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ANALYSIS OF BIURET IN STOCKFOODS USING GEL FILTRATION

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SUMMARY

The separation of biuret from proteins and other interfering compounds was achieved using gel filtration with "Sephadex G-10." Good recoveries and reproducible results were produced on a wide variety of stockfoods. Biuret is estimated colorimetrically by the standard copper-biuret reaction.

I. INTRODUCTION

Biuret is increasingly being used as a non-protein nitrogenous supplement in animal feeds. The commonly used method of determining biuret is the reaction with copper sulphate in alkaline tartrate solutions (Ellis and Formaini 1955). This so-called "biuret test" depends on the peptide grouping —NHCONH—, and therefore is also given by all proteins.

In order to estimate the concentration of biuret in stockfoods, it is necessary to separate it from proteins and ammonium ions, the latter forming cupric tetra-ammonium complexes which result in added solution colour and erroneously high biuret results.

II. MATERIALS AND METHODS

Apparatus

(i) Chromatographic column (1·9 cm i.d.) fitted with teflon stopcock or preferably a gel filtration column manufactured by Pharmacia Fine Chemicals.

(ii) Spectrophotometer with 4 cm glass cells.

Reagents

(i) Alkaline tartrate solution: Dissolve 50 g of laboratory reagent grade sodium potassium tartrate in 1 litre of 1N sodium hydroxide. Let stand 1 day before use.

(ii) Copper sulphate solution: Prepare a 0·06N solution of penta-hydrated copper sulphate in carbon-dioxide free distilled water.

(iii) Biuret standard solution: 1 mg/ml Biuret, B.D.H. laboratory reagent grade, was recrystallized from water, m.p. 190°–191°C. Prepare a fresh solution each time just before use.

(iv) "Sephadex G-10": Pharmacia Fine Chemicals, 40-120μ, No. 4252.

Preparation of Sephadex column

The gel should be allowed to swell in excess distilled water and allowed to stand for at least 3 hr at room temperature. Half fill the column with distilled water and then add enough gel slurry to make a bed height of 20 cm. After allowing the gel to settle, place a piece of filter paper on top of the bed to protect the upper surface. Adjust the flow rate to 1 ml/min. To stabilize the bed, the elution medium, in this case distilled water, should be allowed to run through until at least two bed volumes have passed.

Preparation of standard solutions

From the standard biuret solution of 1 mg/ml take aliquots of 5, ... 25 ml and add to a 100 ml volumetric flask giving 5, ... 25 mg biuret/100 ml. Add 20 ml of alkaline tartrate solution (i) and 20 ml of copper sulphate solution (ii) to each flask and adjust to volume.

Prepare a reagent blank at the same time.

Read the absorbance of each solution against the reagent blank at 555 nm using 4 cm glass cells, according to the Official Methods of Analysis of the Association of Official Agricultural Chemists (1965, p. 20). Plot the absorbance against biuret (mg)/100 ml.

Method

Weigh an amount of stockfood containing 0·2 to 0·8 g of biuret into a 200 ml volumetric flask. Add approximately 150 ml of distilled water, shake for 1 hr and adjust to volume. Filter a portion of the solution through a Whatman No. 2 filter paper. Take a 5 ml aliquot which contains biuret within the standard range and add it to the top of the Sephadex column. Commence collecting the eluate in a 50 ml measuring cylinder, adjusting the flow rate if necessary to 1 ml/min.

Wash the sides of the column with distilled water when the sample solution has entered the column and then continue to add distilled water to the column. Discard the first 50 ml of eulate and collect the next 30 ml in a premarked 100 ml volumetric flask. Develop the colour as for the standard solutions and read off their biuret concentration from the standard curve.

N.B. Each column prepared should be tested to find at what volume the biuret is eluted, since different column sizes and varying bed heights of Sephadex will cause variations in the elution volume of biuret.

The following experiments were carried out to find the suitability of the column.

(*a*) Recovery of biuret alone through the column.
(*b*) Recovery of biuret added to a sample of stockfood not containing biuret.
(*c*) Recovery of biuret added to a sample of stockfood containing biuret.
(*d*) Reproducibility of method.
(*e*) Interference from ammonium ions.

III. RESULTS AND DISCUSSION

(*a*) *Recovery of biuret alone through the column.*—In recovering biuret from the column, 5 ml aliquots containing 5, 10 and 15 mg of biuret were passed through the column, colour was developed and biuret concentration was read from the standard curve. The results are shown in Table 1.

TABLE 1

RECOVERY OF BIURET FROM COLUMN

Biuret Solution (mg)	Biuret Found* (mg/5 ml)	Percentage Recovery
5	5·1	102·0
10	9·8	98·0
15	15·0	100·0

* The figure in this column is the mean of two results.

(*b*) *Recovery of biuret added to a sample of stockfood not containing biuret.*—A general stockfood (1 g) was analysed three times according to the proposed method and was found to contain no biuret. To this stockfood sample was added an amount of biuret to give 5, 10, 15 and 20 mg biuret in a 5 ml aliquot of the sample solution. The stockfood plus added biuret was then analyzed. The results were as shown in Table 2.

TABLE 2

RECOVERY OF ADDED BIURET FROM SAMPLE NOT CONTAINING BIURET

Sample + Biuret Solution	Biuret Found* (mg/5 ml)	Percentage Recovery
1 g stockfood + 5 mg ..	5·1	102·0
1 g stockfood + 10 mg ..	9·9	99·0
1 g stockfood + 15 mg ..	15·0	100·0
1 g stockfood + 20 mg ..	19·8	99·0

*The figure in this column is the mean of two results.

(*c*) *Recovery of biuret added to a sample of stockfood containing biuret.*—Two stockfoods containing different amounts of biuret were analyzed in duplicate. A known amount of biuret was then added and the stockfoods were analyzed again in duplicate to give the results shown in Table 3.

TABLE 3

RECOVERY OF ADDED BIURET FROM SAMPLE CONTAINING BIURET

Sample	Amount Biuret Added (mg/5 ml)	Amount Biuret Found (mg/5 ml)	Amount Recovered (mg)	Percentage Recovery
10993 (1)	..	8·8	..	..
10993 (2)	..	9·0	..	..
10993 (1)	5	13·8	4·9	98·0
10993 (2)	5	14·0	5·1	102·0
10994 (1)	..	9·6	..	..
10994 (2)	..	9·7	..	..
10994 (1)	10	19·5	9·85	98·5
10994 (2)	10	19·5	9·85	98·5

(*d*) *Reproducibility of method.*—The reproducibility of the method was tested by analysing two samples of differing biuret content at least six times. In all cases a 1 g sample weight was taken. The results are shown in Table 4.

TABLE 4

PRECISION OF METHOD ON TWO STOCKFOOD SAMPLES

SAMPLE A		SAMPLE B	
Biuret Found (mg/5 ml)	Percentage Biuret	Biuret Found (mg/5 ml)	Percentage Biuret
8·8	35·2	6·1	24·4
9·0	36·0	6·0	24·0
9·1	36·4	6·0	24·0
9·0	36·0	6·1	24·4
8·9	35·6	5·8	23·2
9·1	36·4	6·1	24·4
8·9	35·6		
9·1	36·4		
9·0	36·0		
8·9	35·6		
Mean = 35·9 S.D. = 0·413 Coefficient of variation = 1·15%		Mean = 24·1 S.D. = 0·468 Coefficient of variation = 1·94%	

(*e*) *Interference from ammonium ions.*—The interference due to ammonium ions was shown by examining the effect on the absorbance of solutions with and without added mono ammonium phosphate and also by using and omitting the column clean-up procedure. The results are shown in Table 5.

TABLE 5

EFFECT OF AMMONIUM ION INTERFERENCE

Solution	Absorbance, Column	Absorbance, No Column
1 g stockfood	·002	·039
1 g stockfood	·003	·026
1 g stockfood + 10% M.A.P.	·003	·044
1 g stockfood + 10% M.A.P.	·004	·051
1 g stockfood + 10% M.A.P. + 10 mg biuret	·087	·135
1 g stockfood + 20% M.A.P. + 10 mg biuret	·087	·147
1 g stockfood + 10 mg biuret	·086	·104

IV. DISCUSSION

It is seen from the above results that biuret can be recovered quantitatively from the Sephadex column by the collection of the appropriate fraction while at the same time it can be separated from interfering proteins and ammonium ions. The combined[14] recovery results in Tables 1 and 2 give an average recovery of $99 \cdot 7 \pm 0 \cdot 8$ at the 95% confidence limit. Table 4 shows the reproducibility of the method by giving a pooled standard deviation of $\pm 0 \cdot 433$ obtained from the standard deviations of the two samples.

The method is rapid, requiring approximately 2 hr for complete analysis, and is suitable for determining biuret in stockfoods provided the biuret content in the sample weight is within the range used.

REFERENCES

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS (1965).—"Official Methods of Analysis of the Association of Official Agricultural Chemists" 10th Ed. (A.O.A.C.: Washington D.C.).

ELLIS, G. C., and FORMAINI, R. L. (1955).—*J. Agric. Fd Chem.* 3:615.

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