Determination of Chromium in Plants and Other Biological Materials

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An atomic absorption method for the determination of submicrogram quantities of chromium combines wet digestion of biological materials, isolation of chromium III from other metals by solvent extraction, and extraction of the chromium III chelate of 2,4-pentanedione into chloroform. The chloroform is evaporated, and the chromium compound is taken up in 4-methyl-2-pentanone. Recovery of added chromium-51 in the digestion step when silver nitrate was present was 100%. Overall recovery was 95%. This method allows the estimation of 4 ng of chromium per milliliter of solution and is free from interferences.

The role of chromium in biological processes and evidence that chromium is a required element in human and animal nutrition has been recently reviewed (Mertz, 1969). This evidence poses a need for a rugged and generally applicable method of measuring the concentration of chromium in foods and other biological materials.

A review of recent methods for chromium analysis is given by Bachman and Banks (1969). Problems encountered in determination of chromium in biological materials include the losses of chromium during ashing and interference from other elements that are normally more abundant than chromium in these materials. These problems have been especially severe in the analysis of plant material, since plants are very slowly oxidized by low-temperature plasma methods, and they frequently contain sufficient iron, alkaline earths, and phosphates to interfere with the isolation of chromium from alkaline solutions. Techniques for quantitative detection of chromium have included gas chromatographic isolation and detection of the complexes formed by chromium with fluorinated analogs of 2,4-pentanedione (Ross and Sievers, 1969), atomic absorption (Midgett and Fishman, 1967), X-ray fluorescence (Beyermann *et al.*, 1969), emission spectrography utilizing inert atmospheres (Hambidge, 1970), and colorimetric procedures based on the absorption of the complex formed by reaction of chromium VI with diphenylcarbazide (Saltzman, 1952), and of the complex formed by the reaction of chromium III with 2,4-pentanedione (McKaveney and Freiser, 1958).

The method described here utilizes wet ashing of biological materials by nitric, perchloric, and sulfuric acids in the presence of silver nitrate to prevent losses of chromium. Many potentially interfering elements are removed from the digest by complexing and prompt solvent extraction from a cold solution. Chromium is then reacted with 2,4-pentanedione at elevated temperature for 12 hr, and concentrated by solvent extraction prior to measurement by atomic absorption.

EXPERIMENTAL

Chromium-Free Nitric Acid. Reagent grade nitric acid must be distilled in glass to remove traces of chromium. The first 50 ml of HNO_3 distilled over is discarded, as well as the last 400 ml of HNO_3 remaining in the 5-l. distillation flask.

Purified 2,4-Pentanedione (HAA). HAA is distilled and the fraction boiling from 135 to 137° C (745 mm Hg; Steinbach and Freiser, 1953) is collected. This is mixed with chloroform (1:1) and the solution is extracted twice with 10-ml portions of distilled water.

Chromium Standard. Prepare a solution containing 0.1 μg of chromium per milliliter from reagent grade potassium chromate dissolved in water. The pH should be adjusted to pH 6–7 (Beyermann, 1962).

SPECIAL APPARATUS

A Perkin-Elmer atomic absorption spectrometer (Model 303) equipped with a Perkin-Elmer recorder (Model 165) and recorder readout was used.

Glass. New glass is cleaned with a sodium dichromate "cleaning" solution. After the glass is free draining, it is washed with detergent, rinsed in distilled water, and soaked overnight in 10% (v/v) HCl. Then it is rinsed in distilled water and allowed to drain dry on plastic.

PROCEDURE

Weigh out a sample containing at least 0.1 μg of chromium and transfer it to a 100-ml micro-Kjeldahl flask. Add two glass beads and 10 ml of concentrated nitric acid per gram (dry weight) of sample. Heat slowly until the vigorous reaction between nitric acid and the organic matter subsides. Then increase the temperature and allow the mixture to boil for 5 min. Cool, and add 2 ml of concentrated sulfuric acid, 2 ml of 70% perchloric acid, and 0.2 ml of 10% (w/v) silver nitrate. Reheat the mixture and reflux until all of the nitric acid has distilled off. As the last traces of nitric acid distil off, the solution should remain clear. If it does not, cool the flask and its contents and add 2 ml of concentrated nitric acid. Then resume the digestion. Repeat this step until the solution remains clear. Continue boiling the solution for 5 min after the appearance of perchloric acid fumes.

After the digest has cooled, add 3 ml of H_2O and 1 ml of 10% (w/v) sodium sulfite. Allow several minutes for the sodium sulfite to reduce the chromium VI to chromium III before adding 2 ml of 0.1 *N* hydrochloric acid to complete the precipitation of silver. After the digest has cooled to room temperature, remove the silver chloride by filtering the digest into a 125-ml separatory funnel. Filter paper contains acid-soluble chromium and care must be taken to wash the paper well before proceeding with the filtering step.

Adjust the pH to about 3 using cresol red and 80% (v/v) ammonium hydroxide for the initial adjustment. Then use a pH meter equipped with a combination microelectrode for final pH adjustment in the separatory funnel.

Cool the separatory funnel and its contents to 4° C before adding 1 ml of 3% (w/v) ammonium pyrrolidinodithiocarbamate and 2 ml of 2,4-pentanedione (Mansell and Emmel,

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Table I. Retention of Added ^{51}Cr during Wet Digestion of Biological Materials

Digestion mixture	Sample	Average % ^{51}Cr recovery
$HNO_3 + HClO_4 + H_2SO_4 + AgNO_3$	Alfalfa (12)[a]	101
$HNO_3 + HClO_4 + H_2SO_4 + AgNO_3$	Wheat (4)	100
$HNO_3 + HClO_4 + H_2SO_4 + AgNO_3$	Blood (2)	99
$HNO_3 + HClO_4 + H_2SO_4 + AgNO_3$	$CrCl_3$ (2)	101
$HNO_3 + HClO_4 + H_2SO_4 +$ ...	Alfalfa (4)	89
$HNO_3 + HClO_4 + H_2SO_4 +$ ...	$CrCl_3$ (2)	88
$HNO_3 + HClO_4 +$ ... + ...	Alfalfa (2)	89
$HNO_3 + HClO_4 +$ ... + ...	$CrCl_3$ (2)	84
$HNO_3 +$ ... + ... + ...	$CrCl_3$ (2)	95

[a] Numbers in parentheses indicate the number of samples analyzed.

Table II. Recovery of ^{51}Cr Added to Wheat Straw and Whole Blood (One 2-g Sample of Each Material)

Procedural step	Blood	Wheat
	100%[a]	
Digestion	101	104
Filtering (2 × H_2O wash)	98	97
Preextraction	98	96
88° C treatment	98	96
Cr Extraction ($CHCl_3$ phase)	97	95

[a] These values are based on a beginning activity of 18,000 counts per minute.

1964; Slavin, 1964; Steinbach and Freiser, 1953). Using a mechanical shaker, shake the system for 90 sec. Then extract the solution twice with 5-ml portions of cold chloroform. Shake for 1 min per extraction, and discard the organic phase.

Transfer the sample to a 125-ml reagent bottle fitted with a ground glass stopper. Add 6 ml of chloroform-2,4-pentanedione solution and adjust the pH to 7 using 10% (v/v) ammonium hydroxide. Place the bottle and its contents in a constant temperature oven set at 88° C. Heat the solution to about 50° C; then, using extreme care, shake the bottle and release any pressure that has built up. Replace the stopper and allow the mixture to stand in the 88° C oven overnight. Next, transfer the sample to a 125-ml separatory funnel and cool to 4° C. Extract the aqueous phase twice with 5-ml portions of cold chloroform. Use 3-min shaking periods on a mechanical shaker. Collect the chloroform phase in an evaporating dish and evaporate off the chloroform in a hood under an air stream at room temperature. The presence of chloroform in the solution to be aspirated will depress the chromium signal. Therefore, care must be taken to evaporate all of the chloroform at this point. Take the residue containing chromium up in 4-methyl-2-pentanone and make to an exact volume (5 to 10 ml). Aspirate this solution through an atomic absorption spectrometer at approximately 5 ml per min.

RESULTS

Recovery of Added Chromium-51. Digestion of biological material can result in partial loss of chromium, especially if the digestion mixture contains perchloric acid (Table I; Gorsuch, 1959). There is no completely satisfactory standard to use for checking the recovery of chromium from biological samples. However, the formation and subsequent volatilization of chromyl chloride is often suspected as being a major source of chromium loss during digestion (Gorsuch, 1959). Thus, the recovery of chromium-51 added as the chloride to biological samples is a necessary though not sufficient test for a chromium method. The addition of silver nitrate to the digestion mixture, as mentioned by Udy (1956), was an effective way of avoiding the loss of chromium during a perchloric acid digestion (Tables I and II). When silver nitrate was omitted during digestion, up to 16% of the chromium-51 was lost.

Table III. Contamination of Wheat Grain Samples by Grinding (Grinder was a Standard Wiley Mill)

Treatment (screen material)	Avg μg Cr
Unground	0.26
Ground:	
20-Mesh brass	0.38
40-Mesh brass	0.30
20-Mesh stainless	0.34
20-Mesh chrome	0.24
40-Mesh chrome	0.28

The recovery of chromium-51 for all steps in the method is 95% or better (Table II). The amount of chromium retained by the filter paper and silver chloride can be reduced to 1% of the total by washing the filter paper six times with 3 ml of water per wash.

PRECISION

Nineteen 2-g subsamples of a wheat stem and leaf sample were analyzed for chromium over a 7 week period by two analysts. The mean value (±S.D.) for all 19 subsamples was 0.29 ± 0.03 μg chromium. Twelve of these subsamples were included in a single run. The standard deviation for the single run was 0.02 μg of chromium.

Twenty-four 1-g subsamples of an oak leaf and stem sample were analyzed by two analysts over a period of 4 weeks. The mean value (±S.D.) for all 24 subsamples was 2.17 ± 0.31 μg of chromium.

SENSITIVITY

Seven standard aliquots, each containing 20 ng of chromium, and seven reagent blanks were carried through all steps of this method. The reagent blanks gave an average signal (±S.D.) increase of 8.2 ± 4.8 mm over the average background signal, and the average signal (±S.D.) increase for 20 ng of chromium in 5 ml of solution was 16.8 ± 4.8 mm over the average background signal.

SAMPLE PREPARATION AND CONTAMINATION PROBLEMS

Two important problems in sampling and grinding have been noted. First there appears to be more chromium in older portions of plants than in the younger tissues. Therefore, one has to be concerned with tissue age when sampling, as well as uniform particle size after grinding a plant. Second, the grinding operation may contaminate the sample (Table III). Stainless steel equipment for grinding or homogenizing samples should be avoided. Chrome-plated materials are less likely to contaminate dry sample materials than are stainless steel materials, probably because of the abrasion resistance of chrome plating. Many reagent grade chemicals, especially Fe salts, phosphates, and HNO_3 contain sufficient Cr to cause serious errors.

Plastics have been very useful as sample containers and as laboratory ware because of their durability, low contamination qualities, and flexibility. These may not be used in any phase of chromium analysis where solutions of chromium III will come in contact with the plastic. Relatively large amounts of chromium III can be sorbed on such materials.

Table IV. Effect of Preextraction on Chromium Determination

Sample				Preextraction	No preextraction
Metals, μg per sample				μg Cr/sample	
Cr	Fe	Al	Ni		
1	20	20	5		0.987
1	80	80	20		0.996
1	100	100	40		1.00
1		1000	80		1.00
1	1000		500		1.01
1	500				1.03
1		500			1.01
1			500		0.991
1					1.00
Wheat, grain, 2 g				0.24	0.27
				(Range) 0.24–0.25	0.22–0.32
Wheat, straw, 2 g				0.12	0.11
				(Range) 0.10–0.14	0.08–0.13
Blood, 2 ml				N.D.[a]	N.D.

[a] N.D. = not detectable.

Table V. Comparison of Methods for the Determination of Nanogram Amounts of Chromium in Human Serum and Plasma

Method	Units	Serum	Plasma[a]
Emission arc	ng/ml	5.0	7.5
Neutron activation	ng/ml	...	5.7
Atomic absorption	ng/g	8.2	6.3

[a] The density of plasma at 25° C was 1.0270 g/ml.

INTERFERENCES

A number of chromium determinations were carried out to see if preextraction of metals from samples was necessary (Table IV). Various amounts of iron, aluminum, and nickel were added to 1-μg chromium standards, and chromium was determined without preextracting these metals. There was no suppression or enhancement of the signal in the air–acetylene flame. The interferences noted by Slavin (1968) apparently do not occur in this system. Chromium was also determined on samples of wheat and blood with and without the preextraction step (Table IV). The values found were not different from one another. However, the variation among samples was greater for samples that had not been preextracted than for those that had been preextracted. Preextraction at pH 3 does protect against the possibility of a precipitate forming when the solution pH is adjusted to pH 7.

If it is necessary to isolate chromium further after extracting it as the chromium III–2,4-pentanedione complex into chloroform, the chloroform phase may be washed with 3 *N* sulfuric or 3 *N* hydrochloric acid (McKaveney and Freiser, 1958). Ninety-nine percent of the chromium remained in the organic phase after three hydrochloric acid washings.

COMPARISON WITH OTHER METHODS

Human serum and plasma samples were collected by R. J. Doisy (Upstate Medical Center, Syracuse, N.Y.), and pooled to form two homogeneous samples of biological tissue to be used for comparative method studies. Subsamples were sent to several laboratories for chromium determination. The results obtained, using this atomic absorption method, agreed well with the results obtained at other laboratories using emission arc or neutron activation methods (Table V).

DISCUSSION

The concentration of chromium in many biological materials is very low, and the dangers of contamination of samples with chromium are appreciable. Since contamination problems are not always proportional to sample size, the use of fairly large samples is desirable when this is possible.

Chromium methods that are based on the reaction or separation of the chromate anion in acid solution are not well suited to biological materials because minute amounts of organic material may cause the reduction of chromium VI to chromium III, resulting in low values (Pilkington and Smith, 1967). The relatively slow rate of reaction of the hydrated chromium III cation with organic ligands and the stability in acidic solution of the complexes formed make this cation easy to separate from other metal cations. The chromium III–2,4-pentanedione complex is unaffected by acid washing a chloroform solution containing this complex. Therefore, pH adjustment just prior to extraction of the chromium complex with chloroform is not necessary. This is an additional advantage in preparing a relatively pure chromium compound to use in atomic absorption spectrometry.

Wet ashing was used in preference to dry ashing because chromium volatilized under some dry ashing conditions. Fusion or bonding of chromium to glassware was also noted in some cases.

Direct extraction of chromium from plant material in a manner similar to the procedure used by Hansen *et al.* (1971) was not found to be useful. Plant particles tended to be dispersed in the organic phase and extraction of added chromium-51 was not consistent.

It is possible to extract the chromium complex from the aqueous phase with 4-methyl-2-pentanone. However, chloroform was used because the partition coefficient between organic and aqueous phases was more favorable. Also, extraction with chloroform, subsequent volatilization of the chloroform, and solution of the residue in 4-methyl-2-pentanone allows the final volume to be easily controlled.

Ammonium pyrrolidinodithiocarbamate and 8-hydroxyquinoline were also tested as chelating agents for chromium. Ammonium pyrrolidinodithiocarbamate was inferior, while 8-hydroxyquinoline was as good as 2,4-pentanedione. The latter was used because pH control was not as critical.

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