Determination of Inorganic Sulfate in Plasma with a Centrifugal Analyzer

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Turbidimetry of inorganic sulfate, after precipitation with barium sulfate, can be done simply in a Cobas Bio centrifugal analyzer. Polyethylene glycol is used as the precipitate-stabilizing agent. Reproducibility of precipitation is enhanced by the presence of BaSO₄ particles, which function as seed nuclei. There is no interference by normal or above-normal concentrations of phosphate, heparin, bilirubin, hemoglobin, or erythrocyte contents, or by lipemia (triglyceride concentrations up to 6.5 mmol/L). Analytical recovery of added inorganic sulfate was found to be quantitative. Precision is similar to that for other methods for inorganic sulfate in plasma. This method is suitable for the rapid, routine analysis of plasma inorganic sulfate, and it is simple and less expensive to perform than alternative methods.

Inorganic sulfate, the major end product of sulfur metabolism, is excreted mainly via the kidney, its concentration in plasma being increased in renal insufficiency. Some investigators (1–3) have indicated that information on the concentrations of sulfate in plasma is equal or superior to information on the concentrations of urea, urate, phosphate, or creatinine in plasma as an indicator of renal insufficiency. Plasma sulfate is also increased in various other conditions, including hyperthyroidism and pregnancy, at the middle of the menstrual cycle, and after ingestion of protein (4).

Several methods have been described for the determination of plasma inorganic sulfate (reviewed in 4). The techniques used have included gravimetry (1), colorimetry (5), turbidimetry (6, 7), nephelometry (8), indirect flame photometry (9), atomic absorption spectrophotometry (10), and radiometry (11). Most of these are time consuming, require relatively large sample volumes, and are inadequately sensitive. In methods of continuous-flow analysis, dialysis has been used to remove interfering substances and background plasma absorbance (12), but such methods have the disadvantages of a low signal/noise ratio, extensive baseline drift, and undesirably low and varying sensitivity throughout the analytical run. Thus a method better suited for routine use is needed, for use in further investigations of the homeostasis and chemical pathology of this anion. Moreover, volume requirements should be small so that samples from neonates can be analyzed.

The method we describe is based on a procedure for measuring inorganic sulfate in urine (7). It involves the use of a precipitate-stabilizing agent, polyethylene glycol 6000 (PEG). In this study we used a Cobas Bio centrifugal analyzer (Roche Analytical Instruments, Nutley, NJ 07110), because its design ensures that all of the barium sulfate precipitate remains in the light path. Sample volumes can be as little as 25 µL.

Materials and Methods

All measurements of plasma inorganic sulfate were performed, in duplicate, according to the instrument settings shown in Table 1. The centrifugal analyzer was calibrated with an aqueous 0.8 mmol/L (NH₄)₂SO₄ standard, prepared by serially diluting a 1.0 mol/L (NH₄)₂SO₄ stock standard. After correcting for background sample absorbance by using 130 mmol/L HCl (instrument setting 12, Table 1), we determined inorganic sulfate content from the absorbance changes produced by adding a "start" reagent (instrument setting 14, Table 1), prepared as follows:

Dissolve 9.77 g of BaCl₂·2H₂O and 200 g of polyethylene glycol 6000 in glass-distilled water and dilute to 1.0 L. Transfer 100 mL of this solution to a 100-mL measuring cylinder, and add 800 µL of 50 mmol/L (NH₄)₂SO₄ to "seed" the mixture. This reagent must be mixed thoroughly before use. Stored in glass, it is stable for two months at room temperature. The concentration of reactants in the final assay mixture (Table 1) and the pH are in accordance with previous recommendations (13).

Quality-control material was obtained from Nyegaard and Co., Oslo 4, Norway ("Pathonorm"), and Wellcome Reagents Ltd., Beckenham, Kent, U.K. ("Wellcomtrol").

Results and Discussion

Our preliminary investigations verified the reported need for a stabilizer for BaSO₄ precipitates (7). We found that omission of the stabilizer led to poor linearity and precision, and that PEG was more easily pipetted and gave a broader range of linearity than glycerol or sucrose. At the high mixing speed and final running speed of the Cobas Bio rotor (approximately 3000 and 1000 revolutions/min, respectively) the centrifugal force causes the BaSO₄ particles to precipitate too rapidly on the cuvette window. To prevent this we increased the viscosity of the reaction mixture with polyethylene glycol.

Previous work (6) indicated that acidic conditions are

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<th>Table 1. Instrument Settings for Measuring Inorganic Sulfate in Plasma in the Cobas Bio Centrifugal Analyzer</th>
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Concentrations of the constituents of the final assay mixture are: HCl, 50 mmol/L; BaCl₂·2H₂O, 7.5 mmol/L; PEG 6000, 37.7 g/L; (NH₄)₂SO₄ seed, 75 µmol/L.
required for turbidimetry of inorganic sulfate, to avoid interference from other anions such as phosphate. Accordingly, we acidified samples with HCl, chosen because of its weak precipitating action on proteins. Equally important was the presence of a small amount of pre-formed BaSO₄ in the barium–PEG reagent (7).

Precision studies were undertaken to determine both the optimal BaSO₄ seed volume to be added to the stabilizing solution (PEG) and the viscosity of the stabilizing solution itself (Figure 1). The optimal seed volume was that specified above, as shown in Figure 1. Larger amounts of seed resulted in impaired precision and excessive background absorbance. Smaller amounts of seed also resulted in impaired precision, and when no seed was added to the reagent the within-run CV of the assay was as high as 30%. Although similar precision and linearity resulted when we used 175 and 200 g of PEG per liter, the latter concentration provided the optimal compromise between sensitivity, linearity, and reagent viscosity.

For linearity studies we prepared a series of aqueous standards by serially diluting a 1.0 mol/L (NH₄)₂SO₄ stock solution (132.14 g/L). We used an aqueous 0.8 mmol/L standard for routine analyses. Under the optimized conditions (Table 1) calculated results were linearly related to concentration up to 1.4 mmol/L (Figure 2). Obtaining such linearity for turbidimetric methods performed on centrifugal analyzers presents significant problems. In this study, absorbance was maximum in a very short time for samples containing high concentrations of inorganic sulfate, because of the greater seeding effect. Then the absorbance decreased as individual particles centrifuged down to form a thin deposit on the peripheral wall of the cuvettes. In contrast, samples containing low concentrations of inorganic sulfate required longer to reach maximum absorbance. Thus linearity depends on the reaction interval; the analytical conditions we specify represent the best compromise. Linearity can be increased by using additional "seed" in the stabilizing solution, but at a cost in sensitivity and precision. This same problem has been encountered with other turbidimetric methods.

Within-run precision was assessed by analysis of four aqueous standards with inorganic sulfate concentrations ranging from 0.40 to 1.26 mmol/L and two commercial quality-control materials with normal and above-normal concentrations of inorganic sulfate. Pathonor was diluted twofold before analysis and Wellcomtrol was reconstituted as directed. On each of 20 occasions, 25 samples were analyzed in the same run. Table 2 gives the precision data for each material.

Using Pathonor and Wellcomtrol, we assessed between-run precision from results of analyses performed over 20 runs. The between-run SD was only marginally greater than the within-run SD (Table 2).

Phosphate interference was investigated by serially diluting a phosphate-enriched plasma pool with the original phosphate-depleted plasma pool (obtained from a diabetic ketoacidotic patient being treated with insulin). Phosphate concentrations (below 5 mmol/L showed no interference.

Interference from heparin has also been reported (6, 12). We investigated this by serially diluting a serum pool containing 150 int. units of lithium heparin per milliliter with the original serum pool. Lithium heparin concentrations = 150 int. units/mL (14) showed no interference.

Bilirubin interference was assessed by serially diluting a bilirubin-enriched serum pool with the original serum pool (low bilirubin concentration). The six samples so prepared had total bilirubin concentrations ranging from 50 to 500 µmol/L. No interference with the measurement of inorganic sulfate was noted.

To assess possible triglyceride interference, we prepared serial dilutions of a pooled specimen of hyperlipidemic plasma. Part of the hyperlipidemic plasma pool that had been clarified by ultracentrifugation was used as the diluent. Plasma inorganic sulfate analyses were unaffected by plasma triglyceride concentrations up to 6.5 mmol/L. Greater concentrations gave a positive interference.

We measured inorganic sulfate in plasma from a blood sample that had been repeatedly frozen and thawed and

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**Fig. 1.** Effect of volume of 50 mmol/L (NH₄)₂SO₄ seed (added to 100 mL of stabilizing solution) on the within-run precision of the assay (a) when no (NH₄)₂SO₄ seed was added to the stabilizing solution, the within-run CV of the assay was 30%.

**Fig. 2.** Linearity obtained under optimized conditions

- Least-squares regression equation: y = 0.994x - 0.001
- r = 0.999
- n = 9
- Sₑₑ = 0.023
plasma from a blood sample maintained at room temperature for 24 h. Neither showed any difference from controls.

Analytical recovery experiments were performed in which 0.50 mmol/L and 1.00 mmol/L of inorganic sulfate was added to a plasma pool containing 0.40 mmol/L of inorganic sulfate. On analysis, about 99% of the added sulfate was accounted for.

A reference interval was derived from data on samples from 274 hospital patients and from 30 healthy laboratory volunteers. Before these two sets of data were merged, each sample was statistically analyzed. The means and ranges did not differ significantly. Analysis of the merged data by the Hoffmann technique (15) gave a mean value of 0.33 mmol/L, and a 95% reference interval of 0.22–0.49 mmol/L. These values compare favorably with a previously published turbidimetric reference interval (6) and with all-method reference intervals (4, 10).

We have confirmed earlier observations that plasma inorganic sulfate is increased (with respect to an adult-based reference interval) in newborn infants (16) and in women in the third trimester of pregnancy (4). Moreover, detailed correlations with age, sex, plasma urea, and plasma creatinine are currently being established.

This method has performed satisfactorily in our laboratory during a year. Its suitability for routine inorganic sulfate analyses is enhanced by the following advantages over previously published methods: it is simple (only three aqueous reagents require preparation), economical (none of the reagents are expensive), and fast (10 patient and quality-control samples can be analyzed in duplicate in 15 min); it has adequate linearity and good precision; and it is the first method that can measure inorganic sulfate in micro-scale samples.

References