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Total nitrogen determination in plant material by means of the indophenol-blue method

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Summary

A modification of the indophenol-blue method for the determination of total nitrogen in plant material is described. The method is quick and simple since no heating is required for colour development. A straight calibration curve is obtained allowing direct automatic registration of the results.

Introduction

Within recent years there has been a growing interest in the Berthelot colour reaction for the determination of ammonia. The use of the catalytic effect of sodium nitroprusside (Lubochinsky & Zalta, 1954) considerably increased the sensitivity of the determination and the stability of the colour. Weatherburn (1967) reported on optimum reagent concentration and time as well as temperature conditions influencing the development of the colour. Beecher & Whitten (1970) checked this procedure and found that several biochemicals used as buffers affected colour development. It was confirmed that, with sodium nitroprusside as a catalyst, a linear calibration line is obtained and that colour development is rather pH-dependent.

Attempts to apply the method to the determination of ammonia in digests of plant material called for a dilution step because of the sensitivity of the method and the use of a phosphate buffer to suppress pH influence caused by slight variations in the acid content of the digests. Furthermore, EDTA will prevent precipitation of hydroxides and phosphates in this alkaline region.

The digestion procedure is based on a destruction with H_2SO_4 and H_2O_2 in the presence of salicylic acid (van Schouwenburg & Walinga, 1974) which will quantitatively transform nitrates, if present in the sample, via nitrosalicylic acid into aminosalicylic acid. The nitrosalicylic acid temporarily formed during the first stage of the process is rather volatile and even a moderate rise in temperature should then be avoided. For this reason some water has been incorporated in the digestion mixture. Addition of the acid mixture to the sample will consequently only result in a negligible rise in temperature.

Reagents

– Sulphuric acid solution 0.7 *M*: add 40 ml H_2SO_4 (sp.gr. 1.84) to 500 ml distilled water in a volumetric flask of 1 litre. Cool and make up to the mark.

– Standard solution 3000 mg N/litre: dissolve 1.4159 g of $(NH_4)_2SO_4$ in about 50 ml of sulphuric acid solution 0.7 *M*. Transfer quantitatively into a 100-ml volumetric flask and make up to the mark with the same sulphuric acid.
– Standard series: pipet in 50-ml volumetric flasks 0 - 1 - 2 - 3 - 4 - 5 ml of the standard solution 3000 mg N/litre. Make up to the mark with sulphuric acid 0.7 *M*. This series contains 0 - 60 - 120 - 180 - 240 - 300 mg N/litre.
– Sodium hydroxide 1 *M*: 40 g/litre.
– Alkaline phenolate solution: dissolve 22 g of phenol in 250 ml of NaOH 1 *M* and make up to 1 litre. The solution is stable for at least one week.
– Sodium nitroprusside solution 0.05 %: A fresh solution should be prepared daily.
– Di-sodium EDTA solution 4 %.
– Phosphate buffer solution: dissolve 13.35 g of $Na_2HPO_4.2\ H_2O$, add 50 ml of NaOH 1 *M* and make up to 1 litre. If necessary adjust the pH to 12 $\pm$ 0.2.
– Sodium hypochlorite solution: dilute 20 ml of sodium hypochlorite solution (BDH: approximately 1 *M* hypochlorite solution in 0.1 *M* NaOH) to 100 ml. The final solution should contain 0.7 % $\pm$ 0.1 of active chlorine. It is advisable to check the chlorine content in each new batch of sodium hypochlorite solution purchased.
– Mixed reagent I: mix 100 ml of alkaline phenolate solution with 200 ml of sodium nitroprusside solution and 10 ml of the EDTA solution. Prepare immediately before use.
– Mixed reagent II: mix 400 ml of phosphate buffer with 100 ml sodium hypochlorite solution.
– Diluted sulphuric acid: add 100 ml H_2SO_4 (sp. gr. 1.84) to 18 ml of distilled water.
– Acid mixture: dissolve 6 g of salicylic acid in diluted sulphuric acid and make up to a final volume of about 110 ml.
– Hydrogen peroxide 30 %.

Procedure

Digestion: Weigh accurately about 0.3 g of air-dry, finely ground plant material in a 50-ml volumetric flask. Add 3.3 ml of the acid mixture, mix well and wait, at least one hour, for the nitration reaction to proceed. Use 3.3 ml of the acid mixture as the blank.

Heat moderately on a hot plate and swirl gently now and then to minimize foaming. Should foam enter the neck of the flask, add 1 or 2 drops of hydrogen peroxide. Wait 60 minutes, then add at 10-minute intervals 5 drops of hydrogen peroxide and raise the temperature to about 280 °C. Repeat this procedure until the resulting solution remains clear after 10 minutes at 280 °C.

Cool and fill to the mark with distilled water. Filter the solution.

Analysis

Dilute aliquots of digest and standard series 1 + 9 with distilled water. Take 0.2 ml of these diluted solutions and add 3.0 ml of mixed reagent I and 5 ml of mixed reagent II. Mix after each addition; wait 90 minutes and measure at 630 nm.

Discussion

The use of the alkaline phenolate and the phosphate buffer brings the pH of the final solutions to the desired value in spite of variations in the acidity of the digests. A

variation in acidity from 0.12 – 0.18 *N* in the diluted digest results in a pH change of 11.2 – 11.1.

Since nitroprusside is unstable in an alkaline medium, it is considered necessary to prepare a fresh solution before the analysis. A relatively high concentration of this reagent has been chosen to increase the reaction rate of the colour reaction.

Once developed the blue colour is stable for at least 24 hours. The colour is virtually completely developed in 60 minutes and the method has a molar extinction coefficient of 15 000. Beer's law is followed in the range of the standard series.

The pH dependence of the colour intensity is in accordance with the experiences of Beecher & Whitten (1970). Because of the higher buffer capacity of the reagents used, extreme pH values could not be tested.

The interference of some cations was examined at concentrations which were a multiple of those actually present in plant material. For that purpose solutions were prepared containing next to 300 mg N, 240 mg Al^{3+}, 240 mg Fe^{3+}, 480 mg Ca^{2+}, 1200 mg Mg^{2+}, 200 mg Cu^{2+}, 200 mg Zn^{2+}, 200 ppm Co^{2+}, 200 ppm Ni^{2+} and 300 ppm Mn^{2+} per litre, either alone or present simultaneously. No effect could be registered.

The precision of the method, tested upon 20 samples, was within 1.5 %. Mathematical evaluation of the results from analysis of 330 digests with this colorimetric and a titrimetric procedure proved that no significant difference existed (see Table 1).

Table 1. Mean difference between the results of the colorimetric and titrimetric method $\bar{d}$, standard deviation of this difference $s_{\bar{d}}$ and the number of samples analysed n in the different concentration groups.

	Concentration group (mg/litre)						
	51-100	101-150	151-200	201-250	251-300	301-350	351-400
n	12	81	86	41	27	68	15
$\bar{d}$	–0.25	–0.47	0.163	–1.22	0.556	0.044	–0.467
$s_{\bar{d}}$	1.76	2.46	0.512	0.831	1.31	0.605	1.37
t(n-1)	–0.49	–1.72	0.318	–1.47	0.424	0.07	–0.34
P(+(n-1)	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

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