CHARACTERIZATION OF RESIDUAL ORGANICS

FROM BIOLOGICAL TREATMENT

CHARACTERIZATION OF RESIDUAL ORGANICS FROM BIOLOGICAL TREATMENT

by

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A Thesis

Submitted to the Faculty of Graduate Studies

In Partial Fulfillment of the Requirements

For The Degree

MASTER OF ENGINEERING

McMaster University

April, 1972

MASTER OF ENGINEERING
(Chemical Engineering)

McMASTER UNIVERSITY
Hamilton, Ontario

TITLE: Characterization of Residual Organics From Biological Treatment

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NUMBER OF PAGES: xiii, 110

ABSTRACT:

Gel filtration chromatography on Sephadex gels G15 and G50, was used to characterize the residual organic materials found in effluents from biological treatment. Molecular weight distributions were determined as the equivalent molecular weight distribution of a homologous series of sugars and alcohols. The homologous series was also used to determine equivalent molecular radii, based on Corey-Pauling-Koltun space filling models of the homolgous series.

To determine the distributions of residual organics from mixed cultures grown on simple, pure substrates, laboratory batch studies were performed. For this purpose, media containing glucose or glutamic acid substrates and a bicarbonate-phosphate buffer system were innoculated with activated sludge. Both high and low substrate and microorganism concentrations were used at constant temperature and pH. As a comparison for the

mixed cultures, a representative strain of Flavobacterium sp. isolated from activated sludge was grown in pure culture on glucose.

Both the laboratory mixed culture effluents and treatment plant effluents contained material of equivalent molecular weight less than 1500. However, little similarity appeared to exist between the low molecular weight (<1500) distributions of treatment plant effluents and those from the mixed cultures. A significant fraction of the treatment plant and batch effluents had equivalent molecular weights of greater than 10,000. The pure culture studies showed that a single strain of bacteria can produce material of equivalent molecular weight both greater and less than 1500.

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ACKNOWLEDGEMENTS

I would like to thank all the people who contributed directly or indirectly to the production of this thesis.

In particular, my thanks to my supervisor, Dr. K. L. Murphy, for his unflagging good advice and enthusiasm for the project despite many setbacks. My thanks also go to Dr. G. A. Vigers, for his advice in matters both biological and biochemical.

My gratitude is extended to Alina Latoszek for her microbiological work, without which parts of this thesis would not have been possible.

Anna Addie deserves particular thanks for her efficient help in the maintenance of sometimes trouble-prone instruments.

Finally, but not least, my thanks are extended to Ingrid Ellis for the typing of the manuscript.

Financial assistance received from the Canada Department of the Environment and the National Research Council was greatly appreciated.

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CHAPTER 1

1. INTRODUCTION

As the sources of clean natural surface waters which supply our municipal water systems diminish, and as the amount of recycle of "used" water increases, the removal of small quantities of "refractory" residual organics from these waters becomes important. Some of the refractory organics are known to be of natural origin, while others are generated within the biological treatment plants used to "purify" domestic and industrial wastes. The nature of these refractory residual organic materials has been the subject of considerable controversy and scientific study for many years. Knowledge of their chemical composition, structure, and size should prove important to their removal in tertiary treatment systems, or pretreatment in water purification.

Identification and quantitation of the major chemical groups making up the residual organics from biological treatment has been generally difficult and incomplete. Identification of all the individual compounds found in biological treatment plant effluents would be effectively impossible because of the low concentrations and complexity of the mixtures. Some method of characterization by which an overall evaluation of each effluent could be obtained is therefore desirable.

Gel filtration chromatography has been applied extensively by biochemists in separating proteins and other biological

compounds on the basis of molecular weight and size. This method has recently been applied on a limited scale to the analysis of the residual organics in biological treatment plant effluents and in natural surface waters. There is disagreement in the literature over the molecular weight distributions of the residual organics from biological treatment as determined on Sephadex gels. In addition, there is still doubt whether or not residual organics of molecular weight less than 1200 are produced in the activated sludge process. Similarly, the effects of different substrates, substrate concentrations, and microbial populations on the molecular weight distributions of effluents from biological treatment have not been clarified.

To date, all determinations of residual organics from biological treatment have been on the basis of molecular weight as established by calibration with a homologous series of sugars and alcohols. The numbers thus obtained have apparently been interpreted as the "true" molecular weights, with little regard for the slightly different behaviours of different types of organic compounds on the chromatographic gels and for the adsorptive and ionic interactions that occur in the gel bed during separation. In addition, no attempt has been made to estimate the dimensions of the residual organic molecules, parameters which are of interest in activated carbon adsorption where pore size distribution is

is important and in membrane processes (i.e., reverse osmosis) where molecular dimensions are the basis of removal.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Sources of Residual Organics

Four sources have been postulated for the residual organic material found in the effluents from biological treatment, both in laboratory studies and in actual biological sewage treatment plants (Murphy, Sedivy, and Vigers, 1971). These are:

i) relatively non-degradable material entering the biological reactor and subsequently appearing in the effluent,

ii) partially degradable material which is not entirely removed during treatment,

iii) by-products of microbial metabolism, and

iv) material resulting from cell lysis.

That at least part of the residual organic material is generated within the biological process itself has been demonstrated (Sedivy, 1971). Some of the factors influencing the concentration of the residuals have been evaluated in laboratory studies, using both a variety of pure "simple" organic compounds and raw sewage, as substrates for activated sludge (Chudoba, 1967, 1968). Regardless of the strength of the influent to biological treatment, some dissolved organic

residue will remain. Chudoba (1969), developed an empirical equation relating the initial soluble Chemical Oxygen Demand (COD) concentration to the residual COD. This has been modified to relate initial soluble Total Organic Carbon (TOC) to Mixed Liquor Suspended Solids (MLSS) to residual TOC (Murphy et al, 1971).

Sedivy (1971) showed that minimization of the residuals within the biological process is possible. However, complete removal must be carried out by tertiary treatment or replacement of the biological process by physico-chemical treatment of some form. In order to remove residual soluble organics in tertiary treatment, some knowledge of their nature must be gained.

2.2 Composition of Sewage - Chemical Analysis

Many attempts have been made to characterize the organic components of treatment plant effluents and sewage itself. Painter et al (1961) were able to identify 80% of the total soluble carbon by weight in raw sewage, while identifying only 26% of the residuals after treatment in a trickling filter. The major soluble components of the raw sewage were identified as carbohydrate, soluble acids and anionic surfactants together with small amounts of protein and amino acid. The particulate portion of the raw sewage contained relatively large amounts of proteins, fatty acids and esters, and some carbohydrates. Of the whole sewage, soluble

plus particulate, 20% was fatty acids, another 20% was carbohydrate, and 10% was protein. In another analysis, Hunter and Heukelekian (1965) found in the particulate fraction 24% carbohydrates, 10% amino acids, while the soluble portion was mainly organic acids (55% of the ether extractable material which in turn constituted 65% of the total volatile dissolved solids (VDS)). This contrasts to the previous analysis where the major portion appeared to be carbohydrate. Carbohydrates, either soluble or particulate, are a major constituent of raw sewage and hence a major substrate in the biological treatment process. Proteins and their basic components, amino acids, also appear to be major components of raw sewage. Hunter and Heukelekian (1965) found that amino acids and total sugars (as hexoses and pentoses) constituted 12.5% and 14.6% respectively of the volatile dissolved solids. Some of the acids found in the ether extractable portion were gallic, citric and lactic acids. Acids corresponding to gallic and lactic, as well as relatively large quantities of acetic acid were found in raw sewage by Meuller et al (1958).

2.3 Composition of Final Effluents - Chemical Analysis

Several studies have been made on final effluents, resulting in the identification of a great many individual compounds. However, complete identification has not been successful. Painter et al (1961) detected small amounts of protein, amino acids, carbohydrates, surfactants, and soluble

acids in an effluent after 16 hours of aeration, of which the soluble acid portion was the largest. Bunch et al (1961), also detected protein, carbohydrate and surfactants in final effluents, of which the protein and surfactant portions were the largest. In neither case did the carbohydrate portion contain simple sugars. Helfgott et al (1970) confirmed these findings.

Organic acids have been variously found to make up 56% to 65% of the ether extractable portion. The significance of the ether extractable portion is that less polar, less oxidized material is preferentially removed from the aqueous sample, while those compounds which are relatively insoluble in ether because of high molecular weight or size (proteins), or are more polar and more oxidized are not extracted. The implication is that these organic acids are not very polar and at the same time are not highly oxidized or of high molecular weight (i.e., not comparable to proteins and therefore <10,000). Mueller et al (1958) identified some of the soluble organic acids in river water, final effluents and raw sewage by silica gel chromatography. In final effluents they found acids which corresponded to low molecular weight compounds such as acetic acid, lactic acid, citric acid, gallic acid, together with lauryl hydrogen sulfate. This method was extended by Painter et al (1961), for identification of some of the organic acids found in sewage solids. It is interesting

to note that lactic, citric, and gallic acids were also present in the ether soluble portion of raw sewage as determined by Hunter and Heukelekian (1965). Since these acids are products of microbial metabolism, they may not be "original" components of sewage, but rather, products of the microbial activity in sewage.

Extensive tests by Vallentyne (1957) showed the presence of many compounds of biological origin in the soluble residual organics. Biotin, dehydroascorbic acid, thiamine, Vitamin B_{12} and some lower sugars were found in solution. As hydrolysates, several amino acids were detected, indicating the probable presence of proteins as well as the above compounds. In sewage and in activated sludge itself, the studies of Hunter and Heukelekian (1965), and Painter et al (1961), confirmed the findings of Vallentyne with the exception that the presence of biotin was not reported.

Instead of using vacuum distillation or freeze-drying as most of the previous authors used for concentrating samples of secondary effluent, Johnson and Sletten (1968) used activated carbon adsorption in their determination of the groups of chemical compounds which constitute residual organics. The organics were recovered by solvent extraction. Sufficiently pure samples were obtained for analysis by ultraviolet (UV), infrared, and nuclear magnetic resonance spectroscopy. Some of the groups of organics determined were biodegradable on

concentration and contained:

i) strongly acidic, easily oxidizable compounds of molecular weight 630 (possibly salicylic in nature),

ii) strongly acidic, easily oxidizable compounds with phenolic and aliphatic portions,

iii) basic secondary or tertiary amines which were easily oxidizable, and

iv) a group of neutral aliphatics and aromatics.

In addition, a non-biodegradable, not easily oxidizable compound containing ether groups, ester and possible carboxylic groups was isolated and assigned an empirical formula of $C_{48-52}H_{94}O_{22}$ with an average molecular weight of 1050. Various polyhydroxy aromatics with alkane and ether type linkages were also described. The authors concluded, significantly, that some of the "refractory" residual organics were indeed biodegradable when in sufficient concentration. However, their determinations may be incomplete, or somewhat altered by possible loss or change of the organics on the adsorbent. Andelman et al (1965) reported recoveries of 65-85% of acetophenone from activated carbon, depending on the number of steps in the solvent extraction process. Since only two successive extractions yielded 85% recovery, the authors concluded that the kinetics of the extraction should be studied

before it is assumed that oxidation on the activated carbon caused the loss in recovery. The authors further warned against quantitative measurements using activated carbon for concentrating organics, since the rate of adsorption and equilibrium amount of a solute will be reduced by the precence of other solutes.

The determinations by Painter et al (1961), Bunch et al (1961), and Helfgott et al (1970), all left a large fraction of the final effluents unidentified. The determinations by Vallentyne (1957) and Johnson and Sletten (1968) gave some idea of what these unidentified portions might be. Rebhun and Manka (1971), theorizing that so-called "humic substances" which are formed during natural breakdown of plant and animal substances, should also appear in final effluents, found in fact that about 40% of the total COD of the effluent could be classified as "humic substances". These were isolated using a modification of the method of Bunch et al (1961), and that of Kononova (1961) (see Rebhun and Manka, 1971), for isolation of humic substances from water and soil. By this method the authors succeeded in identifying nearly 100% of the total and soluble COD. However, the authors did not state the criteria by which they identified the "humic substances". Vallentyne (1957) gave the following criteria for "purity" of humic materials:

1) soluble in base,

2) insoluble in acid, and

3) insoluble in organic solvents.

Humic substances from samples taken from secondary effluents, river water and sediments were found to give variable composition when analyzed for C, N, H, acetyl, methoxyl, hydroxyl, and carbonyl. Black and Christman (1963) identified the different components of humic substances by the following method devised by Oden (1919) (see Black and Christman (1963)):

1) soluble in alkali, not precipitated by acid - fulvic acid,

2) soluble in alkali, precipitated by HCl, insoluble in alcohol - humic acid,

3) soluble in alkali, precipitated by HCl, soluble in alcohol - hymatomelanic acid, and

4) insoluble in alkali - humin.

This appears to be the system used by Rebhun and Manka (1971).

As suggested by the variable compositions found by Vallentyne (1957), Schreiner and Shorey (1908) found that the humic and hymatomelanic acid fractions were actually composed of a range of compounds such as vanillin, salicylic aldehyde, dihydroxystearic acid and various resin acids. Johnson and Sletten (1968) described components of residual organics which are strikingly similar to the components isolated by Schreiner and Shorey (1908). It would appear

that the non-biodegradable compound given the general formula $C_{48-52}H_{94}O_{22}$ by Johnson and Sletten (1968), containing ether linkages, ester and carboxylic groups, seems to fit the requirements of a "humic substance", although it may be a tannin or lignin compound.

The so-called "humic substances" may be formed, according to Kononova and Alexandrova (1959) (see Rebhun and Manka, 1971), by a complex two-step mechanism; decomposition of original plant and animal matter to simpler compounds followed by subsequent synthesis of high molecular weight "humic substances". Since raw sewage contains an appreciable amount of vegetable and animal matter, it is not unreasonable to expect a similar mechanism to occur in a sewage treatment plant. Whether this process is aerobic or anaerobic is not clear.

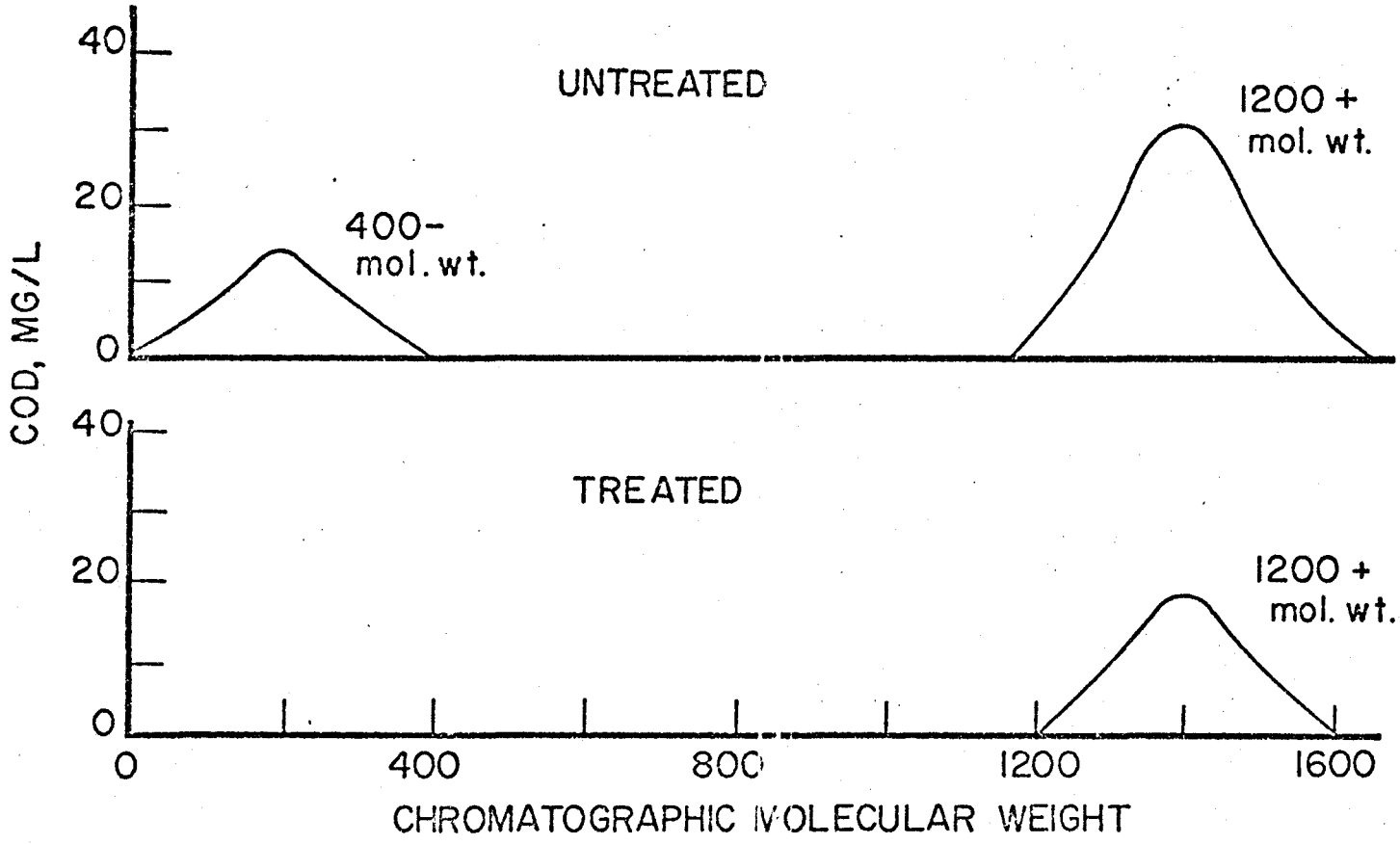
A summary of the results of different analyses of final effluents is presented in Table I.

TABLE I. Final Effluent Composition

	Helfgott et al % Total COD	Bunch et al % Soluble Organics (weight basis)	Painter et al % Soluble Organic Carbon	Rebhun & Manka (3 sam.) Total Effluent % Total COD	Rebhun & Manka (3 sam.) Soluble % Soluble COD
Protein	10	10	2	22.4	28.4
Carbohydrates	5	5	1.5	11.5	13.7
Anionic Surfactants	10	10	10	13.9	11.3
Soluble Acids	-	-	12	-	-
Amino Acids	-	-	-	-	-
Ether Extractables	10	10	-	8.3	8.5
Humic Substances	-	-	-	41.6	36.6
Tannins and Lignins	5	5	-	1.7	1.5
Unidentified	65	65	74	0.2	0

2.4 Gel Filtration Analysis of Sewage and Final Effluents

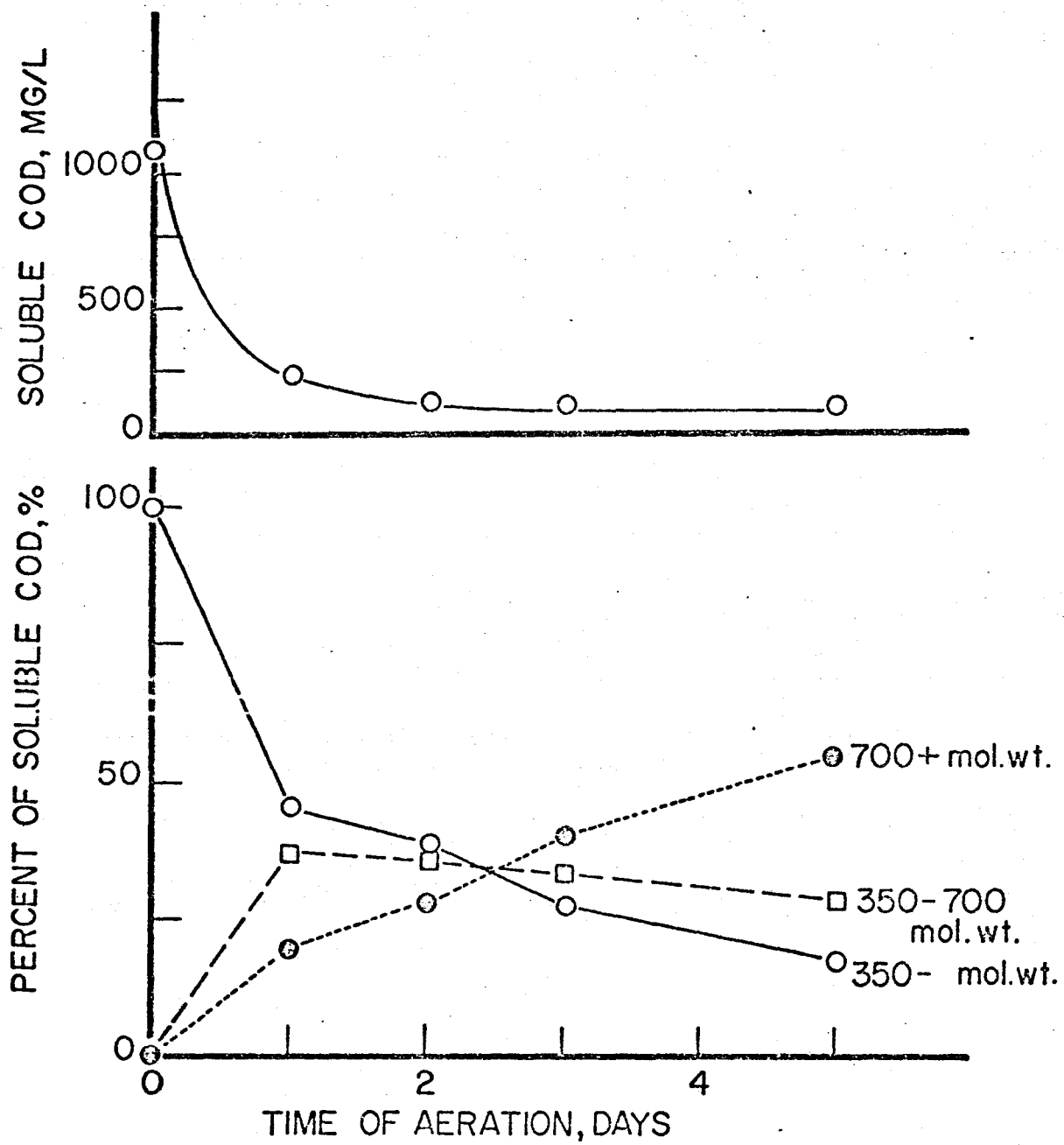
Many of the classes of compounds, including many individual compounds, present as residual organics in final effluents following biological treatment have been identified. However, since final effluents have been shown to vary considerably (Table I), the task of identifying and possibly quantifying even the major components of each effluent for the purpose of further treatment would be staggering. For this reason, gel filtration chromatography has been used with some success in monitoring the components of raw sewage (Zuckerman, 1969), (Hardt et al, 1971), final effluent (ibid) and natural waters (Gjessing and Lee, 1967), (Ghassemi and Christman, 1968). Zuckerman (1969) attempted to define the molecular weight distribution of raw sewage and final effluents using Sephadex G15. The soluble organic fraction was monitored by a differential refractometer and by automated low-level COD determinations using a Technicon AutoAnalyzer. The influent and biologically treated effluent molecular weight distributions are shown in Figure 1, while the changes in the distribution during aeration are shown in Figures 2 and 3. The data indicated that only the 400^- molecular weight fraction in raw sewage was completely removed during treatment, and that the 1200^+ fraction remained relatively constant after an initial rapid uptake of the 1200^+ fraction. The data presented in Figure 2 showed that compounds of molecular



THE 1-1200 MOLECULAR WEIGHT DISTRIBUTION OF WARDS ISLAND RAW WASTEWATER AND STEP AERATION ACTIVATED SLUDGE PLANT EFFLUENT

(ZUCKERMAN, 1969)

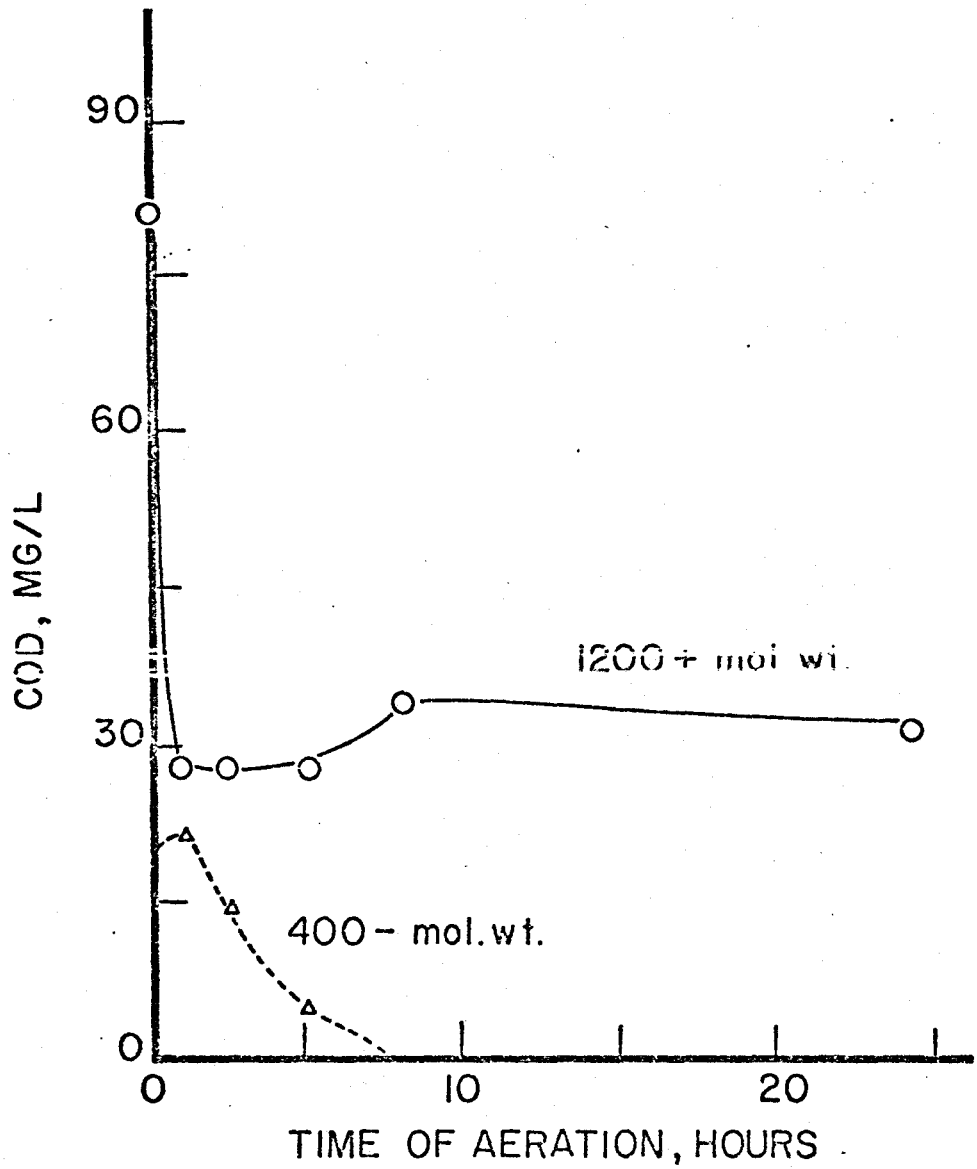
FIGURE 1



THE 1-700 MOLECULAR WEIGHT DISTRIBUTION FROM AERATION OF A GLUCOSE-SETTLED WASTEWATER MIXTURE.

(ZUCKERMAN, 1969)

FIGURE 2



THE 1-1200 MOLECULAR WEIGHT DISTRIBUTION FROM ACTIVATED SLUDGE AERATION OF A WARDS ISLAND RAW WASTEWATER

(ZUCKERMAN, 1969)

FIGURE 3

weight 700^+ were generated during the aeration process. Figure 3 indicated that the 1200^+ fraction was composed of a readily degradable portion. Since there was 700^+ material generated by the biological process, and since there is biological action in raw sewage, one would suspect that the non-degradable portion entering with raw sewage could be a product of biological action.

A correlation between the two monitoring methods, COD and differential refractive index (RI) was attempted as shown in Figure 4. Although the author indicated that the RI monitor was sensitive to NaCl, no attempt was made to explain the obviously non-quantitative nature of the relationship between COD and RI. As a further detection device, the optical density was monitored at 220 mμ (UV) for the raw, biologically treated, and physico-chemically treated samples. UV spectrograms of these samples were also run from 200-400 mμ. This does not, however, provide any information about the individual molecular weight fractions. The use of the COD test as a measure of organic material is also questionable, since the test as modified, does not give complete oxidation of straight chain aliphatic compounds and does not oxidize aromatic hydrocarbons and pyridine. The test is also sensitive to incompletely oxidized inorganics such as nitrites which appear in final effluents. In the use of Sephadex gel, Weber (1970) (see Zuckerman and Molof, 1970), pointed out

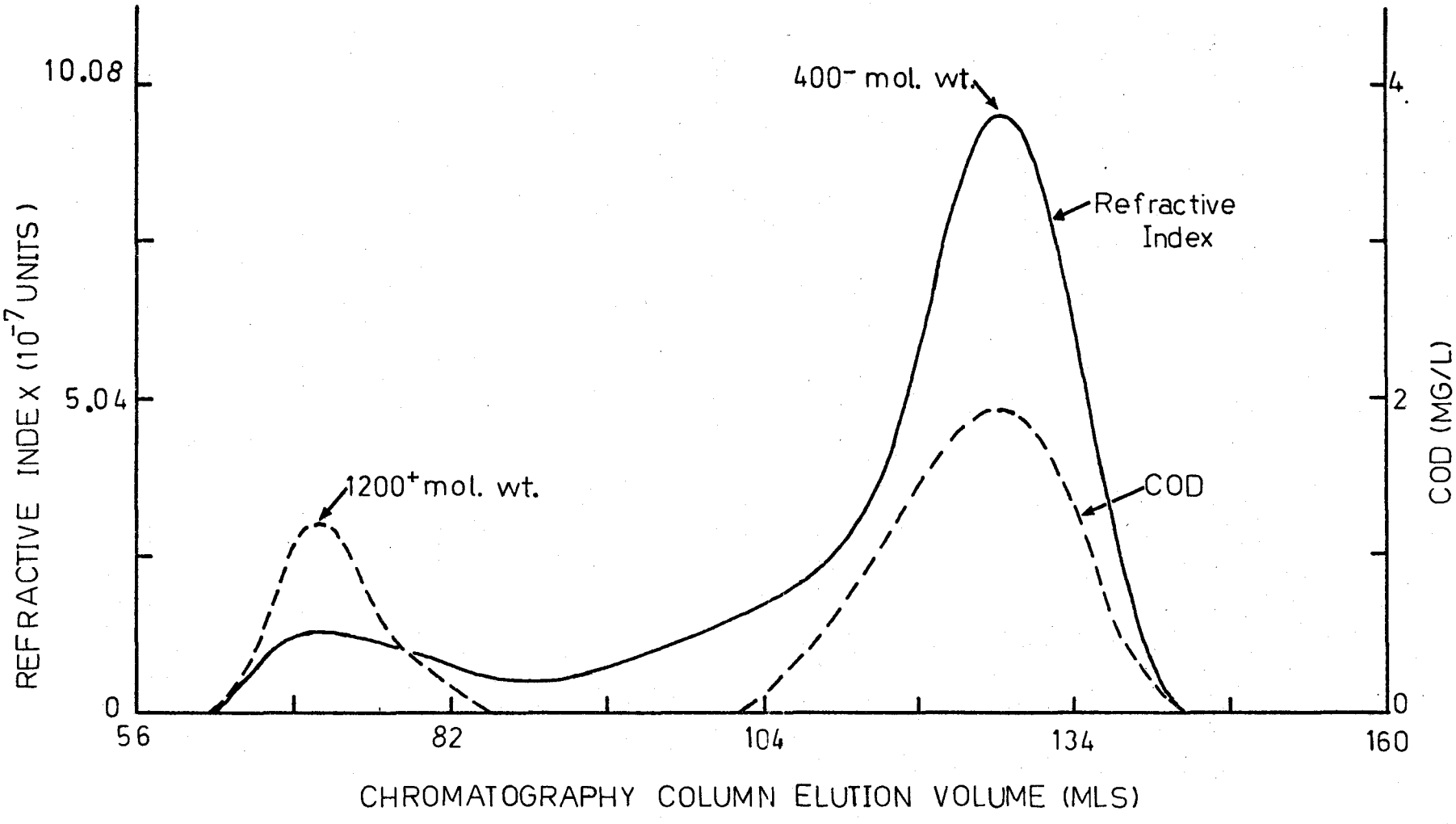


FIGURE 4: EFFLUENT MONITORING OF A CHROMATOGRAPHY COLUMN BY DIFFERENTIAL REFRACTIVE INDEX AND COD (ZUCKERMAN, 1969)

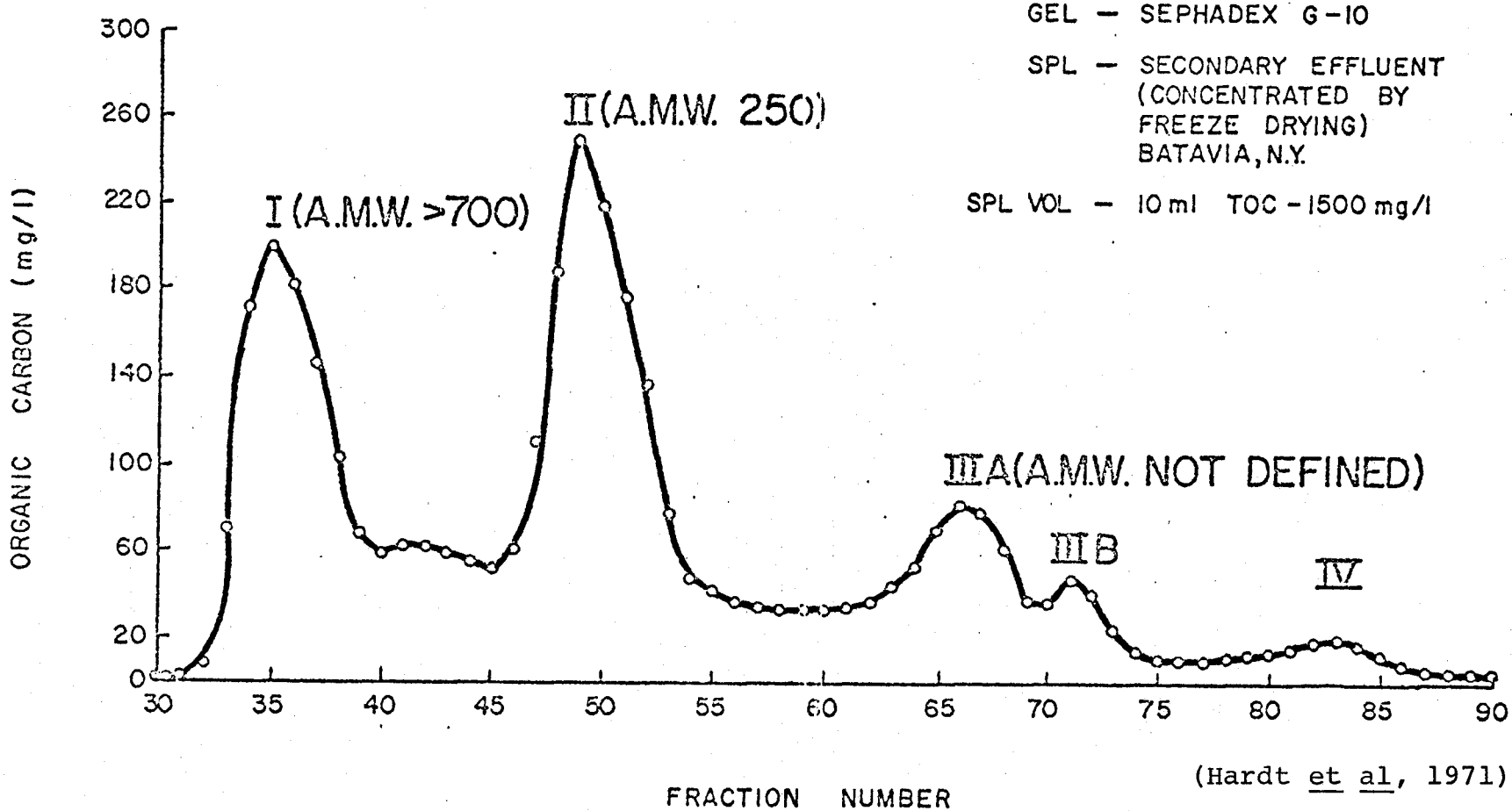
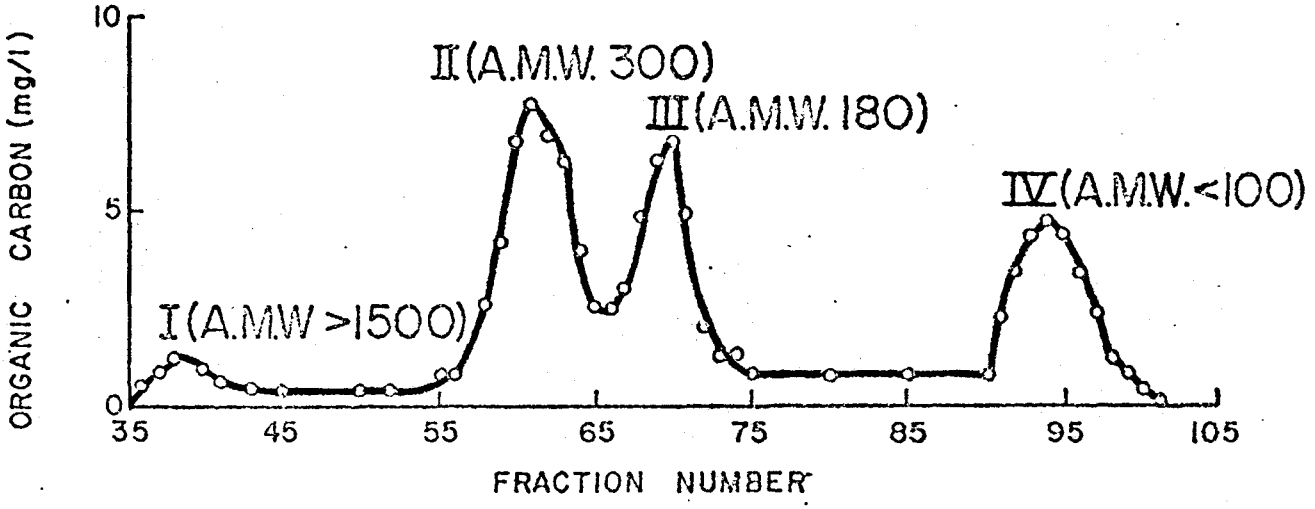
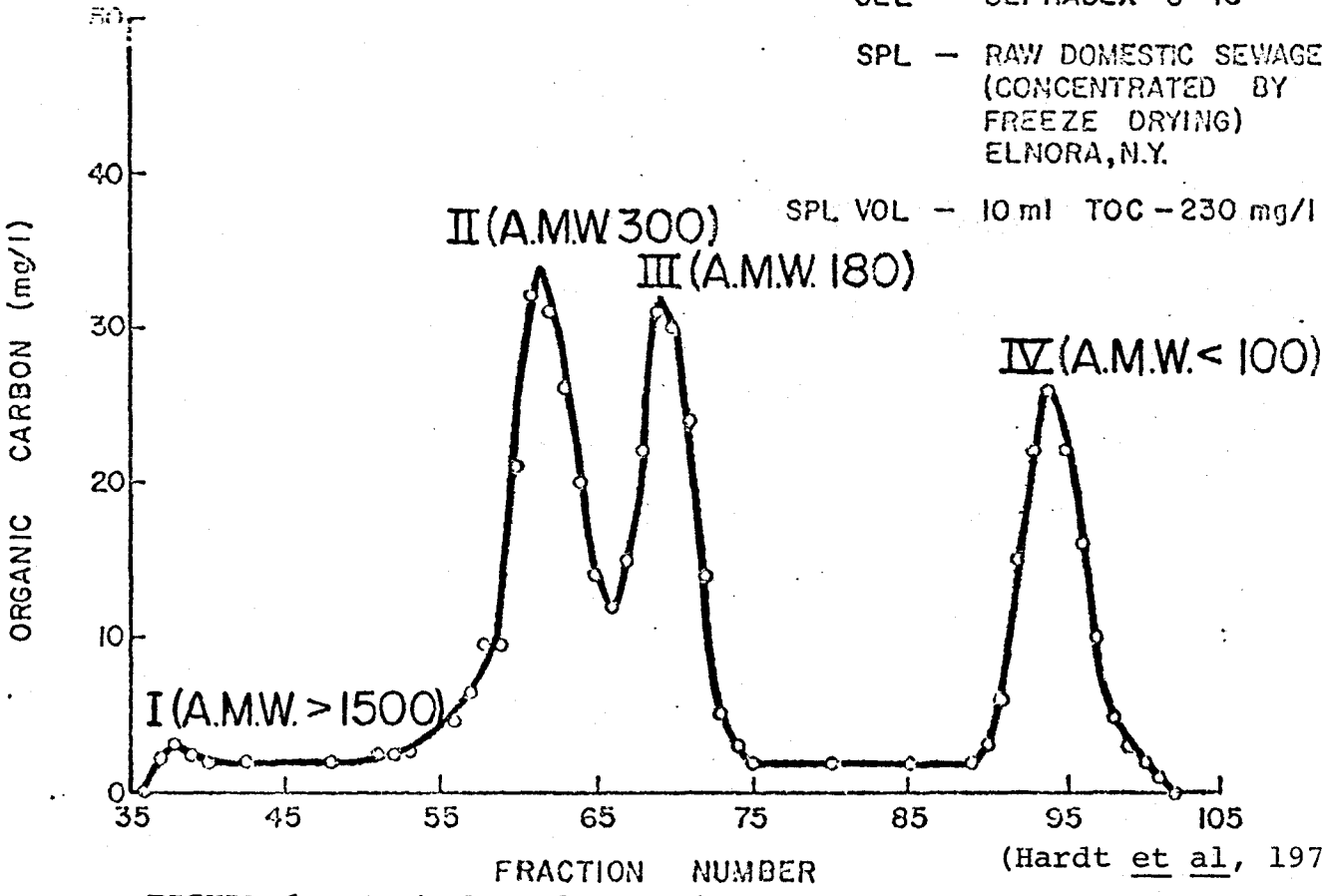


FIGURE 5. Sephadex G10 Elution Profile of a Freeze-Dry Concentrated Secondary Effluent





(Hardt et al, 1971)

FIGURE 6. Sephadex G10 Elution Profiles of a Raw Sewage Before and After Freeze-Dry Concentration

that no mention was made of the effects of pH and ionic strengtn on the molecular weight distribution obtained.

The molecular weight distributions of Zuckerman have been criticized by Hardt et al (1971) who presented data contrary to Zuckerman's contention that only 1200^{+} molecular weight organics remain after treatment (Figure 1). Significant amounts of organic carbon occurred at 700^{+} molecular weight and at 250 molecular weight (see Figures 5 and 6). Greater than 65% of the total organics remaining were classified as under 700 molecular weight for three different sources:

i) a conventional activated sludge plant,

ii) a trickling filter, and

iii) an extended aeration tank.

2.5 Gel Filtration Analysis of Surface Waters

Analyzing natural waters on Sephadex gels, Gjessing and Lee (1967) found that the yellow organic material commonly found in surface waters was fractionable. It did not appear to exist as a continuous distribution of molecular sizes, that is, discrete fractions could be isolated. They found that low-coloured waters had most of the colour in the low molecular weight range (<5000), while highly or moderately coloured waters had colour predominantly in the high MW range (>5000), as determined on Sephadex G75. A significant portion of the high molecular weight material appeared to be of molecular weight greater than 20,000, with a sizeable

amount greater than 50,000. A highly coloured stream originating in a swamp gave the following analysis:

Molecular Weight	% of Total COD
<700	10
100-5000	17
3000-5000	12
20,000-30,000	11
50,000-100,000	6
>100,000	10

The reliability of the highest molecular weight fraction is questionable, since a 1.0 μ filter was used in preparing the samples and some colloidal material was probably included.

Ghassemi and Christman (1968) in a similar study found the greatest portion of coloured materials in the 700-10,000 range, with one fraction of greater than 50,000 molecular weight relative to dextran, providing confirmation of the previous results. They found that the colour and apparent size of the yellow organic material were pH dependent. The apparent size of the molecules increased with increasing pH, a change ascribed to increasing ionization of functional groups. These poly-hydroxy-methoxy-carboxylic acids were found to fluoresce in a broad band centred at 450-460 mμ with maximum excitation at 360-370 mu. The fluorescence-to-carbon and

fluorescence-to-colour ratios changed from sample to sample, possibly due to differences in chemical structure or changing mineral content. Gjessing (1966) found that these compounds also gave good absorbance in the UV at 254 mμ.

2.6 Theory of Gel Filtration Applied to Wastewater Analysis

Gel filtration is a method of fractionation used to separate mixtures of compounds on the basis of molecular size. A "reverse sieving" process in a bed of porous gel granules causes the large molecules to be eluted from the column first, with the smaller molecules following according to size. The technique has variously been called gel filtration, molecular sieve chromatography, gel chromatography, or gel permeation chromatography. The last name generally refers to the process when hydrophobic gels and non-aqueous solvents are used, while the former names refer to aqueous eluants and hydrophilic gels (Andrews, 1970).

Gel filtration can be regarded as a form of partition chromatography in which separation is effected according to differences in solute distribution between the mobile solvent phase flowing in the voids of the gel granules, and the stationary solvent phase held within the gel matrix. Retardation of solute molecules occurs depending on the extent to which they enter the gel pores. On this basis the molecules should theoretically emerge from the bed in order of decreasing size. Since the elution volume of a given component does not vary

greatly with flow rate over a wide range, the partition of the solute seems to be due to a concentration equilibrium of the solute between the fixed and stationary solvent phases.

Several mathematical models have been proposed, based on exclusion of molecules from pores of different geometries (depending on the model) according to the Stokes' radius of the molecule (Pharmacia, 1970). These "steric exclusion" mechanisms are reasonable models for gel filtration, but experimental data are inadequate to determine the relative merits of the theories (Andrews, 1970).

Since Stokes' radius is the parameter on which the separation is dependent, direct determinations of molecular weight cannot be made unless a series of standards of a homologous series have been prepared, and the unknowns are similar in chemical composition and structure. Stokes' radius reflects only the hydrodynamic properties of the molecule in solution. This may be very nearly the actual molecular radius for a near-spherical molecule, but will deviate considerably if the geometry of the molecule is other than spherical. The relation between the behaviour of a molecule in gel filtration and Stokes' radius is independent of the chemical nature of the molecule. Successful application of this method does require, however, that no adsorptive or ionic interactions between the solute and the filtration medium occur (Andrews, 1970).

Molecules larger than the largest pores in the gel matrix do not penetrate the gel and can only occupy the volume between gel particles. This is the void volume V_o. The volume of all the pores within the gel is defined as V_i. The entire volume can only be penetrated by molecules smaller than the smallest pores. Such molecules would elute from the column at a volume of $V_o + V_i$. The behaviours of most solutes which can penetrate only a part of the pore size distribution, can be characterized by their elution volume, V_e, on a particular column. For a specific gel, the bed volume will differ from column to column, and V_e, V_i, and V_o will differ. This problem can be largely overcome by the use of the ratio of V_e/V_o for comparison of results from different columns. A more significant description of chromatographic behaviour is given by the distribution coefficient K_d defined by the relation:

$$V_e = V_o + V_i K_d$$

$$\text{or} \quad K_d = (V_e - V_o)/V_i$$

where K_d represents the fraction of V_i available to a particular size of molecule.

For solutes excluded from the gel pores, $K_d = 0$, while $K_d = 1$ for those molecules able to penetrate into the smallest gel pores. If $K_d > 1$, then absorptive or ionic interactions have occurred between solute and gel.

Sephadex is a highly crossed-linked dextran polymer supplied in bead form. Because of the high content of hydroxyl groups in the polysaccharide (dextran) chains, Sephadex is very hydrophilic and thus swells in water and aqueous solutions. The amount of swelling determines the fractionating ability of the gel. The more water absorbed by the gel, the larger will be the pores in the gel, and the larger the molecular size which can be fractionated. The various grades of Sephadex and their molecular weight fraction ranges are shown in Table II. For Sephadex gels of designation above G25, V_i can be quite accurately determined from:

$$V_i = V_f - V_o$$

where V_f is the elution volume at which separation ceases to occur.

For Sephadex G10 and G15, this method is prone to error because of steric factors, adsorptive and ionic effects. In this case, a sufficiently accurate calculation of V_i is obtained from the specified water regain of the gel and the dry weight of gel in the column. V_o is found by determining the elution volume of a molecule too large to penetrate the gel pores. Alternatively, the coefficient K_{av} can be used where:

$$K_{av} = \frac{V_e - V_o}{V_t - V_o}$$

where V_t is the total bed volume, that is, V_i plus the volume of the gel structure itself (Pharmacia, 1970).

TABLE II. Physical Data for Sephadex Gels (Pharmacia, 1970)

Sephadex Type	Dry Particle Diameter μ	Fractionation Range (Molecular Weight)	
		Peptides & Globular Proteins	Dextrans
G10	40-120	Up to 700	Up to 700
G15	40-120	Up to 1500	Up to 1500
G25 Medium	50-150	1,000 - 5,000	100 - 5,000
Fine	20-80		
G50 Medium	50-150	1,500 - 30,000	500 - 10,000
Fine	20-80		
G75	40-120	3,000 - 70,000	1,000 - 50,000
G100	40-120	5,000 - 400,000	1,000 - 150,000
G200	40-120	5,000 - 800,000	1,000 - 200,000

Sephadex contains about 10-30 μ-equivalents of acid groups per gram of gel, a property evident only if pure distilled water or solutions of very low ionic strength are used as eluants (Andrews, 1970). Solute molecules which are acidic will tend to be accelerated because of repulsion by the acid groups in the gel. Solutes which are aromatic or heterocyclic, conjugated polyenes, and other low molecular weight compounds which contain extended coplanar π-electron systems are reversibly adsorbed by Sephadex due to an affinity for the ether linkages in the gel matrix. Compounds such as these often exhibit K_d values greater than one (Andrews, 1970).

Occasionally, in gel filtration, negative peaks are obtained with the RI detector. There are two causes for this phenomenon. One is that the compound being measured has a refractive index less than that of the eluant. The second cause is an eluant effect. If the eluant contains a solute which is not present in the sample applied, a negative peak will be observed at the elution volume which would be observed if the solute were in the sample instead of the eluant. This observation is true for both organic and inorganic solutes, (Nedermeyer, 1968).

2.7 Microbiology of Activated Sludge

The identity of the bacteria which form activated sludge has been the topic of numerous research papers and considerable controversy. The bacterial growth was given the

name "Zoogloea ramigera", (literally, "living glue") in 1867 when it was first found in a culture of decaying algae, and was subsequently described in mixtures of decomposing plant matter and sewage. It can best be described as very small rod-shaped cells (1 by 2 to 4 μ), embedded in transparent, gelatinous, clustered masses (Breed et al, 1957).

Since the original identification of Zoogloea sp., considerable controversy has arisen as to whether it is a single species or simply a growth form. It is well known (Brock, 1970), (Stanier et al, 1970), that many procaryotic organisms form capsules or slime layers, including some of the more common bacteria such as Bacillus subtilis (Aiba et al, 1965), various streptococci (Aiba et al, 1965), and Aerobacter aerogenes (Stanier, et al, 1970). The slime layers produced by these organisms could under the right conditions lead to flocculant growth such as observed in "Zoogloea ramigera". Both Bacillus subtilis and Aerobacter aerogenes have been found in activated sludge. Many other bacteria have since been isolated from activated sludge, causing the speculation that Zoogloea sp. may be a growth form rather than a distinct species. Among the other genera noted consistently were: Flavobacter sp., Achromobacter sp., Pseudomonas sp., Bacillus sp., Escherichia sp., and Comamonas sp., Of these, Pseudomonas sp., and Escherichia sp., (as well as about a dozen others) have been shown to produce flocculent growth when grown in pure culture on sterilized

raw or synthetic sewage. A recent determination (Lighthart and Ogelsby, 1969), using the numerical taxonomy approach with about 90 characteristics has not identified any Zoogloea ramigera, but instead has indicated various types of Flavobacterium as the dominant organisms in activated sluge. Achromobacter sp., and a group resembling pseudomonads have also been isolated (Lighthart and Ogelsby, 1969).

CHAPTER 3

3. EXPERIMENTAL DESCRIPTION

3.1 Apparatus

a) Biological Reactors

For all biological studies, 5-litre, 3-necked, round-bottom flasks were used as reaction vessels. Each neck was fitted with a bored rubber stopper, enabling insertion of a combination electrode pH probe, a 10 mm diameter glass tube approximately 8 cm long, with loose fitting cap, and a coarse fritted glass bubbler. Air supplied to the bubbler was prefiltered through a sterilized glass fibre and cotton filter.

Variable speed laboratory magnetic stirrers were placed under each vessel with a ¼ inch air space between to insulate against heat transfer to the flask from the stirrer. Teflon-coated magnetic stirring bars were used to mix the contents.

The reactor design is illustrated in Figure 7. The reactors and pH meters were held in a laboratory frame, in a controlled temperature room fixed at $20.0^{\circ}C \pm 0.5^{\circ}C$. The number of simultaneous experiments was limited to two, because of a shortage of pH meters.

b) Gel Filtration Equipment

Molecular weight distributions for molecules of molecular weight less than 1500 (based on dextran calibration),

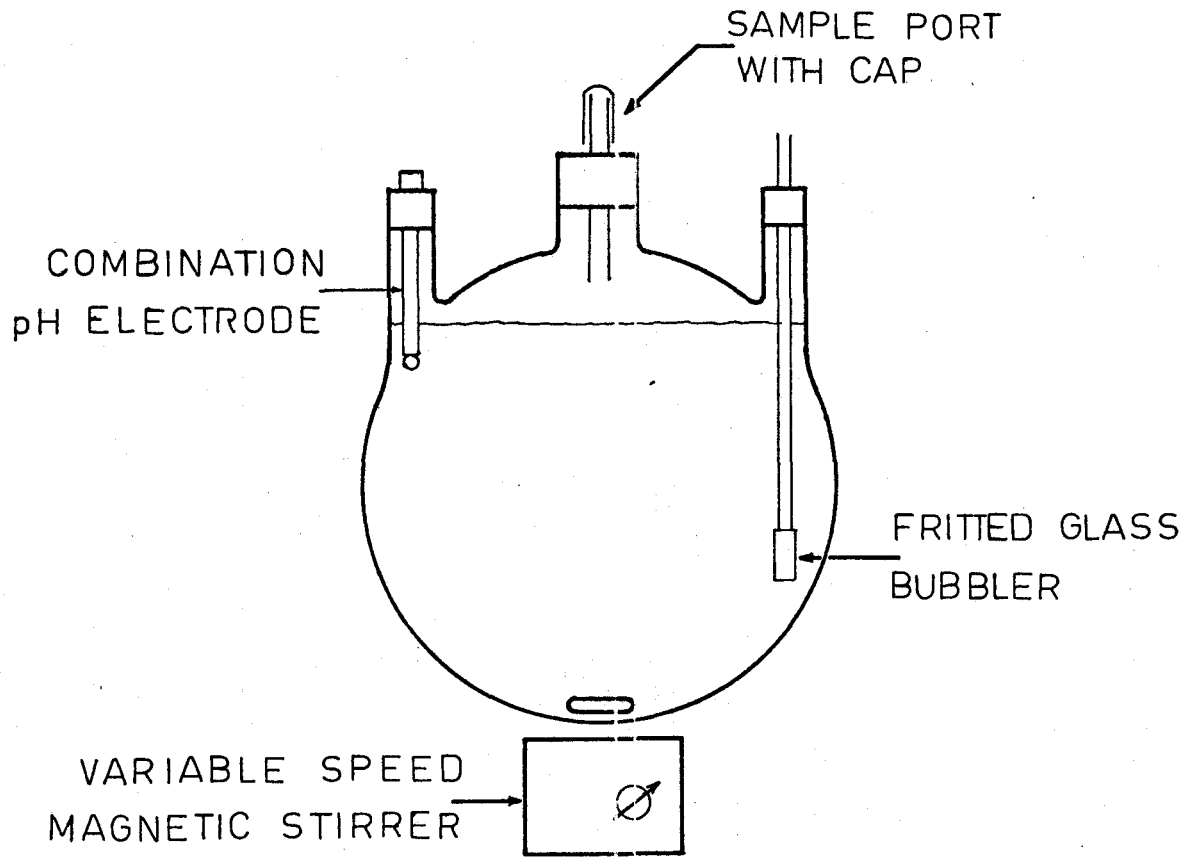


FIGURE 7. Biological Batch Reactor

were determined using Sephadex G15. The chromatographic column was a water-jacketed, gravity-feed, down-flow column supplied by Pharamacia (Canada) Limited (K16100 with sample applicator). The bed volume (V_t) was 182 ml.

For molecules of molecular weights distributed between 500 and 10,000 (based on dextran) Sephadex G50 was used (bed volume was 153 ml).

Eluant was supplied to the columns at a constant flowrate from a 300 ml Mariotte bottle.

The column effluent was monitored continuously by a differential refractometer and differential UV monitor in series. The refractometer was a Waters Model R403 designed for gravity feed systems and equipped with a flowing reference and heat exchanger. The detector had eight ranges, seven from maximum sensity of 6×10^{-6} RI units full scale deflection (10 mV output) linearly to 1.536×10^{-3} RI units full scale. The eighth was a low-sensitivity, non-linear range. Following the refractometer, the column eluant passed through the UV monitor (part of a Waters ALC 202). This detector was a dual-beam (single-source), air-referenced unit equipped with the 254 mμ UV source. The sensitivity ranged in six steps from 0.02 O.D. (optical density) full scale deflection (10 mV output), to 0.64, with a non-linear range also available. Although limited specifically to the detection of compounds which absorb UV light at 254 mμ, this detector was useful

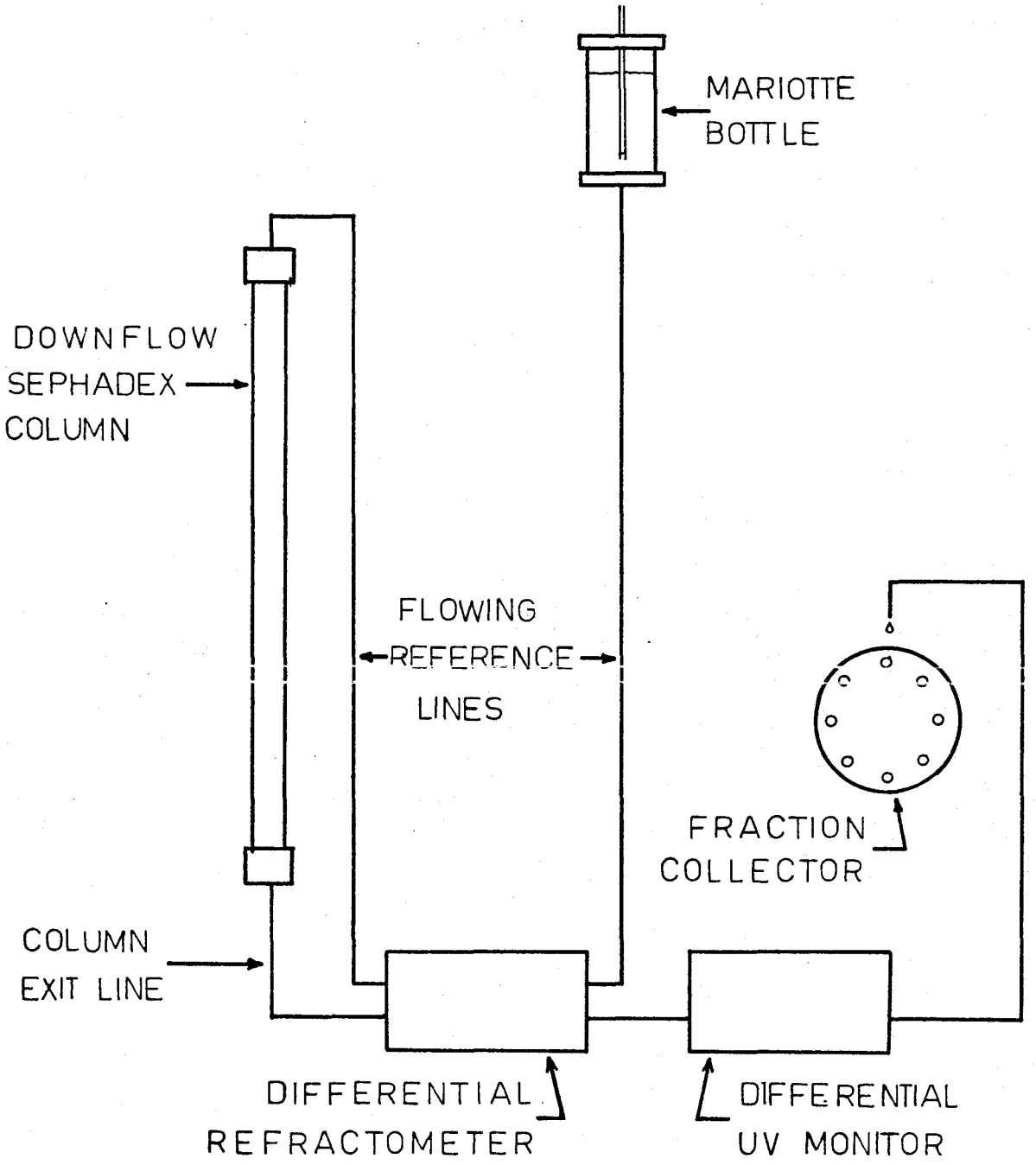


FIGURE 8. Flow Diagram for Gel Filtration Apparatus

for picking out materials often masked in the refractometer response.

Figure 8 shows the flow diagram for the chromatographic equipment.

3.2 Procedure

a) Preparation of Media

Analytical grade dextrose and L-glutamic acid were used as the carbon sources for the laboratory studies. For most instances, the concentration used was 200 mg/l as organic carbon.

A phosphate-bicarbonate buffer system consisting of 0.2×10^{-3} M KH_2PO_4, 0.4×10^{-3} M K_2HPO_4, 0.2×10^{-3} M $Na_2HPO_4 \cdot 7H_2O$ and 207 mg/l $(NH_4)HCO_3$ (130 mg/l alkalinity as $CaCO_3$ added after autoclaving) was normally used giving an initial pH of 7.8-8.0. Additional pH control was necessary only in the activated sludge studies and was accomplished by the addition of 1.0N HCl or NaOH. The pH was held between 7.2 and 8.0.

All media were made up from distilled water, with trace elements supplied in accordance with the procedure outlined in "Standard Methods" for the BOD test, (APHA, AWWA, WPCF, 1971):

$CaCl_2$	27.5 mg/l
$MgSO_4$	22.5 mg/l, and
$FeCl_3$	0.25 mg/l

Prior to use in pure culture studies, the media, reactors, and ancillary equipment, except pH probes were autoclaved at 15 psi and 120°C for 15 minutes. All vessels were stoppered with non-absorbent cotton-wool plugs and capped with aluminum foil. After cooling, the $(NH_4)HCO_3$, previously sterilized by washing with 95% ethanol and vacuum drying, was weighed out and added to the media. The pH probes were sterilized by washing in 95% ethanol and then with sterilized distilled water. This procedure was not deemed necessary for the activated sludge studies.

b) Laboratory Batch Activated Sludge Studies

Activated sludge collected from the Burlington Skyway Sewage Plant aeration tank was used as seed for the activated sludge studies. The sludge was allowed to digest for 6-12 hours after collection. After settling, the supernatant was drawn off and replaced with distilled water containing mineral salts. After one hour aeration, the sludge was again settled and the supernatant drawn off and replaced with distilled water. After the one hour aeration period, the sludge was allowed to settle and the supernatant drawn off. Suspended solids measurements were made on the concentrated sludge. The volume of concentrated sludge required for the experiment was drawn off and placed in the reactor. New sludge was used for each experiment.

Two different initial food-to-microorganism ratios and initial TOC concentrations were used in the activated sludge experiments. For one series of experiments a TOC:MLSS ratio of 2:1 was used with an initial TOC of 225 mg/l. The second series increased the initial TOC to 1000 mg/l and the MLSS to 1000 mg/l, giving an F:M ratio of 1:1. At both concentrations, the pH of the glutamic acid medium had to be adjusted upward to 7.0 with 1.ON NaOH.

A third series of experiments tested the effect of the presence of a micro-biological derivative, yeast extract. Media containing 200 mg/l (as TOC) of glucose and glutamic acid, with an F:M ratio of 2:1, had 200 mg/l (62 mg/l as TOC) of yeast extract added to them. Typical formulations are given in Table III.

c) Preparation of Dominant Cultures

Fresh activated sludge from the Burlington Skyway Sewage Treatment Plant was the source of the microorganisms used in the dominant culture studies. After 5 minutes of vigorous shaking of the sample, 1 ml of the sludge was added to 95 ml of phosphate buffer and shaken vigorously for 5 minutes. After settling for 1 hour, a 0.1 ml of a 1:100 dilution of the supernatant was surface plated in duplicate on Difco Bacto Plate Count Agar. The plates were incubated for 3 days at 20°C. Well-separated colonies were transferred to nutrient agar slants and incubated at 20°C. To confirm

Component	Series 1 F:M = 2:1	Series 2 F:M = 1:1	Series 3 F:M = 2:1
Initial Glucose or Glutamic acid (mg/l as C)	200	1000	200
KH_2PO_4 (moles/l)	2×10^{-4}	2×10^{-3}	2×10^{-4}
(mg/l)	26.8	26.8	26.8
K_2HPO_4 (moles/l)	4×10^{-4}	4×10^{-3}	4×10^{-4}
(mg/l)	69.6	69.6	69.6
Na_2HPO_4 (moles/l)	4×10^{-4}	4×10^{-3}	4×10^{-4}
(mg/l)	107.2	107.2	107.2
$(NH_4)HCO_3$ (moles/l)	2.62×10^{-3}	2.62×10^{-3}	2.62×10^{-3}
(mg/l)	207	207	207
Yeast Extract			
(mg/l)	-	-	200
(mg/l as C)	-	-	62

TABLE III. Typical Biological Media Formulations for Activated Sludge Studies

the purity of the isolated strains, the growth on the slants was resuspended in phosphate buffer and surface plated on plate count agar or nutrient agar (incubation at 20°C). The pure strains were given number designations and transferred to nutrient agar slants incubated at 20°C. The growth on the slants served as stock cultures for the batch experiments. A Gram negative Pseudomonas sp. and a Gram negative Flavobacterium sp. isolated by this procedure were used in the batch studies.

d) Dominant Culture Studies

For innoculation of the 5 litre reactors, small shake flasks containing 75 ml of the reactor medium were prepared. Innoculation of the shake flasks was made from the nutrient agar streak plates prepared in the isolation procedure. After 3-4 days growth in the shake flasks, the contents were transferred aseptically to the large reactors, keeping a small amount (<1 ml) for purity checks. This same procedure was used when transferring a different organism to a reactor already in progress.

Growth tests were performed with all bacteria isolated to determine their viability on a simple substrate such as glucose or glutamic acid. Yeast extract was introduced into the medium in concentrations ranging from 0 mg/l to 500 mg/l. The TOC of each was determined after 3-4 days growth. The concentrations of yeast extract for which the lowest TOC remaining was observed was taken as the optimum concentration.

e) Sampling

After mixing the organism seed and biological medium for ten minutes, a 10 ml sample was withdrawn using a wide-mouthed pipette. The sample was filtered through a 0.45 μ Sartorius membrane filter. The residue was used as a measure of suspended solids and a portion of the filtrate analyzed for TOC. A further portion was used for chromatographic analysis. If it was not possible to place the sample on the chromatograph immediately, it was stored at 3^{o}C. Further samples were taken at intervals throughout the experiment, the time between samples depending on the rate of carbon removal.

For purposes of aseptic sampling from the pure culture reactors, the wide-mouth pipettes were flamed almost to red heat, wrapped in aluminum foil while hot, and stored in an oven at 105^{o}C for at least 3 hours before use. The pipettes were allowed to cool before use.

Treatment plant samples were obtained from three plants in the Hamilton area. Final effluent samples were taken from the Burlington Skyway and Dundas Municipal sewage treatment plants. The samples were taken at the weirs of the final settling tanks in stoppered glass bottles. Raw sewage samples were obtained directly from the grit chambers of the Dundas and Burlington Elizabeth Gardens plants. All samples were obtained between 11 a.m. and 2 p.m. of weekdays.

3.3 Analyses

a) Sample Preparation for Gel Filtration

Samples (~300 ml) were collected from the biological reactors when the organic carbon concentration in the reactor had remained constant for 6-12 hours. The samples were immediately centrifuged at 5000 rpm (~3200 g) for 15 minutes at 5°C on a Lourdes automatic refrigerated centrifuge and filtered through a 0.45 μ Sartorius membrane filter. Samples of raw sewage and final effluents were centrifuged (if required) and filtered within one to two hours of collection.

Concentration of the samples was carried out by freeze-drying on a Virtis Unitrap freeze-dryer, at 0.01 - 0.02 mmHg. The samples were shell-frozen in the vacuum flasks in liquid nitrogen. To avoid excessive losses of volatile materials, the samples were not sublimated to complete dryness. Since some material was formed which was insoluble in the liquid remaining, some distilled water was added to try to redissolve the material. The insoluble portion was left in contact with the liquid for 24 hours but did not completely redissolve.

Since all the samples were slightly alkaline, and to avoid pH effects the chromatographic columns, neutralization was carried out with 1.0N HCl, while the samples still contained the insoluble matter. After neutralization, the samples were again filtered through a 0.45 μ Sartorius membrane filter, and stored at 3°C. Before being applied to the chromatographic columns, the samples were brought to room temperature.

b) Gel Filtration Methods

To apply a sample to a column, the eluant above the top of the gel bed was mostly removed with a Pasteur pipette. The remaining eluant was allowed to "just" drain into the bed by opening the column outlet. The sample (2 ml on G15; 1.5 ml on G50) was then layered carefully on top of the gel bed, and allowed to "just" drain into the bed. A small amount of eluant (~1 ml) was placed on top of the bed and this too was allowed to drain into the bed. After the top of the column had been refilled with eluant, the system was closed and the eluant started flowing. Flow-rate measurements were made by collecting the eluant at the detector system exit and recording the volume against time.

Calibration of the G15 column was accomplished using Blue Dextran 2000 (Pharmacia Limited) to define the void volume V_o, and a homologous series of sugars and alcohols: raffinose (MW 504), sucrose (MW 342), dextrose (MW 180), and ethylene glycol (MW 62). Sodium chloride, disodium hydrogen phosphate, sodium carbonate (pH adjusted to 7.0 with HCl), and sodium sulphate were used to standardize the behaviour of some inorganic compounds on the G15.

The Sephadex G50 column was standardized with Blue Dextran 2000 to determine the void volume, raffinose (MW 504), and NaCl to determine the internal volume V_i.

The ionic strength of eluant for both columns was maintained at 0.02 using a phosphate buffer consisting of 5×10^{-3}M potassium dihydrogen phosphate (KH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) at a pH of 6.9. To inhibit microbial growth sodium azide (200 mg/l) was added to the eluant. The eluant was not degassed before use.

To test the effect of washing the activated sludge with distilled water, samples taken just after mixing with the substrate solution were compared chromatographically for low and high suspended solids concentrations. The effect of the pH of the sample, when applied to the column was checked in one sample, although carbon analysis was not used as the basis of comparison. The effect of filtering samples through 0.1 μ filters was also evaluated for one sample.

c) Analysis of Organic Carbon Fractions

Total organic carbon was monitored by discrete sampling using a Braun Simplex fraction collector and a Beckman 915B Carbon Analyzer. Because of the low levels of carbon in the chromatographic column effluent stream, the Carbon Analyzer normally had to be run at high gain (7.0 Total Carbon Channel; 6.0 of inorganic Carbon Channel) using relatively large (30-50 μ-litre) sample injections.

After carbon analysis, those fractions containing sufficiently large amounts of carbon (>5 mg/l TOC) were

subjected to UV analysis in the range 200-400 mμ using a Cary 14 spectrophotometer.

CHAPTER 4

4. GEL FILTRATION COLUMN CALIBRATIONS

Calibration of the G15 column for molecular weight on the basis of a homologous series of sugars and alcohols was done to compare the results with previous work. A typical calibration elution profile is shown in Figure 9. Because of the difficulty and inherent inaccuracies in determining the internal volume V_i of Sephadex G15 for calculation of K_d, the alternative distribution coefficient, K_{av}, was used for determining molecular weight distributions. As all the fractionations were performed on the same G15 column, elution volumes V_e could have been used to estimate molecular weights without much loss in accuracy. However, K_{av} is theoretically more valid. The relation between molecular weight and K_{av} is shown graphically in Figure 10.

The data of Goodson *et al* (1971) for a similar homologous series is presented in Figure 10 together with the calibration used in this study. The dashed portion of the curves is an extrapolation at MW 1500, the exclusion limit for G15, where both K_{av} and K_d should be zero (Pharmacia Limited, 1970). The data was fitted with straight lines, as a linear relation between molecular weight and K_{av} had been reported for the separation, on Sephadex G25, of a homologous series of oligosaccharides from cellulose hydrolysis (Laurent and Killander, 1964). A similar linear relation is expected for the homologous series

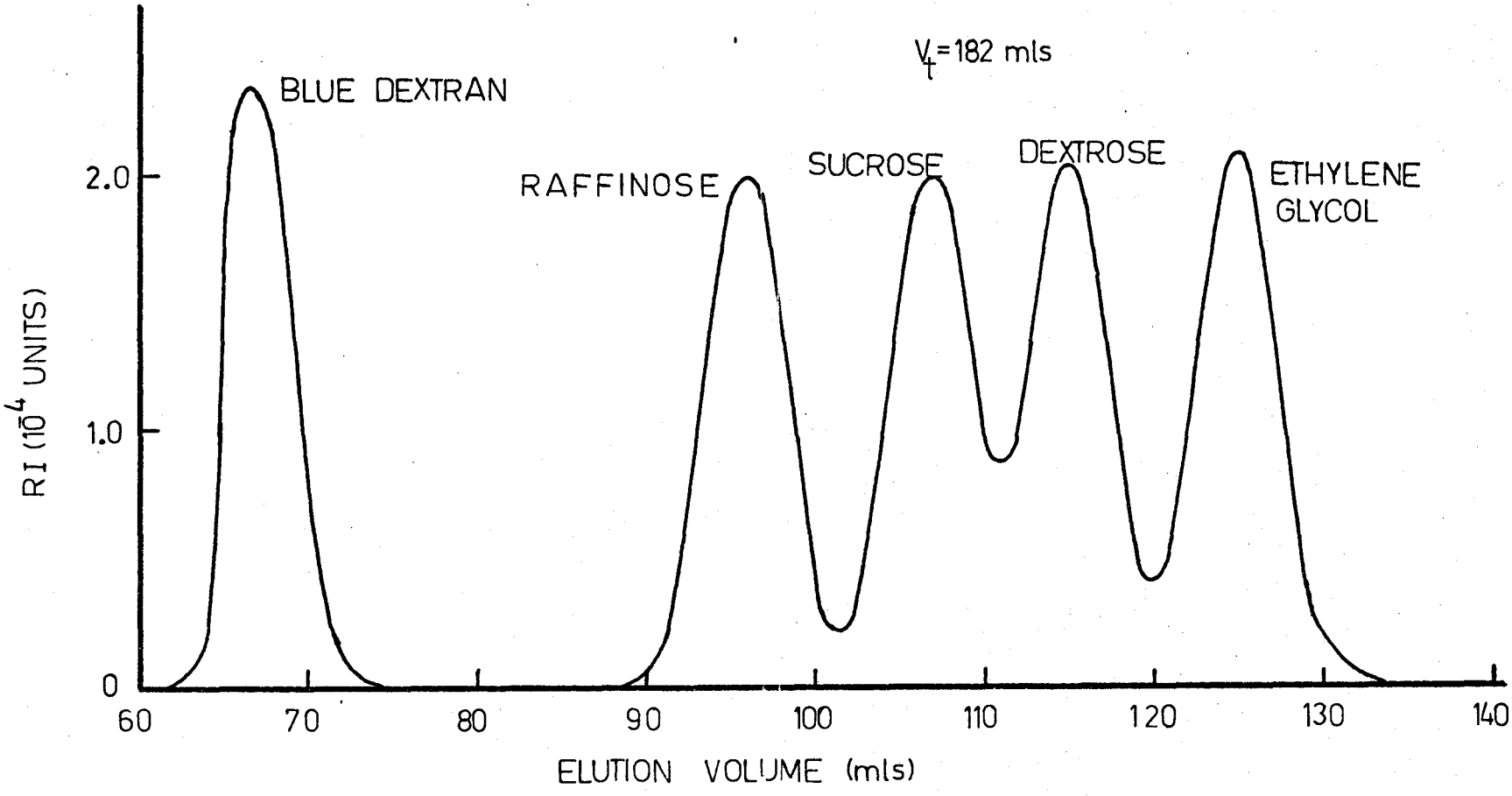


FIGURE 9. Calibration of G15 with a Homologous Series of Sugars and Alcohols

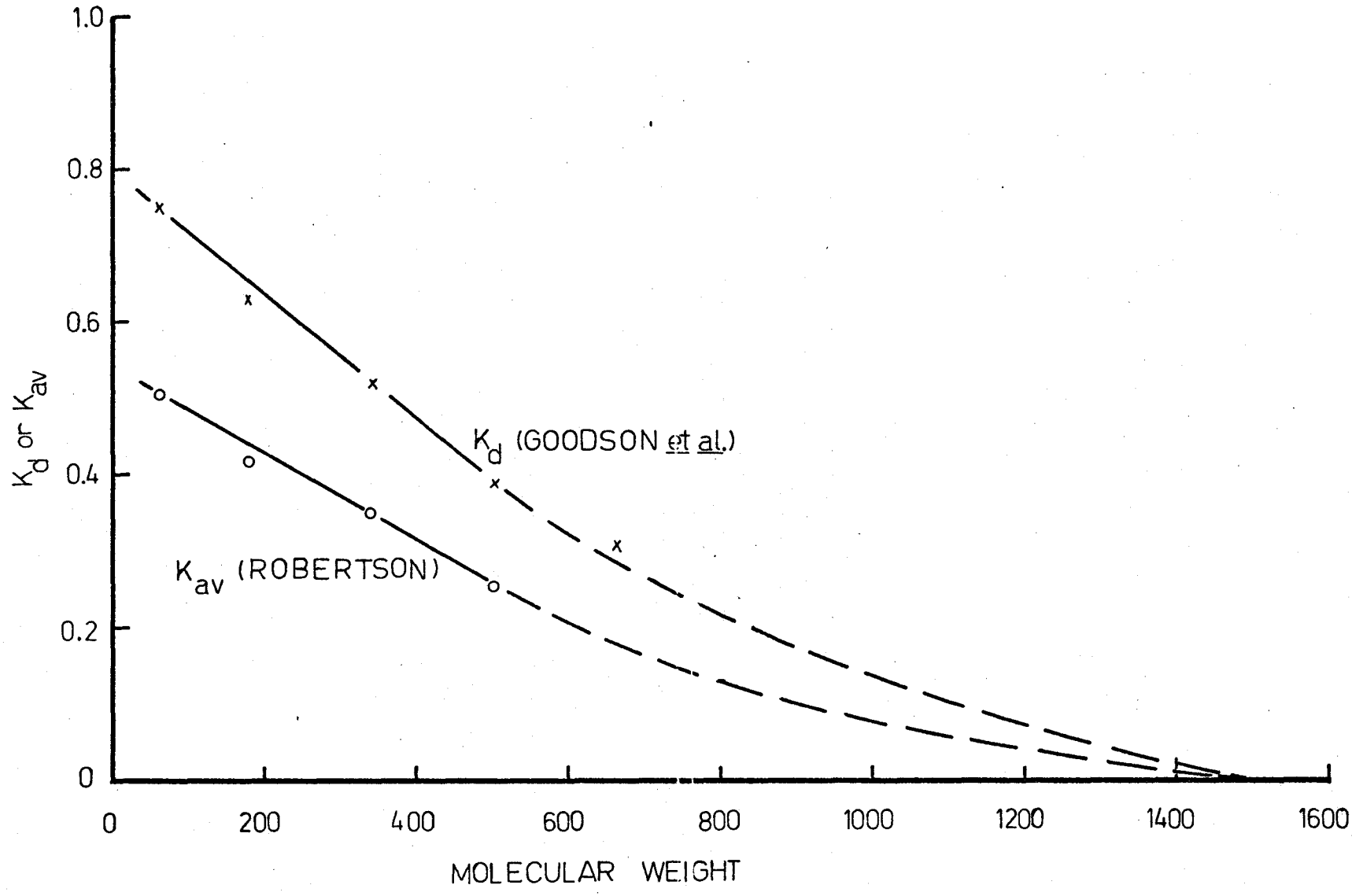


FIGURE 10. Molecular Weight Calibration for G15

on Sephadex G15, as indicated by the similarity between the data obtained in this study and that of Goodson et al. (1971). Calculation of K_d values, instead of K_{av}, from an estimate of the internal volume obtained from the gel water regain and the published range of volumes of swollen gel per gram of dry gel (Pharmacia Limited, 1970), resulted in good agreement between the K_d values obtained here and those found by Goodson et al (1971). For a swollen gel volume of 2.5 ml per gram of dry gel the calculated K_d values are coincident with the $K_d$values plotted in Figure 10.

Calibration of the G15 on the basis of molecular size was accomplished using the data of Goodson et al (1971). Space-filling Corey-Pauling-Koltrun (CPK) models of the homologous series stachyose, raffinose, maltose, glucose, and ethylene glycol were used to determine the average unhydrated radii of these molecules. The average radius was found by measuring the smallest radius, r_g (min), and the maximum radius, r_g (max), determined as the geometric average radii along three mutually perpendicular axes, and then algebraically averaging the two. This method appears to give more realistic results than the alternative of calculating the radius of a sphere of equivalent weight and density (Goodson et al, 1971). The data in Table IV were taken from Goodson et al (1971) with the exception of the K_{av} values which were determined in this study. The

	MW	$r_{g(min)}$ (Å)	$r_{g(max)}$ (Å)	r (Å)	K_d	K_{av}
Stachyose	667	—	—	—	0.31	-
Raffinose	505	4.83	5.73	5.28	0.39	0.256
Maltose	342	4.28	5.01	4.64	0.52	0.352 (sucrose)
Glucose	180	3.46	3.67	3.56	0.63	0.420
Ethylene Glycol	62	2.24	2.76	2.50	0.75	0.506

TABLE IV. Molecular Radius Data for a Homologous Series of Sugars and Alcohols

K_d and K_{av} values in Table IV are plotted against the radius "r" in Figure 11. The values of r_g(min), and r_g(max) are indicated by the T-shaped lines extending horizontally from each data point. Extrapolation of the relationship is uncertain since the exact nature of the relationship between K_{av} and r is unknown in this region. However, both the figures obtained in this study and those obtained by Goodson et al (1971) intersect $K_{av} = 0$ or $K_d = 0$ at very nearly the same value of r (i.e., $r \approx 7.7$ A). Extrapolation of the log r_{Stokes} versus log MW graphs (Figure 15) for dextrans and proteins give values of r_{Stokes} of 8.7 and 7.5 respectively at MW 1500, the molecular weight for which $K_d = 0$ for Sephadex G15 (Andrews, 1970). Although the extrapolations may be questionable, it is encouraging that the figures agree within 10-15%.

Whether or not the molecules used for calibration are hydrated in solution is still not clear. Goodson et al (1971) found the r value from the CPK model of stachyose to be greater than the radius as determined by Robinson and Stokes (1950), and concluded that hydration was probably not significant in the gel filtration behaviour of these molecules.

The apparent size of the molecules therefore can be assumed to lie somewhere within the limits established by r_g(min), and r_g(max), if not at the calculated value of r. Although these limits are quite broad, the values of r determined from Figure 11 can be taken as approximate sizes (ignoring

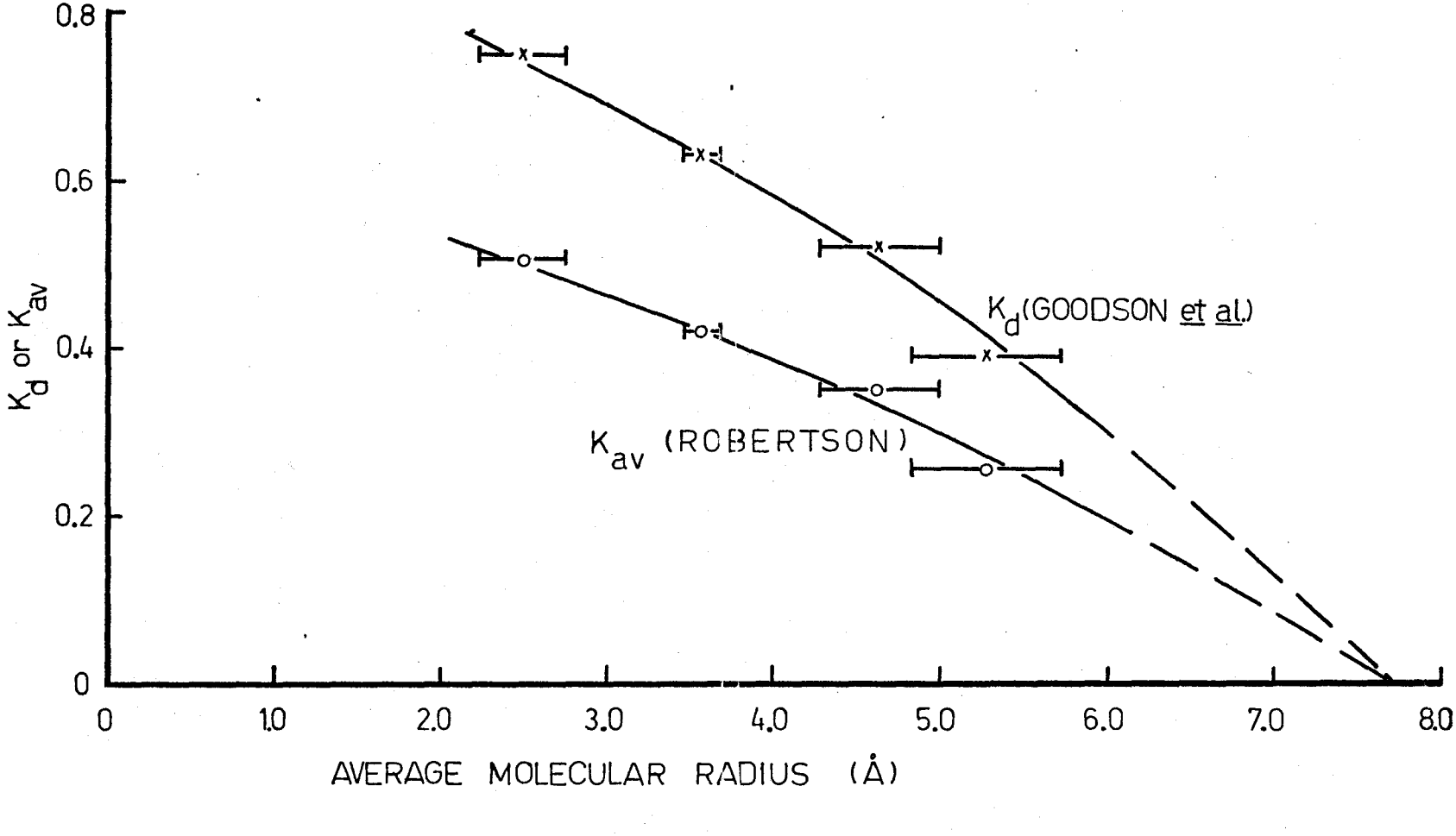


FIGURE 11. Average Molecular Radius Calibration for G15

gel-solute interactions) for molecules resulting from biological treatment. Equivalent molecular weights (EMW) and Equivalent Molecular Radii (EMR) based on the homologous series of sugars and alcohols are reported in this study. No assessment of interactions in the column can be made except where K_{av} exceeds the value at the limit of separation. Because it is more highly cross-linked than Sephadex G50, Sephadex G15 may be expected to be more susceptible to gel-solute interactions.

A correlation between molecular weight and size of a homologous series of sugars and alcohols has been established by Goodson et al (1971):

$$r = 0.65 (M_w)^{0.336}$$

where r is the radius in Angstroms (Å), and

M_w is the molecular weight

No such correlation should be attempted for the residual organics found in this study as their structure and chemical composition are unknown. Accordingly, the size and molecular weight of the residuals are reported in terms of the equivalent values for the homologous series. Molecular size is the parameter according to which separation in gel filtration occurs and should therefore be the more meaningful to report.

Calibration of the G15 with various inorganics was carried out in an attempt to define some of the large peaks obtained in the refractive index (RI) profiles of the treatment plant studies. The calibration elution profile is shown in

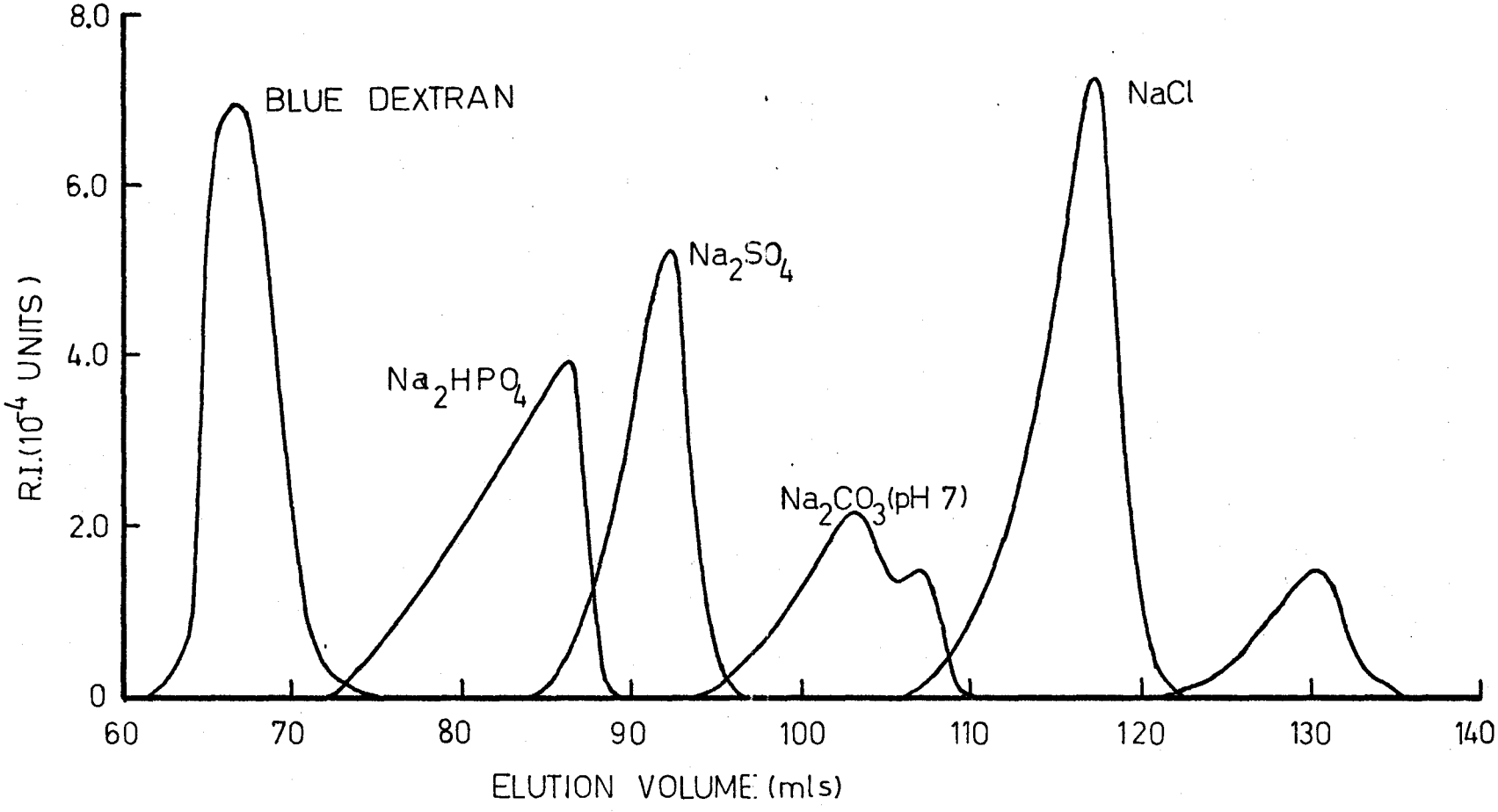


FIGURE 12. Elution Profiles of Inorganic Salts on G15

in Figure 12. The small peak at V_e = 130 ml is anomalous in that it occurred when NaCl and Na_2CO_3 (pH = 7.0) were run separately, and showed UV absorbance where none was expected. No significant amounts of carbon could be detected in this fraction. Negative UV peaks were found to correspond to the RI peaks of all the inorganic salts used. This behaviour is attributed mainly to dilution of the UV-absorbing azide, added to the eluant.

The inorganic elution profile in Figure 12 shows a strong resemblance to the refractive index profile of the concentrated Dundas effluent shown in Figure 33.

A typical calibration elution profile for the Sephadex G50 column is shown in Figure 13. The raffinose peak has a K_d = 0.89, very close to the value K_d = 0.90, reported by Bhatti and Clamp (1968). The molecular size as determined by Goodson et al (1971) is approximately 5.3 Å ± 0.5 Å. Since the experimental and literature values of K_d for raffinose are so close, calibration of the low end of the molecular weight scale (i.e., <1500) is possible by adopting the K_d versus molecular weight calibration given by Bhatti and Clamp (1968), as in Figure 14. This can be roughly converted to K_d versus molecular size (Figure 14) by using the relation developed by Goodson et al (1971) to correlate molecular weight and mean radius for homologous series of sugars and alcohols.

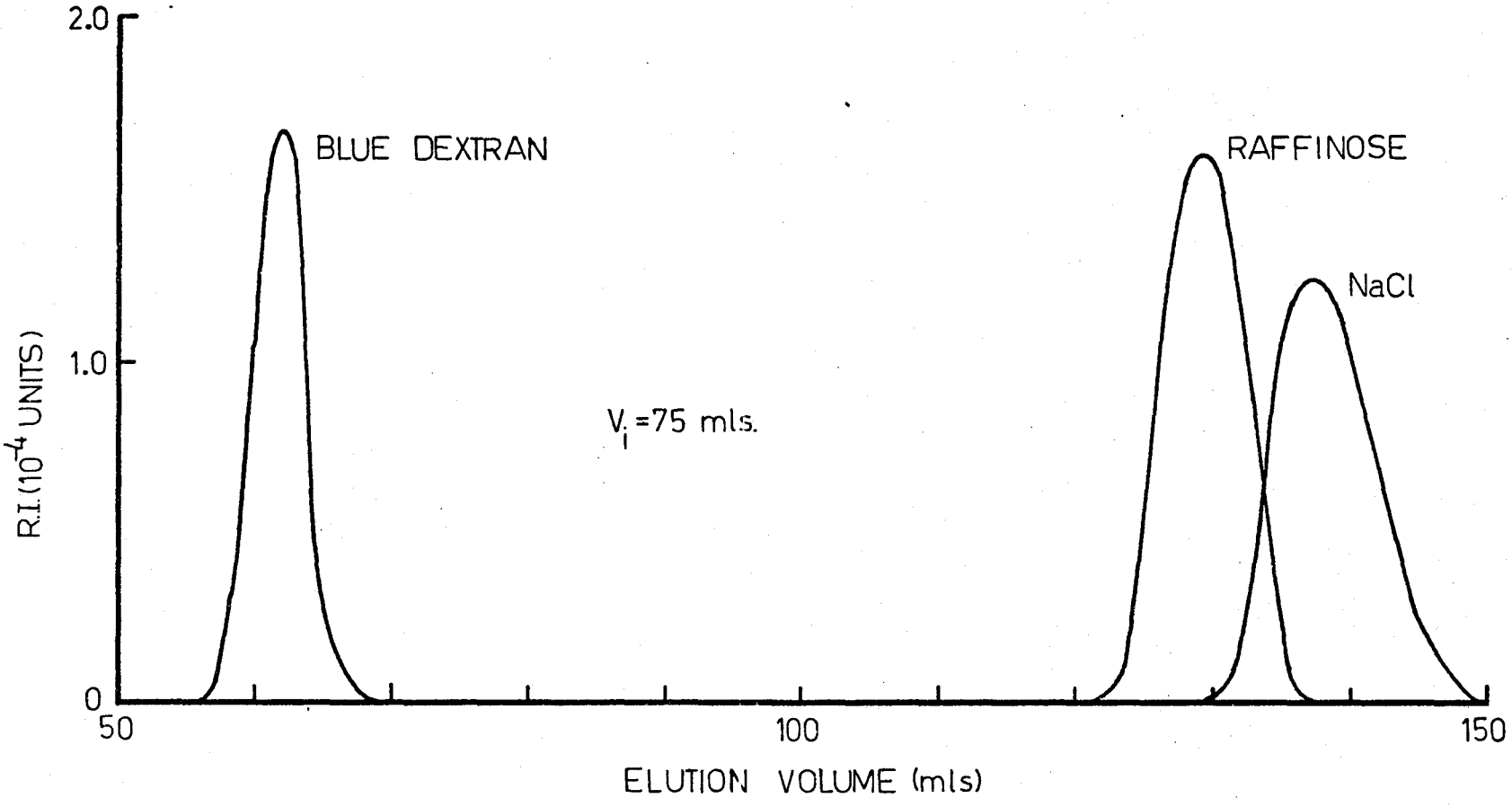


FIGURE 13. G50 Calibration Elution Profile

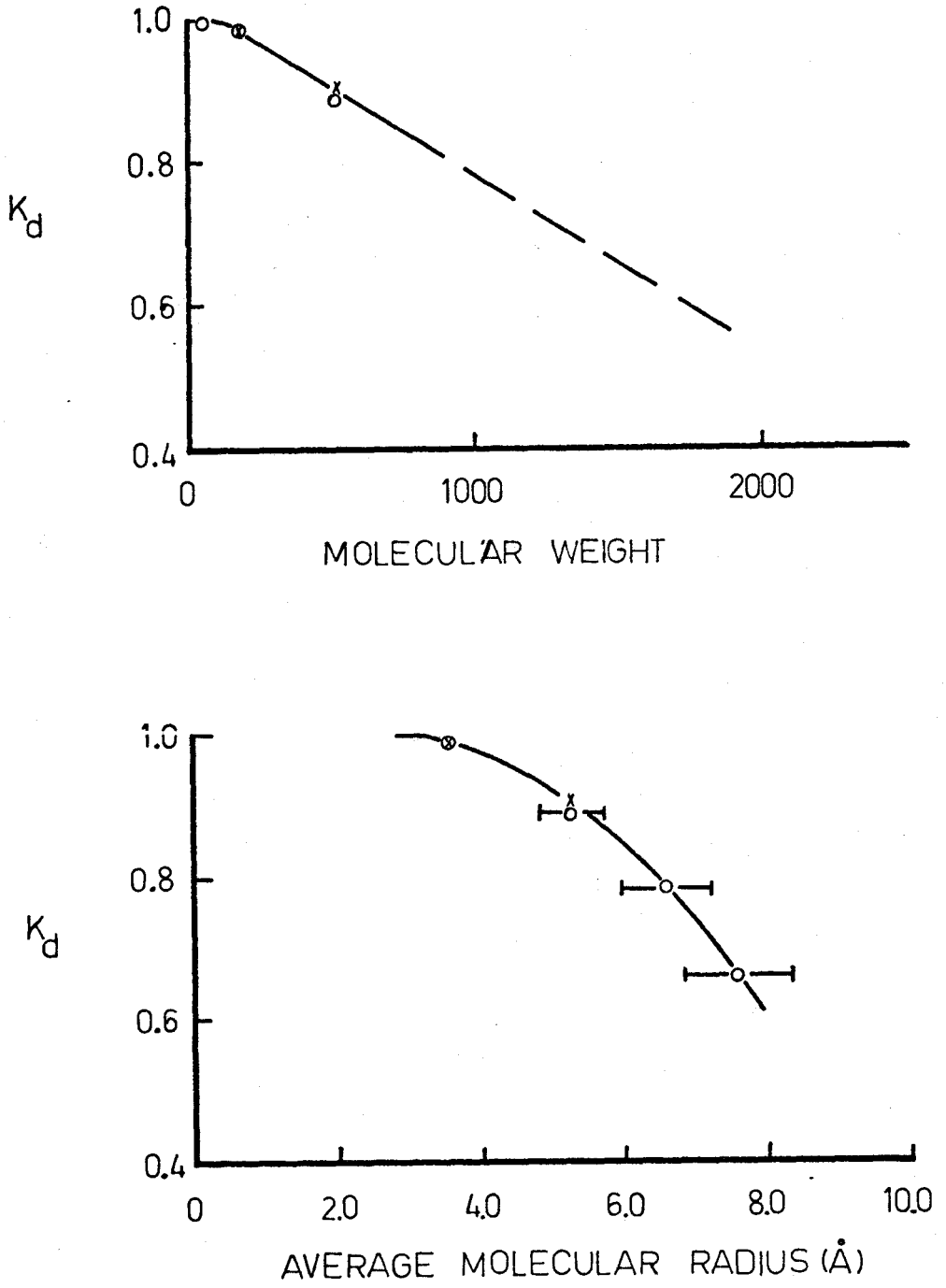


FIGURE 14. G50 Calibrations for Molecular Weight and Average Molecular Radius

Calibration of the central region of the molecular weight distributions was not considered necessary, since only negligible carbon could be detected in any samples between the G50 exclusion limit and the low molecular weight range already calibrated. The exclusion limit for Sephadex G50 occurs at MW 10,000 for dextrans and MW 30,000 for globular proteins, which correspond to molecular sizes of 22.4 Å and 24.5 Å, respectively, as determined from Figure 15. The molecular weight and Stokes' radius data for proteins, on which Figure 15 is based, is given in Table V.

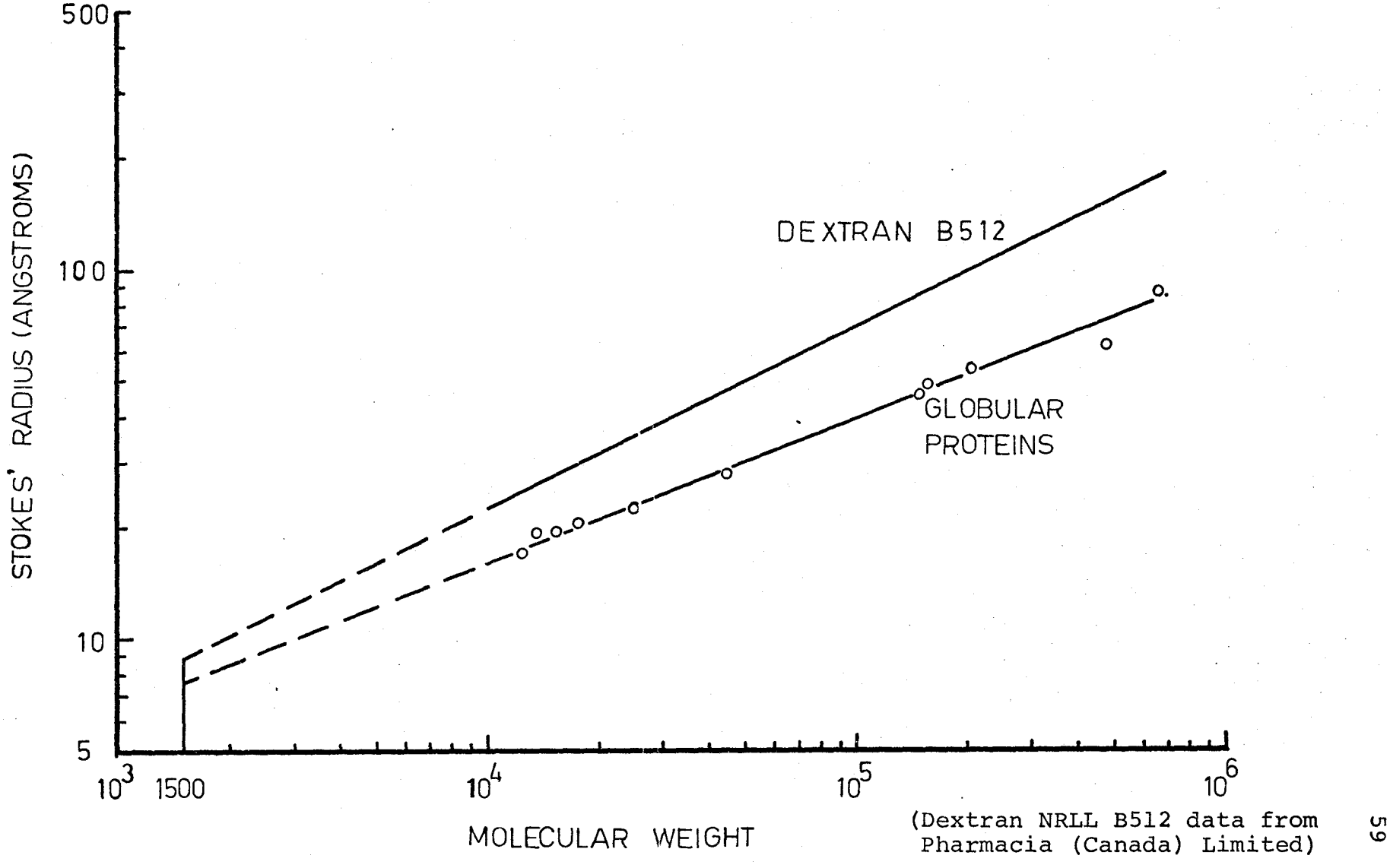


FIGURE 15. Stokes' Radius versus Molecular Weight for Dextrans and Globular Proteins

Protein	Molecular Weight	Stokes' Radius (Å)
Cytochrome C	12,400	16.4 - 17.4
Ribonuclease A*	13,700	19.2
α-Lactoalbumin	15,500	19.4
Myoglobulin	17,800	20.7
Chymotrypsinogen A*	25,000	22.4
Ovalbumin*	45,000	27.3
Serum Albumin (Bovine)	67,000	35.5
Alcohol Dehydrogenase	150,000	45.3
Aldolase*	158,000	46.0
γ-Globulin	205,000	52.0 - 55.5
Apoferritin	480,000	61.0
Thyroglobulin	670,000	85.0

* Pharmacia (Canada) Limited. All other values from Andrews (1970)

TABLE V. Molecular Weight and Stokes' Radius Data for Globular Proteins

CHAPTER 5

5. RESULTS AND DISCUSSION

5.1 Effects of Sample Preparation on Elution Profiles

Before determining the elution profiles of any of the systems studied, the effects of sample pH, washing of the activated sludge with distilled water, membrane filter pore size and freeze-drying on the elution profiles from gel filtration columns had to be evaluated.

a) Effect of pH

That significant differences occurred in elution profile depending on the pH at which a sample was applied to the columns is demonstrated well by comparing Figures 16 and 23. Even without testing the effect on the TOC profile, the changes in the UV and RI profiles were sufficient to point out the need for careful neutralization of each sample before application to the chromatographic columns.

b) Effect of Sludge Washing

Washing of bacterial cells with distilled water is known to cause cell lysis (Lamanna and Mallette, 1965). To ensure that these products did not affect the quantity of residuals appearing at the G15 exclusion limit, at both high and low initial MLSS concentration, the starting solutions of representative batch activated sludge runs were compared to the final effluents. Figure 17 and 18 show the starting and final elution profiles of a Series II glucose run at 1000 mg/l TOC and 1000 mg/l MLSS, in which there is clear indication that considerable residual material is formed at the G15

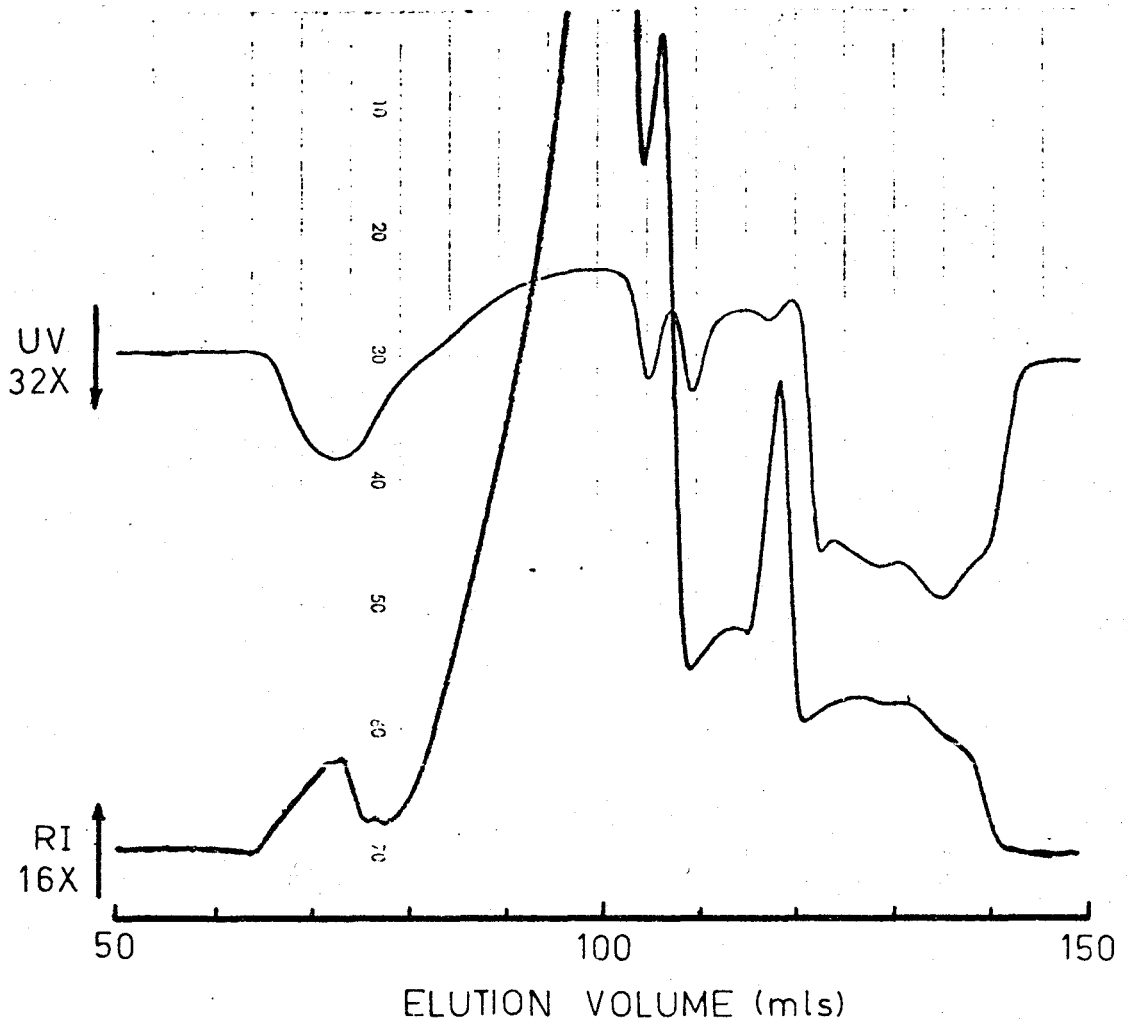


FIGURE 16. G15 Elution Profile of Concentrated Effluent from Series II (Glucose) at pH 7.8

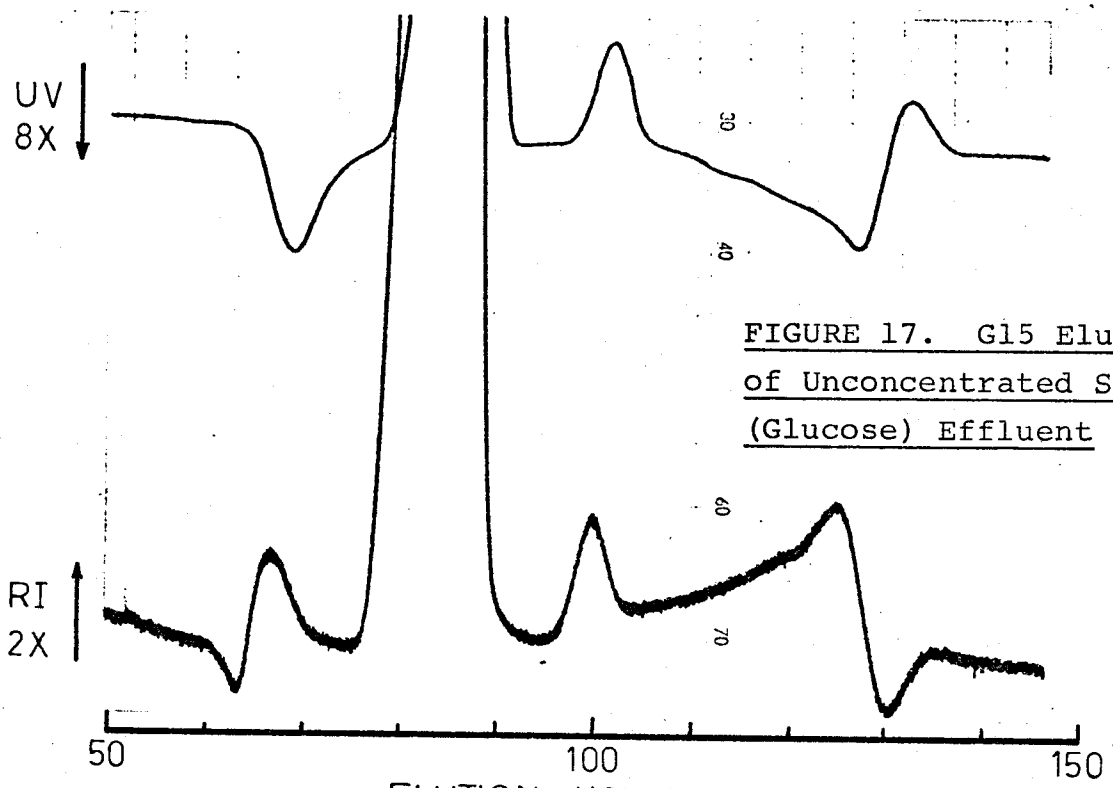


FIGURE 17. G15 Elution Profile of Unconcentrated Series II (Glucose) Effluent

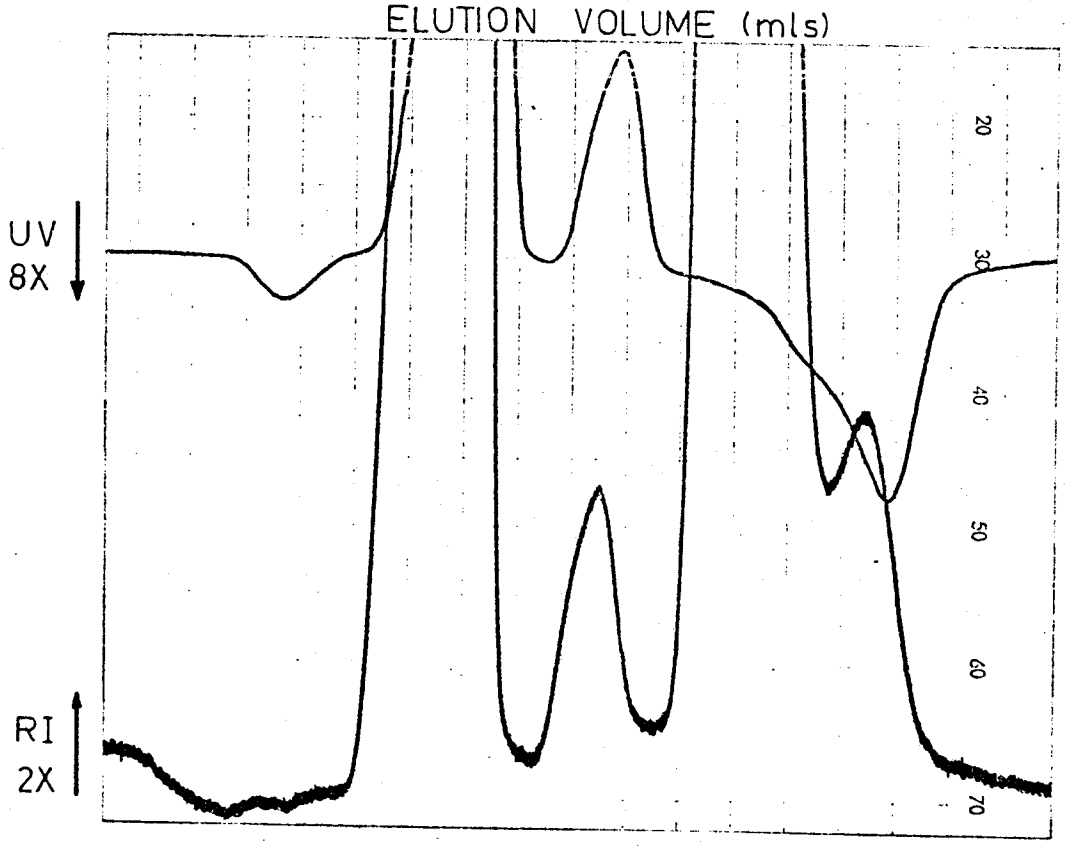


FIGURE 18. G15 Elution Profile of Unconcentrated Series II (Glucose) Starting Medium

exclusion limit above and beyond the small amount already present. Figures 21 and 22, in which the starting and final elution profiles of a low solids and TOC concentration glutamic acid run are shown, indicate again the minor effect of washing the activated sludge with distilled water.

c) Effect of Membrane Filter Pore Size

To check on Weber's criticism (1970) of Molof and Zuckerman (1970), that part of the material excluded from G15 was colloidal, a sample of the unconcentrated effluent from the Series II glucose run (TOC_i = 1000 mg/l) was filtered through a 0.1 μ Sartorius membrane filter and compared chromatographically to a sample filtered through the standard 0.45 μ membrane filter. Comparison of the UV traces in Figures 18 and 19 show no significant differences. Comparison of the RI traces is invalid since the RI detector was cleaned between runs. It is felt that the UV profile gives a good quantitiative measure of the compounds at the G15 exclusion limit, and is therefore sufficient for the comparison.

d) Effect of Freeze-Drying

Because of the insoluble matter formed during the freeze-drying process, the recovery of the organic matter becomes important. Hardt et al (1971) reported by way of diagrams that the freeze-drying process had no effect on the distribution of organic carbon. No details of the procedure used were provided. In this study it was found that up to

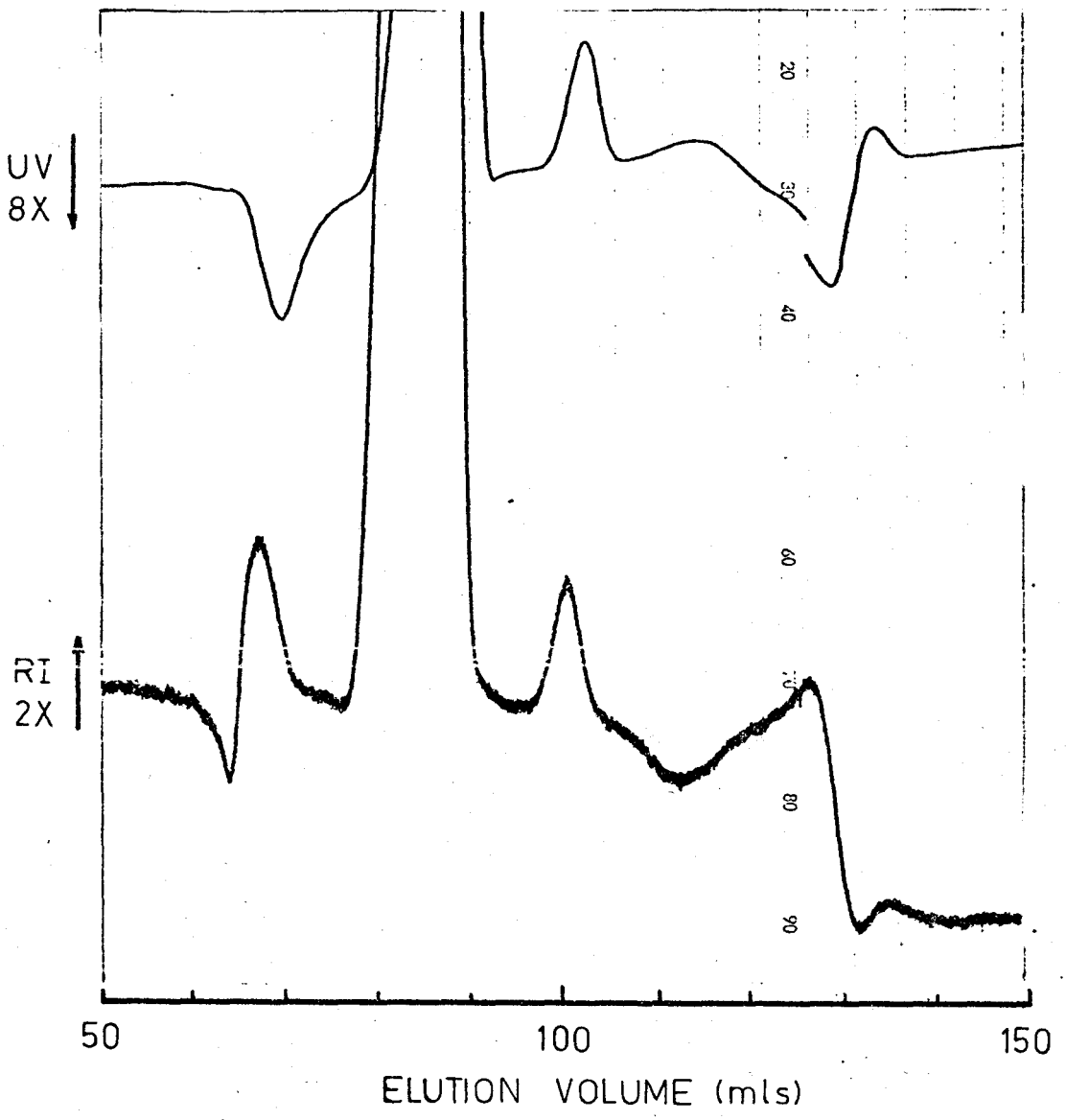


FIGURE 19. G15 Elution Profile of 0.1 μ Membrane Filtered Concentrated Effluent from Series II (Glucose)

40% of the TOC could be lost during freeze-drying. For instance, after concentrating a raw sewage sample, prefiltered through a 0.45 μ Sartorius membrane filter, a sonicated suspension of the freeze-dried material gave a TOC of 400 mg/l, while a gravity-settled sample contained only 330 mg/l. Filtering through a 0.45 μ filter further reduced the carbon level to 252 mg/l. Approximately 40% of the inorganic carbon content is removed by settling and filtering. A large portion of the organic material which is lost appears to be removed by joint precipitation with inorganic materials. It must be realized that concentrated samples may not be representative of the "unconcentrated" samples because of these losses in the concentration procedure.

The findings of Hardt et al (1971) regarding the similarity in relative compositions of samples before and after concentration, lead to the unlikely hypothesis that material of all molecular weights is precipitated equally. In general, as molecular weight increases, solubility in water decreases, unless the number of ionizable sites and hydrogen bonding sites in the molecule increases in proportion to the increase in molecular weight. This is unlikely to happen in the chemical systems under study, because of the diversity of compositions and structures known to exist. In addition, compounds of a particular molecular weight are unlikely to have the same solubilities because of different chemical structure and composition.

5.2 Activated Sludge Batch Studies

a) Series I

Activated sludge grown on a glucose only substrate at a concentration of 200 mg/l as TOC showed that ~40% of the residual carbon occurred at the G15 exclusion limit, indicating an equivalent molecular weight (EMW) of greater than 1500 (dextran and protein calibration) and equivalent molecular radius (EMR) greater than 7.5 Å. A further 15% appeared at an EMW ~660 corresponding to an EMR of ~6.1 Å. The remaining carbon was distributed down to EMW 210 and equivalent radius of 3.5 Å. Recovery of the TOC applied as concentrated effluent to the Sephadex G15 column was 98% ± 4%. The TOC, RI and UV elution profiles are shown in Figure 20.

The reactor effluent showed that ~31% of the TOC was excluded from the G50 column indicating that this portion had an EMW of greater than 10,000 (dextran calibration) and an equivalent molecular radius of greater than 22.5 Å. The remainder appeared at MW ~900 or less ($r < 7.0$ Å) confirming the G15 results and indicating negligible amounts of TOC between 900 and 10,000 molecular weight (dextran basis).

Growth of activated sludge on 200 mg/l (as TOC) of glutamic acid resulted in residual levels considerably lower than when grown on glucose, with the result that no reliable carbon analysis could be performed on the effluent from this

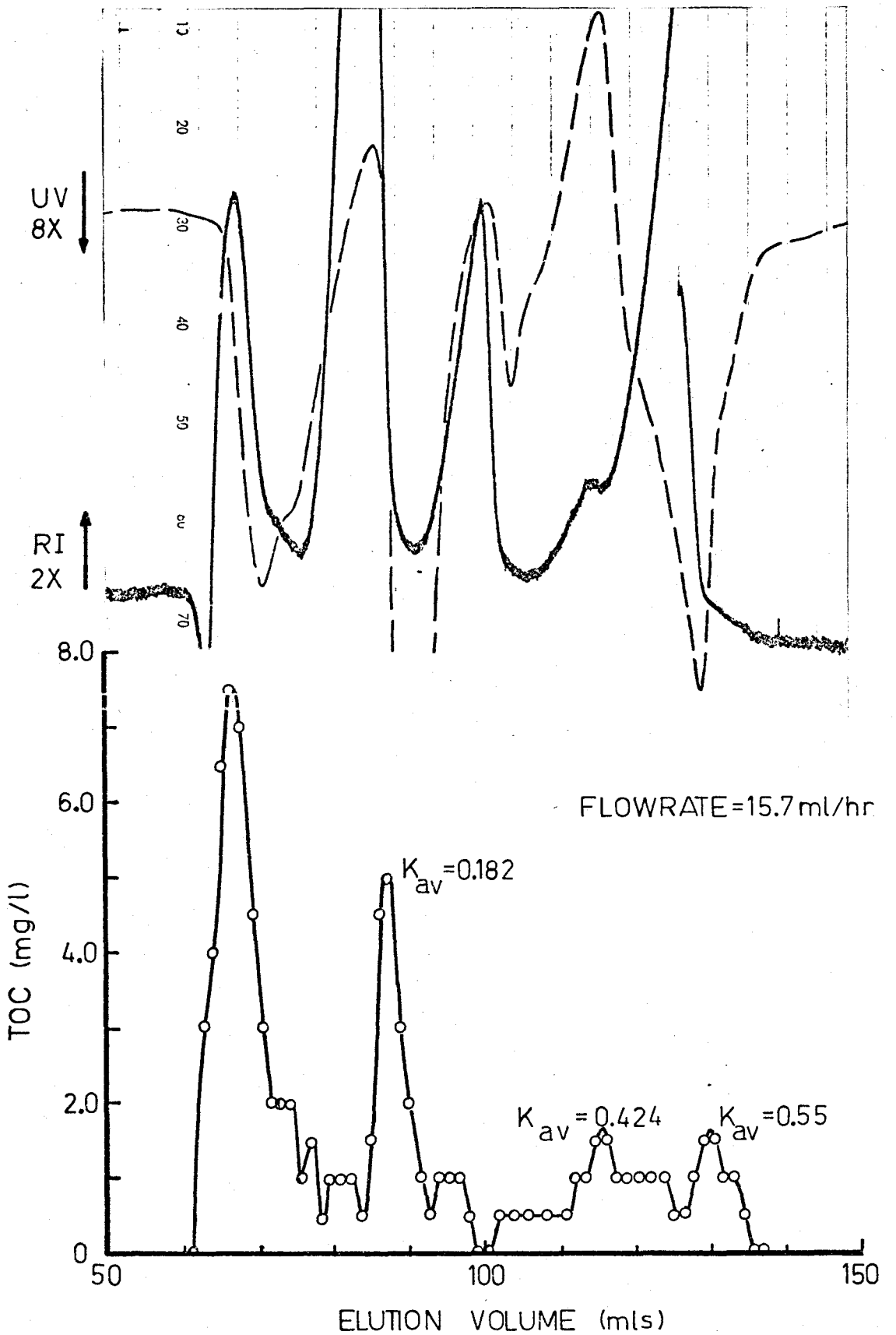


FIGURE 20. G15 Elution Profile of Concentrated Effluent from Series I (Glucose)

reaction. However, examination of the initial and final UV and RI traces in Figure 21 and 22 showed the formation of 1500 + EMW (7.7^{+} Å radius) material during the course of the reaction.

Although not detailed well on the TOC versus elution volume trace, both the RI and UV traces show the presence of a fraction just below the G15 exclusion limit which is formed during the biodegradation of both glutamic acid and glucose.

b) Series II

When the initial concentration of total organic carbon was increased by a factor of five and initial MLSS concentration by a factor of ten, significant changes in the residual carbon distribution resulted. For glucose as the substrate, 70% of the TOC applied to the G15 column (in concentrated form) appeared at or just below the exclusion limit as can be seen from the TOC versus elution volume trace in Figure 23. (This broad distribution is also evident in the UV and RI traces.) This corresponds to an equivalent molecular weight greater than about 1300 (polysaccharide calibration) and equivalent molecular radius of greater than 7.3 Å. A further 18% of the TOC appeared at approximately 370 EMW and 4.7 Å EMR. Recovery of applied TOC on the column was 101.6% $\pm$ 3%. On Sephadex G50, 70% of the TOC applied was distributed at and just below exclusion limit indicating an equivalent molecular

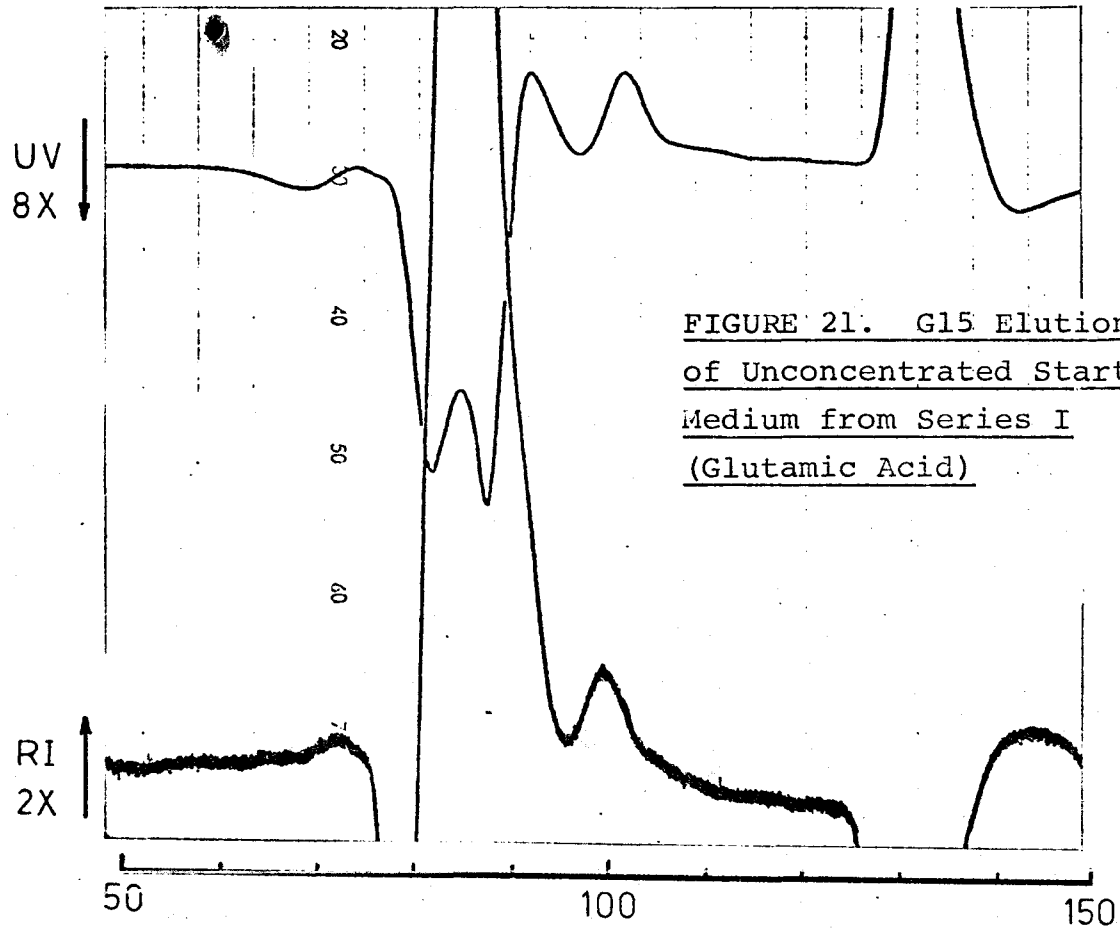


FIGURE 21. G15 Elution Profile of Unconcentrated Starting Medium from Series I (Glutamic Acid)

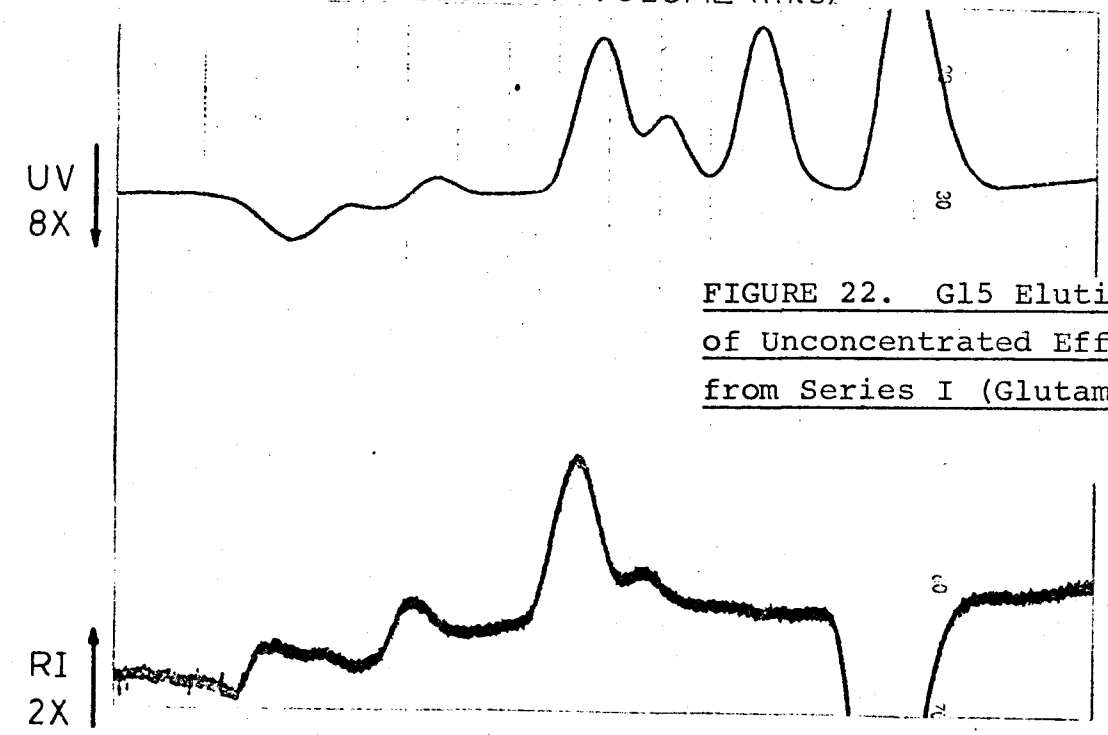


FIGURE 22. G15 Elution Profile of Unconcentrated Effluent from Series I (Glutamic Acid)

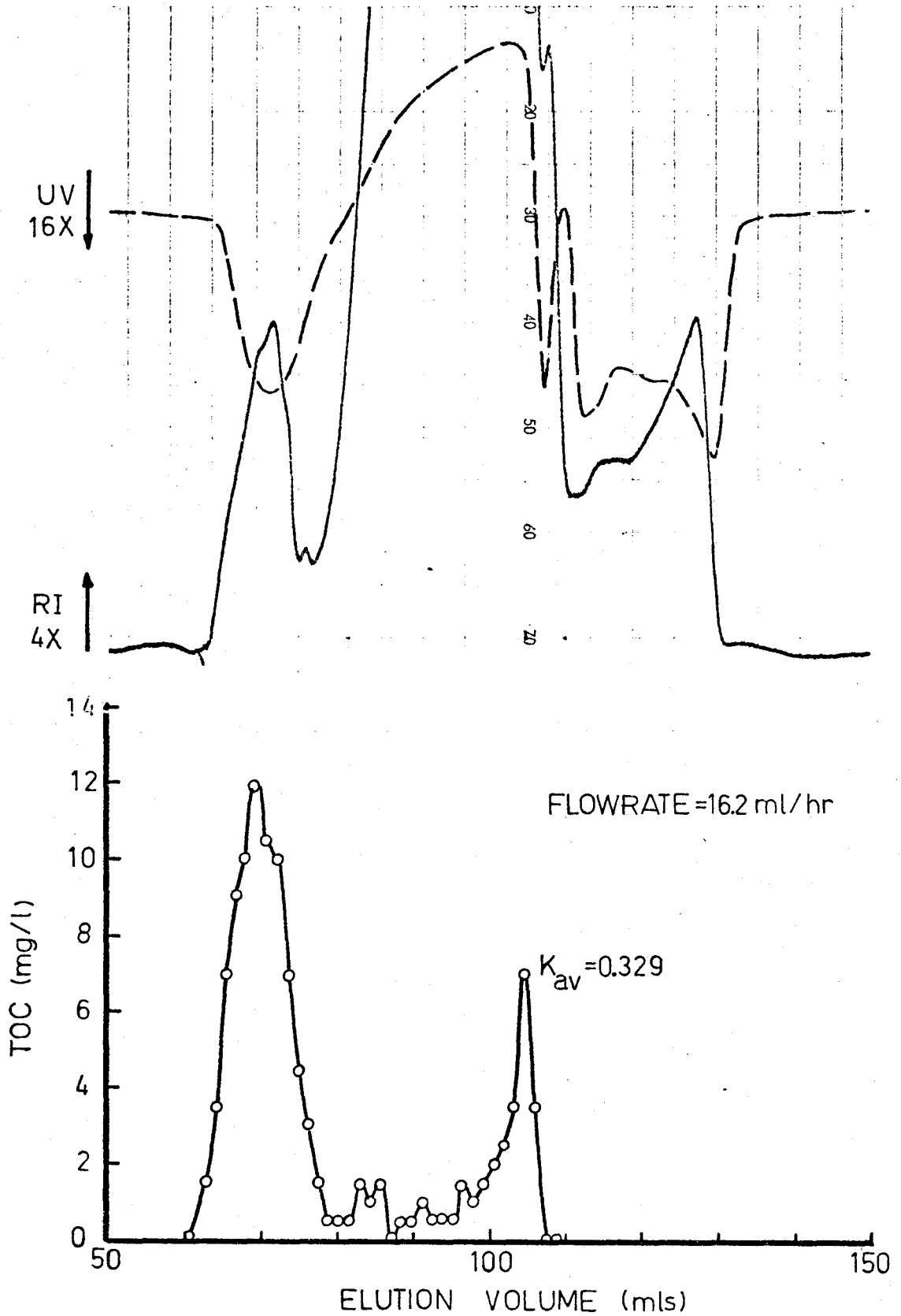


FIGURE 23. G15 Elution Profile of Concentrated Effluent from Series II (Glucose) at pH 7.0

weight of approximately 9000^{+} (dextran basis) and equivalent molecular radii of greater than 20 Å. The remainder of the TOC appeared at EMW < 400 with negligible quantities between EMW 400 and the exclusion limit, confirming the G15 results. Recovery from the G50 column was 102% ± 5%.

When activated sludge was grown on glutamic acid at high initial TOC solids concentrations, the molecular weight and size distribution of the residual organics is similar to that when activated sludge is grown on glucose. Approximately 69% of the residual TOC appeared at the G15 exclusion limit while a further 11% appeared at EMW ~ 380 (equivalent molecular radius 4.75 Å) and another 9% at lower EMW, as shown in Figure 24. The fraction at EMW ~ 380 can be considered chromatographically equivalent to the EMW 370 fraction from the glucose experiment within the limits of experimental error. Both fractions showed strong UV absorbance at 254 mμ indicating possible similarities in chemical composition. There is a significant difference in the breadth of the exclusion limit fractions, the one from the glucose run being considerably broader than that from the glutamic acid run. Although not evident in the TOC versus elution volume trace, the RI versus elution volume graph for the glutamic acid run does show a slight separation of two different fractions near the exclusion limit.

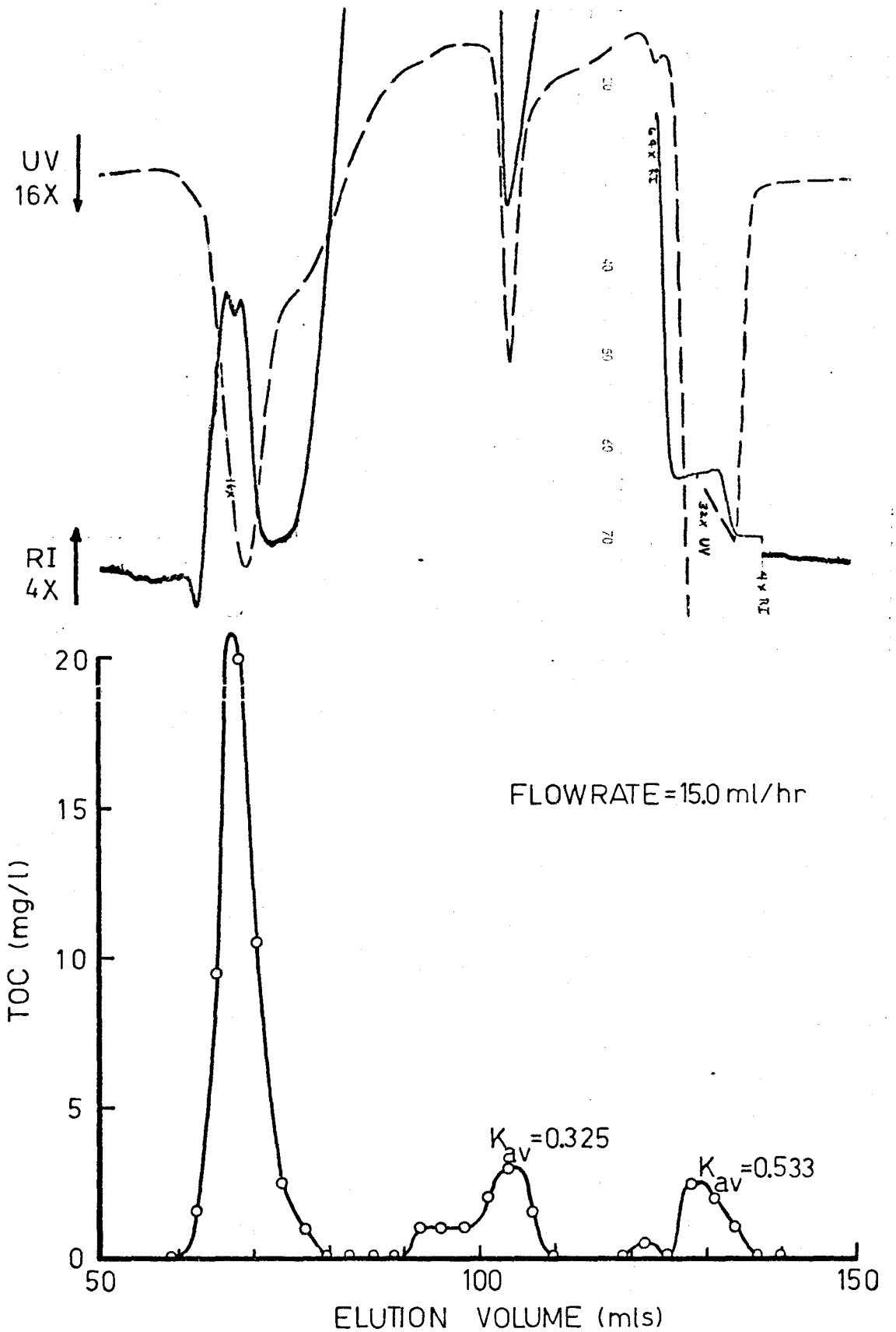


FIGURE 24. G15 Elution Profile of Concentrated Effluent from Series II (Glutamic Acid)

Unlike the glucose run, the glutamic acid run produced unequal amounts of TOC at the exclusion limits of both G15 and G50. The glutamic acid run showed 59% at the G50 exclusion limit, 10% less than at the G15 limit, with an almost continuous low TOC level between the exclusion limit and EMW 630. Approximately 21% of the TOC appeared at EMW less than 630, very close to the G15 result of 19% for the same EMW range. Recovery on the G50 column was 100.7% $\pm$ 6%.

c) Series III

Addition of 200 mg/l of yeast extract to the starting solution for the batch tests at 200 mg/l TOC resulted only in slight changes in the MW distribution (Figure 25). When glucose and yeast extract were the substrates, 40% of the residual carbon appeared at or just below the G15 exclusion limit, the same proportion as for glucose alone. A further 20% appeared at an equivalent molecular radius of ~5.8 Å (EMW ~580). This fraction, as well as the exclusion limit fraction, exhibited a strong absorbance in the UV at 254 mμ.

When glutamic acid plus yeast extract was the substrate, an elution profile very similar to that of the equivalent glucose run was obtained (Figure 26). At or just below the G15 exclusion limit about 40% of the TOC appeared, while another 18% occurred at an EMW of 570 (EMR 5.7 Å). Only these two fractions showed any appreciable UV absorbance at 254 mμ.

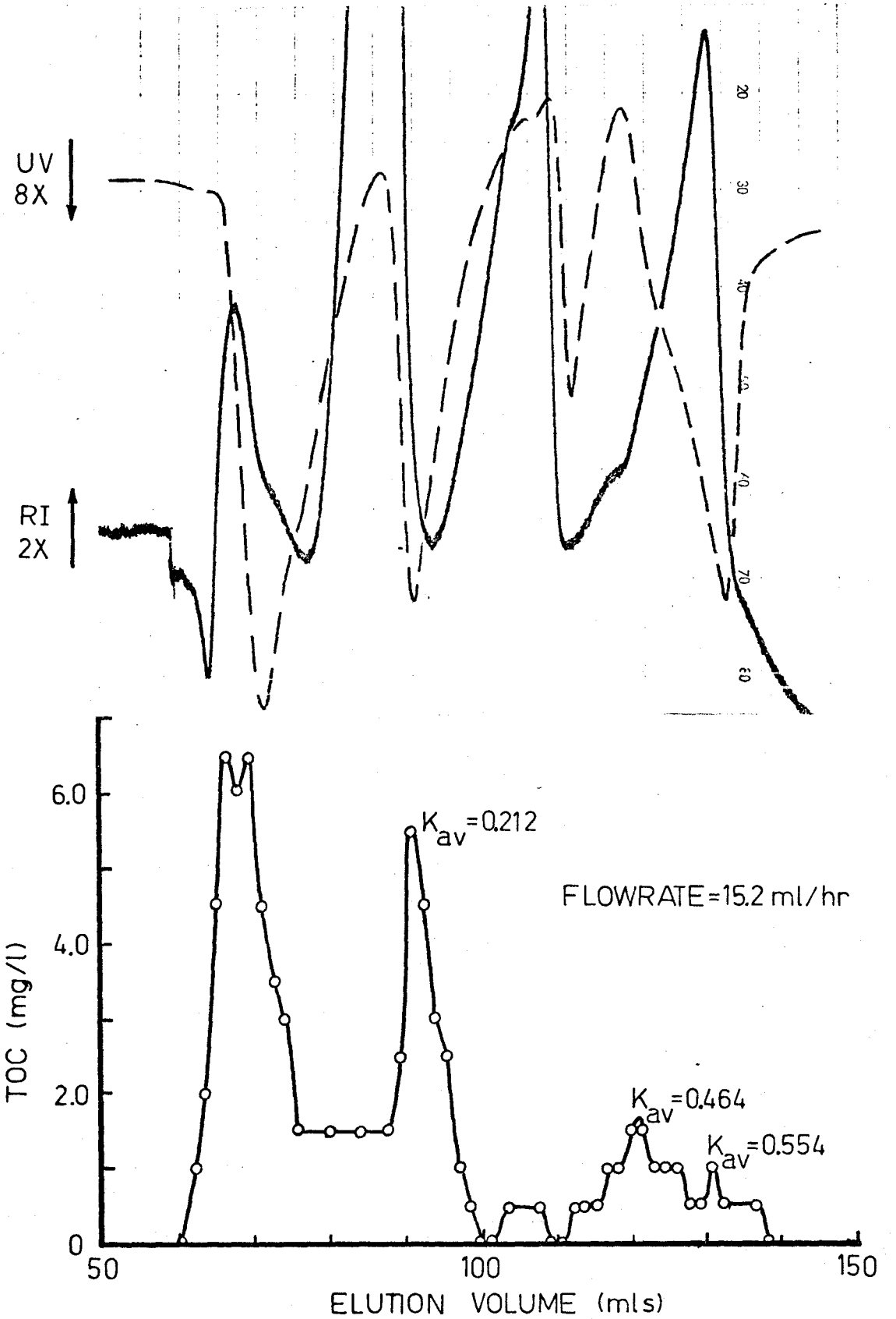


FIGURE 25. G15 Elution Profile of Concentrated Effluent from Series III (Glucose)

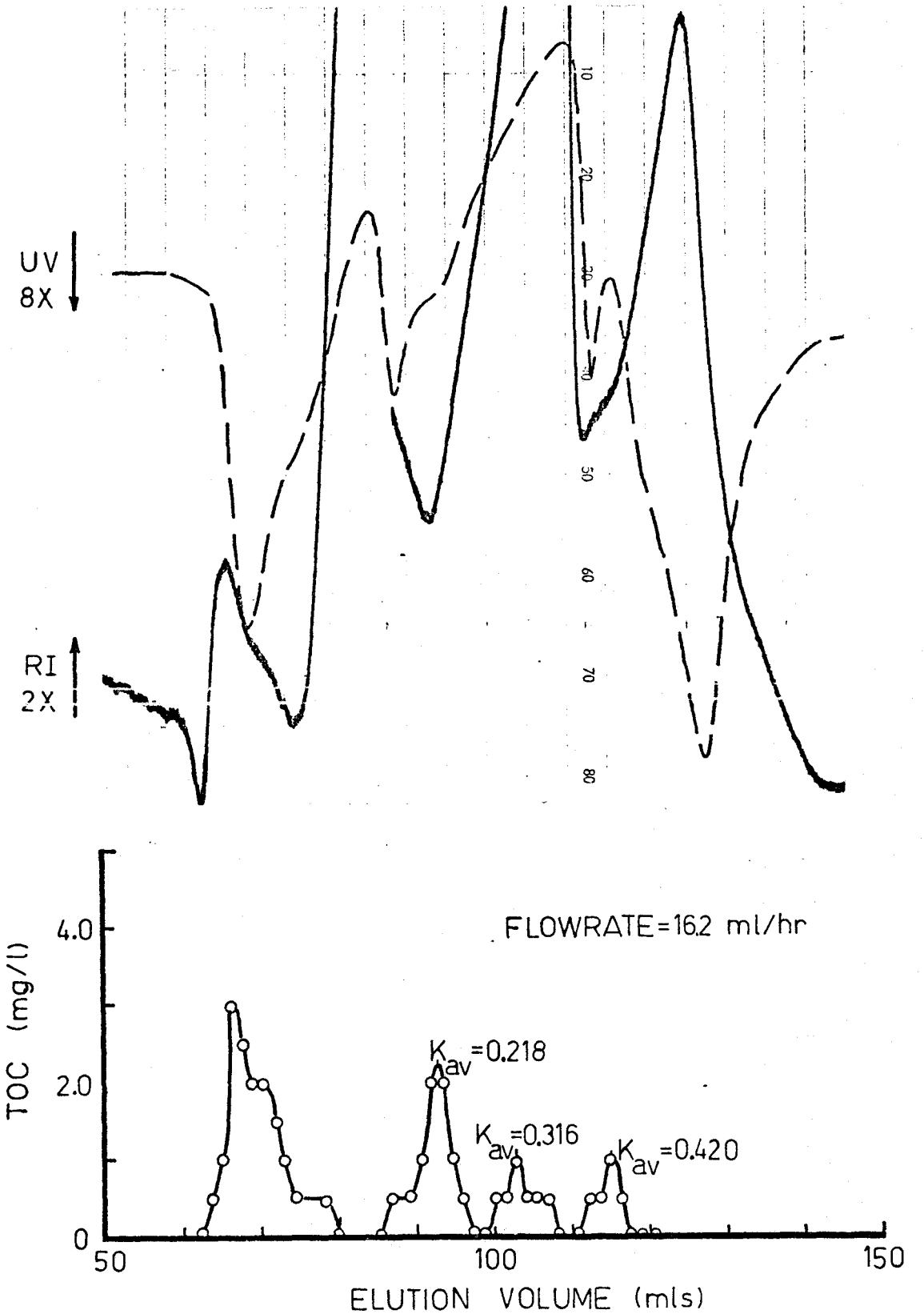


FIGURE 26. G15 Elution Profile of Concentrated Effluent from Series III (Glutamic Acid)

Although the TOC versus elution volume profiles are similar in percentage of TOC at a given molecular weight or size, the actual components of each peak and their relative concentration could be different as suggested by the differences in the UV and RI traces shown in Figures 25 and 26.

Because of the very low carbon concentration of the glutamic acid effluent, only the glucose effluent could be run on the G50 column. Although TOC recovery on the column was poor (~65% in this instance) just slightly more than 10% of the TOC recovered appeared at the G50 exclusion limit. The remainder of the recovered TOC occurred at the lower limit of separation.

5.3 Dominant Culture Studies

Although only one strain of bacteria was grown successfully in pure culture, some indication is given of the differences in effluent molecular weight and size distributions as a result of different microbial populations growing on the same substrate.

A yellow-pigmented bacterium belonging to the genus Flavobacterium sp. was successfully grown in pure culture. Tezuka(1969) found that approximately 60% of the bacteria in a Tokyo activated sludge were yellow pigmented and appeared to be Flavobacterium sp. Similarly, Lighthart and Ogelsby (1969) found that Flavobacterium sp. appeared to be predominant in an American activated sludge. In the isolation

procedure used in this study, Flavobacterium sp. was found to be important in the ecology of the Burlington Skyway Treatment Plant activated sludge. The species isolated and used here exhibited growth characteristics similar to those reported for the species of Flavobacterium sp. studied by Tezuka (1969) and is therefore considered representative.

This strain of Flavobacterium sp. showed good utilization of glucose when grown with 200 mg/l of yeast extract, as can be seen by comparing the initial and final elution profiles in Figures 27 and 28. Total organic carbon removal, however, was poor. Of the starting 267 mg/l of TOC, approximately 170 mg/l remained after the completion of the reaction. Of the TOC remaining just slightly greater than 55% was present as a strongly UV-absorbing compound of equivalent molecular weight ~560 and equivalent molecular size 5.7 Å (Figure 28). This fraction strongly resembles in chromatographic behaviour, the 570 - 580 EMW fractions obtained when activated sludge was grown on glucose and glutamic acid plus yeast extract (Series III) and, within the limits of experimental error, the 660 EMW fraction produced when activated sludge was grown on glucose (Series I; initial TOC = 200 mg/l). All these fractions showed very strong absorbance in the UV at 254 mμ. The UV monitor showed the formation of another fraction at EMW ~1000 (EMR ~7.1 Å) indicating that high molecular weight material is formed by single bacterial species. Comparison

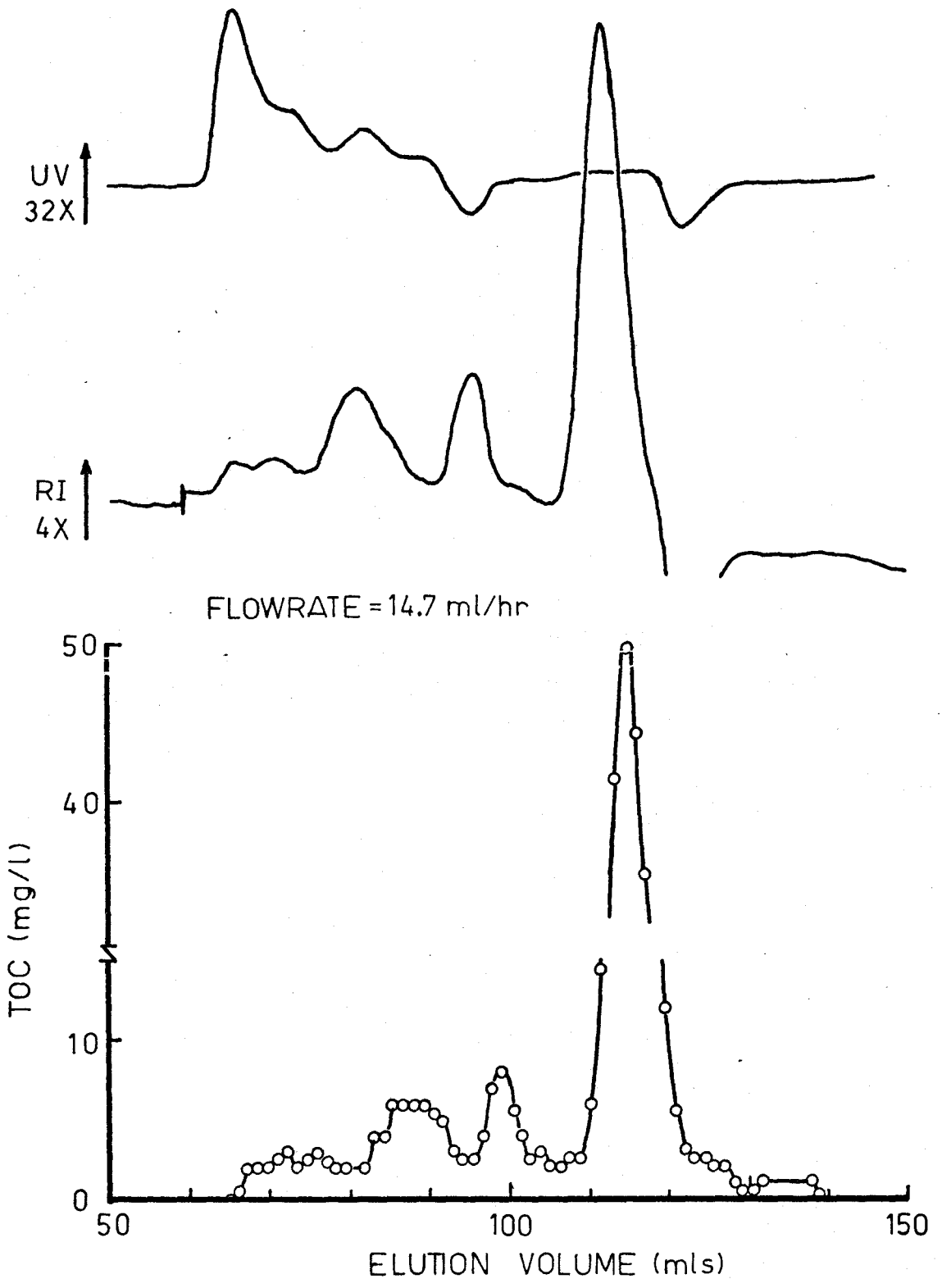


FIGURE 27. G15 Elution Profile of Unconcentrated Glucose Starting Medium for Flavobacterium sp.

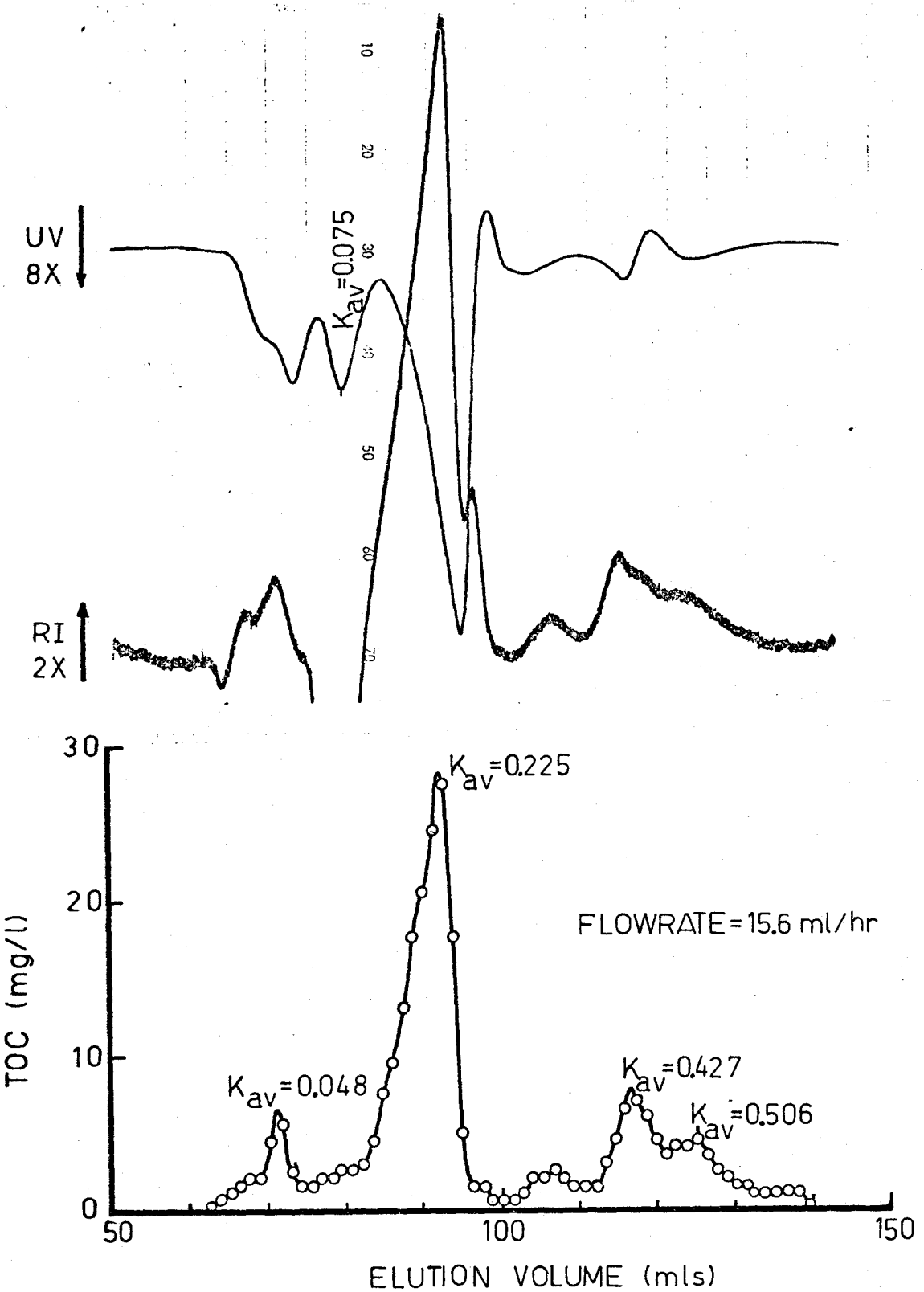


FIGURE 28. G15 Elution Profile of Unconcentrated Effluent from a Pure Flavobacterium sp. Batch Test

of the percentage of the TOC present in the elution volume range 68-75 mls, shows that the fraction of carbon in this volume increased by two-fold from the initial profile (3.4%) to the final profile (6.8%). This confirms that some relatively high molecular weight material is formed even by a pure strain of bacteria. TOC recovery on the G15 column was 101.1% $\pm$ 0.6%.

Attempts to innoculate the final reaction mixture from the Flavobacterium sp. studies with a second pure culture suffered from contamination by yeasts, but the results are nevertheless instructive. Innoculation of one reactor was attempted with a Pseudomonas sp. isolated from activated sludge. Upon completion of the run, plating and microscopic examination of live specimens showed no Pseudomonas sp., but large amounts of Flavobacterium sp. and budding yeasts, and some Gram positive short rods tentatively classified as Brevibacterium sp. A small number of sporulated rods tentatively classified as Bacillus mycoides were also found. This mixture of microorganisms reduced the TOC of the Flavobacterium sp. effluent from 170 mg/l to 63 mg/l. The TOC, UV and RI elution profiles are shown in Figure 29. Comparison with the pure Flavobacterium sp. effluent shows a decrease in the fraction at K_{av} = 0.225 and the formation of material just under the G15 exclusion limit (EMR ~7.3 Å) and at K_{av} = 0.502 corresponding to an EMW of ~65 (EMR ~2.4 Å).

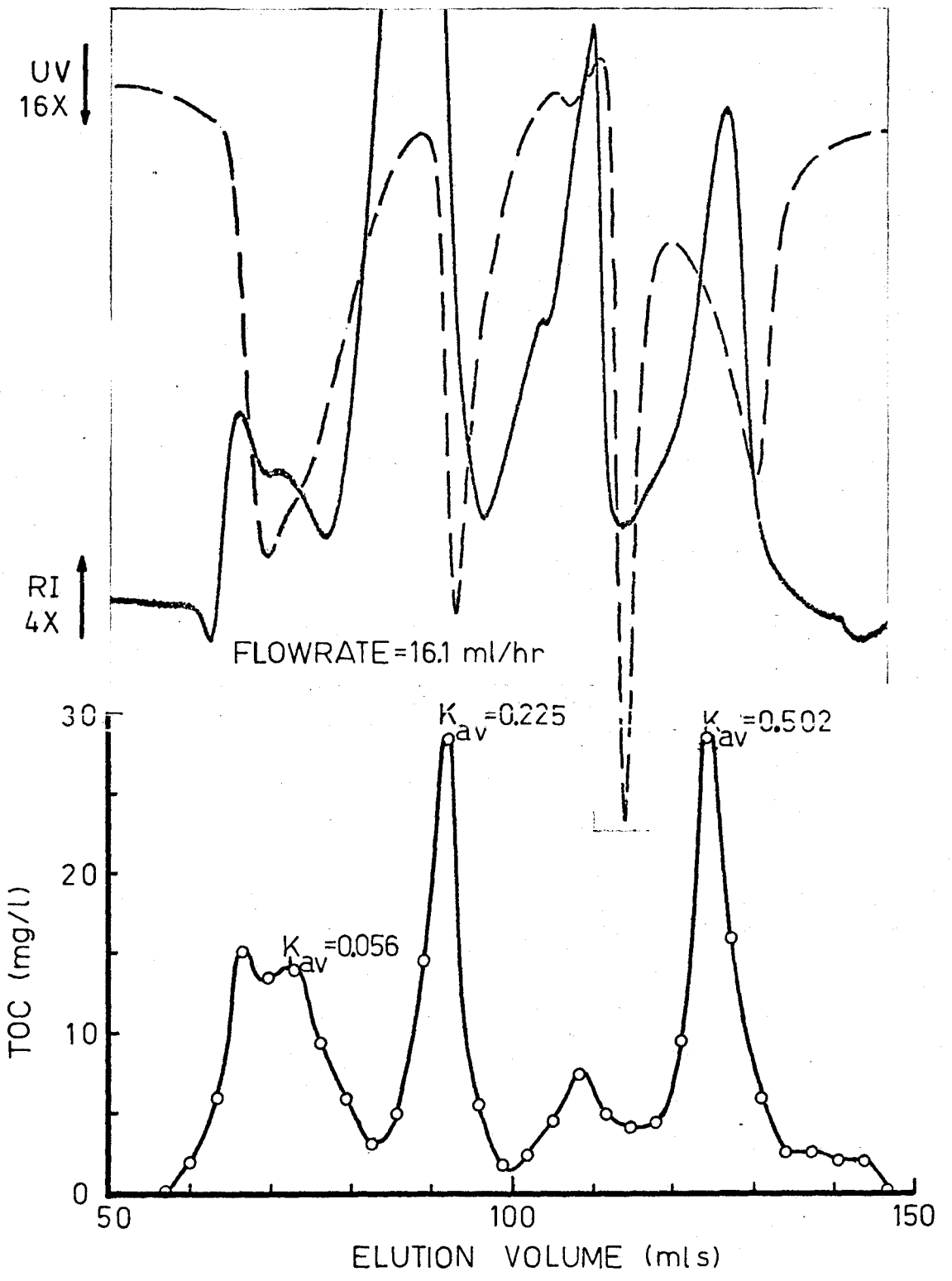


FIGURE 29. G15 Elution Profile of Concentrated Effluent from a Flavobacterium sp. Batch Test Re-innoculated with Pseudomonas sp.

Innoculation of a second portion of the pure Flavobacterium sp. effluent with an unidentified strain of Gram negative rods resulted in reduction of TOC to ~125 mg/l. This reactor was found to contain yeast and Flavobacterium sp. in predominance with some of the unidentified Gram negative rods and a small amount of B. mycoides (tentative identification). The elution profile of this run is very similar to that of the previous one, with the exception of the fraction at K_{av} = 0.056 which is missing in this effluent (Figure 30). The UV spectra of the large peaks at MW ~ 65 in the two mixed culture runs show that the component or components of the peaks are very similar if not identical. They are probably produced by the yeasts since these are the only new organisms in high concentration common to both runs.

The microbial relationship demonstrated here, in which the end-product of the action of one microorganism serves as the substrate for another microorganism occurs extensively in the complex ecology of activated sludge. Further, the somewhat different microbial populations of the two laboratory studies reported, were responsible for differences in the final composition of the effluents. Extending these two concepts to activated sludge where relative microbial populations can differ considerably, it is concluded that the composition of effluents will vary among activated sludge from different sources.

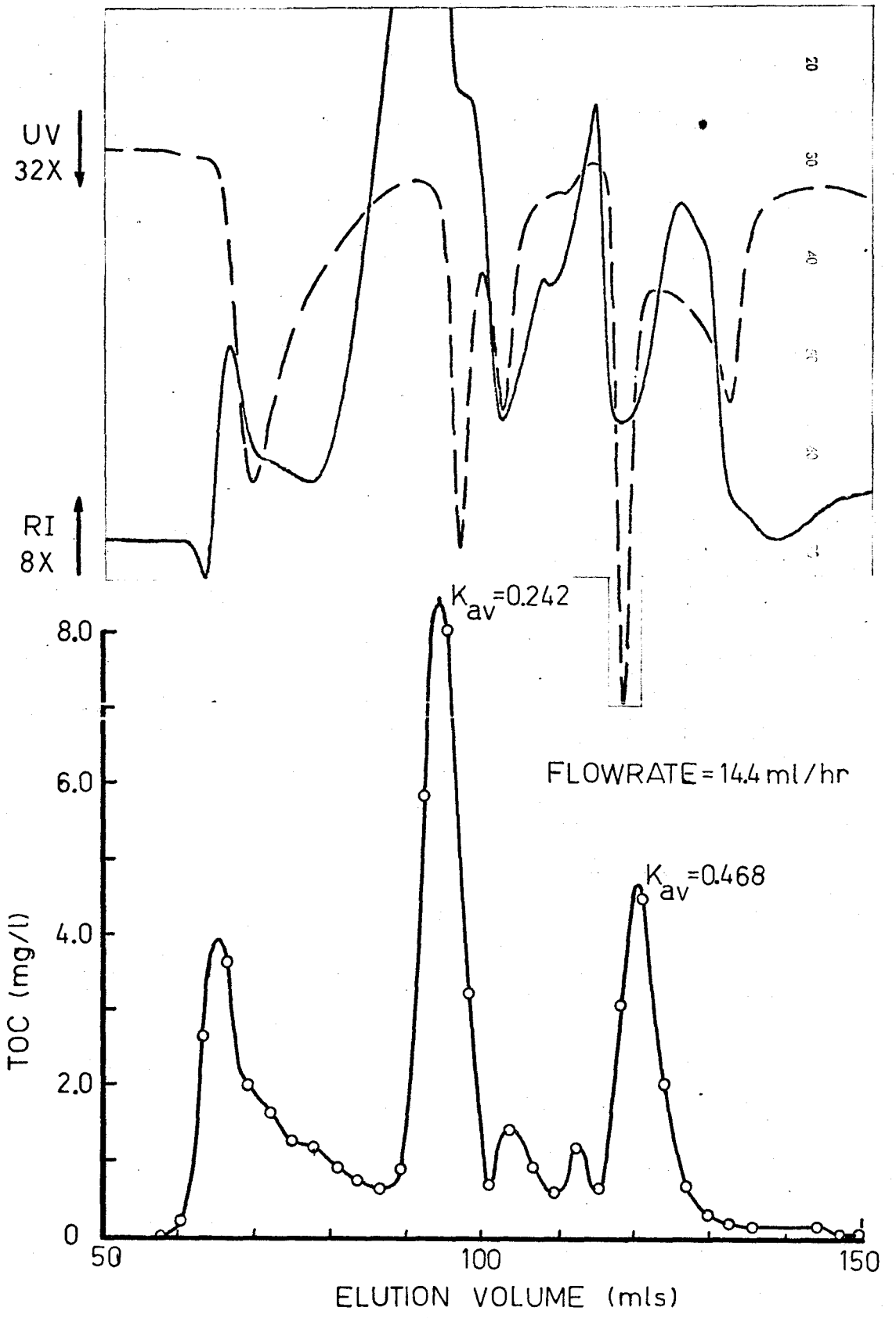


FIGURE 30. G15 Elution Profile of Concentrated Effluent from a Flavobacterium sp. Batch Test Re-innoculated with an Unidentified Strain Gram Negative Rod.

5.4 Treatment Plant Studies

a) Raw Sewage

To provide comparisons for the laboratory batch studies with activated sludge, and in an attempt to resolve the controversy over the equivalent molecular weight distributions of secondary effluents and raw sewage, samples obtained from local sewage treatment plants were analyzed.

Figures 31 and 32 show the elution profiles obtained for raw sewages from the Dundas and Elizabeth Gardens Sewage Treatment Plants. It is immediately obvious that the quantities of TOC to be found at any given equivalent molecular weight or radius differ between sewages. The Elizabeth Gardens plant serves a strictly residential area, while the Dundas plant serves residential, commercial and light industrial areas, which may account for part of the difference. Microbial action in the raw sewage before reaching the treatment plant probably accounts for further changes. Since the sewages are sampled at the treatment plant, different residence times in the sewers will probably result in different distributions. Despite this most obvious difference, both profiles show the presence of carbonaceous matter contrary to the less than 400 and greater than 1200 molecular weight distribution of Zuckerman (1969) and similar to the distribution of Hardt et al (1971). The Dundas raw sewage has major components at equivalent molecular weights (EMW) of ~950 and 790 (equivalent molecular

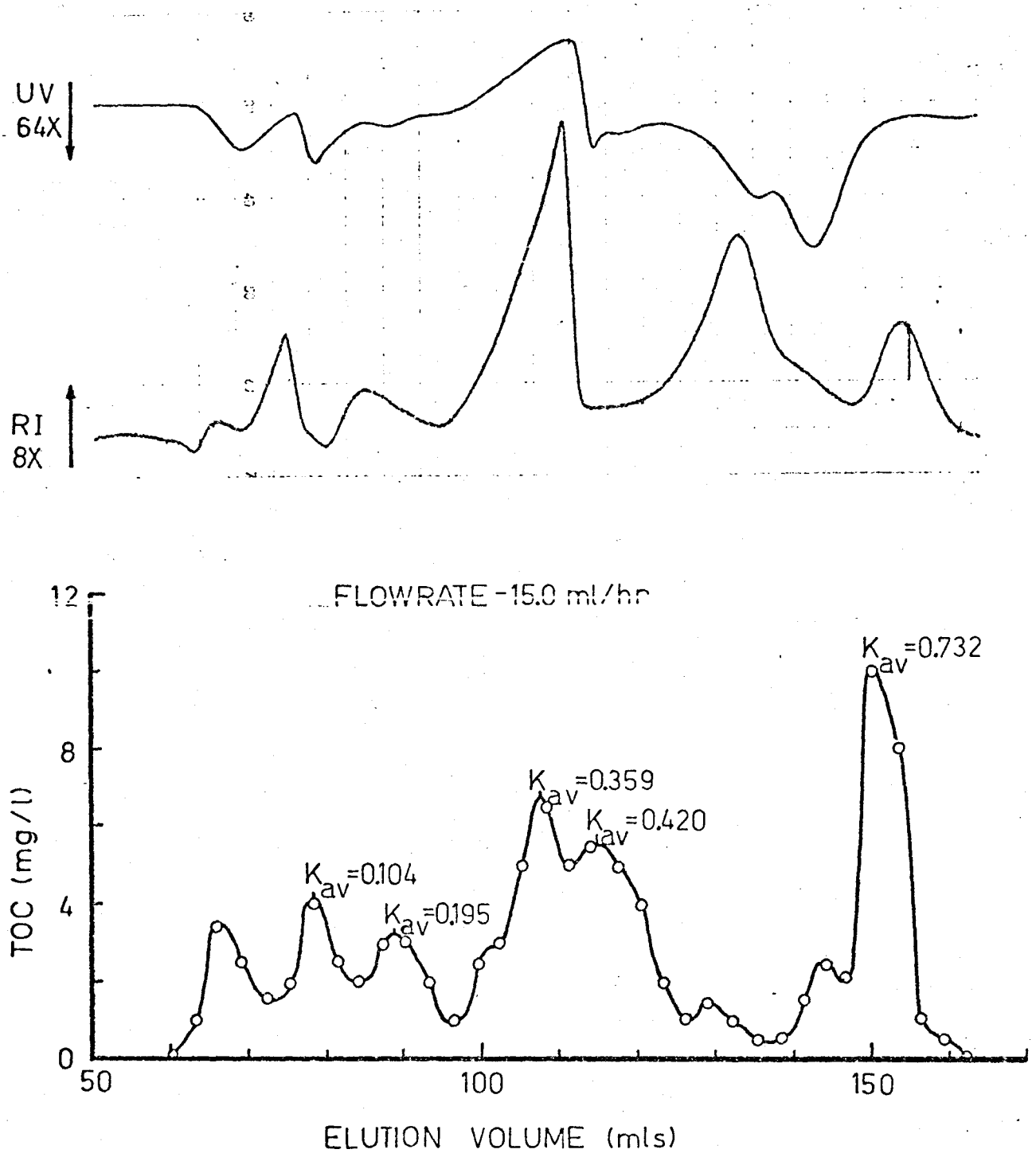


FIGURE 31. G15 Elution Profile of Concentrated Elizabeth Gardens Raw Sewage

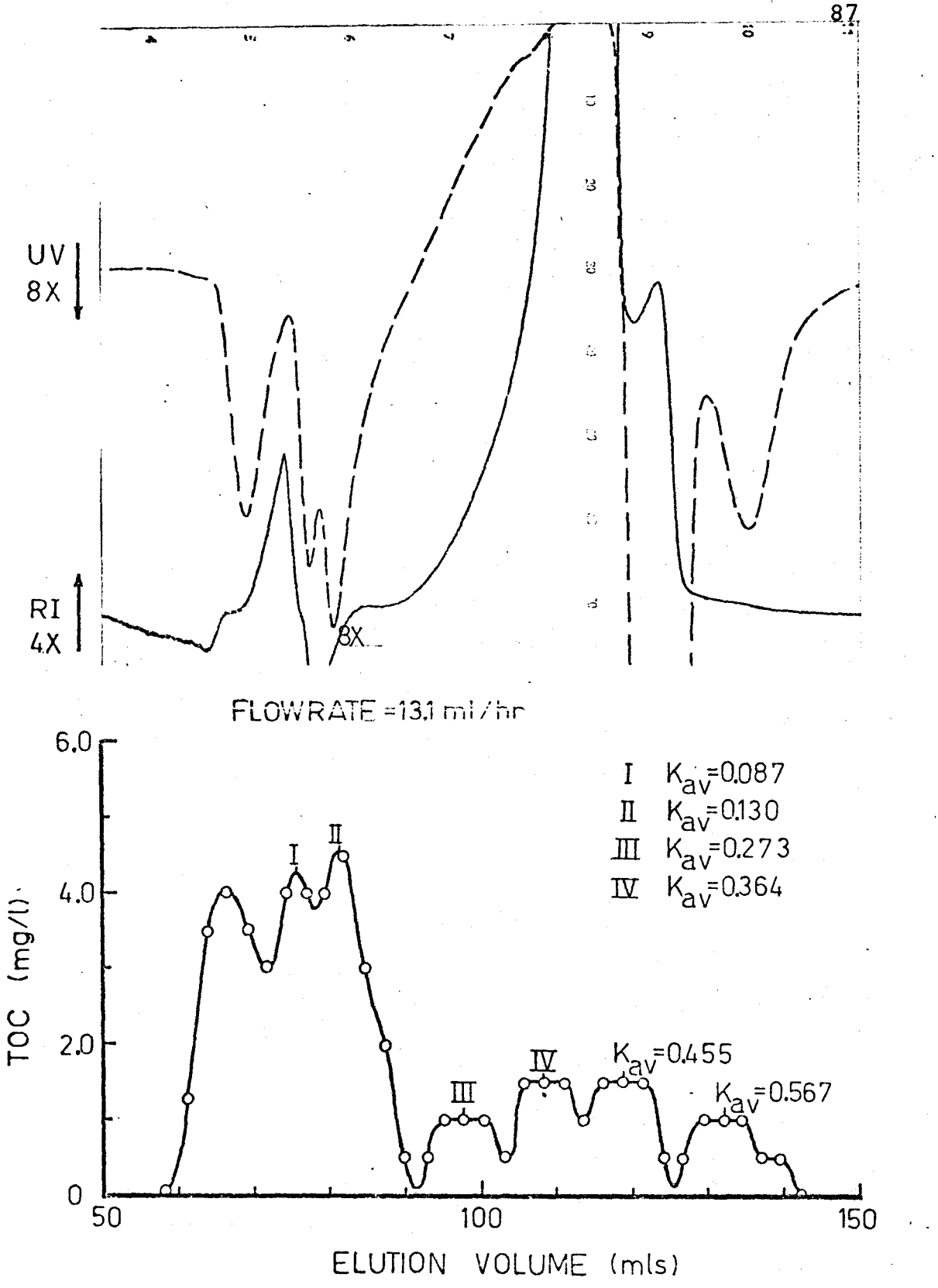


FIGURE 32. G15 Elution Profile of Concentrated Dundas Raw Sewage

radii (EMR)~7.0 Å and 6.7 Å, respectively) while the Elizabeth Gardens sewage contains fractions at ~880 and ~620 EMW (EMR 6.8 Å and 6.0 Å, respectively). The Elizabeth Gardens sewage exhibits a major fraction at K_{av} = 0.732 which is greater than the value for zero radius, indicating some sort of gel-solute interaction in the column. A possible explanation of this phenomenon is postulated by Andrews (1970).

b) Secondary Effluents

The elution profiles of secondary effluents from Dundas and Burlington Skyway shown in Figures 33 and 34 indicate that significant portions of the residual organics in secondary effluents occur at equivalent molecular weights of less than 1200. The Dundas effluent contained fractions with equivalent molecular weights of ~570, ~360, and ~160 (equivalent molecular radii of 5.8 Å, 4.6 Å, and 2.5 Å, respectively), while the Burlington Skyway effluent contained fractions at ~630 and ~530 EMW (equivalent molecular radii of 6.0 Å and 5.5 Å, respectively). This is in agreement with the results of Hardt et al (1971) and contrary to the results of Zuckerman (1970). Interestingly, the Skyway effluent contained fractions that seemed to exhibit gel-solute interactions but which behave differently than the fraction in the Elizabeth Gardens raw sewage. Neither effluent elution profile resembles the other or the raw sewage profiles, indicating that the residuals differ from plant to plant, and

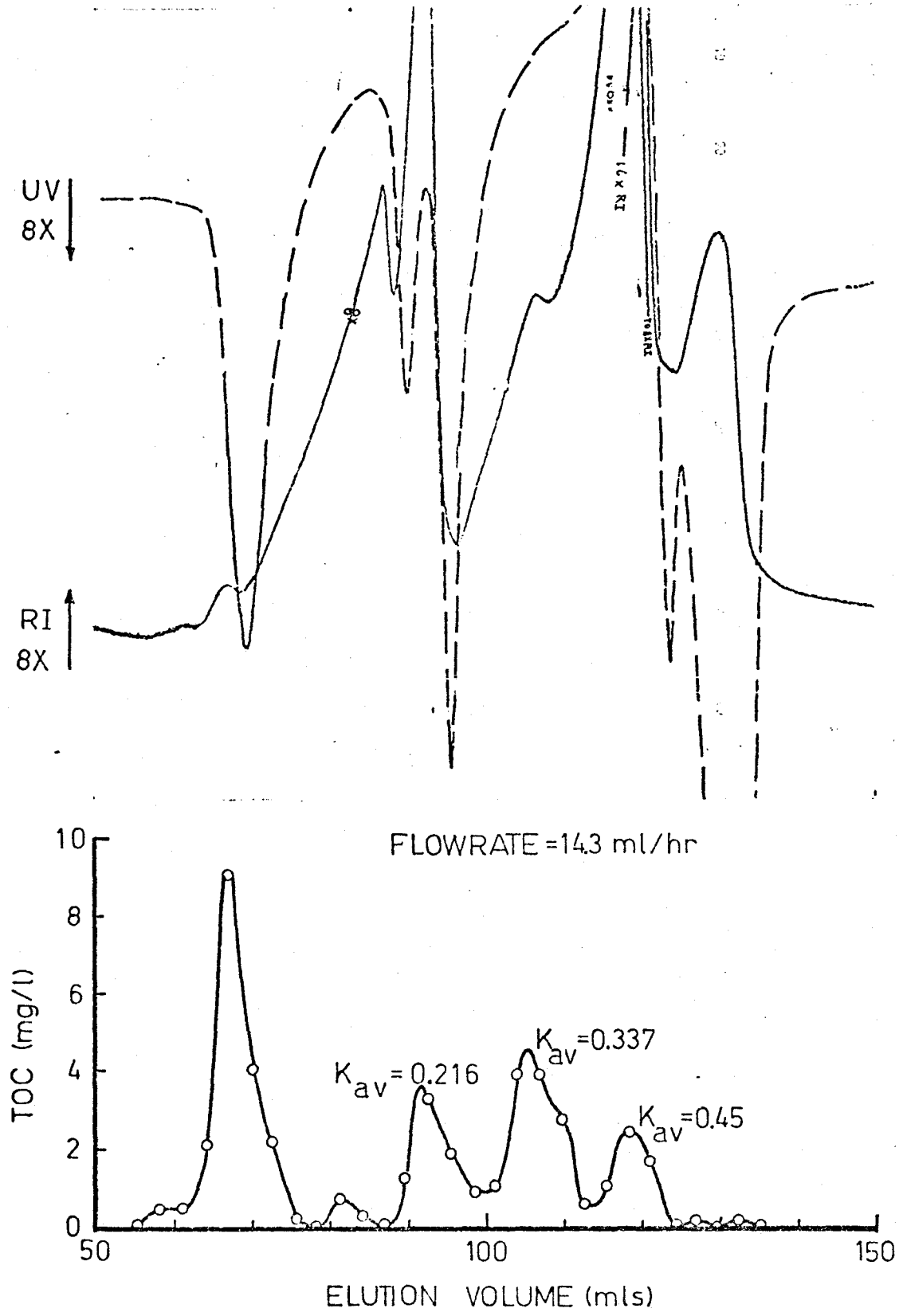


FIGURE 33. G15 Elution Profile of Concentrated Dundas Final Effluent

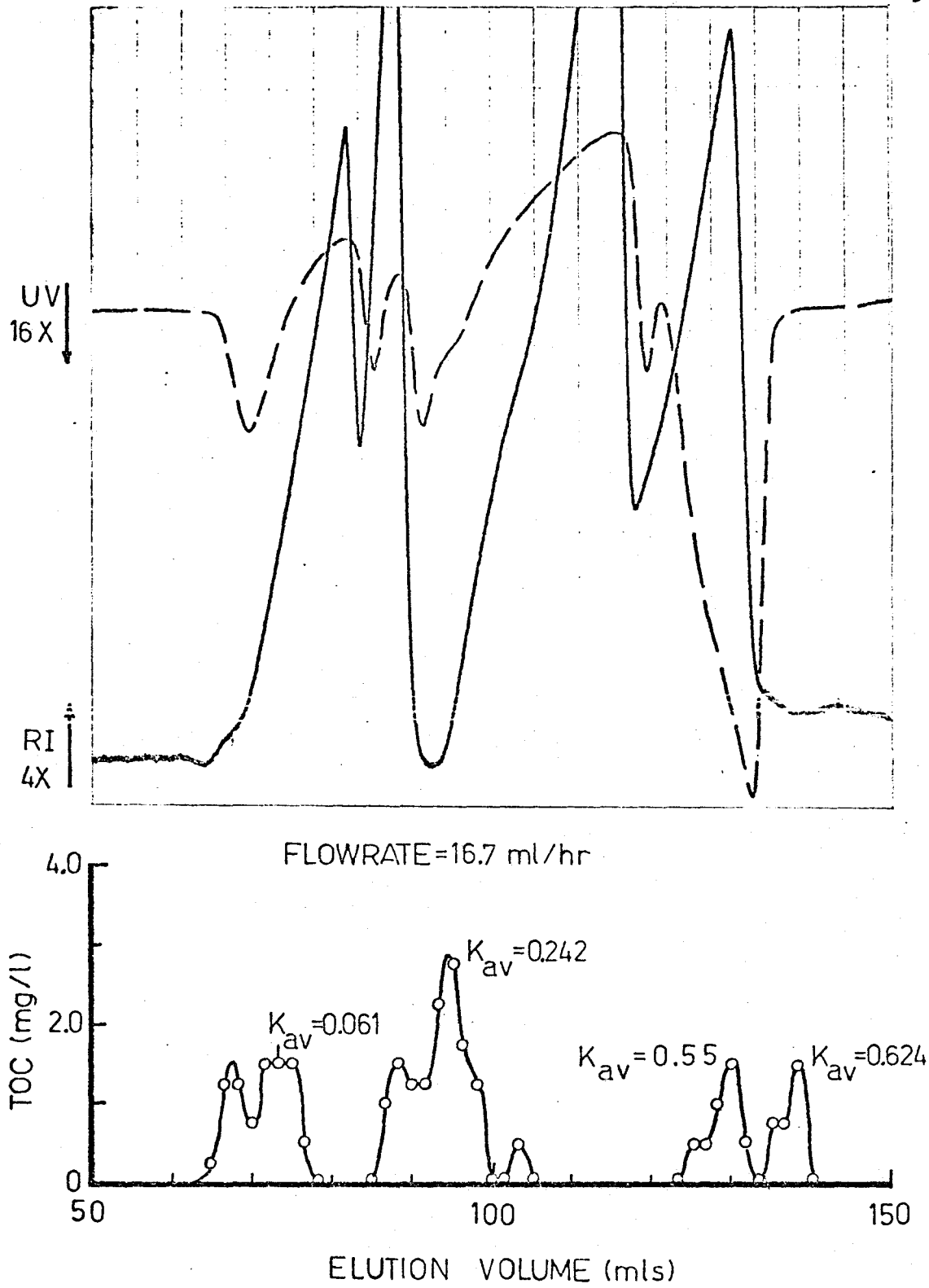


FIGURE 34. G15 Elution Profile of Concentrated Burlington Skyway Final Effluent

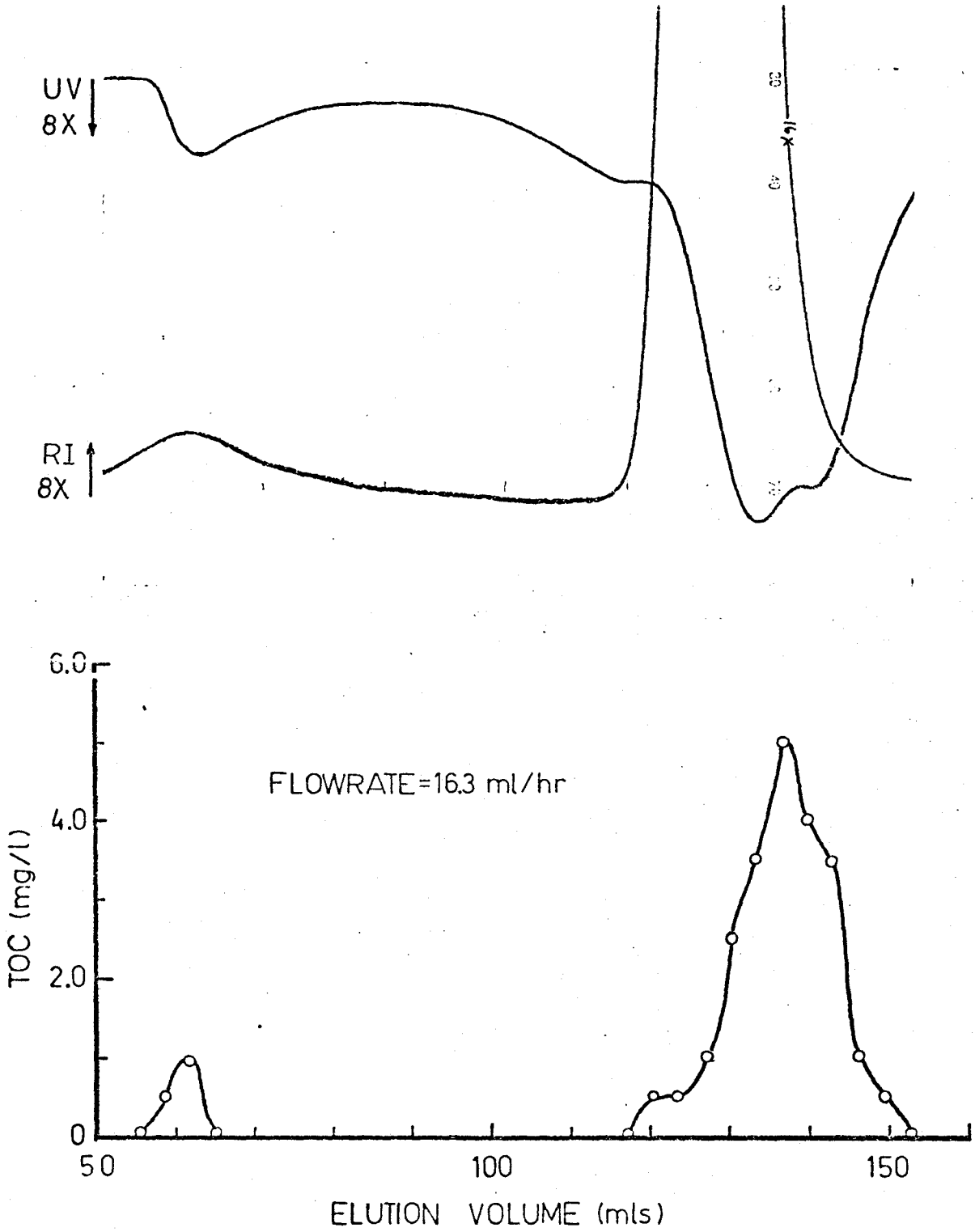


FIGURE 35. Sephadex G50 Elution Profile of Concentrated Dundas Final Effluent

that generalizations regarding their composition should be avoided.

Analysis of the Dundas effluent on G50, Figure 35, (Burlington Skyway effluent was too weak to be reliable) showed only 6% of the TOC recovered from the column to be greater than 10,000 MW (dextran calibration, molecular radius >22.5 A). Recovery of organic carbon from the column was 82% $\pm$ 9%. As shown in Figure 35, negligible TOC was found between the exclusion limit and EMW ~950 (K_d = 0.80), while the remaining TOC appeared at EMW <950. This confirms the results already described for the G15 analysis.

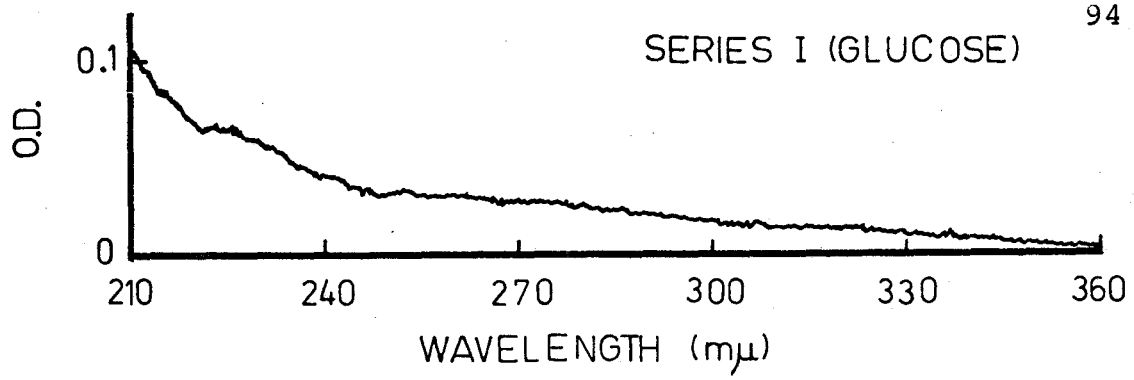
c) Comparison to Laboratory Studies

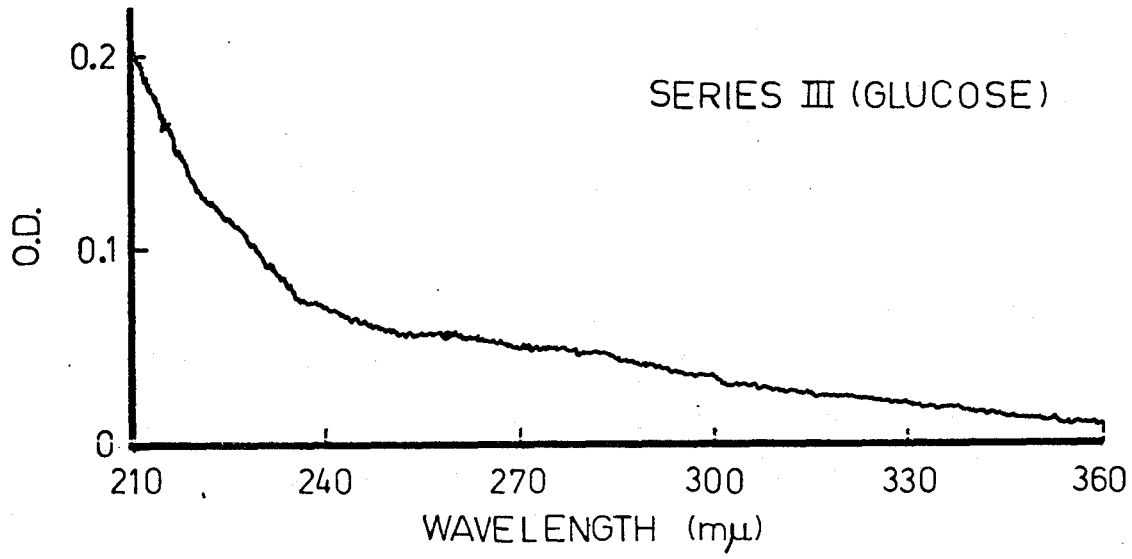
Comparisons of the elution profiles on Sephadex G15 of treatment plant effluents to those from the batch activated sludge tests on pure substrates show few similarities. For treatment plant effluents, the portion of the TOC at the G15 exclusion limit varied from 10% to 40% while in the batch activated sludge tests the fraction varied from 40% to 70% by weight. Between the exclusion limit and K_{av} = 0.55 few similarities are observed, except that peaks overlap to some extent. At K_{av} = 0.55 small amounts of carbon were observed in the Burlington Skyway effluent as well as when activated sludge was grown on glucose (200 mg/l TOC with and without yeast extract) and glutamic acid (1000 mg/l TOC). The inconsistency of this fraction with regard to substrate and concentration makes conclusions difficult.

The appearance of high molecular weight material and a distinct yellow colour in the effluents from batch tests on pure substrates and from actual treatment plants suggest the presence of "humic substances" in the effluents. The molecular weight distributions determined are not inconsistent with those found for humic materials from natural sources. However, because of the dilute nature of the fractions from the chromatographic columns, no estimate of the colour of each fraction was attempted.

5.5 UV Spectra

Ultraviolet spectroscopy was used primarily as a comparative tool for the purpose of indicating similarities between compounds or groups of compounds occurring in various fractions of the elution profiles of samples from different sources. The G15 exclusion limit fractions were compared for the Series I and III batch activated sludge studies and were found similar but not identical (Figure 36). Decreasing from 400 mμ, optical density increased gradually to a broad peak or shoulder centered in the 260-280 mμ range. At about 240 mμ the optical density increased rapidly to a sharp break at 225 mμ and thereafter increased slowly at first and then quite rapidly as wavelength decreased to 200 mμ. A similar, but again not identical, spectrum was reported by Sedivy (1970) for the whole unfractionated effluent from activated sludge grown on glucose. Because of the lack of distinct maxima





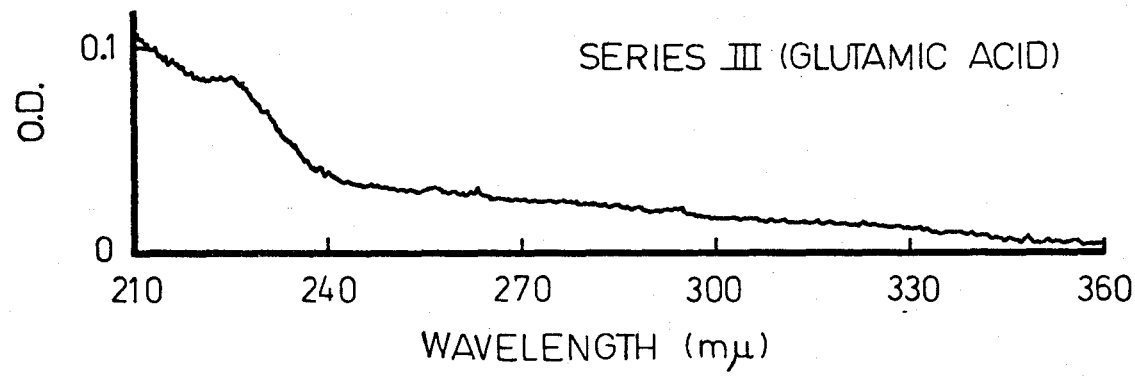
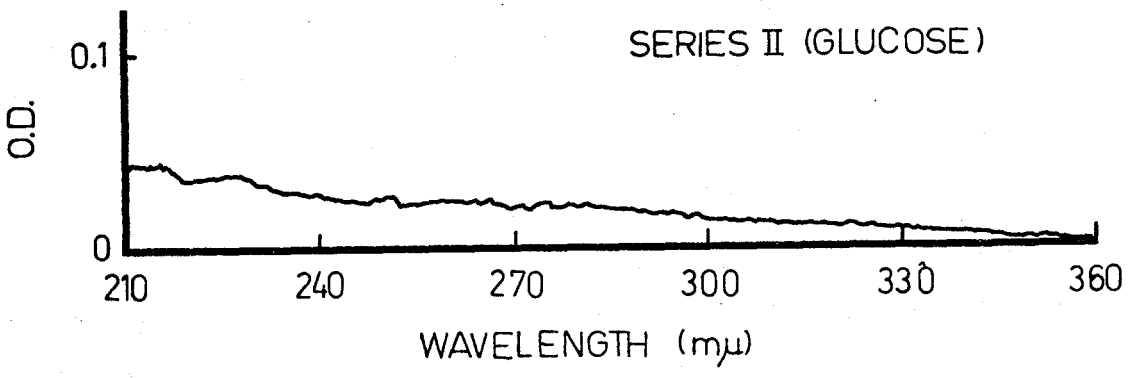


FIGURE 36. Comparison of UV Spectra of G15 Exclusion Limit Fractions from Series I and Series III

in the spectra, it is concluded that a complex mixture of compounds is present in these G15 exclusion limit fractions.

Comparison of the G15 exclusion limit fractions for the Series II (high initial TOC and MLSS concentrations), showed little similarity (Figure 37). When glucose was the substrate only a slight shoulder was observed in the 260-280 mμ range, and the optical density increased only slightly as the wavelength decreased to 200 mμ. This spectrum is quite similar to that from the Series I (glucose) batch test, indicating possible similarities in composition. However, when glutamic acid was the substrate, a definite shoulder centered at 270 mμ was observed, whereafter the optical density increased rapidly as the wavelength decreased to 200 mμ. The rapid increase in optical density as wavelength decreases to 200 mμ is reportedly typical of the yellow organic acids classified as humic substances in natural surface waters (Black and Christman, 1963).

The UV spectra of the G15 exclusion limit fractions from Elizabeth Gardens raw sewage and the Dundas final effluent are shown in Figure 38 for comparison. The broad peak centered at 270-280 mμ in the Series II (glutamic acid) spectrum appeared again in the Dundas final effluent spectrum. This may indicate some similarities in chemical composition.



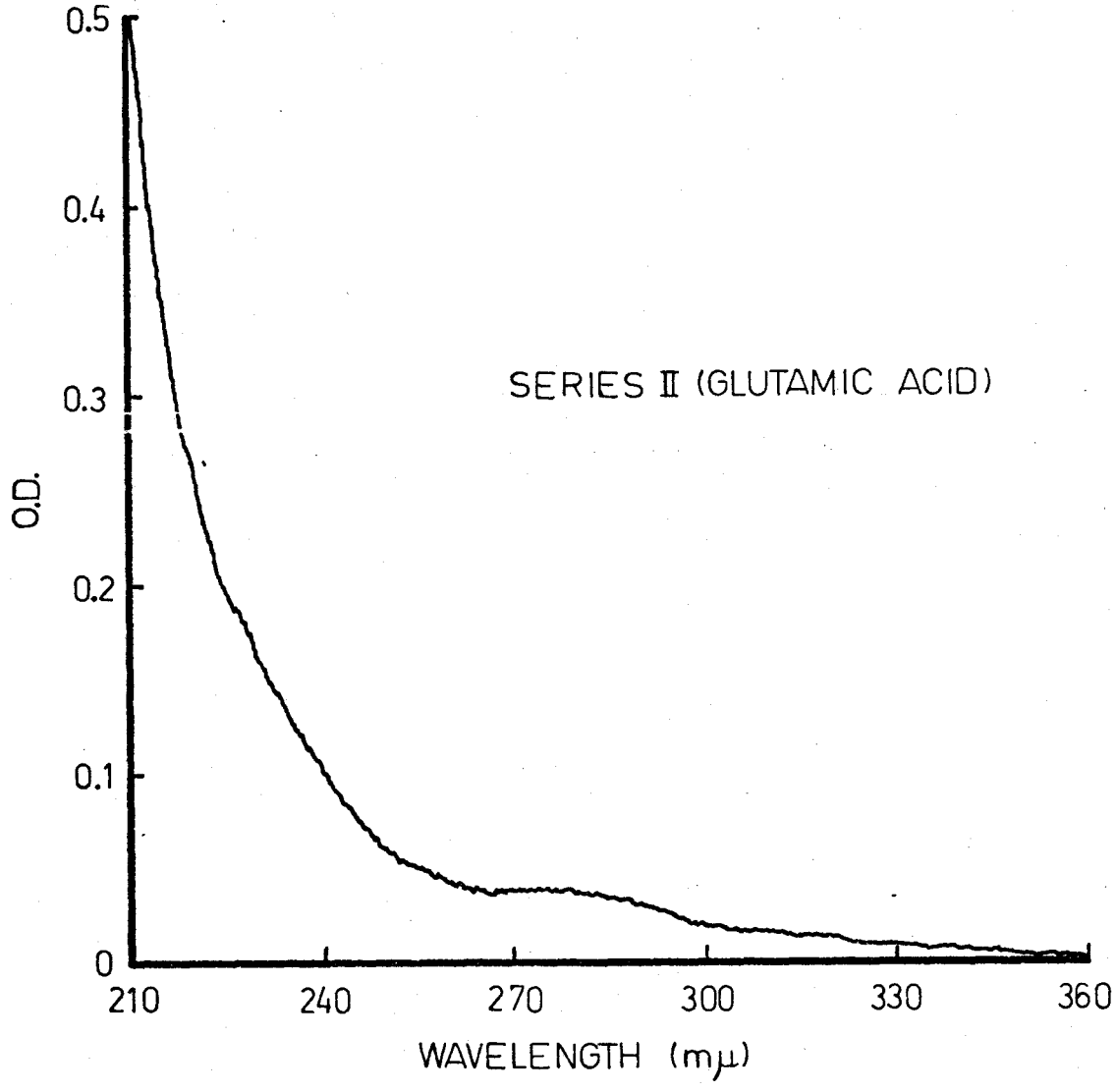


FIGURE 37. Comparison of UV Spectra of G15 Exclusion Limit Fractions from Series II

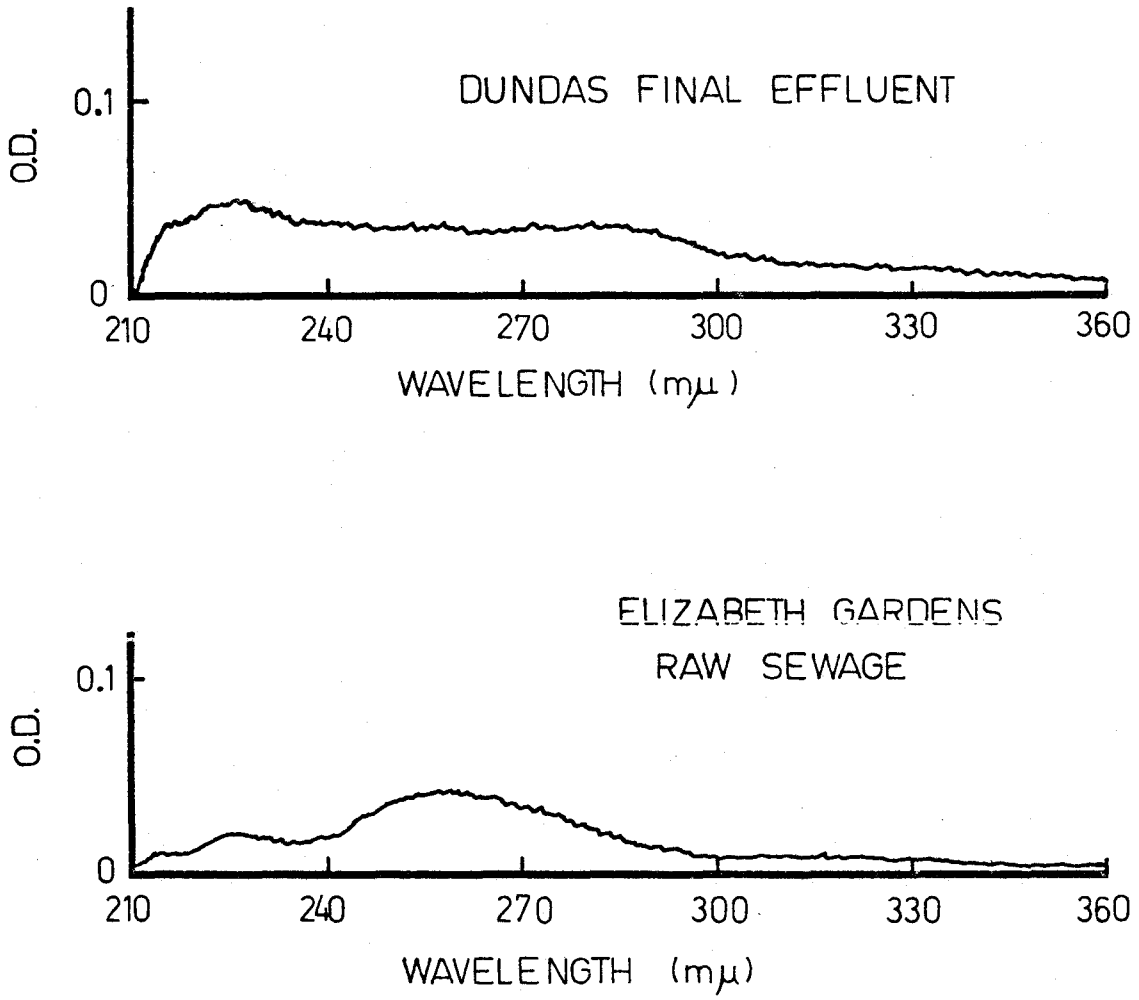


FIGURE 38. Comparison of UV Spectra of G15 Exclusion Limit Fractions from a Raw Sewage and a Final Effluent

On the basis of comparison of the UV spectra, it appears that a wide range of different compounds and not just a few, make up the G15 exclusion limit fractions from the various sources.

CHAPTER 6

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. The usefulness of Sephadex gel filtration for the partial characterization of residual organic material from biological treatment has been reconfirmed. However, the limitations of the method must be kept in mind when interpreting the results. It is for this reason, that the terms "equivalent molecular weight" and "equivalent molecular radius" have been used, based on calibration with a homologous series of sugars and alcohols.

2. Calibration of Sephadex G15 for average molecular radius, based on space-filling models of a homologous series of sugars and alcohols, has been applied to the estimation of residual organics from biological treatment. This method therefore provides approximations of molecular radii of small molecules, for use in designing tertiary treatment processes in which knowledge of molecular size is important to the success of the process. Activated carbon adsorption and membrane processes (i.e., reverse osmosis) are two instances where such information would be of use.

3. Determination of the elution profile of various inorganic salts on Sephadex G15 showed that gel filtration could be used to determine inorganic as well as organic compounds in water. However, in this study the response of the RI and UV detectors to inorganics was considered a nuisance. When the inorganic concentration was high, relative to the organic concentration (as it was in most concentrated effluents) the RI response to organics was masked by the response to inorganic materials. Despite the negative UV response to inorganic compounds, any UV-absorbing compounds which eluted from the column at the same time, showed positive peaks. Thus, the UV monitor was useful in picking out some compounds which were masked in the RI response. The masking problem was serious for the G15 column only below the exclusion limit. When monitoring the G50 column, all the inorganics appeared around $K_d = 1$, so that the RI detector responded solely to organics at $K_d < 0.8$.

4. Both laboratory batch tests and treatment plant studies showed the presence of significant amounts of organic material of low molecular weight (EMW<800) and size (EMR<7 A) as residual organics. This is contrary to the contention of Zuckerman (1969) that organic material of molecular weight greater than

1200 only, remains after biological treatment. Few similarities appeared to exist between the effluents from different treatment plants, which in turn were different from the results obtained in the batch studies. This indicates that complex mixtures of compounds, rather than a few compounds, comprise the residual organics. In addition, raw sewages were found to differ between treatment plants, and contained organic material ranging in equivalent molecular weight from 1500^{+} down to ~60. This is again contrary to the distributions of Zuckerman (1969) in which the organic materials in raw sewage were classified as being greater than 1200 or less than 400. These conclusions are limited to the portion of the residual organics remaining after concentration.

5. Within the limited number of experiments performed, the molecular weight and size distributions appeared to be influenced by substrate type and concentration, and by differences in microbial populations. Organic material of EMW >10,000 is produced by activated sludge even when grown on pure, simple substrates. One pure strain of bacteria was shown to produce material of EMW >1500 when grown on glucose.

6.2 Recommendations

1. Since the UV monitor is specific to UV-absorbing materials only, and since the refractive index detector is sensitive to inorganic compounds which tend to mask the response to organics, especially in freeze-dry concentrated samples, alternative methods of monitoring the organic carbon concentration should be evaluated. Continuous COD has been used (Zuckerman, 1970) but a continuous TOC monitor would be more valuable than the COD method and would be considerably less time-consuming than discrete sampling as used in this study.

2. The usefulness of Sephadex G15 for separation in the low molecular weight range has been demonstrated. However, the method is somewhat limited by the length of time required for each sample run (often up to 12 hours). Pumping of the lower Sephadex gels is known to be practicable, without loss in resolution. It is recommended that pumping of the Sephadex G15 instead of gravity flow be evaluated.

3. Preparative scale gel filtration studies should be carried out to obtain quantities of various molecular weight fractions large enough for elemental analysis (i.e., carbon, total nitrogen, oxygen, hydrogen, and total phosphorus) and spectroscopic analysis. Spot

checks for various chemical groups could also be performed on the fractions if sufficiently concentrated. If large sufficiently concentrated fractions could be obtained, Warburg biodegradability studies could be performed. Very pure distilled water eluant would be required for these preparations. Heavy metal analyses by atomic absorption spectroscopy to determine the effects of metal complexing with the organics could also be performed.

4. Gel filtration studies could be used to monitor the effects of further treatment on the distribution of residual organics. Chlorination, ozonation and activated carbon adsorption are treatment processes which could be easily analyzed by gel filtration.

5. Extensive treatment plant surveys should be performed to monitor changes in the molecular size and weight distributions of raw sewage and final effluents over a period of time.

CHAPTER 7

7. APPREVIATIONS AND SYMBOLS

7.1 Abbreviations

EMR	equivalent molecular radius
EMW	equivalent molecular weight
hr	hour
mg	milligrams
mg/l	milligrams per litre
mls	millilitres
MLSS	mixed liquor suspended solids
No.	number
O.D.	optical density
RI	refractive index
sp.	species
TOC	total organic carbon
UV	ultraviolet

7.2 Symbols

Å	angstrom
mμ	millimicron
μ	micron
%	percent
>	greater than
<	less than
~	approximately
≡	is equivalent to

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APPENDIX A

SAMPLE CARBON BALANCE ON SEPHADEX G15 COLUMN

The following carbon balance was done on the fractions (see Figure 24) from the G15 column for a freeze-dried concentrated sample of the effluent from the Series II glutamic acid run (initial TOC = 1000 mg/l; initial MLSS = 1000 mg/l). The concentration of the concentrated sample as applied to the column was 92 mg/l. The 2 ml sample applied to the column therefore contained 0.184 mg TOC. At a flow-rate of 15.0 ml/hr in the column, with fractions collected at the rate of 5 per hour, each fraction contained 3.0 ml. The TOC of each fraction in mg/l was converted to milligrams of TOC per fraction by multiplying by the volume of each fraction in litres. Recovery of TOC from the column is then quoted as the recovered weight of TOC (mg) as a percent of the applied TOC (mg). The probable error was determined from the equation:

$$E_{sum} = \sqrt{(E_i)^2 n}$$

where E_{sum} is the probable error over all measurements,

E_i is the experimental error, and

n is the number of samples taken.

The experimental error in TOC measurements was $\pm$ 0.5 mg/l for each sample. This was converted to mg. TOC by the method previously described and used in the above formula to calculate

probable error in mg. This was then expressed as a percent of the recovered TOC.

The calculations are shown in Table A-1.

TABLE A-1. Carbon Balance on Sephadex G15 for A Series II (Glutamic Acid) Effluent

Sample TOC: 92 mg/l = 0.184 mgTOC

Flow-rate: 15 ml/hr = 3 ml/sample

Sample No.	Elution Volume (mls)	TOC (mg/l)	TOC (mg x 10^3)
1	59.5	0	0
2	61.5	1.5	4.5
3	65.5	9.5	28.5
4	68.5	20.0	60.0
5	71.5	10.5	31.5
6	74.5	2.5	7.5
7	77.5	1.0	3.0
8-11	80.5-89.5	0	0
12	92.5	1.0	3.0
13	95.5	1.0	3.0
14	98.5	1.0	3.0
15	101.5	2.0	6.0
16	104.5	3.0	9.0
17	107.5	1.5	4.5
18-21	110.5-119.5	0	0
22	122.5	0.5	0.0015
23	125.5	0	0
24	128.5	2.5	7.5
25	131.5	2.0	6.0
26	134.5	1.0	3.0
27	137.	0	0
28	140.5	0	0
			181.5

$$\% \text{ Recovery} = \frac{0.1815}{0.184} \times 100 = 98.5\% \pm 3.4\%$$

$$\text{Probable Error} = \sqrt{(1.5 \times 10^{-3})^2 \times 28}$$

$$= 6.3 \times 10^{-3}$$

$$\equiv 3.4\%$$