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AUTOMATED ANALYSES OF POLLUTED WATER

Sidney Katz
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CHEMICAL TECHNOLOGY DIVISION

AUTOMATED ANALYSES OF POLLUTED WATER*

Sidney Katz
W. W. Pitt, Jr.

*This work was supported by the Environmental Protection Agency under Project #16020 EGY, Contract #14-12-833.

NOTICE: This document contains information of a preliminary nature and was prepared primarily for internal use at the Oak Ridge National Laboratory. It is subject to revision or correction and therefore does not represent a final report.

AUGUST 1972

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EPA Review Notice

This report has been reviewed by the Office of Research and Monitoring, EPA, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

PREFACE

This is the completion report on the first year's work in the Water Pollution Control Research Series. Although it was originally prepared for the Water Quality Office of the Environmental Protection Agency, it was not issued by this agency because the contract for the work was extended for an additional year and, in turn, the information documented was considered only an interim report. In order to make the details available locally, we have adapted the format of the report to that of an ORNL-TM Report.

ABSTRACT

Two anion exchange chromatographs, one for ultraviolet-absorbing compounds (UV-Analyzer) and the other for carbohydrates (carbohydrate analyzer), were adapted to the problem of analyzing the residual organic compounds present in municipal and industrial sewage effluents at μg/liter levels.

It was necessary to concentrate the samples of effluent 50- to 1000-fold prior to their analysis on the chromatographs. A two-step procedure, consisting of 10- to 30-fold concentration by vacuum distillation, followed by freeze-drying to attain the desired final volume, was developed. Loss of noncarbonate carbon with this procedure was less than 15%.

Both analytical procedures were demonstrated to be reliable and reproducible. Significantly different chromatograms were obtained of samples from different sources as well as samples from the same sources at different times. Normally, 70 to 100 ultraviolet (UV)-absorbing chromatographic peaks were resolved from samples of primary sewage treatment effluents; 30 to 50 were found for secondary effluents. Identities of 13 of the peaks occurring in primary effluent have been established. The carbohydrate analyzer chromatograms of primary effluent contained up to 38 peaks, while those of secondary effluent contained up to 19 peaks.

A UV-Analyzer for the Advanced Waste Treatment Research Laboratory of the Office of Research and Monitoring was constructed at ORNL at a cost of $13,200 and delivered in November 1970 to the Robert A. Taft Sanitary Engineering Center in Cincinnati, Ohio. Its use in the analysis of samples of both treated and untreated sewage has been satisfactory. An operating manual was supplied with the analyzer.

A modification of the UV-Analyzer has reduced the analysis time from 40 hr to about 6 hr.

This report was submitted in fulfillment of Contract No. 14-12-833 between the Office of Research and Monitoring, EPA, and the Atomic Energy Commission.

SECTION I

CONCLUSIONS

1. At least 50 to 100 refractory organic compounds are present in effluents from municipal sewage treatment plants at μg/liter levels; many more refractory compounds are present at higher concentrations in effluents from industrial sewage treatment plants.

2. High-resolution anion exchange chromatography provides a reliable and useful tool for determining refractory organic compounds present at low μg/liter levels in sewage effluents and various other polluted waters.

3. 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 analyzer for detecting sources of pollution, testing 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 program to adapt existing analyzers be continued and be expanded to include a more complete study of the carbohydrate analyzer and another analytical system for analysis of ninhydrin-positive compounds. Further, it is recommended that the range of usefulness of the UV-analyzer be extended and its operability improved.

To fit the special needs of this program, a "second-generation" UV-analyzer should be designed to meet the requirements as defined in field testing of the existing analyzer.

It is recommended that the identities of as many as possible of the residual stable organic compounds being discharged to surface waters be determined and their potential hazards be evaluated.

A very active program of analyzer application evaluation should be undertaken in the field to determine sources of pollution, the effectiveness of sewage treatments, and the fate of organic pollutants. 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 about problem areas that would lead to necessary modifications of the instruments.

SECTION III

INTRODUCTION

The discharge of stable organic compounds in sewage effluents to surface waters and the buildup of organic constituents by recycle can seriously affect water quality.[1,2] Studies aimed at developing methods to improve sewage treatment have been hampered by the lack of suitable analytical tools, and reliance on nonspecific and indirect determination has frequently been necessary. These determinations include measurements of total carbon, oxygen demand, total UV absorption, and selective fractional extraction.[3] As a result, potentially useful information regarding the chemical forms of the stable organic compounds, their concentration levels in discharged effluents, and the effectiveness of the various treatment methods for their degradation or removal has not been obtained.[2,4]

Because of their sensitivity and capability for detecting and quantifying many of the constituents in body fluid samples, two high-resolution anion exchange chromatographs recently developed at Oak Ridge National Laboratory[5-9] appeared to be adaptable for use in analyzing for the molecular components of residual organic compounds present in water at low concentrations. One of these chromatographic systems developed for the analysis of UV-absorbing constituents (UV-analyzer) is shown schematically in Fig. 1; it utilizes an acetate buffer as the eluent and ultraviolet photometry for detection to determine more than 200 compounds of biological origin in the concentration range of 100 μg/liter to 100 mg/liter. The second chromatograph, a carbohydrate analyzer, is shown schematically in Fig. 2; it uses a borate eluent and a phenol--sulfuric acid color development system to determine more than 50 carbohydrate compounds of biological origin in the concentration range of 1 to 100 mg/liter. Many of these compounds and their degradation products are probably present in sewage effluents. Additional organic compounds of concern from industrial and agricultural sources[2,4] could also be determined by these instruments.[10]

In this report, the following topics are discussed: progress in the preparation of sewage effluent samples for analysis by high-resolution anion exchange chromatography; evaluation of the method for reproducibility and sensitivity to sample differences; identification of specific organic compounds in effluent samples; initial observations on the relationship of the analyses to operating parameters of the sewage treatment plant; the construction of a UV-analyzer for use at the Advanced Waste Treatment Research (AWTR) Laboratory, and cooperative studies with the AWTR Laboratory; and possible applications of new separation systems to the pollution problem.

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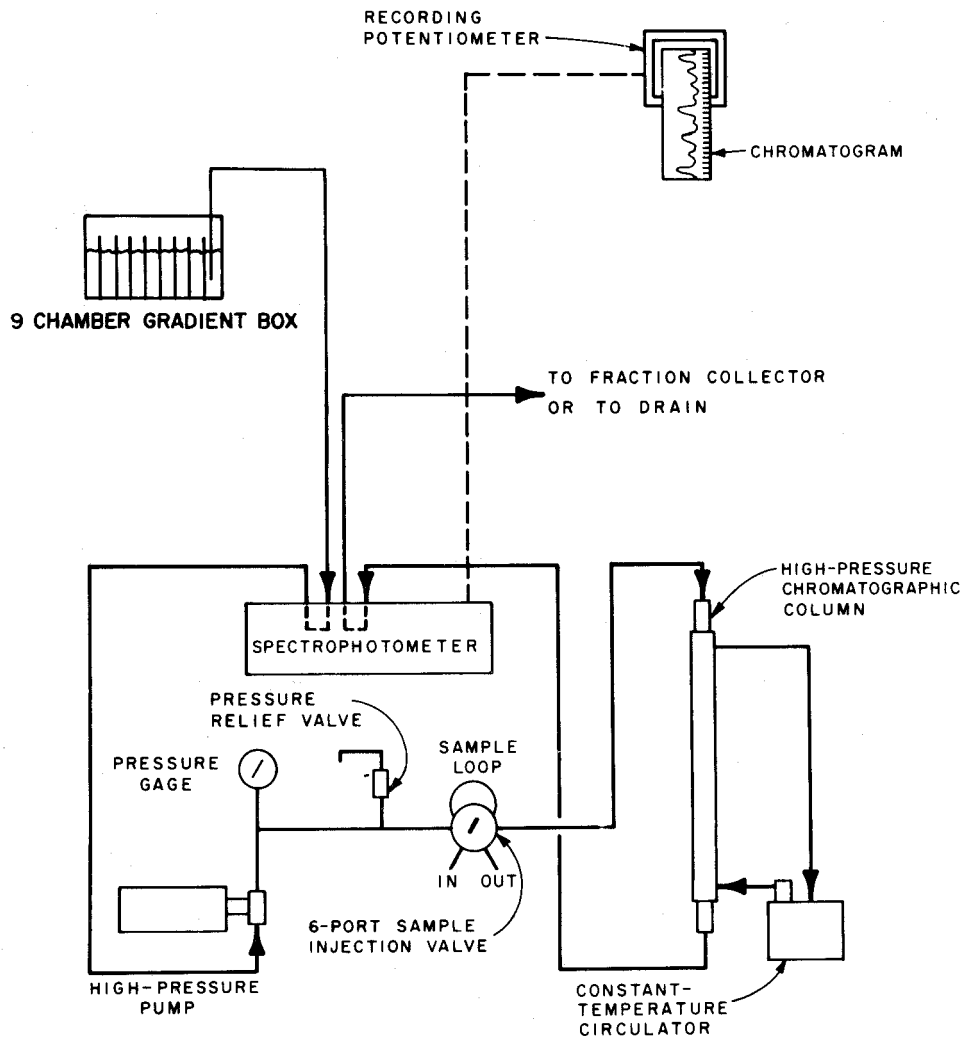

Fig. 1. High-pressure anion exchange chromatograph for the UV-absorbing constituents of body fluids.

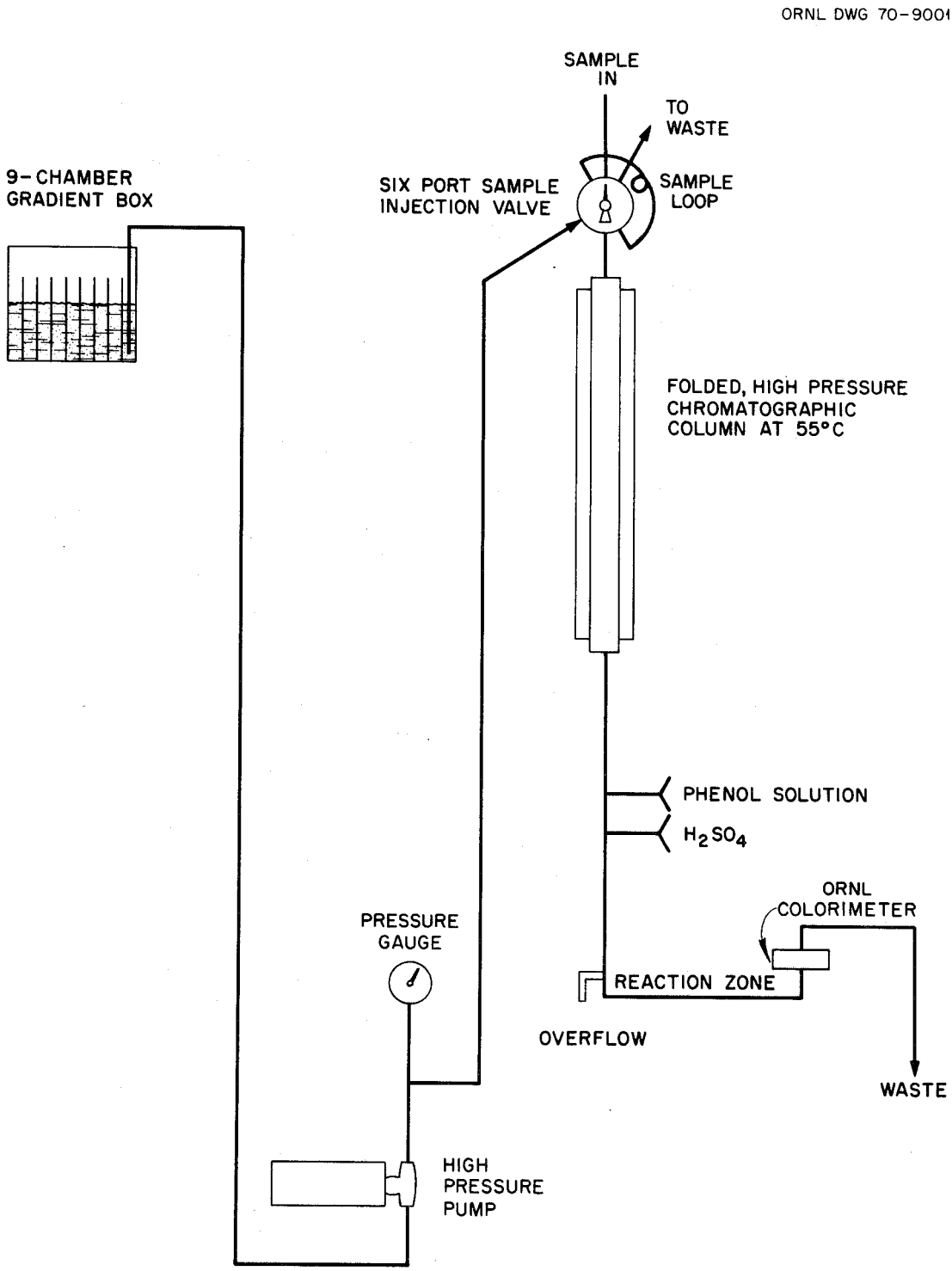

Fig. 2. High-pressure anion exchange chromatograph for carbohydrates in biochemical mixtures.

SECTION IV

INITIAL ADAPTATION AND TESTING OF ANALYZER

The beginning period of this study, from May through August of 1970, was primarily concerned with the development of a sample concentration technique. Work during the period from September 1970 through April 1971 was primarily aimed at demonstrating the quality and usefulness of the combined concentration and analysis method. Concurrent with the development of the concentration technique, the resulting concentrates were analyzed; however, since these analyses served mostly as a means for judging the effectiveness of the concentration method and were variable because of differences in techniques, actual data and details have been omitted. The contents of this section have been arranged to correspond largely with the order in which the work was done, as follows: (1) a listing of sample sources, and the development of a sample concentration technique; (2) the demonstration of reproducibility and sensitivity of the technique; (3) the identification and measurement of specific stable organic compounds in effluents; and (4) the results of various studies of actual sewage plant operating parameters.

Sources of Sewage Treatment Plant Effluent

Samples of sewage plant effluent after primary and secondary treatment were collected from the East Sewage Treatment Plant, Oak Ridge, Tennessee (essentially a municipal source with little industrial waste) and from the Third Creek Treatment Plant, Knoxville, Tennessee (80% industrial sources).

Concentration of Sewage Samples Prior to Analysis

Since the lower limit of detection for the UV-analyzer is 100 μg/liter to 100 mg/liter, depending upon the ultraviolet absorptivity of the individual compound, and the concentrations of specific contaminants in effluent samples may be 10 μg/liter or less, concentration of sewage effluents by factors up to 1000 may be necessary prior to analysis. As shown in later sections, the concentration factor depends upon the history of the effluent. Concentration methods[2,4,11] 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[12] (Fig. 3), a two-step concentration 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 10-μ frit 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 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 acetic acid (for the UV-analyzer) or borate buffer solution (for the carbohydrate analyzer) is added to attain the desired liquid volume and to adjust the pH to ~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.

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 secondary effluent and the separated phases of the final concentrates. The method used for carbon analysis involves oxidation with persulfate in acid solution, collection of the carbon dioxide in a caustic solution, and titration of the resulting carbonate. Unfortunately, this method is not sufficiently sensitive to permit accurate determination of the carbon concentration (22 ng/ml ± 100%) in the starting secondary effluent. However, the method is satisfactory for analyzing the liquid concentrate and the separated solids. The carbon contents of the liquid and solid portions of two samples of concentrate (3 liters of secondary effluent concentrated to 6 ml) were as follows:

	Carbon Content (mg ± 10%)		Fraction of Carbon
Sample	Liquid Portion	Solid Portion	in Solid Portion, %
1	120	6.0	5.0
2	150	8.4	5.3

The carbon found in the solid portion (and therefore not recovered for analysis) may be biased high since the solid could not be washed and thus undoubtedly retained some liquid concentrate.

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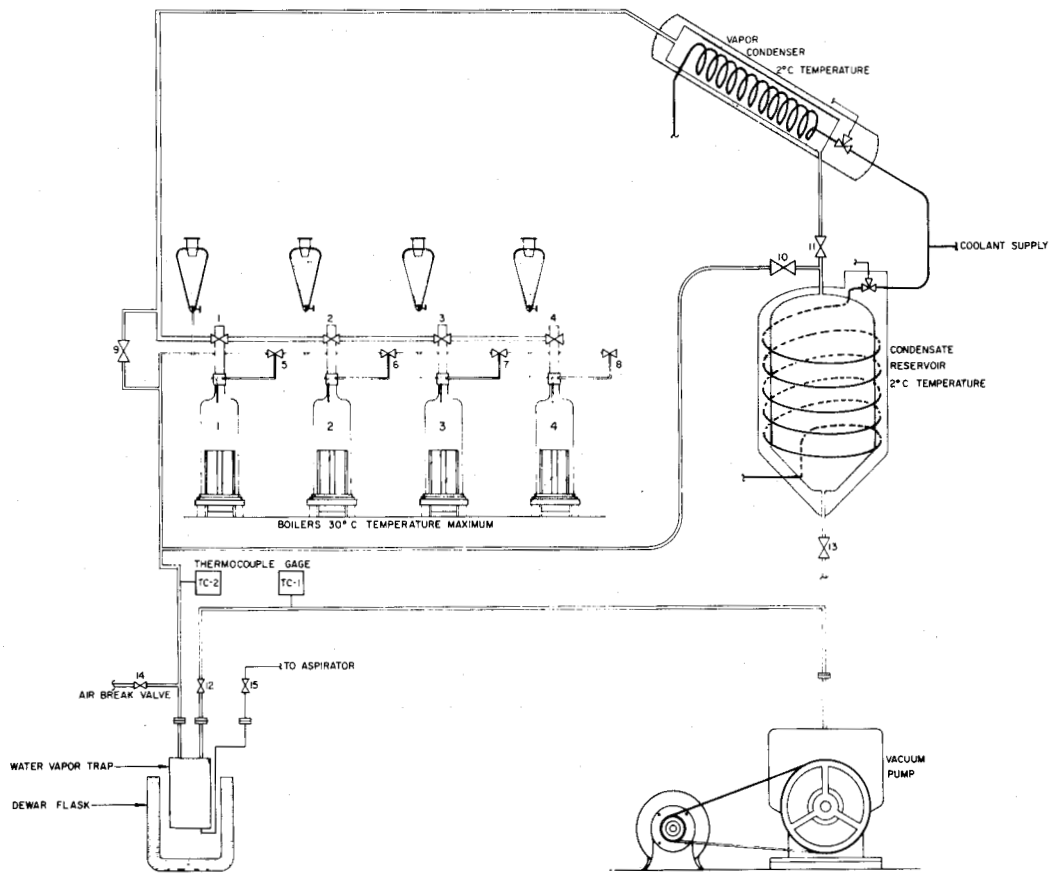

Fig. 3. Schematic diagram of vacuum distillation system.

Analytical Reproducibility and Capability of the UV-Analyzer

Several sets of duplicate analyses were made to demonstrate the reliability and reproducibility of the sample preparation and analytical procedures (Fig. 4). For each chromatogram shown, a separate 3-liter sample was individually prepared and the concentrate was chromatographed on a Mark II UV-analyzer, using the procedure described in the Appendix A. The replicate chromatograms for a primary municipal effluent collected on August 14, 1970, show good agreement. The two chromatograms for a primary municipal effluent collected on September 14, 1970, are in good agreement except that, in the second, the resolution of peaks at an elution volume of 130 ml was slightly better. While chromatograms for duplicate samples of primary municipal effluents are similar, large differences are noted in effluents collected on different days. The two chromatograms for secondary effluent collected on September 14 are similar except for the resolution of the peaks at an elution volume of approximately 320 ml.

Analysis of Municipal Sewage Effluents with the UV-Analyzer

Many of the peaks in UV chromatograms obtained from samples of primary municipal effluent are similar to those obtained from urine and other physiologic fluid samples (see the comparison in Fig. 5). Seventy-seven peaks were found in the chromatogram shown for a sample of primary effluent collected on August 19, 1970; about the same number of peaks appeared in other chromatograms of primary effluent. The compounds represented by these peaks are estimated to be present in the original samples at concentrations below 100 μg/liter. The UV chromatograms from secondary municipal effluent contain considerably fewer peaks than the normal urine chromatogram, although most of the 38 peaks present are commonly found in chromatograms for primary effluent. However, two of the peaks in the secondary effluent chromatogram are significantly larger than the corresponding peaks in the primary effluent chromatogram, indicating the production of some compounds during the secondary treatment. The concentrations of individual compounds noted in the secondary effluent are estimated to be less than 20 μg/liter.

Tentative identification of compounds indicated by the chromatographic peaks may be made from chromatographic properties (elution positions) and from relative UV-absorption at the several wavelengths measured. However, positive identification requires the collection of separated fractions from a preparative-size anion exchange system and subsequent examination of the fractions by such techniques as cation exchange chromatography, UV absorption, gas chromatography, and mass spectroscopy. Such treatments are tedious and become increasingly difficult as one progresses from more to less abundant constituents. The chromatogram from a preparative system showing the elution positions of the 13 compounds which have been identified is shown in Fig. 6. The identified compounds are also listed in Table 1 and in cases where the separation from other peaks permitted, quantitative results are given.

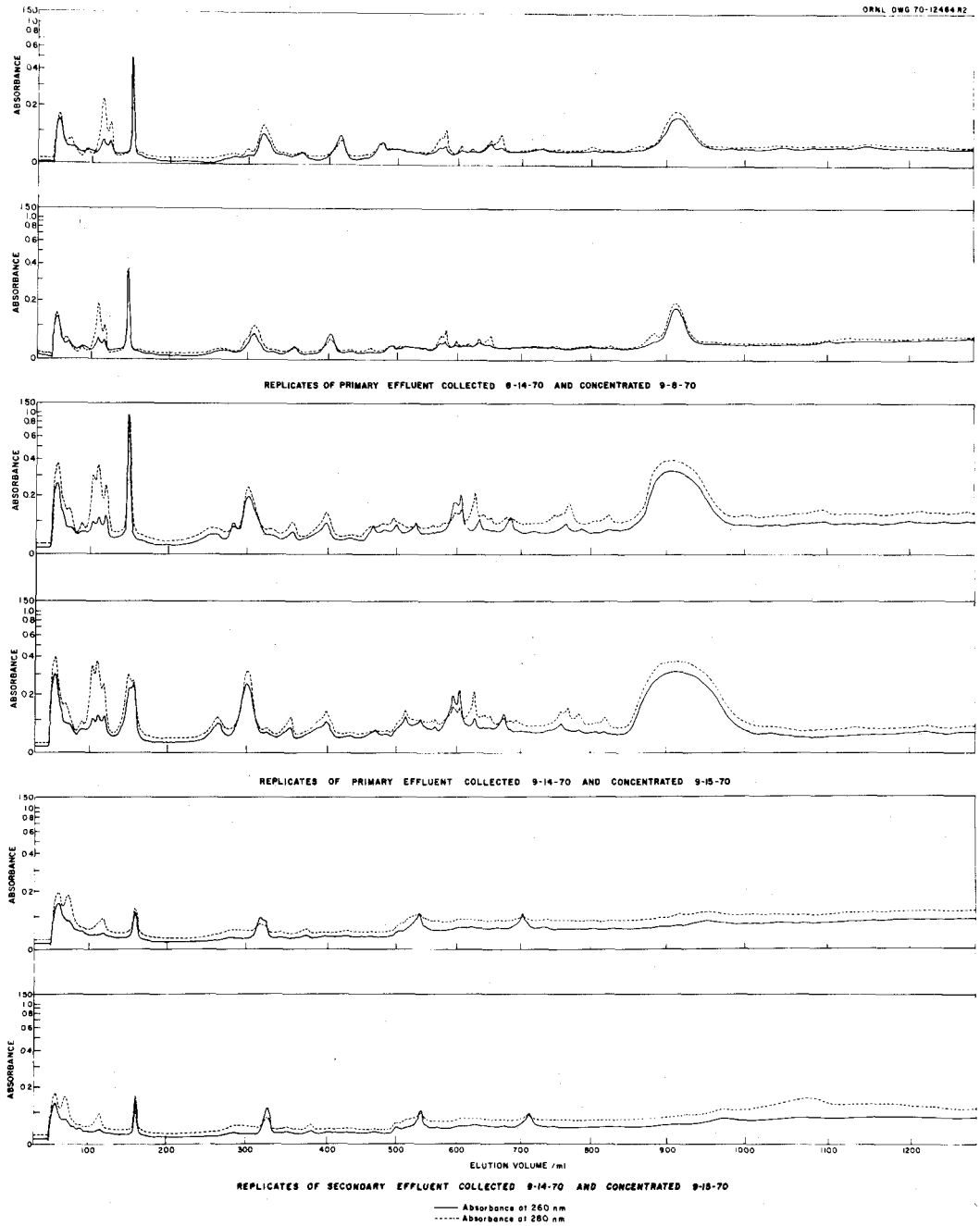

Fig. 4. Duplicate chromatograms of three sewage plant effluents analyzed by the UV-Analyzer.

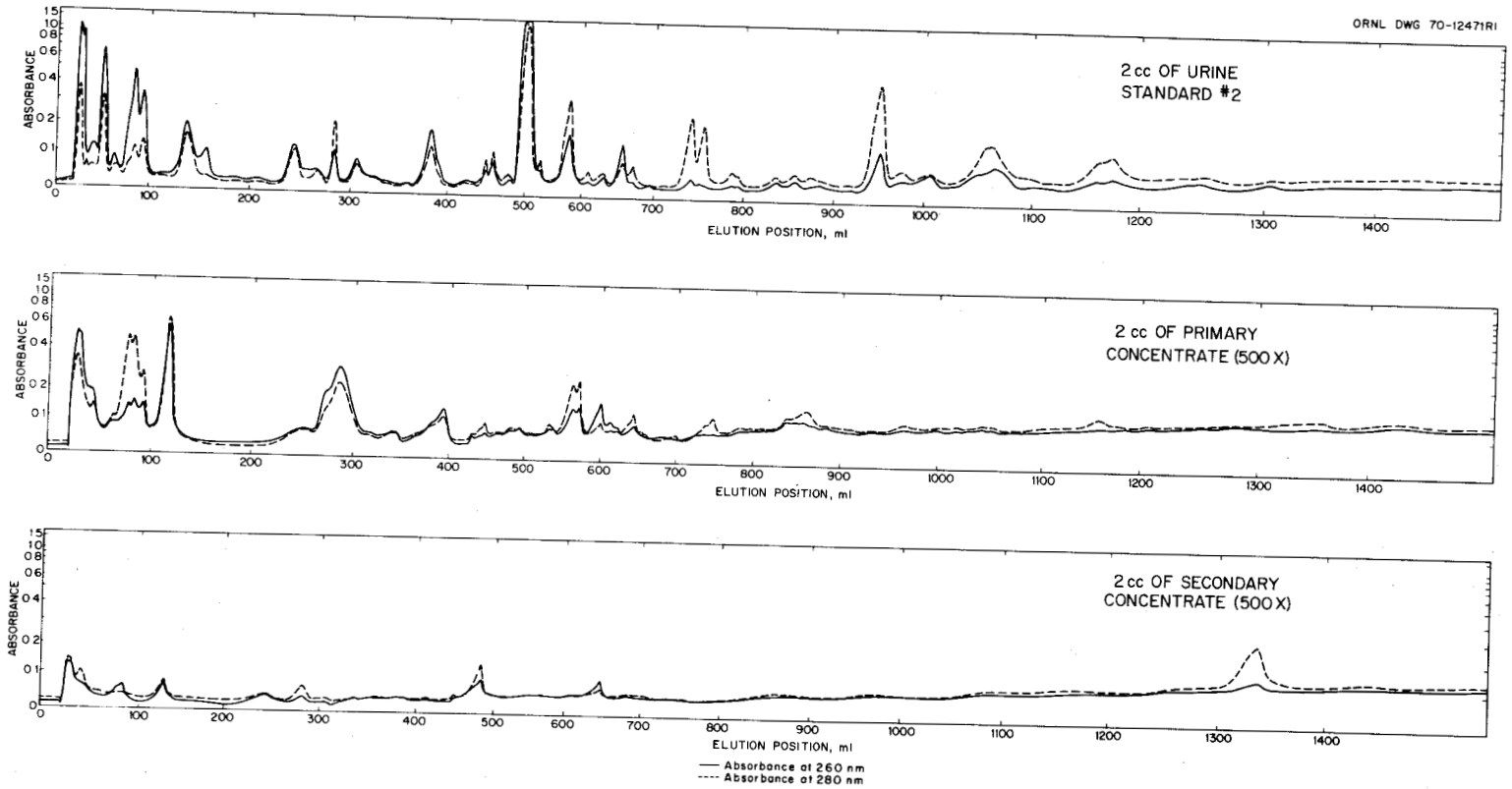

Fig. 5. Comparison of UV chromatograms of primary and secondary sewage plant effluents with a UV chromatogram of human urine.

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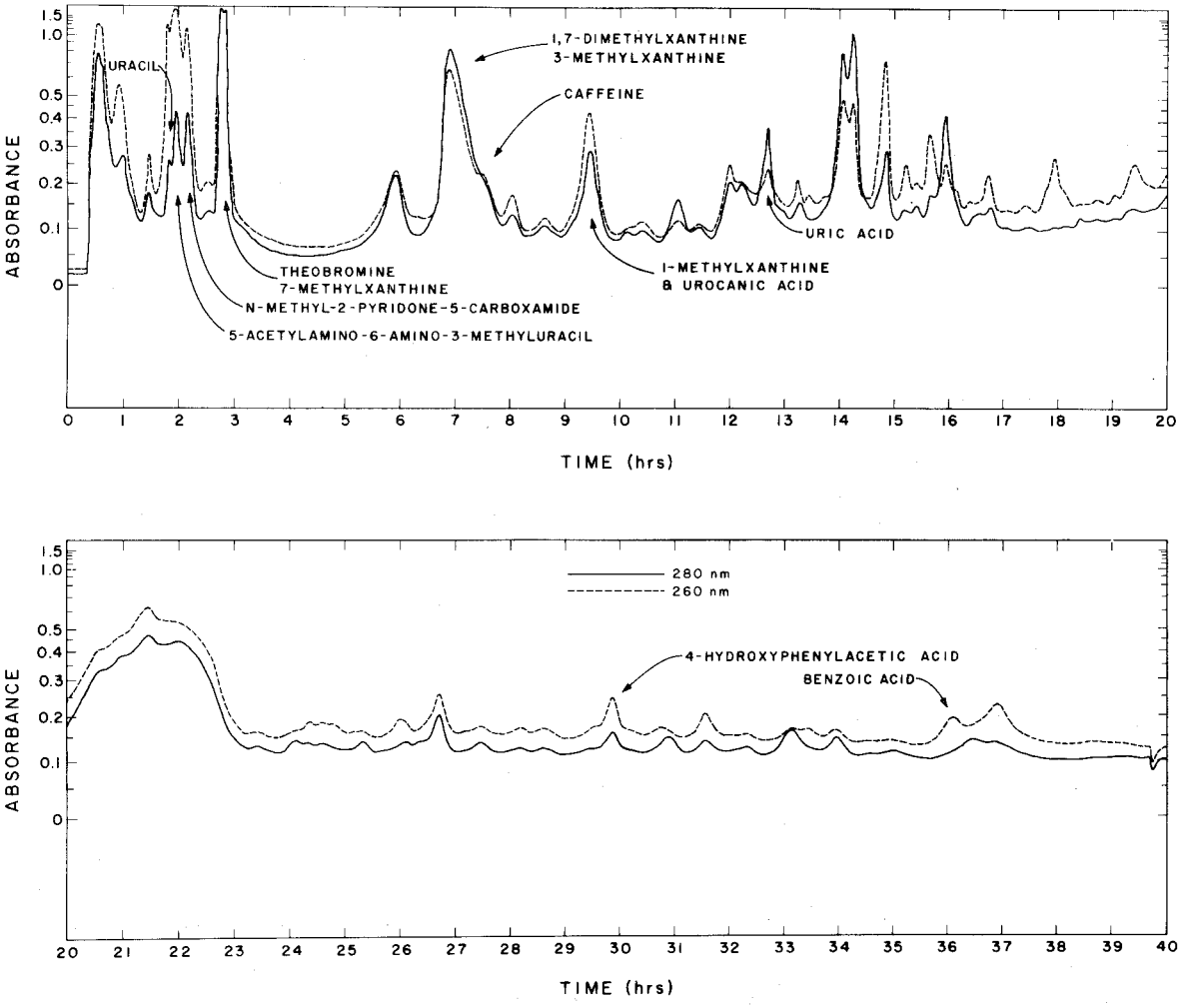

Fig. 6. Chromatogram of primary sewage effluent analyzed on the preparative UV-Analyzer.

Table 1. Compounds Identified in Sewage Effluents

Compound	Identification Methods[a]	Concentration[b] (μg/liter)
Uracil	AC, CC	13
5-Acetylamino-6-amino-3-methyluracil	AC, CC	30
N^1-Methyl-2-pyridone-5-carboxamide	AC, CC	10
Caffeine	AC, GC, MS	
Theobromine	AC, CC	
7-Methylxanthine	AC, CC	
1,7-Dimethylxanthine	AC, CC	
3-Methylxanthine	AC, CC	
1-Methylxanthine	AC, CC	17
Urocanic acid	AC	
Uric acid	AC, GC, MS	
4-Hydroxyphenylacetic acid	AC, GC, MS	
Benzoic acid	AC, GC, MS	

[a]AC - anion exchange cochromatography; CC - cation exchange cochromatography; MS - mass spectroscopy; GC - gas chromatography.

[b]For peaks sufficiently resolved to allow quantification.

Analysis of Municipal Sewage Effluents with the Carbohydrate Analyzer

A new, more sensitive Mark III Carbohydrate Analyzer[9] 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 for urine and reference sugar standard samples in Fig. 7. In the first one and one-half hours 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 upon elution positions. Several of the simple sugars also appear in the urine sample; the others, if actually present, may be products of chemical or biological reactions during the treatment. The peaks appearing at 14 hr in the chromatogram of primary effluent (and to a lesser extent in the chromatogram of secondary effluent) and the very large peak appearing only in the secondary chromatogram at 17 hr (Fig. 7) are not due to simple sugars.

Analysis of Industrial Sewage Effluents with the UV-Analyzer

Chromatograms for effluents from a plant treating industrial wastes (see Fig. 8) are significantly different from those for effluents from a municipal treatment plant (see Figs. 4 and 5). Furthermore, the sample used to obtain the chromatograms shown in Fig. 8 was concentrated only 40-fold because of its higher content of organic compounds (estimated to be up to 1 mg/liter) and other dissolved salts; in contrast, municipal-type effluents are usually concentrated 500-fold.

In the case of the industrial sewage, the secondary treatment was largely ineffective for a large number of organic compounds, and the chromatogram for the secondary effluent closely resembled the chromatogram for the primary effluent, as shown in Fig. 8.

Effects of Operating Conditions at the Treatment Plant on Analyses

A qualitative relationship appeared to reflect seasonal and weather conditions. During hot and dry periods, the size and the number of peaks found in UV chromatograms from municipal sewage treatment effluents were diminished as compared with those found during cold or wet periods. This effect may be due to more efficient treatment at higher temperature and with the longer residence time in the treatment plant.

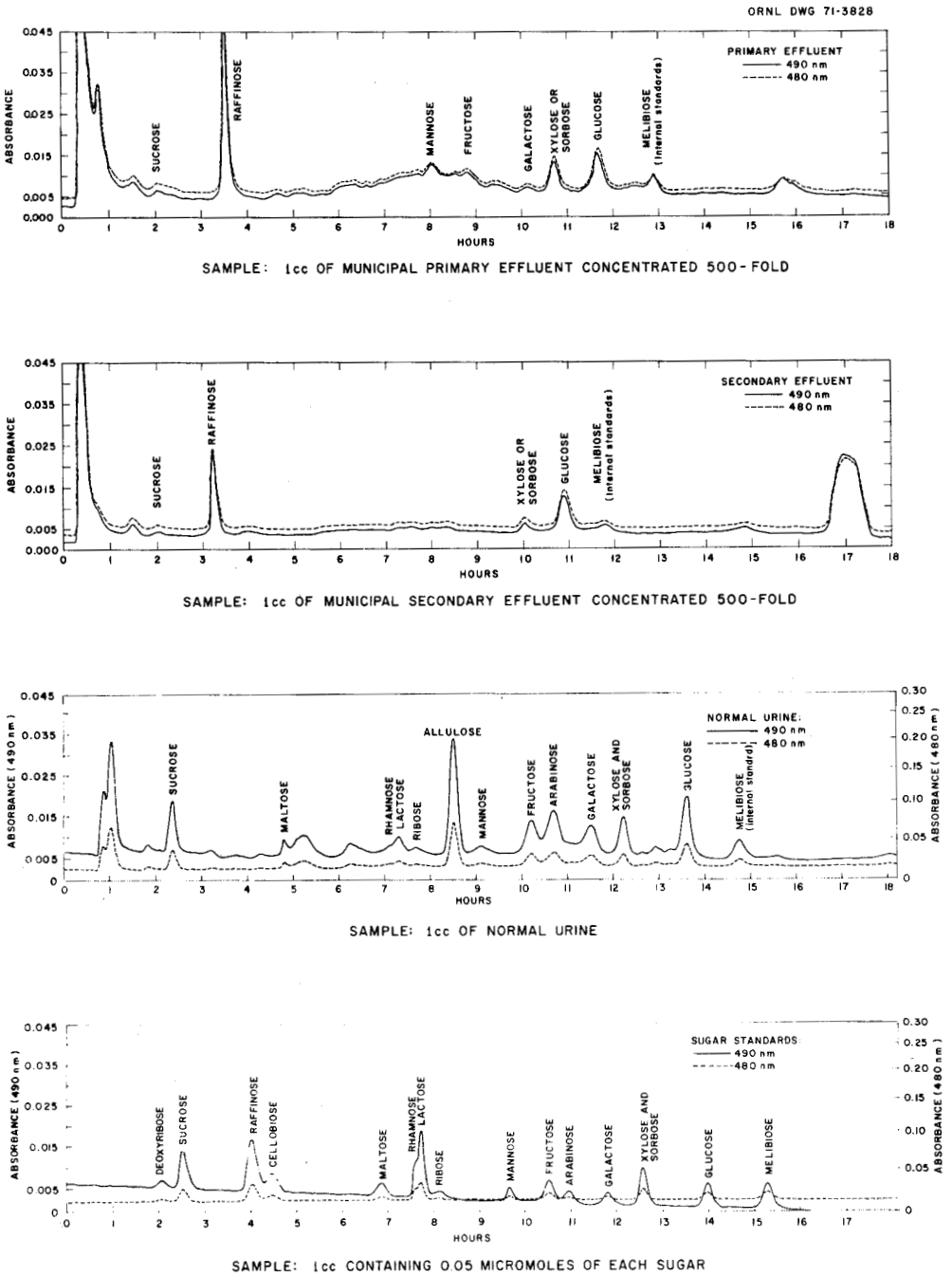

Fig. 7. Chromatograms of primary and secondary sewage effluents, human urine, and sugar standards obtained on the carbohydrate analyzer.

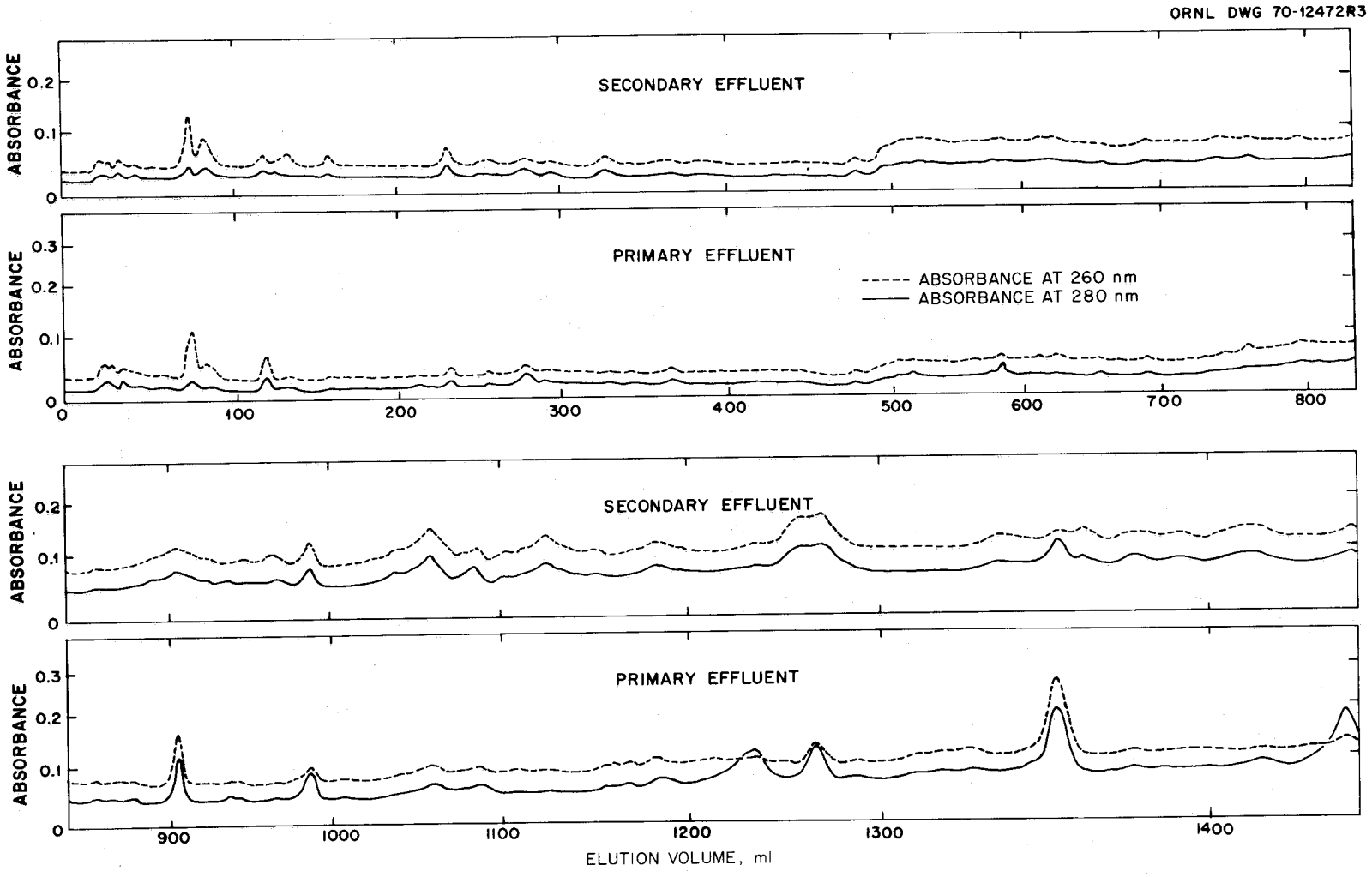

Fig. 8. UV chromatograms of effluents of primary and secondary plants treating industrial wastes.

SECTION V

ADVANCED ANALYZER DEVELOPMENT

A large part of the developmental effort involving high-resolution separations at ORNL, exclusive of that devoted to the Automated Analysis of Polluted Water, is aimed at decreasing the time required for analysis and at providing means for analyzing multiple samples in parallel at a significantly lower cost but with no loss in resolution. Similar requirements appear to exist in the water pollution area, and work has begun along these lines. To provide perspective, it should be noted that the Mark II UV-analyzer (the model at the AWTR Laboratory) was developed in its present form in 1968; however, a more compact Mark III model, using a 0.22-cm-diam instead of a 0.62 cm-diam column but with essentially the same operating time requirement and output, was developed in 1969-1970. Design and operating parameters that have been under continuing investigation are length and/or diameter of the column, the steepness of the concentration gradient, the operating pressure, the coupling of anion to cation columns, and the assembly of multiple columns into a single analyzer. Based on the information obtained to date on the interactions of these parameters, we believe that we could build an analyzer that would accept multiple samples and resolve 90% of the peaks found in each sample in a normal 40-hr run within a 5-hr period.

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:

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

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

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

Van Deemter's[13] 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 obtained thus far have confirmed the first proportionality (Fig. 9). 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 might be eluted between any two peaks. (Indeed, it is known that other compounds elute between the two pairs shown in Fig. 9.) 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. 9, 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,

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

where A = cross-sectional area of the column. Thus, the separation of pseudouridine and uracil (Fig. 9) 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.

Development of Parallel Multicolumn Systems

Another means of increasing the sample handling capacity of a chromatographic system is by the use of multiple columns in parallel. A conceptual design for operating eight columns in parallel on the Mark II UV-analyzer was made (Fig. 10). To test the concept, a chromatographic system with two columns in parallel was constructed (Fig. 11), and various pairs of samples (both duplicate and different) were analyzed. The resulting chromatograms (two pairs of which are shown in Figs. 12 and 13) indicate that this method of operation is not only feasible but desirable.

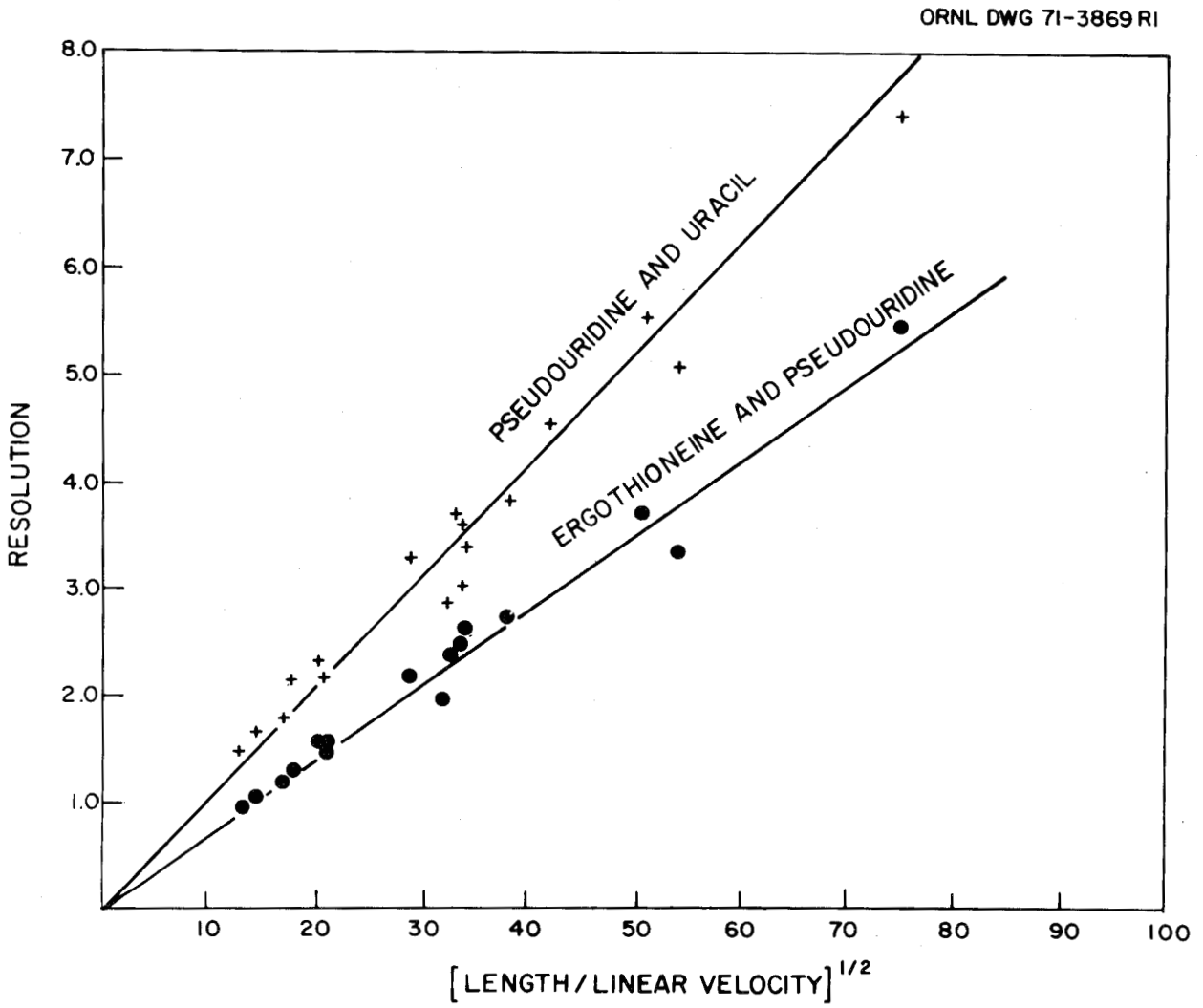

Fig. 9. Dependence of chromatographic resolution on column length and linear velocity of eluent.

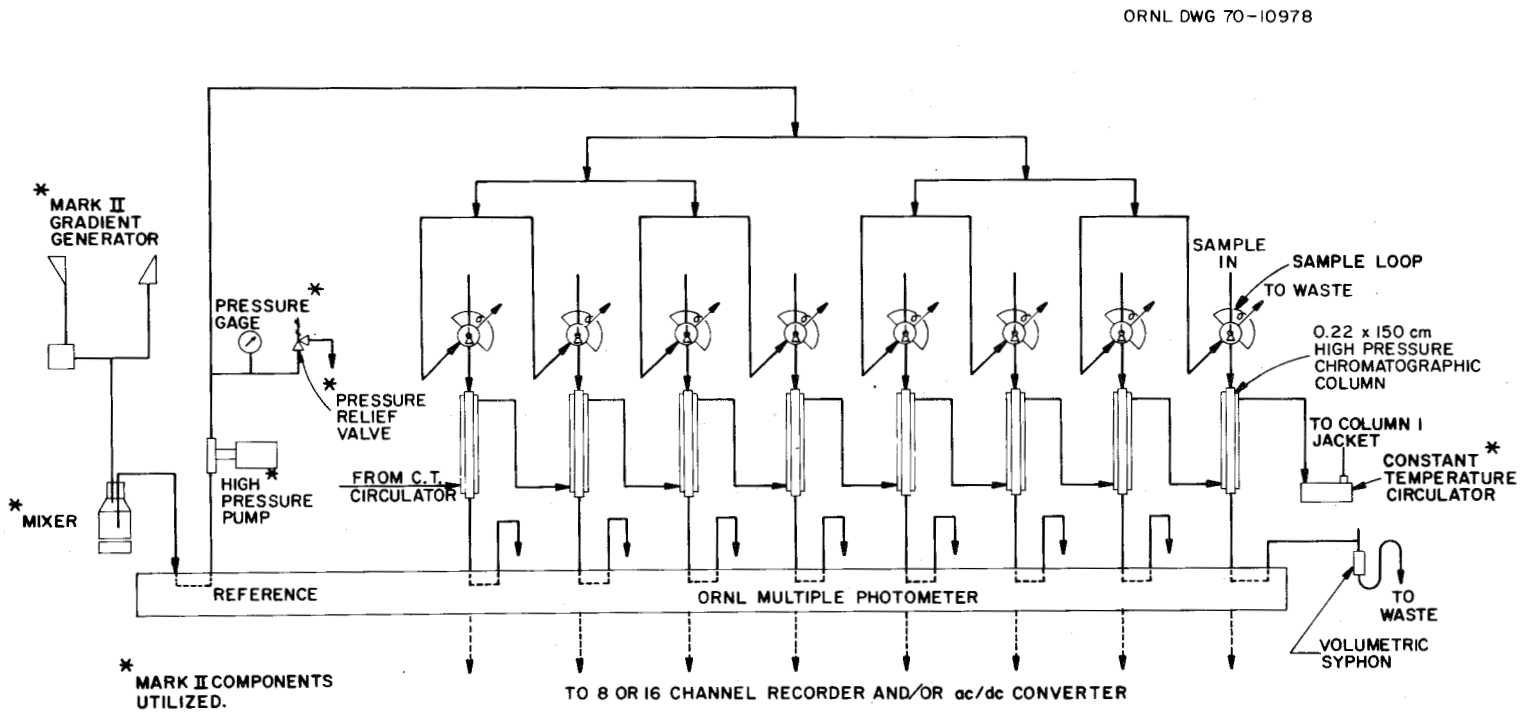

Fig. 10. Schematic diagram of parallel-multicolumn UV-Analyzer utilizing Mark II prototype components.

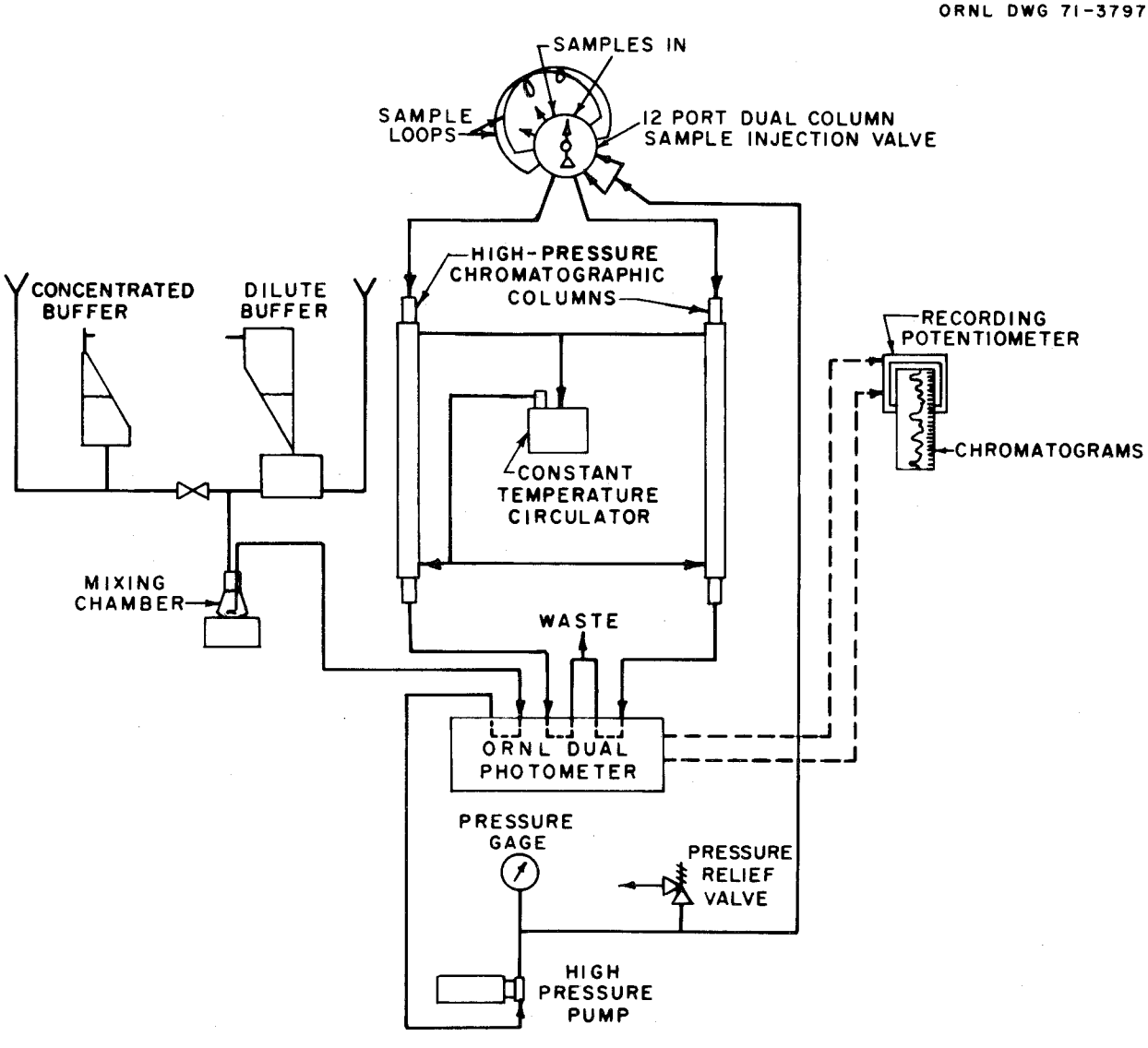

Fig. 11. Schematic Diagram of parallel-dual-column UV-Analyzer.

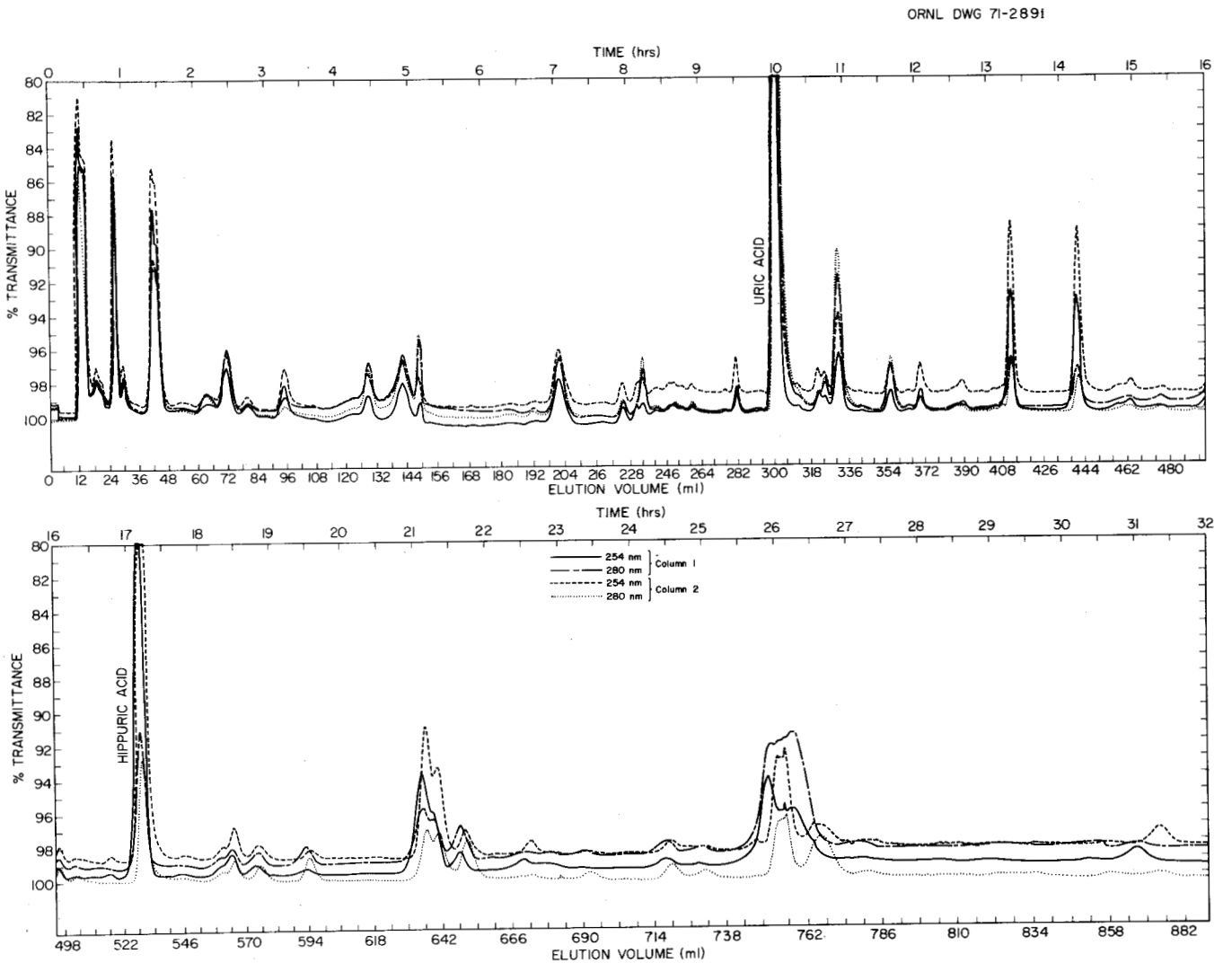

Fig. 12. Chromatograms of duplicate urine samples obtained on the parallel-dual-column UV-Analyzer.

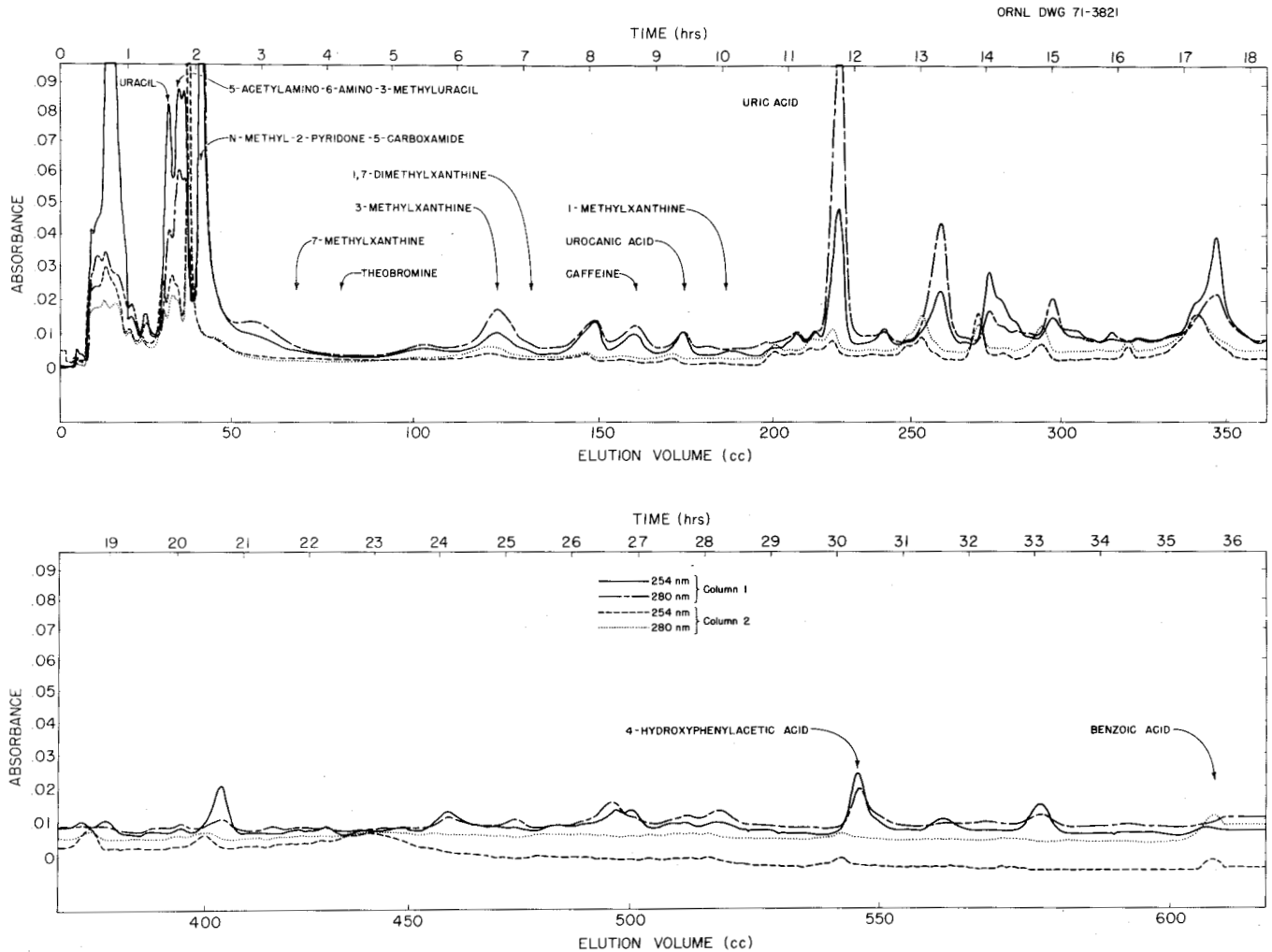

Fig. 13. Chromatograms of primary (Column 1) and secondary (Column 2) sewage effluents obtained on the parallel-dual-column UV-Analyzer.

Before routine parallel-column analyzers can be built, however, two auxiliary components -- a multiple sample injection valve and a multiple column detector -- need to be developed. A two-column sample injection valve with 12 ports has been designed, built, and tested on the parallel-column system. This valve can simultaneously inject two different samples into two fluid streams at pressures up to 5000 psi. The design principle can be utilized to construct valves for the simultaneous injection of more than two samples. A conceptual design of a multicolumn, ultraviolet photometer was also made (Fig. 14). This design provides for monitoring several flowing streams at two wavelengths, referenced to another flowing stream.

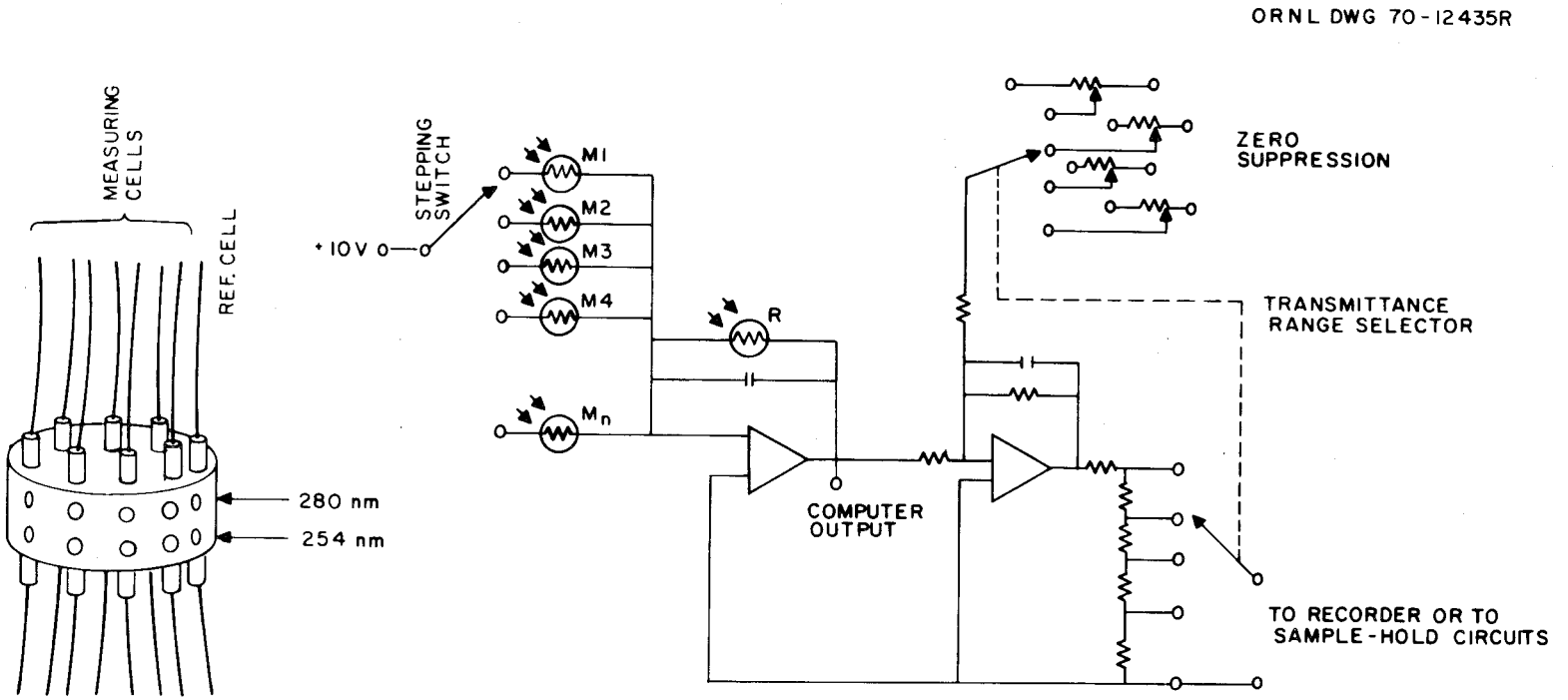

Fig. 14. Conceptual design of a multicolumn, dual-wavelength ultraviolet photometer.

SECTION VI

COOPERATIVE EFFORTS WITH THE ADVANCED WASTE TREATMENT RESEARCH LABORATORY

In accordance with the specific aims stated in the program proposal, a high-resolution chromatograph for the analysis of stable organic compounds was designed, constructed, tested, and then delivered to the Advanced Waste Treatment Laboratory. Supporting efforts in applying the instrument to field problems have included assistance in the areas of operation, maintenance, and minor modifications but have principally consisted of studies to identify compounds.

Construction of a UV-Analyzer for the Advanced Waste Treatment Research Laboratory

A significant part of this program has been concerned with the construction of a UV-analyzer for use at the AWTR Laboratory. The design of the Mark II UV-analyzer (from the Body Fluids Analyses Program) was selected. However, a nine-chamber Varigrad was substituted for the original two-chamber gradient generator to provide flexibility in choosing a gradient. The use of this analyzer design will simplify 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 will be directly applicable.

Fabrication of the analyzer was completed in November 1970 at a cost of $13,200. Six test samples were run at ORNL to ensure satisfactory operation. Excellent results were obtained, and the resolution of peaks was equal to that obtained with other Mark II UV-analyzers. During the test period, Mr. Charles Mashni of the ORM Laboratory was given three days of training with the instrument. Finally, the analyzer was disassembled, shipped, reassembled (at the Robert A. Taft Sanitary Engineering Center), and, with the assistance of personnel from ORNL, placed in operation. The operation of the analyzer has been very satisfactory; only minor maintenance has been required. In the initial tests, samples of untreated and treated sewage available at the Cincinnati facility were used. A cooperative effort to identify stable organic constituents of effluents collected and separated on the ORM analyzer is discussed in the section that follows.

An operating manual for the Mark II UV-analyzer (see Appendix A) was prepared and sent to the ORM Laboratory. An additional column, 50 cm long and 0.45 cm in inside diameter (as described in the preceding section of this report) was supplied in order to permit shorter operating periods with only a small loss in resolution.

Identification of Organic Constituents in AWTR Laboratory 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 ORM. He concentrated

the sample by vacuum distillation and freeze-drying to 30 ml. A 2-ml portion of the concentrate sample was introduced into the UV-analyzer at the ORM, and the separated fractions were collected. Fractions corresponding to five major peaks were sent to ORNL for identification studies. Two of the fractions contained compounds that were sufficiently separated to give good gas chromatographic traces; however, the elution positions of the peaks indicated that the compounds were different from those in our library of compounds, in physiologic fluids, or in sewage effluent samples examined to date. The mass spectrographic data indicated that a more clearly defined separation was needed.

In order to provide a larger quantity of unknown compound for examination, a 5-ml portion of concentrate was injected into our preparative system. The fractions corresponding to the larger peaks shown in the resulting chromatogram (Fig. 15) are being studied.

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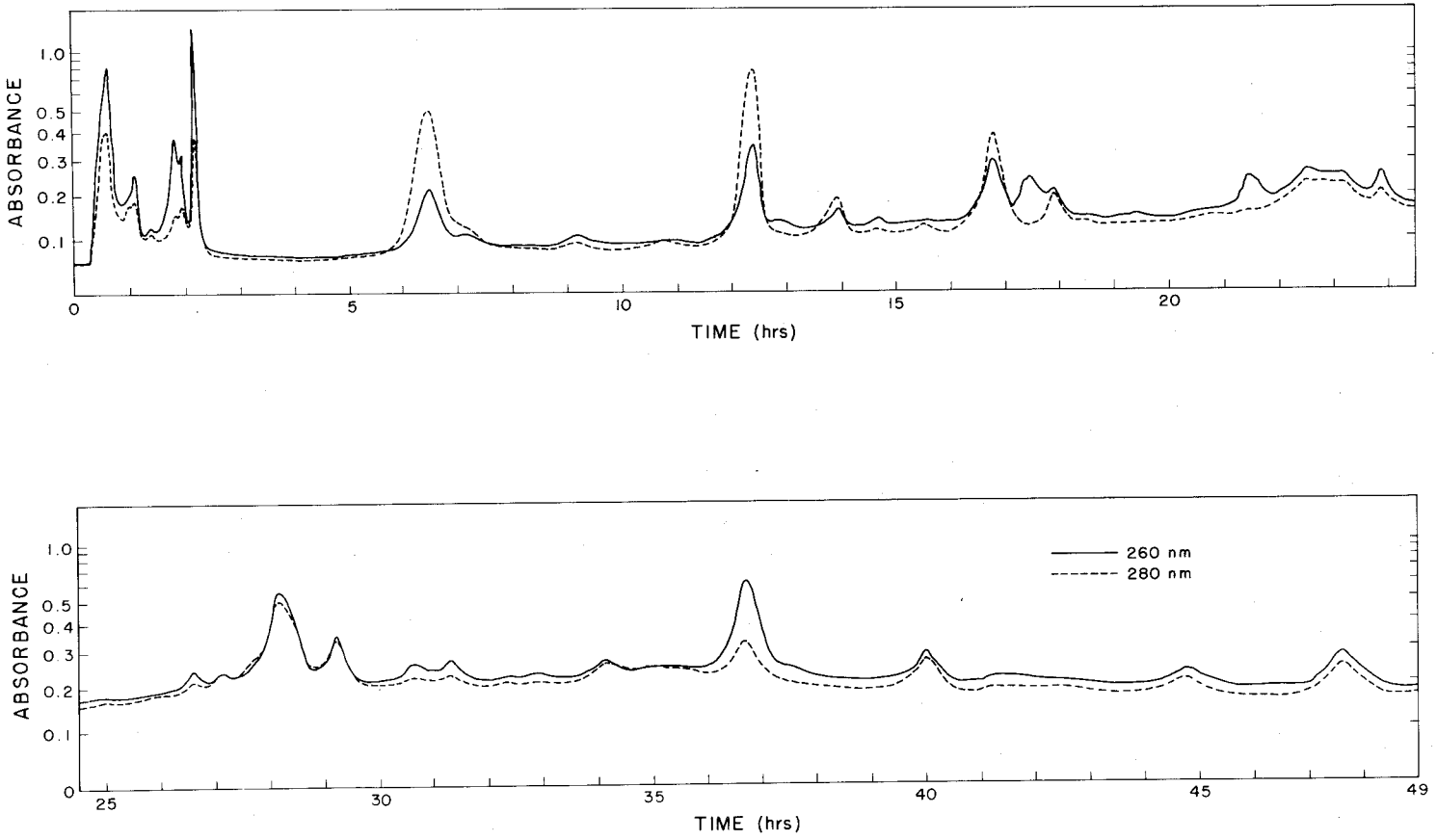

Fig. 15. Preparative column chromatogram of sample from Mill Creek Sewage Treatment Plant.

SECTION VII

DISCUSSION AND AIMS OF FUTURE WORK

In its present state of development the UV-analyzer, with the alternate design and operating features that have been evaluated, appears to have significant potential in the field of water pollution. As an analytical tool, it can be used to identify sources of pollution, to help evaluate new processing steps and operational methods, and to routinely monitor treatment plant operational effectiveness. In each of these analytical applications, the need for resolution, the time requirement, and the sample load would determine what combination of column(s), column dimensions, operating parameters, and methods for handling data would be most desirable. As a research tool, the UV-analyzer can be used to study the conditions that would be favorable to degeneration of stable organic compounds during and after sewage treatment, to follow the chemical changes in stable organic compounds as a result of test treatments, to gain a better understanding of the chemical and biological processes during the various treatment steps, and to develop an understanding of chemical structural units and arrangements which favor stability of organic compounds during treatment.

At present, the carbohydrate analyzer can separate and quantify about one-third as many compounds as the UV-analyzer. Because of the more recent development of the carbohydrate analyzer in its more sensitive form, the application, testing, and identification of compounds have not progressed to the same extent as has been possible with the UV-analyzer.

Some of the more important contributions that this project could make to an understanding of the problem of water pollution are: (1) identification of the individual stable organic compounds that are being discharged to surface waters, (2) determination of the fate of such compounds during chlorination and upon dispersal, and (3) establishment of a background for studies of the possible harmful effects upon the ecology. Thus, a continuing effort is being directed toward the identification of stable organic compounds that are separated on the UV- and the carbohydrate analyzers. Application testing of the UV-analyzer is expected to continue in cooperation with the Advanced Waste Treatment Research Laboratory. Application testing of the carbohydrate analyzer will also be conducted to make the status of this instrument equal to that of the UV-analyzer. In addition, concurrent developmental work to accelerate the analyses and make the instruments more reliable and more sensitive will be carried out.

SECTION VIII

ACKNOWLEDGMENTS

The suggestion that high-resolution chromatography might be useful in water pollution studies emerged from a conversation between Dr. A. A. Rosen of the Division of Field Investigation, Environmental Protection Agency and Dr. C. D. Scott of the Oak Ridge National Laboratory. Both have been intimately involved with the maturation of the project and have maintained frequent contact with ongoing developments. Dr. Rosen was especially helpful during his seven-month tenure as project officer. Dr. Scott, as overall director of the Biochemical Separation Systems Development Section of the Molecular Anatomy Program at ORNL, has been in close contact with the development effort. The principal development effort and report preparation has been by Dr. S. Katz and Dr. W. W. Pitt, Jr.

The assistance and the cooperation of Mr. Charles Mashni, Office of Research and Monitoring, Environmental Protection Agency, throughout the program, and especially during the closing five months when he was project officer, have been exceedingly helpful.

Samples from the East Sewage Treatment Plant were collected in cooperation with Mr. T. C. Stephens and Mr. J. Robinson, Jr., operating under the supervision of Mr. O. K. Rickman, the City of Oak Ridge Director of Public Works.

Samples from the Third Creek Sewage Treatment Plant were collected in cooperation with Mr. O. Johnson, operating under the supervision of Mr. J. W. Whisman, the engineer in charge of wastewater control, Knoxville, Tennessee.

The four-place vacuum distillation equipment was made available by Dr. W. Davis, Jr.

The construction of the Mark II UV-analyzer for the Advanced Waste Treatment Laboratory of the Office of Research and Monitoring was under the direction of Mr. W. F. Johnson. Mr. G. Jones assisted in the checkout of the analyzer.

A number of samples were run on the UV-analyzer and the carbohydrate analyzer by Mr. S. R. Dinsmore and Mr. G. Jones.

The support of the Chemical Identification Group working under Dr. J. E. Mrochek and including Dr. W. C. Butts, Mr. M. L. Freeman, and Dr. W. T. Rainey, was of particular importance to the success of the program.

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SECTION X

PUBLICATIONS

1. Katz, S., Pitt, W.W., Jr., Scott, C.D., and Rosen, A.A., "The Determination of Stable Organic Compounds Present in Water at ppb Levels by Automatic High-Resolution Ion Exchange Chromatography," 161st American Chemical Society National Meeting, Los Angeles, Calif., March 29 to April 2, 1971.

2. Katz, S., Pitt, W.W., Jr., Scott, C.D., and Rosen, A.A., "The Determination of Stable Organic Compounds in Water Effluents at μg/liter Levels by Automatic High-Resolution Ion Exchange Chromatography," Environmental Science and Technology (submitted for publication).

APPENDIX

OPERATION MANUAL FOR THE FWQA UV ANALYZER, PROTOTYPE MARK II

W. Wilson Pitt, W. F. Johnson, C. D. Scott

1. INTRODUCTION

A major goal of the Body Fluid Analyses Program is to develop automated, high resolution analytical systems for the clinical laboratory. Two such machines are under development-- an analyzer for the UV-absorbing constituents of body fluids (UV-analyzer) and a carbohydrate analyzer.[1,2] Development has progressed to the point where prototype instruments have been built and are being tested. This memorandum describes the design and operations of the UV-Analyzer Prototype Mark II as modified for application to sewage effluent concentrates.

The instrument uses a heated high-pressure anion exchange column for separation, elution with an acetate buffer whose concentration increases with time for separating and transporting the constituents of the sample, and a recording spectrophotometer for detection. A sample is introduced into the column via a six-port injection valve in the high pressure eluent stream just ahead of the separation column. The chromatogram, which shows the absorbance of the column effluent as a function of time, is developed by gradient elution with an acetate buffer, increasing the acetate concentration from 0.015 M to 6.0 M during the elution, and consecutively measuring the absorbance of the effluent at 260, 270, 280 and 290 mμ (Fig. 1).

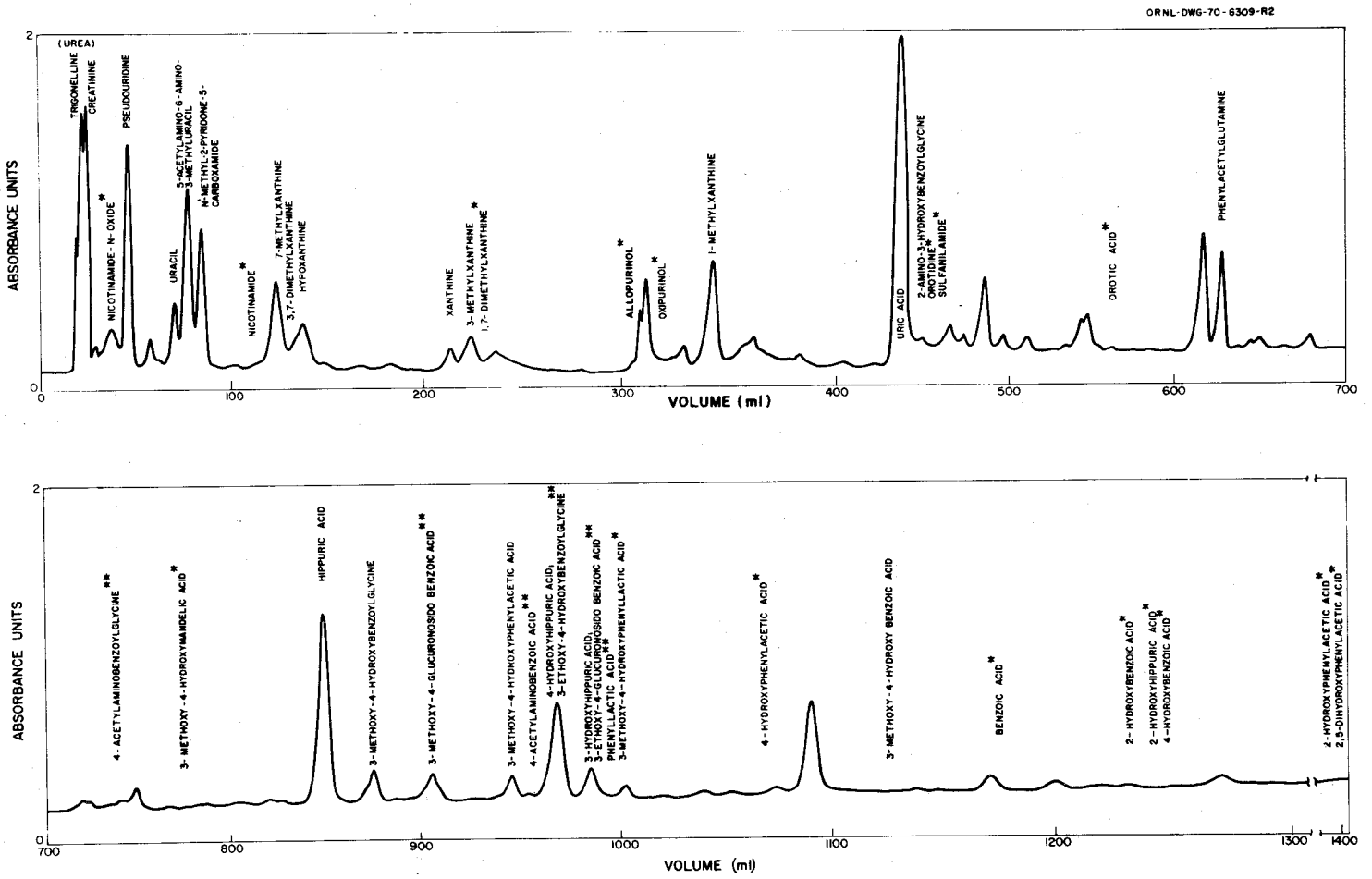

Fig. 1. Standard Urine Chromatogram From UV Analyzer Showing Identified Compounds

2. GENERAL DESCRIPTION OF THE UV ANALYZER

A schematic diagram of the UV analyzer is shown in Fig. 2. The eluent buffer concentration gradient is produced from two coupled reservoirs--one containing dilute buffer (0.0047 M $NH_4C_2H_3O_2$ · 0.0103 M CH_3COOH) and the other containing concentrated buffer (1.86 M $NH_4C_2H_3O_2$ - 4.14 M CH_3COOH). These reservoirs are shaped to deliver a fixed program of a 1300 cc nearly linear gradient, preceded by 150 cc of dilute buffer, and followed by 100 cc of concentrated buffer. This gradient is then followed by 250 cc or more of the dilute buffer to equilibrate the column prior to the next run. The eluent is pumped to the resin column through a 6-way valve that contains a 2-ml sample loop (replaceable) and a Luer-type loading fitting for sample insertion. Rotation of the valve stem through 60° causes the sample loop to be inserted into the eluent system and, thus, delivers the sample onto the resin bed. The resin column consists of the acetate form of a strongly basic anion exchange resin, equilibrated prior to sample injection with dilute acetate buffer. The column is jacketed and a controlled temperature fluid (ethylene glycol-water) is circulated through the jacket.

At the time of sample injection, the buffer reservoirs of the gradient generator are coupled via an electric solenoid valve and the gradient is started. The resin column eluate containing the progressively eluted sample constituents flows through the sample cell of a dual beam spectrophotometer which is cycling between the four wavelengths 260, 270, 280, 290 mμ at ∿6 cycles/min, the eluate then flows to a 50 cc volumetric siphon which activates the recorder event marker each time it siphons. The buffer solution from the gradient generator passes through the reference cell of the

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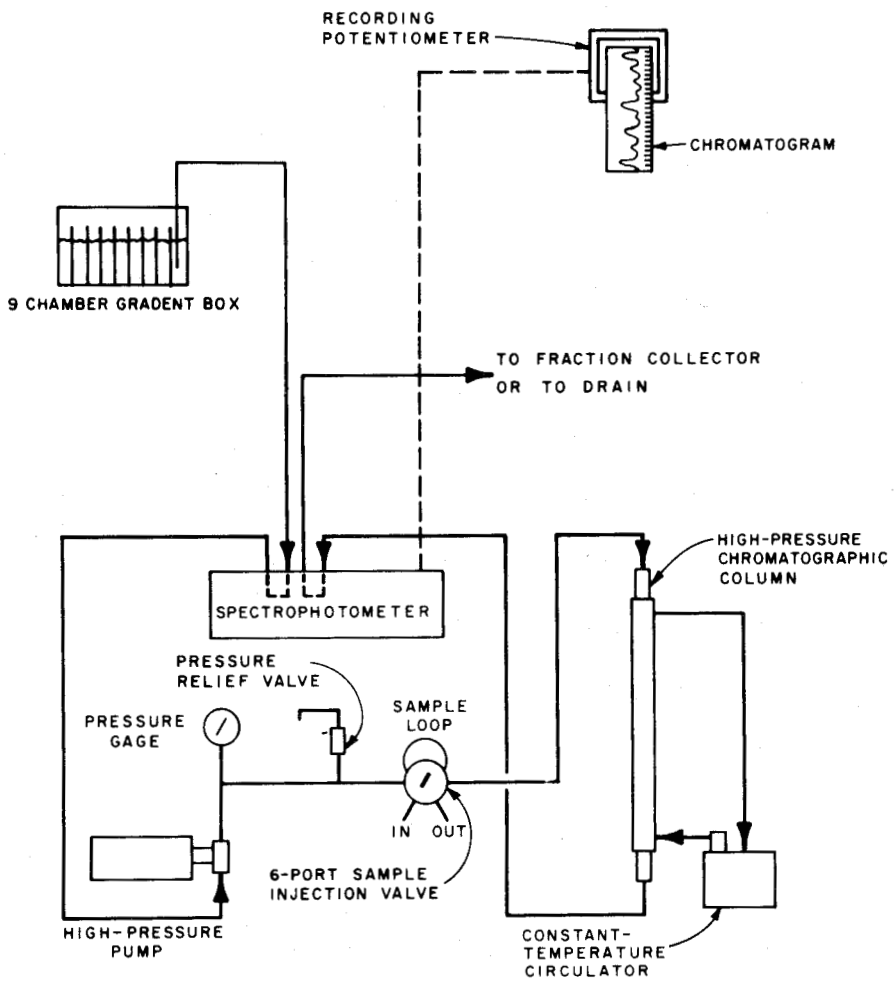

Fig. 2. High-Pressure Anion Exchange Chromatograph for the UV-Absorbing Constituents of Body Fluids

spectrophotometer before going to the pump, thus providing a balancing signal for the acetate absorbance.

3. DETAILED OPERATION OF COMPONENTS

A doors open view of the instrument cabinet showing the major components (except for the gradient box) of the UV analyzer is shown in Fig. 3. (A detailed set of construction prints are also available; these are ORNL prints Q-2862-10 through Q-2862-27.)*

3.1 Electrical Controls

The wiring diagram for the instrument is shown in Figure 4. Electrical power to the entire analyzer is controlled by the CABINET POWER switch (S5),and the pilot light immediately above this switch is illuminated when power is being supplied to the cabinet. Normally, this switch is left in the ON position.

The ELUENT PUMP switch (S2) controls power to the eluent pump, and the GRAD. SYSTEM STIRRER switch (S4) supplies power to the gradient box stirrer motor. During normal operation, both of these switches are left in the ON position.

The CHART DRIVE switch (S1) controls power to the recorder chart drive and printing mechanism and the volumetric marking pen. These items can be operated continuously (switch at ON), completely turned off (switch at OFF), or controlled by timer No. 2 (switch at PROGRAM). Normally this switch is at the PROGRAM position which saves chart paper

*Available as CAPE-1753 from the Clearing House for Federal Scientific and Technical Information, U. S. Department of Commerce, 5285 Port Royal Road, Springfield, Virginia 22151.

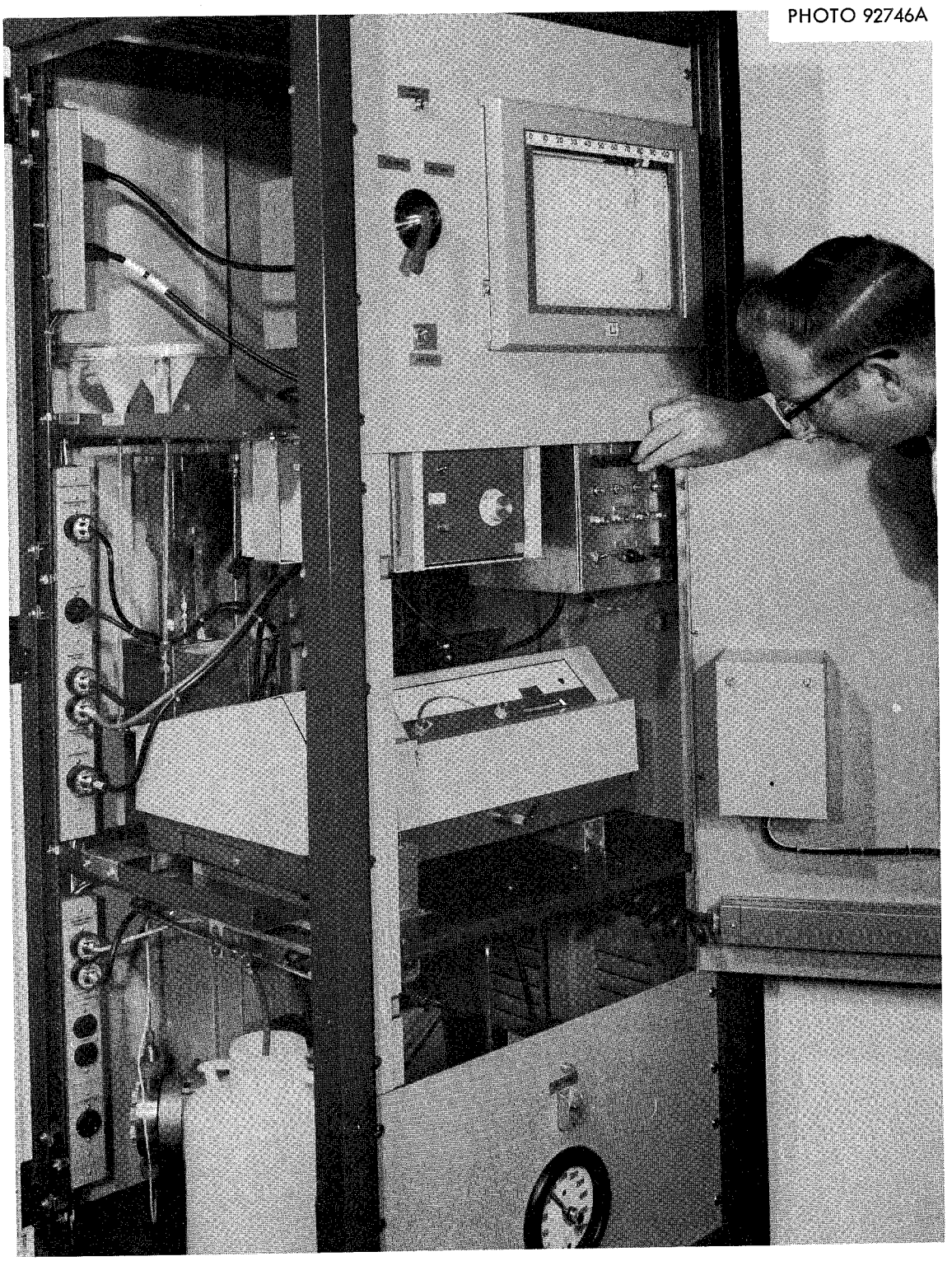

Fig. 3. Mark II UV-Analyzer.

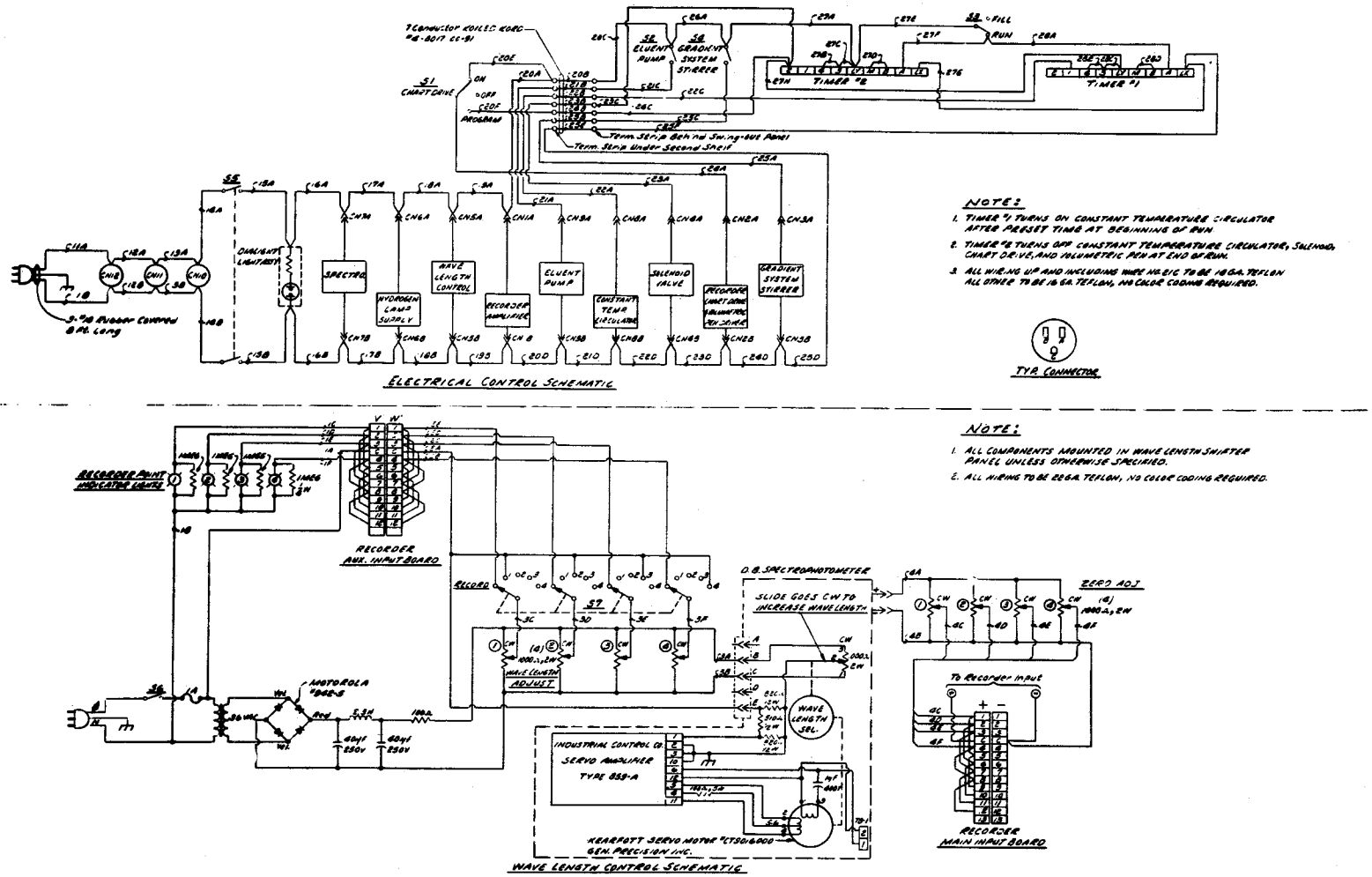

Fig. 4. Electrical Wiring Diagram for Mark II UV Analyzer

since the chart drive is stopped at the end of the run. It may be desirable to switch it to ON for a short period of time prior to starting a new run to be sure that the system has properly equilibrated (printing a smooth continuous baseline).

The FILL-RUN switch (S3) resets and activates the two timers. It should be in the FILL position while the gradient generation system reservoirs are being filled and while the sample is being loaded into the sample loop and injected into the column. The switch should then be placed in the RUN position and left there for the duration of the run. Switch S3 should be in the FILL position when the settings on the timers are being adjusted because they will be damaged if adjustments are attempted while they are energized (switch at RUN).

Timer No. 1 turns on the constant temperature circulator (column heater) when the preset time interval has elapsed after the beginning of a run. This setting is usually 11.5 hours.

Timer No. 2 controls power to the constant temperature circulator (through timer No. 1), the gradient generation system solenoid, the recorder chart drive and printing mechanism, and the volumetric marking pen. This timer determines the duration of a run, and at the end of this time period, deactivates those components it controls to allow the system to be equilibrated in preparation for the next run. The length of a run is usually about 40 hours. At the end of the run the column cools to ambient temperature, concentrated buffer is shut off from the gradient generation system mixing chamber, and the recorder is stopped.

3.2 Gradient Generation System

The gradient generation system provides a means of producing an elution buffer solution with a concentration that gradually increases with volume pumped through the resin column. It consists of a 9 chamber, varigrad box which feeds the high-pressure eluent pump (Figure 2). During a run (FILL-RUN switch at RUN) the solenoid valve connecting the concentrated buffer solution (chamber with narrow top) to the mixing vessel is open, and it remains open for the duration of the run. At the end of the run (timer 2 has timed out), the valve closes and only dilute buffer is pumped through the column to equilibrate the system. This continues until the timers are reactivated by switching the FILL-RUN switch to FILL and then to RUN at the beginning of the next run.

One hand valve is provided in the gradient generation system to clear the "dip" tube line of concentrated buffer after a run.

3.3 Eluent Pumping System

The eluent pump is controlled by switch S2 (ELUENT PUMP) on the front panel. The pumping pressure and thus flow rate is adjusted by means of a 10-turn dial located on the pump. This can be adjusted through the front door of the cabinet while watching the pressure gauge. The system normally operates at a pressure of 2000-3000 psi with a corresponding flow rate of 38 cc/hr and a pump dial setting of 2.1. Because of variations in column pressure drop and pump characteristics the dial setting is adjusted to the desired flow rate. The pressure relief valve opens at 4000 psi, and the sample injection valve seals to about 4000 psi also. Therefore, it should be safe to operate the pump up to about 3750 psi pumping pressure, although

the Lapp pump used in this prototype analyzer is rated at 3500 psi. For pumping problems, e.g., air locking, refer to the pump manual (Bulletin 457, Lapp Insulator Co., Inc., Le Roy, New York).

3.4 Sample Injection Valve

The sample injection valve located immediately above the CHART DRIVE switch provides for the loading of the sample into the column eluent, while the eluent is being pumped through the column and the system is under pressure. The valve should be rotated clockwise to the stop in the FILL LOOP position, and the sample can then be injected into the sample loop through the SAMPLE LOADING PORT.

When the chromatographic run is started, the valve should be rotated counterclockwise to the stop in the LOAD COLUMN position. In this position, the sample is introduced to the top of the resin column. The valve is left in this position for the duration of the run. This valve is a Hoke 6-way Model 30213-1 valve which has been modified by additional machining to provide polished mating surfaces for its moving parts. If it is necessary to disassemble the valve, care should be taken that the internal surfaces are not scratched. During reassembly, the nut at the rear of the valve stem should be torqued to 130 in.-lbs torque.

3.5 Resin and Resin Column

Strongly basic anion exchange resin, e.g., Dowex 1-X8 or Biorad Aminex A-15, was fractionated[3] and the 5-10-μ-diam fraction retained for analyzer use. The resin was converted to the acetate form by mixing with

the appropriate reagent and then filtering and washing the filter cake using the following procedure: 1 hr each 1 N HCl two times followed by washing with distilled H_2O; 1 hr each with 1 N NaOH first at 40°C and then at ambient temperature followed by washing with distilled H_2O; and finally, 1 hr with 6 M acetate buffer and stored in that buffer. The resin is then dynamically packed into the column using concentrated acetate buffer.[4] After packing the column, 6 M buffer at 60°C is pumped through the column for 12 to 24 hours. The resin is then equilibrated with dilute buffer in preparation for a chromatographic run.

The high-pressure anion exchange column was fabricated from standard, type 316 stainless steel tubing and has a stainless steel jacket for the circulating heating fluid. A porous metal filter supports the ion exchange resin (See Fig. 5). The column provided with the analyzer is 150 cm long x 0.62 cm I.D. The instrument cabinet is designed to accommodate longer columns if desired.

3.6 Constant Temperature Circulator

The constant temperature circulator is controlled by the timers. When Timer No. 1 times out, the resin column is heated to 60°C (or any other desired temperature) and maintained at that temperature for the remainder of the run by the use of a constant temperature circulator. The unit supplied with the analyzer is a HAAKE Model FE Circulator. This circulator has been set to maintain the temperature of the ethylene glycol being circulated through the column heating jacket at 60°C. The main power switch on the circulator should be left ON and the heater switch should be adjusted to provide about equal on and off times for the heater.

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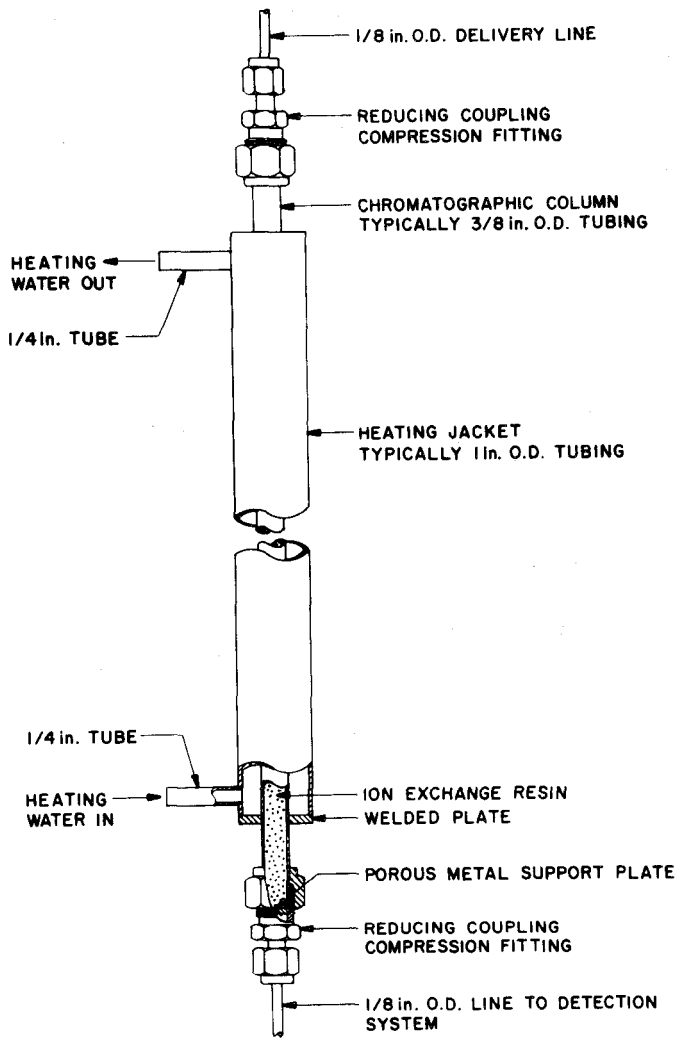

MATERIAL: TYPE 316 STAINLESS STEEL

Fig. 5. High-Pressure Chromatographic Column Fabricated From Stainless Steel Tubing.

3.7 Spectrophotometer and Wave Length Shifter

The spectrophotometer (Beckman Model DB-G) and its wave length shifting control are located behind the hinged door at the front of the cabinet. All of these items should normally be left ON continuously. The wave length shifting control allows the recording of the absorbance of the column effluent at up to four different wave lengths either sequentially or continuously at any one of the four. The four wave lengths are designated points 1 through 4 and individual potentiometers (labeled WAVE LENGTH ADJUST) allow the adjustment of the specific wave lengths at each point. These potentiometers should be adjusted by switching the CHART DRIVE switch to ON, and the selector switch to the particular point being adjusted and watching the spectrophotometer wave length control wheel indexing pointer for an indication of the desired wave length. After all four points have been adjusted, the selector switch should be moved to the RECORD position, and any final adjustments of the WAVE LENGTH ADJUST potentiometers should be made in that mode. The four wave lengths normally used are 260, 270, 280 and 290 mμ.

The potentiometers designated ZERO ADJUST allow the positioning of the recorder baseline for each of the four points. These are usually set after equilibration to a near zero absorbance but providing for some separation between the four printed baselines.

The instruction manual for the Beckman DB-G should be consulted for operation of the spectrophotometer. The slit program selector is normally left in the MAN position and the manual micrometer slit adjust should be set at 1.00.

4. MATERIALS

4.1 Concentrated Acetate Buffer

To make 24 liters of 6 M buffer, add 2,962 cc of 28.5% NH_3OH to 8 liters of double distilled water. Slowly add 8,275 cc of acetic acid (99.8%). Make up to 24 liters with stirring. It should have a pH of 4.4 and a SG of 1.059.

4.2 Diluted Acetate Buffer, 4 M, 1 M, and 0.015 M

Dilute appropriate volumes of the 6 M acetate buffer with doubly distilled water to make 4 M, 1 M and 0.015 M. (Dilution factors of 1.5, 6, and 400, respectively.) The pH should be approximately 4.4.

5. INITIAL AND PERIODIC MAINTENANCE

1. Fill and maintain the ethylene glycol level in the constant temperature circulator at about 1/2 in. below the edge of the filling hole.
2. Refill dilute buffer reservoir as needed.
3. Empty waste receiver as needed.

6. INITIAL STARTUP OF THE ANALYZER

1. Move fill-run switch to fill.
2. Turn Cabinet Power Switch on.
3. Turn on the spectrophotometer and its lamp power supply and start the deuterium lamp.

4. Start the eluent pump and adjust the system flowrate to about 38 cc/hr.

5. Turn the Chart Drive switch on. Adjust baselines to be within 2 to 5 chart divisions without overlapping.

6. Proceed with PREPARATIONS FOR RUN starting with step 2.

7. PREPARATIONS FOR RUN

1. Move the fill-run switch to fill position to deactivate the timers.
2. Flush sample valve and fill sample loop as follows:
   (a) Flush valve in load position with dilute buffer.
   (b) Turn valve to fill loop position and flush loop.
   (c) Load sample into loop.
3. Make up gradient box and flush line as follows:
   (a) Turn off stirrer.
   (b) Transfer dip tube from gradient box to dilute reservoir.
   (c) Flush out dip tube line by flipping solenoid valve switch for 1/2 minute.
   (d) Flush out connecting line by moving fill-run valve to run position for at least 1 minute.
   (e) Refill gradient box after thorough rinsing with distilled water as follows: (Leave valves 2-3, 4-5, and 6-7 closed.)

Chambers	1 & 2	380 gr of 0.015 M acetate buffer
	3 & 4	380 gr of 1.0 M acetate buffer
	5 & 6	380 gr of 4.0 M acetate buffer
	7, 8, & 9	570 gr of 6.0 M acetate buffer

(f) With fill run switch in fill position replace the dip tube into the gradient box.

(g) Turn on stirrer.

4. Adjust timers if necessary. Fill-run switch must be in fill position. The normal positions are 11.5 hrs for timer 1 and 40 hrs for timer 2.

8. START OF RUN

1. If desired that recorder stop at end of run, move Chart drive switch to program position; if desired that recorder not stop, switch to on position. Adjust recorder chart to start at major line.

2. Turn sample valve to load position.

3. Open valves on gradient box.

4. Move fill-run switch to run position.

5. Overflow the waste syphon.

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