor of 2 and the geometrical error caused by differences in the distribution of the activity is minimized. Less than 1% of the radiation from the lower half of the paper is absorbed. The total error caused by both effects is smaller than 3%.

The slit width and the scanning speed are regulated in accordance with the activity on the paper; usually a slit of 5 mm and a speed of 2 to 32 cm/h are sufficient to obtain a statistical error which is smaller than 3%. The total of all errors is about 3 to 4%.

The resulting curve is planimeted. After evaluation of the half-life correction, the amount of lead is found by comparison with standards.

#### PROCEDURE

(for case 1)

The procedure is given for a sample containing from 1 to 4  $\mu\text{g}$  of lead and (per  $\mu\text{equiv.}$  of lead) not more than the following amounts of foreign metals (in  $\mu\text{equiv.}$ ): 250 Ca, 70 Sr, 20 Ba\*, 0.1 Hg<sup>+</sup>, 0.1 Tl<sup>+</sup>\*\*, 0.1 Ce<sup>+3</sup> and 0.1 Th<sup>+4</sup>\*\*\*. Less than 1 mg of inorganic material should be present altogether†.

Dissolve in hydrochloric and/or nitric acid and evaporate to 0.05–0.1 ml. Prepare strips of acid-washed Whatman No. 1 paper, fold them at 6 cm from one end and dip the fold in the solution until the spot is about 5 cm<sup>2</sup>††. Dry while hanging on a glass frame in a clean atmosphere and dip again until all the solution is taken up (twice is mostly sufficient).

Dry for 60 min, condition in the vapor of 9:1 ethanol–5 N hydrochloric acid for 16 h and chromatograph with this solvent in the ascending way for 4 h (about 20 cm).

Dry for 60 min, neutralize in the vapor of concentrated ammonia for 30 min and ventilate for 15 min.

Spray the region of  $R_F$  0.1 to 0.2 with the Na<sub>2</sub>H<sup>32</sup>PO<sub>4</sub>-solution (b) in a dry box the inside of which is covered with absorbing paper. Spray on both sides and to thorough dampness; about 1 ml is required per 80 cm<sup>2</sup>†††.

Dry again and allow the borax–oxalate buffer to ascend in the direction of the chromatogram. Dry, and determine the radioactivity in the region of  $R_F$  0.1 to 0.2.

#### RESULTS

The blank amounted to 0.51  $\mu\text{g}$  of lead (St. dev. 0.06) per cm<sup>2</sup> of acid-washed paper. This blank went up with (1) the surface area of the spot, (2) the amount of phosphate-<sup>32</sup>P, (3) the amount of nitric acid–hydrogen peroxide applied. For factors (1) and (2) it did not go up in equal ratio to their increase. The blank for unwashed paper was 1  $\mu\text{g}$  of lead (St. dev. 0.7) per cm<sup>2</sup>.

The ions Fe<sup>+3</sup>, Cu<sup>+2</sup>, Zn<sup>+2</sup>, Mn<sup>+2</sup>, MoO<sub>4</sub><sup>-2</sup>, Co<sup>+2</sup>, Al<sup>+3</sup>, Hg<sup>+2</sup>, Tl<sup>+3</sup> and Mg<sup>+2</sup> in 100- $\mu\text{g}$  amounts had no influence on the blank. The same amounts of Ca<sup>+2</sup>, Sr<sup>+2</sup> and

\* 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 subtracted. Alternatively another solvent should be sought for chromatography.

\*\* The interference of Hg<sup>+</sup> and Tl<sup>+</sup> is easily prevented by oxidation to Hg<sup>+3</sup> and Tl<sup>+3</sup> respectively.

\*\*\* With larger amounts of Ce<sup>+3</sup> and Th<sup>+4</sup> another solvent should be sought for chromatography.

† With thicker papers (e.g. Whatman No. 3 mm) larger samples can be handled.

†† With amounts of lead larger than 3  $\mu\text{g}$  unwashed papers can be used.

††† Solution (a) is used for 4 to 16  $\mu\text{g}$  of lead.

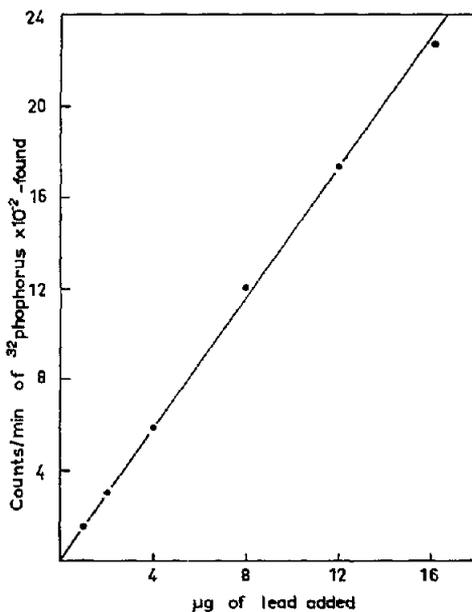


Fig. 4. Results with 1 to 16  $\mu\text{g}$  of lead. (Mean values of 4 experiments each).

TABLE I  
DETERMINATION OF 1  $\mu\text{g}$  OF LEAD IN A CHROMATOGRAM  
(Mean values and standard deviations of 3 experiments each)

Exp. No.	Foreign ions ( $\mu\text{g}$ )	Subtracted blank ( $\mu\text{g}$ of Pb) <sup>a</sup>	Lead found ( $\mu\text{g}$ )	Standard deviation (%)	Lead found, corrected value <sup>b</sup> ( $\mu\text{g}$ )
1	Ca 100 Sr 20 Ba 20	0.91	1.03	11.5	1.12
2	Fe 300 Cu 100 Zn 200	0.51	0.89	9.5	0.98
3 <sup>c</sup>	Na 150 Mn 1 K 400 Fe 15 Ca 15 Cu 5 Mg 10 Zn 10 Mo 1 Co 0.1	0.54	0.95	11.0	1.04
4 <sup>c</sup>	As in the preceding experiment above: PO <sub>4</sub> <sup>-3</sup> 30 SO <sub>4</sub> <sup>-2</sup> 20	0.54	0.91	9.5	1.00
5	PO <sub>4</sub> <sup>-3</sup> 100 SO <sub>4</sub> <sup>-2</sup> 100	0.51	0.92	8.5	1.01

<sup>a</sup> An extra blank, equivalent to 0.40 (St. dev. 0.06)  $\mu\text{g}$  of lead, can be calculated for experiment 1, and an extra of 0.03  $\mu\text{g}$  of lead for experiments 3 and 4.

<sup>b</sup> For the loss of 0.09  $\mu\text{g}$  of lead in the paper chromatographic separation.

<sup>c</sup> Cations (and in experiment 4: 2% of the anions) in the ash of 50 mg of dry cow liver, which generally contains more than 2  $\mu\text{g}$  of lead.

Ba<sup>+2</sup> produced a blank corresponding to 0.2, 0.3 and 0.7  $\mu\text{g}$  of lead respectively (St. devs. 0.05, 0.08 and 0.17).

Results with 1 to 16  $\mu\text{g}$  of lead are shown in Fig. 4. Mean values of 4 experiments are given. Acid-washed papers were used throughout; the results for 8, 12 and 16  $\mu\text{g}$  of lead obtained with unwashed paper were similar but the standard deviations were higher.

In Table I some examples are given of the determination of 1  $\mu\text{g}$  of lead in a simulated biological ash.

The amount of lead is calculated from the <sup>32</sup>P-value found (in a spot of 1 cm<sup>2</sup>) in the absence of foreign ions and hence by omission of the paper chromatographic separation. Thus a correction for the loss of 0.09  $\mu\text{g}$  of lead in this separation had to be made. On the other hand only the variations found in the loss of lead phosphate had an influence, while a correction for the 10% loss itself was not required.

Results with larger amounts of lead show smaller standard deviations, while a larger excess of foreign ions is allowed. As the latter tend to disturb the chromatogram by overloading\*, these results are not very interesting: an aliquot of the sample containing at least 1  $\mu\text{g}$  of lead should be taken.

#### DISCUSSION

Combination with paper chromatography makes the radioactive reagent more selective and more sensitive. It now becomes applicable to several cations simultaneously.

Phosphate-<sup>32</sup>P is a very useful reagent in the quantitative paper chromatography of trace elements. The sensitivity is comparable with the polarographic and spectrophotometric (dithizone) techniques but the method is 5 times less sensitive than the spectrographic method. Our technique has the distinct advantage, however, that it is directly applicable to the paper. For metals like Fe, Mn, Zn, Ca, Mg etc. the sensitivity in  $\mu\text{g}$  will probably compare favourable with densitometric practise.

The ratio P/Pb can be calculated from the specific activity of the phosphate-<sup>32</sup>P. As a mean of 10 experiments with 16  $\mu\text{g}$  of lead, the value 1.10 (St. dev. 0.06) was found. Probably PbHPO<sub>4</sub> is precipitated at pH 4.5 (see above\*\*) and excess <sup>32</sup>PO<sub>4</sub><sup>-3</sup> is adsorbed or occluded. Repetition of the separation of phosphate-<sup>32</sup>P was found to decrease the P/Pb-ratio to 1.06 (St. dev. 0.035). The blank and its standard deviation were also lowered but 0.05 to 0.12  $\mu\text{g}$  of lead was lost. Thus repetition of the separation did not increase the overall accuracy.

The effect of the borax-oxalate buffer is obviously due to the precipitation of alkaline earth oxalates.

When the phosphate-<sup>32</sup>P is sprayed on the "neutralized" chromatogram, lead phosphate precipitates but the pH of 4 to 5 will largely prevent the precipitation of the alkaline earth-phosphates. Separation of excess reagent with the borax-oxalate buffer of pH 8.0 causes precipitation of calcium, strontium and barium, partly as the phosphates but mostly as the oxalates. As the solubility products of the latter are smaller (for calcium 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 alkaline earth phosphates is finally transformed to the oxalates.

\* Larger amounts are permitted with Whatman No. 3 mm paper.

\*\* НУВИСКИ *et al.*<sup>17</sup> found the solubility product of PbHPO<sub>4</sub> to be minimal at pH 4.5. The addition of alcohol decreased the solubility. Pb<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> was precipitated only above pH 6.

Owing to the change in the concentrations of the two anions, part of the lead phosphate is also transformed to 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 a precipitate of the lead.

The experimental standard deviations can be totally accounted for by the factors known to contribute to them: (1) 0.015  $\mu\text{g}$  is due to variations in the transformation of lead phosphate described above, (2) 0.03  $\mu\text{g}$  is due to variations in the amount of lead lost in the paper chromatographic separation, (3) 0.06  $\mu\text{g}$  is due to the variable paper blank\*, (4) 0.04  $\mu\text{g}$  is due to the error made in the measurement of  $^{32}\text{P}$  and (5) 0.06  $\mu\text{g}$  is caused by the adsorption of the reagent on (or occlusion in) the precipitate. In experiment 1 (Table I) a factor (6) of 0.06  $\mu\text{g}$  due to variations in the alkaline earth blanks has to be added to this list.

#### ACKNOWLEDGEMENT

The author is indebted to MRS. W. CLINÉ-THEIL for valuable technical assistance.

#### SUMMARY

A method is described for the selective determination of lead in paper chromatograms, down to 1  $\mu\text{g}$  (standard deviation 11%). After development and drying, the lead spot is sprayed with a  $\text{Na}_2\text{H}^{32}\text{PO}_4$  solution and dried. Excess reagent and alkaline earth phosphates are eluted with a borax-oxalate buffer of pH 8.0. Eventually, the radio-activity of the lead spot is measured. The filter paper renders the method about 40 times more sensitive than existing radiometric techniques with phosphate- $^{32}\text{P}$ . The method can be modified for the simultaneous determination of many cations.

#### RÉSUMÉ

Une méthode radiométrique est proposée pour le dosage du plomb par chromatographie sur papier, au moyen de phosphate disodique, marqué au phosphore-32. La sensibilité est meilleure que celle obtenue avec les autres techniques radiométriques, à l'aide de  $\text{Na}_2\text{H}^{32}\text{PO}_4$ .

#### ZUSAMMENFASSUNG

Beschreibung einer radiometrischen Methode zur Bestimmung von Spuren von Blei durch Papierchromatographie unter Verwendung von Dinatriumphosphat- $^{32}\text{P}$ . Auf mögliche Modifikationen zur gleichzeitigen Bestimmung mehrerer Kationen wird hingewiesen.

#### REFERENCES

- 1 P. C. VAN ERKELENS, *Thesis*, Utrecht, 1960.
- 2 P. SUE, *J. Phys. Chem.*, 45 (1941) 639; *Bull. soc. chim. France*, 13 (1946) 102.
- 3 A. LANGER, *J. Phys. Chem.*, 45 (1941) 639.
- 4 TH. MOELLER AND G. K. SCHWEITZER, *Anal. Chem.*, 20 (1948) 1201.
- 5 G. BARCIA GOYANES, *Bol. radiactividad*, 24 (1951) 34.
- 6 I. V. TANANAEV, *Izvest. Akad. Nauk S.S.S.R.*, (1955) 5; (*Anal. Abstr.*, 4 (1957) 367).
- 7 N. MEVEL, *Intern. Congr. Pure and Appl. Chem.*, Lisbon, 1956, Paper V, p. 20.
- 8 W. O. PHILLIPS, *Anal. Chem.*, 28 (1956) 280.
- 9 D. I. FRENCH AND M. R. GIBSON, *Anal. Chem.*, 29 (1957) 1166.
- 10 R. E. THIERS, J. F. WILLIAMS AND J. H. YOE, *Anal. Chem.*, 27 (1955) 1725.
- 11 M. LEDERER, *Anal. Chim. Acta*, 5 (1951) 185.
- 12 W. R. WALKER AND M. LEDERER, *Anal. Chim. Acta*, 5 (1951) 191.
- 13 F. H. BURSTALL, G. R. DAVIES, R. P. LINSTAD AND R. A. WELLS, *J. Chem. Soc.*, (1950) 516.
- 14 E. C. HUNT, *Report CRL/AE 114*, Chemical Research Laboratory, Teddington (UK), 1954.
- 15 F. H. POLLARD AND J. F. R. MCOMIE, *Chromatographic Methods of Inorganic Analysis*, Butterworths, London, 1953.
- 16 J. P. EBEL AND Y. VOLMAR, *Compt. rend.*, 233 (1951) 415.
- 17 W. HUBICKI, B. FRANK AND J. TRAU, *Ann. Univ. Mariae Curie-Sklodowska, Lublin-Polonia, Sect. A.A.*, 5 (1950) 53; *C.A.*, 47, 9211<sup>a</sup>.

\* It can be shown<sup>1</sup>, that the greater part of this blank is due to the adsorption of phosphate- $^{32}\text{P}$  to the paper.