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Alkaline Azobilirubin Color Reaction to Determine Sodium Azide

To the Editor:

The interference of sodium azide in the determination of bilirubin by diazotized sulfanilic acid (Ehrlich’s reagent) has been well documented (1–3). However, because of its bacteriostatic property and unresponsiveness of interference from sodium azide in the measurement of several constituents of biological fluids by use of certain reagents, this preservative is still frequently used in various biological samples.

We have seen a substantial decrease in the color yield of the alkaline azobilirubin color reaction in the presence of sodium azide (4). Azide inhibits the formation of azopigment in the reaction between bilirubin and diazotized sulfanilic acid. However, this inhibition is not specific for azide, and ascorbic acid (e.g.) has a similar effect on this reaction.

Recently we detected sodium azide in our bilirubin-erythrocyte binding assay, in which albumin samples (containing 0.2 g/L sodium azide as preservative) used for bilirubin extraction from erythrocytes interfered in the color reaction. Extending these studies to determine the effect of sodium azide on the alkaline azobilirubin color reaction for bilirubin determination, we found a linear decrease in color yield on increasing azide concentration. We thus used this finding for the determination of azide concentrations in biological samples and, as we expected, the method worked well. A standard bilirubin (Sisco Research Labs., Bombay, India) solution (74 mg/dL or 1.27 mmol/L) was prepared as described by Bratlid (5), and its concentration was determined by the method of Fog (6). A stock solution (0.8 g/L) of sodium azide (SD’s Fine Chemicals, Bombay, India) was prepared in the same solution. Fog’s reagent I (caffeine reagent), reagent II (diazoo reagent), and reagent III (alkaline tartrate) were prepared as described by Fog (6).

The method is as follows: To 75–313 μL of solution containing 60–250 μg of sodium azide, we added 100 μL of 1.27 mmol/L bilirubin solution and brought the volume to 1.0 mL with 38 mmol/L sodium carbonate solution containing 5 mmol/L EDTA, pH 11.0 (use of 0.07 mol/L sodium phosphate buffer, pH 8.0, instead of sodium carbonate solution did not affect the color intensity). Then we added 2 mL of Fog’s reagent I, followed by 0.5 mL of reagent II, and mixed well. After 10 min of incubation at room temperature, we added 1.5 mL of Fog’s reagent III and recorded the absorbance at 600 nm. The percent decrease in the color intensity of bilirubin in the presence of sodium azide was calculated as \( \frac{(A_1 - A_2)}{A_1} \times 100 \), where \( A_1 \) and \( A_2 \) are the absorbances in the absence and the presence of sodium azide, respectively.

A plot of the amount of azide (within the range 60–250 μg) vs percent decrease in color intensity (Fig. 1) showed a linear relationship. When we used two different bilirubin concentrations (56 and 127 μmol/L), the results fit the following linear equations, respectively:

% decrease in color =

\[ 0.55 \text{ sodium azide (μg)} - 33.6 \]

% decrease in color =

\[ 0.41 \text{ sodium azide (μg)} - 28.4 \]

Because the range of linearity of the curve varied at different bilirubin concentrations, we used the curve obtained with the lower concentration (56 μmol/L) of bilirubin to determine azide concentration in the range 60–200 μg, and the curve obtained with 127 μmol/L bilirubin for azide in the range 70–250 μg. In fact, we found the bilirubin concentration of 127 μmol/L to be more appropriate for determining the concentration of sodium azide in various biological samples. Bilirubin at concentrations <56 μmol/L and >127 μmol/L did not yield good results, owing to either deviation from linearity or high color intensity in the control. To use this method with biological samples already containing bilirubin, we recommend exposing the samples to light to degrade the bilirubin already present.

References


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