

## Determination of Molybdenum in Biological Materials with Dithiol. Control of Copper Interference

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When molybdenum is determined in biological material with dithiol, interference from up to 1000  $\mu\text{g.}$  of copper can be prevented by the use of thiourea. This eliminates the need for preliminary separation of copper and therefore extends the applicability of the direct determination of molybdenum to a wider range of materials.

IN 1959, Bingley (1) described the determination of molybdenum in plant material without preliminary separation and indicated that up to 30  $\mu\text{g.}$  of copper did not interfere. This relatively large amount of copper is only rarely found in plant samples. However, in animal tissues appreciably greater amounts of copper are frequently encountered.

When analysis of these samples is attempted with dithiol, a heavy black precipitate is formed, and results for molybdenum are low and nonreproducible. The thiocyanate method of Dick and Bingley (3) offers an alternative for the determination of molybdenum in such cases, but, as a matter of convenience, the use of the one method for the widest range of materials is desirable.

To overcome the interference from some element, the element is usually either removed from the mixture or chelated with some sequestering agent which is without influence on the quantitative recovery of the element sought. The pH of the reaction mixture, after the addition of dithiol in the determination of molybdenum, is about 0.6. Consequently, if the second course of interference control is adopted, the chelating agent must be able to constrain the undesirable reaction at a comparatively low pH. Thiourea has found use as a chelating agent (6) and offers a useful means of controlling the interference of some elements, particularly copper, without precipitate formation.

The purpose of this article is to demonstrate the successful application of thiourea as an agent in preventing interference of up to 1000  $\mu\text{g.}$  of copper, and thereby extending the applicability of the previously described method to a wider range of materials.

### Reagents

Dithiol, 0.2% solution prepared as previously described (1).

Ascorbic acid, 5% solution in glass-distilled water, prepared immediately before use.

Tartaric acid, 50% solution in glass-distilled water.

Ferric ammonium sulfate, 9.1% solution of the dodecahydrate in 2% sulfuric acid.

Thiourea, 10% solution in glass-distilled water; prepared and filtered immediately before use.

Isoamylacetate, b.p. 136° to 142° C.

Potassium iodide, 50% solution in glass-distilled water.

### Method

The sample is digested with sulfuric, perchloric, and nitric acids as described earlier (1). After addition of potassium iodide and liberation of iodine, the iodine is cleared from the solution by dropwise addition of 5% ascorbic acid solution. This is followed by 0.25 ml. of tartaric acid solution and then 2 ml. of 10% thiourea. The contents of the tube are then mixed by swirling, and 2 ml. of 0.2% dithiol reagent is added. After standing for 30 minutes, 5.0 ml. of isoamylacetate is added and the tube vigorously shaken for 30 to 40 seconds. After separation, the isoamylacetate layer is removed, centrifuged to remove droplets of water, and the absorbance measured at 680  $m\mu$  in a 1-cm. cuvette.

### Results

When standard solutions were prepared containing 0, 25, 50, 250, 500, and 1000  $\mu\text{g.}$  of copper together with 2  $\mu\text{g.}$  of molybdenum, and the molybdenum-dithiol complex was developed in the customary way, there was a noticeable black precipitate at the 25- $\mu\text{g.}$  level of added copper, increasing in intensity to a heavy dense precipitate at the 1000- $\mu\text{g.}$  level. Where the isoamylacetate extracts of these solutions were examined after protracted centrifugation, the recovery of molybdenum was progressively less as the amount of copper increased. This effect is shown in Table I.

The same standard solutions as used above were then submitted to an identical course of analysis except that 2 ml. of 10% thiourea solution was added

Table I. Effect of Thiourea and Copper on the Recovery of 2  $\mu\text{g.}$  of Molybdenum

Cu, $\mu\text{g.}$	Thiourea Added (ml. of 10% Aq. Soln.)				
	0	0.5	1.0	2.0	5.0
	Mo RECOVERED, $\mu\text{g.}$				
0	2.06	2.01	1.93	1.97	1.82
25	2.03	...	...	1.93	...
50	1.90	...	...	1.95	...
250	0.96	1.99	2.01	1.99	1.82
500	0.38	...	...	1.95	...
1000	0.3	1.76	1.98	1.91	1.84

immediately before the dithiol reagent. When the isoamylacetate extracts were examined, the recovery of molybdenum was quantitative up to 500  $\mu\text{g.}$  of copper, and 96% was recovered when 1000  $\mu\text{g.}$  of copper was present. This effect is also noted in Table I.

To make some assessment of the amount of thiourea which needs to be present to control interference from up to 1000  $\mu\text{g.}$  of copper, an examination was made of the effects of graded amounts of thiourea on the recovery of 2  $\mu\text{g.}$  of molybdenum in the presence of 0, 250, and 1000  $\mu\text{g.}$  of copper. The results (Table I) showed that 0.5, 1.0, and 2.0 ml. of 10% thiourea solution added to the systems was without effect on the recovery of molybdenum when copper was absent, but 5 ml. of 10% thiourea solution tended to produce low recovery. Apart from this effect of low recoveries of molybdenum when more than 2 ml. of thiourea solution was used, recoveries were unaffected by the presence of 250  $\mu\text{g.}$  or 1000  $\mu\text{g.}$  of copper at all levels of thiourea addition except that of 0.5 ml. to the system containing 1000  $\mu\text{g.}$  of copper. At this point, inadequate control of copper interference was evident, both by the lower recovery of molybdenum recorded and by the observation that the aqueous phase had a gray appearance which suggested incomplete chelation of copper, and consequently scope for reaction with dithiol. For general use, an amount of

**Table II. Increasing Weights of Adult Sheep Liver Sample Analyzed for Molybdenum by Modified Dithiol Method**

Sample Wt. (Grams)	0.237	0.293	0.377	0.445	0.494	0.633
Cu present, $\mu\text{g.}$	378	470	600	710	790	1005
Mo per gram found, $\mu\text{g.}$	4.0	3.9	3.9	3.9	3.9	3.8

**Table III. Comparison of Methods of Analysis**

(Dry weight molybdenum p.p.m.)

Adult Sheep Liver Sample No.	Modified Dithiol Method	Unmodified Dithiol Method	Thiocyanate Method	Cupferron Extraction, Unmodified Dithiol Method	Liver Cu, P.P.M., Dry Wt.
5085	3.0, 3.1	0.4, 0.5	...	3.3, 3.4	1000
5093	5.2, 5.3	1.6, 1.8	...	5.4, 5.6	190
5097	5.1, 5.1	0.6, 1.0	...	5.2, 5.4	400
5502	5.5, 5.6	3.9, 4.3	5.5, 5.6	...	420
5506	2.2, 2.3	1.5	2.2, 2.2	...	500
5514	4.1, 4.1	0.7	4.4, 4.4	...	1400

2 ml. of 10% thiourea solution was chosen to control interference of up to 1000  $\mu\text{g.}$  of copper.

To test whether a finite time was required for chelation of copper with thiourea or the possible interference of thiourea in the quantitative extraction of the dithiol-molybdenum complex, a series of experiments was undertaken in which 0, 10, and 30 minutes elapsed after addition of thiourea and before the addition of dithiol and periods of 5 and 30 minutes after the addition of dithiol but before extraction of the complex. The result of this investigation was to show that in none of these circumstances was any influence exerted on the recovery of molybdenum. Absorbance measurements of the isoamylacetate extracts indicated long stability.

When it was proposed to use thiourea as a masking agent for copper, the thiourea was first added before reduction with potassium iodide, but this led to low recoveries of molybdenum in the absence of copper and even lower recoveries if the amount of copper exceeded 300  $\mu\text{g.}$  Addition of the thiourea immediately before the dithiol did not result in lower recoveries and was therefore routinely employed.

When it had been demonstrated that interference from graded amounts of copper added to inorganic solutions of molybdenum could be controlled with thiourea, the procedure was then applied to graded weights of a liver sample containing up to 1000  $\mu\text{g.}$  of copper. Samples were decomposed by a wet digestion technique (4) and then analyzed for molybdenum with dithiol. Results are given in Table II.

Two series of comparative analyses were made by alternative procedures for the determination of molybdenum in liver tissue.

In one series, analyses were made by

the proposed method and by the thiocyanate method (3), which is free from interference by copper.

In the other series, analyses were made by the proposed method, and by the dithiol method of Bingley (1), after separation of the molybdenum from the acid digest with cupferron and chloroform (7). After quantitative removal of the chloroform phase, the aqueous phase, which had been adjusted to approximately 2N with respect to acid, was analyzed for copper. At the same time, the sample was analyzed for copper directly. No difference was found in the copper content before and after extraction of molybdenum.

When the samples were analyzed without the addition of thiourea, low and erratic results were obtained. These results are presented in Table III.

Sodium thiosulfate solution was specified in the original method as the reagent for discharging iodine from the acid solution of the digest after addition of potassium iodide reagent. On a few occasions, however, variable results were reported for some samples which, when repeated, confirmed the higher of the two results. Since no information was available as to the effect of excess thiosulfate on the reaction, some tests were carried out which suggested that thiosulfate in excess of that required to discharge the iodine color often leads to the formation of a precipitate of sulfur and that it is in such systems that results for molybdenum tend to be low. The cause of this low result was not pursued, but it was found that ascorbic acid solution could replace thiosulfate at this point in the reaction and be added in considerable excess without producing a low result. No formation of a precipitate is evident, and this reducing agent has now replaced thiosulfate for routine use.

## Discussion

Interference from copper in the reaction of dithiol with molybdenum was noted when the reagent was first described, and in using it as an analytical tool for molybdenum, it has sometimes been necessary to remove copper from the mixture before proceeding to the molybdenum determination (5). Some workers, however, do not appear to have encountered this difficulty for Clark and Axley (2) were able to determine 1  $\mu\text{g.}$  of molybdenum in the presence of 100  $\mu\text{g.}$  of copper. Bingley (7) found that up to 30  $\mu\text{g.}$  of copper was without effect on the determination of micro amounts of molybdenum, but when copper was present in excess of this limit low recoveries resulted. Subsequently, when the method was applied to liver samples containing up to 1000 p.p.m. of copper, results for molybdenum were low and erratic. Analysis by the alternative method of Dick and Bingley (3) showed how low these results were.

The data in Table I indicate that thiourea is an effective reagent for controlling this interference from copper, and at the same time does not affect the molybdenum-dithiol complex except when present in somewhat excessive amounts. Complete absence of any black precipitate in the aqueous phase after addition of dithiol in systems containing copper is a clear indication of the strong chelating properties of thiourea. Protracted contact of thiourea with the reactants does not appear to exert any competitive effect as indicated by complete recovery of molybdenum after various time periods before and after adding dithiol.

The upper limit of 1000  $\mu\text{g.}$  of copper chosen to be present in the tests enables concentrations of up to 2000 p.p.m. of copper to be tolerated by appropriate selection of the sample weight. It would be unusual to find liver copper concentrations in excess of this.

## Literature Cited

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