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A sensitive procedure for determining cyanate in urea solutions

Cyanate reacts readily with proteins[1], and its adventitious accumulation in urea solutions therefore presents a serious risk of unwanted protein modification[2–6]. As a first step in studying this possibility, a sensitive method for estimating cyanate is needed. The following procedure, devised to meet this requirement, depends on the formation of copper pyridinium cyanate[7]. The absorption maximum at 700 nm of this blue complex in chloroform is the basis of a quantitative test for cyanate[8]. In the present method the much higher absorption at 315 nm (Fig. 1) is used to provide about thirty times more sensitivity than the earlier method[8].

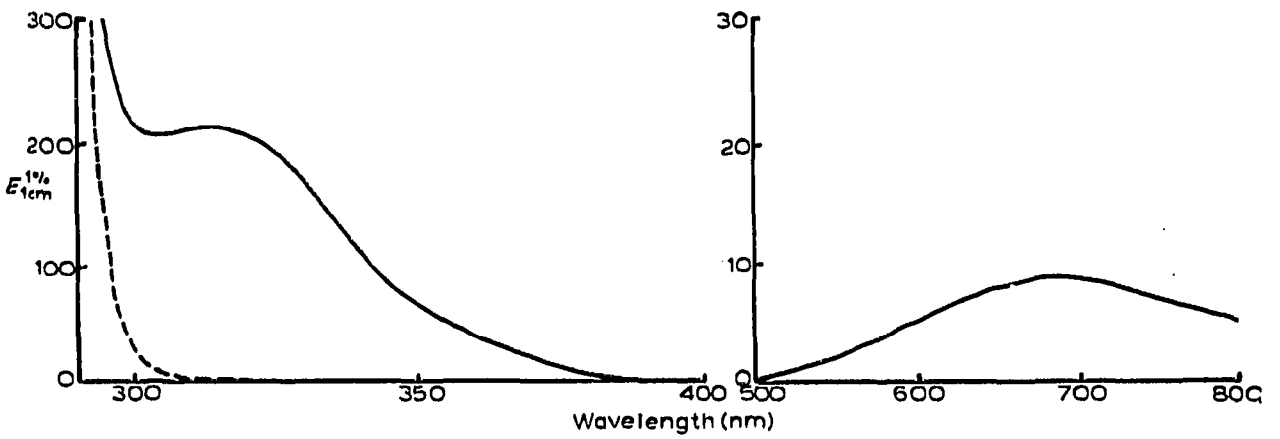

Fig. 1. Absorption spectrum of copper pyridinium cyanate (solid line) in chloroform, prepared as described under *Procedure*. The dotted line represents the absorption of the blank. The extinction coefficient refers to the concentration of cyanate ion.

Procedure

Mix the sample, made up to 3.0 ml in a test-tube, with 0.4 ml of copper–pyridine reagent (containing pyridine and 10% $CuSO_4 \cdot 5H_2O$, premixed in equal volumes). Add 3.0 ml of chloroform with vigorous mixing. After 5 min, mix again and stand or centrifuge to separate the layers. Withdraw the lower chloroform layer and determine its absorbance at 315 nm. Compare this absorbance with those of solutions of known cyanate concentration. 1 μmole of cyanate in the above test produces an absorbance of about 0.3 (1-cm light path).

Procedures for preventing cyanate formation and for removing cyanate from urea solutions have been suggested[8–10]. This sensitive estimation method should prove useful in testing the effectiveness of these measures in the particular case under study.

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1 G. R. STARK, in C. H. W. HIRS (Editor), *Methods in Enzymology*, Vol. XI, Academic Press, New York, 1967, pp. 125 and 590.
2 R. D. COLE, *J. Biol. Chem.*, 236 (1961) 2670.
3 W. MANSON, *Biochim. Biophys. Acta*, 63 (1962) 515.

4 E. G. COLE AND D. K. MECHAM, *Anal. Biochem.*, 14 (1966) 215.
5 F. R. HUEBNER, J. A. ROTHFUS AND J. S. WALL, *Cereal Chem.* 44 (1967) 221.
6 D. BOBB AND B. H. J. HOFSTEE, *Anal. Biochem.*, 40 (1971) 209.
7 E. A. WERNER, *J. Chem. Soc.*, 123 (1923) 2577.
8 J. R. MARIER AND D. ROSE, *Anal. Biochem.*, 7 (1964) 304.
9 G. R. STARK, W. H. STEIN AND S. MOORE, *J. Biol. Chem.*, 235 (1960) 3177.
10 E. HABER, *Proc. Nat. Acad. Sci.*, 52 (1964) 1099.

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