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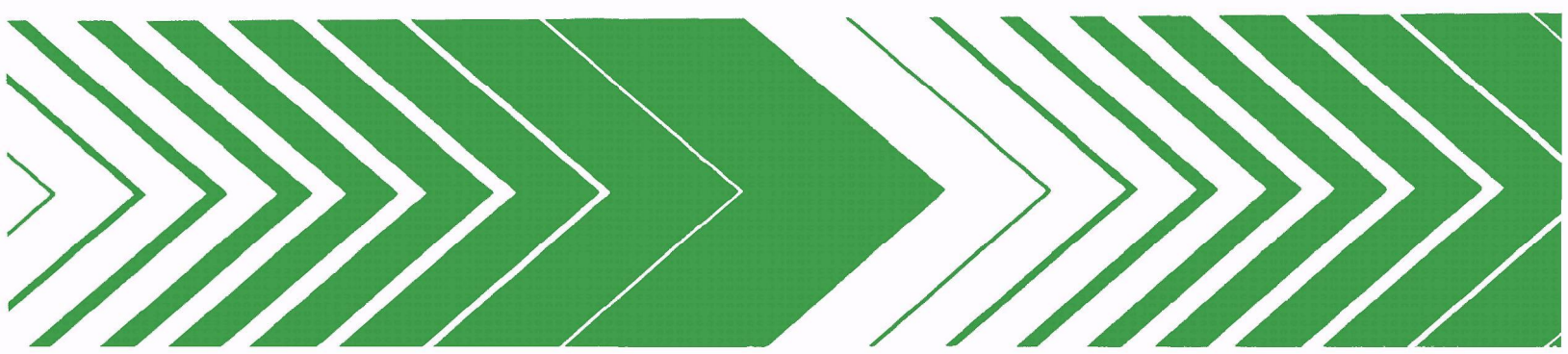
Determination of Trace Quantities of Sulfate Ion

New Approaches to an Old Problem



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May 1979

DETERMINATION OF TRACE QUANTITIES OF SULFATE ION
New Approaches to an Old Problem

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ABSTRACT

Several analytical methods for possible use in measuring trace amounts of water soluble sulfate anions were reviewed and evaluated. Enzymatic sulfate determination does not appear to be a viable approach until the required enzymes can be obtained commercially. Gas chromatographic analysis of bis(trimethylsilyl)sulfate was studied and, with further development, may be a selective method for the determination of sulfate as well as other oxy-anions. A direct kinetic method, based on the ability of sulfate to catalyze the depolymerization of zirconyl species, was investigated. The method can detect 0.2-20 ppm sulfate and, with a minimum of sample preparation, is relatively selective.

CONTENTS

FIGURES

TABLES

ACKNOWLEDGEMENT

The authors wish to thank Dr. Kenneth Monty for his advice and for providing the Salmonella typhimurium culture used in our studies.

SECTION 1

INTRODUCTION

There are many methods currently available for the determination of water soluble sulfate. The majority of these techniques are based on the reaction of sulfate with a barium salt to form the precipitate, barium sulfate. Direct sulfate analysis has been achieved by quantitatively determining the amount of precipitate formed either turbidimetrically, gravimetrically (1), or nephelometrically (2). Sulfate concentration has also been determined indirectly by measuring excess barium ion, or its inorganic counter ion, in solution using atomic absorption (3), ion selective electrode (4, 5), or titrimetric (6) procedures. The above techniques usually exhibit only a modest sensitivity and reproducibility, and are often not applicable to the analysis of samples containing less than 2 ppm sulfate. Alternatively, several colorimetric procedures have been developed which employ barium salts composed of organic bases to slightly improve the sensitivity of the barium sulfate reaction (1). In the latter case, the change in free counter ion concentration is measured.

Even with this and numerous other modifications in the above methods, sulfate analysis is still subject to a number of difficulties which are inherent to the barium sulfate reaction. The sensitivity of the analysis based on this reaction is limited by the solubility of barium sulfate in solution. Additionally, other divalent anions and cations react with either barium or sulfate, respectively, and bias results. These interferents therefore must be removed from the sample prior to

analysis. Aqueous samples often contain sulfite and sulfide, in addition to sulfate. These species also react with the barium salts, and thus total sulfur anion rather than sulfate is actually being measured.

Several novel reaction schemes have been evaluated in order to overcome the deficiences encountered with the barium sulfate raction. Sulfate analysis using an enzymatic reaction was proposed in an effort to establish a more selective technique. Enzymes characteristically react specifically with micromolar concentrations of their substrates. Hence, the anions and cations which interfere with the barium sulfate reaction should not affect the enzymatic sulfate reactions. Additionally, the enzyme will react with only the single sulfur anion for which it is specific. Two enzymatic reaction schemes were investigated; one procedure used sulfite oxidase to indirectly determine sulfate and the other required a three-enzyme "sulfate transferring system" to form a chromogen which is directly related to sulfate concentration.

Reaction selectivity can also be achieved by using chromatographic techniques to separate sulfate from other anions present in a sample. A gas chromatographic procedure for sulfate was evaluated which is based on the formation of a volatile trimethylsilyl (TMS) derivatives of oxy-anions. Such a technique provides a separation of sulfate from other potentially interfering species, thereby permitting the selective analysis of sulfate and possibly other separated anions contained in the sample as well.

A direct kinetic method for sulfate analysis was evaluated in a third reaction scheme. The kinetic method might be more sensitive and reproducible than those methods based on the barium sulfate reaction, and is amenable to the use of masking agents which might improve the selectivity of the analysis. A colorimetric procedure for sulfate was previously reported, which was based on the catalytic properties of sulfate to depolymerize a zirconyl species (7). Sulfate concentration was found to be linearly proportional to the rate of formation of a free zirconyl-methylthymol blue chromogen. This kinetic procedure has been adapted to the miniature centrifugal fast analyzer, and masking agents have been added to the reaction mixture to improve the selectivity of the kinetic reaction.

This report reviews in detail the three proposed reaction schemes, summarizes the results obtained to date with each procedure, and evaluates their potential for providing a sensitive and selective means for determining sulfate concentration.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

Enzymatic analysis of sulfate ion may represent a selective and sensitive approach for the determination of micromolar concentrations. The enzymes are reported to react specifically with sulfate rather than total sulfur anion, as in the barium sulfate procedures. If sufficient enzyme activity is present in the reaction mixture, and if the reaction proceeds toward equilibrium any enzymatic inhibition by other anions or cations in the sample will be negligible. The enzymatic analysis of sulfate appears feasible since the same reaction sequences have been previously used in the laboratory, although for other applications. However, if the enzymatic analysis of sulfate is to have wide application in the general chemistry laboratory, a reliable and stable source of the enzymes must be available commercially. Until such time, additional development of this procedure does not appear fruitful.

Selective sulfate analysis can also be achieved by using chromatographic procedures to separate sulfate from the interfering anions. The gc analysis of TMS-SO_4 may be easily adapted to the analysis of sulfate and be easily performed in a general chemical laboratory. To date, 0.2 μg ammonium sulfate in aqueous solutions can be analyzed reproducibly if the sample is evaporated to dryness and silylated in the presence of bis(trimethylsilyl)trifluoroactamide and methylene chloride. For more dilute environmental samples, sulfate ion may be concentrated on an anion exchange column and subsequently eluted by a concentrated ammonium salt. Thus, a concentration of sulfate and conversion to its

ammonium salt might be performed in a single step with an ion exchange column to analyze less-concentrated environmental samples. Precipitating agents might also be useful to concentrate sulfate and remove water prior to the silylation reaction. The sensitivity of the gc procedure might also be improved by investigating other gc column supports and liquid phases. A silylized glass capillary column would probably improve the detection limit by eliminating the need for a solid support. Finally, either selective or non-selective gc detectors may be used to analyze the TMS-SO_4 or all volatile oxy-anion derivatives, respectively, in a sample. Further studies in the gc analysis of sulfate appear profitable, since the technique promises to provide a procedure which is significantly more selective than the barium sulfate methods and is applicable to several other anions as well.

The kinetic Zr-MTB procedure is both more sensitive and selective than the barium sulfate methods. A detection limit of 0.3 ppm sulfate can be measured and reproducibilities of 2% are obtainable if care is taken in loading the rotor. The major cation interferences are removed with batch cation exchange treatment and fluoride and sulfide can easily be masked with Al(III) and Hg(II), respectively. Highly colored samples may be accurately analyzed with a minimum of interference. The instrumentation permits the simultaneous processing of standards and samples in the 0.3-20 ppm sulfate range. The linear reaction data is located and the reaction rate calculated by a linear regression computer routine, limiting the computing time required of the analyst. Further studies should be directed toward locating an appropriate masking agent for phosphate and arsenate ions. This would eliminate the need to use

magnesium oxide adsorption in samples containing these interferents. By removing cations and adding a mixture of masking agents to both samples and standards, the kinetic Zr-MTB technique would provide a procedurally simple, semi-automated and selective analysis of sulfate in environmental samples.

SECTION 3

PROPOSED ENZYMATIC SCHEMES FOR SULFATE ANALYSIS

THE SULFITE OXIDASE REACTION

An indirect method for sulfate analysis can be achieved by chemically reducing sulfate to sulfite and coupling this reaction with the following enzymatic reaction:

$$SO_3^{2-} + O_2 \xrightarrow{\text{Sulfite Oxidase}} SO_4^{2-} + H_2O_2.$$

The sulfite concentration is then proportional to the production of hydrogen peroxide, which can be measured by either colorimetric (8), fluorimetric (8b), or chemiluminescent (9) procedures, all of which would provide great sensitivity. A sample would be analyzed by first determining the sulfite concentration initially present in the sample. A second sample aliquot would then be reduced and the total sulfite content determined. The difference between the two measurements would represent the sulfate concentration in the sample. Such a reaction scheme would provide a specific and sensitive means for analyzing both sulfate and sulfite. The procedure may be automated by use of reduction and immobilized enzyme columns in a flow system.

The feasibility of this enzymatic method is dependent upon the reproducibly of the sulfate reduction, and therefore a study of several reducing agents was initiated. The reducing characteristics of various granulated metal reactors were investigated first with the expectation that no undesirable side products would be formed in the reduction

that could interfere with the subsequent enzymatic reaction. Nitrate reduction to nitrite and ammonia has been accomplished by using such reducing columns containing copper-coated cadmium granules (10), Devarda's alloy (11, 12), a Jone's reductor (13), and copper granules (14). Similar columns and conditions were used in an attempt to reduce sulfate to sulfite. Ethylenediaminetetraacetic acid was added to sulfate and sulfite standards to prevent formation of insoluble metal sulfites and sulfides, as well as to protect against subsequent metal poisoning of the enzyme. Although no reduction of sulfate was observed with any of the metal reducing columns, 95% of sulfite standard introduced into the columns was recovered.

Sulfate reduction to sulfite and sulfide was observed using sodium borohydride pellets. Optimizing pH and borohydride concentration may permit the reproducible reduction of sulfate to sulfite; however, unreacted borohydride would have to be removed prior to the enzymatic reaction.

Because sulfate reduction has met with only limited success, further studies in this direction were postponed in order to review the potential of a second enzymatic method for sulfate analysis.

THE SULFATE TRANSFERRING ENZYMES

A direct procedure for sulfate determination was proposed using the three-enzyme "sulfate transferring system." The colorimetric method, outlined in Figure 1, takes place in the presence of excess adenosine triphosphate (ATP). Sulfate is transferred to ATP in the

SULFATE TRANSFERRING SYSTEM

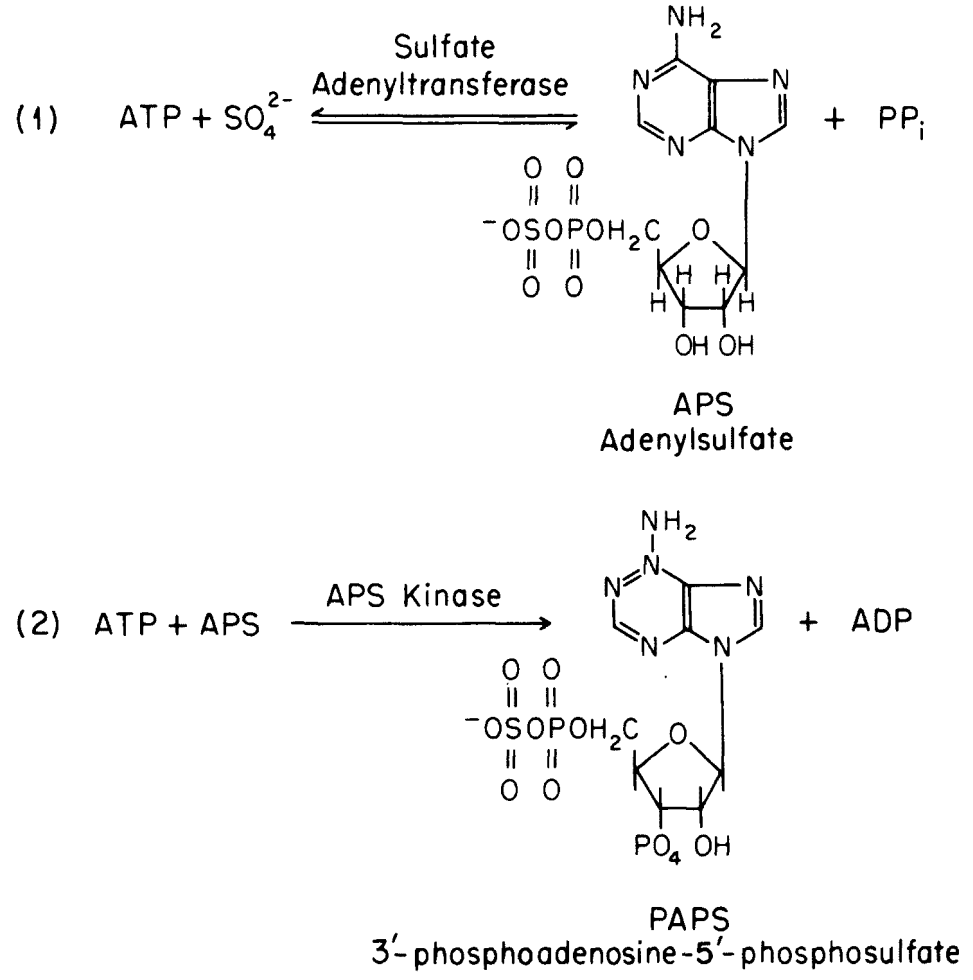

Figure 1. Sulfate Transferring System

presence of sulfate adenyltransferase to form adenylsulfate (APS) and inorganic pyrophosphate (PP_i). APS kinase catalyzes the second reaction in which APS and ATP react to form 3'-phosphoadenosine-5'-phosphosulfate (PAPS) and adenosine diphosphate (ADP). Although it is feasible to monitor reactions 1 and 2 by the consumption of ATP, this would not be specific for sulfate since sulfate adenyltransferase (reaction 1) also reacts with molybdate, tungstate, selenate, chromate, and sulfite. However, none of these other anions produce PAP (reaction 3). Thus, reaction 3 can be used as a specific indicator reaction for the sulfate reaction by monitoring the consumption of the phenol substrate.

Sulfate analysis using the three-enzyme "sulfate transferring system" appears to be a feasible approach, since various individual aspects of the reaction scheme have been successfully employed for other purposes. The first two enzymes are currently being used to prepare both APS and PAPS. The third reaction has been used in combination with the two ATP-dependent reactions to quantitatively determine PAPS and PAP in clinical samples. Literature sources describe the use of various phenol substrates, including p-nitrophenol and m-aminophenol, as well as methylumbilliferone, as spectrometric indicators for the reaction sequence. By appropriate selection of reaction conditions, the overall reaction may be made dependent upon sulfate ion concentration and be used as the basis for a novel approach to sulfate analysis.

To implement this reaction scheme, it was necessary to locate a source of the enzymes. Sulfate adenyltransferase is the only enzyme of the three which is currently available commercially. Several biochemical companies are presently attempting to isolate APS kinase, but

have not, as yet, obtained a stable preparation. We have made several attempts to prepare active APS kinase from yeast (15) and phenol sulfurylase from guinia pig liver (16, 17). No measurable enzyme activity could be demonstrated in any of the preparations isolated from these sources. An attenuated strain of Salmonella typhimurium mutant was also investigated as an enzyme source. The bacteria possesses sulfate adenyltransferase and APS kinase but completes the reaction sequence by reducing the transferred sulfate to free sulfite, as shown in reaction 3'. A method for sulfate analysis was studied in which intact cells are used as the enzyme source to microbiologically reduce sulfate to sulfite. The liberated sulfite is then measured via the West-Gaeke colorimetric procedure (18) and is proportional to the original sulfate concentration in the sample. The feasibility of such a procedure is demonstrated by the fact that an identical procedure has previously been used to study genetic coding in Salmonella mutants (19). A culture of the mutant was obtained and initial studies were made to demonstrate proof of principle. Although the culture could be grown and cells harvested, second generation cells did not exhibit the ability to reduce sulfate to sulfite. Until a suitable source can be obtained for the three enzymes, further assessment of sulfate analysis based on the sulfate transferring system can not be made.

SECTION 4

THE GAS CHROMATOGRAPHIC ANALYSIS OF BIS(TRIMETHYLSILYL)SULFATE

PREVIOUS INVESTIGATIONS

A gas chromatographic (gc) technique was previously developed for the determination of milligram quantities of oxy-anions contained in aqueous samples (20, 21). The procedure required the conversion of anions to their ammonium salts, followed by reaction with a silylating reagent to form the corresponding trimethylsilyl (TMS) derivatives of the anions. The volatile derivatives were subsequently separated and detected by gc. This procedure was modified for the analysis of microgram levels of sulfate in environmental samples.

PREPARATION OF BIS(TRIMETHYLSILYL)SULFATE STANDARDS

Preliminary studies were concerned with the preparation of reliable bis(trimethylsilyl)sulfate (TMS-SO_4) standards. Butts and Matthews prepared TMS-SO_4 samples by adding 5-10 mg sulfate as either solid or aqueous ammonium sulfate to 200 µl bis(trimethylsilyl)trifluoroacetamide (BSTFA). Butts, et al. also included 200 µl dimethylformamide to the reaction mixture. The resulting solution was shaken and then allowed to react overnight at 25°C. The TMS-SO_4 yield was often irreproducible; occasionally, no TMS-SO_4 could be detected. Several variables, including water content, organic solvent, reaction time and reaction temperature, were investigated in order to optimize the chemical reaction conditions for maximum, reproducible TMS-SO_4 yields at the microgram sulfate concentration range.

We found that aqueous sulfate samples must be evaporated to dryness prior to silylation. If greater than 10 µl of water is present in the reaction mixture, a precipitate will form and no TMS-SO_4 will be produced. The presence of smaller quantities of water will result in less than maximum yield of the derivative, as well as severe gc peak tailing and irreproducible peak heights.

The reaction yield is also affected by the presence of organic solvents in the reaction mixture. The percent yield of TMS-SO_4 and the production of secondary products were compared when using BSTFA alone, or in combination with dimethylformamide, acetonitrile, pyridine or methylene chloride. We found that the presence of an organic solvent, in addition to BSTFA, was required to form TMS-SO_4. The greater the solvent-to-BSTFA volume ratio, the more quantitative the yield and the faster the reaction progressed. A ratio of 2.5 was found to be optimum. Methylene chloride was found to be the solvent of choice. In addition to producing the greatest yield of TMS-SO_4, this volatile solvent elutes the gc column rapidly, resulting in a low gc background (Figure 2). Methylene chloride does not produce secondary products, as do both dimethylformamide and pyridine. No side reactions were formed in the presence of acetonitrile; however, the TMS-SO_4 yield was relatively low. Optimum sample composition therefore includes the addition of 250 µl methylene chloride and 100 µl BSTFA to an evaporated ammonium sulfate standard.

The effect of reaction temperature on the percent-yield of TMS-SO_4 was also investigated. No TMS-SO_4 is produced if the temperature is less than 25°C. Fifty percent of the maximum yield is obtained at 45°C,

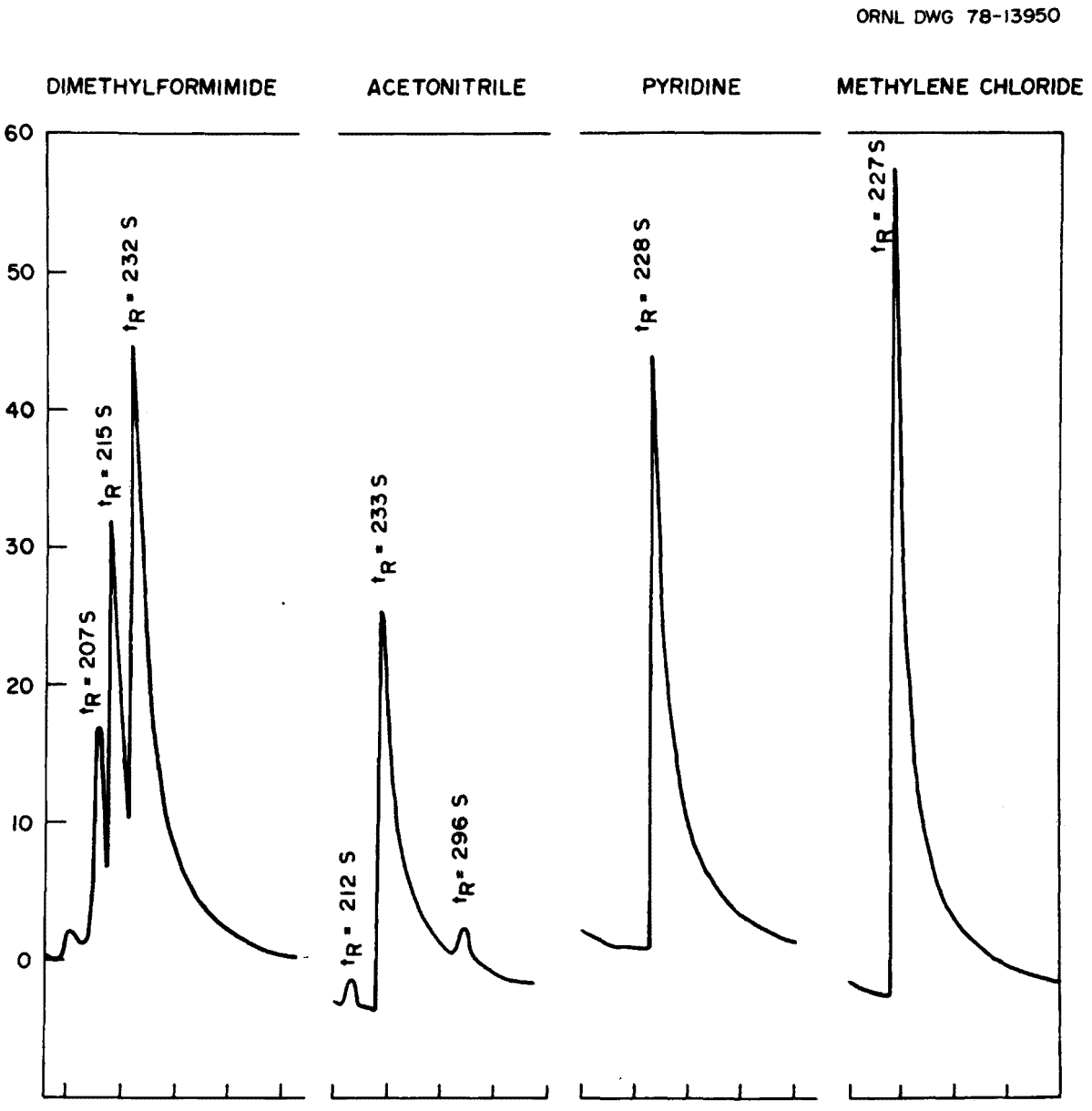

Figure 2. Effect of Solvent on Silylation of Ammonium Sulfate

whereas maximum yield is obtained above 55°C. A 60°C reaction temperature is used to prepare sulfate standards. At reaction temperatures above this value, solvent loss due to leakage during sample preparation becomes a problem. Even at elevated temperatures, an overnight reaction time is required for maximum yield. Using the above optimized reaction conditions, reproducibility in the preparation of standard replicates is ±2% and day-to-day reproducibility in standard preparation is ±5%.

GC SEPARATION AND ANALYSIS OF TMS-SO_4

The gas chromatographic conditions were also optimized for microgram quantities of TMS-sulfate. The derivatized oxy-anions were previously separated using a 5% SE-30 coating on Chromosorb G(HP) (21). The analysis of sulfate on this support was found to be very non-reproducible, due to irreversible adsorption and/or decomposition of TMS-sulfate on the column. The detection limit of the analysis was reported as 2 μg sulfate.

Several columns have been prepared to determine the gc parameters which contribute to the loss of TMS-SO_4. Table I describes five columns which either differ in the solid support used or in the percent loading of the liquid phase, SE-30. The performance of these columns was compared with respect to the amount of tailing of the TMS-SO_4 peak, the detection limit, and the linearity of the calibration curve. Peak tailing is described using a tailing factor (T), defined as

$$T = 100 \times W_1/W_2$$

TABLE 1
COMPARISON OF GC COLUMNS FOR THE
SEPARATION AND DETECTION OF TMS-SO_4

GC Column #	%SE-30 Loading (w/w)	Type of Support*	Optimum Column Temperature	Retention Time (sec)	Tailing Factor	Detection Limit ($\mu g\ SO_4^{2-}$)	Relative Sensitivity	Correlation Coefficient*
1	5	Gas Chrom Q	130°C	93	15	1	0.54	0.973
2	5+0.5% Carbowax 20M	Gas Chrom Q	140°C	135	8	1	4.74	0.992
3	5	Chromosorb W(HP)	140°C	103	33	0.8	33.1	0.990
4	1	Chromosorb W(HP)	95°C	102	80	0.25	32.5	0.997
5	1	Chromosorb 750	90°C	93	100	0.2	37.3	0.998

*80/100 mesh support, 6' glass column, 0.25 in OD, 60 ml argon flow

where W_1 is the base width of the first half of the gc peak and W_2 is the base width of the second half. The base widths are measured at 10% of peak height. Since the gc column tends to absorb TMS-SO_4, the practical limit of detection was estimated by calculating the x-intercept of the TMS-SO_4 calibration curve. The relative sensitivity was determined by comparing the slope of the calibration plots at identical amplifier attenuations. The linearity of the calibration curves was estimated by comparing the correlation coefficients obtained from a linear regression analysis of sulfate standards. Results of such a comparison should suggest alternate columns which would improve the reproducibility and detection limit of TMS-SO_4 analysis.

Gas Chrom Q is similar to Chromosorb G(HP), used in the original study (20), except that it is distributed by another manufacturer. Product specifications state that the support should be used to minimize tailing and to eliminate catalytic decomposition of sample constituents. Gas Chrom Q, with 5% SE-30 (column 1), performed similarly to the initial column using Chromosorb G(HP). The column had to be "loaded" with several injections of microgram quantities of TMS-SO_4 before any peak could be observed. Once the column was conditioned, 4 µg sulfate and greater could be analyzed reproducibly if samples were injected at equal time intervals. The TMS-SO_4 peak width broadened considerably and the retention time increased by at least 15 seconds as the amount of TMS-SO_4 injected was decreased. The least concentrated standards gave no response unless at least 4 µg sulfate was injected just prior to their analysis. The 1 µg detection limit observed was slightly improved over that reported for the Chromosorb G(HP). Peak tailing was relatively severe on this support (see Figure 3).

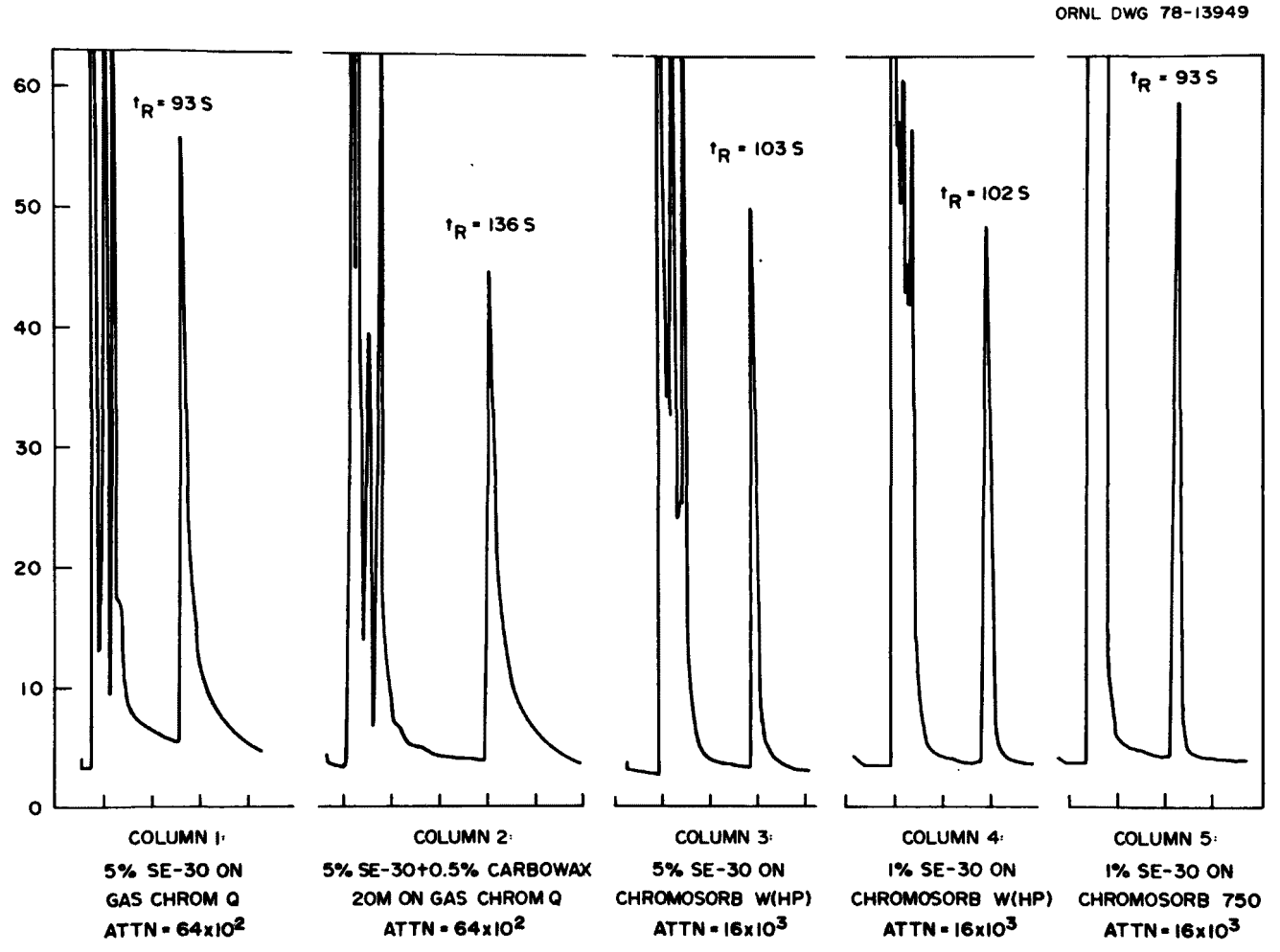

Figure 3. Separation of TMS-SO_4 on Various GC Columns

A 0.5% Carbowax 20M coating was used on Column 2 to cover any active sites present on Gas Chrom Q, prior to loading the support with 5% SE-30. This procedure has frequently been used to reduce peak tailing and to increase the inertness of a column. Peak broadening at low TMS-SO_4 concentration was not as severe on this column, although the retention time increased by as much as 50 seconds for dilute samples. Tailing of the sulfate peak was more severe than without the Carbowax coating. It was still necessary to "load" the column before elution of the anion could be observed. The detection limit was not improved with the 0.5% Carbowax 20M coating.

Chromosorb W(HP) was used as the support in column 3. Tailing of the sulfate peak was reduced and the sensitivity of the analysis was markedly improved. The column exhibited fewer "loading" effects and the detection limit was somewhat better (0.8 μg sulfate).

A 1.0% SE-30 loading was used on Chromosorb W(HP) to determine the effect of the liquid phase. The tailing factor improved to 80 and the retention time of the lower concentrated samples differed by only a few seconds from that of the more concentrated TMS-SO_4 standards. The column still required "loading" if a sample containing less than 0.5 μg SO_4 was to be analyzed.

The final column support investigated was Chromosorb 750, which has recently been placed on the market. The support is reportedly more inert than Chromosorb W(HP). No peak tailing was observed as indicated by a tailing factor of 100 (Table 1), and retention times were identical regardless of sample concentration. The sensitivity is the greatest of all columns investigated and the calibration curve is the most linear, whether based on peak area or peak height.

In summary, these results suggest that the percent loading of the liquid phase is the primary factor affecting detection limit, peak tailing and linearity of the calibration curve. Minimizing the amount of SE-30 will reduce the TMS-SO_4 which is adsorbed or decomposed on the gc column. Reducing the reactivity of the solid supports appears to increase the relative sensitivity of the analysis. Under optimum conditions, a detection limit of 0.2 μg sulfate in aqueous solution can be achieved using 1% SE-30 on Chromosorb 750. This detection limit will have to be reduced by at least a factor of 10 if gc analysis of sulfate is to be conveniently used for the analysis of environmental samples. Further assessment of this procedure should include the investigation of columns prepared with a few-tenths percent of SE-30, as well as other silicon oils, on silylated glass beads or in a glass capillary column. Using Chromosorb 750 with a larger mesh size may reduce the reactivity of the gc column further without adversely affecting the gc separation of TMS-SO_4.

SECTION 5

A SPECTROKINETIC PROCEDURE FOR SULFATE ANALYSIS

PREVIOUS INVESTIGATIONS

Hems, et al. (7) introduced a direct kinetic method for sulfate determination based on the reactions presented in Figure 4. Sulfate increases the depolymerization of the aged zirconyl species in acidic media, and the rate of formation of the free zirconium ion-methylthymol blue chromophore is proportional to sulfate concentration. Hems followed the reaction of macro-volumes of sample at 586 nm and related the absorbance at a single 60 minute observation interval to sulfate concentration.

This procedure has been modified to provide a true kinetic analysis for use with a miniature centrifugal fast analyzer. Such a technique permits the relatively rapid, semi-automated analysis of micro volumes of samples and standards, all of which can be analyzed simultaneously. Sub-ppm detection limits are noted and, with appropriate sample pretreatment, can be made relatively specific for sulfate ion.

REAGENTS AND INSTRUMENTATION

Stock methy thynol blue (MTB) is prepared by dissolving 0.0378 g MTB in 100 ml water. Although the basic form of this indicator is relatively unstable, we found that this reagent is stable indefinitely if it is acidified with one drop of 1M HCl. The working MTB solution is prepared by adding 1 ml of 6M HCl to 3.45 ml of the stock MTB solution and diluting the mixture to 5 ml with water.

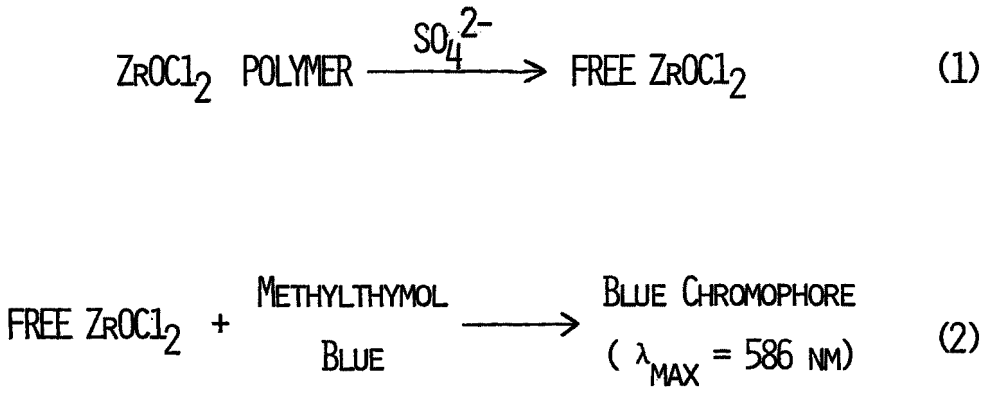

Figure 4. ZR-MTB Reaction

The zirconium polymer (ZR) is formed by dissolving 0.0805 g $ZrOCl_2$ (A. D. Mackay) in 25 ml of 0.0125 M HCl. Two days are required to adequately polymerize the zirconium. This stock solution may then be used for one week. Fresh working zirconium polymer is prepared daily by diluting 1 ml of the stock solution to 10 ml with 0.05M HCl.

The instrumentation of the miniature fast analyzer and the automatic rotor loading station are described elsewhere (22, 23).

EXPERIMENTAL PROCEDURE AND DATA ANALYSIS

A 17-place multicuvet rotor is used to simultaneously analyze a combination of 16 sulfate standards and samples and a water blank. A two-step procedure is used to load the ZR polymer, MTB, and sulfate sample into the rotor. The ORNL Sample-Reagent Loader is used to preload 20 µl of the working ZR solution (+ 25 µl water) into one of the two reagent wells associated with each cuvet. The ZR polymer is then transferred to each of the 16 cuvets by placing the rotor on the Analyzer, accelerating it to 4000 rpm and allowing the rotor to coast to rest. The rotor is then carefully replaced on the Loader and 20 µl of sulfate standard or sample (+ 25 µl water) and 20 µl MTB working solution (+ 25 µl water) are loaded into the two reagent wells. This two step loading scheme prevents premixing of the ZR polymer with either the sulfate or the MTB prior to reaction initiation.

The rotor and its contents are subsequently placed on the Analyzer and brought to 30.0 $\pm$ 0.2°C by radiant heating with an infrared lamp.

The kinetic reaction is initiated when the sulfate and MTB are transferred into the cuvets containing the ZR by rapidly accelerating the rotor to 4000 rpm. The three reaction components are then thoroughly mixed by instantly braking the rotor. The rotor speed is then adjusted from rest to 1000 rpm for the remainder of the reaction.

After a 100-s delay period, 30 consecutive absorbance measurements are taken at 30-s intervals. Each measurement represents the average of 25 consecutive passes of the appropriate cuvet past the stationary phototube. Figure 5 shows the oscilliscope trace of the optical transmission of each cuvet at one such observation interval. The percent transmission (and thus absorbance) is reproducible within replicates of the standards and samples. However, contrary to Hems' report, the absorbance measurement at a single reaction time was not found to be linear with sulfate concentration. Sequential absorbance measurements are displayed in Figure 6 for five sulfate standards. After an approximately 4-minute induction period, the absorbance change for each standard is linear with time and proportional to sulfate ion concentration.

The kinetic data for all 17 cuvets are stored on a laboratory computer. A computer routine (Figure 7) has been written to retrieve the data from computer memory, locate the linear portion of the reaction data for each cuvet, and calculate the reaction rate. The linear least squares regression analysis subroutine locates the linear portion of the data by computing the rate and linearity criterion (correlation coefficient) for the first 6 data intervals of a given cuvet. The correlation coefficient computed is compared to a "minimum acceptable

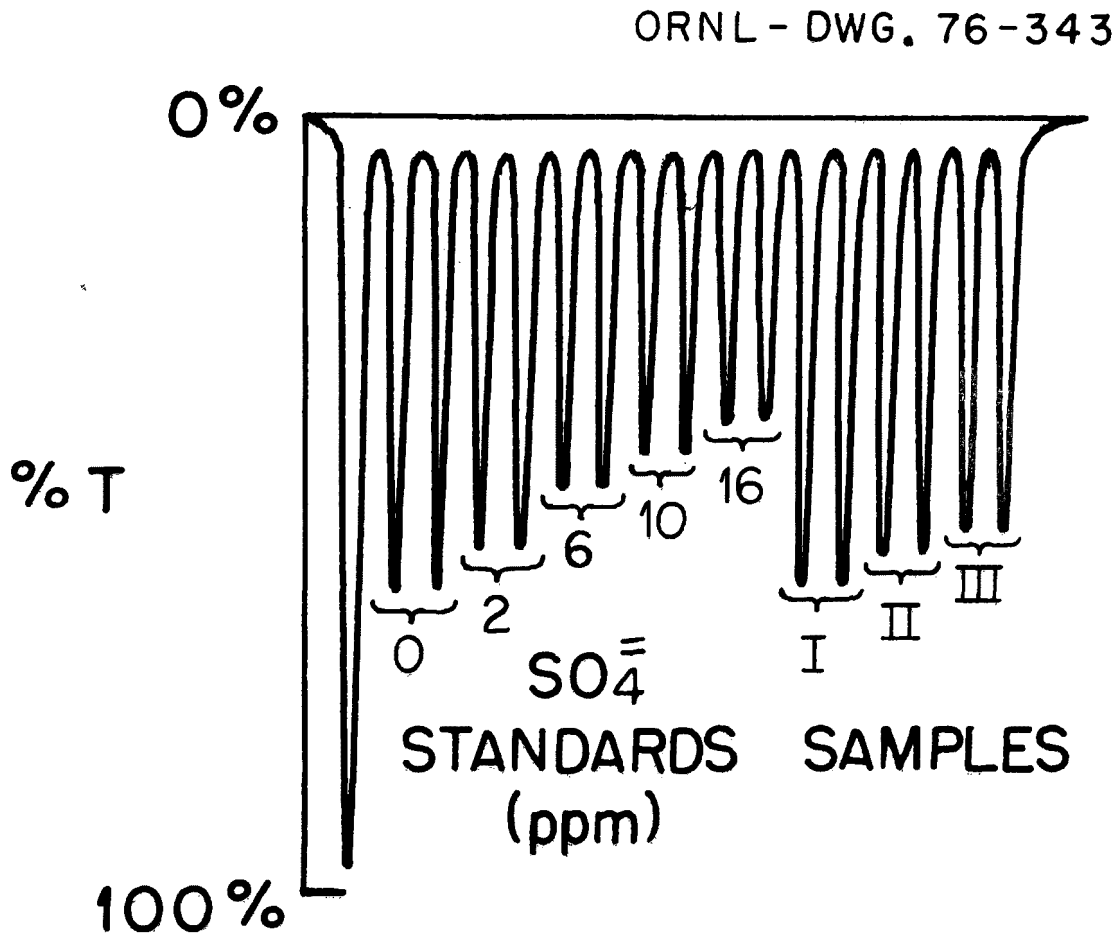

Figure 5. Optical Transmission of Sulfate Standards and Samples in Multicuvet Rotor

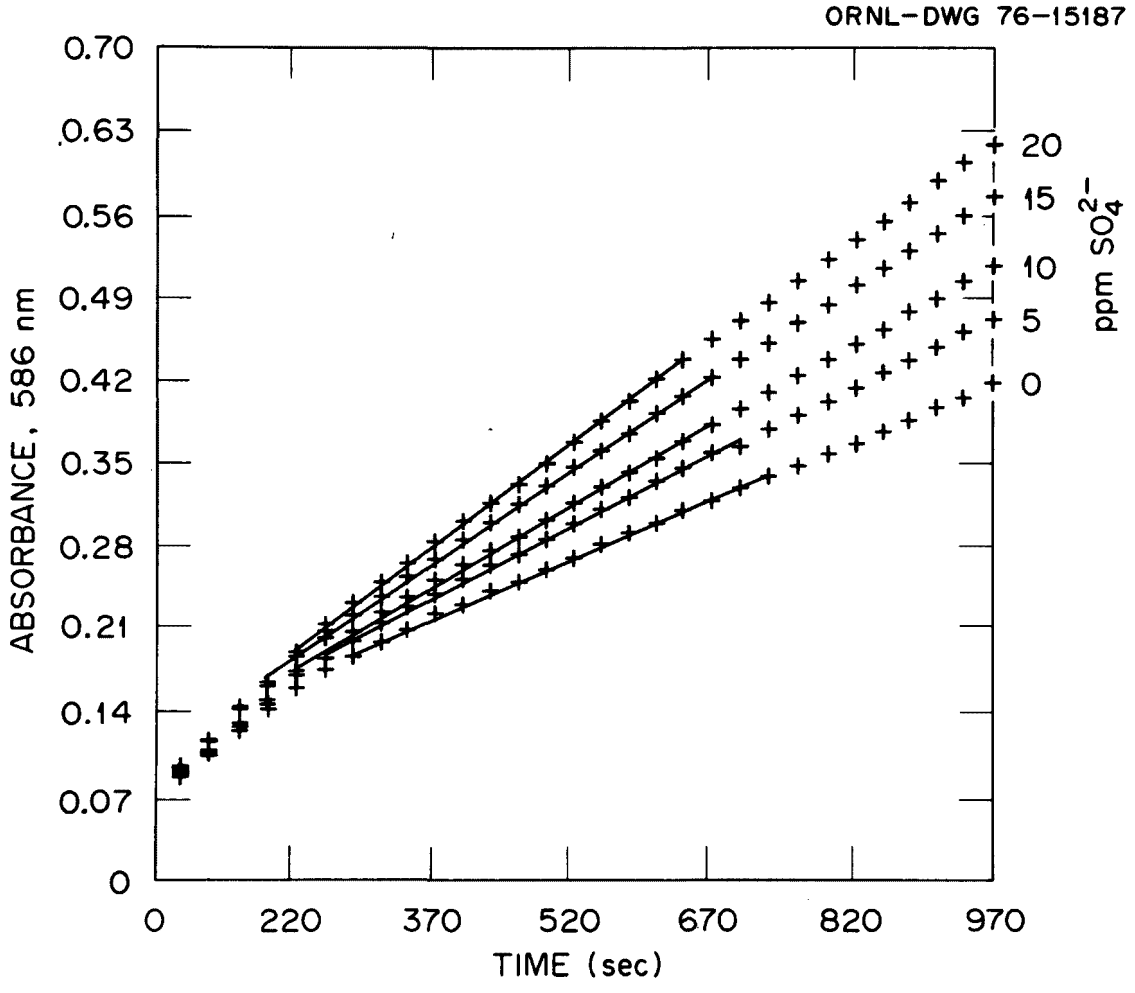

Figure 6. Reaction Progress of Sulfate Ion Standards

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01.02 E
01.03 A ! "NUMBER OF SETS OF OBSERVATIONS",NI
01.04 A ! "DELAY INTERVAL",DI
01.05 A ! "OBSERVATION INTERVAL",SE
01.06 A ! "RUN NUMBER",RN
01.07 A ! "GEMSAEC UNIT",UN
01.08 A ! "WHICH CUVET CONTAINS BLANK?",RC
01.09 A ! "MINIMUM ACCEPTABLE CORRELATION COEFF.?",CX
01.10 A ! "NUMBER OF CUVETS UNDER CONSIDERATION",NK,!

02.01 S ND=FITR(NI/2)
02.07 T !,"CUVET     INTERVAL (SEC)            SLOPE  (A/MIN)        CC"
02.10 S NC=17; S ST=(FITR((UN-NC)*10+0.5)-1)*50

03.01 F J=RC+1,1,NK; S I=1; S M=0; D 10
03.99 QUIT

07.10 S S3=(NC+1)*(N-1);S S1=FGET(S3+1);S S2=FGET(S3+2)-S1
07.30 S SN=FGET(S3+J+1)-S1
07.40 I (-SN)7.5;S AB(J)=9999;R
07.50 S AB(J)=.4343*FLOG(S2/(SN*FSTR(ST+J)))
07.60 I (1-RC)7.7,7.99
07.70 S SB=FGET(S3+RC+1)-S1
07.75 S AD=.4343*FLOG(S2/(SB*FSTR(ST+RC)))
07.80 S AB(J)=AB(J)-AD
07.99 R

10.01 S SX=0; S SY=0; S XY=0; S X2=0; S Y2=0
10.05 S H=I+ND-1
10.06 F N=I,1,H; D 7; D 11
10.07 S M=M+1
10.10 S MM(M)=60*FABS(XY-SX*SY/(ND))/(X2-SX*SX/(ND))
10.15 S CO(M)=FABS((ND)*XY-SX*SY)/FSQT(((ND)*X2-SX*SX)*((ND)*Y2-SY*SY))
10.20 S I=I+1
10.30 I (((I+ND)-NI)10.01,10.01; D 12
10.40 R

11.05 S SY=SY+AB(J)
11.10 S X=DI+( (N-1)*SE)
11.13 S SX=SX+X
11.15 S XY=XY+X*AB(J)
11.17 S X2=X2+X*X
11.19 S Y2=Y2+AB(J)*AB(J)
11.30 R

12.01 S MAX=MM(1);S  CC=CO(1);S  IN=1
12.05 F M=2,1,(NI-1); D 13
12.08 S TO=(IN-1 )*SE+DI; S TF=TO+(ND-1)*SE
12.30 I (CC-CX)12.35,12.5,12.5
12.35 I ((NI-1)-IN)12.4,12.45
12.40 S MM(IN)=0; G 12.01
12.45 T !,%4.0,J," DATA DO NOT MEET LINEARITY CRITERION"; R
12.50 T !,%4.0,J,"  ",TO," - ",TF,%20.06,MAX,CC;R

13.01 I (MAX-MM(M)) 13.1,13.1,13.2
13.10 S MAX=MM(M); S CC=CO(M); S IN=M
13.20 R
*
```

Figure 7. Linear Search Computer Subroutine

correlation coefficient" selected by the operator. If the computed value is less than this criterion, the slope is set to zero; otherwise, the computed value is retained. The interval used, slope, and correlation coefficient are stored in computer memory. The process is repeated for intervals 2-18, 3-19, ..., 14-30. The list of computed slopes is searched for the maximum rate, and the result is presented on a teletype. If none of the intervals meet the linearity criterion selected by the operator, the teletype flags the result by typing "DATA DO NOT MEET LINEARITY CRITERION." The computer program then proceeds to calculate the reaction rate of the next cuvet.

The format of the program output is illustrated in Figure 8. The value of the minimum acceptable correlation coefficient is typically selected between the values 0.9990 to 0.9995; this permits discrimination from the initial rapid, but relatively nonlinear, reaction "induction" phase (see Figure 6). This initial sulfate-independent reaction phase is presumably due to the presence of residual free Zr in the polymeric reagent, and its duration is a function of the age (hence extent of polymerization) of this reagent. For a given polymeric reagent, it is found that the initiation of the sulfate-dependent reaction phase occurs at shorter time for higher sulfate concentration in the sample. The interval selected by the linear search program for compution of reaction rate is indicated in Figure 6 by the joined data points, and illustrates the versatility of the linear search subroutine. By its use, a single set of observation conditions may be used for a wide range of sulfate ion concentration and degree of zirconyl polymerization in the reagent, since the computer automatically selects the data interval used for computation of sulfate-dependent rate.

ORNL-DWG. 76-14949

RUN NUMBER: 3.
NUMBER OF CUVETS UNDER CONSIDERATION: 17.
GEMSAEC UNIT: 17.2.
DELAY INTERVAL: 100.
OBSERVATION INTERVAL: 30.
NUMBER OF SETS OF OBSERVATIONS: 30.
MINIMUM ACCEPTABLE CORRELATION COEFFICIENT: 0.9993.

CUVET	INTERVAL (SEC)	SO_4^{2-} Conc.	RATE (A/MIN)	CC
2	280 - 730	0 ppm	0.020339	0.999620
3	280 - 730		0.020260	0.999530
4	250 - 700	5 ppm	0.024149	0.999428
5	250 - 700		0.024459	0.999391
6	220 - 670	10 ppm	0.028198	0.999355
7	220 - 670		0.028117	0.999367
8	220 - 670	15 ppm	0.031905	0.999488
9	220 - 670		0.032066	0.999407
10	190 - 640	20 ppm	0.036377	0.999476
11	190 - 640		0.036486	0.999581
12	250 - 700	Sample #1	0.024886	0.999544
13	250 - 700		0.024344	0.999585
14	250 - 700		0.024938	0.999488
15	220 - 670	Sample #2	0.038488	0.999478
16	220 - 670		0.038599	0.999571
17	220 - 670		0.037926	0.999471

Figure 8. Data Output Format for Linear Search Computer Subroutine

The results of the computer output are plotted in Figure 9. The method is linear over a range of 0-20 ppm (0-400 ng) sulfate. The slope of the calibration curve is 0.010 absorbance units/min/ppm sulfate. The detection limit, equivalent to twice the standard deviation of the lowest standard divided by the slope, is 0.3 ppm sulfate.

OPTIMIZATION OF REACTION CONDITIONS

The reaction concentration of the Zr polymer was found to affect both the linearity and sensitivity of the sulfate calibration curve. At a zirconium reaction concentration of 1.1×10^{-4} M, the reaction blank exhibits little change in absorbance (Figure 10). As the zirconium reaction concentration increases, the reaction rate of the reagent blank becomes significant and the sensitivity of the sulfate calibration curve increases. Above 1.8×10^{-4} M zirconium, the calibration curve is no longer linear. A reaction concentration of 1.5×10^{-4} M zirconium is presently used for sulfate analysis.

The age of the stock ZR solution also affects the sensitivity of the analysis. As the zirconium ages, it becomes more polymerized and subsequently longer times are required to form free zirconium during the reaction. The sensitivity decreases by approximately 30% as the stock ZR solution ages over a period of a week. The working Zr polymer exhibits no significant influence on sensitivity as it ages during the day.

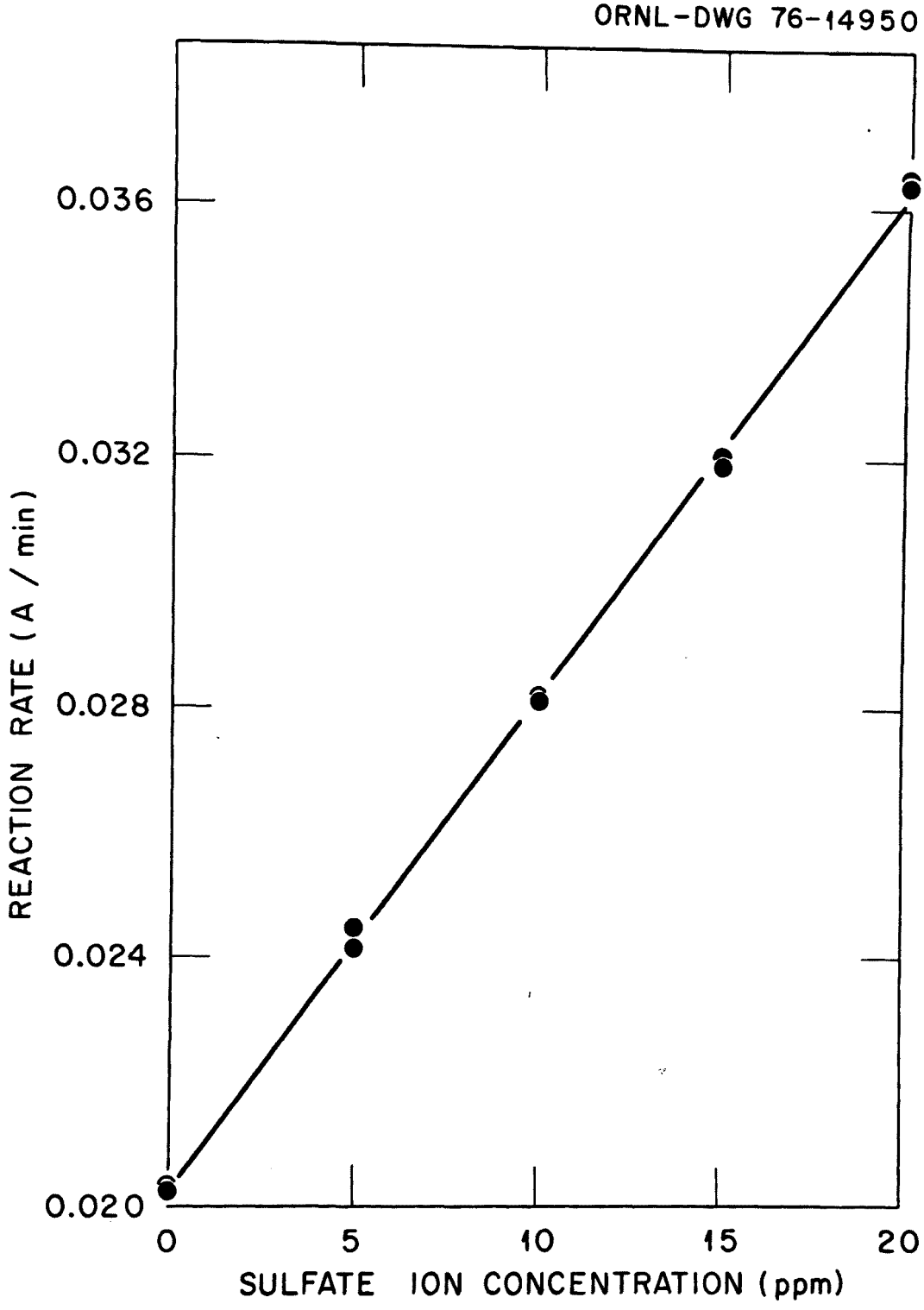

Figure 9. Sulfate Ion Calibration Curve

ORNL DWG. 75-8717

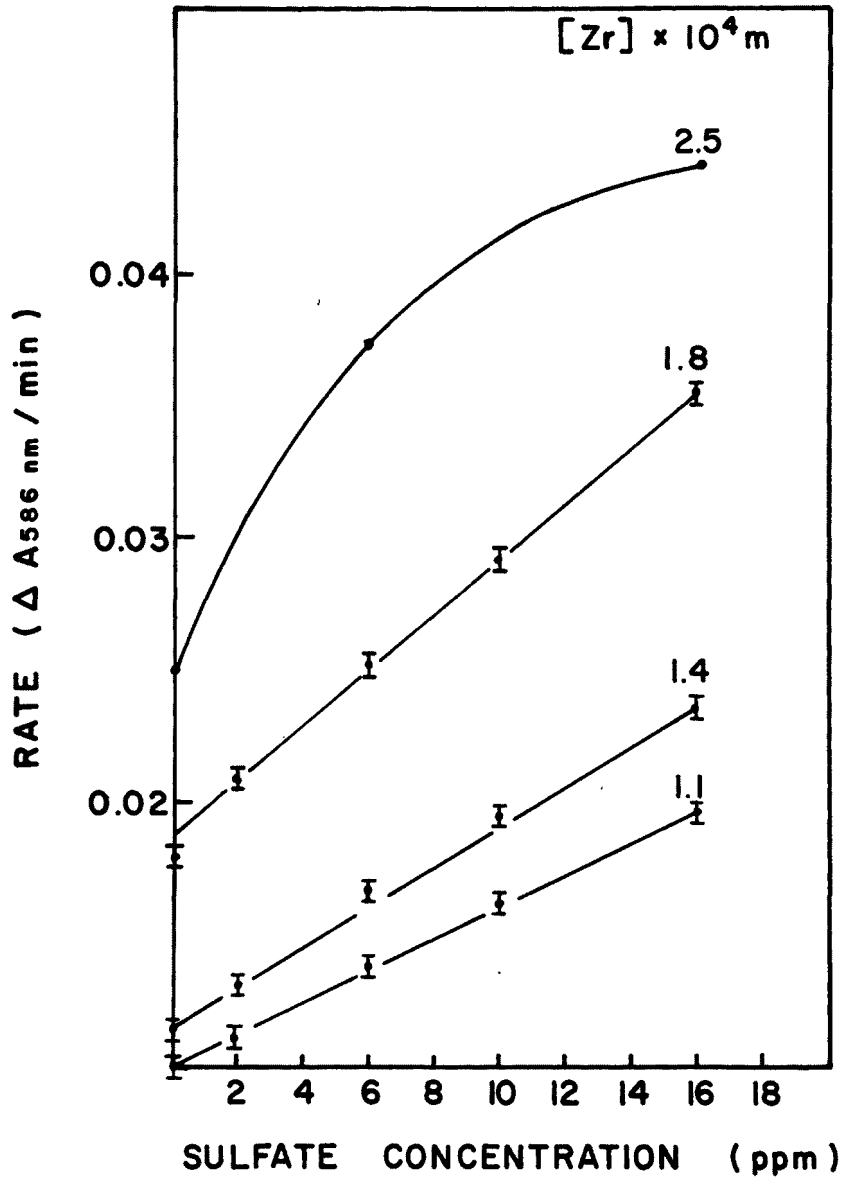

Figure 10. Effect of ZR Polymer Concentration on Sulfate Ion Reaction Rate

The concentration of HCl in the zirconium reagent and in the final zirconium-methylthymol blue (Zr-MTB) reaction mixture determines the degree of polymerization of the zirconyl species. Consequently, the reaction acidity also affects the sensitivity and linearity of the kinetic data for the sulfate analysis. Optimum acid concentration was determined for the 0.05-0.36 M HCl concentration range. The sensitivit of the Zr-MTB procedure approaches zero outside the 0.1-0.3 M HCl range. At low HCl concentration the absorbance of the reagent blank increases, as do both the reaction rate and sensitivity of the analysis. The reaction rate of a given sample is more linear for higher HCl concentrations. An optimum concentation of 0.2 M HCl was selected to obtain a modest sensitivity with a minimal reaction time and an adequate reproducibility for the reaction rate of a given sample.

The sensitivity of the reaction was also studied in the presence of several organic solvents. Previous techniques using methylthymol blue dye as an indicator have employed the addition of organics to increase the absorption coefficient of MTB, thereby improving the sensitivity of the procedure. We examined the effect of formaldehyde, ethanol and methanol on the kinetic procedure for sulfate ion determination. Formaldehyde was selected because it is a common masking agent for sulfite, and therefore, its presence might also make the Zr-MTB method selective for sulfate. However, calibration curves prepared in the presence of 5% formaldehyde were not proportional to sulfate concentration. This response remained non-linear even when the MTB concentration was varied. Ethanol and methanol are the most common

organic solvents used to improve sensitivity. Reaction concentrations of 5-50% of these solvents served only to decrease the sensitivity of the Zr-MTB method, although the calibration curves remained proportional to sulfate concentration. Presently, a reaction concentration of 0.52 M MTB is used in the absence of any organic solvents.

REACTION INTERFERENCES

Table II summarizes the major cation and anion interferences in the Zr-MTB reaction reported by Hems, et al. They proposed the use of Amberlite IR-120 (H) for the removal of cation interferents. Because acidity influences the sensitivity of the Zr-MTB reaction, the sodium form of the resin is presently used. Batch-wise ion exchange with approximately 200 mg resin was found to remove cation interferences from 2 ml of sulfate sample. Results from a study comparing sulfate determination in the presence and absence of ionic interferences are presented in Table III. Sulfate analysis using the Zr-MTB procedure was compared with results obtained using a Technicon Autoanalyzer. The Autoanalyzer system removes cation interferences with a flow-through cation exchange column and indirectly determines sulfate concentration colorimetrically by reacting excess barium with methylthymol blue. The untreated samples in Table III all exhibit a positive bias compared to the analysis performed with the Autoanalyzer. Samples in which cations have been removed and analyzed with the Zr-MTB procedure are in much closer agreement with those results obtained with the Autoanalyzer. Recovery of added sulfate in these treated samples averaged 102% using the kinetic procedure.

TABLE 2

INTERFERING IONS AND METHODS FOR THEIR REMOVAL

Cations	Removal
Bi, Ce, Fe, Mn, Sb, Se, Sn, Sr, Te, Th, V	cation-exchange on Amberlite IR-120 (Na)
Anions	
F^-	mask with 30 ppm Al^{+3}
S^{2-}	centrifuge with 30 ppm Hg^{2+}
PO_4^{3-}, AsO_4^{3-}	adsorb with MgO
SO_3^{2-}	
Oxalate, tartrate	

TABLE 3

COMPARISON OF TREATED AND UNTREATED SAMPLES

Sample	Zr-MTB Procedure			Technicon Autoanalyzer
	Untreated	Cations Removed	Cations & Anions Removed	
1	8.9	8.1	7.7	10.4
2	4.7	2.9	2.7	2.8
3	5.2	3.0	2.9	2.8
4	9.3	7.3	7.6	6.7

Anions, including fluoride, phosphate, and arsenate, interfere in Hems' method at even trace levels. A 1 ppm concentration of fluoride or arsenate has been found to interfere in the kinetic Zr-MTB procedure by producing reaction rates comparable to 15 and 2 ppm sulfate, respectively. Phosphate varies in its effect from 0-9 ppm sulfate, depending on the age of the zirconyl polymer. Hems, et al. suggested the use of magnesium oxide (24, 25) to remove the above anions from sulfate samples. We have investigated several masking agents which might be added to the reaction mixture to remove the anions and thus eliminate lengthy pretreatment of samples. Boric acid, La(III), or Al(III) were added to sulfate samples in an attempt to mask up to 2 ppm concentrations of the anions. Neither boric acid nor La(III) decreases the interference of any of the three anions. However, 2 ppm fluoride can be masked if at least 30 ppm Al(III) is present in the sample. Because this quantity of Al(III) slightly increases the reaction rate of sulfate, a similar concentration of the masking agent should be added to standards for accurate sulfate analysis.

Sulfite and sulfide also produce a positive interference in the ZR-MTB procedure. The effect of these two anions must be removed if the procedure is to be selective for sulfate anion. The interference from sulfite and sulfide is not due to these anions per se, but was found to be the result of anion auto-oxidation to sulfate prior to sample analysis. Mercuric ion was used to stabilize the anions. A 30 ppm concentration of the cation was added to samples containing 5 ppm sulfide and 10 ppm sulfate. The presence of the resulting HgS precipitate and color formation did not interfere in the kinetic analys

of sulfate ion in the samples, since the precipitate is centrifuged out of the light path during the induction period of the reaction. Mercuric ion at this concentration level is sufficient to remove sulfite interference if the samples are analyzed within 1-2 days after the masking agent is added. If samples are to be stored, the HgS precipitate should be removed by centrifugation before sample storage to prevent the masked sulfide from slowly oxidizing during the elapsed time.

Sulfite anion was not stabilized in the presence of 30 ppm Hg(II), even for a relatively short period of time. A thousand-fold increase in mercuric ion concentration is used to prevent the oxidation of sulfite in the West-Gaeke sulfite technique (18). However, mercuric ion concentrations of this magnitude would interfere with the Zr-MTB procedure.

Table III also includes the analysis of the four samples in which cations were removed by batch ion exchange and fluoride and sulfide interferences were removed by adding 30 ppm each of Al(III) and Hg(II) to the samples. The results are generally somewhat lower than when just the cations are removed. However, the analysis indicates that the major interference in the water samples are primarily due to the presence of cations. There is a discrepancy between results obtained for sample #1 using the Zr-MTB and Autoanalyzer procedures. The sample was highly colored with organic material. Because the Autoanalyzer used a single absorbance measurement to determine sulfate concentration, any absorbing species will also interfere with the analysis. However,

since the kinetic Zr-MTB procedure is based on a change in absorbance, the presence of a constantly absorbing background does not interfere with the determination. This represents a significant improvement over Hems' single measurement method.

PRECISION AND ACCURACY OF RESULTS

The precision and accuracy of the Zr-MTB procedure was evaluated by analyzing rain water samples and comparing the results with those obtained with the Technicon Autoanalyzer. A within-run precision of better than 2%, as demonstrated in Figure 8, can be achieved with the present method if the reagents are reproducibly loaded into the rotor. The major source of error appears to be in the loading of the MTB, which acts as a wetting agent and forms beads of the reagent on the outside of the pipet of the loader. This beading is minimized if the pipet exterior is cleaned with methanol prior to loading the rotor.

Run-to-run reproducibility was demonstrated by analyzing a series of standards and samples in five runs made in a single day. Results of the five runs are given in Table IV. The standard deviations observed, both within-run and run-to-run, are typically lower than the detection limit of the method (0.3 ppm SO_4^{2-}).

The same four samples were analyzed on four separate days to determine day-to-day reproducibility in sample analysis. Results for this study are given in Table V. Results for a given day were calculated from the average of 3-5 runs, performed on that day. Again, the standard deviations for day-to-day sample analysis do not exceed the detection limit of the Zr-MTB procedure.

TABLE 4

RUN-TO-RUN REPRODUCIBILITY IN SAMPLE ANALYSIS

Sample #	Run 1	Run 2	Run 3	Run 4	Run 5	Average
1	2.9 ± 0.1	2.6 ± 0.4	2.8 ± 0.1	2.3 ± 0.1	2.2	2.6 ± 0.3
2	3.1 ± 0.1	3.0 ± 0.3	3.1 ± 0.1	2.7 ± 0.1	3.6 ± 0.0	3.1 ± 0.3
3	5.3 ± 0.1	5.3	5.3 ± 0.3	5.1	5.5 ± 0.3	5.3 ± 0.1
4	20.9 ± 0.0	21.0 ± 0.2	20.1 ± 0.2	21.4 ± 0.2	21.3 ± 0.4	29.9 ± 0.5

TABLE 5

DAY-TO-DAY REPRODUCIBILITY IN SAMPLE ANALYSIS

	Sample #			
Day #	1	2	3	4
1	2.7 ± 0.3	2.9 ± 0.2	5.6 ± 0.1	21.1 ± 0.2
2	2.9 ± 0.7	3.1 ± 0.3	5.4 ± 0.3	21.4 ± 1.3
3	2.6 ± 0.3	3.1 ± 0.3	5.3 ± 0.2	20.9 ± 0.5
4	3.2 ± 0.4	3.4 ± 0.3	5.5 ± 0.3	21.3 ± 0.4
Average	2.8 ± 0.3	3.2 ± 0.2	5.4 ± 0.1	21.2 ± 0.2

The accuracy of the method was estimated by comparing results obtained by the Zr-MTB procedure and the Technicon Autoanalyzer. Ten rain water samples and a double-blind control sample were analyzed in the study. The samples were analyzed independently by the Environmental Analysis Laboratory, Analytical Chemistry Division, ORNL, using the Technicon system. Table VI lists the results of the correspondence study. Except for sample #10, results from the two techniques are in close agreement. A linear regression analysis, based on all sample data, calculated a slope of 1.01 $\pm$ 0.02, a y intercept of -0.15 $\pm$ 0.5 ppm and a correlation coefficient of 0.998. The standard error of the estimate was equivalent to the detection limit. The results of the two methods agreed within a confidence limit greater than 99.5% for 9 degrees of freedom and students' T = 46.

TABLE 6

CORRESPONDENCE OF THE ZR-MTB WITH A REFERENCE METHOD FOR THE DETERMINATION OF SULFATE ION

Sample Number	SO_4^{2-} Found, ppm: Technicon Autoanalyzer (X)	SO_4^{2-} Found, ppm: CFA Kinetic (Y)
1	2.8	2.8
2	2.8	3.1
3	21.0	21.2
4	2.8	3.1
5	1.4	1.6
6	3.5	3.3
7	4.0	4.2
8	2.5	2.2
9	1.4	1.0
10	3.5	2.7
11	6.3	5.8

Linear Least Squares Regression Analysis:

Slope = 1.01 $\pm$ 0.02
Y intercept = -0.15 $\pm$ 0.47 ppm
Correlation coefficient = 0.9979
Std error of estimate = 0.35 ppm
Student's t = 46

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15. SUPPLEMENTARY NOTES

16. ABSTRACT

Several analytical methods for possible use in measuring trace amounts of water soluble sulfate anions were reviewed and evaluated. Enzymatic sulfate determination does not appear to be a viable approach until the required enzymes can be obtained commercially. Gas chromatographic analysis of bis(trimethylsilyl)sulfate was studied and, with further development, may be a selective method for the determination of sulfate as well as other oxy-anions. A direct kinetic method, based on the ability of sulfate to catalyze the depolymerization of zirconyl species, was investigated. The method can detect 0.2-20 ppm sulfate and, with a minimum of sample preparation, is relatively selective.

17. KEY WORDS AND DOCUMENT ANALYSIS

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