NASA TECH BRIEF

NASA Pasadena Office

Increasing the Sensitivity of the Jaffe Reaction for Creatinine

The classic Jaffe reaction for the determination of creatinine is predicated on the formation of a transient red-colored substance with alkaline picrate. In typical analytical procedures, the intensity of color is measured at a narrow bandwidth in the vicinity of 490 nm.

A plot of absorbance *vs* concentration of creatinine is linear over a narrow range of creatinine concentrations; the plot characteristically curves downward at higher creatinine concentrations, and the position of the "break" in linearity is a function of procedural variables such as total concentration of base and picric acid, temperature, and the presence of other substances.

The Jaffe reaction is the basis for an automated analysis of urine in the Biosatellite Program; the creatinine content of primate urine is used to assess the effects of weightlessness in space. Creatine is an alkaloid which is an end product of amino acid catabolism, and it exists in relatively large amounts in the muscle tissue of all vertebrates. The anhydride of creatine, creatinine, is a normal constituent of mammalian urine and its presence is an indicator of muscle waste. When creatine is heated in an acidic media for a short period of time, it is converted to creatinine.

First a mixture of urine and alkaline picrate is prepared; the color intensity of this mixture is related to the creatinine content of the urine sample. A second sample of urine is heated in an acidic (H_2SO_4) solution at 100°C for 25 minutes to convert creatine to creatinine and then made alkaline in the presence of picrate; the incremental color intensity is linearly related to the creatine content of the urine sample. If the creatine content of the sample is too large, the absorbance of the creatinine-picrate reaction product falls on the nonlinear portion of the calibration curve; in ordinary laboratory work, a smaller sample or a larger dilution is used to bring the absorbance into the linear region of the calibration curve. However, for automated analysis in space, it is desirable to use an analytical procedure which covers the expected range of creatinine concentrations.

A systematic study of the analytical procedure has revealed that the linearity of the creatinine calibration curve can be extended by using a 0.03 molar picric acid solution made up in 70-percent ethanol instead of water; three to five times more creatinine concentration can be encompassed within the linear portion of the calibration curve. In a typical procedure, the reference solution is made by mixing 0.100 ml of urine sample, 2.00 ml of 0.03 *M* ethanolic picric acid solution, and 1.00 ml of 2.25 *M* sodium hydroxide solution, and then diluting to 10 ml with water. The creatinine conversion is accomplished by heating a mixture of 0.100 ml of urine sample and 0.54 ml of 2 *M* sulfuric acid; then, the mixture is treated with 1.96 ml of 2.25 *M* sodium hydroxide and 2.00 ml of 0.03 *M* ethanolic picric acid solution and diluted to 10 ml. With ethanolic picric acid, the color intensity of the Jaffe reaction product obeys Lambert-Beer's law at an absorbance as high as 3.4.

Note:

Picric acid of high purity must be used.

(continued overleaf)

Patent status:

NASA has decided not to apply for a patent.

Source: Ho Yet Tom of
Caltech/JPL
under contract to
NASA Pasadena Office
(NPO-11587)