Biophysical and Physiological Investigations on Cartilage and other Mesenchymal Tissues

IV. A Semimicro Method for Conductometric Determination of Sulfur *

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The common method of determining sulfur in organic compounds by combustion¹ is inapplicable when the sample is a tissue, because then some of the sulfur remains in the ash as sulfate. When the sample contains a metal it is necessary to choose another way to decompose it. It is possible — more or less easy — to oxidize the sulfur containing compounds with nitric acid, and the sulfur can then be determined as sulfate. When the amount of sulfur to be determined is about 0.1 mg, the gravimetric method demands great care and very good balances, whereas a conductometric titration based upon precipitation of barium sulfate is rapid and less laborious. For references to similar methods see ^{2,3}.

Apparatus. The conductivity bridge did not deviate from the conventional Wheatstone AC type. In the detector circuit a narrowband amplifier tuned to the frequency (1000Hz) supplied by the RC-oscillator was used. A broadband amplifier can also be used, although the balance point of the bridge is then obscured by harmonics. The conductance change could be read directly on an electronic voltmeter. The voltmeter reading was very nearly a linear function of the conductance. A Wagner earth was applied, although it was not indispensable. The oscillator as well as the amplifier and the voltmeter were supplied with a stabilized voltage.

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The conductance cell was a glass cylinder, 6 cm in length and 2 cm in diameter. The two platinum electrodes (area 2 cm²) were placed just at the bottom of the cell in order to avoid impedance changes originating from slapping of waves on the surface of the solution. The stirring was provided by a motor driven glass rod rotating in the center axis of the cell. The handling of the cell was facilitated by the fact that it was not necessary to platinize the electrodes. The voltage over the cell was about half a volt.

In spite of the rather large temperature dependence of the conductivity, the only thermostating necessary is to place the cell in a vessel with a large volume (2 l) of water. If the solution is titrated after an hour, the temperature usually does not change more than one hundredth of a degree during the titration.

Titration. The titration should be rapid especially because of the simple thermostat used. The precipitation of barium sulfate, however, is a slow process, but if the solution is seeded with about a hundred times as much barium sulfate as to be precipitated and the stirring is sufficient, the precipitation rate is accelerated considerably. The barium sulfate used in seeding was precipitated from hot barium hydroxide solution with sulfuric acid (excess) and then thoroughly washed. The same volume of seed suspension was always used and correction applied for the small amount sulfate in excess. Attention should be paid to the small increase in this correction term particularly during the first time. This increase can probably be explained as a liberation of occluded sulfuric acid.

To prevent precipitation of compounds other than barium sulfate, acetic acid was added to the barium sulfate suspension such that the final solution contained 0.2 % acetic acid.

The standard solution, 0.15 *M* barium chloride, was standardized against potassium sulfate and thus the sulfur content could be directly calibrated to the graduations of the micrometer of the micro buret. From a diagram of the voltmeter reading plotted against the micrometer reading (Fig. 1) it is easy to determine the equivalence point.

EXPERIMENTS

Upon standardization of the barium chloride standard solution duplicate tests agreed within 0.3 %.

It is always possible to burn the sample in a bomb tube with fuming nitric acid according to Carius and then evaporate the nitric acid in excess. In several cases the tedious manipulation with the bomb tubes can be avoided and the process performed at atmospheric pressure in a test tube placed in a small open electric furnace at 140° C. To avoid evaporation of sulfuric acid enough sodium hydroxide or sodium nitrate must be supplied. Because only a fraction of a milliliter conc. nitric acid is required there is little risk of bumping but for safety a little glass powder was added.

Fig. 1. Voltmeter reading (ordinate) plotted against volume barium chloride standard solution (abscissa). The sample contained 0.0759 mg sulfur.

Procedure: A dried sample containing about 0.1 mg sulfur is weighed. It is then heated together with 0.5 ml conc. nitric acid, 1 mg sodium nitrate and a little glass powder in a test tube for a few hours. The heat supply is increased and the evaporation carried on to dryness. The salt crust is dissolved in 4 ml water, transferred to the conductance cell and titrated together with 1.00 ml of barium sulfate sol.

In a tissue sulfur appears in appreciable amounts as ester sulfate of polysaccharides and in derivatives of the amino acids cystine, cysteine and methionine and as sulfate ions. To control the reproducibility and validity of the method some of these substances were tested:

1. *Reproducibility test.* A chondroitin sulfuric acid preparation. Five tests on different amounts burned three to six hours at atmospheric pressure gave the sulfur percentage: 5.62, 5.64, 5.72, 5.62 and 5.61 %.

2. *Validity test.* L(-)Cystine, Merck, analytical reagent. Burned seven hours at atm. pressure. Found 26.3, 26.7 and 26.6 % sulfur. Calculated 26.68 %.

3. DL-Methionine, Merck. Burned seven hours at atmospheric pressure. Only a small fraction of the sulfur had been oxidized to sulfate. To decompose methionine within a reasonable time it is thus necessary to apply higher temperature.

An experiment to accelerate the oxidation rate with a drop of perchloric acid failed, because then the sulfuric acid partially evaporated.

Cartilage⁴, protein free hyaluronic acid containing an admixture of ester sulfate bearing polysaccharides⁵ and the cystein containing polypeptide bacitracin hydrochloride⁶ may be mentioned as examples of samples determined by this method.

Interference of Calcium.

In the usual gravimetric sulfur determination method calcium is coprecipitated. Even in conductometric methods errors due to calcium have been reported². Under the conditions mentioned above, however, calcium in moderate amounts does not interfere. Sulfate samples with up to 0.4 equivalents calcium per equivalent sulfate were tested and gave correct sulfur values within the normal limits of error. The calcium ions adsorbed on the seed crystals do not seem to change during the titration and the solution is so dilute that it is very far from saturation with respect to calcium sulfate.

DISCUSSION

From the experiments performed it can be concluded that it is possible to determine down to 0.1 mg sulfur with 1 % error with this method. Before the titration the sulfur must be converted to sulfate. The oxidation with nitric acid can be performed at ordinary pressure when the sample is a polysaccharide sulfate or cystine derivate. When the sulfur is connected to two carbon atoms as in methionine, high pressure methods must be applied.

Many other methods for volumetric determination of sulfate have been reviewed by Thomson⁷. The conductometric method described here, however, seems to be comparable to the best of them.

A conductometric titration method using benzidine dihydrochloride instead of barium has recently been proposed by Fischer *et al.*⁸. This way, however, is more time-consuming.

SUMMARY

Determination of sulfur in tissues is discussed. Conductometric titration in the presence of a very large excess of barium sulfate permits rapid determination of 0.1 mg sulfur with 1 % error. Moderate amounts of calcium do not interfere.

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