

**Figure 2.** Logarithmic extrapolation plot for the same mixture as in Figure 1

Optical path length is different from that in Figure 1

$$\ln \{[\text{NO}]_0/[\text{NO}]_t\} = \ln R_{\text{NO}} = k_{\text{NO}}(t - t_0) \quad (3)$$

and

$$\ln \{[\text{NO}_2]_0/[\text{NO}_2]_t\} = \ln R_{\text{NO}_2} = k_{\text{NO}_2}(t - t_0) \quad (4)$$

it is found that at  $t = 1$  sec,  $R_{\text{NO}} \sim 20$  and  $R_{\text{NO}_2} \sim 1.01$ , while at  $t = 2$  sec, corresponding values are 400 and 1.02, respectively. Consequently, if for an equimolar mixture of NO and NO<sub>2</sub> the absorbance at  $t = 1$  sec is taken as a measure of  $[\text{NO}]_0$ , an error of -4% would result, and at  $t = 2$  sec, the error would be +2%. For  $[\text{NO}]_0/[\text{NO}_2]_0$  ratios other than unity, the errors naturally would be different.

Typical results obtained in the stopped-flow determination of NO in NO-NO<sub>2</sub> mixtures are listed in Table II; concentrations of NO<sub>2</sub> were obtained by difference from the limiting absorbance which was closely approached at  $t = 4$ -5 min. The same mixtures were also analyzed by the "logarithmic extrapolation" method (12), using a recording spectrophotometer. In this case, the concentration of NO<sub>2</sub> was determined and that of NO was obtained by difference. At a time  $t$ , after virtually all NO has reacted, it is readily shown that in the presence of a large excess of Fe(II)

$$-\ln(A_\infty - A_t) = k_{\text{NO}_2}t - \ln A_\infty \quad (5)$$

so that a plot of  $-\ln(A_\infty - A_t)$  vs.  $t$  should be linear with a slope  $= k_{\text{NO}_2}$  and an intercept  $= -\ln A_\infty$ , where  $A_\infty$  refers to NO<sub>2</sub> alone. A typical plot is given in Figure 2 and the results are also entered in Table II. Agreement with the stopped-flow results is satisfactory.

The main limitation of the methods described here is that their sensitivity is only moderate (near  $10^{-5}M$ , depending on the sensitivity of the photometer used). In their present form, these methods appear to have potentialities for the analysis of "grab" samples collected at NO<sub>x</sub> emission sources. Further work would be required to test sampling procedures for ambient air.

Received for review February 22, 1973. Accepted May 4, 1973. We thank the National Science Foundation for financial support under Grant GP-16342 as well as Grant GU-3184 under the University Science Development program.

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## Fast Analytical Procedure for the Separation and Determination of the Polythionates Found in Wackenroder's Solution by High Speed Liquid Chromatography

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Since the 1920's, there has been interest in determining polythionates in solution. The reaction of H<sub>2</sub>S with SO<sub>2</sub> in solution yields Wackenroder's solution, which contains S<sub>2</sub>O<sub>3</sub><sup>2-</sup>, S<sub>3</sub>O<sub>6</sub><sup>2-</sup>, S<sub>4</sub>O<sub>6</sub><sup>2-</sup>, and S<sub>5</sub>O<sub>6</sub><sup>2-</sup> but no other polythionates or S<sub>2</sub>O<sub>6</sub><sup>2-</sup> (1).

Many methods have been developed for determining polythionates contained in this mixture, but there is no satisfactory way of separating and quantifying them that is not lengthy and time-consuming (2). Because the time involved in these separations is long, the stability of the polythionates from the beginning to the end of analysis is in

doubt. Karchmer (3) writes, "There appear to be no satisfactory methods for the determination of individual polythionates in the presence of each other."

The method described in this paper will separate and quantify the above named polythionates in 15 minutes. The method depends on the adsorption and elution from activated carbon by high-speed liquid chromatography. The factors governing the separation are described.

### EXPERIMENTAL

**Apparatus.** All development and analytical work was performed on the Waters ALC-100 Analytical Liquid Chromato-

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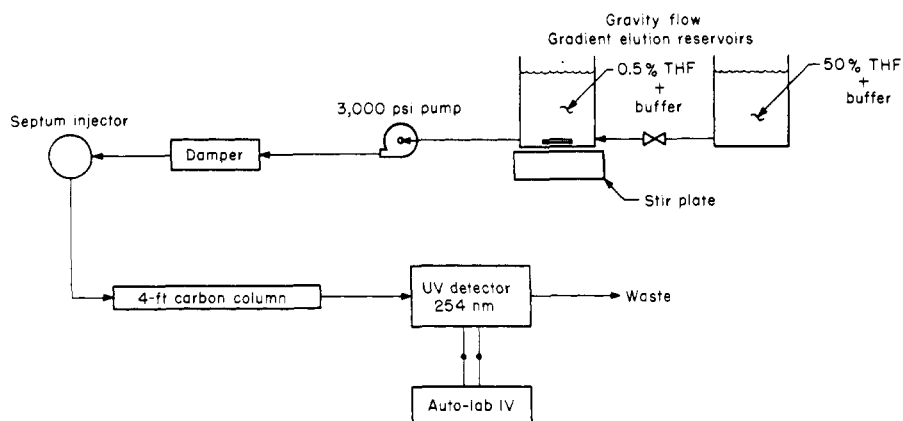


Figure 1. Block diagram of liquid chromatographic flow system

graph. Detection of eluate samples was monitored by the Waters Model 77 UV detection module, which emits a voltage signal proportional to the absorbance of the sample at 254 nm. The Autolab System IV Digital Peak Analyzer was used to quantify peak areas. Solvent gradients were generated in a 50-ml Glenco Gradient Elution Apparatus, a simple gravity flow device. A stainless steel column 0.085 × 48 inches packed with 200 to 270 mesh Darco Red Label activated carbon was selected for compound separation. System pressures exceeding 2000 psi required the use of a Milton-Roy pump, Waters Part No. 98750, rated to 3000 psi. A flow diagram of the above system is shown in Figure 1. The polythionate salts were checked for purity on a Coleman Model 124 D Double Beam Spectrophotometer.

**Reagents.** *Solution A.* A mild buffer passing UV radiation at 254 nm consists of 11 grams per liter of  $\text{Na}_2\text{HPO}_4(\text{anh})$ , 0.5 volume-per cent analytical grade tetrahydrofuran (THF) adjusted to pH  $10.0 \pm 0.1$ . This was used as the primary phase.

*Solution B.* A 50 volume-per cent THF in primary phase buffer was used as the secondary gradient phase. This phase was mixed just prior to use to avoid salting out  $\text{Na}_2\text{HPO}_4$ .

Solution A was stoppered at all times to avoid any evaporation and checked daily for pH.

Five-gram-per-liter samples of  $\text{Na}_2\text{S}_2\text{O}_3$ ,  $\text{K}_2\text{S}_3\text{O}_6$ ,  $\text{K}_2\text{S}_4\text{O}_6$ , and  $\text{K}_2\text{S}_5\text{O}_6 \cdot 1\frac{1}{2} \text{H}_2\text{O}$  were dissolved in  $\text{H}_2\text{O}$ . Aliquots of each were drawn into the syringe just before injection to minimize chemical interactions. Samples were replenished daily as a precaution against unobservable decomposition of the polythionates. The three potassium salts of the polythionates that were synthesized (4, 5) at this station were checked for purity on a UV spectrophotometer according to a previously published method (6). The  $\text{K}_2\text{S}_3\text{O}_6$  and  $\text{K}_2\text{S}_4\text{O}_6$  used were 100% pure; the  $\text{K}_2\text{S}_5\text{O}_6 \cdot 1\frac{1}{2} \text{H}_2\text{O}$  was 78% pure. No UV absorbing material was contained in the impurity of the pentathionate compound.

**Procedure.** The column was dry-packed by a recommended procedure (7). Fine particle removal was necessary to eliminate plugging and unnecessary back pressure in the system. Elimination of adsorbed air from the freshly packed carbon took place by solvent purge for 30 minutes before connection to the flow circuit. The UV detector was set at 0.08 absorbance full scale with an attenuator setting of 1 on the digital peak analyzer. Sample peaks were referenced against an air stream. Matching of background solvents during gradient elution was impossible and precluded the use of a solvent reference. The flow rate was set at 1.62 ml/min and maintained through pressure increases of 400 psi caused by gradient elution above the normal 1600-psi system pressure. Fifty- $\mu\text{l}$  sample injections were made into an EPR rubber septum by a stop-flow injection technique at 900 psi. A Precision syringe equipped with a Teflon (Du Pont) plunger tip to avoid high-pressure blowback was used. An exponential gradient (8) was generated by gravity flow in which 20 ml of solution B in the secondary

Table I. Precision of Wackender's Sulfur Species by Peak Areas Determination

Sulfur species	Rel std dev, %	$\mu\text{g}$ injected in 50 $\mu\text{l}$
$\text{Na}_2\text{S}_2\text{O}_3$	2.5	50
$\text{K}_2\text{S}_3\text{O}_6$	3.6	100
$\text{K}_2\text{S}_4\text{O}_6$	3.0	50
$\text{K}_2\text{S}_5\text{O}_6 \cdot 1\frac{1}{2} \text{H}_2\text{O}$	2.3	39

reservoir was mixed with 20 ml of solution A in the primary reservoir. Gradient startup was initiated 2 minutes prior to sample injection. This permitted the separation of  $\text{S}_2\text{O}_3^{2-}$  and  $\text{S}_3\text{O}_6^{2-}$  to depend solely on solution A and the separation of  $\text{S}_4\text{O}_6^{2-}$  and  $\text{S}_5\text{O}_6^{2-}$  to depend on solution B in A. Thus, a 9-minute gradient flow through time from primary reservoir to detector resulted in reasonable spacing of  $\text{S}_3\text{O}_6^{2-}$  and  $\text{S}_4\text{O}_6^{2-}$  peaks with a complete elution time of 15 minutes (Figure 2). Reestablishment of system equilibrium including regeneration time for the column and damper flushing of the pump was 25 minutes minimum.

## RESULTS AND DISCUSSION

**Precision.** Relative standard deviations for 25 separate chromatograms were taken as a measure of the precision of the determinations. Table I gives the results. The column relating the number of micrograms injected in 50  $\mu\text{l}$  reports the values based upon salt purity determinations mentioned previously. The precision for each species represents "normal" values for a syringe-septum method of sample introduction.

**Limits of Determination.** Limits were based on the least UV sensitive species,  $\text{S}_3\text{O}_6^{2-}$ . At an injection level of 25  $\mu\text{g}$ , a small peak resulted. The relative standard deviation increased to 8% based upon 15 sample injections. Since each of the thionates follows Beer's law (6), a linear detector response can be assumed. Thus, injected amounts of 1.6, 0.39, and 0.26  $\mu\text{g}$  of for  $\text{Na}_2\text{S}_2\text{O}_3$ ,  $\text{K}_2\text{S}_4\text{O}_6$ , and  $\text{K}_2\text{S}_5\text{O}_6 \cdot 1\frac{1}{2} \text{H}_2\text{O}$ , respectively, would produce nearly the same number of counts on the peak analyzer as for 25  $\mu\text{g}$  of  $\text{K}_2\text{S}_3\text{O}_6$ , assuming a closely proximate per cent relative standard deviation for each.

**Optimization of Operating Conditions.** The optimum pH for the separation of  $\text{S}_2\text{O}_3^{2-}$  and  $\text{S}_3\text{O}_6^{2-}$  was first investigated using mild buffer solutions. In the absence of an organic modifier such as THF, total peak resolutions meant long retention times with resultant broadness for  $\text{S}_4\text{O}_6^{2-}$  and  $\text{S}_5\text{O}_6^{2-}$  peaks. Addition of THF as a gradient after the separation of  $\text{S}_2\text{O}_3^{2-}$  and  $\text{S}_3\text{O}_6^{2-}$  decreased the retention times of  $\text{S}_4\text{O}_6^{2-}$  and  $\text{S}_5\text{O}_6^{2-}$  and sharpened the peaks. Addition of small concentrations of THF as a car-

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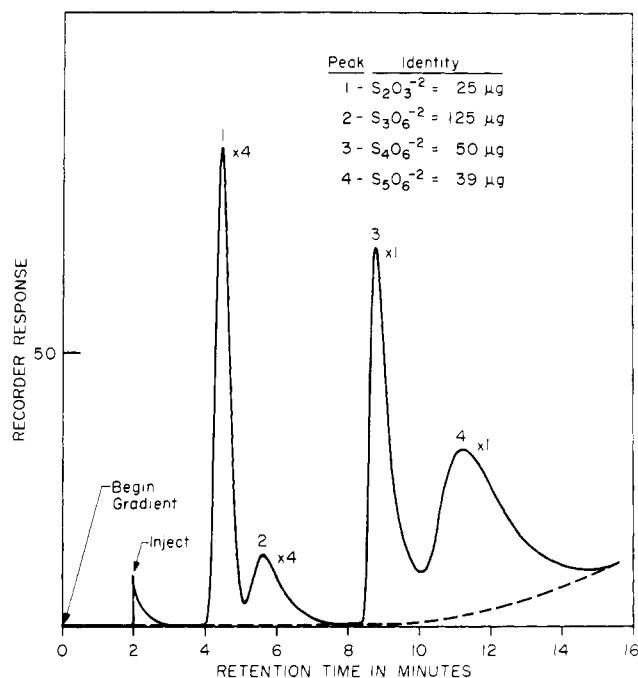


Figure 2. Separation of thionates in Wackenroder's solution

bon modifier to sharpen the  $\text{S}_3\text{O}_6^{2-}$  peak in its separation from  $\text{S}_2\text{O}_3^{2-}$  had a similar effect to increasing the pH with no modifier. However, above 1 volume-per cent THF in buffer, the separation became difficult to control. At pH 10.0 and 0.5 volume-per cent THF, an adequate  $\text{S}_2\text{O}_3^{2-}$ ,  $\text{S}_3\text{O}_6^{2-}$  separation occurred with a nearly Gauss-

ian  $\text{S}_3\text{O}_6^{2-}$  peak. The  $\text{S}_4\text{O}_6^{2-}$  and  $\text{S}_5\text{O}_6^{2-}$  peaks were then positioned on the chromatogram by the 2-minute injection-time lag. The finished chromatogram is shown in Figure 2. This solvent matrix continuously increasing in THF concentration over a 15-minute span produced a slight positive base-line drift as indicated in Figure 2 by the dotted line. The drift, however, has no effect if peak areas are measured by a digital peak analyzer.

Since increased concentrations of THF in buffer may raise the apparent pH of the buffer, it may be desirable to monitor the detector effluent with a pH meter and assume nearly complete removal of THF from the column during regeneration when the pH has returned to a value of 10.0. Our experience, however, has shown that 10 ml of solution A will return the pH to 10.0, yet 40 ml are required to restore the carbon to the condition of polarity required to effect a good separation of  $\text{S}_2\text{O}_3^{2-}$  and  $\text{S}_3\text{O}_6^{2-}$ .

## CONCLUSIONS

High-speed liquid chromatography offers a fast determination of the polythionates with no measurable decomposition during analysis. When samples of individual thionates were injected, one and only one peak resulted. The total sample is eluted from the column, and no column deterioration has been observed over a 2-month period. This method can be streamlined with a more sophisticated gradient generator and solvent turnover system.

Received for review May 21, 1973. Accepted June 28, 1973. Reference to specific equipment is made for identification only and does not imply endorsement by the Bureau of Mines.

# Quantitative Chromatographic Determination of Maleic Hydrazide in Cigarette Smoke

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Maleic hydrazide (MH; 6-hydroxy-3(2H)-pyridazinone) is widely used to control tobacco sucker as demonstrated by the fact that at least 80% of all tobaccos harvested in the USA in 1968 and 1969 were treated with this agent (1). MH is commonly found in processed tobacco in concentrations of between 30 to 100 ppm (1, 2). G. J. Stone has assayed the fate of  $\text{MH-}^{14}\text{C}$  when cigarettes are smoked and found that 22% of the activity appeared in the mainstream smoke and that half of that  $\beta$  activity was located in the particulate phase. MH itself has not been identified in tobacco smoke prior to this investigation (3-5).

Maleic hydrazide is a proved mutagenic agent (6, 7), and is cytotoxic and antimitotic to mammalian cells *in vitro* (8), although it appears to be inactive as a carcinogen in adult rats and mice or as a tumor initiator on mouse skin (9). Epstein and Mantel reported that male mice treated in infancy with MH (0.4% hydrazine impurity) developed a high incidence of liver tumors (10).

This study reports a quantitative method for determining maleic hydrazide in tobacco and cigarette smoke. The determination employs a variety of techniques, including ion exchange chromatography, reaction with 4-chlorobenzyl chloride, absorption chromatography, and, finally, gas

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