

activity of each copper chelate reaches the highest level when the mixing ratio of the metal to the organic compound is 1:2. In copper (II)-neocuproin, however, this optimal ratio is about 1:1.5; this is probably due to the steric hindrance of the methyl groups in the 2- and 9-positions. The above results, which were obtained by manometric methods, were also confirmed by the direct determination of dihydroxyphenylalanine itself by Arnow's method³.

From these results it may be considered that all the bonds of copper in copper (II)-dipyridyl and -phenanthroline are fully co-ordinated with nitrogen atoms of the chelating agents when the catalytic activity of these complexes is at the maximum. During the course of reaction, however, the dihydroxyphenylalanine will also chelate, if temporarily, with the copper.

Among the copper complexes investigated, copper (II)-neocuproin showed the highest activity, as shown in Figs. 1 and 2. Comparing the structure of this complex with that of copper (II)-phenanthroline, it seems that the high catalytic activity has been acquired under the influence of the electron-releasing methyl groups in the 2- and 9-positions. On the other hand, copper (II)-neocuproin strongly inhibited the oxidation of the substrate if the chelating agents were present in excess, that is, if the mixing ratio exceeded 1:2. The competition between chelating agents and substrates for the metal would be responsible for this.

The copper seems to act as an electron carrier in this reaction, for copper (II)-chelates could be extracted with chloroform when copper (II)-neocuproin or -cuproin reacted with dihydroxyphenylalanine in an excess of chelating agents. One of the extracts was purple in colour and was found to be copper (I)-cuproin⁴, having the maximal absorption band at 545 m μ ; the other was orange-yellow and was identified as copper (I)-neocuproin⁵, with the maximal absorption band at 457 m μ . It is likely that a reversible change of valency takes place in the copper atom under oxidation.

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¹ Isaka, S., and Ishida, S., *Nature*, **171**, 303 (1953). Isaka, S., and Akino, M., *Nature*, **177**, 184 (1956).

² Calvin, M., *Naturwiss.*, **8**, 387 (1956).

³ Arnow, L. E., *J. Biol. Chem.*, **118**, 531 (1937).

⁴ Felgl, F., "Spot Tests", **1**, 87 (1954).

⁵ Gahler, A. R., *Anal. Chem.*, **26**, 577 (1954).

A New Micromethod for the Estimation of Cellulose

THE only recorded micromethod for the estimation of bacterial cellulose is that of Schramm and Hestrin¹, which requires the acetolysis of a previously purified sample and careful neutralization of the hydrolysate with sodium hydroxide prior to colorimetric estimation of the reducing power of this neutralized solution. This method is rather long—a disadvantage when several samples are to be assayed—and the neutralization step is critical. A micromethod has now been devised which halves the time taken for analysis, by eliminating acetolysis, hydrolysis and neutralization. This new method is an adaptation of that reported

by Mendel, Kemp and Myers² for estimating blood glucose. These workers found that their method was also applicable to such polysaccharides as starch and glycogen. Although cellulose is much more difficult to hydrolyse than either of these polysaccharides, adaptation of their method has proved successful in estimating cellulose directly without initial hydrolytic treatment.

Samples of bacterial cellulose are prepared by treatment with 4 per cent (w/v) sodium hydroxide to remove cells, washed and centrifuged as described by Schramm and Hestrin¹. Talc is added to weight the cellulose fibres in the centrifuge tubes as recommended by these workers. Each dried sample is suspended in a definite volume of water in a large test-tube to bring the concentration of cellulose within the correct range of 0–150 μ gm. per ml. Three times this volume of 96 per cent (w/v) sulphuric acid is added dropwise to the tube while it is shaken. (The commercial origin of the sulphuric acid used should be one of those recommended by Mendel and his co-workers².) The tube is then heated for exactly 6.5 min. in a boiling water-bath, and cooled under running water. The pink solution is centrifuged to remove any suspended talc, and its optical density is measured at 520 m μ in a 'Coleman Junior' spectrophotometer. The standard curve uses glucose as a reference substance. This is a straight line between values of 0 and 150 μ gm. of glucose per ml.

In a typical experiment to test the method, the cellulose formed by a static culture of *A. xylinum* was purified, air-dried, weighed and homogenized to give a uniform suspension of a concentration falling within the limits of the method. The cellulose content of a series of duplicate samples of the suspension was then estimated as described above. The results are given in Table 1.

Table 1. RECOVERIES OF SAMPLES OF BACTERIAL CELLULOSE (IN μ GM.)

Weighed (air-dried)	Found	Recovery (per cent)
205	210	102
308	280	91
411	395	96
514	500	97
616	595	97

The method also works well for filter paper, and possibly also for other suitably purified forms of cellulose.

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¹ Schramm, M., and Hestrin, S., *Biochem. J.*, **56**, 163 (1954).

² Mendel, B., Kemp, A., and Myers, D. K., *Biochem. J.*, **56**, 639 (1954).

Action of Solvents on Dried Linseed Oil Films

THE action of a range of organic solvents on aged linseed oil films, both pigmented and unpigmented, has been investigated¹. A specially designed apparatus based on the parallel plate viscometer was used² to make precise measurements of changes in thickness of films coated on a rigid substrate, while free films (these proved to be isotropic in behaviour) were observed under a measuring microscope. The results