

THE ASSAY OF LEPTAZOL

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UNTIL recent years the determination of pentamethylenetetrazole presented great difficulties due to the absence of typical "functional groups"; furthermore it was found¹ that the estimation of the nitrogen content by the usual Kjeldahl process gave results up to 60 per cent. too low. Ultra-violet absorption measurements, suitable in the case of some tetrazoles, were also useless for leptazol as solutions were transparent in all utilisable parts of the spectrum.²

The compound is now determined by taking advantage of the sparingly-soluble nature of complexes formed with salts of the heavy metals, especially mercuric chloride³ and cuprous chloride and that formed with the latter was thoroughly investigated by Dister.⁴ The precipitate formed upon adding a solution of cuprous chloride to a solution of leptazol, under certain conditions, he found to have the composition $8C_6H_{10}N_4 \cdot 7Cu_2Cl_2$, and to be practically insoluble in 1 per cent. acetic acid, which was therefore used for washing the complex. Dister assayed the complex for cuprous copper by dissolving it in warm ferric alum solution and then estimating the ferrous iron thereby produced by titrating with potassium permanganate solution. Good results were obtained.

The present author has found that the complex soon turns blue in the air, due to oxidation, and hence the assay must be completed quickly; to obviate this the method has been modified, the total copper in the filtrate being estimated after oxidising with hydrogen peroxide. By using a pipetted quantity of cuprous chloride solution for the assay and performing a "blank" the amount of copper removed as complex can readily be calculated. As total copper is estimated in each case, two or three precipitations and filtrations can be performed together and the filtrates assayed at leisure, as air oxidation will not vitiate the result once the complex has been removed.

At first great difficulty was experienced in obtaining consistent figures both by Dister's method, and by the modified method described; the fault was eventually traced to the cuprous chloride used in preparing the reagent. Not only must the reagent be freshly prepared but the cuprous chloride itself must be of good quality and should assay well for cuprous chloride by the ferric alum/permanganate method described below. Old samples of cuprous chloride are likely to consist largely of cupric compounds and although the reagent contains sodium metabisulphite (which would be expected to reduce all the copper to the cuprous state) poor results are obtained. The reason for this is not clear for although reagent made with an old sample was more acidic (*pH* 1.6) than one made with freshly prepared cuprous chloride (*pH* 2.7) the hydrogen ion concentration

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of the reagent does not appreciably affect the results. This was readily ascertained by reversing the *pH* values of the two reagents by adding a sufficient quantity of N sodium hydroxide to the "old" reagent to raise its *pH* from 1.6 to 2.7, and N hydrochloric acid to the other to lower its *pH* from 2.7 to 1.6 and then repeating the assay with both of them. (See Table I.)

TABLE I
RESULTS OBTAINED WITH DIFFERENT SAMPLES OF CUPROUS CHLORIDE REAGENT

| Reagent | <i>pH</i> | C ₆ H ₁₀ N ₄ per cent. |
|--|-----------|--|
| (1) Prepared with fresh Cu ₂ Cl ₂ | 2.7 | 99.8 |
| (2) Prepared with old Cu ₂ Cl ₂ | 1.6 | 77.5 84.2 68.0 |
| (3) Prepared with fresh Cu ₂ Cl ₂ but <i>pH</i> adjusted with HCl .. | 1.6 | 97.5 |
| (4) Prepared with old Cu ₂ Cl ₂ but <i>pH</i> adjusted with NaOH | 2.7 | 84.2 |

EXPERIMENTAL

Assay of Leptazol. Dissolve about 0.1 g. of sample, accurately weighed, in 25 ml. of water, and add slowly with stirring, 25 ml. of solution of cuprous chloride. Stopper the flask and allow to stand for 3 hours with occasional shaking. Filter through a sintered-glass crucible (No. 3) and wash the precipitate and flask with 30 ml. of water containing 1 per cent. w/w of glacial acetic acid. To the mixed filtrate and washings add 10 ml. of solution of hydrogen peroxide, and if necessary, a few drops of dilute sulphuric acid to clarify the solution. After effervescence has ceased, bring gently to the boil and continue boiling for 10 minutes to decompose the excess of hydrogen peroxide. Cool to room temperature add solution of ammonia drop by drop to obtain an opalescence and clear by addition, drop by drop, of acetic acid. Add 5 g. of potassium iodide, titrate with 0.1N sodium thiosulphate until the liquid is pale brown, add mucilage of starch and 3 g. of ammonium thiocyanate and continue the titration until the colour is discharged. Note the number of ml. required for the whole titration. Repeat the determination with 25 ml. of solution of cuprous chloride commencing with the words ". . . add 10 ml. of solution of hydrogen peroxide. . . ." The difference between the two titrations is equivalent to the amount of copper present in the precipitate. Each ml. of 0.1N sodium thiosulphate is equivalent to 0.007891 g. of C₆H₁₀N₄.

Cuprous chloride. This should be freshly prepared⁵ or of reagent purity.

Assay. Dissolve by warming about 0.3 g., accurately weighed, in 20 ml. of a solution of ferric ammonium sulphate (5 per cent.) in sulphuric acid (20 per cent. w/w). Cool to room temperature and titrate with 0.1N potassium permanganate. Each ml. of 0.1N potassium permanganate is equivalent to 0.009903 g. of Cu₂Cl₂.

Solution of Cuprous Chloride (Dister's formula⁴). Dissolve 1.25 g. of cuprous chloride in 100 ml. of solution of ammonium chloride containing

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1 g. of sodium metabisulphite. When solution is complete, filter if necessary. The solution should be freshly prepared.

Results. 0.1205 g. of sample gave (a) 100.1, (b) 99.5, (c) 99.8, (d) 99.8 per cent. of $C_6H_{10}N_4$.

SUMMARY

1. The assay of leptazol introduced by Dister has been modified, the filtrate being titrated for total copper instead of the complex being assayed for cuprous copper. It is claimed that the method is quicker and more convenient, potassium permanganate being replaced by the more stable sodium thiosulphate as volumetric reagent.

2. It has been shown that the cuprous chloride used for preparing the precipitant must be either freshly prepared or of reagent purity.

REFERENCES

1. Berman, *J. Assoc. off. agric. Chem. Wash.*, 1942, 25, 790.
2. Schueler *et al.*, *J. Pharmacol.*, 1949, 97, 266.
3. Horsley, *Analyst*, 1946, 71, 308.
4. Dister, *J. Pharm. Belg.*, 1948, 190.
5. Vogel, *Textbook of Practical Organic Chemistry*, Longmans, Green & Co., 186.