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Environmental Protection Technology Series

Automated Analysis of Individual Refractory Organics in Water Polluted



**Office of Research and Development
U.S. Environmental Protection Agency
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AUTOMATED ANALYSIS OF INDIVIDUAL REFRACTORY ORGANICS
IN POLLUTED WATER

by

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ABSTRACT

High-resolution anion-exchange chromatography has been applied to the problem of analyzing for the residual organic compounds present in municipal sewage plant effluents at microgram-per-liter levels. Two different chromatographic systems have been used: one capable of analyzing for compounds which are uv-absorbing and/or oxidizable with sulfatoceric acid, and the other for carbohydrate analysis.

It was necessary to concentrate the samples of effluent 50- to 3000-fold prior to their analysis on the chromatographs. A two-step procedure, consisting of 10- to 30-fold concentration by vacuum evaporation, followed by freeze-drying to the desired final volume, was developed. Loss of noncarbonate carbon with this procedure was generally less than 15%.

Samples of sewage plant effluents were concentrated and analyzed. Techniques of positively identifying compounds present in sewage were established. Using these techniques, 56 organic compounds have been identified in samples of effluent from a primary treatment plant and 13 organic compounds have been identified in samples of effluent from a secondary treatment plant.

The concentration, chromatographic, and identification procedures were also applied to the analysis of chlorinated effluent from primary and secondary sewage plants. More than 60 chromatographic peaks containing chlorine have been found, and specific chlorinated compounds were tentatively identified by cochromatography and quantified at the 0.5- to 4- $\mu\text{g}/\text{liter}$ level.

A detector system for liquid chromatography based on cerate oxidimetry was adapted as a rapid, sensitive continuous monitor for measuring the COD of water. Analysis of COD levels as low as 100 micrograms-per-liter can be obtained in a few minutes by using perchloratoceric acid as the oxidant and measuring the resulting Ce(III) fluorometrically.

An experimental study of the effects of column geometry and operating parameters on chromatographic resolution was made to permit optimization of the ion exchange resin systems.

One high-resolution, ion exchange chromatograph (UV-Analyzer) was constructed for the Advanced Waste Treatment Research Laboratory and another for the Southeast Environmental Research Laboratory. These instruments are being used in the analysis of treated sewage effluents and other polluted waters.

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CONTENTS

	<u>Page</u>
Abstract	ii
List of Figures	v
List of Tables	vii
Acknowledgements	ix

Sections

I	Conclusions	1
II	Recommendations	2
III	Introduction	3
IV	Methods and Instrumental Development	5
V	Identification of Stable Organic Pollutants	42
VI	Effects of Chlorination of Sewage Plant Effluents	69
VII	Cooperative Efforts with EPA Laboratories	89
VIII	Discussion	93
IX	References	94
X	Publications	97

FIGURES

<u>No.</u>		<u>Page</u>
1	Procedure for Concentrating Sewage Plant Effluent Samples	7
2	Schematic Diagram of Vacuum Distillation System	8
3	High-Resolution Anion Exchange Chromatograph for UV-Absorbing Compounds (UV-Analyzer)	10
4	High-Resolution Anion Exchange Chromatograph for Carbohydrates (Carbohydrate Analyzer)	11
5	Reference UV-Analyzer Chromatogram of 1000X Concentrate of Primary Sewage Treatment Plant Effluent	12
6	Reference UV-Analyzer Chromatogram of 2000X Concentrate of Secondary Sewage Treatment Plant Effluent	13
7	Carbohydrate Analyzer Chromatograms of Primary and Secondary Sewage Treatment Plant Effluents, Human Urine, and Sugar Standards	15
8	Schematic Diagram of Dual-Column, High Resolution, Liquid Chromatograph for Analyzing Two Polluted Water Samples Simultaneously	16
9	Dual-Column Sample Injection Valve	17
10	Electrical Heating System for Chromatographic Columns	19
11	Dual-Column Chromatograms of Two Identical Samples	20
12	Dependence of Chromatographic Resolution on Column Length and Linear Velocity of Eluent	22
13	Schematic of Cerate Oxidative Monitor	26
14	Schematic of Flow Fluorometer	27
15	Exploded View of Flow Fluorometer Body	28
16	Calibration Curve for Flow Fluorometer	30
17	Chromatogram of Primary Sewage Plant Effluent Analyzed by the UV-Analyzer with a Cerate Oxidative Monitor	31
18	Chromatogram of Secondary Sewage Plant Effluent Analyzed by the UV-Analyzer with a Cerate Oxidative Monitor	32

<u>No.</u>		<u>Page</u>
19	Cerium Fluorescence Oxidimetry	35
20	Schematic of Continuous Chemical Oxygen Demand Analyzer	37
21	Recorder Trace from Continuous COD Analyzer	39
22	Ultraviolet Absorption Spectra of Unknown Sample Taken at Different pH Values	49
23	Ultraviolet Absorption Spectra of Guanosine Taken at Different pH Values	50
24	Gas Chromatograms of TMS Derivatives of Reference Compound (Guanosine) and Liquid Chromatographic Fraction from Primary Sewage Treatment Plant Effluent	52
25	Mass Spectra of TMS Derivatives of the Reference Compound (Guanosine) and Liquid Chromatographic Fraction from Primary Sewage Treatment Plant Effluent	53
26	UV-Analyzer Chromatograms of Primary Sewage Treatment Plant Effluent Chlorinated with Different Amounts of Calcium Hypochlorite	71
27	Schematic of Chlorine Generator and Sample Chlorinator	73
28	Schematic of Apparatus Used for Concentration of Radioactive Samples	74
29	Dual-Column UV-Analyzer Chromatograms of Chlorinated Primary Sewage Treatment Plant Effluent	76
30	Dual-Column UV-Analyzer Chromatograms of Chlorinated Effluent from a Secondary Sewage Treatment Plant	79
31	Chromatograms of Secondary Sewage Treatment Plant Effluent Chlorinated with Hypochlorite Solution for 15-, 45-, and 90-min Reaction Times	80
32	Chromatograms of UV-Absorbing Constituents Developed on the Southeast Environmental Research Laboratory UV-Analyzer a) 0.25 ml of Reference Urine (URS-IV) b) 0.25 ml of Primary Effluent (1000X)	91

TABLES

<u>No.</u>		<u>Page</u>
1	Resolution of Coupled Anion-Cation Columns During Chromatography of ORESP Secondary Sewage Effluent Samples	25
2	Oxidation Potential Available with Various Oxidants Used in COD Analysis	34
3	Oxidation of Organic Compounds and Polluted Waters by Perchloratoceric Acid COD Method	40
4	Physical Characteristics and Operating Parameters for the Analytical and Preparative Anion Exchange Columns	44
5	Guanosine Identification Data	51
6	Description of Samples	54
7	Status of Sewage Plant Effluent Samples (5/1/74)	55
8	Identification of Molecular Constituents in 1000- to 3000-Fold Concentrates of Primary Domestic Sewage	57
9	Data on Unknown Compounds Found in Unchlorinated Primary Effluent (SPJ-1) From the Oak Ridge East Sewage Plant	60
10	Data on Unknown Compounds Found in Chlorinated Sewage Plant Effluent (SPJ-3) From the Oak Ridge West Sewage Plant	61
11	Data on Unknown Compounds Found in Chlorinated Effluent (SPJ-8) from the Oak Ridge West Sewage Plant	62
12	Data on Unknown Compounds Found in SPJ-11, Composite of SPJ-3 and SPJ-8	66
13	Identification of Molecular Constituents in 1000- and 2000-fold Concentrates of Secondary Domestic Sewage Effluent	67
14	Data on Unknown Compounds Found in Secondary Effluent (SPJ-9 and SPL-1) From a Domestic Sewage Treatment Plant	68

<u>No.</u>		<u>Page</u>
15	Stable Chlorine-Containing Organic Constituents in Chlorinated Effluents from Domestic Sanitary Sewage Treatment Plants. (Concentrations of the Constituents, ng Cl/liter of original effluent)	82
16	Tentative Identifications and Concentrations of Chlorine-Containing Constituents in Chlorinated Effluents	86
17	Percentage Chlorination Yield of Chlorine-Containing Constituents with Respect to Reaction Time for the Chlorination of Effluents	88

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SECTION I

CONCLUSIONS

1. Over 100 refractory organic compounds can be present in effluents from municipal sewage treatment plants at microgram-per-liter levels; some of these refractory compounds are chlorinated under conditions existing when effluents from sewage treatment plants are chlorinated.
2. High-resolution anion exchange chromatography provides a reliable and useful tool for determining refractory organic compounds present at low levels in sewage effluents and various other polluted waters.
3. In addition to uv-absorbing compounds, numerous other compounds can be detected by sulfatoceric acid oxidimetry.
4. The detection of sources of pollution, the testing of the effectiveness of sewage treatment steps, including possible tertiary steps, and the determination of the ultimate disposition of pollutants are obvious end uses of the high-resolution anion exchange chromatographic systems.

SECTION II

RECOMMENDATIONS

This program has been limited to the adaptation of existing analytical systems for use in the determination of refractory organic compounds in polluted waters with a minimum of instrumental development. The scope of the program did not include exploiting the capabilities of the analyzers for detecting sources of pollution, testing the effectiveness of sewage treatment plants, or determining the ultimate fate of pollutants; nor did it include positive identification of all the separated organic constituents.

It is recommended that the use of high-resolution liquid chromatographs with uv photometers and cerate oxidative monitors for determining refractory organic compounds in industrial waters and other polluted waters be significantly expanded. This can be accomplished either by fabricating additional UV-Analyzers as described herein or by modifying commercially available high-pressure chromatographs. A vigorous effort should be continued to determine the identities of as many as possible of the residual stable organic compounds being discharged to surface waters. Also, potential hazards of these compounds, particularly those which are chlorinated, should be evaluated. High-resolution analyzers have been developed to the point that they could be used by appropriate agencies to determine sources of pollution, the effectiveness of sewage treatments, and the fate of organic pollutants - all on a molecular level. This effort should be closely coordinated with the analytical development program to take advantage of improvements as they are made and to "feed back" information relating to problem areas that would lead to necessary modifications of the instruments.

SECTION III

INTRODUCTION

The continuing discharge of stable organic compounds in sewage effluents to surface waters and the buildup of organic constituents by recycle constitute a serious threat to our water quality.^{1,2} Furthermore, as such water contamination becomes excessive, additional processing steps may become necessary to permit reuse of the water. Because present analytical techniques for sewage effluents are non-specific and often indirect, they are not adequate for use in evaluating new developments of advanced treatment processes. These analytical techniques do not provide sufficient information to determine the chemical forms or concentrations of the stable organic compounds that resist degradation. Thus, we do not know the effectiveness of various treatment steps for specific refractory compounds; nor do we know what harmful effects might result if these refractory compounds continue to build up in the water supply.

In an effort to better define the pollution problem, this program to determine the specific organic compounds present in sewage plant effluents was undertaken. An important step in providing the analytical information to answer pertinent questions and permit rational development of additional sewage treatment steps has been the demonstration that the high-resolution anion exchange analytical system can provide reliable measurement of refractory organic compounds at microgram-per-liter levels in sewage effluents and other polluted waters. In the first year under this contract,^{3,4} we demonstrated that at least 50 to 100 specific refractory organic compounds are present in effluents from municipal sewage treatment plants at microgram-per-liter levels and that many more refractory compounds are present at higher concentrations in effluents from industrial sewage treatment plants.

It was demonstrated that previously developed automated, high-resolution liquid chromatographs would be useful in the (a) detection

of sources of pollution, (b) testing of the effectiveness of sewage treatment steps including possible tertiary steps, and (c) determination of the ultimate disposition of pollutants. Subsequently, emphasis of this program has been directed toward the exploitation of this type of instrumentation, primarily by identifying specific compounds found in the plant effluents but also by improving the chromatographic separation system.

A major effort has been to define more clearly the magnitude of the pollution threat by identifying and quantifying the specific stable organic compounds that are difficult to degrade during common sewage treatment steps. This has resulted in establishing the identities of 56 of the organic compounds detected in primary sewage plant effluents and 13 of the separated organic compounds in secondary sewage plant effluents. An effort has also been made to determine whether the chlorination of sewage effluents produces undesirable levels of stable chlorinated organic compounds. Additionally, several detection systems for increasing the sensitivity and detection capability of the existing high-resolution chromatographs were evaluated. One detector was adapted for use as a continuous oxygen demand (COD) analyzer that is several times more sensitive than any presently available.

In the interest of more closely coordinating the direction and emphasis of the program with other activities in EPA Laboratories, several informal meetings of the contributing personnel were held. The participants at the meetings included personnel from the Southeast Environmental Research Laboratory and the Advanced Waste Treatment Research Laboratory.

SECTION IV

METHODS AND INSTRUMENTAL DEVELOPMENT

The primary developmental effort of this program has been directed toward adaptation of existing high-resolution ion exchange chromatographs to the analysis of individual refractory organic compounds in effluents from sewage treatment plants. To achieve this objective, it was necessary to develop a technique to easily and reliably concentrate sewage plant effluent samples by as much as several thousand-fold with little loss of noncarbonate carbon and to modify and improve the existing UV-Analyzer and carbohydrate analyzer. In addition, an oxidative detector which was developed as a liquid chromatograph monitor was modified for use as a very sensitive continuous COD monitor.

CONCENTRATION OF SEWAGE SAMPLES PRIOR TO ANALYSIS

Since the lower limit of detection for the UV-Analyzer is 100 $\mu\text{g/liter}$ to 100 mg/liter , depending on the ultraviolet absorptivity of the individual compound, and the concentrations of specific contaminants in effluent samples may be 10 $\mu\text{g/liter}$ or less, concentration of sewage effluents by factors up to 3000 may be necessary prior to analysis. As shown in later sections, the concentration factor depends on the history of the effluent. Concentration methods^{5,6} such as freezing, extraction, adsorption, and low-temperature distillation were considered. Freezing is inadequately understood for the large number of compounds of interest and would require multiple stages to achieve the concentration required in most cases. Extraction and adsorption may not quantitatively concentrate all compounds of interest. Therefore, the method of low-temperature distillation appears to be the most convenient and should provide adequate recovery of stable, nonvolatile organic compounds.

Low-temperature distillation may be carried out by freeze-drying, rotary evaporation, or vacuum distillation. Considerations relative

to the choice of equipment are: the desirability of maintaining the sample at a low temperature to avoid decomposition, the necessity of collecting the solids that separate during the concentration step in order to redissolve coprecipitated organic compounds, and the need to reduce the volume of sample from several liters to a few milliliters in a reasonable time. Although freeze-drying provides maximum sample integrity and simplified collection of separated solids, it is not well suited to the reduction of volumes in excess of 200 ml. Rotary evaporation is also unwieldy for large volumes; in addition, it exposes the sample to temperatures higher than ambient and leaves the precipitate distributed over a large surface. Vacuum distillation appears to be the best method in that it is more rapid than either of the other distillation methods, the temperature of the sample is maintained at or below ambient, and the precipitated solids are well concentrated; however, reduction to a final volume less than 50 ml is difficult in existing equipment. Based on these considerations and the availability of a vacuum still,⁷ a two-step concentration (Fig. 1) procedure was adopted. First, a concentration of 10- to 30-fold is effected in the vacuum still; then the resulting volume (150 ml) of concentrate is further reduced by freeze-drying.

Normally, a volume of several liters of waste effluent is reduced to a few milliliters to provide for a working sample and a spare sample, and to allow for minor losses. In the first step, the effluent is filtered through a 0.45- μ m membrane to remove suspended matter; only negligible uv-absorbing material is lost in this separation. The volume of the filtrate is then reduced in the vacuum still (Fig. 2) to about 150 ml of liquid, plus some separated solids. The concentrated liquid, the solids, and a rinse solution are transferred to the freeze-dryer, where the final reduction in volume is made. Water and either acetic acid (for the UV-Analyzer) or borate buffer solution (for the carbohydrate analyzer) are added to attain the desired liquid volume and to adjust the pH to \sim 4-5 for the UV-Analyzer or to 8.5 for the carbohydrate analyzer. Finally, the resulting sample is well mixed, and the solids are separated by centrifugation.

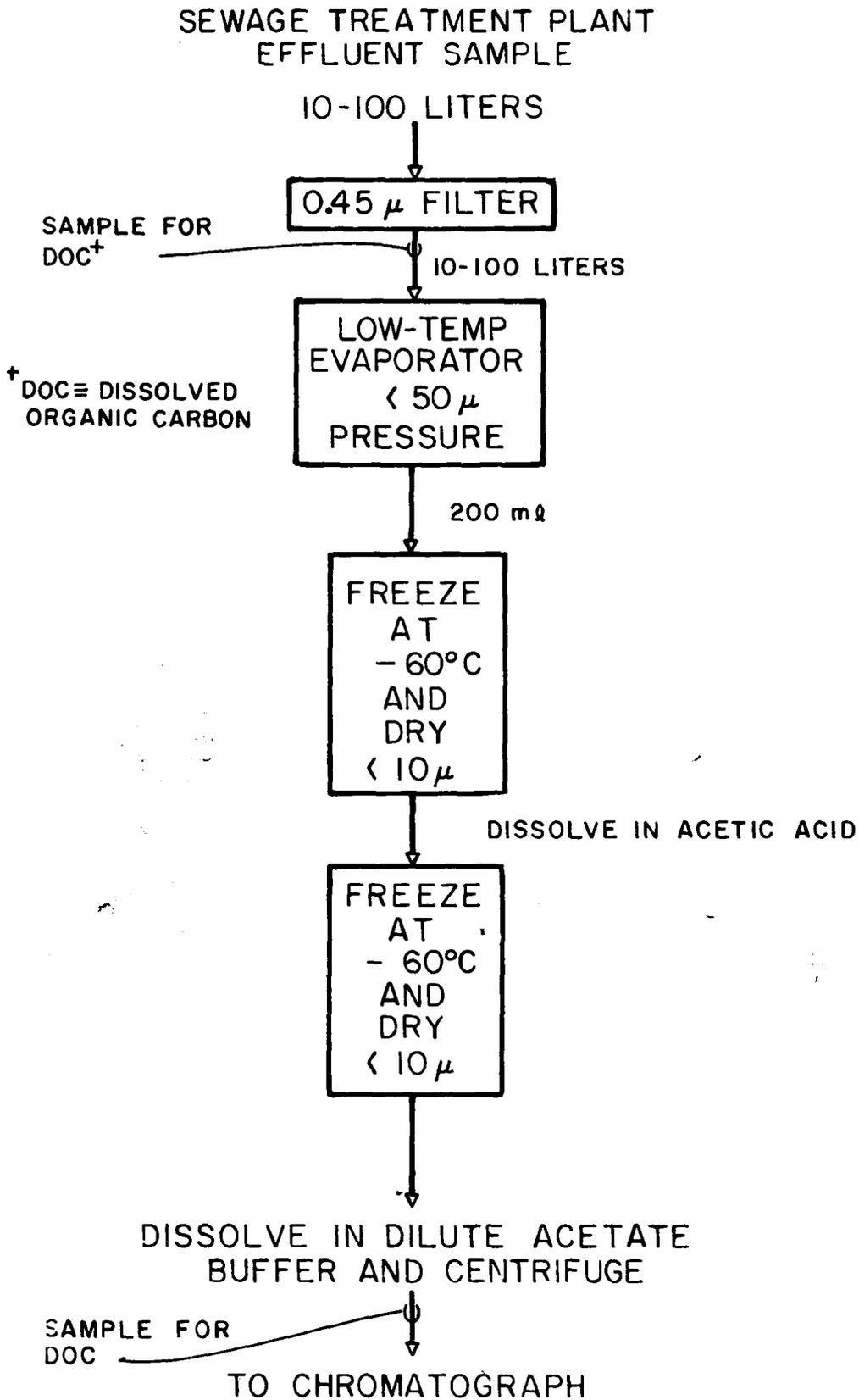


Fig. 1. Procedure for Concentrating Sewage Plant Effluent Samples.

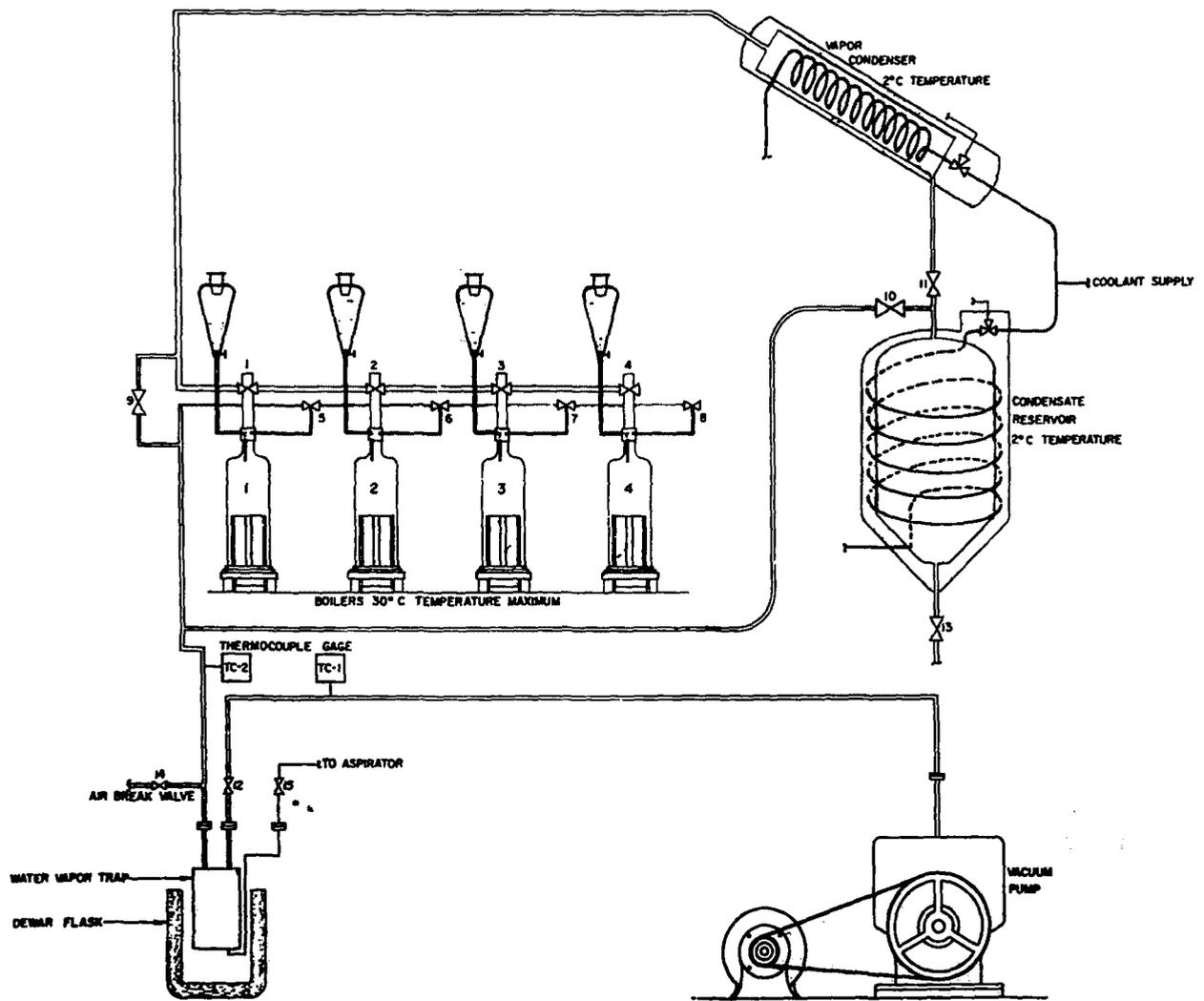


Fig. 2. Schematic Diagram of Vacuum Distillation System.

The recovery of the noncarbonate organic compounds in the final concentrate appears to be satisfactory (greater than 85%), as determined by carbon analyses of the effluent and the separated phases of the final concentrates.

HIGH-RESOLUTION ANALYZERS

Because of their sensitivity and capability for detecting and quantifying many individual organic compounds in complex aqueous samples, two high-resolution anion exchange chromatographs⁸⁻¹² have been adapted for use in analyzing for the molecular components in sewage plant effluents and other water supplies. Each of these systems, the UV-Analyzer for ultraviolet-absorbing compounds (Fig. 3) and the carbohydrate analyzer (Fig. 4), consists primarily of a heated, high-pressure, anion exchange column; a sample injection valve; a concentration-gradient generating and pumping system; a two-wavelength dual-beam photometer; and a strip-chart recorder. The ion-exchange column for each system is a 150-cm length of type 316 seamless stainless-steel tubing (0.22 to 0.62 cm ID) packed with strongly basic anion exchange resin. A 0.05- to 2.5-ml sample (the volume depending on the inside diameter of the ion exchange column and the nature of the sample) is applied to the column by a 6-port injection valve mounted as near to the top of the column as possible in order to minimize peak broadening.

On the UV-Analyzer the chromatograms are developed by eluting the sample constituents with an ammonium acetate--acetic acid buffer solution (pH 4.4) whose acetate concentration gradually increases from 0.015 to 6.0 M. The eluent is pumped through the ion exchange column at the rate of about $250 \text{ ml cm}^{-2} \text{ hr}^{-1}$ with a pressure drop of 100 to 200 atm. The absorbances of the column effluent at 254 and 280 nm, referred (at the same wavelengths) to the stream entering the column, are monitored by a two-wavelength, dual-beam flow photometer and recorded on a strip chart. Typical UV-Analyzer chromatograms of municipal sewage treatment plant effluent samples are shown in Fig. 5 (primary effluent) and Fig. 6 (secondary effluent). The compounds

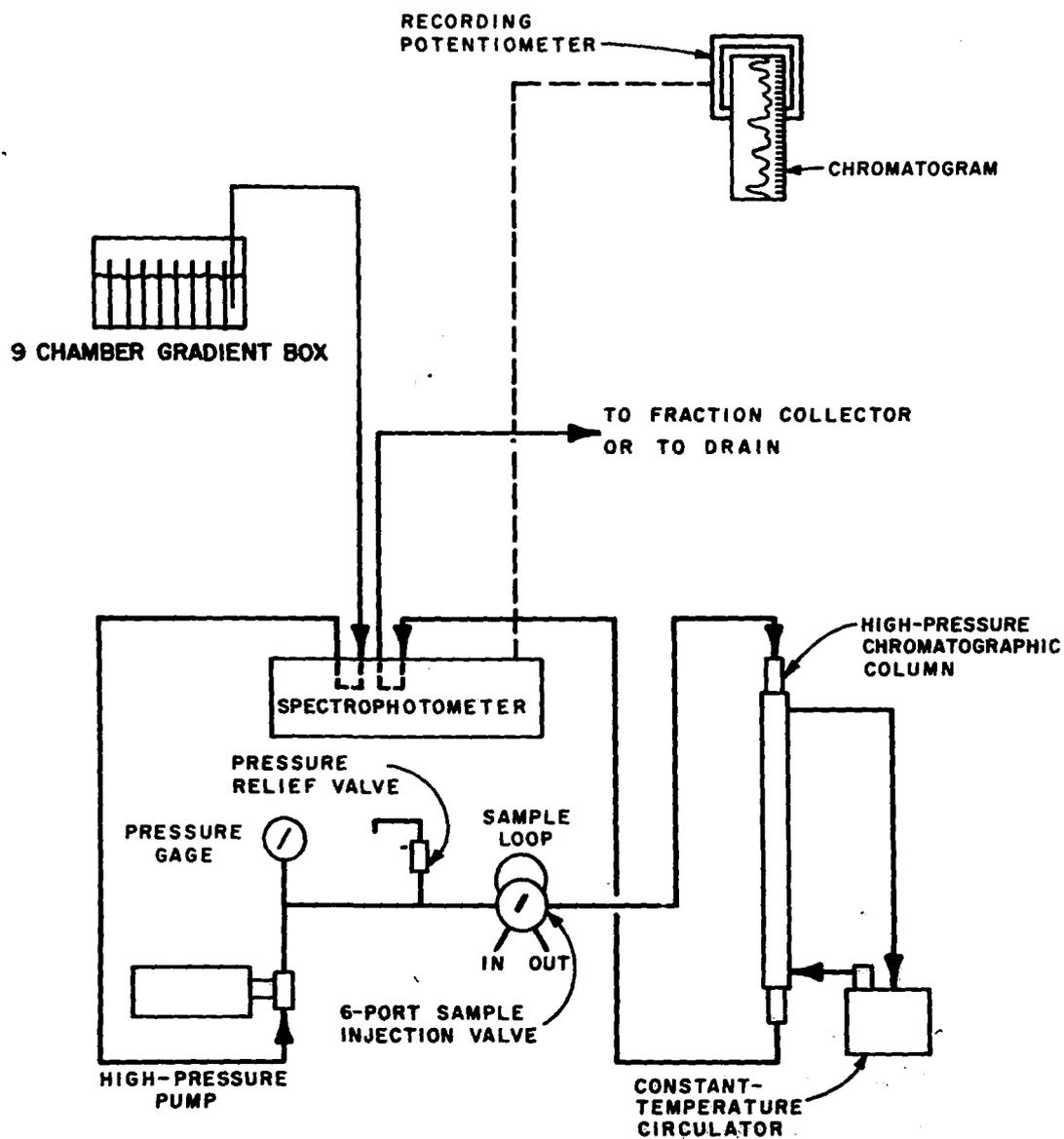


Fig. 3. High-Resolution Anion Exchange Chromatograph for UV-Absorbing Compounds (UV-Analyzer).

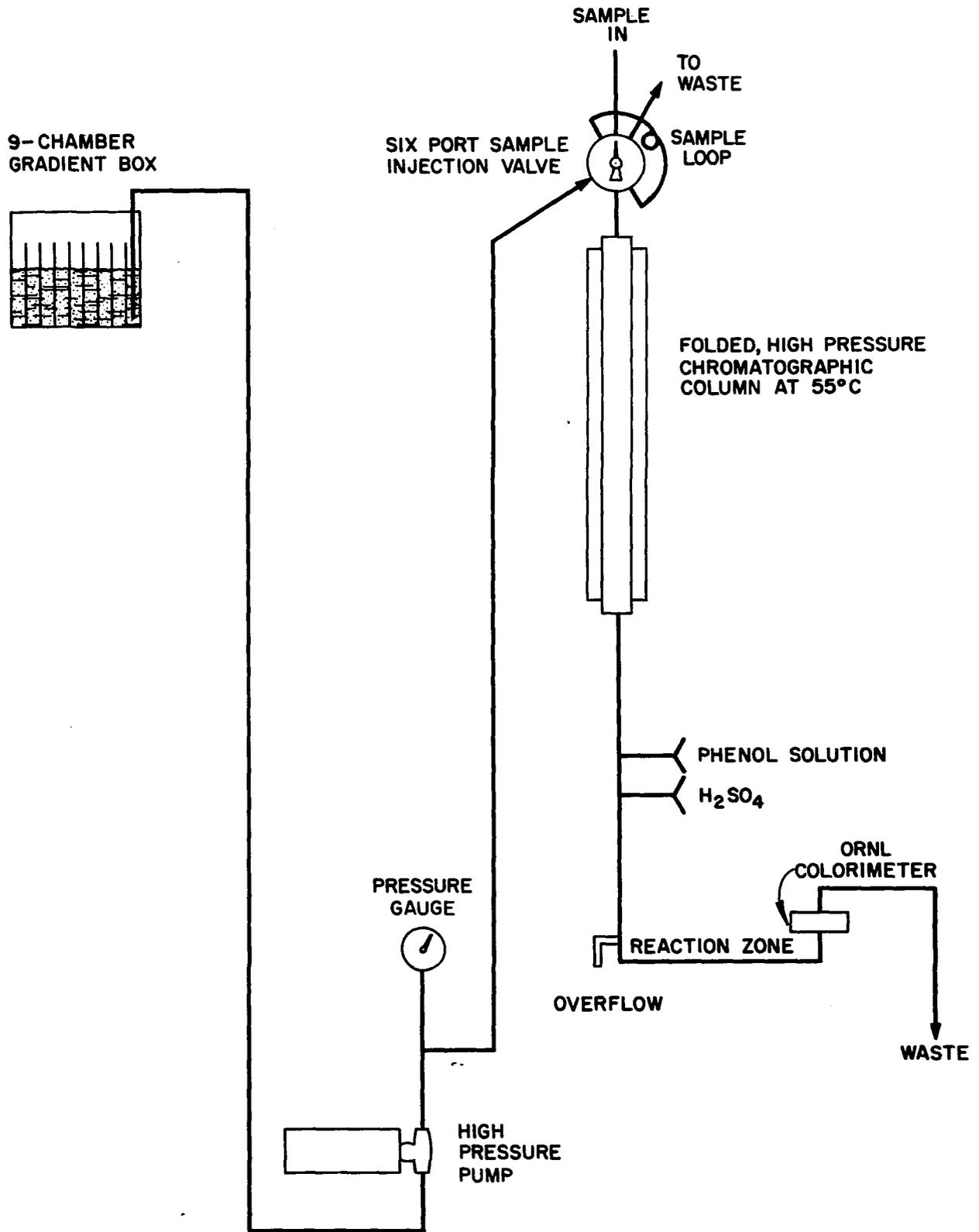


Fig. 4. High-Resolution Anion Exchange Chromatograph for Carbohydrates (Carbohydrate Analyzer).

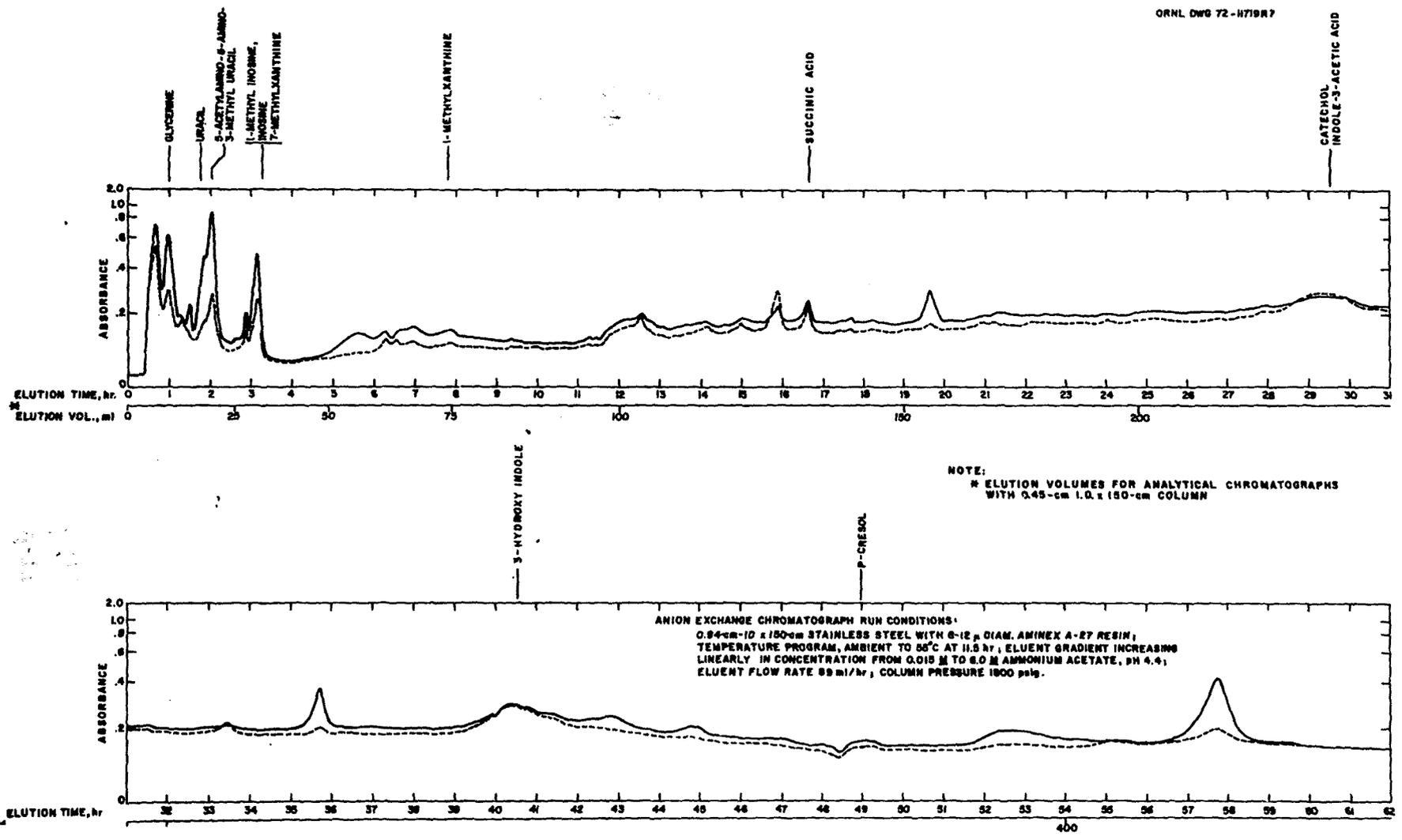


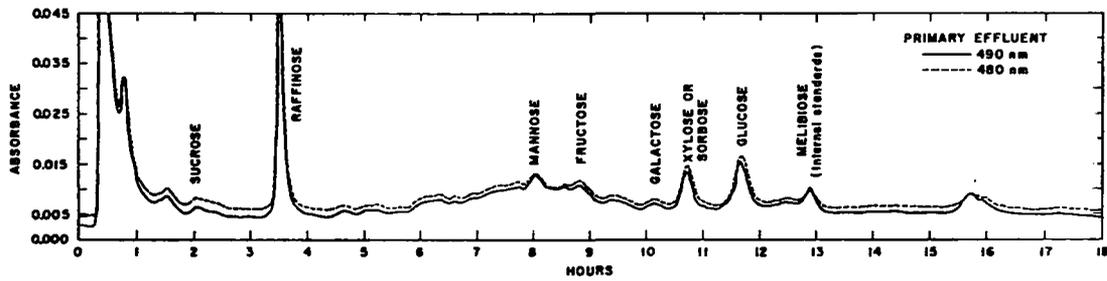
Fig. 6. Reference UV-Analyzer Chromatogram of 2000X Concentrate of Secondary Sewage Treatment Plant Effluent.

shown on the chromatograms are those that have been identified by the techniques described in Section V.

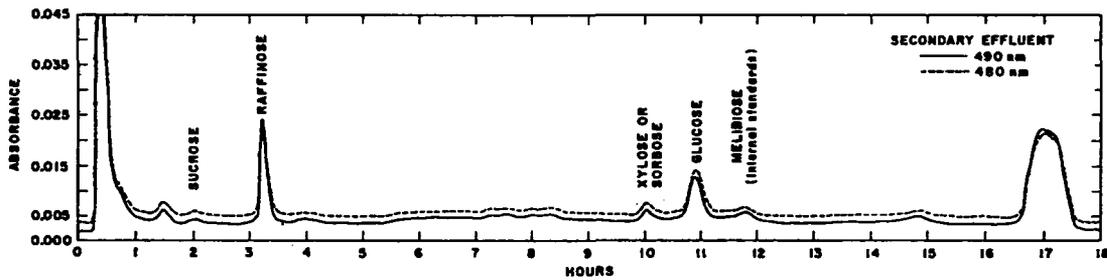
The carbohydrate analyzer utilizes a sodium borate--boric acid buffer (pH 8.9) as the eluent. This eluent, whose boron concentration increases from 0.085 to 0.845 M during an analysis, is pumped through the column at the rate of about 350 ml cm⁻² hr⁻¹, also with a pressure drop of 100 to 200 atm. The separated carbohydrate compounds are monitored with a phenol--sulfuric acid color development system which mixes the column effluent with 5% phenol and concentrated sulfuric acid and subsequently measures the absorbance, at 480 and 490 nm, of the reaction mixture after reaction for 3 min at 100°C. The more-sensitive Mark III Carbohydrate Analyzer,¹³ which was originally developed for physiologic fluids, has been applied to municipal primary and secondary effluents concentrated 500-fold by the technique described earlier. The resulting chromatograms contained 38 and 19 peaks, respectively, and are compared to those obtained for urine and reference sugar standard samples in Fig. 7. In the first 1-1/2 hr of elution, the primary and secondary effluent chromatograms contain several large peaks, similar to those in the urine chromatogram, that are probably due to complex sugars. Among the simple sugars appearing in the primary and secondary effluent samples are sucrose, raffinose, allulose, mannose, fructose, xylose or sorbose, and glucose. These are tentative identifications based on elution positions, but several of these sugars have been definitely identified in UV-Analyzer fractions.

DUAL-COLUMN UV-ANALYZER

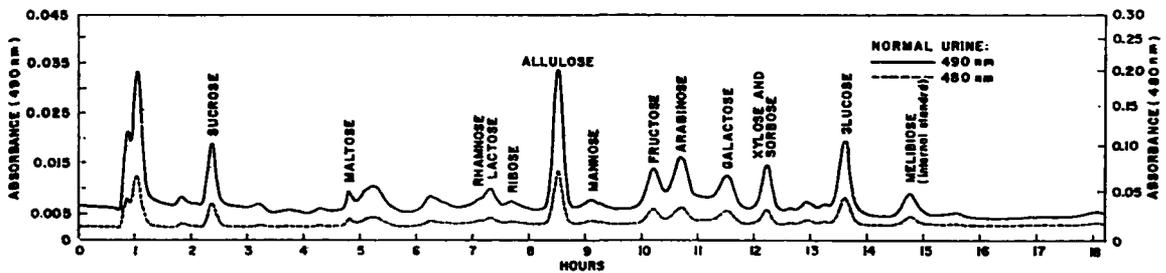
A dual-column UV-Analyzer, which has two columns operating in parallel, has been demonstrated to be a powerful tool for comparing the chromatograms of related samples. The system, although primarily developed for biomedical applications,¹² has been adapted for use in the water pollution effort. The final design (shown schematically in Fig. 8) was based on the Mark II-A UV-Analyzer design but incorporates two recent improvements and a dual-column sample injection valve (Fig. 9). This valve, which essentially consists of two,



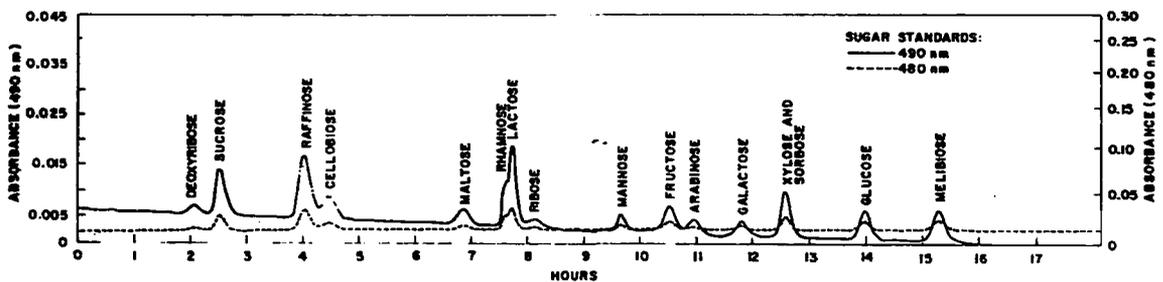
SAMPLE: 1cc OF MUNICIPAL PRIMARY EFFLUENT CONCENTRATED 500-FOLD



SAMPLE: 1cc OF MUNICIPAL SECONDARY EFFLUENT CONCENTRATED 500-FOLD

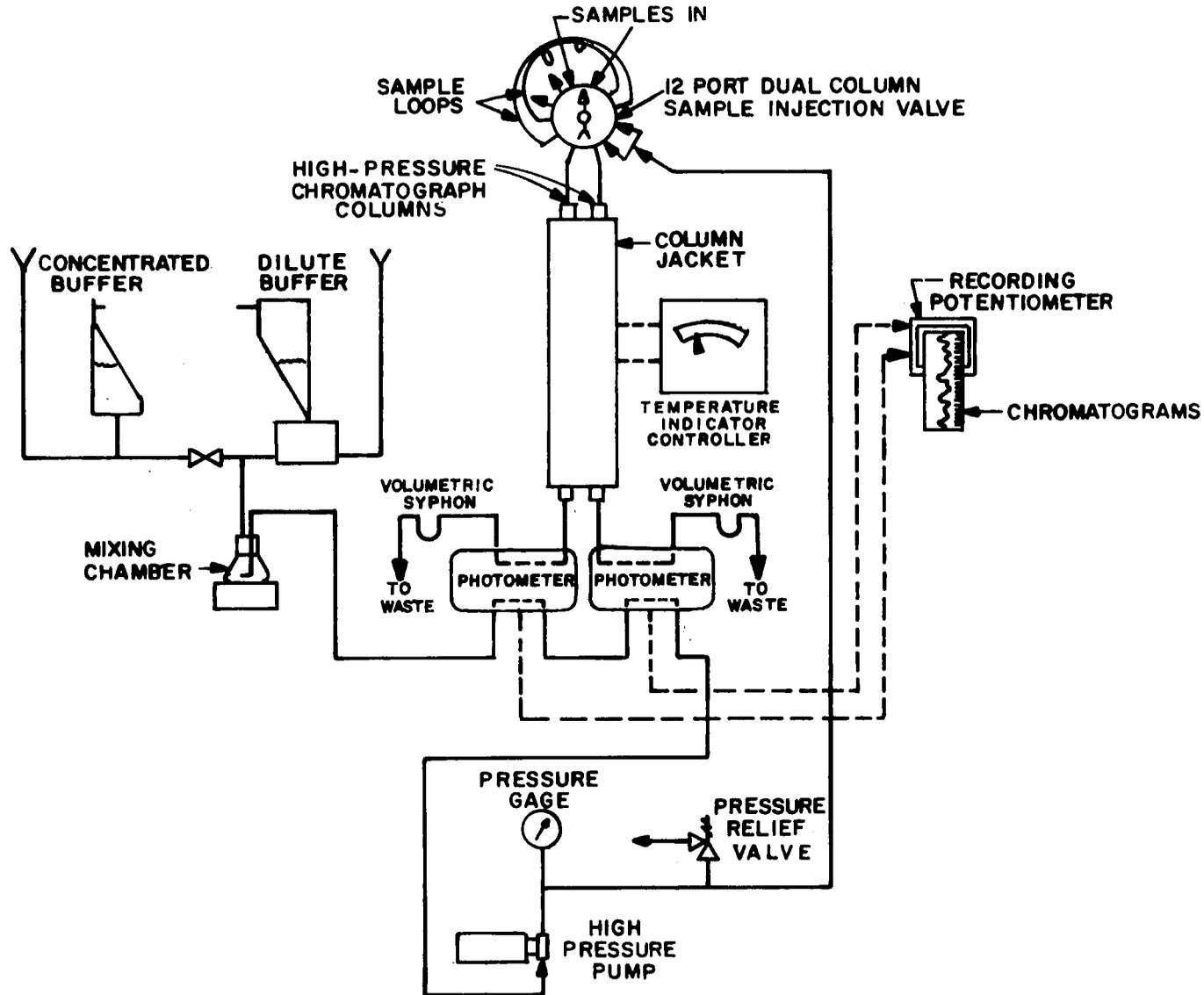


SAMPLE: 1cc OF NORMAL URINE



SAMPLE: 1cc CONTAINING 0.05 MICROMOLES OF EACH SUGAR

Fig. 7. Carbohydrate Analyzer Chromatograms of Primary and Secondary Sewage Treatment Plant Effluents, Human Urine, and Sugar Standards.



16

Fig. 8. Schematic Diagram of Dual-Column, High Resolution, Liquid Chromatograph for Analyzing Two Polluted Water Samples Simultaneously.

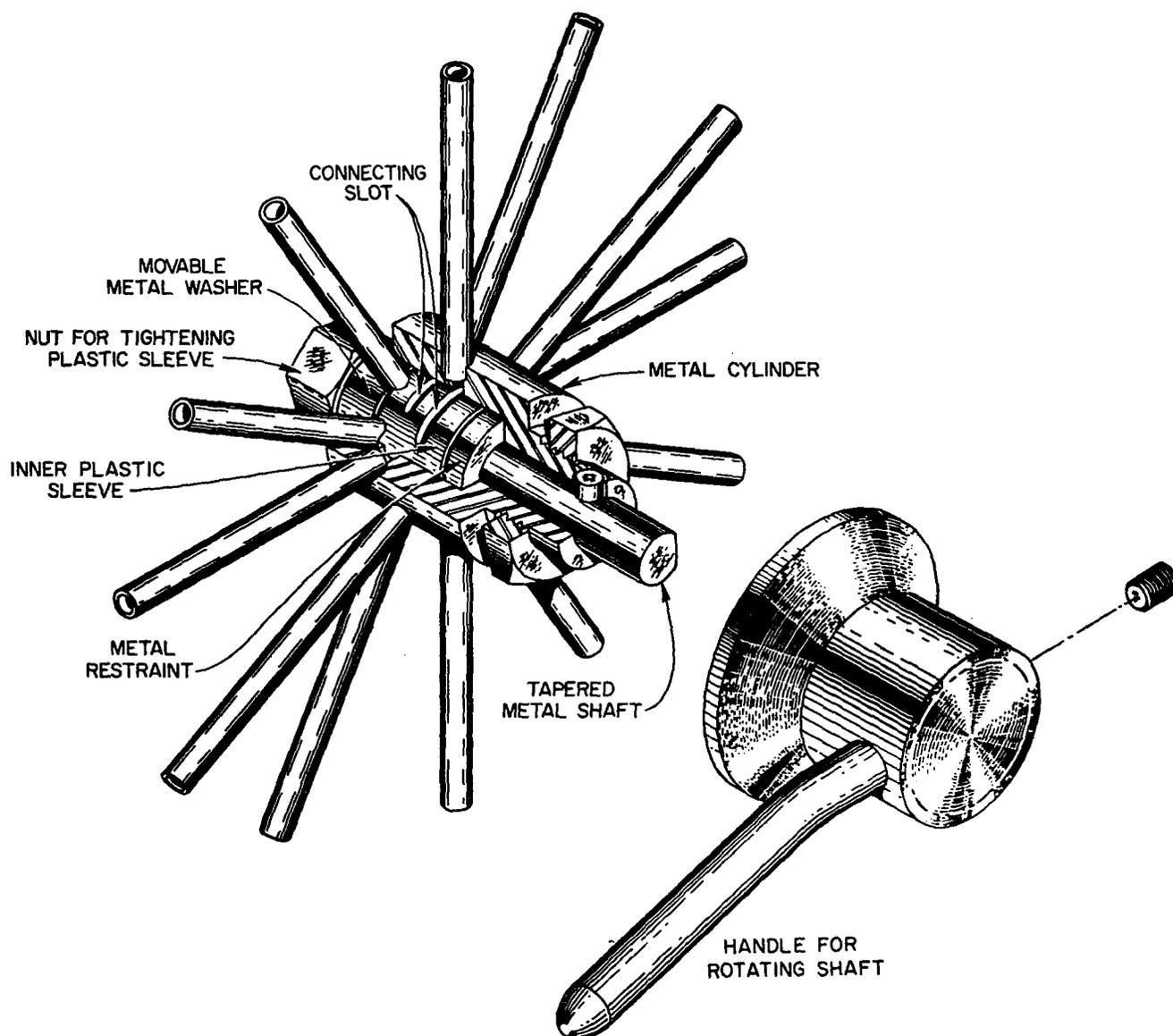


Fig. 9. Dual-Column Sample Injection Valve.

ganged 6-port sample injection valves, provides simultaneous injection of two different samples onto two columns; the same design principle could be used to construct multicolumn injection valves. The improvements consisted of: (a) replacement of the constant-temperature circulator with a more reliable thermistor-controlled, resistance heater wound around the outside of the column jacket (Fig. 10); and (b) replacement of the metal gradient generator with a clear-plastic device which produces a more gentle transition into the gradient.

To convert the Mark II Analyzer to a dual-column system, the single 0.62 cm x 150 cm ion exchange column in the Mark II unit was replaced initially with two 0.45 cm x 150 cm columns, then later with two 0.30 cm x 150 cm columns. Each of the smaller pair of columns contained one-fourth the volume of the single large column; thus the amounts of resin and eluent required for the two columns are only one-half of the amounts needed for the single column in the Mark II prototype. The effluent from each column was monitored with a two-wavelength, dual-beam photometer, and the absorbances at 254 and 280 nm were recorded on a single strip chart.

The operating procedure for the dual-column UV-Analyzer is identical with that used for the Mark II UV-Analyzer except that, in the dual-column system, two samples are loaded into the 12-port sample injection valve and are simultaneously injected, one onto each chromatographic column.

The dual-column Analyzer yields chromatograms that are almost superimposable when identical samples are injected onto each column (Fig. 11); however, the patterns strikingly show even small dissimilarities when different samples are injected. The dual-column analyzer is particularly useful for comparing sewage samples before and after treatment. The results obtained from routine use of the dual-column UV-Analyzer have demonstrated the usefulness of the multicolumn mode of operation. In addition to the higher sample capacity, the capability for comparing samples simultaneously represents a significant improvement over single-column operation. With relatively minor modification, the existing Mark II UV-Analyzers can be adapted to dual-column operation.

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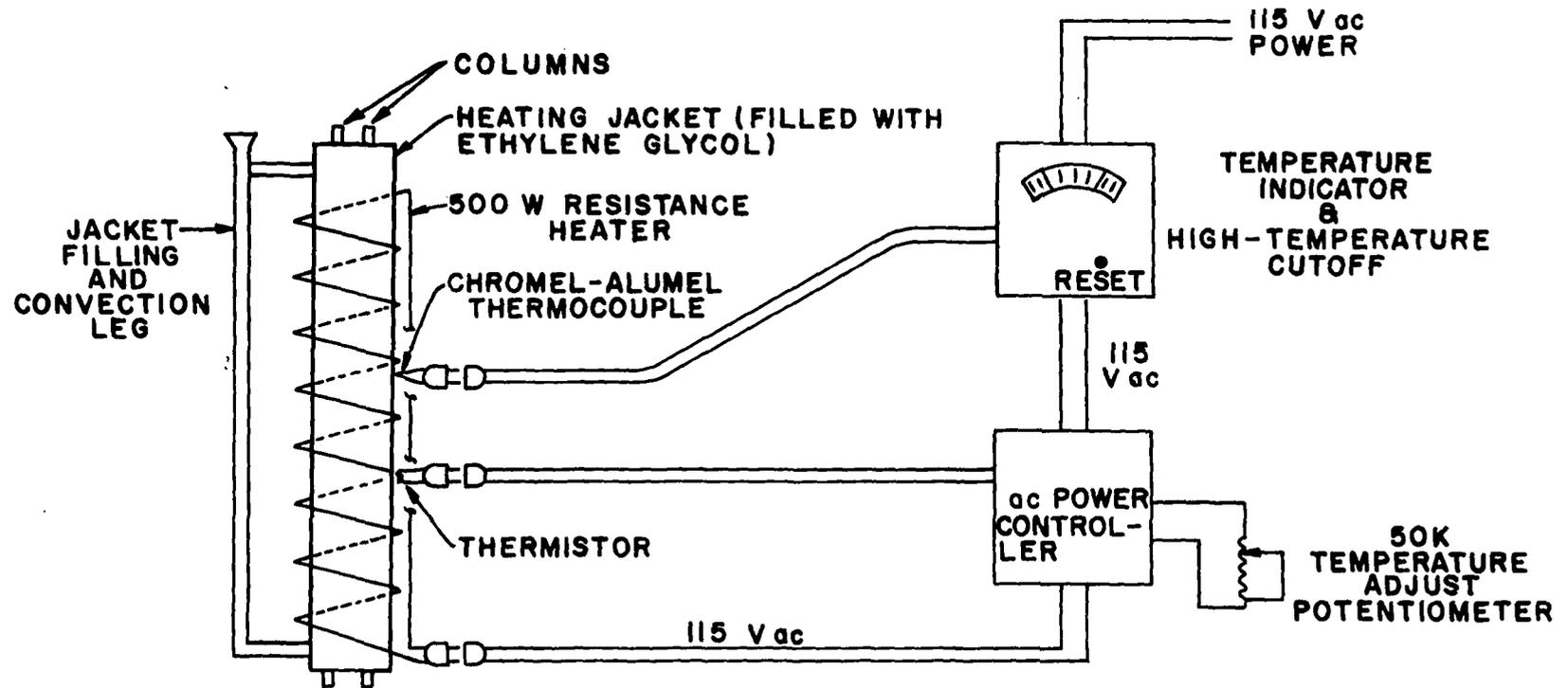


Fig. 10. Electrical Heating System for Chromatographic Columns.

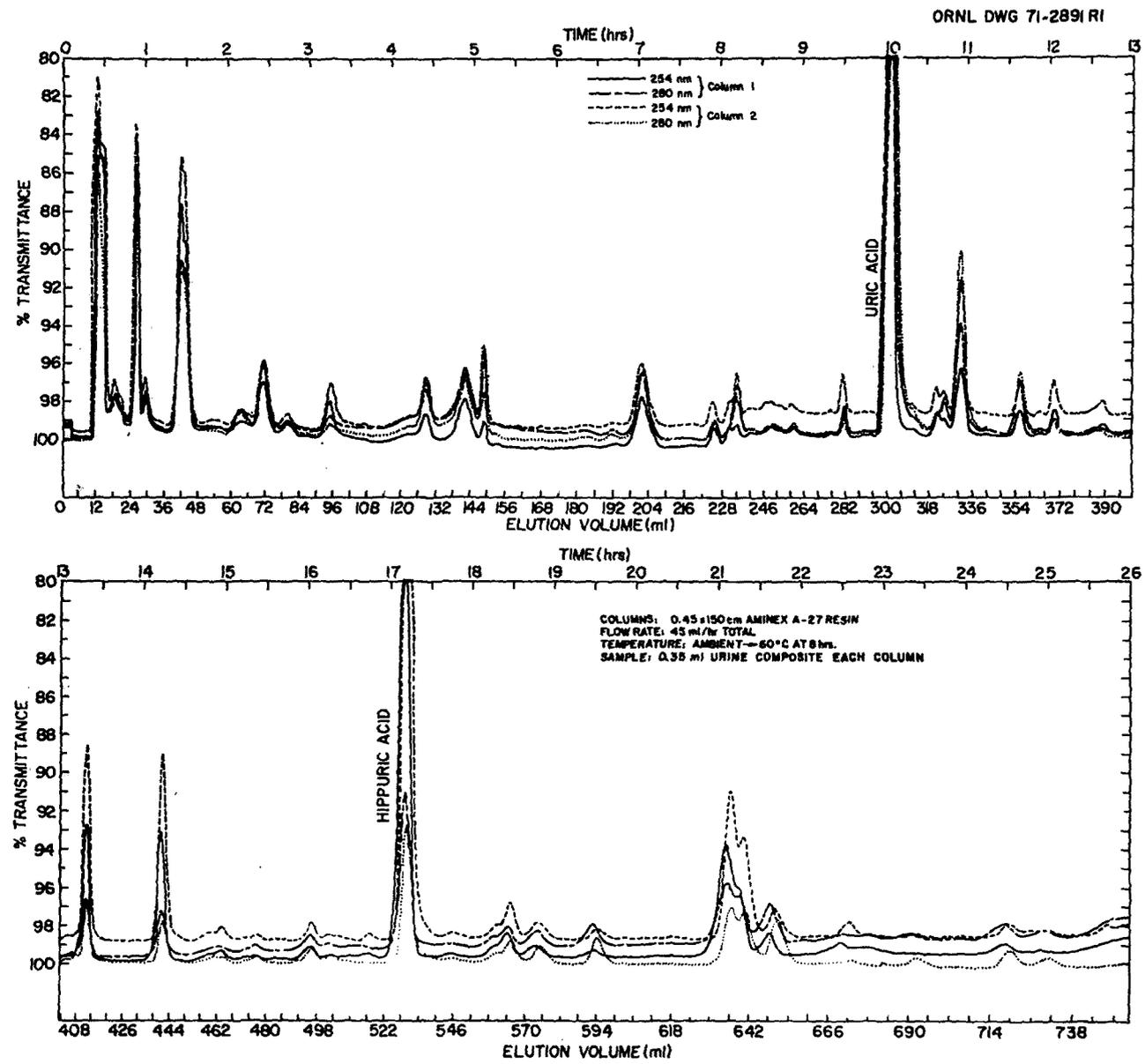


Fig. 11. Dual-Column Chromatograms of Two Identical Samples.

COLUMN GEOMETRY AND OPERATING PARAMETER STUDIES

An experimental study of the effects of column geometry and operating parameters on chromatographic resolution has been made in cooperation with the Body Fluids Analyses Program with the goal of determining the best combination of column dimensions and operating parameters. According to theoretical considerations, faster analyses and higher resolution are conflicting requirements for a given chromatographic system. One can define the resolution, R_s , of peaks 1 and 2 by:

$$R_s \equiv (\bar{v}_2 - \bar{v}_1) / 4\sigma,$$

where \bar{v}_1 and \bar{v}_2 = elution volumes of peaks 1 and 2,

σ = average standard deviation of the peaks in volume units.

Van Deemter's¹⁴ differential model for chromatography results in:

$$R_s \propto \frac{1}{\sqrt{2}} \left(\frac{L}{U_o} \right)^{1/2};$$

but, on the other hand, analysis time, t_a , will have the following proportionality:

$$t_a \propto \frac{L}{U_o},$$

where L = column length,

U_o = linear velocity of eluent.

The experimental results confirmed the first proportionality (Fig. 12). One fact should be borne in mind here; that is, although higher values for resolution (as defined above) are generally desired, each system has an upper limit above which any increase would be superfluous and, in fact, would only increase separation time. For example, two adjacent peaks are almost completely separated if their R_s is greater than about 1.0. Of course, in the separation of a very complex mixture such as sewage plant effluent, it is always possible that additional compounds

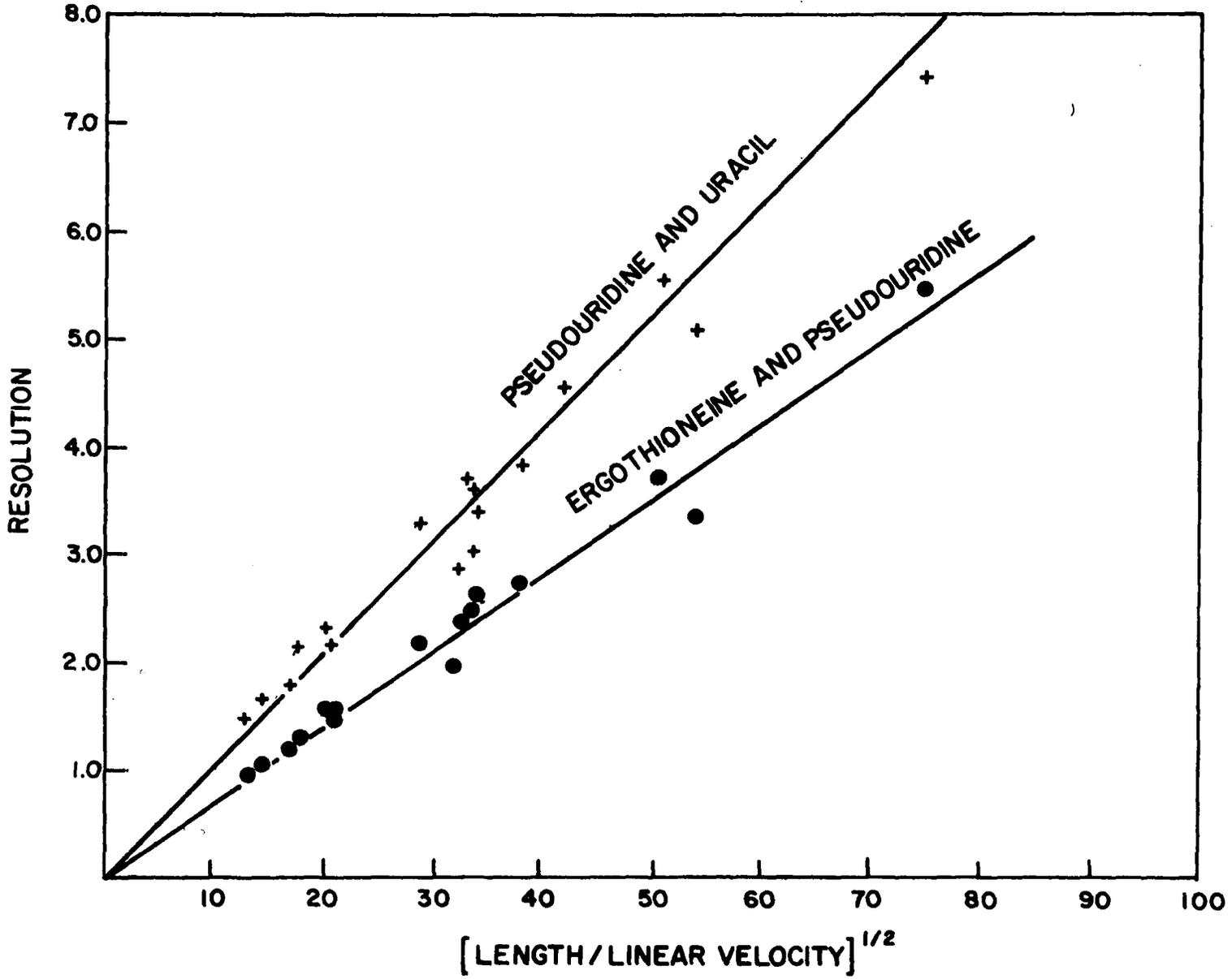


Fig. 12. Dependence of Chromatographic Resolution on Column Length and Linear Velocity of Eluent.

might be eluted between any two peaks. (Indeed, it is known that other compounds elute between the two pairs shown in Fig. 12). However, one can reach a point of diminishing returns when trying to improve resolution. It was the purpose of this investigation to determine where that point is. From plots such as the one in Fig. 12, it is possible to establish the minimum time required to separate two given compounds. This is done by multiplying the square of the abscissa at $R_s = 1.0$ by the ratio of the elution volume to the column geometric volume; that is,

$$t_{\min} = \left[\left(\frac{L}{U_0} \right)^{1/2} \right]^2_{R_s = 1.0} \times \frac{\bar{v}_2}{AL},$$

where A = cross-sectional area of the column. Thus, the separation of pseudouridine and uracil (Fig. 12) would require a minimum time of

$$(10)^2 \times \frac{9.5}{5.7} = 167 \text{ sec.}$$

Considering the above data and typical sewage effluent chromatograms, it became apparent that a shorter column operated at a higher linear velocity could be used effectively. Hence, a 0.45-cm-diam by 50-cm-long column was fabricated and tested at a linear velocity of 0.1 cm/sec. The results showed that sewage effluent samples would be analyzed in 6 to 8 hr with little, if any, loss of resolution. This column was then sent to the AWTR Laboratory for incorporation in their UV-Analyzer.

CATION-ANION COLUMNS IN SERIES

A coupled anion-cation exchange column system was devised¹⁵ which vastly improves the resolution of the UV-Analyzer. The improved resolution results from a greater separation between the compounds that elute early from the standard anion-exchange column. In order to optimize this coupled-column system for analysis of sewage samples, a sample of ORESP secondary effluent was analyzed using three different combinations of

0.45-cm-ID columns whose lengths totaled 100 cm. The results shown in Table 1 indicate that equal lengths of cation and anion exchange columns provide the best resolution.

OXIDATIVE DETECTOR

An oxidative system, which has been under development in the Body Fluids Analyses Program¹⁶ for use in monitoring the eluates from liquid chromatographs (Fig. 13), appeared to have potential as a monitor for dissolved aqueous pollutants. This system relies on the reduction of tetravalent cerium reagent to fluorescent, trivalent cerium by compounds in the column effluent and is thus capable of monitoring many non-uv-absorbing compounds. Although its primary application has been in the analysis of samples of human body fluids, it has also been used to analyze several sewage plant effluent samples for oxidizable compounds. The resulting chromatograms showed that this detector has a high potential for ion-exchange chromatography of polluted waters. One of these detectors was also installed on a small (~ 1%) side stream of the preparative column effluents, where it acted as a monitor for those fractions which contained oxidizable compounds.

When used as a column monitor in conjunction with a uv photometer, the cerate fluorescence monitor is placed immediately downstream from the photometer. The column effluent, after passing through the photometer, is continuously combined with an equal volumetric flow of the reagent [2.5×10^{-4} N Ce(IV) in 4 N sulfuric acid] in a small glass jet mixer. The reaction mixture then flows, with a 10-min residence time, through a coiled tube reactor immersed in boiling water. This stream passes through a flow fluorometer,¹⁷ shown schematically in Fig. 14. It consists of a fluorometer body, an electronic chassis, and a high-voltage power supply. The fluorometer body (Fig. 15) is a machined aluminum block which contains a low-pressure mercury lamp, a 254-nm interference primary filter, quartz tube flow-cell, two Corning 7-60 secondary filters, photomultiplier tube and housing, and a photoconductor for compensation of changes in lamp intensity. In

Table 1. RESOLUTION OF COUPLED ANION-CATION COLUMNS
 DURING CHROMATOGRAPHY OF ORESP SECONDARY
 SEWAGE EFFLUENT SAMPLES

Column length, cm		Resolution (No. of chromatographic peaks)
Anion	Cation	
75	25	40
50	50	45
25	75	35

26

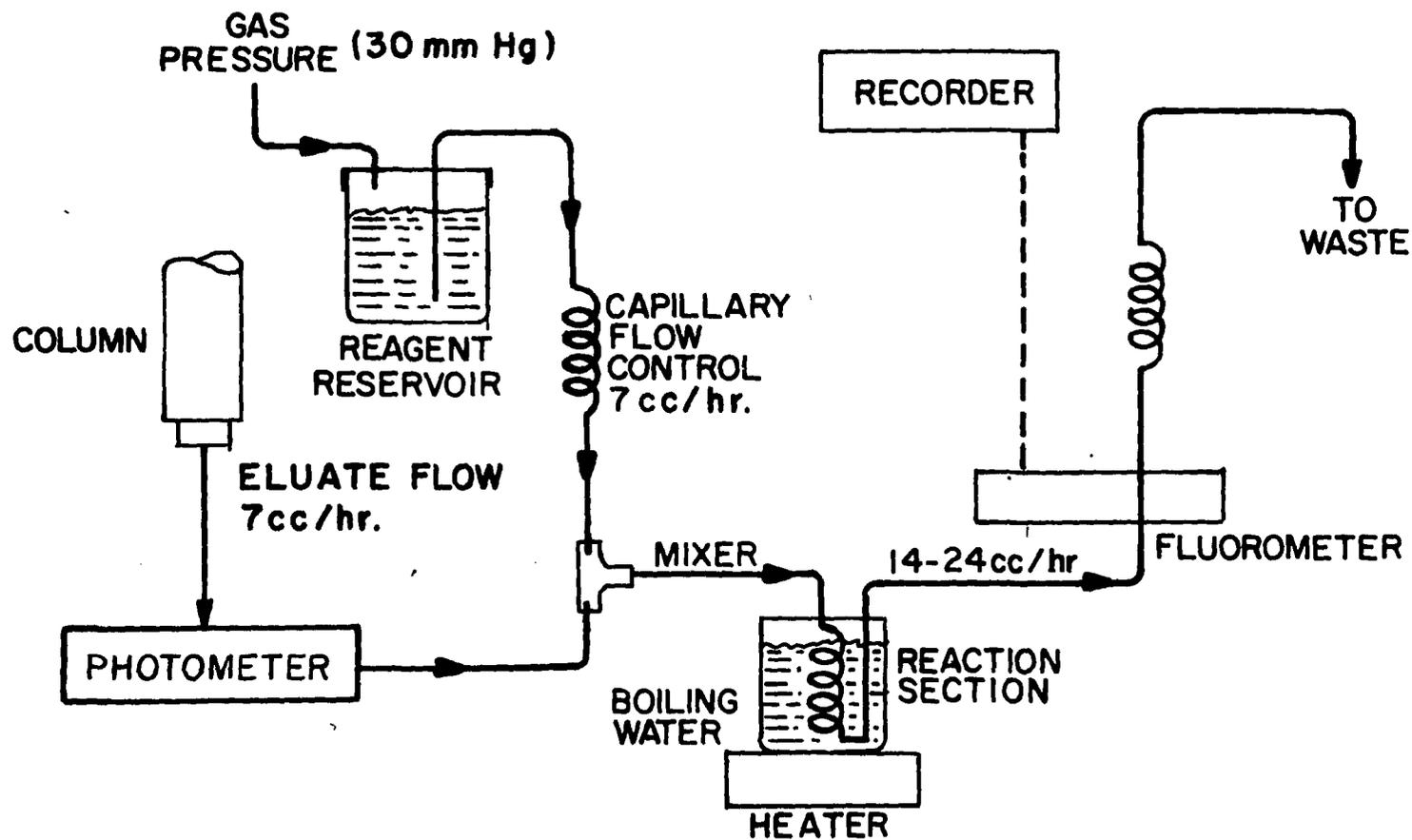


Fig. 13. Schematic of Cerate Oxidation Monitor.

27

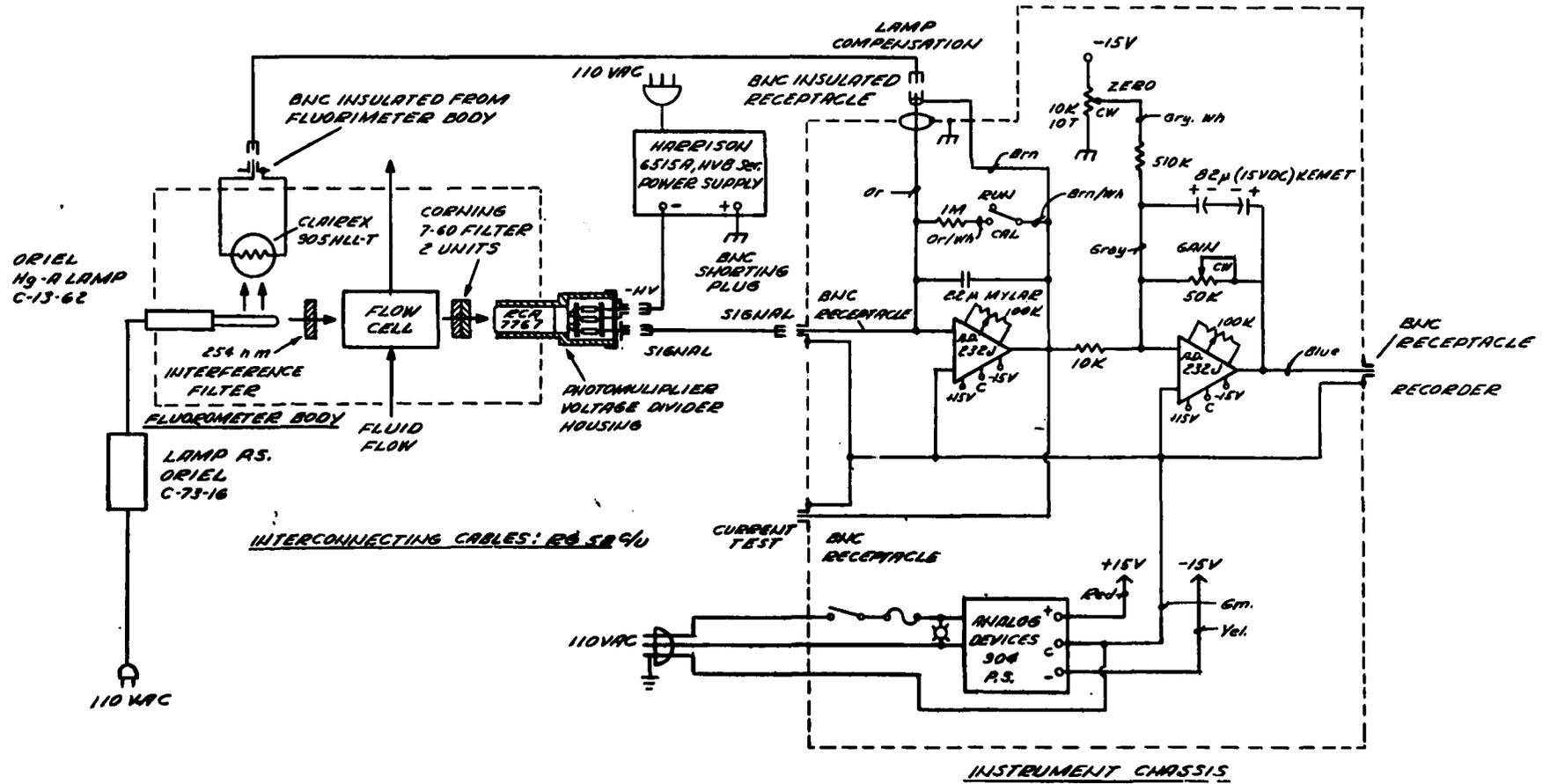


Fig. 14. Schematic of Flow Fluorometer.

28

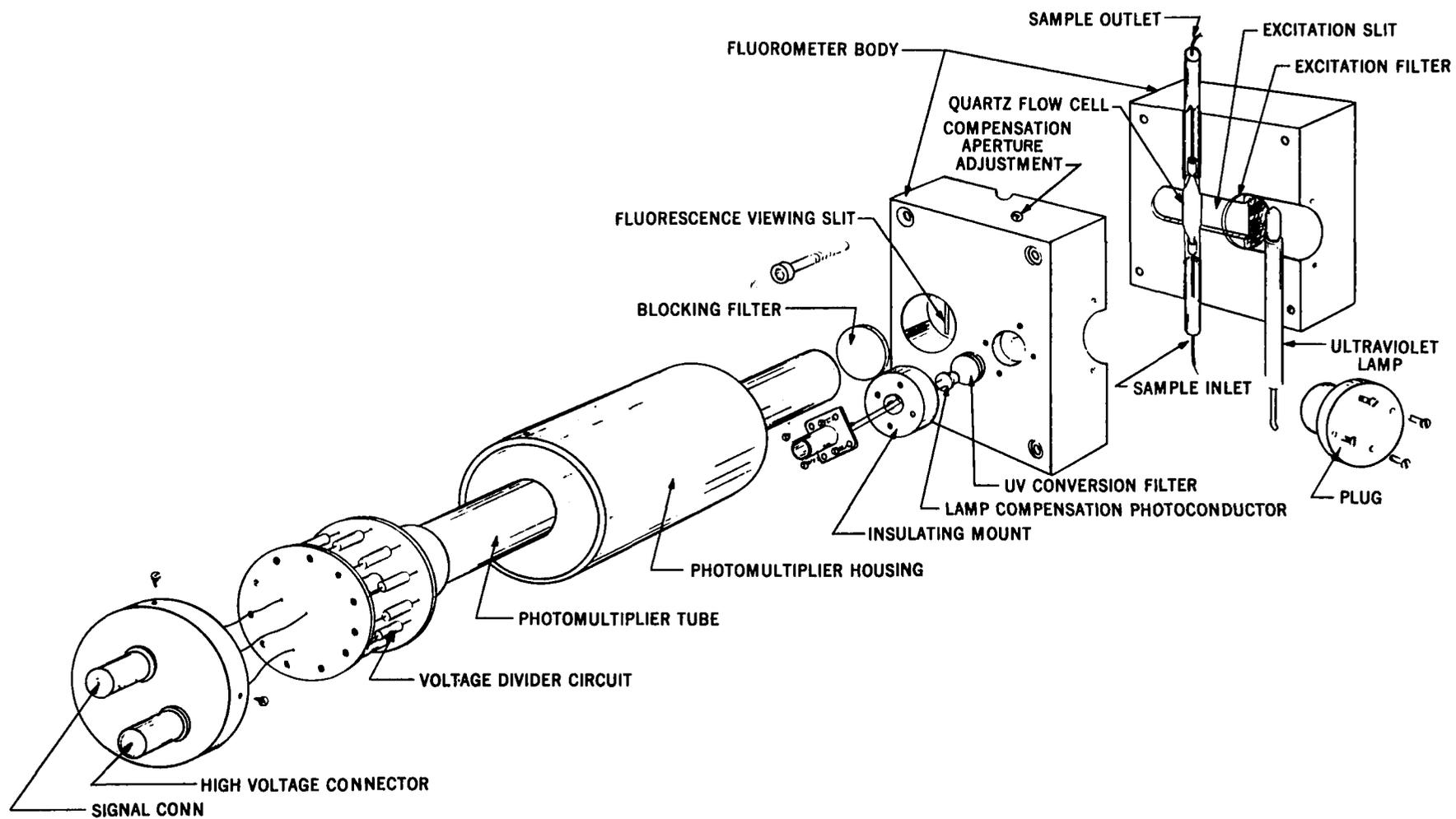


Fig. 15. Exploded View of Flow Fluorometer Body.

this situation, nature was exceptionally benevolent because the excitation maximum for trivalent cerium is very close to the most intense emission line of the low-pressure lamp, 254 nm, and the emission spectrum of Ce(III) corresponds almost precisely with the band-pass of the Corning 7-60 filters. These characteristics plus the photo response characteristics of the S-11 photomultiplier provided an exceedingly low background due to reflected light and allowed the construction of a very sensitive instrument from relatively inexpensive components. The calibration curve for this fluorometer with Ce^{3+} solutions is shown in Fig. 16. As can be seen, the fluorometer has a linear signal output up to about 2×10^{-4} M Ce^{3+} and a slight decrease in a slope up to 10^{-3} M. Although the sensitivity of this detector depends on the oxidizability of the substance being determined, moderately oxidizable compounds can be detected at levels of about 100 ng/ml in column effluents. Chromatograms of primary and secondary sewage effluents analyzed on the Mark III UV-Analyzer with an oxidative monitor are shown in Figs. 17 and 18.

OXYGEN DEMAND MONITOR

The cerate-fluorescence monitor, which was developed as an alternative detector system for liquid chromatography, has been adapted as a rapid, sensitive cerate oxidative monitor for measuring the COD of waters. The pollutants are oxidized with perchloratocerate reagent, and the resulting Ce(III) is determined fluorometrically. Analysis requires only a few minutes for determinations at levels as low as 100 μg oxygen demand per liter.

Cerate oxidimetry has been in general use in volumetric oxidimetry for several decades,¹⁸ and its applicability to the analysis of COD in polluted waters was suggested as early as 1941.¹⁹ For valid reasons, this method was not adopted as the standard in the United States; however, the intrinsic fluorescence of trivalent cerium coupled with recent advances in fluorescence analysis and cerate oxidimetry made reconsideration of cerate oxidation methods for COD analyses seem warranted.

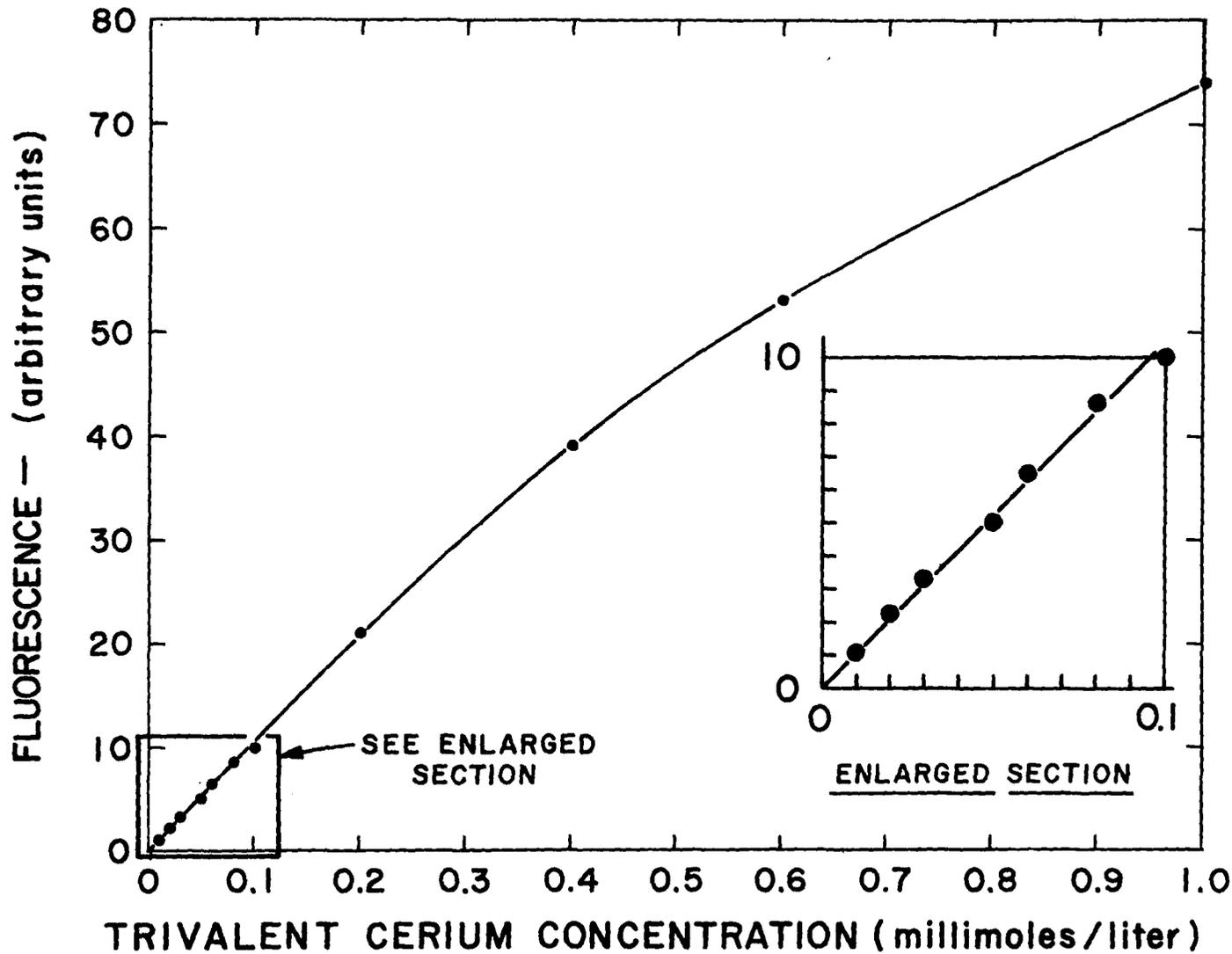


Fig. 16. Calibration Curve for Flow Fluorometer.

FLUOROMETER CALIBRATION CURVE

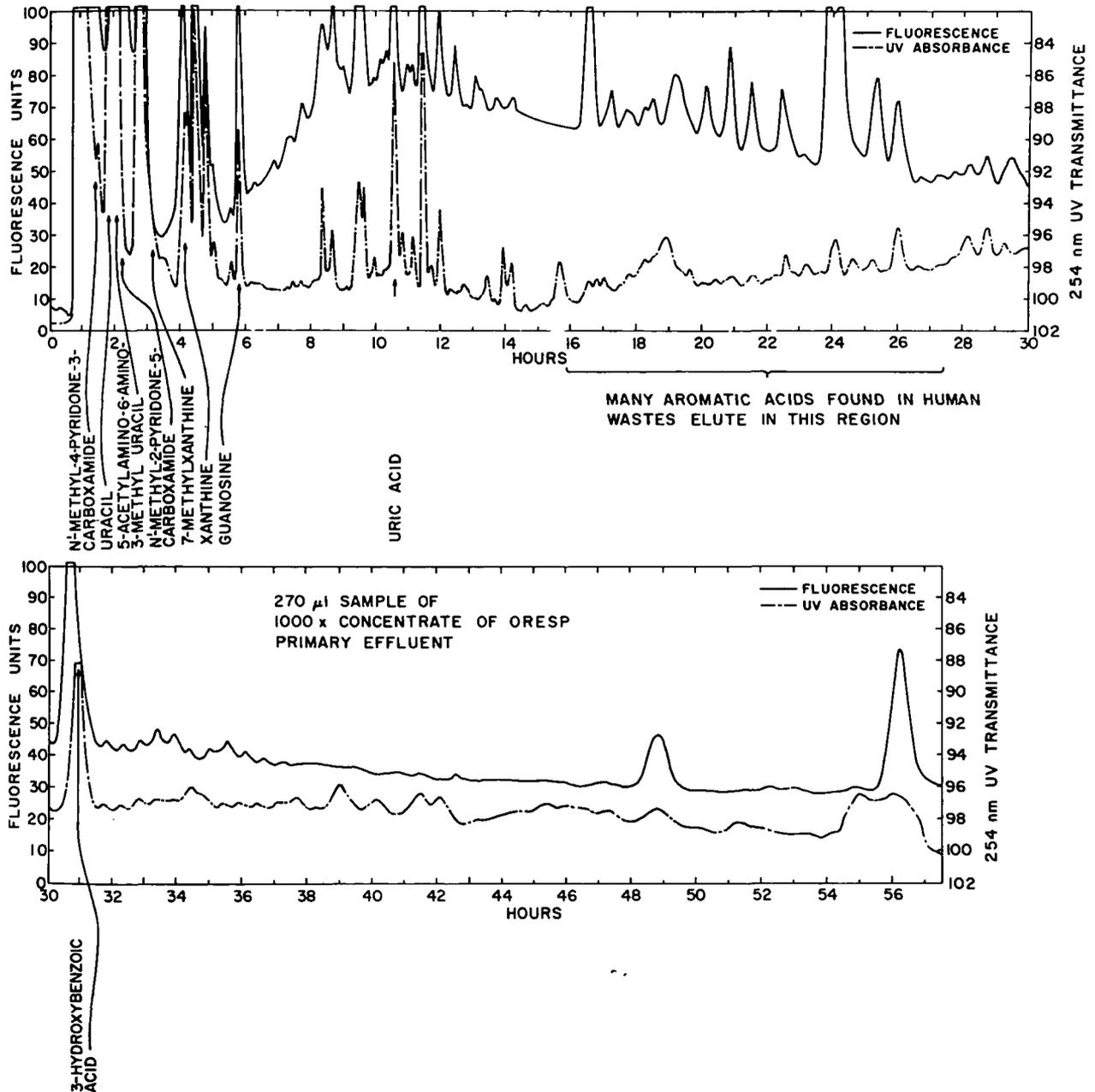


Fig. 17. Chromatogram of Primary Sewage Plant Effluent Analyzed by the UV-Analyzer with a Cerate Oxidative Monitor.

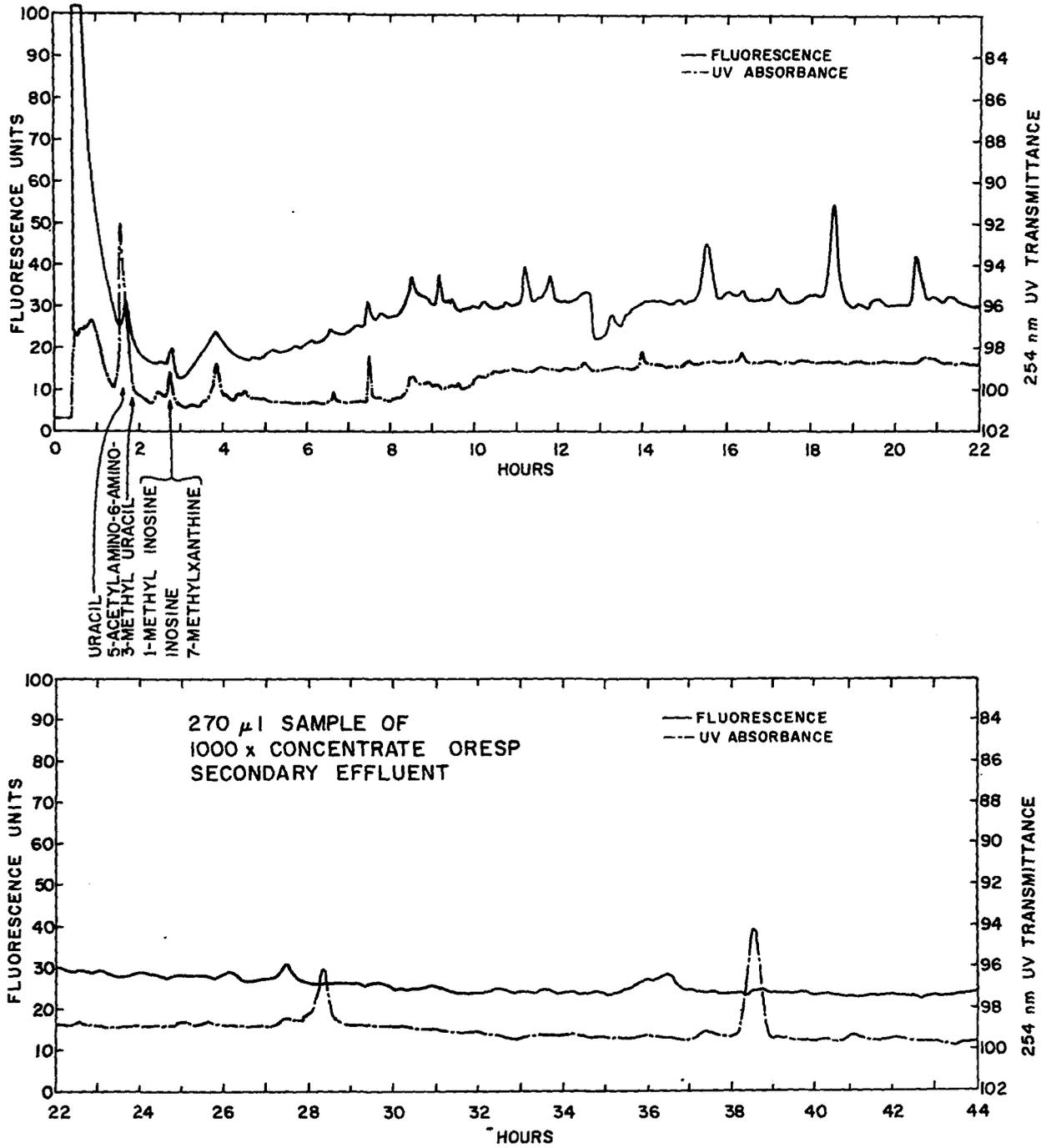


Fig. 18. Chromatogram of Secondary Sewage Plant Effluent Analyzed by the UV-Analyzer with a Cerate Oxidative Monitor.

Many of the organic compounds normally found in sewage and industrial wastes are not oxidized as efficiently with ceric sulfate (sulfatoceric acid) as with dichromic acid, particularly when silver is used as a catalyst with the dichromate. This is understandable when one compares the available oxidation potentials as shown in Table 2; as can also be seen from the table, perchloratoceric acid provides an oxidation potential approaching that of silver-catalyzed chromic acid and thus should also oxidize many of the same organic compounds.

An attractive feature of a cerate oxidation method is that the trivalent cerium which results from any redox reaction with compounds in the water sample is strongly fluorescent (Fig. 19). Concentrations of Ce(III) from 10^{-8} to 10^{-4} M can be readily determined fluorometrically by excitation with ultraviolet light (254 nm) and measuring the 90° light emission at 350 nm.²⁰ With this information in mind, a method which requires only a few minutes for measuring the COD of waters at levels as low as 100 μ g per liter was developed. This method is quite amenable to automation and can be used for continuous, remote stream analysis.

Several organic compounds representative of the various constituents of waste and natural waters were analyzed by this method as a means of testing its applicability. The classes of such compounds included carbohydrates, alcohols, amino acids, surfactants, straight-chain acids, volatiles, and others. In addition, samples of sewage plant effluents and a river water sample were analyzed.

The "perchloratoceric acid" method involves the mixing of the waste sample with 10^{-3} to 10^{-2} M perchloratoceric acid reagent and heating at 100°C for 5 min. The reagent concentration and the ratio of reagent to sample are selected so that about 10 to 50% of the Ce(IV) will be utilized. After this, the mixture is diluted to a total cerium concentration of about 10^{-3} M with cold 0.2 N sulfuric acid, which halts the reactions. The fluorescence of the mixture resulting from trivalent cerium is then measured.

Preliminary tests of the perchloratoceric acid method were carried out with conventional laboratory glassware and a Perkin-Elmer Model 203

Table 2. OXIDATION POTENTIALS AVAILABLE WITH VARIOUS OXIDANTS USED IN COD ANALYSIS

$\text{Cr}_2\text{O}_7^{2-}$	$\xleftrightarrow{18 \text{ M } \text{H}^+}$	Cr^{3+}	<-1.5 V
Ag^+	$\xleftrightarrow{\hspace{2cm}}$	Ag^{2+}	-1.98 V
Ce^{3+}	$\xleftrightarrow{\text{H}_2\text{SO}_4}$	Ce^{4+}	-1.42 V
Ce^{3+}	$\xleftrightarrow{\text{HClO}_4}$	Ce^{4+}	-1.70 to -1.87 V

ORNL DWG 72-8759

CERIUM(IV) + ELUTED COMPOUND →
(NONFLUORESCENT)

CERIUM(III) + OXIDIZED PRODUCTS
(FLUORESCENT)

FLUORESCENCE EXCITATION MAXIMUM: 260 nm

FLUORESCENCE EMISSION MAXIMUM: 350 nm

35

Fig. 19. Cerium Fluorescence Oxidimetry.

fluorescence spectrophotometer. However, once the general procedure was worked out, a continuous-flow apparatus of two pressurized reagent reservoirs, a sample pump (peristaltic), two jet mixers, a boiling water bath, a flow fluorometer, and a strip-chart recorder (Fig. 20) was assembled. This apparatus can be used as a continuous stream monitor or, as described here, as a discrete sample analyzer. In the discrete mode of operation, it is capable of analyzing about ten samples per hour.

Pressurizing the reagent reservoirs is a very simple but reliable method for metering a low flow of the reagents. Using a simple gas pressure regulator and a length of capillary tubing provides a constant flow in the range of a few milliliters per hour.²¹ The sample stream and the perchloratoceric acid stream are mixed in the first jet mixer, the sample entering through the jet and the reagent entering through the annulus. The cross sections of the jet and annulus are designed so that the linear velocities of the two fluids at the jet exit are nearly equal, thus minimizing back-mixing. From this mixer the stream flows through a coil of thin-walled Teflon tubing immersed in a boiling-water bath. The residence time in the bath is 5 min. This mixture is then diluted tenfold in the second jet mixer with 0.2 N sulfuric acid, which effectively stops the reaction. The concentration of trivalent cerium in this diluted stream is continuously measured by the flow fluorometer described in the previous sections and recorded on a strip-chart.

Three or more samples of nine organic compounds dissolved in triply distilled water and three samples of polluted waters were run using this apparatus in the discrete sample mode. A blank of triply distilled water was run between each sample. Periodically, a calibration standard containing a known amount of trivalent cerium in distilled water was also analyzed.

A quantity of each organic compound which would require about 10 mg of oxygen for complete combustion to carbon dioxide and water is weighed out and dissolved in distilled water. At approximately 5-min intervals, the sample tube is placed into one of these solutions and a 1-min sample

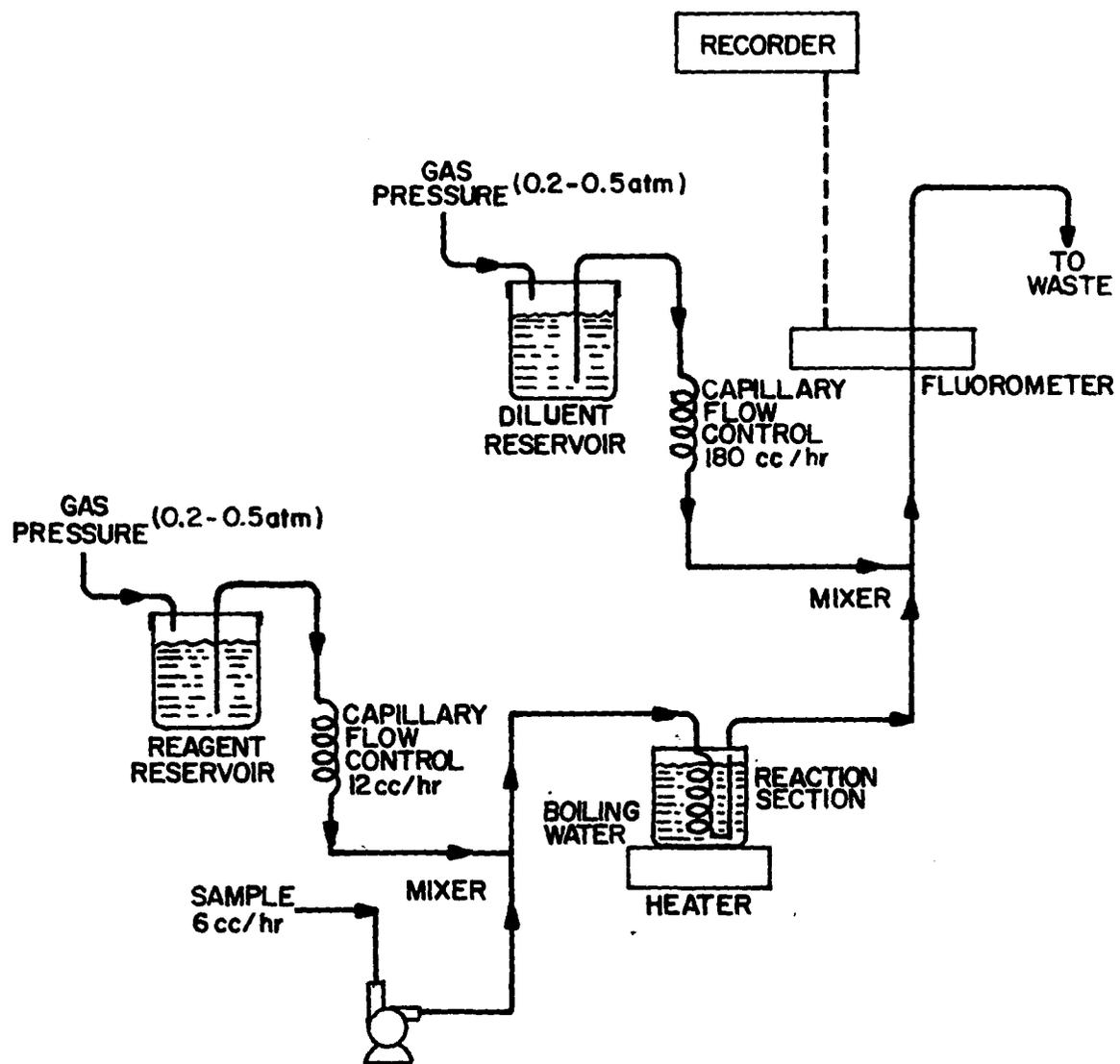


Fig. 20. Schematic of Continuous Chemical Oxygen Demand Analyzer.

is withdrawn. During the remainder of the cycle, the sample tube is inserted into distilled water. The COD of the sample is indicated by the height of a peak on the strip-chart recorder 10 min after sampling. Once each day, usually at the start of the operation, the system is calibrated with three samples of a Ce(III) solution of known concentration (1 to 2×10^{-5} M or 8 to 16 mg oxygen demand per liter). An example of the recorder trace is shown in Fig. 21. The results of several series of runs using the selected organic compounds and pollution samples are shown in Table 3.

Although some compounds were not as completely oxidized as with the catalyzed chromic acid, all of the samples with the exception of acetone, benzene, and pyridine were oxidized to a sufficient level after 5 min at 100°C. Note that, under these conditions, pyridine and benzene were oxidized to a greater extent than by the Standard Methods²² procedure. If greater levels of oxidation are required, longer reaction times or a higher temperature can be used. Temperatures above 125°C should not be used because rapid degradation of the cerate occurs.

The results obtained from analyses of polluted water samples show that the perchloratoceric method is indeed rapid and sensitive, and should be a useful complement to the standard method. A proportionality factor relating this method to the standard method may be determined by running several samples by each procedure. Such a factor would probably be necessary in cases where samples contain significant quantities of compounds which are oxidized to a lesser extent than with the standard method. This factor can then be applied to later samples run by the perchloratoceric acid method, if its accuracy is ensured by an occasional check by the standard method.

The major advantages of this method are rapidity, sensitivity, and little need for manual attention. Even though no effort has been made to optimize conditions for maximum sample throughput, 10 to 12 samples per hour can be analyzed on the apparatus described here. With the addition of an automatic sampler, operator attention would be reduced to about 1 hr per day for charging the reagent

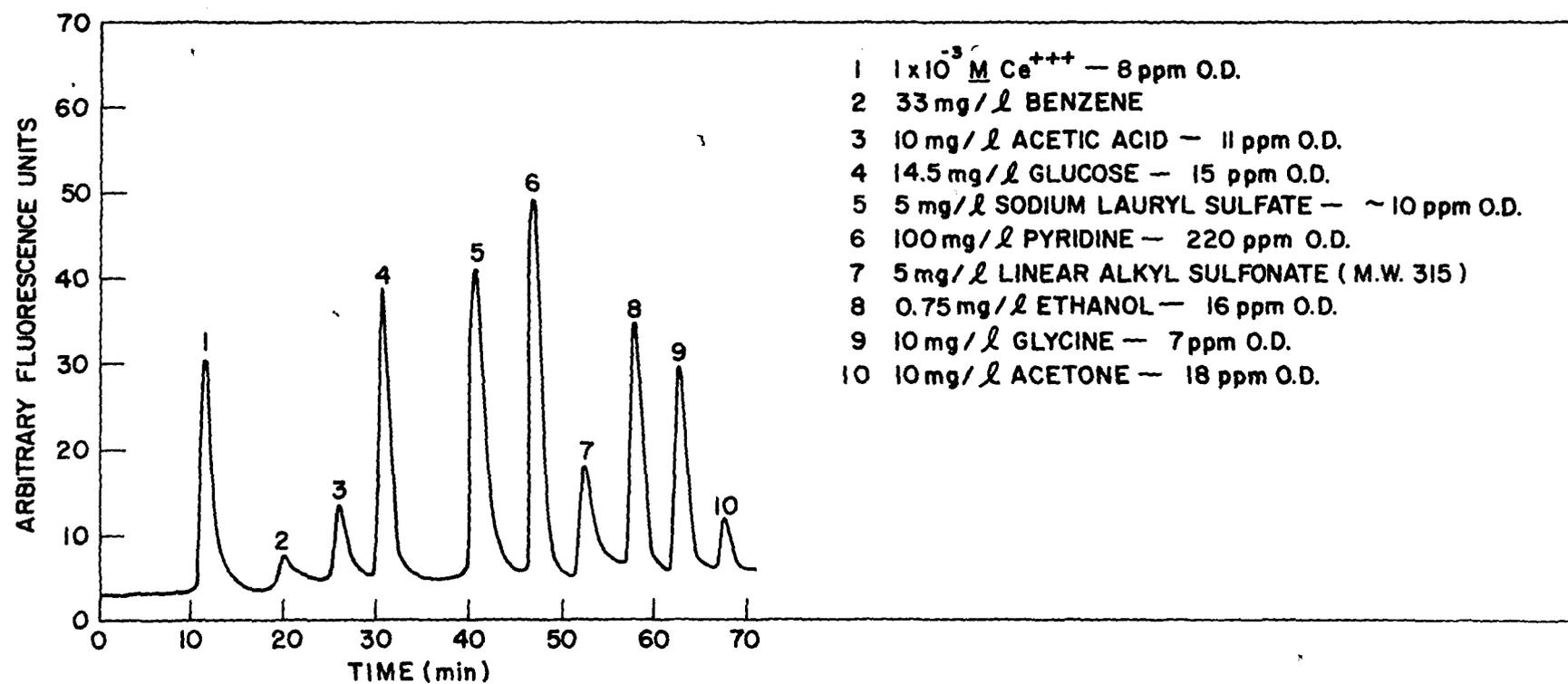


Fig. 21. Recorder Trace from Continuous COD Analyzer.

Table 3. OXIDATION OF ORGANIC COMPOUNDS AND POLLUTED WATERS
BY PERCHLORATOCERIC ACID COD METHOD

of

Sample	Concentration, mg/liter	Number of determinations	Oxygen consumed, mg/liter		% of theoretical COD
			Average	Range	
Acetic acid	10	4	6.1	5.2 - 7.4	57
Acetone	8	5	3.2	3.0 - 3.3	18
Benzene	3.3	4	2.8	2.4 - 3.5	26
Ethanol	7.5	3	11.0	10.8 - 11.1	69
Glucose	14.5	4	17.4	15.9 - 17.9	112
Glycine	10	4	3.0	2.8 - 3.2	46
Linear alkyl sulfonate	5	5	6.9	6.3 - 7.4	105
Pyridine	10	6	3.5	3.2 - 3.6	16
Sodium lauryl sulfate	5	5	13.4	12.8 - 14.6	135
Sewage plant effluent (industrial plant)	19 COD	5	16.5	14.8 - 18.1	
Secondary sewage plant effluent (Oak Ridge, Tenn.)	6 BOD ₅	5	25.9	24.2 - 27.4	
Clinch River water	8 COD	3	7.8	7.5 - 8.0	

reservoirs and samples and for reading the strip chart. The disadvantages of the method are its relative low dynamic range (less than two decades) for a given set of conditions and the necessity for running a standard.

SECTION V

IDENTIFICATION OF STABLE ORGANIC POLLUTANTS

Thirteen samples of "domestic" sewage plant effluents, five from the primary stage and eight from the secondary stage, were obtained and concentrated during this investigation. Fifty-six compounds were identified in the primary effluent, while thirteen compounds were identified in secondary effluent.

IDENTIFICATION TECHNIQUES

The preparation of samples for analysis, the separation of constituents, and the application of analytical methods to separate fractions involve an integrated and complex series of manipulations and investigative techniques.²³ A brief description of this process is given below.

Preparation of Sewage Effluent Samples for Analysis

Concentration of sewage effluents by factors up to 3000 is necessary prior to analysis. Of the several concentration techniques considered, low-temperature distillation described in Section IV was selected as the most desirable. One sample was concentrated on XAD-4 macroreticular resin; however, since this technique gave disappointingly poor results, it was not investigated further.

Anion Exchange Chromatography

The major effort during the program was concerned with the identification of uv-absorbing molecular components of samples of sewage plant effluents. The UV-Analyzer used for the analysis of these samples employs a 0.30 cm x 150 cm column and is restricted to sample volumes of less than 1 ml. Thus, repeated 40-hr chromatographic runs would be required to obtain sufficient quantities of many of the molecular constituents for identification purposes. A preparatory system with several times the capacity of the analytical system has been used to

increase the quantities of compounds collected per run. This system, which is coupled to a fraction collector, is capable of chromatographing 5 ml of sample with a resolution approaching that of the smaller analytical column. By combining several adjacent 5- or 10-min fractions from a single chromatographic run, sufficient quantities of many chromatographed compounds were collected to allow identification by suitable analytical techniques. Comparative data on column sizes, resin, and operating parameters for the analytical and preparative systems are listed in Table 4.

Rechromatography Using a Cation Exchange Resin

For most separations, chromatographic peaks that eluted earlier than uric acid were further purified using a 0.62 cm x 150 cm jacketed column packed with cation exchange resin (Aminex A-7, Bio-Rad Laboratories, Richmond, California). The fractions corresponding to individual chromatographic peaks from the preparative anion-exchange system were lyophilized and then redissolved in 0.3 M ammonium acetate--acetic acid buffer (pH, 4.65). Each fraction was loaded on the column and chromatographed at 50°C using the same buffer that was used for dissolution. The effluent was monitored at 254 nm with an ultraviolet photometer. In addition to purification, the use of cation exchange established a second characteristic elution position for individual organic compounds, providing an additional means of identification.

Preparation of Fractions for Analyses

Adjacent 5- or 10-min eluate fractions corresponding to individual chromatographic peaks from either the anion or cation exchange separations were combined, frozen at -70°C, and then lyophilized in a freeze-dryer at -60°C and a pressure of 50 microns for removal of the ammonium acetate--acetic acid buffer. The samples were then dissolved in 2 to 3 ml of spectroscopic-grade methanol and subjected to uv spectral, gas chromatographic, and mass spectral analyses.

Table 4. PHYSICAL CHARACTERISTICS AND OPERATING PARAMETERS FOR THE ANALYTICAL AND PREPARATIVE ANION EXCHANGE COLUMNS

Parameters	Columns ^a	
	Analytical	Preparative
Column dimensions, cm	0.30 x 150	0.94 x 150
Resin	Bio-Rad A-27	Bio-Rad A-27
Resin particle size, μ	8-12	12-15
Sample size, ml	0.28	5
Run time, hr	40	42
Temperature program	Ambient, increasing to 60°C after 11.0 hr	Ambient, increasing to 60°C after 11.5 hr
Operating pressure, psig	1900	1600
Flow rate, ml/hr	10	88
Eluent	Ammonium acetate--acetic acid buffer, pH 4.44	Ammonium acetate--acetic acid buffer, pH 4.44
Concentration gradient	0.015 <u>M</u> - 6.0 <u>M</u>	0.015 <u>M</u> - 6.0 <u>M</u>
Gradient generator	2-chamber ^b	9-chamber ^c

^aColumns were constructed of stainless steel and jacketed for temperature control.

^bOne chamber was filled with 0.015 M buffer, and the other was filled with 6.0 M buffer.

^cTwo chambers contained 874 ml of 0.015 M, two chambers contained 851 ml of 1.0 M, two chambers contained 828 ml of 4.0 M, and three chambers contained 1208 ml of 6.0 M buffer.

Ultraviolet Spectrometry

Ultraviolet spectra were obtained for each of the collected fractions in methanol solution from 320 to 210 nm on a Beckman DB-G recording spectrophotometer, and compared with uv spectra of reference compounds obtained in the same manner.

Gas Chromatography

A 1-ml aliquot of the methanol solution of the fraction to be identified was evaporated to dryness under a stream of dry nitrogen. Trimethylsilyl (TMS) derivatives were then formed by adding 50 to 100 μ l of dry pyridine (Pierce Chemical Company) and 50 to 100 μ l of bis(trimethylsilyl)-trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (Regis Chemical Company) and heating the reaction mixture overnight at 50°C. Separate aliquots (4 μ l) of the reaction mixture were injected directly onto the two columns of a MicroTek MT-220 gas chromatograph equipped with dual flame ionization detectors and dual electrometers. The two columns (6-ft x 0.25-in.-O.D. Pyrex tubing packed with 3% OV-1 or OV-17 on 80/100 mesh Chromosorb W-HP) were placed in the same oven, which was programmed for a temperature increase from 100 to 325°C at the rate of 10°C/min. The helium carrier gas flow rate was 85 cc/min for each column.

Mass Spectrometry

Mass spectrometry was performed on 2- μ l aliquots of the TMS-derivatized samples described above or on nonderivatized samples. In each case, 1-ml aliquots of the methanol solution of the samples were reduced in volume, transferred to the glass insert of the mass spectrometer probe, and evaporated to dryness in a vacuum desiccator. In the case of samples containing compounds that eluted later than uric acid in the anion exchange chromatogram, a drop of 6 N HCl was added prior to evaporation to ensure that the compounds remained in the acidic form.

Three mass spectrometers were available for use. The TMS-derivatized sample was run routinely on a Finnigan model 3000 gas chromatograph--mass spectrometer using a 3-ft x 0.25-in. glass column packed with 2% Dexsil on 80/100 mesh Chromosorb W-HP for additional resolution. This usually provided data concerning the molecular weights of the constituents and the number of active hydrogen atoms per molecule. Comparison of the fragmentation pattern with that for reference standards was necessary for absolute identification.

A second mass spectrometer, constructed at Oak Ridge National Laboratory, was used for low-resolution spectra ($N/\Delta m \approx 800$). This instrument was a single-focusing mass spectrometer with a 12-in. radius, 90°-sector magnet, operating at an accelerating voltage of 4 kV. Solid samples were introduced via a vacuum-lock direct insertion probe operating at the lowest temperature required to produce a suitable spectrum, generally between 150 and 250°C and the spectra were recorded with electron energies of 70 and 15 eV. These spectra were then manually transferred from the oscillograph output to punched cards for subsequent computer calculation of metastable data, relative intensities, and plotting.

A Varian Aerograph model 1200 gas chromatograph was coupled with this instrument for gas chromatographic--mass spectrometric (GC-MS) studies. The gas chromatograph column consisted of a 3-ft x 0.25-in. glass tube packed with 2% Dexsil on 80/100 Chromosorb W-HP. Effluent from the column was divided, with approximately 10% being directed to the flame ionization (FI) detector. The remaining effluent was fed through a heated stainless steel capillary to the helium separator, contained in a separate oven. The separator was a Biemann-Watson type, constructed of porous stainless steel tubing as described by Krueger and McCloskey.²⁴ A micro-needle valve located between the chromatograph and separator was adjusted to allow operation of the FI detector at atmospheric pressure. The enriched sample passed from the separator through a cutoff valve and heated stainless steel capillary into the ionizing region of the mass

spectrometer. At a helium carrier flow rate of 30 ml/min, the separator operated at approximately 0.5 torr, and the source can at $< 1 \times 10^{-5}$ torr (uncorrected). For normal operation, the column temperature was programmed such that the GC resolution was adequate but retention time for the peak of interest was minimized. The separator and interconnecting tubing were maintained at 250°C. The mass spectra for the GC-MS system were obtained using an ionizing electron energy of 30 eV and manually transferred from the recording of the oscillograph to punched cards.

High-resolution spectra were obtained using an instrument which was constructed at the Oak Ridge National Laboratory. This instrument had a Nier Johnson configuration, with a 17-in. radius and 90°-sector magnetic stage. A 14-stage electron multiplier served as the collector, and its output was obtained in the form of an oscillogram of the integrated ion current. An ion accelerating voltage of 5000 V and an ionizing electron energy of 70 eV were used in routine operation. Samples were introduced by means of a direct inlet probe, while the mass standard perfluorokerosene was introduced from a reservoir through an adjustable leak. An IBM 1130 computer was used to acquire data from the spectrometer, to process the data, and to print the exact masses and possible empirical formulas of selected peaks.

Fluorescence Spectrometry

The use of fluorescence spectrometry, which might prove advantageous as an identification aid, was explored with several reference compounds and used in the identification of indican. Although it did not conclusively prove the existence of indican (this was primarily established by anion exchange position and gas chromatographic MU values), it did show that several other fluorescent compounds were present in the eluate fraction, and that indican was a minor constituent of this fraction. The extreme sensitivity of fluorescence spectrometry should prove useful in detecting constituents present at less than microgram-per-liter concentrations.

Example of Typical Identification Data

The information which resulted in the identification of guanosine is presented as an example of the identification data and

procedure. Eluate fraction 95 from the chromatographic separation of SPJ-1 concentrate of primary effluent was further chromatographed on the cation exchange system. The elution positions determined for both the original anion exchange separation and the cation exchange separation suggested that the unknown might be guanosine. The fraction collected from the cation separation was then lyophilized, and uv absorbance scans from 220 to 320 nm of the methanol solution were obtained for the residue dissolved in methanol (Fig. 22). Comparison of these scans with spectra of a reference standard (Fig. 23) further supported the identification of guanosine. The spectra of the unknown determined at different pH values (Fig. 22) showed a spectral shift at alkaline pH similar to that of guanosine. The methanol solution was evaporated under nitrogen gas and derivatized in preparation for gas chromatography. Retention values determined for the unknown compared very well with those for the guanosine reference standard (Table 5). The gas chromatograms for the OV-17 column are shown in Fig. 24. Absolute confirmation as guanosine was obtained from the mass spectra as determined by the low-resolution mass spectrograph. Figure 25 shows the mass spectra of the unknown offset 0.5 mass unit and superimposed on the mass spectra of the reference standard.

SAMPLE STATUS

Thirteen effluent samples were taken from a domestic sewage plant, concentrated, and separated on a preparative-scale anion exchange system. Five of these were of primary effluents, some of which were chlorinated; the remaining eight were of secondary effluents, also both unchlorinated and chlorinated (Table 6). The status of analytical work on these samples at the close of the report period is given in Table 7. One sample of primary effluent and one sample of secondary effluent were involved in the ^{36}Cl tracer chlorination study described in Section VI. Two primary samples were taken for identification of carbohydrates, while the others were obtained for identification of uv-absorbing and other organic compounds.

67

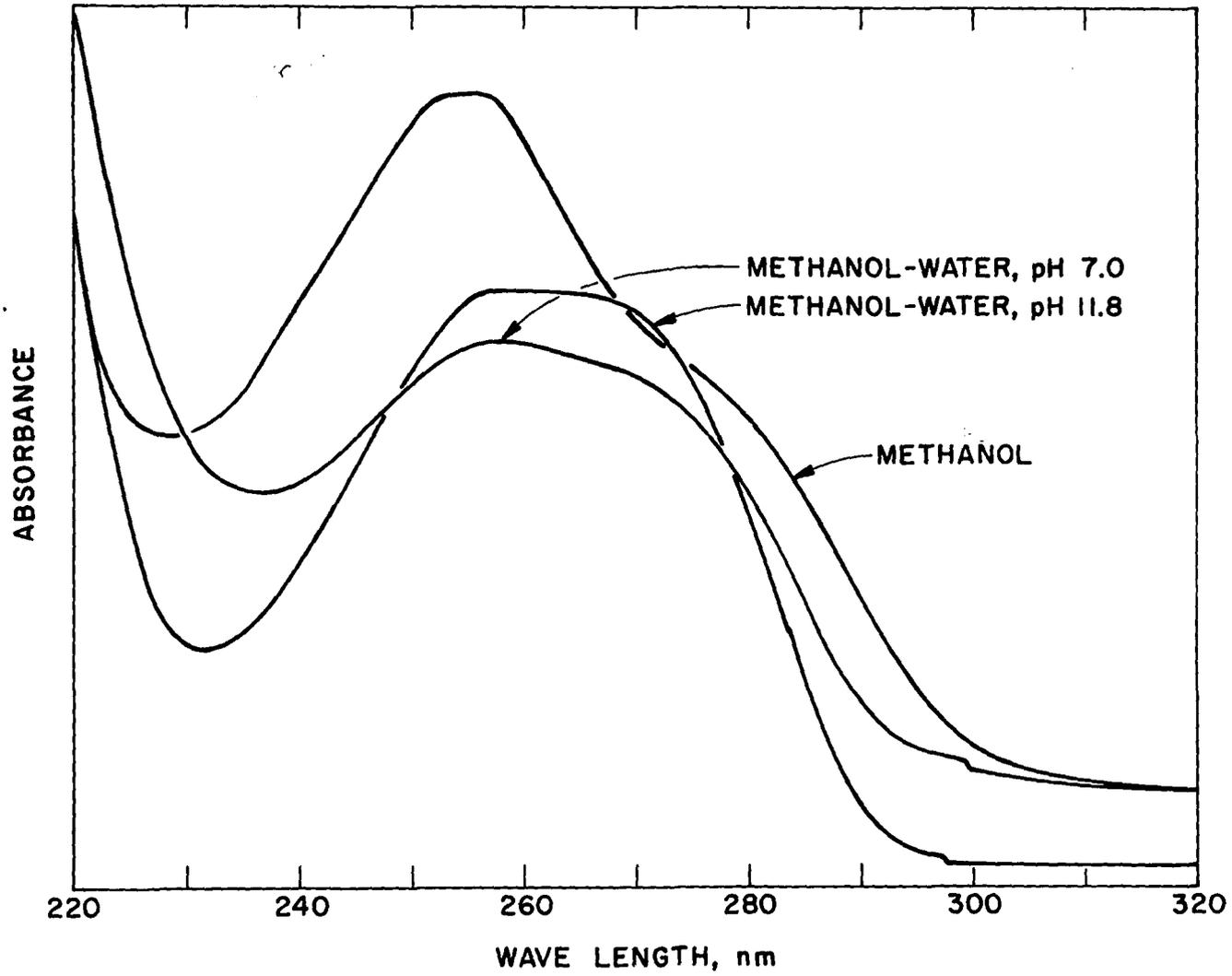


Fig. 22. Ultraviolet Absorption Spectra of Unknown Sample Taken at Different pH Values.

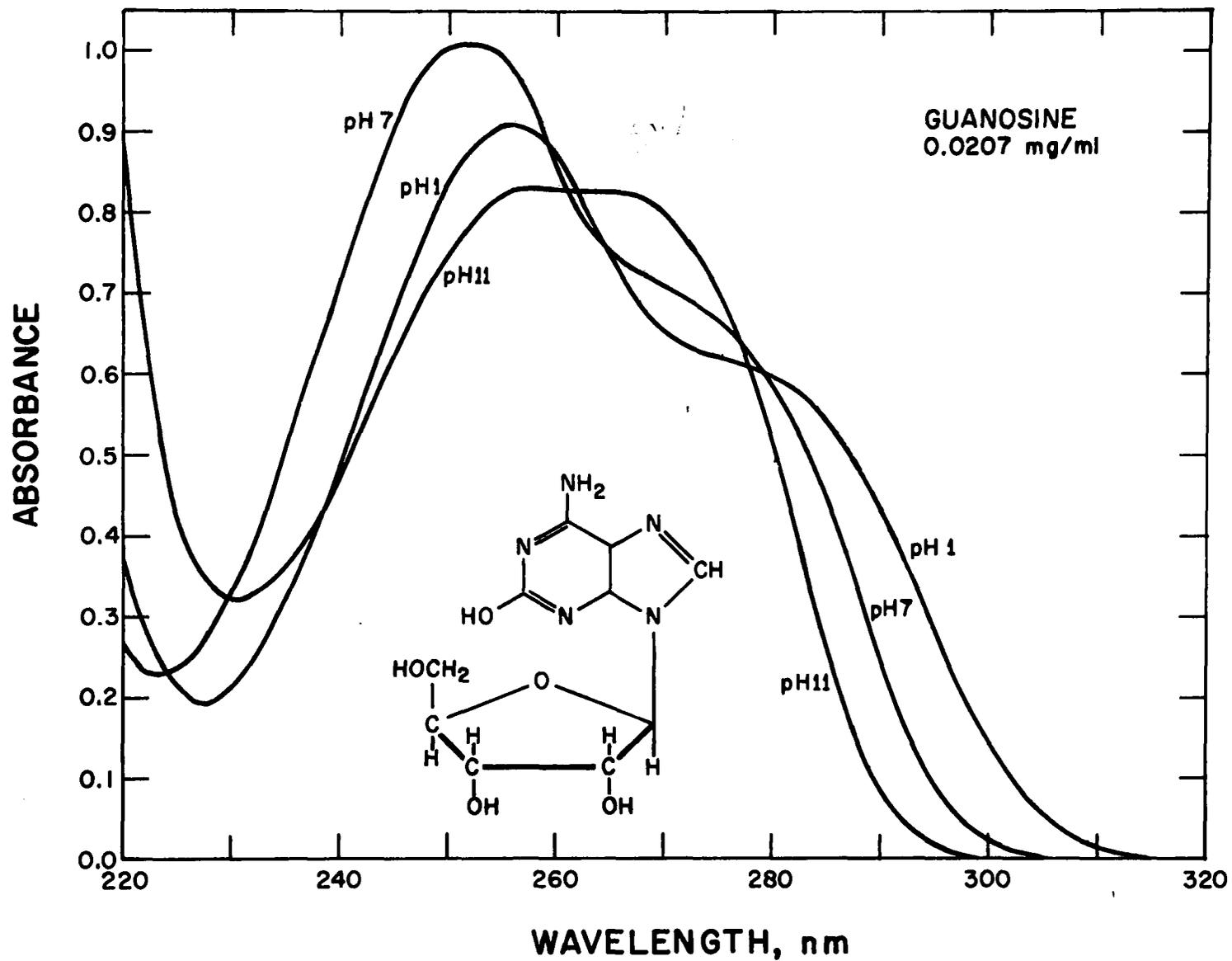


Fig. 23. Ultraviolet Absorption Spectra of Guanosine Taken at Different pH Values.

(Data from Circular OR-10 Sixth Printing, 1969, Pabst Laboratories, Division of Pabst Brewing Co.)

Table 5. GUANOSINE IDENTIFICATION DATA

	Guanosine, SPJ 1-95-2 unknown	Guanosine, reference standard
Anion exchange elution position, hr	8.3 (prep.)	7.5 (urine, analyt.)
Cation exchange elution position, ml	13.8	13.8
Ultraviolet spectra	255 (max), 275 (sh)	254 (max), 275 (sh)
Gas chromatographic retention value, M.U.	28.00, 29.54	28.00, 29.42
Mass spectra		
Molecular weight, TMS derivative	643	643
Molecular weight, minus TMS groups	283	283
Major fragments, m/e	245, 324, 368, 410, 556, 571, 628	245, 324, 268, 410, 556, 571, 628

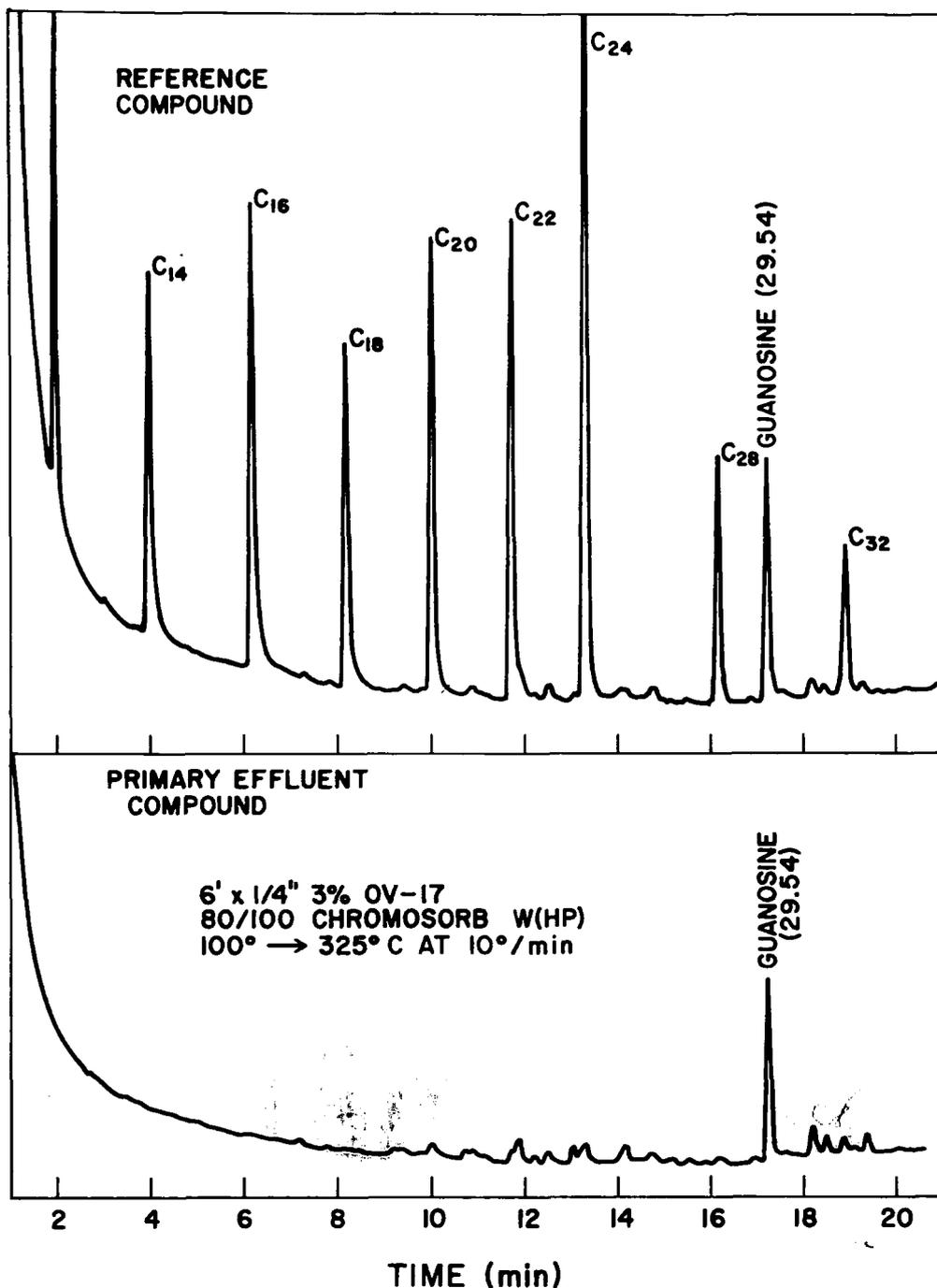


Fig. 24. Gas Chromatograms of TMS Derivatives of Reference Compound (Guanosine) and Liquid Chromatographic Fraction from Primary Sewage Treatment Plant Effluent.

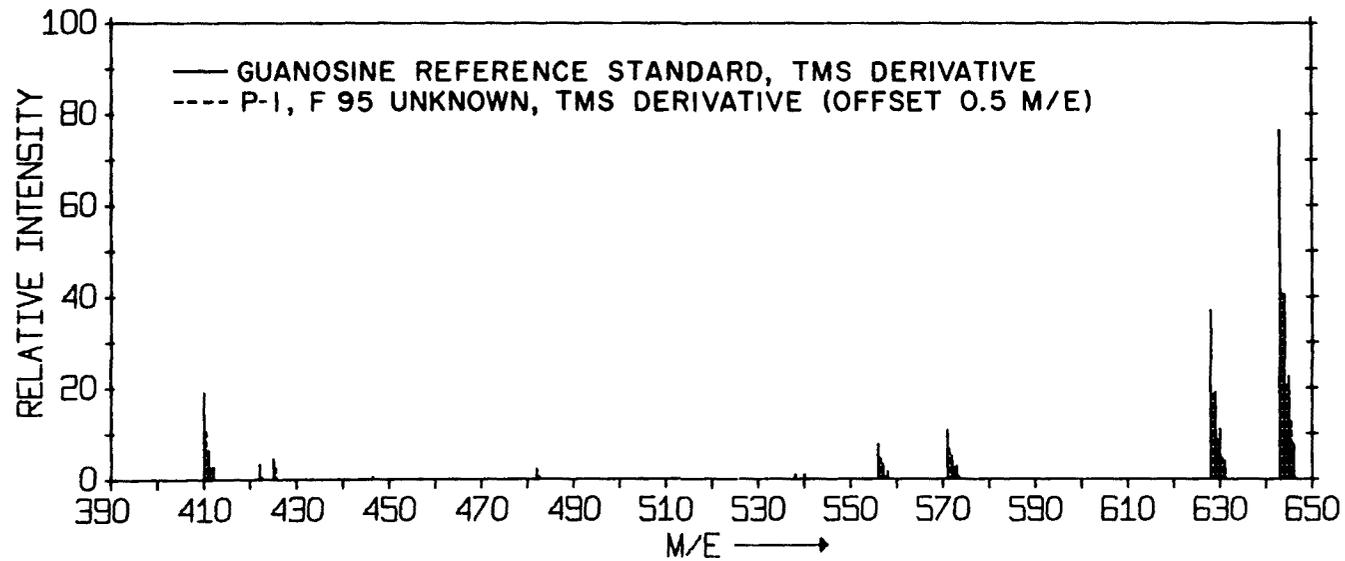
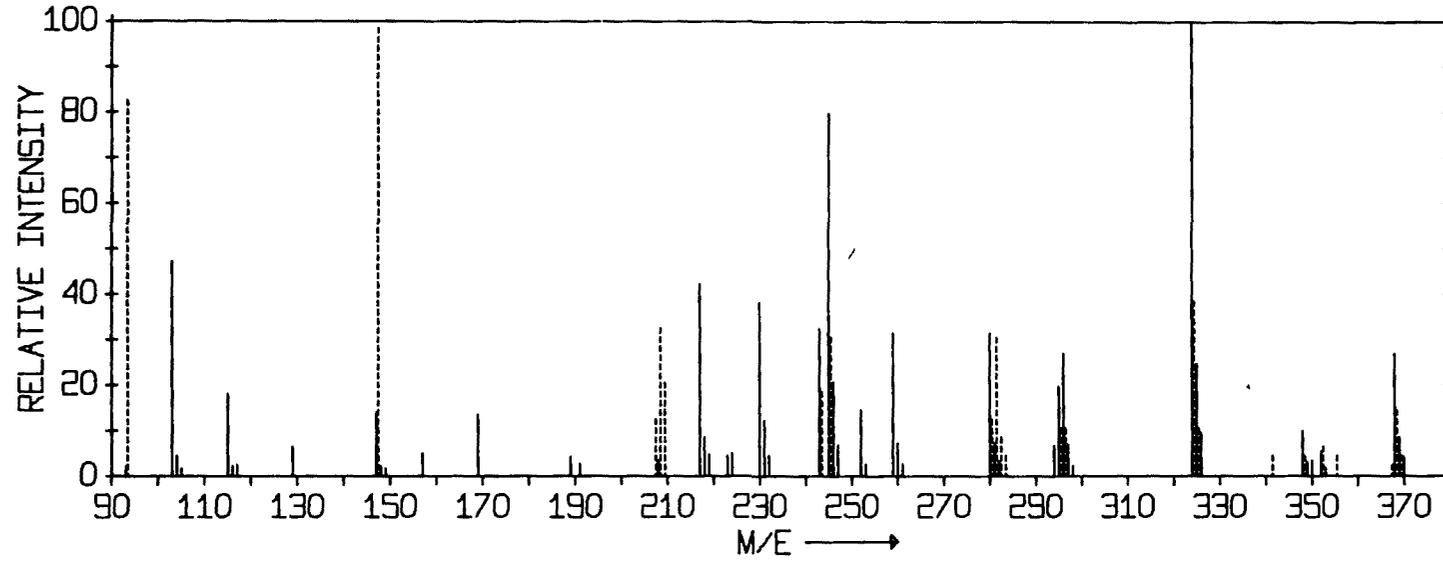


Fig. 25. Mass Spectra of TMS Derivatives of the Reference Compound (Guanosine) and Liquid Chromatographic Fraction from Primary Sewage Treatment Plant Effluent.

Table 6. DESCRIPTION OF SAMPLES

Sample	Description
SPJ-1	ORESP primary effluent (10-5-71).
SPJ-2	ORESP primary effluent (10-5-71) chlorinated in the laboratory.
SPJ-3	ORWSP chlorinated primary effluent (10-28-71); 2 ppm residual chlorine (orthotolidine).
SPJ-4	ORESP secondary effluent (11-22-71).
SPJ-5	ORESP chlorinated secondary effluent (11-22-71); 0.5 ppm residual chlorine (orthotolidine).
SPJ-6	ORESP secondary effluent (1-18-72).
SPJ-7	ORESP chlorinated secondary effluent (1-18-72); 0.75 ppm residual chlorine.
SPJ-8	ORWSP chlorinated primary effluent (2-23-72); 0.5 ppm residual chlorine (orthotolidine).
SPJ-9	ORESP secondary effluent (4-19-72).
SPJ-10	ORESP chlorinated secondary effluent (4-19-72); 1 ppm residual chlorine (orthotolidine).
SPJ-11	Composite of SPJ-3 and SPJ-8.
SPK-1	ORESP primary effluent (3-1-72) for carbohydrate determination.
SPK-2	ORESP secondary effluent (4-24-72) for carbohydrate determination.
SPL-1	ORESP secondary effluent (12-12-73).
H-1	ORWSP primary effluent (2-23-72) chlorinated in the laboratory with ^{36}Cl tracer as Cl_2 gas to approximately 2 ppm residual chlorine (orthotolidine).
H-2	SPJ-9 chlorinated in the laboratory with ^{36}Cl tracer as Cl_2 gas to 1.0 ppm residual chlorine (orthotolidine).
H-5	SPJ-9 spiked with ^{36}Cl tracer as chloride.
H-6	SPJ-9 chlorinated in the laboratory with ^{36}Cl tracer as Cl_2 gas to 1.0 ppm residual chlorine (orthotolidine). Chlorine residual destroyed with potassium thiosulfate after 90 min.
H-8	SPJ-9 chlorinated in the laboratory with ^{36}Cl tracer as hypochlorite to 1.0 ppm residual chlorine (orthotolidine). Chlorine residual destroyed with thiosulfate after 15 min.
H-9	Same as H-8. Chlorine residual destroyed after 45 min.
H-10	Same as H-8. Chlorine residual destroyed after 90 min.

Table 7. STATUS OF SEWAGE PLANT EFFLUENT SAMPLES (5/1/74)

Sample	Concentration factor	Chromatographed on analytical column	Chromatographed on preparative column	Number of anion exchange column fractions collected	Number of anion column fractions chromatographed on cation exchange column	Number of cation column fractions collected	Number of fractions ultra-violet spectra obtained	Number of fractions chromatographed on dual-column gas chromatograph	Number of fractions mass spectra obtained	Number of analyses for ³⁶ Cl
SPJ-1	1000	X	X	27	11	27	41	35	27	
SPJ-2	1000	X	X	10	4	4	17	3	1	
SPJ-3	1000	X	X	55	14	48	88	35	14	
SPJ-4	1000	X	X	24	14	49	36	43	6	
SPJ-5	1000	X	X	21			16	17	1	
SPJ-6	1000 ^a	X								
SPJ-7	1000	X								
SPJ-8	1000	X	X	59			59	59	31	
H-1	570	X		250						286
SPK-1	1000	X ^b	X	20						
SPJ-9	2000	X	X	48			48	29	25	
SPJ-10	2000	X	X	40			40	7		
SPK-2	2000									
SPJ-11	3000		X	59	2	10	59	13	15	
SPL-1	3400		X	35	17	40	41	42	29	
H-2	1040	X		290						310
H-5	880	X		290						310
H-6	1110	X		290						310
H-8	640	X		290						310
H-9	580	X		290						310
H-10	490	X		290						310

^aConcentrated using XAD-4 macroreticular resin.

^bAnalyzed on cerate oxidative monitor.

IDENTIFICATION RESULTS

The primary effluent samples in which 56 compounds were identified included both unchlorinated and chlorinated samples; several of the constituents were also quantified. The identities and concentrations of these molecular pollutants are given in Table 8. Thus far, the sugars galactose, glucose, and maltose have been found in fractions collected from the carbohydrate analyzer. The remaining compounds were found in fractions collected from the UV-Analyzer. The copper (II) acetate (binuclear) identified using mass spectrometry in a cooperative effort with the Southeast Environmental Research Laboratory at Athens, Georgia, may have resulted from the reaction of copper ion in the sewage plant effluent with the acetate ion of the chromatographic column eluent. It is worth noting that the fractions containing the copper (II) acetate (binuclear) were blue, probably because of the presence of Cu(II), and that this blue color has appeared only in chlorinated sewage effluents. The copper may have been present as the result of corrosion in the concentration apparatus. A standard reference uv chromatogram of primary sewage plant effluent has been prepared (shown with identified compounds in Fig. 5). It will be used for reporting additional identifications and elution positions. The elution positions for identified compounds are given in Table 8.

In addition to the 56 identified compounds, other chromatographic fractions have been partially characterized. Tables 9 and 10 list the molecular weights obtained by mass spectrometry and gas chromatographic retention data for 15 unknown compounds found in five chromatographic fractions of unchlorinated domestic sewage plant effluent, and 8 unknown compounds found in four chromatographic fractions of chlorinated sewage plant effluent. Thirty-eight unknown compounds were found on more extensive characterization of a chlorinated sewage effluent which had been concentrated 2000-fold. These compounds are listed in Table 11 according to increasing molecular weight. It is obvious that, as more interpretative information regarding TMS-mass spectra becomes available, numerous additional compounds will be identified. Thirty unknown compounds were characterized in the SPJ-11 separation. SPJ-11 was a

Table 8. IDENTIFICATION OF MOLECULAR CONSTITUENTS IN 1000- TO 3000-FOLD CONCENTRATES OF PRIMARY DOMESTIC SEWAGE

Compound	Identification method ^a	Concentration, $\mu\text{g/liter}$	Anion exchange elution position, ml
Ethylene Glycol ^b	AC, GC, MS		8
Maltose	AC, GC		8
Galactose	AC, GC		8
Glucose	AC, GC		8
Glycerine	AC, GC, MS		10
Galacitol ^b	AC, GC, MS		10
Erythritol ^b	AC, GC, MS		10
Urea ^b	AC, GC, MS		10
N ¹ -Methyl-4-pyridone-3-carboxamide	AC, UV, GC	10	14
57 Phenylalanine ^b	AC, GC, MS	90	16
Uracil	AC, CC, UV, GC, MS	40	18
5-Acetylamino-6-amino-3-methyl uracil	AC, CC, UV, GC	140	20
N ¹ -Methyl-2-pyridone-5-carboxamide	AC, CC, UV, GC	20	21
Tyrosine ^b	AC, GC, MS		23
Thymine	AC, CC, GC, MS	~7	29
Theobromine	AC, CC		29
7-Methylxanthine	AC, CC	~90	29
Inosine	AC, CC, UV, GC, MS	50	29
Hypoxanthine ^b	AC, GC, MS	25	30
Xanthine	AC, CC, UV, GC, MS	70	55
Copper (II) acetate (binuclear) ^c	MS		60
Adenosine	AC, CC, UV, GC, MS		64
1,7-Dimethylxanthine	AC, CC		64

Table 8 (continued). IDENTIFICATION OF MOLECULAR CONSTITUENTS IN 1000- TO 3000-FOLD CONCENTRATES OF PRIMARY DOMESTIC SEWAGE

Compound	Identification method ^a	Concentration, $\mu\text{g/liter}$	Anion exchange elution position, ml
3-Methylxanthine	AC, CC		64
Caffeine	AC, CC, UV, MS	~10	67
Guanosine	AC, CC, UV, GC, MS	50	76
2-Deoxyglyceric acid ^b	MS		80
3-Hydroxybutyric acid ^b	GC, MS		80
3-Deoxyarabinoheptonic acid ^b	MS		80
Quinic acid ^{b,d}	MS		80
1-Methylxanthine	AC, CC, UV	70	82
2-Deoxytetronic acid ^b	MS		85
Glyceric acid ^b	MS		90
4-Deoxytetronic acid ^b	MS		90
3-Deoxyerythropentonic acid ^b	MS		90
2,5-dideoxypentonic acid ^{b,d}	MS		90
3,4-Dideoxypentonic acid ^b	MS		90
Ribonic acid ^b	MS		95
Oxalic acid ^b	AC, GC, MS		95
2-Hydroxyisobutyric acid ^b	GC, MS		95
Uric acid	AC, GC, MS	20	106
Orotic acid ^b	AC, UV, GC, MS	5	145
Succinic acid ^b	AC, GC, MS		205

Table 8 (continued). IDENTIFICATION OF MOLECULAR CONSTITUENTS IN 1000- TO 3000-FOLD CONCENTRATES OF PRIMARY DOMESTIC SEWAGE

Compound	Identification method ^a	Concentration, $\mu\text{g/liter}$	Anion exchange elution position, ml
Phenol ^b	AC, GC, MS	6	205
3-Hydroxyphenylhydracrylic acid ^b	AC, UV, GC, MS	10	205
Phenylacetic acid ^b	AC, GC	~10	230
4-Hydroxyphenylacetic acid	AC, UV, GC, MS	190	235
Benzoic acid	AC, GC, MS		260
2-Hydroxybenzoic acid	AC, GC, MS	7	290
4-Hydroxybenzoic acid	AC, GC		295
3-Hydroxybenzoic acid	AC, GC, MS	~40	295
3-Hydroxyphenylpropionic acid	AC, GC, MS	~20	295
Indican ^b	AC, GC, F	~2	325
3-Hydroxyindole ^b	MS		340
<u>o</u> -Phthalic acid ^e	AC, UV, MS	200	400
<u>p</u> -Cresol ^b	AC, GC, MS	20	400

^aAC - anion exchange chromatography; CC - cation exchange chromatography; UV - ultraviolet spectrum; GC - gas chromatography on two columns; MS - mass spectroscopy; F - fluorescent spectrum.

^bFrom chlorinated effluent.

^cIdentified by A. W. Garrison, Southeast Environmental Research Laboratory.

^dNo reference spectra available. Structure deduced from mass spectra analysis.

^eIdentified in Mill Creek sewage effluent.

Table 9. DATA ON UNKNOWN COMPOUNDS FOUND
 IN UNCHLORINATED PRIMARY EFFLUENT (SPJ-1)
 FROM THE OAK RIDGE EAST SEWAGE PLANT

Elution time, hr	Molecular weight	GC retention data, M.U.		
		OV-1	OV-17	Δ
0.6	135	14.99	15.90	+0.91
0.6	130	15.0	-	
0.6	202	17.5	-	
0.6	107	20.0	-	
0.9	100	15.42	-	
0.9	130	18.00	17.94	-0.06
0.9	146	17.58	18.17	+0.59
0.9	158	13.13	13.89	+0.76
1.8	114	13.88	15.36	+1.48
1.8	130	15.0	-	
1.8	136	15.0	-	
15.8	118	13.50	14.96	+1.46
15.8	131	16.92	19.05	+2.13
15.8	211	21.68	24.74	+2.88
46.5	150	13.51	15.00	+1.49

Table 10. DATA ON UNKNOWN COMPOUNDS FOUND IN CHLORINATED SEWAGE PLANT EFFLUENT (SPJ-3) FROM THE OAK RIDGE WEST SEWAGE PLANT^a

Elution time hr	Molecular weight	GC retention data, M.U.		
		OV-1	OV-17	Δ
1.05	221	17.58	17.86	+0.28
1.4	98	13.1	-	
1.4	127	14.79	15.49	+0.70
1.4	113	15.45	16.04	+0.59
14.0	110	14.10	14.88	+0.78
14.0	138	15.0	-	
58	138	15.04	16.24	+1.20
58	236	25.64	27.10	+1.46

^a2 ppm chlorine residual.

Table 11. DATA ON UNKNOWN COMPOUNDS FOUND
 IN CHLORINATED^a EFFLUENT (SPJ-8)
 FROM THE OAK RIDGE WEST SEWAGE PLANT

Molecular weight	Elution time, hr	GC retention data, M.U.		
		OV-1	OV-17	Δ
61	0.8	11.0	-	-
74	3.6	14.5	15.3	0.8
95	3.2	11.4	12.8	1.4
95	9.8	10.8	-	-
97	16.5	14	15.3	1.3
105	10.5	13.6	15.7	2.1
107	40.5	18.9	20.0	1.1
108	67	16	-	-
112	1.4	15.4	16.0	0.6
113	0.8	17.7	-	-
113	0.8	11.2	-	-
116	2.0	15.2	16.0	0.8
124	16	15.0	-	-

Table 11 (continued). DATA ON UNKNOWN COMPOUNDS FOUND
 IN CHLORINATED^a EFFLUENT (SPJ-8)
 FROM THE OAK RIDGE WEST SEWAGE PLANT

Molecular weight	Elution time, hr	GC retention, data M. U.		
		OV-1	OV-17	Δ
125	3.0	15.4	-	-
127	1.4	14.7	-	-
128	12.7	12.2	12.8	0.6
129	3.0	16.0	17.5	1.5
130	10.0	17.8	-	-
130	1.4	14.5	-	-
132 ^b	10.0	20.2	20.5	0.3
132	16.0	16.1	16.4	0.3
140	40.5	15	16.2	1.2
146	3.2	14.5	15.2	0.7
147	10.5	20	20.8	0.8
148	18	15.8	16.5	0.7
151	16	17.7	20.6	2.9
152	16	13.5	14.9	1.1
154	40	17.5	18.6	1.1
159	10	22.4	-	-
160	18	16.9	17.6	0.7

Table 11 (continued). DATA ON UNKNOWN COMPOUNDS FOUND
 IN CHLORINATED^a EFFLUENT (SPJ-8)
 FROM THE OAK RIDGE WEST SEWAGE PLANT

Molecular weight	Elution time, hr	GC retention data, M. U.		
		OV-1	OV-17	Δ
166	40.5	16.8	18.4	1.6
177	2.0	18.7	-	-
184	16	20.4	20.6	0.2
218	10.5	16.4	17.1	0.7
219	3.0	14.8	15.3	0.5
233	12.7	12.2	12.8	0.6
284	40.5	23	-	-
419	10.0	24.8	26.1	1.3

^a0.5 ppm residual.

^bAglycon molecular weight. Probably present as a glucuronide.

ERRATA SHEET

AUTOMATED ANALYSIS OF INDIVIDUAL REFRACTORY
ORGANICS IN WATER POLLUTED

EPA-660/2-74-076

Should read

AUTOMATED ANALYSIS OF INDIVIDUAL REFRACTORY
ORGANICS IN POLLUTED WATER

composite of SPJ-3 and -8 samples concentrated 3000-fold. These compounds, along with pertinent identification data, are listed in Table 12.

Thirteen organic compounds were identified in samples of unchlorinated secondary effluent from a domestic sewage plant. Most of these constituents have been quantified, and their concentrations and anion exchange elution positions are given in Table 13. A standard reference chromatogram of secondary sewage plant effluent for reporting elution positions and identifications is given in Fig. 6. In addition, 20 unknown compounds were characterized in 2000- and 3400-fold concentrates of secondary effluent. These compounds, along with the characterization data, are listed in Table 14.

Table 12. DATA ON UNKNOWN COMPOUNDS FOUND
IN SPJ-11, COMPOSITE OF SPJ-3 AND SPJ-8

Molecular weight	Elution time, hr	TMS peaks				GC retention data, M.U. for OV-1 column
		M	Base (1)	(2)	(3)	
57	53	201	73	116	158	12
76	53	205 ^a	73	75	147	13.5
82	0.8	370	73	117	147	12.4
94	0.8	295 ^a	75	116	149	10.4
101	1.0	245	147	116	73	11.4
106	1.0	235 ^a	147	73	117	12.4
117	385	189	73	70	174	11.8
117	1.5	295 ^a	73	75	174	11.4
128	32	344	259	73	75	14.6
129	38	345	345	330	73	16.0
132	1.5	333 ^a	73	204	217	17.6
147	0.8	276 ^a	146	73	75	16
149	0.8	365	262	350	73	16.4
152	0.8	281 ^a	73	147	117	16
154	0.8	355 ^a	73	188	144	14
157	68	373 ^a	73	75	174	15.2
161	38	290 ^a	203	73	147	14.7
162	44	291 ^a	73	147	129	14.5
163	0.8	292 ^a	189	73	147	19
168	1.5	369 ^a	217	73	116	17.7
170	53	299 ^a	209	224	117	15.4
173	32	374 ^a	274	273	73	17.4
177	68	450 ^a	73	75	129	16.2
192	25	249 ^a	249	75	144	21
208	32	280	75	73	265	18.9
214	68	502	297	73	75	18.5
246	68	591 ^a	73	75	93	20
266	68	482	73	164	245	22
285	53	573 ^a	73	75	117	22
284	53	356	73	75	117	22.5

^aM-15 peak.

Table 13. IDENTIFICATION OF MOLECULAR CONSTITUENTS IN 1000- AND 2000-FOLD CONCENTRATES OF SECONDARY DOMESTIC SEWAGE EFFLUENT

Compound	Identification method ^a	Concentration, $\mu\text{g/liter}$	Approximate anion-exchange elution position, ml
Glycerine	AC, GC, MS		10
Uracil	AC, CC, UV, MS	30	18
5-Acetylamino-6-amino-3-methyl uracil	AC, CC, UV	30	20
1-Methylinosine	AC, CC, UV	80	29
Inosine	AC, CC, UV	20	29
7-Methylxanthine	AC, CC, UV	5	29
1-Methylxanthine	AC, GC	6	82
1,7-Dimethylxanthine	AC, CC, UV	~6	64
Succinic acid	AC, MS		135
Catechol	MS		235
Indole-3-acetic acid	MS		235
3-Hydroxyindole	MS		320
p-Cresol	AC, GC, MS	90	~300

^aAC - anion exchange chromatography; CC - cation exchange chromatography; UV - ultraviolet spectrum; MS - mass spectroscopy.

Table 14. DATA ON UNKNOWN COMPOUNDS FOUND IN SECONDARY EFFLUENT (SPJ-9 AND SPL-1) FROM A DOMESTIC SEWAGE TREATMENT PLANT

Molecular weight	Elution time, hr	TMS peaks				GC retention data, M.U. for OV-1 column
		M	Base (1)	(2)	(3)	
95	0.8	167	98	44	167	10.4
114	12.9	315 ^a	143	147	73	15
115	12.9	331	147	73	262	12.9
124	52.5	196	166	181	196	12.9
131	57.5	203	73	188	174	11.5
133	2.0	349	280	284	147	12.9
157	2.0	373	147	262	304	12.0
161	0.8	305	171	73	166	10.2
163	2.0	379	310	163	379	16.0
166	0.8	310	241	225	73	14.3
177	2.0	393	73	147	324	16.0
190	2.0	406	-	-	-	16.0
202	29.5	346	277	75	73	17.0
203	5.5	347	73	146	52	19.0
208	5.5	352	73	40	52	22
270	5.5	486	73	79	357	29.5
274	40.0	346	331	315	215	18.9
307	52.5	379	75	79	73	15
321	0.8	320 ^{b,c}	99	44	69	11.8
345	5.5	705	131	117	73	27.4

^aM-15 peak.

^bNot TMS derivative.

^cM-1 peak.

SECTION VI

EFFECTS OF CHLORINATION OF SEWAGE PLANT EFFLUENTS

Because little definitive information is available concerning the potential hazards resulting from the chlorination of sewage plant effluents and other waters, a study was undertaken to determine whether chlorinated organic compounds are produced during chlorination of various waters. Indeed, effects on compounds present in both primary and secondary effluents were indicated by preliminary studies on three sewage effluent samples, SPJ-2, -3, and -5, each chlorinated to different residual levels prior to concentration. Comparison of chromatograms before and after chlorination showed that some chromatographic peaks disappeared, while others appeared, apparently as the result of chlorination (see Fig. 5).

It is a matter of some significance whether these effects of chlorination are a consequence of oxidation or result from chlorine addition and/or substitution products. The latter is, of course, of great significance in terms of possible ecological effects, both in the short and long terms. Perhaps the most direct and sensitive technique available to determine whether any of the chromatographic changes are due to chlorine addition or substitution products is the use of a radioactive isotope of chlorine as a tracer. Therefore, the radioactive isotope ^{36}Cl was added as a tracer in the chlorine gas used to chlorinate some of the sewage effluent samples. Chlorine-36 is a 0.71-Mev beta particle emitter with a 3.0×10^5 year half-life.²⁵ The presence of ^{36}Cl in chromatographic fractions other than those containing chloride ion would confirm that some compounds in the sewage had been chlorinated.

Anion exchange chromatography of chlorinated primary sewage effluent tagged with ^{36}Cl and concentrated 500- to 1000-fold revealed as many as 60 radioactive peaks, several of which coeluted with uv-absorbing peaks. These peaks were shown to be mostly chlorine-containing organic compounds. Under the conditions of chlorination

used at the Oak Ridge Sewage Treatment Plants, about 1% of the chlorine dosage was associated with these stable chlorinated organics. Seventeen of the compounds were tentatively identified.

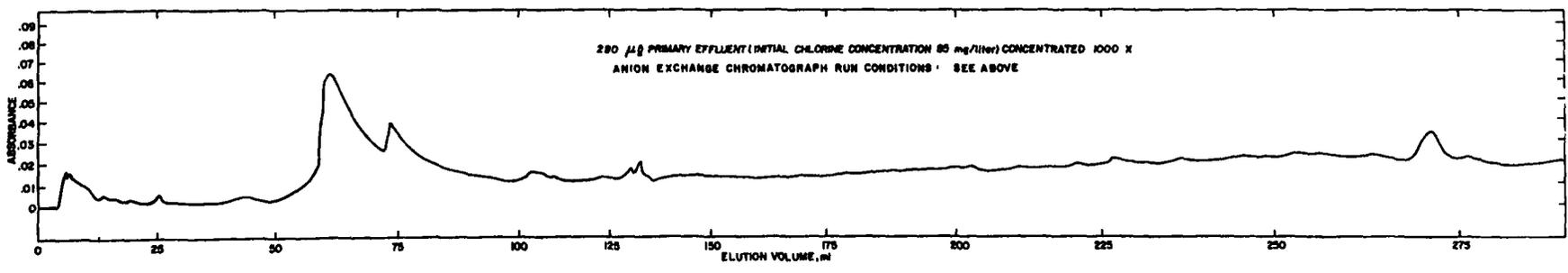
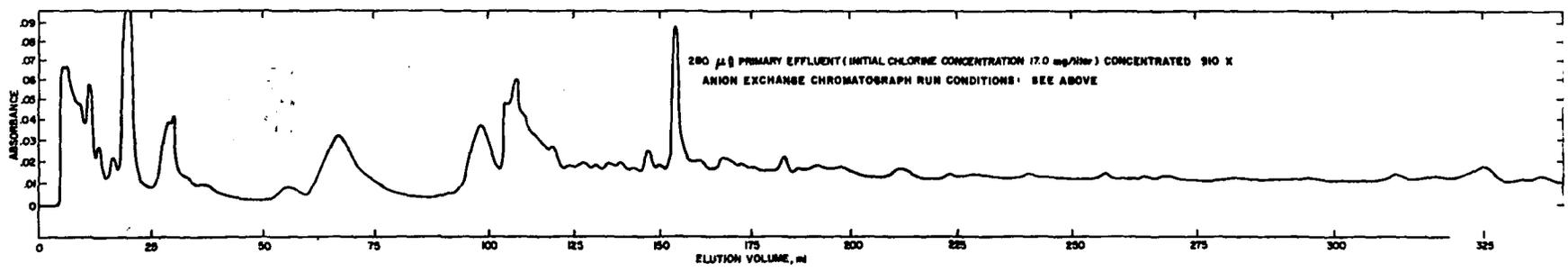
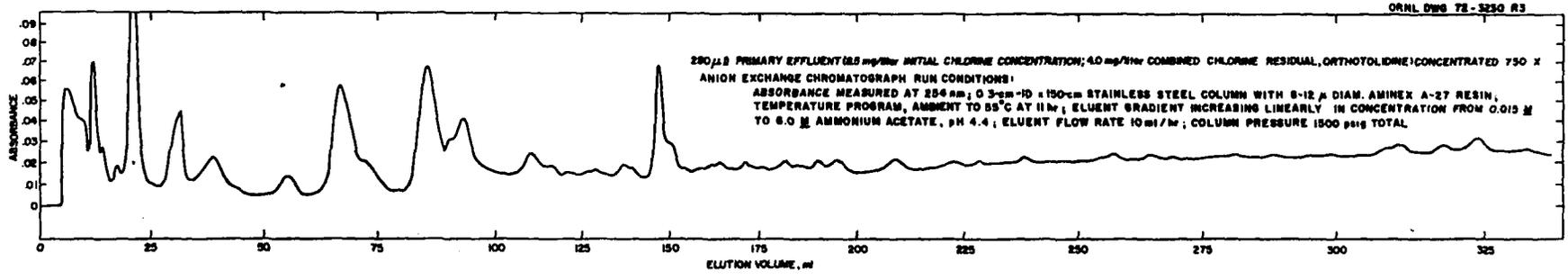
A detailed study of the chlorination effects on organic constituents has been published as a doctoral thesis.²⁶

CHLORINATION OF SAMPLES

In the initial chlorination experiments with nonradioactive chlorine, chlorination was effected by addition of dry calcium hypochlorite to 3-liter aliquots of primary sewage plant effluent at ambient temperature. After being stirred with a Teflon-coated stirring bar for 1 hr, each aliquot was then concentrated by vacuum distillation. The dry calcium hypochlorite used in the chlorination experiments contained 61% available chlorine as determined by the iodometric method for chlorine residual.²⁷ In these scouting experiments, the amount of hypochlorite added to the effluents provided 8.5, 17, and 85 ppm total chlorine. An orthotolidine determination of the combined chlorine residual of the 8.5 ppm experiment indicated an approximate combined chlorine residual of 4 ppm. The effluents were then concentrated 1000-fold and chromatographed (Fig. 26). Comparison of these chromatograms indicates considerable differences, with most of the chromatographic peaks disappearing in the concentrate of the effluent chlorinated at the highest concentration (SPJ-2).

CHLORINATION OF SAMPLES WITH ³⁶Cl

Gaseous chlorine generated by the reaction of KCl in dilute H₂SO₄ with KMnO₄ powder, $10 \text{ KCl} + 2 \text{ KMnO}_4 + 8 \text{ H}_2\text{SO}_4 \rightarrow 2 \text{ MnSO}_4 + 6 \text{ K}_2\text{SO}_4 + 8 \text{ H}_2\text{O} + 5 \text{ Cl}_2$, was used for the radioactive chlorination experiments. A potassium chloride solution tagged with ³⁶Cl and containing an excess of sulfuric acid to convert the chloride ion to HCl was dropped onto potassium permanganate powder, and the chlorine gas was sparged from the reaction tube with nitrogen gas. The nitrogen gas containing



77

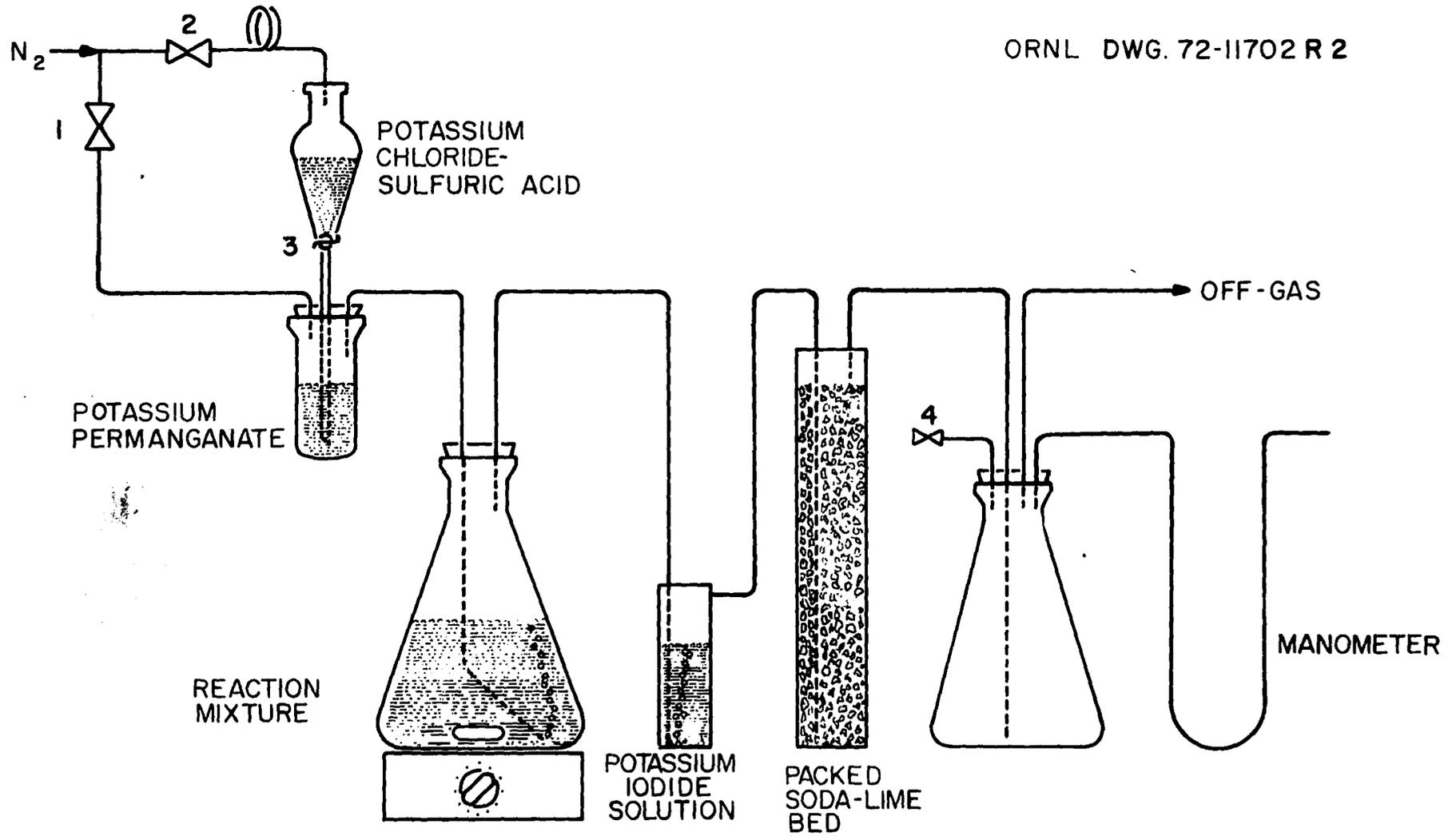
Fig. 26. UV-Analyzer Chromatograms of Primary Sewage Treatment Plant Effluent Chlorinated with Different Amounts of Calcium Hypochlorite.

the chlorine was then bubbled through 2 liters of sewage effluent in a reaction flask at room temperature. A gas trap containing potassium iodide downstream from the reaction flask collected the unreacted chlorine and allowed the efficiency of chlorination to be determined. Milligram quantities of chlorine could be generated at an efficiency of up to 95% using this experimental setup (Fig. 27). To ensure zero leakage of ^{36}Cl to the atmosphere, the experiments were performed in a hood equipped for radioactivity experiments; in addition, the system was operated at a negative pressure (-1 in. H_2O) with a controlled air leak into the "hot" off-gas system. During chlorination, the reaction mixture of sewage plant effluent was sparged with a slow flow of nitrogen gas from the chlorine generator while being stirred with a Teflon-coated magnetic stirring bar. After 1 hr, the combined chlorine residual was determined with orthotolidine.

The remaining chlorinated effluent was taken to dryness using the vacuum distillation apparatus shown in Fig. 28. This equipment was also operated so as to ensure that the radioactivity remained confined within the concentration apparatus. Subsequently, the residue was dissolved in acetic acid to eliminate the carbonates and reevaporated to dryness. The final dissolution was made with dilute ammonium acetate buffer (0.015 M, pH 4.4) in preparation for chromatographic analysis. The pH of the ^{36}Cl tagged concentrate was determined using a pH meter (usually 4.5), and the pH of the untagged sample to be compared with it on the dual-column chromatograph was adjusted to correspond.

A fraction collector was used to serially collect the eluate fractions from the column on which the tagged concentrate had been chromatographed. Half-milliliter aliquots of each of the eluate fractions collected during the chromatographic run, plus samples resulting from various steps of the chlorination, were analyzed for ^{36}Cl by liquid scintillation counting.

73



ORNL DWG. 72-11702 R 2

Fig. 27. Schematic of Chlorine Generator and Sample Chlorinator.

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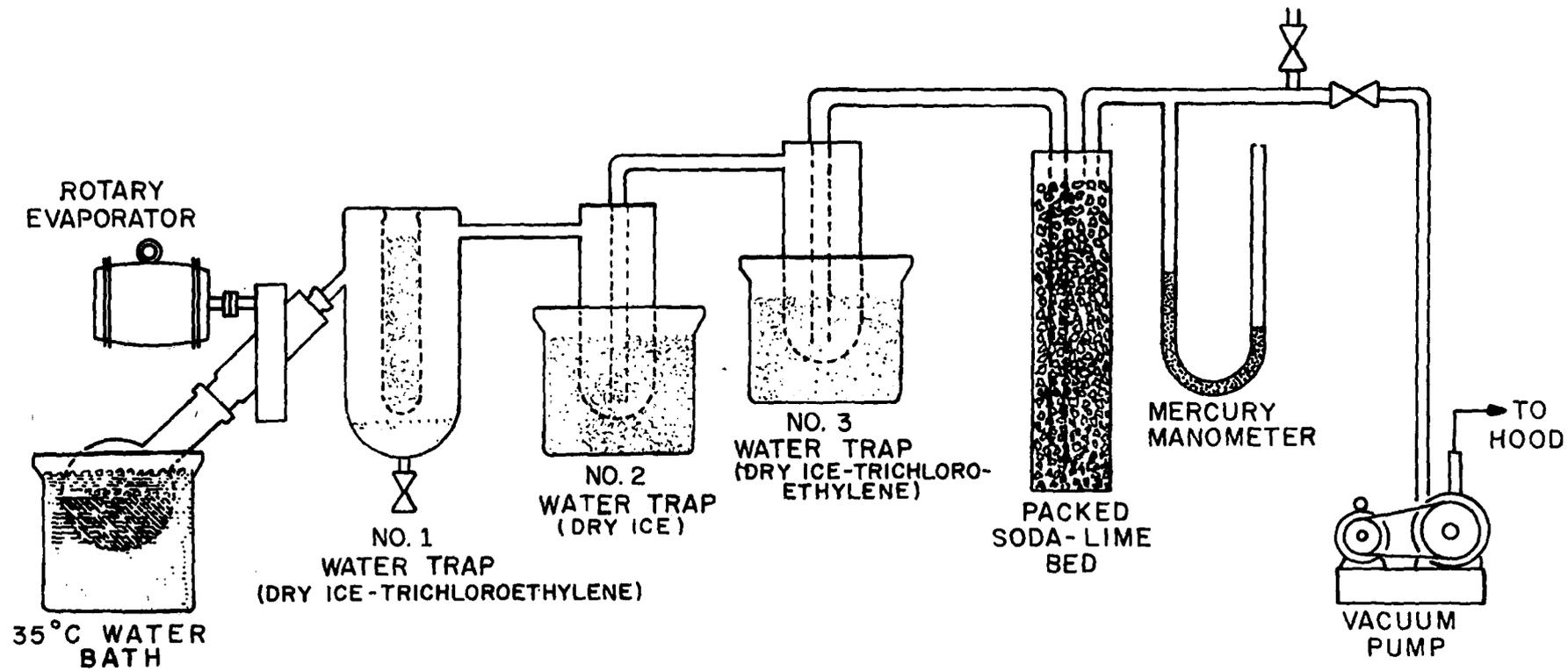


Fig. 28. Schematic of Apparatus Used for Concentration of Radioactive Samples.

EXPERIMENTAL RESULTS

Five samples of effluent from the secondary stage and one from the primary stage of Oak Ridge sewage treatment plants were chlorinated in the laboratory with ^{36}Cl -tagged chlorination agents (see Table 6). The primary effluent sample was chlorinated to 2 ppm chlorine residual (orthotolidine method) using chlorine gas (H-1), and the secondary effluent samples were chlorinated to 1 ppm residual using chlorine gas in two cases (H-2 and -6) and hypochlorite solution in the others (H-8, -9, and -10). Essentially the same results were obtained with each reagent with one major exception. One of the chlorine-containing constituents subsequently separated by anion exchange chromatography was considerably higher in concentration after chlorination with hypochlorite solution than when chlorine gas was used.

In the initial experiment, a 2-liter volume of unchlorinated primary effluent (sample H-1) from the Oak Ridge West Sewage Plant was chlorinated in the laboratory with 18.7 mg of chlorine gas containing approximately 0.12 mCi of ^{36}Cl . The chlorinated mixture was then reduced to a volume of 3.5 ml in a rotary evaporator. Of the initial radioactivity, 48% was found in the concentrate, 10% in the condensate, and the remainder in the chlorine generator, the potassium iodide trap following the chlorination reactor, and the solid residue from the concentration step.

A 15.8-liter sample of primary effluent, chlorinated at the sewage plant, was taken with a slight time delay so as to be representative of the unchlorinated sample used for the tracer experiment. This sample (SPJ-8) was concentrated 1000-fold using the routine concentration techniques previously reported. For comparison purposes, the ^{36}Cl -tagged concentrate and the untagged concentrate were chromatographed simultaneously on the dual-column chromatograph. The chromatograms of the two concentrates are shown in Fig. 29.

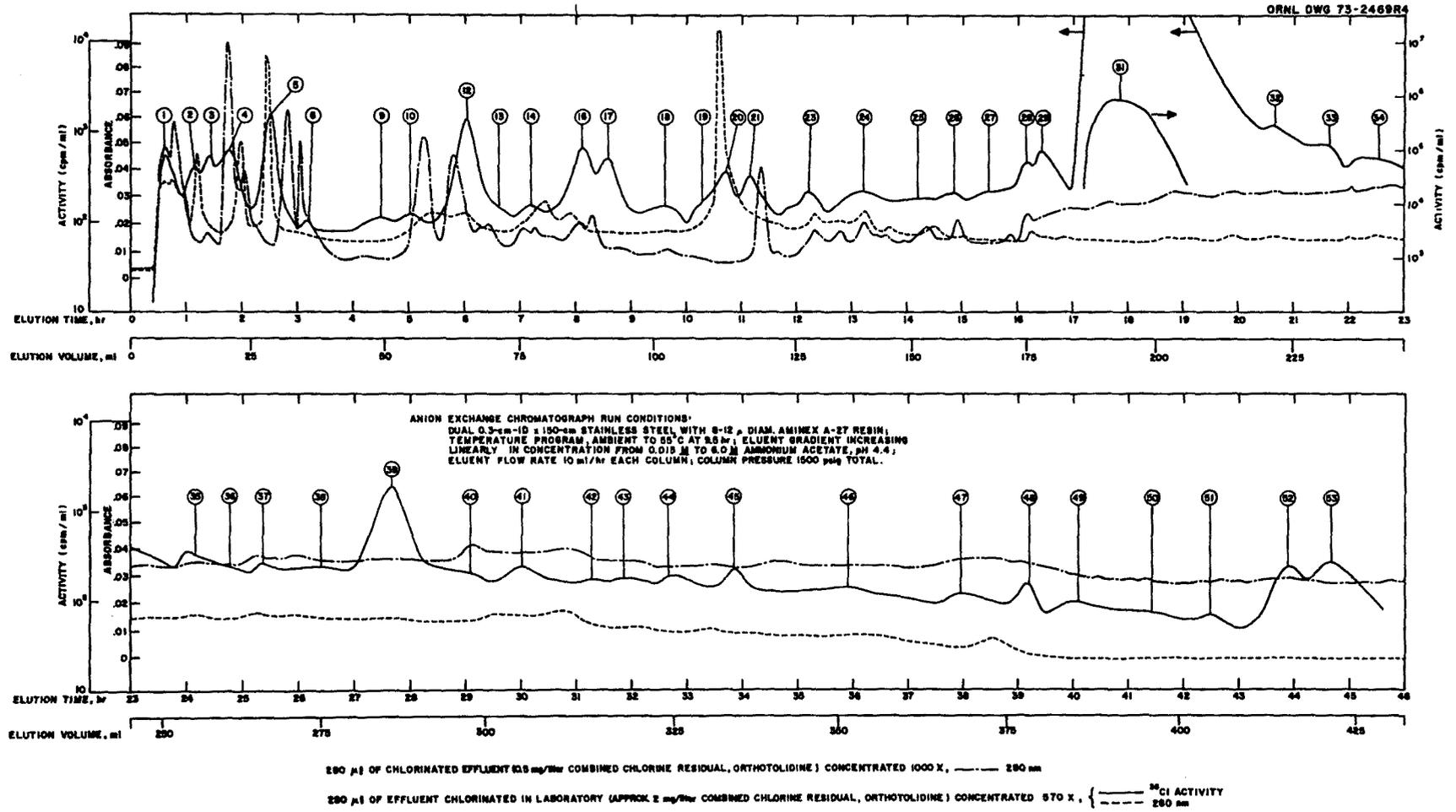


Fig. 29. Dual-Column UV-Analyzer Chromatograms of Chlorinated Primary Sewage Treatment Plant Effluent.

Analysis of the eluate fractions for ^{36}Cl showed 37 radioactive peaks in the chromatogram of the tagged concentrate. Four of the large peaks (peak maximum ≈ 400 cpm/ml) and two of the smaller peaks (peak maximum ~ 50 -400 cpm/ml) were associated with uv-absorbing constituents. The one extremely large peak (peak maximum = 900,000 cpm/ml) eluting at 200 ml was found to be chloride ion. Four moderately large and two small peaks were not associated with uv-absorbing constituents. The elution positions of the remaining peaks were such that no positive conclusion could be made concerning association with uv-absorbing constituents. A material balance of 112% of ^{36}Cl was determined for the chromatographic separation. The ^{36}Cl activity level of this 280- μl sample, which was chromatographed, analyzed 1.11×10^7 cpm. Ninety-nine percent of the activity was found in the chloride peak, 0.6 was found in the remaining eluate fractions, and 0.4 was found on the sacrificial resin cartridge which precedes the ion exchange column and removes constituents that tend to irreversibly adsorb on the resin. The main resin column, although not analyzed, should have little activity on it.

Comparison of the two chromatograms in the dual-column analysis (Fig. 29) revealed that 13 chromatographic peaks in the untagged concentrate (0.5 ppm combined chlorine residual) were apparently common with radioactive peaks in the tagged concentrate (~ 2 ppm combined chlorine residual). Five milliliters of this untagged concentrate was chromatographed on the preparative system, and those fractions which correspond to the radioactive peaks are being processed according to our routine identification procedure in an effort to establish the identities of the chlorinated compounds.

Using similar radioactive tracer experiments with secondary effluent, it was also demonstrated that chlorinated organic residues are produced during chlorination at sewage treatment plants. Several chromatographic experiments were necessary to rigorously prove that: (1) chlorine-containing compounds are found when a concentrate of secondary sewage effluent is chromatographed; (2) these chlorinated

residues are not artifacts of the concentration procedure; (3) the chlorinated residues result from chlorination and not from chloride ion interaction and are, therefore, reasonably stable in aqueous media; and (4) the chlorinated residues are organic in nature.

Several dual-column chromatographic runs were made in which ^{36}Cl -tagged samples were compared with similar samples not tagged. The radioactive sample, H-2, was a 1040-fold concentrate of secondary effluent which had been chlorinated to a chlorine residual of 1 ppm using ^{36}Cl -tagged chlorine gas to determine whether organic constituents in secondary effluent were indeed chlorinated as in primary effluent. This proved to be the case, as is shown in Fig. 30, because many peaks of radioactivity were found in addition to the chloride peak. The radioactive sample, H-6, was a 1110-fold concentrate of secondary effluent chlorinated in the same manner as H-2; however, in this case, the chlorine residual was destroyed with potassium thiosulfate prior to concentration. Because essentially the same results were obtained from this experiment as for H-2, it was concluded that the chlorine-containing peaks were not artifacts of the concentration procedure. It was also concluded that all the chromatographic peaks represent stable compounds and are probably not chloramines, since thiosulfate would probably destroy most chloramines. Radioactive samples H-8, H-9, and H-10 were 640-, 580-, and 490-fold concentrates of secondary effluent chlorinated with ^{36}Cl -tagged hypochlorite to a 1 ppm chlorine residual which was destroyed after 15, 45, and 90 min of reaction time, respectively. Chromatograms of the chlorine-containing constituents in these experiments are shown in Fig. 31. It was concluded from this experimental series that the yield of chlorine-containing constituents increases with increasing reaction time and that chlorination with either hypochlorite or chlorine gas gives essentially equivalent results. A comparison of radioactive chromatographic peaks in secondary sewage effluents with those found in primary sewage effluent

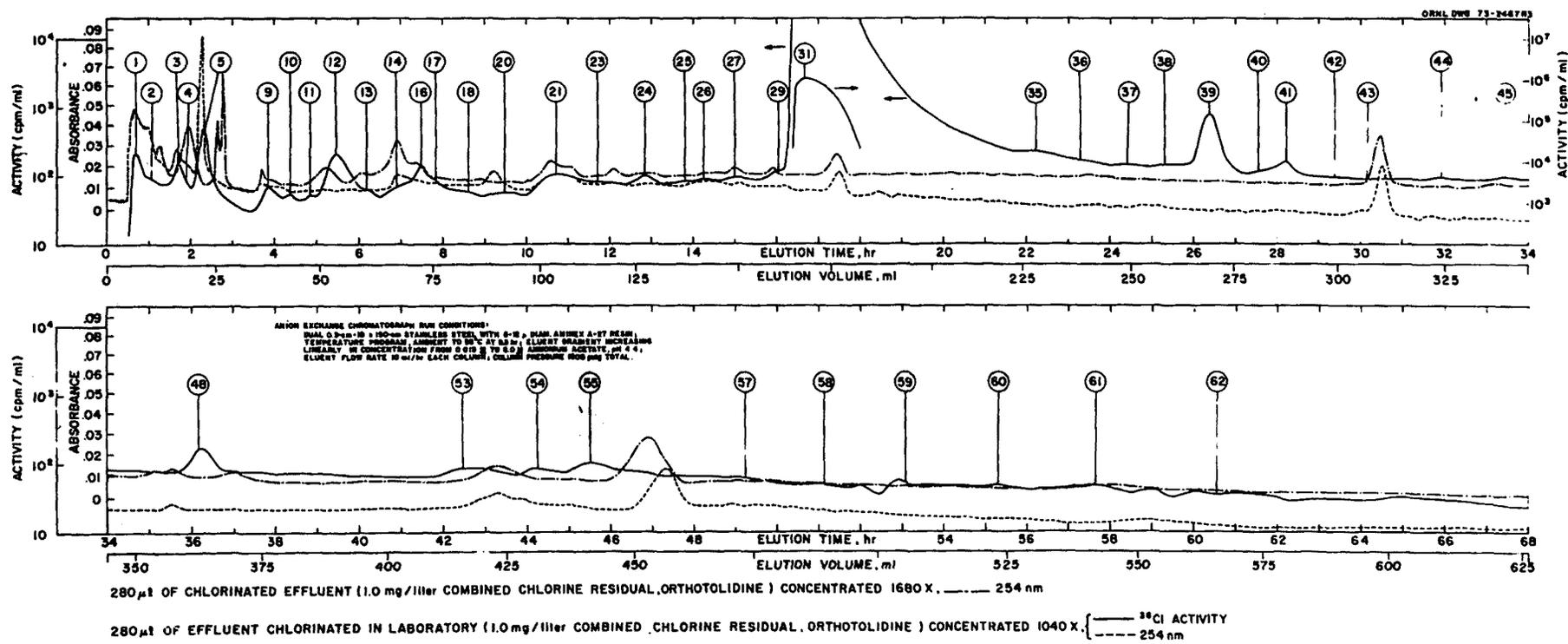


Fig. 30. Dual-Column UV-Analyzer Chromatograms of Chlorinated Effluent from a Secondary Sewage Treatment Plant.

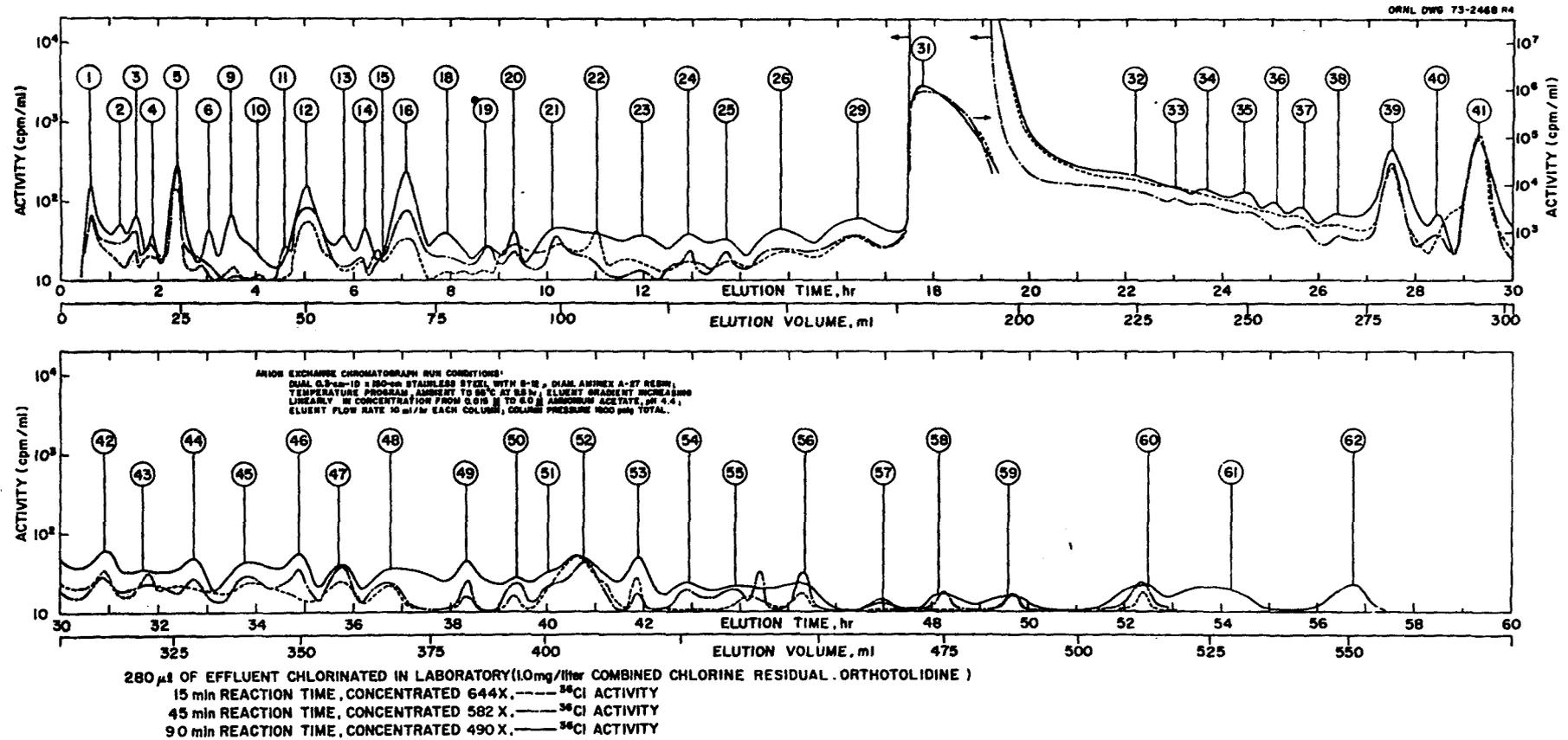


Fig. 31. Chromatograms of Secondary Sewage Treatment Plant Effluent Chlorinated with Hypochlorite Solution for 15-, 45-, and 90-min Reaction Times.

(see Figs. 29-31) indicated that most of the chlorine-containing peaks in the secondary effluent are also found in the primary effluent. As noted in these figures, the chromatographic peaks were correlated and, for purposes of identification, assigned number designations.

Several experiments were required to determine the nature of the chlorine-containing peaks. In two runs (samples H-3 and H-4), only ^{36}Cl -tagged chloride ion was chromatographed, proving that the extremely large chlorine-containing peak (peak 31) was indeed chloride. In one run, H-5, the ^{36}Cl was introduced to the secondary effluent sample as chloride ion and concentrated. The results obtained by chromatographing the 880-fold concentrate conclusively showed by absence of radioactive peaks other than the chloride peak that the chlorine-containing constituents had indeed been created by the chlorination of the secondary effluent, and did not result from chloride-complex formation with inorganic material and/or from isotopic exchange with chlorine-containing constituents which may have been in the effluent prior to chlorination. In one run, H-7, a synthetic effluent containing only inorganic constituents at approximately the same concentrations as that in the secondary effluent, as determined by spark-source mass spectrometry, was chlorinated to a 1 ppm chlorine residual using chlorine gas and concentrated. After chromatographing the 850-fold concentrate, no significant peaks other than chloride were found, thus proving that none of the chromatographic peaks of radioactivity resulted from complexes of hypochlorous acid, hypochlorite ion, or chloramines with inorganic constituents in the secondary effluent. Thus it was concluded from this series of experiments that the large majority of chlorine-containing peaks found in the chlorinated effluents were stable organic compounds.

Analyses of all the chromatograms (Figs. 29-31) showed that at least 62 chlorine-containing constituents which possibly were stable chloro-organic compounds were separated on the chromatograph. The concentrations of these constituents, in nanograms of Cl per liter of original effluent, are given in Table 15.

Table 15. STABLE CHLORINE-CONTAINING ORGANIC CONSTITUENTS IN CHLORINATED EFFLUENTS FROM DOMESTIC SANITARY SEWAGE TREATMENT PLANTS

(Concentration of the constituents, ng Cl per liter of original effluent)

Constituent	Experiment					
	H-1 ^a	H-2 ^b	H-6 ^c	H-8 ^d	H-9 ^e	H-10 ^f
1	3120	1240	1000	460	440	610
2	2240	890	560	150		260
3	3040	760	460	150	240	290
4	4520	630	340	150	350	240
5	7320	2460	1550	120	1040	1070
6	630	560	260	140	250	260
7			130			
8			310			
9	1070	900	600	100	120	360
10	1420	440		110	170	230
11		520	190		210	
12	9260	2760	1340	670	1120	1230
13	980	640	430	140		260
14	2130	700	290	170	200	230
15				240		
16	6200	1410	870	460	1040	1530
17	3950	1000	530			
18	2860	770	580	210	220	470
19	1090		440	190	270	210
20	1940	1210	610	430	240	240
21	3150	2610	1060	230	370	640
22				330	170	680
23	3550	1730	1420	510	320	560

Table 15 (continued). STABLE CHLORINE-CONTAINING ORGANIC CONSTITUENTS
 IN CHLORINATED EFFLUENTS FROM DOMESTIC SANITARY SEWAGE TREATMENT PLANTS
 (Concentration of the constituents, ng Cl per liter of original effluent)

Constituent	Experiment					
	H-1 ^a	H-2 ^b	H-6 ^c	H-8 ^d	H-9 ^e	H-10 ^f
24	4810	1780	590	550	370	490
25	3330	1140	910	560	330	510
26	2170	1210	1010	770	610	910
27	4030	1280				
28	3160					
29	4230	1810	930	910	990	1690
30			850			
31	[21.3 mg] ^g	[20.9 mg] ^g	[17.2 mg] ^g	[24.0 mg] ^g	[21.6 mg] ^g	[17.3 mg] ^g
32	840		390		290	270
33	1500			340	190	120
34	1520			170	70	150
35	910	240	190	230	170	110
36	350	140	90		220	130
37	800	190		140	180	50
38	220	150		50	20	100
39	14400	5610	2450	1670	1750	2600
40	180	120		270	60	140
41	530	490	160	4320	3260	3380
42	90	120	90	80	60	190
43	160		50	60	50	50
44	430	150	120	120	100	170
45	380	90	160	160	210	190
46	503		80		130	180
47	190		70	230	140	150
48	350	790	430	130	170	290

Table 15 (continued). STABLE CHLORINE-CONTAINING ORGANIC CONSTITUENTS
 IN CHLORINATED EFFLUENTS FROM DOMESTIC SANITARY SEWAGE TREATMENT PLANTS
 (Concentration of the constituents, ng Cl per liter of original effluent)

Constituent	Experiment					
	H-1 ^a	H-2 ^b	H-6 ^c	H-8 ^d	H-9 ^e	H-10 ^f
49	170		20	40	250	220
50	210		120	30	100	90
51	120		70		80	100
52	1700		70	730	470	480
53	2430	540	170	130	80	200
54		220	200		170	80
55		650	30	150	250	80
56			90	110	190	220
57		100	60		140	90
58		130	30		220	180
59		260	60	60	300	120
60		140	60	170	260	220
61		210	80		270	340
62		120	180			300
Total	108,183	38,910	22,780	17,800	18,550	23,990
Number of constituents	47	44	52	46	52	54

^aPrimary effluent; 2 ppm chlorine residual; estimated chlorination contact time, 240 min; chlorinating agent, ³⁶Cl-tagged chlorine gas.

^bSecondary effluent; 1 ppm residual; estimated chlorination contact time, 240 min; chlorinating agent, ³⁶Cl-tagged chlorine gas.

^cSecondary effluent; 1 ppm chlorine residual; chlorination contact time, 90 min; chlorinating agent, ³⁶Cl-tagged chlorine gas.

^dSecondary effluent; 1 ppm chlorine residual; chlorination time, 15 min; chlorinating agent, ³⁶Cl-tagged hypochlorite solution.

^eSecondary effluent; 1 ppm chlorine residual; chlorination time, 45 min; chlorinating agent, ³⁶Cl-tagged hypochlorite solution.

^fSecondary effluent; 1 ppm chlorine residual; chlorination time, 90 min; chlorinating agent, ³⁶Cl-tagged hypochlorite solution.

^gTheoretical values are 24.0, 24.8, 25.0, 25.6, 25.6, and 25.6 mg, respectively.

Tentative identifications of 17 of the chlorine-containing constituents were established by comparison of their anion exchange elution volumes with those determined for reference standards. Reasonable agreement of elution volumes is presumptive evidence that the unknown may indeed be the same compound as the reference standard. Table 16 gives tentative identifications of these organic compounds, along with their concentrations as calculated from data for Experiment H-9 in Table 15. Because the extent of chlorination of this sample is approximately equivalent to that expected at the Oak Ridge East Sewage Treatment Plant, the concentrations of the tentatively identified compounds would approximate those expected at that sewage treatment plant. The concentrations were calculated from data which were based on the reasonable assumption that complete isotopic dilution of the ^{36}Cl occurred rapidly during the chlorination process. Therefore, our values may be high if the chlorination reaction by which the compounds are formed proceeds at a rate equivalent to or faster than the chlorination rate for the formation of chloramines.

The magnitude of the chlorination effect is dependent on the chlorine dosage and the total reaction time. In Experiment H-9, with a chlorination reaction time (45 min) and conditions (chlorine residual, 1 mg/liter) approximately equivalent to those at ORESP, 0.6% of the chlorine dose is associated with stable chlorine-containing organic constituents separated chromatographically from the chlorinated effluent; an additional 0.4% is associated with chlorine-containing constituents which did not elute from the resin. Therefore, the total chlorination yield was about 1% of the chlorine dose. The chlorination yields are calculated based on the assumption that during the chlorination, complete isotopic dilution of the ^{36}Cl in the chlorinating agent occurs with the "pool" of nonradioactive chlorine in the effluents. The remainder of the available chlorine (99%) was apparently utilized in oxidation reactions.

Since the experimental conditions are similar to the field conditions at ORESP, chlorination at the sewage treatment plant

Table 16. TENTATIVE IDENTIFICATIONS AND CONCENTRATIONS OF CHLORINE-CONTAINING CONSTITUENTS IN CHLORINATED EFFLUENTS

Constituent	Peak elution volume, ml	Tentative identification	Reference standard elution volume, ml	Concentration of organic compound, µg/liter
16	72.4 ± 2.7	5-Chlorouracil	72 ^a	4.3
18	80.2 ± 4.0	5-Chlorouridine	81	1.7
19	86.2 ± 4.2	8-Chlorocaffeine	86	1.7
22	102 ± 3.3	6-Chloro-2-aminopurine	109	0.9
32	218	8-Chloroxanthine	218	1.5
42	302 ± 5.9	2-Chlorobenzoic acid	307 ± 9 ^b	0.26
43	312 ± 5.9	5-Chlorosalicylic acid	310	0.24
45	334 ± 6.4	4-Chloromandelic acid	338	1.1
52	403 ± 1.7	2-Chlorophenol	400	1.7
53	415 ± 3.8	4-Chlorophenylacetic acid	411	0.38
55	436 ± 5.4	4-Chlorobenzoic acid	434 ± 11 ^c	1.1
56	444 ± 4.4	4-Chlorophenol	446	0.69
57	464 ± 8.7	3-Chlorobenzoic acid and/or 3-Chlorophenol	455 ± 10 ^c 456	0.62 ^d 0.51 ^d
59	496 ± 8.7	4-Chlororesorcinol	495	1.2
61	527 ± 15.6	3-Chloro-4-hydroxybenzoic acid	540	1.3
62	547 ± 20.2	4-Chloro-3-methylphenol	550	1.5 ^e

^aAverage of two determinations.

^bAverage of seven determinations ± standard deviation.

^cAverage of four determinations ± standard deviation.

^dValues based on the assumption that chlorine is present as the pure compound of either, and not a mixture of both.

^eConcentration in sample H-2.

should produce similar chlorination yields; i.e., about 1% of the chlorine dose should be associated with stable chlorine-containing organic constituents. Table 17 summarizes the chlorination yields obtained in the experimental series.

Table 17. PERCENTAGE CHLORINATION YIELD^a OF CHLORINE-CONTAINING CONSTITUENTS WITH RESPECT TO REACTION TIME FOR THE CHLORINATION OF EFFLUENTS

Reaction time, min	Percentage chlorination yield of chromatographable chlorine-containing constituents ^b	Percentage chlorination yield of total chlorine-containing constituents ^c
15	0.55	0.93
45	0.58	0.98
90	0.75-0.87	1.27
240	1.6-1.8	

^aThe yield is expressed as percent of chlorine dose which, after chlorination, is associated with stable chlorine-containing constituents.

^bPercentage chlorination yield of chromatographable chlorine-containing constituents separated as peaks in the radioactive tracer experiments.

^cPercentage chlorination yields of the total chlorine-containing constituents separated from the chlorinated effluent. The total is defined as the sum of both chromatographable constituents and the constituents that did not elute from the resin during the chromatographic separation, prorating the noneluting constituents between H-8, -9, and -10 based on the proportionate amount of chromatographable constituents determined for the respective experiments.

SECTION VII

COOPERATIVE EFFORTS WITH ENVIRONMENTAL PROTECTION AGENCY LABORATORIES

In accordance with the specific aims stated in the program proposal, two high-resolution chromatographs for the analysis of stable organic compounds were designed, constructed, and tested. One was delivered to the Advanced Waste Treatment Research Laboratory (AWTRL), the other to the Southeast Environmental Research Laboratory (SERL). Supportive efforts in applying the instrument to field problems included assistance in the areas of operation, maintenance, and minor modifications.

Other efforts under this contract made in cooperation with personnel from two EPA laboratories included: (1) identification of constituents in samples furnished by C. I. Mashni of AWTRL; and (2) lyophilization and preparative mode chromatography of sewage plant effluent samples for subsequent analysis by A. W. Garrison of SERL.

CONSTRUCTION OF TWO UV-ANALYZERS FOR ENVIRONMENTAL PROTECTION AGENCY LABORATORIES

A significant part of this program has been concerned with the construction of two UV-Analyzers for use at EPA Laboratories. The design of the Mark II UV-Analyzer (from the Body Fluids Analyses Program) was selected for the instrument that was constructed for the AWTRL. This instrument was subsequently converted to a Mark IIA; the analyzer constructed for SERL was an updated Mark IIA version. The use of this analyzer design simplified the problem of peak identification since the identifications that are made and the techniques that are used in the parallel work of the Body Fluids Analyses Program are directly applicable.

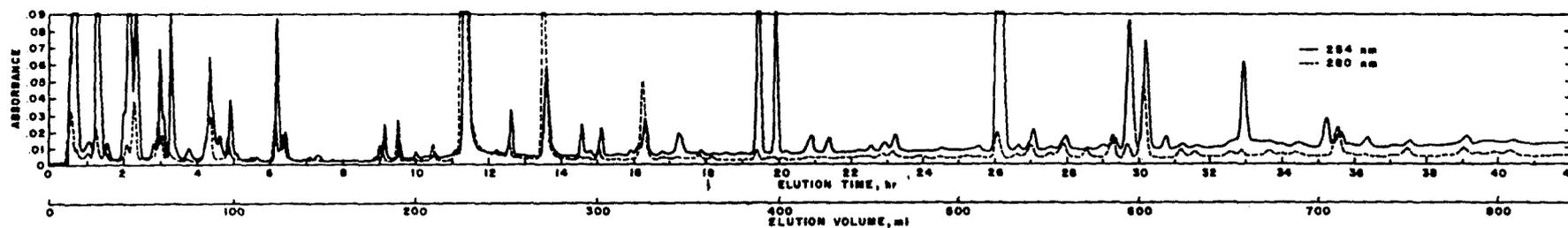
Fabrication of the first analyzer was completed in November 1970 at a cost of \$13,200, and the second was completed in November 1973 at a cost of \$14,500. Both analyzers were tested at ORNL to ensure satisfactory operation, and excellent results were obtained. The

resolution in each case was equal to or better than that obtained with the UV-Analyzers (see Fig. 32). During the shakedown period of each instrument, a scientist from the EPA Laboratory was given three days of training in operation and maintenance of the instrument. After shakedown, the instruments were delivered to the respective EPA Laboratories, where they continue to be operated by EPA personnel.

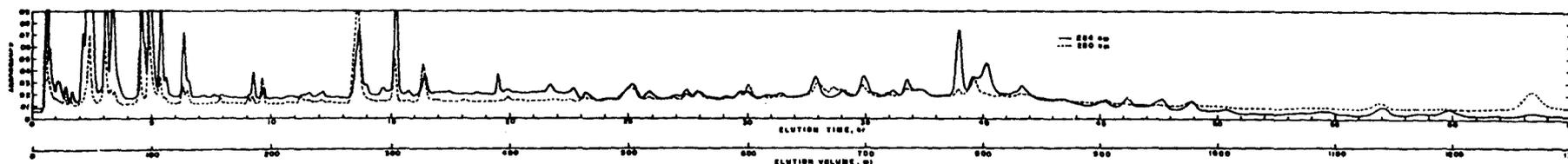
IDENTIFICATION OF ORGANIC CONSTITUENTS IN AWTRL SAMPLES

A 20-liter sample of raw sewage was taken on January 5, 1971, at the Mill Creek Plant in Cincinnati by Mr. Charles Mashni of the AWTRL. He concentrated the sample to 30 ml by vacuum distillation and freeze-drying. A 5-ml portion of concentrate was then shipped to ORNL and chromatographed on our preparative-scale UV-Analyzer. Chromatographic eluent fractions associated with the major peaks were isolated and investigated using gas chromatography and mass spectrometry. One organic compound which eluted from the anion exchange column at 40 hr was identified by mass spectrometry as o-phthalic acid. The molar extinction coefficient of a reference sample of o-phthalic acid was determined to be 1120 absorbance-cm²/g-mole at a wavelength of 280 nm. The chromatographic peak representing this compound was then quantitated to give an o-phthalic acid concentration of 0.2 µg/ml in the original sewage. Although gas chromatographic and mass spectral data on some of the other peaks were obtained, no identifications could be assigned and further characterization was not considered to be profitable.

A sample of raw sewage was chlorinated at AWTRL, and portions of this material were chromatographed, before and after chlorination, on their UV-Analyzer. Fractions containing uv-absorbing peaks were collected, lyophilized, and sent to ORNL for possible identification. One fraction of the raw sewage sample contained xanthine; another contained guanosine. Two others coeluted with adenosine and 1-methylxanthine, but these identifications were discounted by other



(a)



(b)

Fig. 32. Chromatograms of UV-Absorbing Constituents Developed on the Southeast Environmental Research Laboratory UV-Analyzer. a) 0.25 ml of Reference Urine (URS-IV); b) 0.25 ml of Primary Effluent (1000X).

tests. One fraction of the chlorinated sample contained uracil, 5-acetylamino-6-amino-3-methyl uracil, and N-methyl-2-pyridone-5-carboxamide. The remainder of the fractions from both samples did not contain a sufficient quantity of material to obtain gas chromatographic and/or mass spectral data for identification.

ASSISTANCE TO THE SOUTHEAST WATER LABORATORY IN IDENTIFICATION OF COMPOUNDS IN SEWAGE PLANT EFFLUENTS

Concentrates of three samples taken at AWTRL were lyophilized and chromatographed on the preparative-scale UV-Analyzers. Large volumes (20 liters) of raw sewage influent and effluents from the Activated Sludge Sewage Treatment and Physical-Chemical Pilot Plants at the Robert A. Taft Water Research Center were collected and concentrated to less than 200 ml by Wayne Garrison and Frank Allen of SERL. These concentrates were shipped to ORNL, where they were lyophilized to dryness and then redissolved in a minimum volume of dilute ammonium acetate buffer solution. Five-milliliter aliquots of each sample were chromatographed on the preparative-scale UV-Analyzer (0.9 x 150 cm column), and 5-min fractions of the column eluate were collected. The individual fractions that contained uv-absorbing constituents were sent to SERL for subsequent gas chromatographic and mass spectral analyses.

SECTION VIII

DISCUSSION

The value of high-resolution liquid chromatography for analyzing sewage plant effluents has been demonstrated; and, with the identification of specific organic compounds present in effluents, the potential use of the UV-Analyzer, particularly in the dual-column configuration, to monitor and improve sewage plant operations becomes obvious. As additional compounds are identified and their presence or absence noted before and after various treatments, the efficacies of these treatments can be further evaluated. The combination of radioactive tracer techniques using ^{36}Cl with the UV-Analyzer has demonstrated that chlorinated organic compounds result from chlorination of water containing dissolved sewage residues, and this combination can be used to study the effects of chlorination on other waters. The use of the cerate-oxidative system as an additional monitor has further broadened the capability of the UV-Analyzer by adding to the list of detectable compounds and increasing its sensitivity so that its applicability would extend to many other compounds. Perchloratoceric acid is an extremely useful reagent for rapidly determining the chemical oxygen demand at less than 100 $\mu\text{g/liter}$ levels.

Some of the more important contributions that the results of this project could make to an understanding of the problem of water pollution are: (1) identification of most of the uv-absorbing refractory organics and many of the carbohydrates and other oxidizable components found in "domestic" sewage plant effluents; (2) determination of the fate of such compounds, particularly when the effluents are chlorinated, and identification of the stable chlorinated compounds which result; and (3) development of techniques for evaluation of the various methods of removing these pollutants. It is expected that a significant effort will continue to be directed toward the identification of stable organic compounds separated on the UV-Analyzers at AWTRL and SERL.

SECTION IX

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SECTION X

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2. Jolley, R. L. Chlorination Effects on Organic Constituents in Effluents from Domestic Sanitary Sewage Treatment Plants. Ph.D. dissertation, University of Tennessee, Knoxville, Tenn., 1973; Oak Ridge National Laboratory, Oak Ridge, Tenn., USAEC Report ORNL-TM-4290, October 1973. 342 p.
3. Jolley, R. L. Determination of Chlorination Effects on Organic Constituents in Sewage Treatment Plant Effluents: A Coupled ³⁶Cl Tracer--High-Resolution Chromatographic Technique. Presented at the 167th American Chemical Society. Los Angeles, Calif. Mar. 31 - Apr. 5, 1974.
4. Jolley, R. L. Chlorine-Containing Organic Constituents in Chlorinated Effluents from Sewage Treatment Plants. *Journal Water Pollution Control Federation*. Accepted for Publication.
5. Jolley, R. L., S. Katz, J. E. Mrochek, W. W. Pitt, Jr., and W. T. Rainey. A Multicomponent Analytical Procedure for Organics in Complex, Dilute Aqueous Solutions. *Chemical Technology*. Accepted for Publication.
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8. Katz, S., and W. W. Pitt, Jr. A New Versatile and Sensitive Monitoring System for Liquid Chromatography: Cerate Oxidation and Fluorescence Measurement. *Anal Letters* 5: 177, 1972.
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11. Katz, S., W. W. Pitt, Jr., C. D. Scott, and A. A. Rosen. The Determination of Stable Organic Compounds Present in Water at ppb Levels by Automatic High-Resolution Ion Exchange Chromatography. Presented at the 161st American Chemical Society National Meeting, Los Angeles, Calif., Mar. 29 to Apr. 2 1971.
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15. Pitt, W. W., Jr., R. L. Jolley, and C. D. Scott. Determination of Trace Organic Contaminants by High-Resolution Ion Exchange Chromatography. Environmental Science and Technology. Submitted for Publication.
16. Pitt, W. W., Jr., S. Katz, and L. H. Thacker. A Rapid Sensitive Method for the Determination of the Chemical Oxygen Demand of Polluted Waters. Presented at the 73rd National Meeting of the American Institute of Chemical Engineers, Minneapolis, Minn., Aug. 30, 1972; Water 1972. A.I.Ch.E. Symposium Ser. 129, Vol. 69, 1973.
17. Pitt, W. W., Jr., C. D. Scott, and M. D. McBride. Determination of Trace Organic Contaminants by High-Resolution Liquid Chromatography. Presented at the Seventh Annual Conference on Trace Substances in Environmental Health, University of Missouri, Columbia, Mo., June 12-14, 1973.

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16. Abstract					
<p>Residual organic compounds present in municipal sewage treatment plant effluents at microgram-per-liter levels were analyzed using high-resolution anion-exchange chromatography. Effluents were concentrated 50- to 3000-fold by vacuum evaporation and freeze-drying and then analyzed by liquid chromatographs capable of detecting uv-absorbing, oxidizable (with sulfatoceric acid), or carbohydrate constituents. Using techniques such as uv spectroscopy, gas chromatography, and mass spectrometry, 56 organic compounds were identified in primary effluent and 13 organic compounds in secondary effluent. Some of these constituents were quantified.</p> <p>Chromatographic procedures, coupled with radioactive tracer chlorination, were applied to the analysis of chlorinated primary and secondary effluents. More than 60 peaks containing chlorine were found, and specific chlorinated compounds were tentatively identified by cochromatography and quantified at the 0.5- to 4-µg/liter level.</p> <p>A detector system for liquid chromatography based on cerate oxidimetry was adapted as a rapid, sensitive continuous monitor for measuring the COD of waters. The effects of column geometry and operating parameters on chromatographic resolution were studied. Two high-resolution, ion exchange chromatographs (UV-Analyzers) were constructed for U.S. Environmental Protection Agency research laboratories, and are being used in the analysis of treated sewage effluents and other polluted waters. (Jolley - Oak Ridge National Laboratory)</p>					
17a. Descriptors *Pollutant identification, *Sewage effluents, *Chromatography, *Organic compounds, *Chlorination, Water analysis, Water pollution, Pollutants, Municipal wastes, Carbohydrates, Analytical techniques, Separation techniques, Instrumentation, Chemical oxygen demand, Gas chromatography, Spectrometry, Photometry, Fluorometry, Ion exchange, Radioactive tracers, Chlorine, Sewage treatment, Waste water treatment.					
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