Modified Reagents for Determination of Urea and Ammonia

Albert L. Chaney and Edward P. Marbach

Combinations of reagents are described for the catalyzed indophenol reaction for the determination of ammonia, which produces a stable blue color. The procedure is adapted to the determination of urea after hydrolysis with urease.

IN APPLYING the catalyzed indophenol reaction to the determination of urea and ammonia in biological fluids, it has been found advantageous to combine the reagents in the following manner:

Reagent	*Dilute form (gm./L.)*	*Concentrated form (gm./L.)*	*(M./l.)*
	SOLUTION 1		
Phenol	10.0	50.0	0.5
Sodium nitroprusside	0.050	0.25	0.001
	SOLUTION 2		
Sodium hydroxide	5.0	25.0	0.625
Sodium hypochlorite	0.42	2.1	0.03

This reduces the number of reagents required for color development to two, each of which is stable for 60 days or more if kept cool and in amber bottles protected from light.

These reagents are added in equal volumes to the ammonia sample. For example, to 1–5 μg. of ammonia in a volume of 1 ml. or less, 5 ml. each of diluted solutions 1 and 2 are added successively. Maximum blue color is produced at room temperature in about 30 min. and the absorbance remains unchanged for 24 hours or more.

Alternatively, one may use 1 ml. each of the more concentrated reagents and develop the color at a higher temperature—for example, at 50–60°. In this case, color development will be completed in about 3

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min. and the solution is diluted to a convenient volume for spectrophotometric determination.

The absorbance obtained from 10 μg. of ammonia nitrogen in a final volume of 10 ml. is about 1.3 (1-cm. cell) at 625 mμ, which is several times greater than values reported for procedures not using sodium nitroprusside as a catalyst (*1, 4, 5*). This confirms that this catalyst both accelerates the speed of the reaction and increases to a maximum the conversion of ammonia to indophenol (*2, 3, 6*).

The reproducibility of color intensity is excellent from day to day and with different batches of reagents, and is also independent of temperature or the concentration of reagents over the ranges described.

In selecting the composition of the solutions, it should be noted that the phenol and sodium hydroxide should be in approximate equimolar quantities in the final test solution and the amount of sodium hypochlorite somewhat less than 0.1 that of the phenol on a molar basis. The concentration of the catalyst is quite small and may be varied above and below the range given, with the primary effect being the rate of color development. If the concentration is increased very much, the blank will be increased significantly. Sodium hypochlorite has been added in the form of commercial bleach. The other chemicals are of reagent grade. With these, no difficulty has been encountered in keeping the reagent blank to very low values—absorbance of 0.01–0.05 in a 1-cm. cell.

For the determination of urea, 0.01 or 0.02 ml. of serum provide the optimum amount of urea nitrogen for a final colorimetric volume of 10 ml. The serum is first incubated with 0.2 ml. of buffered urease solution (150 mg. urease of activity 1000 U./gm. and 1.0 gm. EDTA per 100 ml., and adjusted to a *p*H of 6.5). The urease solution shoul be kept refrigerated. Under these conditions it may be used for 30 days. If kept frozen till needed, or in a dry or lyophilized mixture, stability is much greater.

After hydrolysis of urea for 20 min. at room temperature, or 5 min. at 50–60°, the color reagents are added according to the description given previously. As has been shown by Fawcett and Scott (*3*), neither the protein nor other normal constituents present in the serum interfere in the color development.

This procedure has been used with marked success for several months in several laboratories. It is believed that the combination of reagents in this manner contributes greatly to the convenience and reproducibility of these determinations. The reagents have also been

prepared in vials,* requiring only dilution with water to volume for preparation.

References

1. Boletter, W. T., Bushman, C. J., and Tidwell, P. W., *Anal. Chem.* **33,** 593 (1961).
2. Brown, R. H., Duda, G. D., Korkes, S., Handler, P., *Arch. Biochem.* **66,** 301 (1957).
3. Fawcett, J. K., and Scott, J. E., *Brit. J. Clin. Path.* **13,** 156 (1960).
4. Noble, E. D., *Anal. Chem.* **27,** 1413 (1955).
5. Riley, J. P., *Anal. Chim. Acta* **9,** 575 (1953).
6. Ternberg, J. L., and Hershey, F. B., *J. Lab. & Clin. Med.* **56,** 766 (1960).

*Hyland Laboratories, Los Angeles, Calif.