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Note

Amino acid analysis

II. Ninhydrin reduction with titanous chloride

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Previous communications describing reduction of ninhydrin with titanous chloride were concerned with a Technicon AutoAnalyzer[1,2]. Difficulty was initially experienced when attempting to use the reducing agent in ninhydrin to be used in Beckman-type amino acid analyzers. This difficulty arose from the inadvertant use of oxidised titanous chloride solution and retention of a ninhydrin filtration in the analyzer. Thus, it was thought necessary to provide additional information on the use of titanous chloride that was applicable to Beckman analyzers, and to describe a rapid test for the reagent to ascertain whether the material at hand has become oxidised.

Ninhydrin reagent is prepared as follows: 60 g of ninhydrin is dissolved in 3 l of methylcellosolve and 1 l of 4 *M* acetate buffer under a nitrogen atmosphere. A 10-ml volume of titanous chloride solution is added. The colour of the preparation should change from yellow-green to a deep red. This red colour will persist for some time after transfer of the reagent to the analyzer reservoir but does not interfere with amino acid analysis which can be commenced immediately. The construction of the ninhydrin reservoir is described elsewhere[2], and is located outside the instrument so as to be cooled by room air conditioners. Adjustment of the rates of buffer and ninhydrin pumps is required. Buffer is to be pumped at 52 ml/h and the ninhydrin pump adjusted to give a combined flow-rate of 84 ml/h. In the older 120B analyzers a modified column connector will have to be installed to withstand the increased column back pressure[3].

Titanous chloride solution, approximately 15% (w/v), low iron content, and containing zinc chloride was obtained from Hopkin and Williams (Chadwell Heath, England). The reagent is purchased in volumes of 250 ml and has always yielded satisfactory results. Upon receipt of a new bottle the reducing potential of the material can be checked with a spectrophotometer. With a Cary 118 recording spectrophotometer the 0–1.0 absorbance scale, a 2 nm/sec wavelength, and a chart speed of 100 nm/in. is used. A scan from a wavelength of 700 nm to 380 nm is carried out and a peak at around 500 nm should be present. A 1-cm cuvette is used into which 0.25 ml of titanous chloride solution with 0.75 ml of water is placed. The reference beam cuvette contained water. When a peak height of 0.98 is recorded, under the conditions stated, the titanous chloride is suitable for reducing ninhydrin.

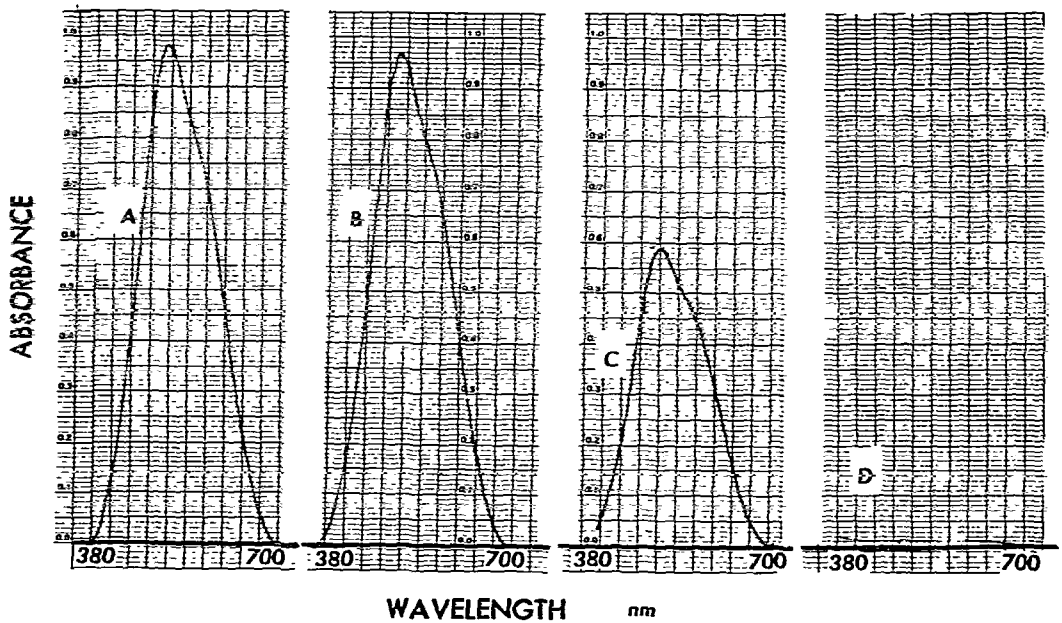

Fig. 1. Spectra of different batches of titanous chloride solutions.

Examples of different batches of titanous chloride solutions analysed with this procedure are shown in Fig. 1. Sample A was made with a new bottle of reagent which gives nearly a full scale deflection. Sample B provided a similar absorbance. This material had been in use for some time, only 1/3 of the original 250 ml remaining at the time of testing. The bottle had been stored at room temperature and 10-ml aliquots had been removed as quickly as possible and the cover replaced. No nitrogen atmosphere had been used. Sample C when tested provided an absorbance of 0.49. Again this material had been in use till 1/3 of the original contents remained. This batch could no longer be used in ninhydrin preparations. With sample D there was no absorption at 500 nm. This last sample had not been obtained from Hopkin and Williams. The reagent is supplied in a bottle which allows a large surface contact with air. Although the contents had only been half used at the time of testing, it was now useless for ninhydrin reduction.

In the case of the material supplied by Hopkin and Williams, the bottle shape is tall form, but these suppliers could safequard their product by initially minimising or eliminating any air space in the bottle. As aliquots of titanous chloride are removed, glass marbles can be rolled into the bottle to return the liquid surface to the narrow neck of the bottle. Forcing a draught of oxygen-free nitrogen into the bottle, after removal of reagent, did not prevent eventual oxidation of the material. Storing the reagent in a refrigerator appeared to hasten the process of oxidation, presumably because the cold liquid takes in more oxygen from the atmosphere. Storing the bottle in a warm place, near an oven, did not have any deleterious effect on the reagent. Eight years ago 7.5 ml of titanous chloride solution was sufficient to obtain optimum condition for the reduction of 60 g of ninhydrin. The volume required some years later was

7.7 ml. It now requires 10 ml of solution. The additional volume required can be explained if the titanous chloride solution currently supplied is more dilute.

When titanous chloride-reduced ninhydrin is used in Beckman analyzers, the ninhydrin filter must be removed. The flow line on the pressure side of the pump is connected directly to the coil or drain via the selector valve. Thus a messy job of replacing glass wool or resin bed filter is eliminated. There occurs no precipitate in the flow lines as is the case when stannous chloride is used for ninhydrin reduction. (Removal of this precipitate which could require an additional step is dispensed with). It is advisable to extend the coil flush cycle from 50 to 80 min especially if high sensitivity cuvettes are installed in the colorimeter. This is readily accomplished by adjustment of the shut-down timer.

Also, if high sensitivity cuvettes are constructed with windows of quartz glass and a 10-mm pathlength in the cuvettes (the alternate 570 nm having a third of the pathlength of the main channel) the integration constant for proline will be 27, for cystine 50, and all other amino acids will be approximately 100. Thus, workers using the manual method of integration will have their task simplified. Further with scale expansion in the recorder it is possible to analyze 5 n*M* amino acid using a Beckman 120B analyzer with a very stable baseline printout.

REFERENCES

1 L. B. James, *J. Chromatogr.*, 59 (1971) 178.
2 L. B. James, *Lab. Pract.*, 21 (1972) 639.
3 L. B. James, *Lab. Pract.*, in press.