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Simplified Spectrophotometric Analysis of Copper from Cupric Sulfide Synthesized in Porcine Fecal Matter

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The presence of cupric sulfide in fecal matter of pigs fed copper sulfate was investigated. The method involves the preliminary isolation of cupric sulfide from soluble copper and oxidation of the sulfide ion with the concurrent dissolution of the insoluble copper. This technique permits the direct analysis of copper from cupric sulfide by atomic absorption spectrophotometry. The method described establishes the feasibility of this approach for obtaining accuracy and precision for the estimation of low amounts of copper from cupric sulfide in fecal matter.

Several reports summarized by Braude (1965) indicate that copper is an effective growth stimulus for pigs. With animals fed copper levels, concern with environmental contamination from soluble copper in fecal matter increases. This contamination, however, may not pose a problem if appreciable amounts of cupric sulfide are present in fecal matter. Cupric sulfide possibly may be synthesized in the intestinal tract by microorganisms. The very low K_{sp} of cupric sulfide (8.5×10^{-45}) indicates that a very high hydrogen ion concentration is not sufficient to dissolve appreciable amounts of it. Copper bound in this form is insoluble and lessens the contamination problem. The necessity for a precise and accurate method for the quantitative estimation of this compound from fecal matter is of prime importance. The proposed method considers the separation of cupric sulfide from soluble copper in fecal matter and uses a combination of nitric–perchloric–sulfuric acid reagents for sample decomposition and the complete dissolution of copper from cupric sulfide. The preparation of the sample for atomic absorption spectrometry requires about 1 hr. Groups of samples can be run simultaneously. Once the insoluble copper is in solution, atomic absorption spectrophotometry offers a sensitive solution for the estimation of copper in low concentrations.

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EXPERIMENTAL SECTION

Standard Curve. A standard copper solution was prepared by dissolving 0.19645 g of $CuSO_4 \cdot 5H_2O$ and diluting to 500 ml with deionized water. Aliquots of this solution (0.25 to 15 ml) were placed in a 100-ml volumetric flask, 8 ml of 0.3 *N* HCl and 2 ml of concentrated H_2SO_4 were added, and the solution was brought to volume with deionized water. Working standards cover a range from 0.25 to 15 μg of copper. Absorbance was measured at 3247 Å with a Model 503 atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn.). A slit setting of 4 (7 Å spectre band width) and a copper hollow cathode lamp utilized at 15 mA were used. The air–acetylene flame was maintained at operating pressures of 30 psi for air and 8 psi for acetylene. Flowmeter settings were regulated to deliver 21.5 l./min of air and 3.5 l./min of fuel to a 4-in. single slot burner.

Cupric Sulfide Determinations. [*Caution:* Conduct determination in well ventilated hood. $HClO_4$ contact with concentrated H_2SO_4 may be explosive. Refer to "Notes on Perchloric Acid and Its Handling in Analytical Work" (1969).] A 200-mg lyophilized sample was placed in a 250-ml digestion flask to which was added 20 ml of 0.3 *N* HCl (analytical reagent grade) and heated to 40° while the flask was swirled to wet the sample. Soluble copper (sample filtrate) was quantitatively removed by filtering through Whatman no. 42 filter paper and washing under vacuum. The paper containing the cupric sulfide (sample residue) was transferred to the original 250-ml volume flask. Concentrated nitric acid (15 ml), 2 ml of concentrated sulfuric

Table I. Recovery of Copper from Cupric Sulfide Samples Containing Copper Sulfate[a]

Compounds	Copper added, μg	Copper recovered, μg		Recovery, %	
		Residual[b]	Filtrate[c]	Residual	Filtrate
CuS, 1 mg +	664.6	665.0 ± 3.6		100.1	
$CuSO_4 \cdot 5H_2O$, 3 mg	762.3		746.7 ± 4.4		98.0

[a] Means and standard errors are based on six analyses. [b] Contains Cu from CuS. Residual copper recovered not significantly different ($P < 0.01$) from copper added. [c] Contains copper from $CuSO_4 \cdot 5H_2O$. Filtrate copper recovered not significantly different ($P < 0.05$) from copper added.

Table II. Recovery of Copper from Cupric Sulfide in Porcine Fecal Matter[a]

Sample[b]	Copper added, μg	Copper recovered, μg		Recovery, %
		Residual[c]	Filtrate[d]	
Fecal matter no. 1	0.0	22.9 ± 0.8	31.7 ± 1.3	
Fecal matter no. 1 plus CuS (1 mg)[e]	664.6	699.6 ± 7.5	32.1 ± 1.00	101.8
Fecal matter no. 2	0.0	112.1 ± 8.0	62.4 ± 1.2	
Fecal matter no. 2 plus CuS (1 mg)[e]	664.6	767.1 ± 12.3	63.7 ± 1.0	98.6

[a] Means and standard errors are based on six analyses. [b] Based on 0.20 g of fecal matter. [c] From cupric sulfide. [d] From soluble copper. [e] Mean difference in residual copper recovered (fecal matter + CuS − fecal matter) was not significantly different at 1% level from mean copper added (664.6).

acid, and 10 ml of 70% perchloric acid were added; the sample was allowed to sit overnight. The contents were gently boiled until the volume was reduced to 2 ml. After the samples were cooled, 10 ml of concentrated nitric acid and 10 ml of 70% perchloric acid were added, and the contents were gently boiled to reduce the volume to 2 ml again. This step was repeated a second time. After the contents were cooled, 8 ml of 0.3 *N* HCl was added, and the flask was swirled to rinse the sides of the glass. The liquid digest was quantitatively transferred to a 250-ml volumetric flask, and the flask was rinsed with 10-ml aliquots of water while the sides of the glass were slightly heated. The contents were quantitatively transferred to the 250-ml volumetric flask. The rinsing process was repeated five times. The digest was brought to volume and filtered through Whatman no. 40 filter paper for analysis by atomic absorption spectrophotometry.

RESULTS AND DISCUSSION

Copper compounds present in fecal matter were analyzed to standardize the analysis procedure (Table I). The data include copper determinations from cupric sulfide (insoluble copper determined from sample residue) and copper sulfate (soluble copper determined from sample filtrate). Practically complete recoveries of residual and filtrate copper from added amounts were achieved.

Table II illustrates the recovery of copper from cupric sulfide originally present in porcine fecal matter. Fecal matter no. 1 is from pigs fed 10 ppm of copper, and fecal matter no. 2 is from pigs fed 500 ppm of copper. Accuracy of the residual copper estimation from insoluble copper originally present in fecal material is based on the recovery of 664.6 μg of copper from a cupric sulfide standard added to fecal matter. Copper recovery averaged 101.8% for fecal matter no. 1 and 98.6% for fecal matter no. 2 (Table II).

The filtrate data of Tables I and II indicate that soluble copper present in the sample is effectively removed from the sample residue. Expected filtrate copper (Table I) and equivalent levels of copper from fecal matter analyzed alone or supplemented with cupric sulfide were recovered.

The results corroborate the use of 0.3 *N* HCl as an effective reagent for the removal of soluble copper. Other insoluble compounds of copper, such as carbonate and phosphate, may be formed in the lower gut of the pig. Copper associated with these compounds could contribute to the error in the final estimation of copper from cupric sulfide in fecal material. Solubility of the compounds in 0.3 *N* HCl, with their subsequent removal from the sample through filter paper, aids in assuring against this potential error. The K_{sp} of cupric sulfide is exceeded in the presence of 0.3 *N* HCl. The data of Table I indicate no significant loss of copper from cupric sulfide due to the dispersion of the sample in 0.3 *N* HCl at 40°.

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