

## Microdetermination of Zinc in Soil Extracts by Paper Chromatography

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Small amounts of zinc are separated from other metals, except mercury, that form coloured complexes with dithizone. The isolated zinc zone on the chromatogram is cut out, and the zinc is eluted and determined colourimetrically with dithizone.

In an investigation on the zinc content of danish soils, the problem of finding a rapid analytical method suitable for routine work arose<sup>1</sup>. In the absence of a good specific colourimetric reagent for zinc, it was desirable to develop a separation method for those small zinc concentrations found in soil extracts.

According to two papers by Venturello *et al.*<sup>2,3</sup> Zn<sup>++</sup> can be separated from Cu<sup>++</sup>, Fe<sup>++</sup>, Fe<sup>+++</sup>, Ni<sup>++</sup>, Pb<sup>++</sup>, Mn<sup>++</sup>, Mg<sup>++</sup> and Al<sup>+++</sup> by means of horizontal circular chromatography. The solvent used was *n*-butanol in equilibrium with an equal amount of 2 *N* HCl. This solvent gives an  $R_F$  value, defined by Le Rosen<sup>4</sup> as the ratio of the distance traversed by a zone to the distance traversed by the developing solvent, of 0.87 for zinc, while the corresponding value for the other metals are about 0.4.

On basis of these results, the present author has tried to include the ordinary metals, which give colour reactions with dithizone (Co<sup>++</sup>, Ag<sup>+</sup>, Bi<sup>+++</sup>, Cd<sup>++</sup> and Hg<sup>++</sup>). In the method outlined below, it was possible to separate zinc from all these metals except from mercury, and the  $R_F$  values for the various metals found under these conditions, are shown in Table 1.

Table 1.  $R_F$  values for the different metals.

Metal	Cu	Ni	Co	Pb	Bi	Ag	Zn	Cd	Hg
$R_F$	0,13	0,06	0,08	0,11	0,12	0,36	0,84	0,68	0,87

### EXPERIMENTAL

The solutions were all made with distilled water from which traces of metals had been removed by passage through an ion exchange column (Amberlite Resin IR—100 (H)).

*Standard Zn-solution, 100 mg Zn/l.* 0.4400 g ZnSO<sub>4</sub> · 7 aq. (Merck p.a.) is dissolved in 1 liter of purified water containing 10 ml 10 % H<sub>2</sub>SO<sub>4</sub>.

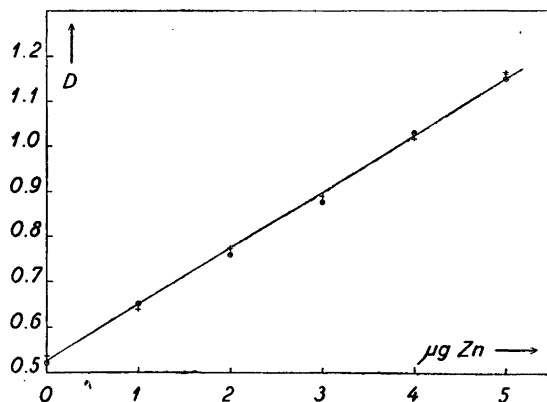


Fig. 1. Standard curve for the zinc-dithizon complexes.  $D$  = optical density measured in 1.0 cm cells at 540  $m\mu$ . The  $\odot$  points mark the  $D$  values of a solution which only contains Zn, while the + points indicate the  $D$  values of a solution, containing Cu, Ni, Co, Pb, Bi, Ag and Cd in addition to Zn.

**Dithizone solution.** 0.04 g dithizone (di-phenylthiocarbazone, Merck p.a.) is dissolved in 200 ml freshly distilled  $\text{CCl}_4$ . This solution is filtered, care being taken to exclude all trace metals from the filter, into a dark bottle and stored at 0 °C. Each day 10 ml of this stock solution is diluted with  $\text{CCl}_4$  to make 100 ml. — All  $\text{CCl}_4$  residues containing dithizone can be purified by a simple distillation in an allglass apparatus.

**Ammonium citrate buffer solution.** 113 g Ammoniumcitrate ( $(\text{NH}_4)_2\text{C}_6\text{H}_6\text{O}_7$ , Merck p.a.) is dissolved in 500 ml purified water, and 150 ml 25 %  $\text{NH}_3$  is added. This solution is repeatedly shaken with 50 ml portions of the dithizone-stock-solution, which are discarded, until the colour of the dithizone solution remains green. The water phase is now yellow from dissolved dithizone, and must be shaken several times with 50 ml portions of  $\text{CCl}_4$ , until both phases remain colourless. The solution is then made up to 1 liter with purified water.

**2 N Hydrochloric acid solution,** was made from constant boiling HCl.

***n*-Butanol-HCl-solvent.** 1 liter of freshly distilled *n*-butanol is shaken for one hour with 1 liter of the 2 N hydrochloric acid solution, and the water phase is discarded.

All these stock solutions must be stored in Pyrex bottles with glass stoppers to avoid contamination by trace metals.

Whatman filter paper no. 1 contains appreciable amounts of metals giving a colour reaction with dithizone and must therefore be eluted before use. This is done by using the following technique. One sheet is cut into 6 smaller sheets of 14 × 23 cm, and each sheet is placed with its long side against the bottom of an aquarium jar 20 cm high and 30 × 30 cm base. A grooved glass-plate is placed on the bottom of the jar, and to keep the sheets upright a grate of parallel glass tubes, 1.5 cm apart, is placed 11 cm above the

Table 2.  $D$  values of the zinc-dithizon complexes in a pure Zn solution (A) and in a metal mixture solution (B).

µg Zn	$D$	
	A	B
0	0.522	0.541
1	0.655	0.640
2	0.760	0.772
3	0.880	0.890
4	1.029	1.015
5	1.147	1.157

bottom. The glass tubes are held together by a wooden frame, supported by four vertical glass tubes.

900 ml of the butanol-HCl-solvent is poured into the jar, one sheet of paper is placed between each glass tube, and the jar is closed with a tight cover to prevent evaporation. By standing overnight the sheets are completely irrigated by the vertical ascending movement of the solvent, and all the impurities of metals contained in the filterpaper like zinc are found in the upper edge of the sheets as indicated by a faint yellow colouration. The sheets are withdrawn from the jar, air-dried and neutralized for one hour in gaseous  $\text{NH}_3$ . (On acid papers the Cd and Zn zones are not distinctly separated.) It is important to handle their upper edges only. With a micropipette a known amount of the metal mixture solution to be analysed is applied in a horizontal stripe about 2 cm from the lower edge and extending almost halfway across the sheet to get two analyses on each sheet. After drying, the sheets are replaced in the jar and left there for about two hours until the solvent front has reached the upper edge of the sheets.

In order to identify the zinc zone, one of the sheets, upon which a pure zinc solution has been applied, is dipped in a solution of dithizone in  $\text{CCl}_4$ . After drying the zinc zone will then be indicated by a reddish colour. By comparison with this sheet, the zinc zone of the other sheets, representing a strip of about  $1 \times 11.5$  cm can be located and cut out.

These strips are cut in 5 pieces and placed in 100 ml Erlenmeyer flasks. The zinc is eluted by pouring a mixture of 1 ml 2 N HCl solution and 15 ml purified water in the flasks and boiling for two minutes. When cold, 3 ml ammonium citrate buffer solution is added together with 10 ml dithizone reagent. The solution is shaken vigorously which makes the  $\text{CCl}_4$ -phase turn red to a degree depending on the zinc content, while the filter-paper fibres remain in the water phase. After 5 minutes the optical density,  $D$ , of the red colour is measured on a Beckman DU spectrophotometer in 1.0 cm Corex cells at 540  $m\mu$ . The reference cell contained pure  $\text{CCl}_4$ .

## RESULTS

To test the reliability of this method, two different solutions were analysed; one containing only Zn, 100 mg/l, and another containing Cu, Ni, Co, Pb, Bi, Ag and Cd in addition to Zn; all 100 mg/l. From these solutions 0, 10, 20, 30, 40 and 50  $\mu\text{l}$ , corresponding to 0, 1, 2, 3, 4 and 5  $\mu\text{g}$  zinc were pipetted onto the paper sheets. The optical densities,  $D$ , were found as previously described, and the results are shown in Table 2 and Fig. 1. There was a good agreement between the zinc concentrations found in the pure zinc solution and in the metal mixture solution, indicating a complete separation of zinc from the other metals.

In this experiment the sulphates, chlorides and nitrates of the metals were used. In case of complexes of the metals stable in the *n*-butanol — HCl solution, however, the  $R_f$  values may vary radically. But in practice the actual metal complexes with ligands from the soil are likely to be so unstable that they will not disturb the analysis.

A report on the application of this method in soil analysis will later appear in *Acta Agriculturae Scandinavica*.

Thanks are due to the director of this laboratory, Professor K. A. Bondorff and to Professor J. Bjerrum, Chemistry Department A of the Technical University, Copenhagen, for suggestions and discussions.

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Received October 17, 1953.

*Acta Chem. Scand.* **7** (1953) No. 10