among phosphorus content of the grain and the various quality measures was toward improved malting quality. All correlations of phosphorus content of the grain with the other criteria were positive, and although most of the coefficients were not sufficiently great to indicate a pronounced increase in the quality criteria from applying phosphorus fertilizer, they were in the desired direction. The associations of protein content of the grain and malt with other quality factors were more variable than the similar associations between phosphorus content and the same factors. Both negative and positive correlations were observed for the relationships with protein content, but again the coefficients did not appear to be sufficiently high for most comparisons to constitute a serious deterrent to good quality. The negative correlations between protein content and malt extract percent and between protein content and 1000-kernel weight were the most undesirable from a quality standpoint.

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AGRICULTURAL PRODUCTS ANALYSIS

Colorimetric Determination of Biuret

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Previous methods for biuret determination—i.e., complexing with copper ion in alkaline media—necessitate removal of hydrated oxide precipitates prior to measurement of the color development. These methods were found to be unreliable and the inaccuracies were attributed to such factors as sorption of the colored complex by the oxides formed and variance in reagent concentration. The procedures were modified and specifically developed for urea pyrolyzate products containing biuret in a wide concentration range. This new method is based on an advantageous equilibrium existing between copper-tartrate and copper-biuret complexes in Fehling's solution. The equilibrium favors the more highly colored copper-biuret complex, oxide precipitation is prevented, and biuret can be directly determined by standard colorimetric methods.

BIURET FINDS POTENTIAL USE in agriculture as a combined herbicide-fertilizer and in the resin industry as an intermediate. It may be conveniently prepared by pyrolysis of urea at moderate temperatures. The solid pyrolyzates contain varying proportions of concomitant materials such as urea, cyanuric acid, ammonia, and triuret.

The development of direct analytical methods for biuret was based on procedures applicable to materials containing the polypeptide linkage or giving the "biuret test" (*2–4, 6*). These methods entail addition of excess sodium hydroxide to dilute copper sulfate solutions of biuret, which results in the development of a colored copper-biuret complex and precipitation of hydrated copper oxides. Oxide removal, by filtration, provides a solution of the complex suitable for colorimetric analysis by conventional methods. The accuracy and precision of these methods were found to be limited by such factors as the sorption properties of hydrated oxides, peptization phenomena, and effects promoted by variance in reagent concentration (Table I).

Experimentation revealed that reversing the order of reagent addition—i.e., addition of copper sulfate solution to biuret solutions of fixed sodium hydroxide

concentration—results in an immediate development of color, with oxide formation apparent only after near depletion of uncomplexed biuret. Addition of an excess of reagent terminates in formation of peptized oxides and suspensions unsuited for colorimetric analysis (Figure 1). Incorporating Rochelle salt in the system prevents oxide formation, yielding solutions of colored complexes ideal for direct colorimetric determination (*1*). Over the biuret concentration range employed, 10 to 80 mg. per 100 ml., the system conforms with Beer's law. Slight deviations are encountered at lower and higher biuret concentrations (Figure 3), but do not appear to reduce the precision or accuracy of the method. The procedure was adapted to the Klett-Summerson photoelectric colorimeter, glass cell model, using a 40-mm. cell path and a No. 54 green filter.

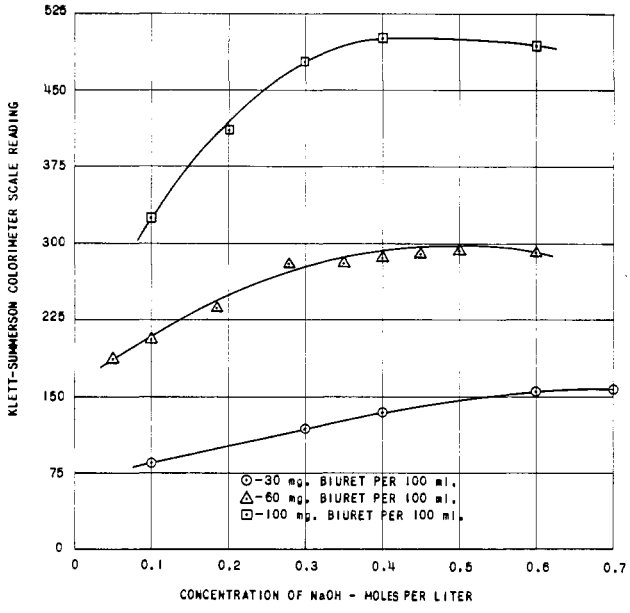


Figure 1. Effect of sodium hydroxide concentration on colorimeter reading

The reagent and biuret concentrations employed were satisfactory for the authors' purposes, but do not necessarily represent the optimum range applicable to the method or other instruments. It is beyond the scope of this paper to postulate whether the system represents a true copper-biuret and copper-tartrate equilibrium, or the formation of a biuret-copper-tartrate complex of undetermined structure. The coordinating characteristics of cupric ion allow prediction of possible intercomplexing in the system.

Reagents and Apparatus

1. Copper Sulfate Reagent. Dissolve 15.0 grams of reagent grade cupric sulfate pentahydrate in distilled water, dilute to 1.0 liter, and age for 1 day before using. This solution is 0.06*M* in copper sulfate.
2. Alkaline Rochelle Salt Reagent. Dissolve 50.8 grams of reagent grade sodium potassium tartrate tetrahydrate in distilled water containing 51.4 ml. of 50% sodium hydroxide (carbonate-free), dilute to 1.0 liter, and age for 1 day before using. Solution is 1.0*N* in sodium hydroxide and 0.18*M* in tartrate salt.
3. Distilled water, 0.1*N* sodium hydroxide and sulfuric acid, prepared by known methods.
4. Biuret Standard Solution. A suitable anhydrous reagent material may be prepared by crystallizing Eastman white label biuret successively from water, 1*N* sodium carbonate, water, and 95% ethyl alcohol (*5*). The standard solution is prepared by dissolving a weighed sample of the reagent biuret (±0.2 mg.) in distilled water, neutralizing to a pH of 7.0, and diluting to 1.0 liter. For convenience the solution should contain 2.0 mg. per ml.
5. Water Bath. A thermostated bath set to operate at a fixed temperature (±1.0° C.), preferably in the 25° to 35° C. range.
6. Colorimeter. A Klett-Summerson photoelectric colorimeter, glass cell model, 40-mm. cells, and No. 54 green filter (490 to 570-mμ spectral range).
7. pH Meter, glass electrode type.

Analytical Procedure

Preparation of Samples for Analysis

Samples Soluble in Water at 25° C. Dissolve a weighed, pulverized sample (±0.2 mg.) containing 0.4 to 1.8 grams of biuret in 500 ml. of water. Neutralize the solution with 0.1*N* acid or base to a pH of 7.0, transfer to a 1000-ml. volumetric flask, and dilute to the mark. Transfer a 50-ml. aliquot of the solution to a 100-ml. volumetric flask and subsequently treat by the procedure for "complex formation."

Samples Insoluble in Water at 25° C. Transfer a weighed, pulverized sample (±0.5 mg.) containing 0.4 to 1.2 grams of biuret to a 1000-ml. beaker with about 700 ml. of water. Heat the suspension to 70° to 80° C. with stirring, and air-cool to 30° C. Neutralize the solution to a pH of 7.0 using 0.1*N* acid or base and filter into a 1000-ml. volumetric flask. Wash the residue with three 50-ml. portions of water, combine washings and filtrate, and dilute the solution to the mark.

Table I. Colorimetric Determination of Biuret in Urea Pyrolyzate Mixtures

Run No.	Grams Solid per 1000 Ml. Solution	Solids Composition, Wt. %				Mg. Biuret per 50-Ml. Aliquot			% Biuret Recovery	
							Observed			
		Urea	Biuret	Cyanuric acid	Triuret	Calcd.	Method 1	Method 2	Method 1	Method 2
1	20.023	80.0	1.7	14.3	4.0	17.0	16.8	16.9	98.9	99.4
2	9.870	73.9	3.1	15.2	7.8	15.3	15.0	15.3	98.0	100.0
3	8.961	71.0	5.0	15.1	8.9	22.4	21.6	22.2	96.4	99.1
4	4.754	60.8	6.9	14.8	17.5	16.4	16.0	16.5	97.5	100.6
5	4.960	51.9	10.4	22.8	14.9	25.8	24.0	25.9	93.0	100.4
6	4.894	44.8	15.0	29.7	10.5	36.7	36.8	36.6	100.3	99.7
7	5.142	42.1	19.7	34.8	3.4	50.6	50.2	50.1	99.2	99.0
8	2.518	34.9	25.1	14.8	25.2	31.6	32.9	32.0	97.6	101.3
9	5.055	31.6	35.7	24.4	8.3	90.2	89.1	90.3	98.8	100.1
10	2.459	25.7	40.1	5.5	28.7	49.3	47.1	49.9	95.6	101.2
11	3.952	21.1	50.3	10.0	18.6	99.4	97.5	100.0	98.1	100.6
12	2.553	7.7	60.0	10.7	21.6	76.6	76.6	77.1	100.0	100.7
13	2.445	5.5	68.4	10.1	15.0	88.2	83.6	88.2	94.8	100.0
14	2.489	3.1	77.7	10.4	8.8	96.7	95.0	96.5	98.2	99.8
15	2.050	1.3	91.4	5.5	1.8	93.7	90.6	93.5	96.7	99.8
							Arithmetic mean		97.5	100.1
							Average deviation		1.5	0.5

Method 1. Add 2.0 ml. of 20% NaOH to solution containing 25 ml. of 0.06*M* $CuSO_4$ and 50 ml. of test solution, dilute to 100 ml. volume, filter free of hydrated oxides. Color by Klett-Summerson colorimeter, 20-mm. cell path, green filter (No. 54).

Method 2. Standard procedure.

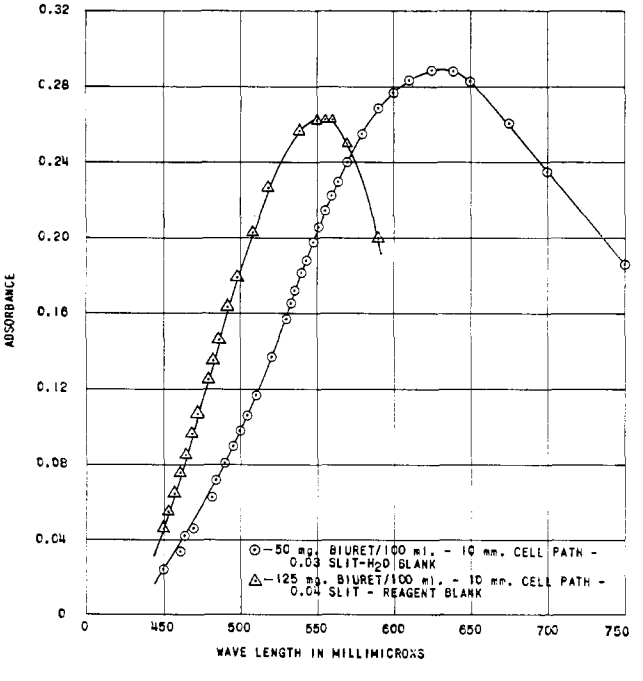
Transfer a 50-ml. portion of the solution to a 100 ml. volumetric flask and subsequently treat by the procedure for complex formation.

Complex Formation **Standard Procedure.** Successively add from burets 20.0-ml. portions of alkaline tartrate and copper sulfate reagents with constant swirling. Dilute the colored solution to the mark, shake vigorously, and suspend in the thermostated bath for 15 to 30 minutes. Shake occasionally during this aging process. Then remove the solution from the bath and determine its colorimetric scale reading with the photoelectric colorimeter.

Preparation of Standard Curve Set the water bath to operate at a fixed temperature (±1.0° C.) in the 25° to 35° C. range. Transfer from a 50-ml. buret varying portions of biuret standard solution (0 to 50.0 ml.) to 100-ml. volumetric flasks. Adjust total volumes to 50 ml. with water and subsequently treat as in the procedure for complex formation. Take colorimetric readings, using either a reagent or water blank.

A rectangular plot of colorimeter scale reading *vs.* milligrams of biuret per 100 ml. of solution gives the standard curve, which is linear in the range of 10 to 80 mg. of biuret per 100 ml. of solution. For any set of colorimetric reagents the curve is fixed and may be used for extended periods. In this laboratory, a plotting scale of 5 mg. of biuret and 25 colorimeter scale units per inch was found most satisfactory. Linear plotting is permissible, as the Klett-Summerson colorimeter scale is logarithmic and directly proportional to absorbance.

Figure 2. Effect of wave length on absorbance



Calculations

Using the colorimeter scale reading, the biuret concentration in the aliquot of sample solution is obtained directly from the standard curve. Then,

$$\frac{(\text{Mg. of biuret})\,(\text{volume of sample solution, ml.})\,(100)}{(1000)\,(\text{sample weight, g.})\,(\text{aliquot, ml.})} = \text{wt. \% of biuret in sample}$$

Discussion

In developing this method of analysis, consideration was given to water solubility of urea pyrolyzate, biuret concentration, and acceptable colorimeter scale limits. Based on the solubility of the least soluble component, a concentration of 2.0 grams of urea pyrolyzate sample per liter was selected as the upper limit. This fixed the biuret content, per 50-ml. aliquot, in the 0- to 100-mg. range. As maximum absorption of transmitted light by copper-tartrate-biuret solution occurs at ca. 550 mμ, a green filter (490 to 570 mμ) was required with the Klett-Summerson colorimeter (Figure 2). Accurate scale readings on this colorimeter can be made in the 0 to 400 unit range (<500 acceptable). A biuret concentration of 100 mg. per 100 ml. represents this upper limit. With the copper content fixed at a minimum copper-biuret mole ratio of 1.0, alkaline tartrate reagent concentrations were varied for maximum color development within the designated experimental limits. Using the reagent concentrations specified, and biuret concentrations of 0 to 100 mg., a 40-mm. cell path was found necessary to obtain the required absorption in the 0 to 500 scale range. The precision of the instrument permitted a plot of 25 colorimeter scale units and 5 mg. of biuret per inch for the standard curve (Figure 3).

Slight deviations from Beer's law were encountered in the range of 0 to 10 mg. of biuret per 100 ml., becoming more pronounced at concentrations greater than 90 mg. However, excellent agreement was obtained in the 10- to 80-mg. range, and proper choice of solution aliquot permits readings within these limits. The deviations could not be reduced or eliminated by choice of reagent concentration or color standard (Figure 3).

The method is rapid, eliminates the filtrations necessary in other methods, reduces operational time, and increases the precision and accuracy obtained in colorimetric determination of biuret. For comparison, biuret was determined in known urea pyrolyzate compositions, using this method and one based on previously reported work (*2*, *3*, *6*). In virtually every case, biuret was more completely recovered by this method (Table I).

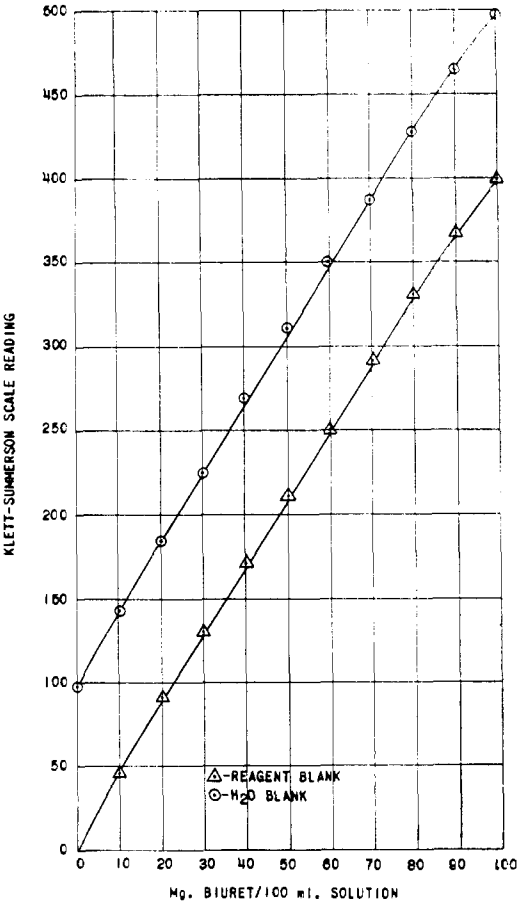


Figure 3. Standard curve

Listed in Table II are data on biuret recoveries from urea pyrolyzate component-biuret mixtures using this method of analysis. Urea and cyanuric acid showed little or no interference even at sample concentrations of 90%. Both ammonia and monoammonium cyanurate showed some interference, the latter at sample concentrations above 50%. The formation of the cupric-tetraammonium complex results in added solution color and erroneously high biuret recovery. The concentrations of ammonia normally encountered in solid urea pyrolyzate are too low to exert any serious effect on analysis. If necessary, ammonia may be removed by treating a water solution of the pyrolyzate sample with cation exchange resin prior to analy-

Table II. Effect of Urea Pyrolyzate Components on Biuret Recovery by Standard Procedure

Run No.	Charge Composition, Wt. %						Mg. Biuret per Aliquot		% Biuret Recovery
	Biuret	Urea	NH_3	Monoammonium cyanurate	Cyanuric acid	Triuret	Calcd.	Obsd.	
1	10.6	89.4	..	..	..	..	10.6	10.5	99.0
2	25.1	74.9	..	..	..	..	25.1	25.0	99.6
3	50.6	49.4	..	..	..	..	50.6	50.2	99.2
4	91.2	8.8	..	..	..	..	91.2	91.0	99.8
5	98.0	..	2.0	..	..	..	98.0	98.2	100.2
6	89.3	..	10.7	..	..	..	50.0	51.0	102.0
7	88.9	..	11.1	..	..	..	80.0	81.9	102.4
8	76.9	..	23.1	..	..	..	50.0	50.9	101.8
9	66.6	..	33.4	..	..	..	20.0	20.8	104.0
10	19.9	..	..	80.1	..	..	19.9	20.4	102.5
11	49.8	..	..	50.2	..	..	49.8	50.3	101.0
12	90.7	..	..	9.3	..	..	90.7	90.5	99.8
13	90.6	..	..	..	9.4	..	90.6	90.9	100.3
14	75.0	..	..	..	25.0	..	75.0	75.0	100.0
15	51.3	..	..	..	48.7	..	51.3	50.8	99.0
16	24.8	..	..	..	75.2	..	24.8	24.5	98.8
17	10.2	..	..	..	89.8	..	10.2	9.7	99.5
18	51.8	..	..	..	..	48.2	51.8	54.5	105.3
19	74.9	..	..	..	..	25.1	74.9	76.5	102.1
20	85.0	..	..	..	..	15.0	85.0	86.7	102.0
21	90.8	..	..	..	..	9.2	90.8	91.4	100.6
22	94.7	..	..	..	..	5.3	94.7	94.6	99.9

sis. This treatment has no apparent effect on biuret recovery. Triuret showed some interference at concentrations of 15 to 50%, but no definite conclusions could be drawn, as the standard triuret sample may have contained some biuret as an impurity. Other materials showing little or no interference with this method are sodium, potassium, chloride, and sulfate ions. From the conditions of analysis, it is evident that marked interferences could be expected by materials which alter solution alkalinity, reduce Fehling's solution, or form complexes with the analytical reagents.

In Table III are recorded data affording an estimation of the precision and accuracy of this method. Precision and accuracy greater than ±0.5% are attained, providing that measurements are made in the linear portion of the standard curve and interfering materials are maintained within acceptable limits (Table II).

This procedure may be of value to those who analyze materials that contain substances giving the biuret reaction.

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Table III. Precision and Accuracy of Standard Procedure

(Biuret standard, 2.0 mg./ml.)

Run No.	Aliquot, Ml.	Mg. Biuret in Aliquot		% Biuret Recovery	Arithmetic Mean	Deviation, Parts per 100	
		Calcd.	Obsd.			Average	Standard
1	5.0	10.0	10.1	101.0			
2	5.0	10.0	10.0	100.0			
3	5.0	10.0	9.9	99.0			
4	5.0	10.0	9.9	99.0			
5	5.0	10.0	10.2	102.0			
6	5.0	10.0	10.1	101.0	100.3	1.0	1.21
7	15.0	30.0	30.1	100.4			
8	15.0	30.0	30.1	100.4			
9	15.0	30.0	29.8	99.3			
10	15.0	30.0	29.9	99.7			
11	15.0	30.0	29.9	99.7			
12	15.0	30.0	29.9	99.7	99.8	0.3	0.45
13	25.0	50.0	49.8	99.6			
14	25.0	50.0	50.1	100.2			
15	25.0	50.0	49.8	99.6			
16	25.0	50.0	49.9	99.8			
17	25.0	50.0	49.8	99.6			
18	25.0	50.0	50.0	100.0	99.8	0.2	0.25
19	40.0	80.0	80.2	100.3			
20	40.0	80.0	80.2	100.3			
21	40.0	80.0	80.0	100.0			
22	40.0	80.0	79.8	99.7			
23	40.0	80.0	80.2	100.3			
24	40.0	80.0	80.2	100.3	100.1	0.2	0.26