not acid, precipitation is incomplete. After this was done it was found that an excess of acid significantly increases the solubility of barium sulphate[2]. In practice, however, the effect seemed to be negligible, and is allowed for if sulphate standards are included in each batch of measurements.

The sample and barium chloride reagent are mixed by shaking, allowed to stand for 1 h, and centrifuged at 4,500 r.p.m. for 10 min. The supernatant is decanted and the tube allowed to drain. The sides of the tube and the surface of the precipitate are then rinsed with 1 ml. of non-radioactive barium chloride reagent. It was found that washing the precipitate decreased the recovery and made the results more irregular. In one experiment the amount of supernatant trapped in the precipitate was measured by precipitating sulphate with a solution of non-radioactive barium chloride containing radioactive silver nitrate. The activity trapped in the precipitate was negligible.

After rinsing, the activity of the precipitate is counted in a scintillation counter with a well crystal. The whole operation is carried out in one tube, so that the possibilities of loss are minimal. The amount of barium-133 added to the reagent is adjusted to give a count-rate of about 2,000 c.p.m./$\mu$M sulphur.

In 21 consecutive measurements of 2–10 $\mu$M sulphate the mean difference between duplicates was 1·4 per cent (max. 3·8 per cent). In 8 consecutive measurements of 0·5–2 $\mu$M sulphate the mean difference was 3·0 per cent, or 1·5 per cent if one aberrant pair of readings, differing by 12 per cent, is excluded.

In 3 experiments to test linearity, the results shown in Table 1 were obtained.

Table 1

| $\mu$M SO$_4$ present | No. of samples | Counts per min | Percentage of counts at 0·5 $\mu$M | Percentage error |
|---|---|---|---|---|
| 0·5 | 4 | 1,028 | 100 | — |
| 1·0 | 6 | 2,058 | 200 | 0·1 |
| 2·0 | 6 | 4,162 | 404 | 1·0 |
| 4·0 | 2 | 7,945 | 772 | 4·0 |

In acid solution the other constituents of urine did not interfere with the precipitation of sulphate. 8 samples of infants' urine were taken at random, and aliquots of 0·5 ml. added to 0·5 ml. of standard solutions containing 10 and 20 $\mu$M sulphate per ml. The mean recovery was 101 per cent.

It appears, therefore, that by this method amounts of sulphate down to 0·5 $\mu$M can be estimated with an accuracy of $\pm$ 2 per cent.

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## Tetrahedral Co-ordination in *Bis-N-isopropyl Salicylaldiminato Nickel II*

SACCONI et al.[1] have recently reported that preliminary investigations indicate a tetrahedral arrangement of ligands about the nickel atom in the paramagnetic *bis-N*-isopropyl salicylaldiminato nickel II. This was based on the isomorphism of this compound with the cobalt and zinc analogues, and the large ($\sim$ 7 Å) distance between nickel atoms, which precludes a polymeric octahedral structure such as found for acetylacetonato nickel II (ref. 2), or *bis*-dimethylglyoximato nickel II (ref. 3).

We have since collected three-dimensional data by the equi-inclination Weissenberg technique and have proceeded with structure determination. We are still refining the structure and at this stage $R = 0.064$. The arrangement of the ligands about the nickel atom is a distorted tetrahedron.

Current bond distances about the nickel atom are:

Average Ni—O distance is 1·90 Å
Average Ni—N distance is 1·97 Å
N$_1$—Ni—O$_1$ angle is 94·0°
N$_2$—Ni—O$_2$ angle is 94·9°
N$_1$—Ni—O$_2$ angle is 112·6°
N$_2$—Ni—O$_1$ angle is 111·7°
N$_1$—Ni—N$_2$ angle is 121·0°
O$_1$—Ni—O$_2$ angle is 125·1°

The two salicylaldimine residues are essentially planar, the mean deviation of atoms from the least square planes of the two being about 0·025 Å. The angle between the planes is 81°.

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## Apparent Molecular Weight of Insulin in Dilute Acid Solution

THE molecular weight of the smallest unit of the insulin molecule occurring in dilute acid solution has been in doubt for some time. Most data from experimental methods, such as ultracentrifugation[1], light scattering[2] and osmometry[3], indicate a minimum value of about 12,000. Some evidence, however, has accumulated for the existence of an insulin molecule of molecular weight of about 6,000 under acidic conditions[4]. This corresponds to the insulin unit the molecular weight of which obtained from the amino-acid sequence is 5,750 (ref. 5). A number of investigators have indeed observed such a value in non-aqueous solvent/water mixtures[6], in media favourable to dissociation[7] and in aqueous solution at alkaline $p$H values[8].

We have measured the apparent molecular weight of insulin in acid solution over a range of concentrations from approximately 0·8 to 0·02 g/100 ml. using the technique of sedimentation equilibrium. A Spinco model E ultracentrifuge, equipped with a Rayleigh interference optical system and both 12-mm and 30-mm cells, was used.

The results of our study are shown in Fig. 1, which refers to four different insulin solutions. The insulin was a crystalline preparation containing an unknown amount of zinc, prepared by the Commonwealth Serum Laboratories (batch number A3). The solvent in all cases was an aqueous glycine–hydrochloric acid–sodium chloride buffered solution of ionic strength 0·1 and $p$H 2·00. Solutions 1, 2, 3 and 4 had initial concentrations of approximately 0·5, 0·2, 0·1, and 0·05 g/100 ml. respectively and were centrifuged at speeds of 17,980, 17,980, 29,500 and 29,500 r.p.m., respectively, until equilibrium was reached. Since column heights of only 2–3 mm were used equilibrium was reached in less than 18 h. The temperature of the rotor was held constant at 25·0 $\pm$ 0·2° C. The four solutions were prepared from a concentrated (1 g/100 ml.) stock solution of insulin that had previously been dialysed for 72 h against a hundred times its volume of buffer with a change of buffer at 48 h. After dialysis the $p$H of both insulin solution and diffusate was 2·04. The refractive index increment of the stock solution compared with the diffusate was determined by means of the refractometer described by Cecil and Ogston[9].