THE EFFECT OF LIME TREATMENT ON THE "SOLUBLE" ORGANICS IN DOMESTIC WASTEWATER

THE EFFECT OF LIME TREATMENT ON THE "SOLUBLE" ORGANICS IN DOMESTIC WASTEWATER

by

James Coss

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AUTHOR: James Coss, B. Eng., (McGill University)

SUPERVISOR: Dr. K.L. Murphy

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ABSTRACT:

Gel Chromatography has been used in this study to investigate the effect of lime treatment on pure compounds and wastewater samples. Using pure compounds, it was found that porous glass gel, even after coating with carbowax, is unsuitable because of its adsorptive and ion exchange tendencies. With Sephadex gel, proteins and wastewater samples showed a strong dependence on sample inorganic content. While use of an ionic eluant provided consistent elution behavior with proteins and carbohydrates on Sephadex; best elution behavior with wastewater samples was obtained with distilled water eluant.

Chemical treatment at high pH of pure compounds revealed that proteins and polysaccharides are not hydrolysed. However, ribonucleic acid is hydrolysed, and some proteins and humic acid can be precipitated from solution. Lime treatment of wastewater has indicated that the removal of "soluble" organics is highly pH and time dependent. Up to 40% organic removal was obtained by precipitation or adsorption mechanisms. Gel filtration analysis of these samples indicated that lime

treatment at different pH levels and residence times has a selective effect on organic fractions. Optimum level for turbidity and phosphate removal with lime is roughly pH 11 with greater than 80% removal achieved.

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CHAPTER I

1. INTRODUCTION

Because of its heterogeneous composition, domestic wastewater has been defined by gross parameters such as suspended solids (SS), biological oxygen demand (BOD), chemical oxygen demand (COD), and total organic carbon (TOC). These parameters are used to design and to define the operating efficiency of treatment units. While this approach apparently has worked satisfactorily, more information may be required for the design and evaluation of more advanced systems such as carbon adsorption, membrane processes and ion exchange.

Chemical classification of domestic wastewater has not been definitive. Advanced treatment processes depend mostly on molecular size and molecular properties such as polarity. A classification based on size and physical properties, therefore, seems more reasonable. One method that provides a separation according to molecular size and has been used extensively in the biochemical field is gel permeation chromatography (GPC). With GPC, one obtains an elution spectrum of different molecular weight distributions. It is possible then to compare distribution spectrums before and after treatment.

In the fractionation of organics by GPC, non-steric effects such as solute-gel interactions and ion exclusion can occur with certain classes of compounds. Because of the largely unidentified nature of domestic wastewater, the impact of these non-steric effects as well as the effect of sample inorganic content and eluant composition warranted preliminary investigation. This was studied with pure compounds and subsequently with

wastewater samples.

Chemical treatment with lime has been used successfully to remove phosphates and turbidity from domestic wastewater and color from pulp mill effluents. Recently, the "Z-M Process" has been brought out where it is proposed that the high molecular weight "soluble" organics in sewage are "hydrolysed" to lower molecular weight organics by raising the pH above 12 with lime. The validity of this claim was checked by applying GPC analysis to pure compounds and domestic wastewater samples treated with lime. In addition, turbidity, phosphate, and organic removal as a function of pH and time was used to establish a practical basis for lime treatment.

It is anticipated that the information gained in this study may lead to an appreciation of the usefulness and limitations of GPC for monitoring wastewater effluents, and a more rational design of chemical treatment processes.

CHAPTER 2

2. BACKGROUND AND LITERATURE REVIEW

2.1 Classification of Domestic Wastewater

Analytical problems caused by the complex mixture, low organic concentration, biological availability and temporal variations of organic material in wastewater, make classification difficult (Faust and Hunter, 1971). Several investigators have provided a comprehensive breakdown of soluble and particulate organics and inorganics in wastewater, but the analysis of specific components awaits better analytical techniques.

Rickert and Hunter (1971) classified sewage into settleable, supra-colloidal, colloidal and soluble fractions. The soluble fraction was defined as the supernatant after centrifuging at 62,500 g. The division of total solids was 68% in the soluble fraction and the remainder in the particulate fraction. The soluble solids, unlike the particulate, were found to be largely inorganic. As a result, the ratio of the organic content of particulate to soluble fractions was roughly 3:2.

Painter et al (1961) defined the soluble organics as those which remained in the supernatant after centrifuging at 1200 g for 10 minutes and filtering through a porcelain candle. They were able to identify 80% of these organics as protein and amino acids, carbohydrate, soluble acids and anionic surfactants (Table 1). They suggested that the unidentified portion was probably made up of small concentrations of a large number of compounds.

Hunter and Heukelekian (1965) defined soluble organics as those present in the filtrate from a high pressure membrane filter. They used

TABLE 1. CLASSIFICATION OF SOLUBLE ORGANIC CONSTITUENTS IN ENGLISH DOMESTIC WASTEWATER*

(AFTER PAINTER ET AL., 1961)

CONSTITUENT	PPM CARBON	% OF TOTAL
PROTEIN	8.0	9.5
AMINO ACIDS	5.0	5.9
CARBOHYDRATE	40.0	47.3
SOLUBLE ACIDS	17.0	20.1
ANIONIC SURFACE ACTIVE AGENTS	11.0	13.0
CREATINE	3.5	4.2
TOTAL BY ADDITION	85	
TOTAL BY ANALYSIS	106	
PROPORTION IDENTIFIED,%	80	

* 24 HOUR COMPOSITE SAMPLE.

different analytical procedures than Painter et al and were able to classify as much as 95% of the soluble organics into ether soluble and ether insoluble components (Table 2). Apart from the differences in classification and units, there is general lack of agreement with Painter et al. These authors found that soluble acids were the major component (33%) with carbohydrate representing only 15% of the total while Painter et al found that carbohydrates represented the major component (47%).

Dobbs et al (1972) used ultraviolet absorbance at 254 nm to monitor the organic content of water and wastewater. They obtained a useful correlation between ultraviolet absorbance and total organic carbon (TOC) and concluded that absorbance may be more reliable in the very low TOC range. They also noted that high concentrations of inorganic ultraviolet absorbers or high concentrations of organics which do not absorb in the ultraviolet range will adversely affect the correlation.

Katz et al (1972) using high-resolution ion exchange chromatography compared the ultraviolet peaks of primary effluent, secondary effluent and urine. They found 77 distinct peaks in primary effluent indicating a complex mixture of compounds. Peak components were estimated to be present at concentrations less than 100 µg/l. Analysis of secondary effluent revealed fewer peaks with many of these occurring also in primary effluent.

Robertson (1972), Hardt et al (1971), and Zuckerman (1969) have studied the molecular size distributions of the soluble organics in raw sewage and secondary effluents using gel permeation chromatography.

Helfgott et al (1970) were able to break down the inorganic content of secondary effluent (Table 3). The inorganic content of raw wastewater should be roughly the same as secondary effluent. Notable is the wide range of inorganics from the 22 treatment plants sampled.

TABLE 2. CLASSIFICATION OF SOLUBLE ORGANIC CONSTITUENTS IN AMERICAN DOMESTIC WASTEWATER

(AFTER HUNTER & HEUKELEKIAN, 1965)

CONSTITUENT	WINTER - SPRING, 1959 mg/l *	% OF TOTAL	FALL - WINTER, 1959-60 mg/l *	% OF TOTAL
ETHER SOLUBLE - I				
ACIDS ¢	22.56	32.9	33.95	39.8
BASES	3.24	4.7	3.55	4.2
AMPHOTERICS	4.80	7.0	7.17	8.4
NEUTRALS @	13.59	19.8	17.31	20.4
ETHER SOLUBLE - II				
DETERGENTS (ABS)	3.94	5.7	4.02	4.7
PHENOLS	0.12		0.10	
CHOLESTEROL	0.03	0.7	0.05	0.5
VOLATILE ACIDS	0.34		0.31	
ETHER INSOLUBLE				
URIC ACID	0.33	0.8	0.34	0.6
CREATINE-CREATININE	0.20		0.17	
AMINO ACIDS	9.05	13.2	9.01	10.5
HEXOSES	9.77		8.65	
PENTOSES	0.77	15.3	0.66	10.9
SUM	68.74	100.0	85.29	100.0
VOLATILE SOLIDS	72.19		87.86	
PROPORTION IDENTIFIED, %	95.2		97.0	

* As constituent concentration.

¢ Corrected for detergent and phenol content.

@ Corrected for cholesterol content.

TABLE 3. INORGANIC CONCENTRATIONS IN SECONDARY EFFLUENTS

(AFTER HELFGOTT, HUNTER & RICKERT, 1970)

INORGANICS	RANGE, mg/l *		
	AVG	MIN	MAX
CATIONS			
Na	124	29	232
K	12	9	20
NH4	17	0	44
Ca	66	33	109
Mg	19	Trace	30
ANIONS			
Cl	143	52	532
NO3	12	0.04	26
NO2	1.5	0.26	2
HCO3	296	110	428
CO3	0	0	0
SO4	84	20	300
SiO3	43	22	80
PO4 (TOTAL)	25	5	50
OTHERS			
HARDNESS (AS CaCO3)	235	100	411
ALKALINITY (AS CaCO3)	242	90	351
pH	7.4	6.5	8.0

* DATA OBTAINED FROM 22 TREATMENT PLANTS.

2.2 Mechanism and Theory of Gel Permeation Chromatography

Only a brief explanation of the basic mechanisms and theories underlying gel permeation chromatography (GPC) will be presented. The book by Deuterrman (1969) and articles by Andrews (1970) and Pescsok and Saunders (1966) provide a more thorough explanation. Gel permeation chromatography has also been referred to as gel filtration, gel chromatography and molecular sieving. A number of different gel types exist including polyacrylamide, agarose, silica, cross-linked dextran (Sephadex) and glass. Only the latter two gel types were used in this study. Glass has the advantage of being more rigid than the organic gels or silica and chemically inert (Haller, 1965). But, as Harmon (1970) points out, polar sites on the glass may require deactivation by a liquid modifier. Sephadex gels are subject to lower flowrates and compression with time. They possess some sorption and ionic properties, but these have been fairly well defined.

As with other forms of chromatography, the separation is based on a selective distribution between the mobile and immobile phases. The mobile solvent percolates through the "void volume" (Vo) in the column (Fig. 1). The immobile phase consists of the solvent within the pores (Vi) and the gel itself (Vg). Solutes emerge from the column in order of decreasing size depending on how much they penetrate the pores.

The total volume within a column (Vt) is given as:

$$Vt = Vg + Vo + Vi$$

where, Vg is the volume of the gel

Vo is the volume of the voids
Vi is the volume within the pores

The elution volume of solute (Ve) is:

$$Ve = Vo + Kd\ Vi$$

where, Kd is the distribution coefficient.

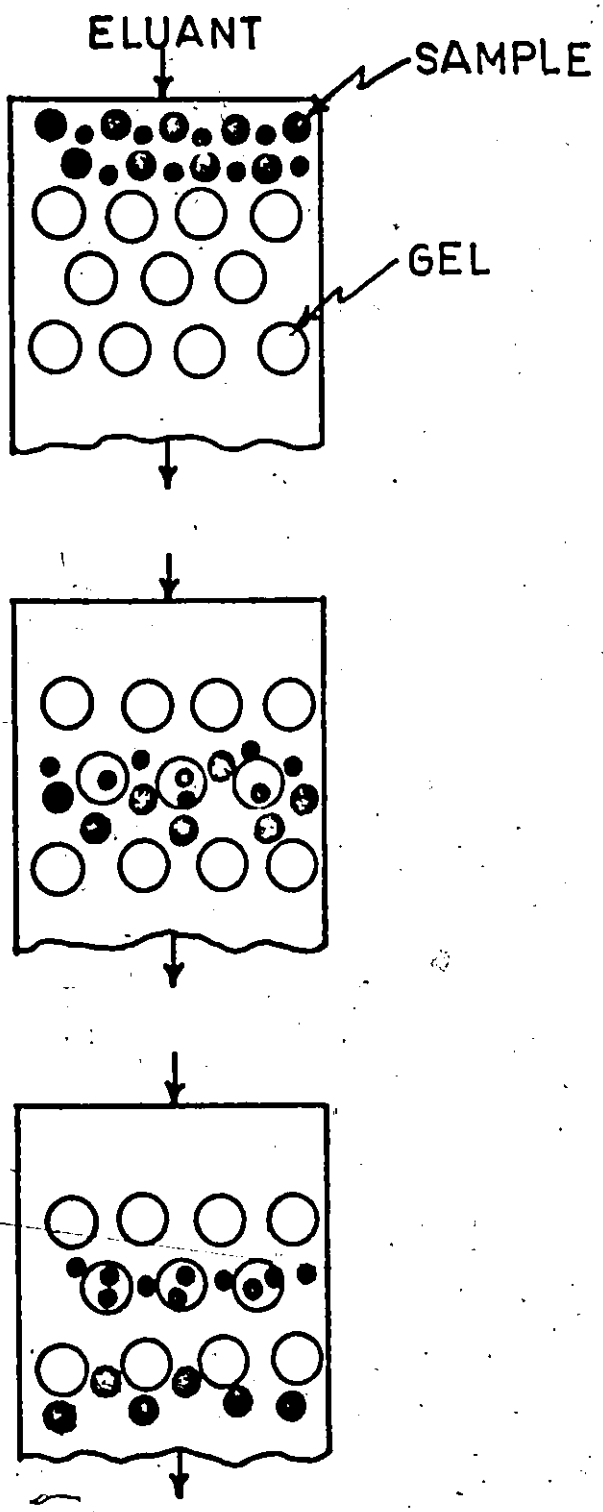


FIGURE 1. SCHEMATIC REPRESENTATION OF GEL CHROMATOGRAPHY

The distribution coefficient, K_d, describes the extent of penetration of solute within the gel. Solutes too large to enter the pores appear in the eluant at a volume equivalent to the void volume ($K_d \equiv 0$). Solutes which can totally penetrate the gel have a K_d of 1. Actual values are closer to 0.8 or 0.9 because of a tightly adsorbed layer of water in the gel surface which is unavailable. Solutes which can penetrate only a fraction of the gel interior will have $0<K_d<1$.

Because of the difficulty in computing Vi based on the water regain of the dry gel, a somewhat different distribution coefficient, Kav, (Laurent and Killander, 1964) is often used instead of K_d.

$$K_{AV} = \frac{Ve - Vo}{Vt - Vo} = K_d \frac{Vi}{Vi + Vg}$$

Pescsok and Saunders (1966) describe a number of the theories that have been proposed to explain gel filtration. They point out that no one has attempted a general equation to predict the elution volume from the characteristic properties of the molecules. Such an expression would be very cumbersome due to many mechanisms involved:

1. sieving,
2. restricted diffusion,
3. adsorption, and
4. the nature of the gel and partitioning.

Andrews (1970) also states that experimental results are largely inadequate to determine the relative merit of different theories and because of uncertainties involved, the method is essentially one of "estimation" rather than "determination" of molecular weights.

Several of these theories are summarized in Table 4. In view

TABLE 4. SUMMARY OF GPC THEORIES

INVESTIGATOR	ASSUMPTIONS	CORRELATION	NOTABLE POINTS
PORATH, 1963	INTERSTICIES ARE HOLLOW CONES $R \propto M^{1/2}$	$K_d^{1/3}$ and $M^{1/2}$	
SQUIRE, 1964	INTERSTICIES HAVE ARBITRARY DISTRIBUTION OF SHAPES $R \propto M^{1/3}$	$\left(\frac{Ve}{Vo}\right)^{1/3}$ and $M^{1/3}$	
LAURENT AND KILLANDER, 1964	MEDIUM AS SYSTEM OF ∞ LONG RODS	$-\log (Kav)^{1/2}$ and R	
ACKERS, 1964	INTERSTICES AS UNIFORM CYLINDERS	USED RENKIN EQUATION $K_d = 1-\left(\frac{r}{A}\right)^2 \left[1-2.104\frac{R}{A} + 2.09\left(\frac{R}{A}\right)^2 - 0.95\left(\frac{R}{A}\right)^3\right]$	TRIED TO ACCOUNT FOR DIFFUSION WITHIN PORES BUT GOT NO DEPENDENCE ON FLOW AND RATE WHICH SHOULD EXIST IF DIFFUSION IS OPERATIVE.
ANDERSON AND STODDART, 1966	REPLOTTED ACKERS' DATA	K_d and log M	SIMILAR PLOTS HAVE BEEN IN COMMON USE.

A = Pore radius.
R = Stokes' radius of molecule.
M = Molecular weight.

of the above considerations, the most commonly used correlation, log (molecular weight) vs. K_{AV}, has been adopted for this study. Keeping in mind that the separation is based on molecular size rather than molecular weight, many authors have substituted for molecular weight effective chain lengths. (Hendrickson, 1968), molar volume (Cazes and Gaskill, 1967), and unhydrated radius based on CPK models (Goodson et al, 1971). Because the molecular shape and chemical composition of the "soluble" organics in wastewater is unknown, an estimation based on molecular weight using a homologous series of proteins and sugars for calibration should prove adequate.

2.3 Solute-Gel Interactions

The theoretical basis of GPC is steric hindrance to diffusion. Non-steric solute-gel interactions are often difficult to recognize unless the size and shape of the molecules are known or can be checked by other means. Many authors have looked into non-steric effects in well defined systems. Choice of operating conditions and definition of non-steric effects with heterogeneous samples such as natural water and wastewater samples is considerably more difficult.

Ion exclusion, Donnan equilibrium and reversible adsorption can result in molecules eluting earlier or later than normal. These effects have been noticed with molecules that are charged or possess sites that are subject to adsorption. They can sometimes be detected by manipulating the ionic strength and pH of the eluant. A K_D value greater than 1.0 is evidence of reversible adsorption. Irreversible adsorption on the gel may also occur and can be determined by measuring recovery of solute from the column.

Concern over adsorptive effects depends on the aim of the fractionation. If one is interested in molecular weight estimation and changes in sample composition or eluant composition alter the degree of solute-gel interaction, molecular weight interpretation is hindered. If one is interested in separation of peaks, possibly for further analysis, then sorption may be beneficial. The exact mechanism of adsorption is often difficult to distinguish due to superimposed effects such as ionic strength and pH.

Ionic effects with Sephadex are believed to be caused by 10-30 μ eq/g of fixed COOH groups in the gel matrix (Andrews, 1970). This causes an exclusion of anions and retention of cations and is most pronounced with the lower porosity gels (De Bersaques, 1967). These electrostatic effects can be overcome by using an eluant with a certain ionic strength depending on how basic the molecules are. Electrostatic effects can also be eliminated by lowering the pH so that ionization of COOH groups is repressed (Eaker and Porath, 1967). However, changes in ionic strength can alter the size and shape of flexible molecules (Skalka, 1965; Brunngraber and Whitney, 1968; Ghassemi and Christman, 1968) and degree of hydration (Eaker and Porath, 1967). A number of authors (Eaker and Porath, 1967; Stenlund, 1970; Lindqvist, 1967) have also demonstrated that use of an ionic background can increase tendency for adsorption since more of the gel is accessible to the solute. Ghassemi and Christman (1968) in working with natural water samples found that organic color was reversibly adsorbed to the Sephadex gel under acidic conditions. Eaker and Porath (1967) note that pH variations can occur in the column if the sample contains buffer salts not present in the eluant.

The GPC behavior of inorganic salts has been investigated by Neddermeyer and Rogers (1968) and Pescsok and Saunders (1968). The former concluded that the Sephadex gel filtration of inorganic salts did not fit a mechanism based on formula weights, but that solute diffusion was probably still the dominant mechanism in the filtration. They showed that ionic interactions and Donnan exclusion could be eliminated by the presence of sufficient electrolyte in the eluant. Pescsok and Saunders demonstrated that even a trace quantity of ionic groups in some gels can have a dramatic effect on elution and resolution. In contrast to Neddermeyer and Rogers they found that sorption rather than diffusion or steric effects mainly determined the separation.

An extensive investigation of Donnan effects using samples of lignosulfonates was carried out by Stenlund (1970). He showed that the polyelectrolyte properties of the non-diffusable high molecular weight lignosulfonates increase the retention of diffusible lignosulfonate fractions. While these effects can be suppressed by using an ionic background, better separation was obtained with distilled water. He concluded that Donnan retardation effects influence the fractionation more than steric effects.

Most authors in talking about adsorption do not distinguish between reversible and irreversible adsorption. Thus, adsorption effects will be presented in a general sense. Pescsok and Saunders (1966) reason that since adsorption of solvent molecules cause the gels to swell, it seems likely that many types of solute molecules will be adsorbed as well. Gelotte (1960) was one of the first to look into sorptive effects with Sephadex gel. He noticed that a slight adsorption of aromatic and hetero-

cyclic substances occurred. Depending on the ionic strength and pH, a strong adsorption of some basic substances and a negative sorption of some acidic substances could occur. He felt that the latter effects could be eliminated by adding electrolyte to the sample or the eluant. Eaker and Porath (1967) have found that the peptide bond, aromatic ring and compounds such as urea are suspectible to adsorption on Sephadex. Jansen (1967) also noted the striking affinity of Sephadex for aromatics.

The problem with characterizing the molecular weight distribution of humic acid, which could also exist with wastewater samples, is that it is not only polydisperse but is also known to contain aromatic, heterocyclic, phenolic and charged compounds which are subject to solute-gel interactions.

Lindqvist (1967) in his investigation of adsorptive effects with humic acid concludes that the presumably polyaromatic and certainly polyelectrolytic character of this organic matter leads to exclusion of the lower molecular weight fractions with decreasing ionic strength and adsorption at higher ionic strengths of eluant. Swift and Posner (1971) also concluded that non-steric effects cannot be eliminated with humic acid. Lindqvist (1967) disagrees with Posner's (1963) recommendation that fractionation of humic acid should be done using distilled water eluant with addition of electrolyte to the sample. Addie et al (1973) using distilled water eluant showed that changes in the electrolyte composition of a wastewater sample can strongly influence the elution positions of certain fractions. Hall (1970) was aware of possible non-steric effects with humic acid, but decided to adopt Posner's method because it resulted in better separation. Brogden (1971) used ultracentri-

fugation to check the molecular weight fractions of humic acid run on Sephadex with O.IN NaCl eluant. He found good agreement and concluded that non-steric effects are negligible under these conditions. The validity of Brogden's conclusion is somewhat questionable in the light of the findings of Cameron et al (1972), that the polydispersity of the humic acid peaks, remaining after gel filtration, and worsened by gel-solute interactions, required additional refractionation and ultrafiltration before ultracentrifugation could be applied.

Stevenson (1968) found that the protein immunoglobulin, could be strongly bound to dextran gel. He suggested passing a large quantity of protein through the column initially to saturate binding sites on the gel. Andrews (1970) argues that protein-gel interactions are not a serious source of error since they are either eastly recognized or avoided by an appropriate choice of conditions. Some doubt that protein-gel interactions can be completely eliminated comes from the statement in the book by Hair (1971) that proteins are more or less universally adsorbed to all surfaces; and, due to their amphoteric and hydrophobic character, may be able to show apparently similar adsorption behavior on either charged or uncharged surfaces.

The non-steric effects described make the choice of proper operating conditions and interpretation of results difficult. Table 5 provides a summary of eluants used by a number of workers and their comments on some of the effects noticed. The list is by no means complete, but includes many of the investigators discussed in this review. Their comments, in general, reaffirm the fact that a background electrolyte provides better estimation of molecular weight but often poorer resolution. There is no

TABLE 5. OPERATING CONDITIONS USED IN GPC BY VARIOUS INVESTIGATORS

AUTHOR	SAMPLE	GEL	ELUANT	AUTHOR'S COMMENTS
ANDREWS, 1970	Proteins + Carbohydrates	Sephadex	.05M Tris-HCl buffer pH 7.5 + 0.1M KCl	
HALLER, 1965	Virus + Protein in saline buffer	glass beads	.01M PO_4 buffer pH 7.0 + 0.85% NaCl	
DeBERSAQUES, 1967	Neucleic acid components	Sephadex	0.13M amm. formate pH 6.0	Increased adsorption with tighter gels.
BRUNNGRABER and WHITNEY, 1968	Sialomucopoly-saccharide	Sephadex	Distilled water and 0.05M Tris-HCl, pH 7.5 + 0.1 M KCl	With background ionic strength got poor separation.
SKALKA, 1965	Heparin + 0.14M NaCl	Sephadex	Phosphate buffer of varying concentration	Elution volume increased with increasing ionic strength.
STEVENSON, 1968	Human Immuno-globulin G	Sephadex	4mM NaAc buffer, pH 5.4	Strong binding of protein. Adsorption sites can be saturated
STENLUND, 1970	Lignosulfonates	Sephadex	Distilled water, $CaCl_2$ and LiCl in varying concentration	Distilled water gave better resolution but electrolyte gives better estimate of molecular weight distribution.
EAKER and PORATH, 1967	Amino acids and other pure compounds	Sephadex	9 eluants of varying composition, ionic strength and pH	Cation exchange capacity of gel reduced by washing with pyridine. Addition of salt increases adsorption.

TABLE 5 - CONTINUED

AUTHOR	SAMPLE	GEL	ELUANT	AUTHOR'S COMMENTS
POSNER, 1963	Ammonium humate	Sephadex	Distilled water. 0.05N NH_4OH	Elution profile is constant if electrolyte concentration of sample is between 0.1 and 0.4N and distilled water eluant is used.
LINDQVIST, 1967	Humic acid	Sephadex	Distilled water. 0.05M NaCl	Must minimize ionic gradients during separation.
BRODSKY et al, 1970	Humic acid	Sephadex	.01M NaCl 0.1M NaCl 0.5M NH_4OH 0.01M $NaHCO_3$	Used 0.01 M NaCl since closest to actual conditions.
HALL, 1970	Natural water	Sephadex	Distilled water	Use of distilled water and adjustment of sample ionic strength gave best separation.
GJESSING and LEE, 1967	Natural water	Sephadex	Distilled water	
GHASSEMI and CHRISTMAN, 1968	Natural water	Sephadex	Various buffers and pH	Color can be reversibly adsorbed on the gel. Elution volume decreases with increasing pH.
ROBERTSON, 1972	Wastewater	Sephadex	.005M KH_2PO_4 + Na_2HPO_4 PH 6.9	

agreement on what is the best electrolyte to use. An interesting discovery was that of Baker and Porath (1967) who found that the cation exchange capacity of Sephadex could be nearly eliminated by washing the gel with pyridine.

2.4 Gel Filtration Analysis of Domestic Wastewater

Zuckerman (1969) analysed the soluble organic material in raw sewage and secondary effluent before and after lime treatment and carbon adsorption. He used Sephadex G-10 and monitored the gel column effluent by differential refractive index (RI) and chemical oxygen demand (COD). His main findings were:

1. the soluble organics in raw sewage are divided into two fractions, one with apparent molecular weight > 1200 and the other with apparent molecular weight < 400,
2. secondary effluent consists of material larger than 1200 apparent molecular weight only, and
3. by high lime dosage the high molecular weight fraction can be broken down to less than 400 apparent molecular weight (Fig. 2).

Addie et al (1973) pointed out that the RI and COD measurements used could be adversely affected by inorganics.

Hardt et al (1971) looked at the molecular weight distribution of the soluble organics in both treated and untreated wastewater using Sephadex G-10 and G-15. Fractions were analysed by total organic carbon (TOC). Their results contradicted Zuckerman on a number of points:

1. both treated and untreated wastewater yielded fractions between 1200 and 400 apparent molecular weight (A.M.W.),

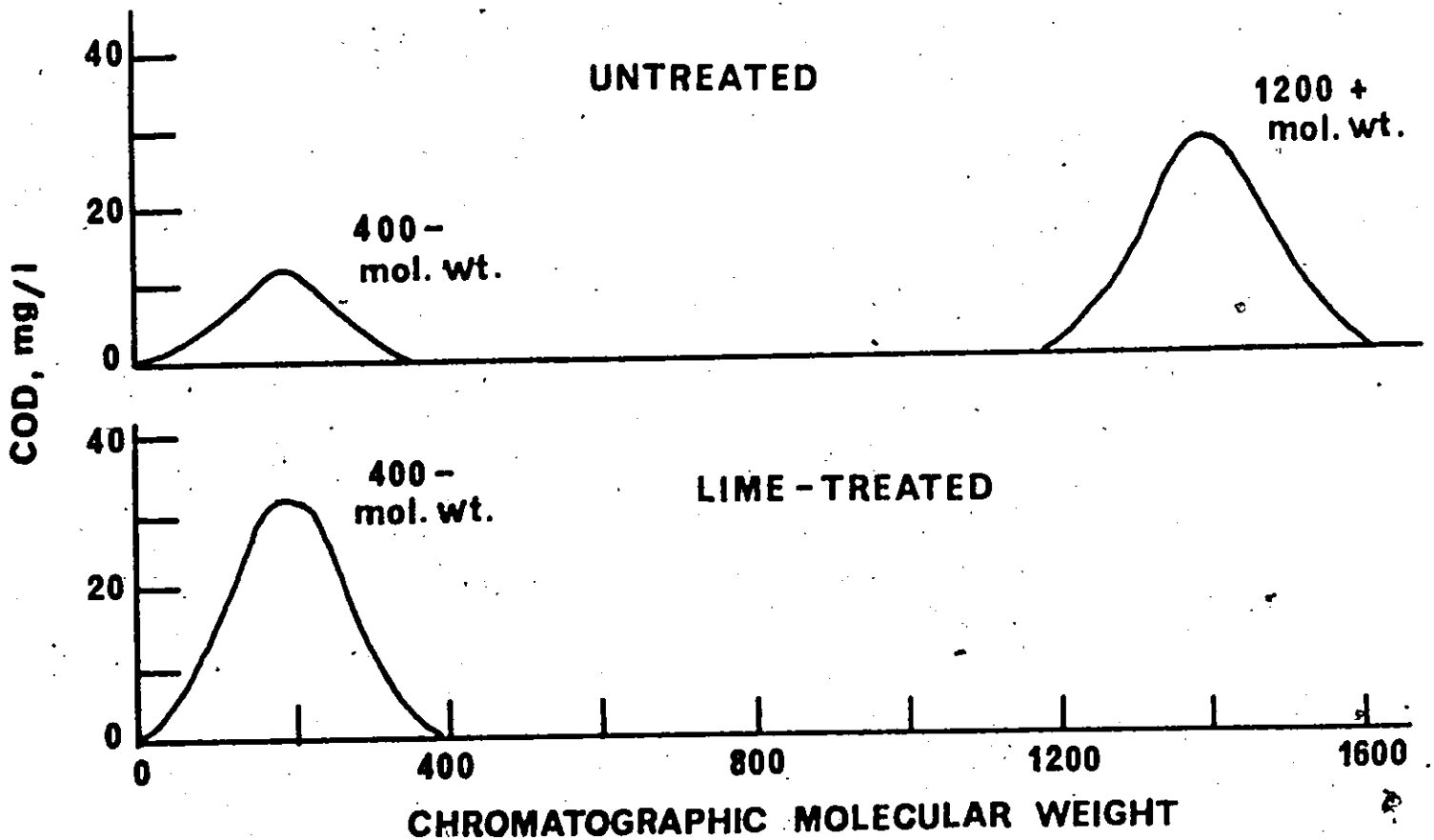


FIGURE 2. THE 1-1200 MOLECULAR WEIGHT DISTRIBUTION OF UNTREATED AND CHEMICAL TREATED WARDS ISLAND RAW WASTEWATER (Zuckerman, 1969)

2. the majority of the organics in untreated wastewater had A.M.W. < 400 with less than 10% having A.M.W. > 1200, and

3. 76% of the organics in secondary effluent had A.M.W. < 1200.

Robertson (1972), using ultraviolet (UV) absorbance, RI and TOC for measurement, also looked at the molecular weight distributions of the soluble organics in raw and secondary effluents. He found that UV correlated well with TOC while the RI response to organics was masked considerably by inorganics in the sample. Again, contrary to Zuckerman he found that raw and secondary effluents contained organic material ranging from 1500+ down to approximately 60 A.M.W.

A comparison of the apparent molecular weight distributions of the soluble organics in untreated domestic wastewater obtained by these investigators is presented in Figs. 2,3,4, and 5. Both Zuckerman and Hardt et al do not state explicitly what eluants they used so that the results may not be strictly comparable.

It is surprising that none of these investigators have questioned whether GPC fractionation of a mixed system such as domestic wastewater does, in fact, provide a valid separation according to molecular weight.

2.5 Gel Filtration Analysis of Natural Water and Humic Material

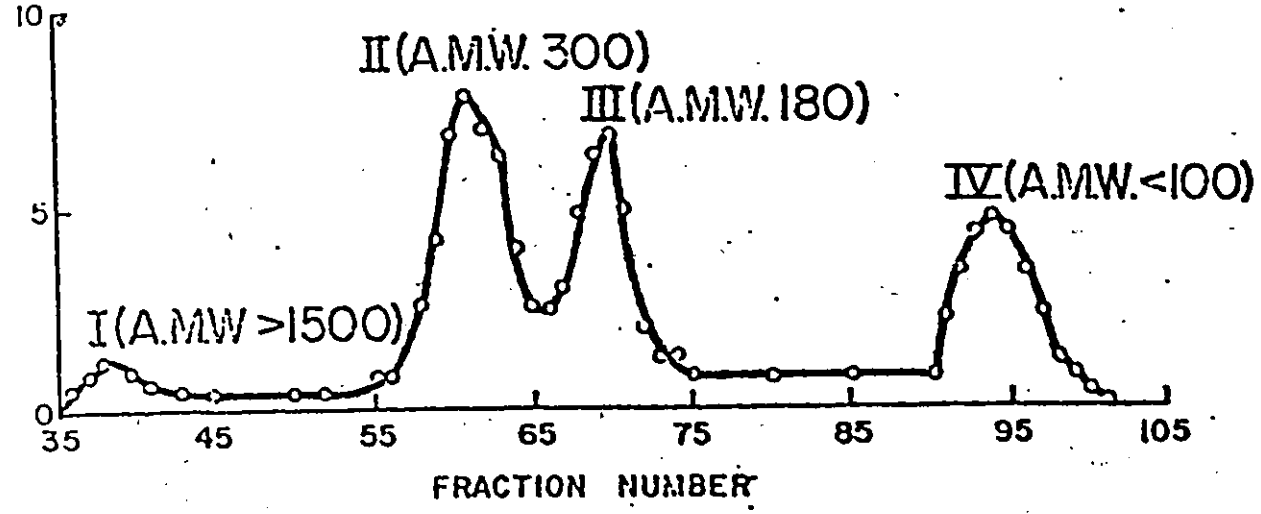
The GPC analysis of organics in natural water and soil materials is important since they may bear some resemblance to those found in wastewater.

Gjessing and Lee (1967) using Sephadex were able to separate the organics in natural lake water into 10 fractions. They concluded that a significant number of the compounds making up color in natural waters have A.M.W. > 50,000. This contradicts Shapiro's (1964) estimate of 456 for

GEL – SEPHADEX G-15

SPL – RAW DOMESTIC SEWAGE (NOT CONCENTRATED) ELNORA, N.Y.

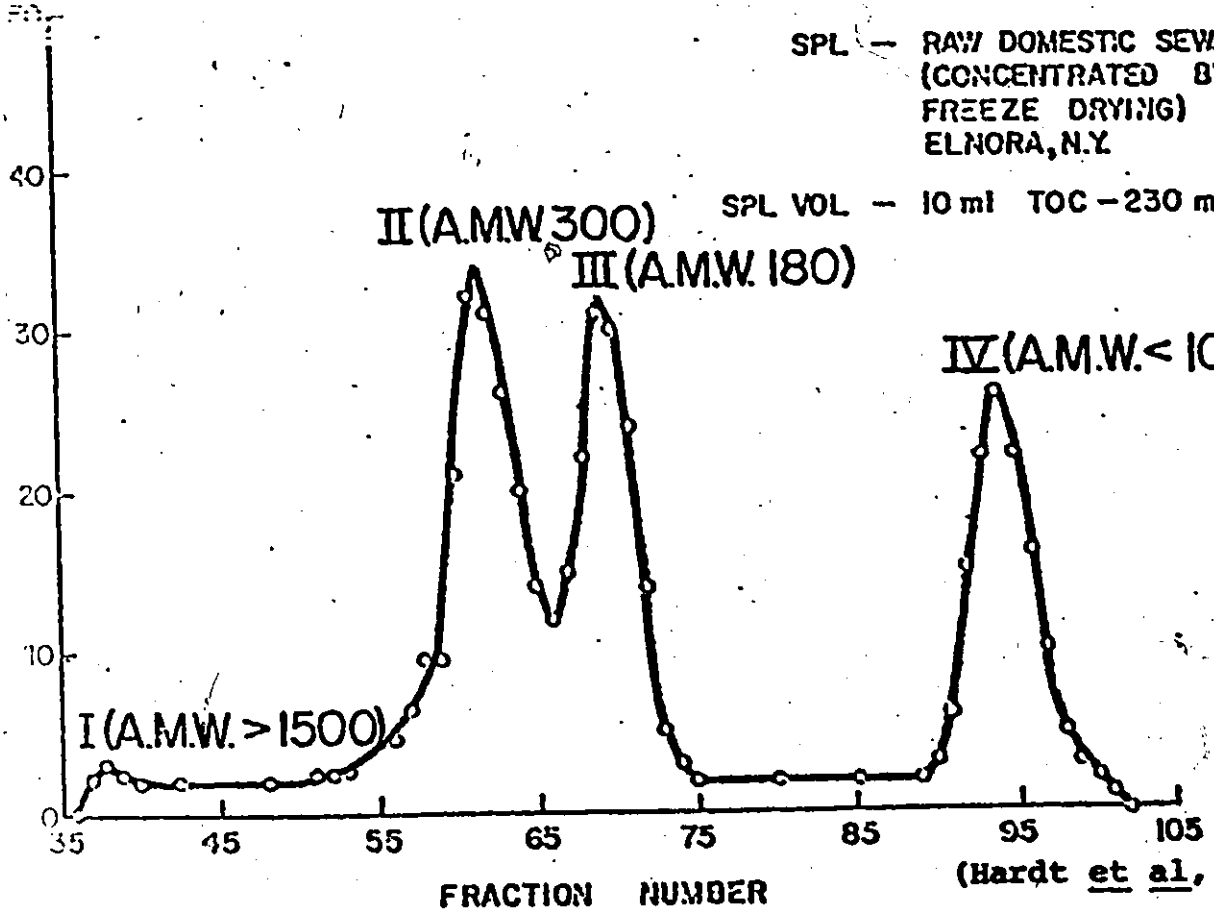
SPL VOL – 10 ml TOC – 62 mg/l



GEL – SEPHADEX G-15

SPL – RAW DOMESTIC SEWAGE (CONCENTRATED BY FREEZE DRYING) ELNORA, N.Y.

SPL VOL – 10 ml TOC – 230 mg/l



(Hardt et al, 1971)

FIGURE 3. Sephadex G10 Elution Profiles of a Raw Sewage Before and After Freeze-Dry Concentration

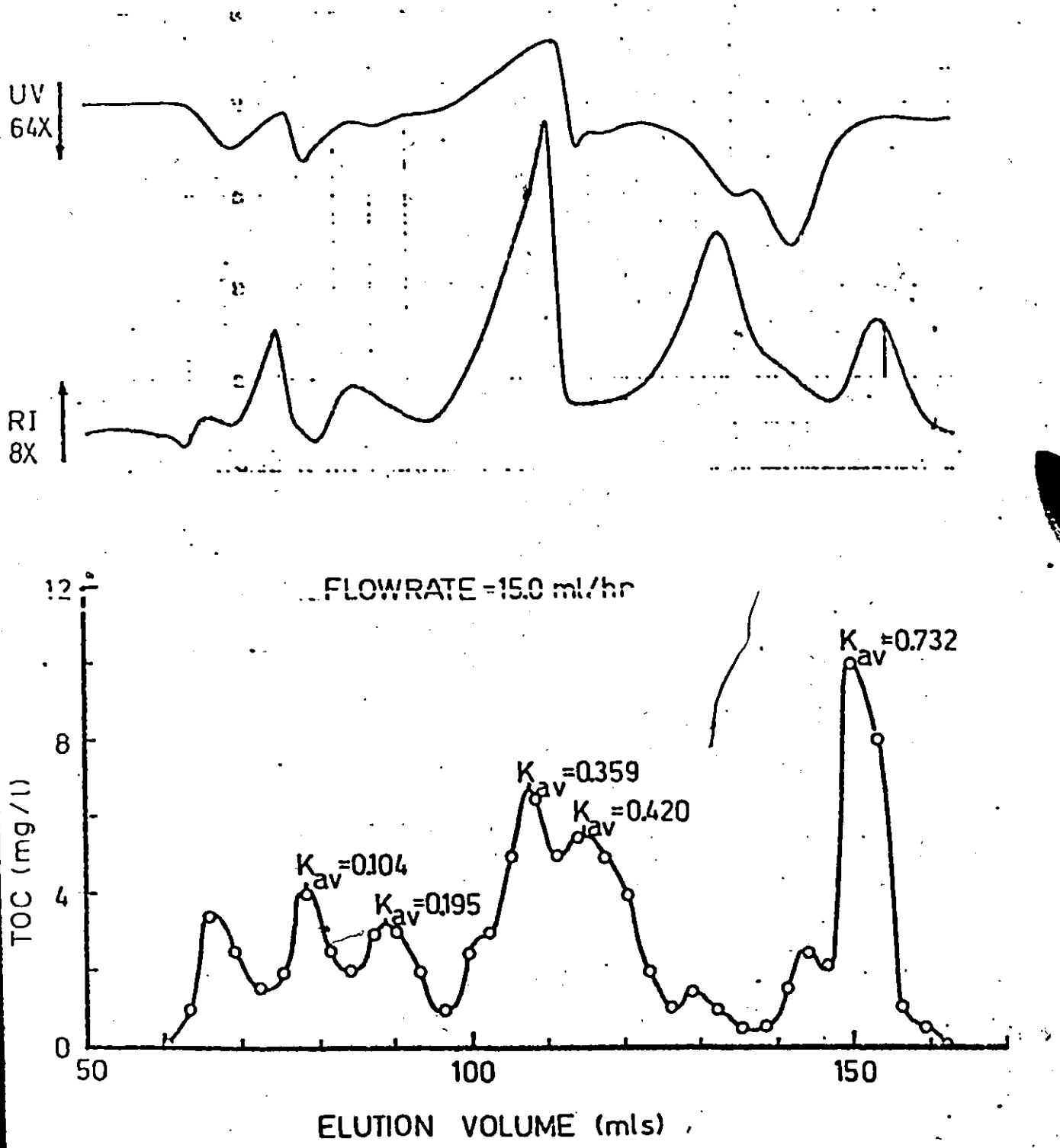


FIGURE 4. G15 Elution Profile of Concentrated Elizabeth Gardens Raw Sewage (Robertson, 1972)

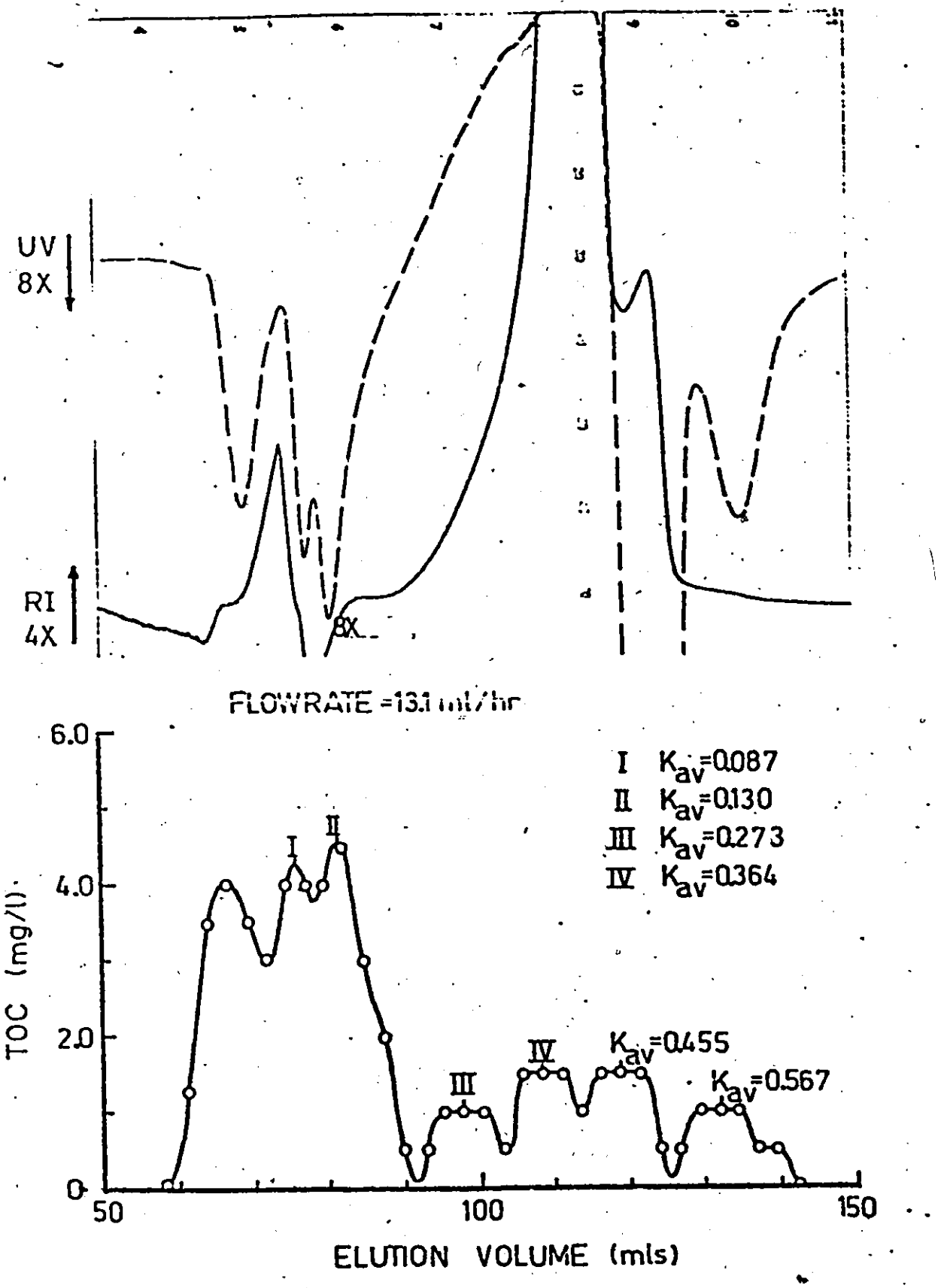


FIGURE 5 . G15 Elution Profile of Concentrated Dundas Raw Sewage (Robertson, 1972)

natural water color. They claim that low colored waters have most of their color in the low molecular weight fractions and highly colored waters have most of their color in the high molecular weight fractions.

Ghassemi and Christman (1968) also studied natural water color. They concluded that color producing molecules were mostly in the A.M.W. range of 700 to 10,000 relative to dextrans. One small fraction seemed to have an A.M.W. > 50,000. They noted that the molecular weight distributions should be verified by other means due to possible solute-gel interaction.

Brodsky et al (1970) and Povoledo and Gerletti (1968) have noted that "humic substances" can play an important part in natural color. Rebhun and Manka (1971) found that up to 50% of the organics in secondary effluents were composed of so-called "humic acids". Gjessing (1965) was one of the first to estimate the molecular weights of these unknown humic-like substances. He obtained two fractions; one with A.M.W. of 100,000 to 200,000 and the other with A.M.W. of < 10,000. Brogden (1971) using gel filtration and ultracentrifugation found that 5 to 13% of natural water humic acids had A.M.W. > 5,000 and the remainder had A.M.W. between 1,000 and 5,000.

The conclusion that can be drawn from this work is that natural water and soil-type organics have a broad range of molecular weight distribution ranging from greater than 100,000 to less than 10,000. Which range is most responsible for natural color is uncertain.

2.6 Lime Treatment Studies

Early uses for chemical treatment were to remove biological oxygen demand (BOD) and suspended solids (SS) from the wastewater or improve the

settling characteristics of activated sludge. The BOD and SS removal were comparable to those achieved by conventional activated sludge, but the process was less economical. Recent interest in chemical treatment has been prompted by concern over nutrient removal and the application of physical-chemical treatment to wastewater.

Stamberg *et al* (1970) indicate that lime addition precipitates the gelatinous calcium hydroxyapatite, granular calcium carbonate and at high pH (>10.5), magnesium hydroxide:

$$Ca^{++} + HCO_3^- + OH^- \rightarrow CaCO_3 + H_2O$$

$$5Ca^{++} + 7OH^- + 3H_2PO_4^- \rightarrow Ca_5(OH)(PO_4)_3 + 6H_2O$$

$$Mg^{++} + 2OH^- \rightarrow Mg(OH)_2$$

They point out that limited information is available on the complex mechanism of the precipitation. Jenkins *et al* (1971) state that calcium carbonate, $CaCO_3$, competes with calcium phosphate, $Ca_5(OH)(PO_4)_3$, precipitation between pH 9 and 10.5.

Most lime precipitation schemes have involved raising the pH to 10.5 or above to obtain fast precipitation, low phosphate residuals and a settleable precipitate. Buzzell and Sawyer (1967) have correlated reported lime doses against alkalinity of the wastewater. Bishop *et al* (1972) state that two options are available:

1. In waters of moderate alkalinity, the high pH (>11.5), 2-stage process gives good solids removal and low phosphate residual, and,
2. In hard waters, with alkalinity greater than 250 mg/l, a single stage low pH (~9.5) gives good results.

Nesbitt (1969) in his summary of the literature has noted a wide variation in lime doses ranging from 280 to 720 mg/l as $Ca(OH)_2$. Jenkins et al (1971) in their review of the literature on precipitation processes also pointed out that wide variations in phosphate residuals, lime dosages and pH's (7.5 to 11.5) existed. They suggested that more emphasis should be put on precipitation at slightly alkaline pH values and singled out solids recycle and destabilization of the colloidal precipitates as the critical parameters in the process. Solids recycle has been used effectively by Albertson and Sherwood (1969) in their Phosphate Extraction Process, where they claim that they can reduce the lime dose required by providing nuclei for precipitation.

A summary of the removals obtained in lime treatment by several investigators is provided in Table 6. On an overall basis BOD removal is in the range of 70% and SS and P removal in the range of 90%. Most authors do not distinguish between soluble and total figures.

A recent patent (Zuckerman and Molof, 1972) involves dosing wastewater with an excess of lime (pH > 12) so as to "hydrolyse" high molecular weight protein and carbohydrate components in the wastewater to lower molecular weight components. Justification is based on a physical-chemical treatment scheme whereby the smaller hydrolysed molecules are more easily adsorbed by actived carbon. Apparent proof of the effectiveness of the scheme is provided by gel permeation chromatography (GPC) analysis of the wastewater before and after treatment (Zuckerman, 1968; Zuckerman and Molof, 1970) and by plant-scale testing (Zuckerman et al, 1972). On plant scale, the authors claim that dosing with 1200 mg/l of $Ca(OH)_2$ (pH 12.2) with 1 hour detention results in "hydrolysis" and a better quality effluent after physical-chemical treatment.

TABLE 6 - APPLICATION OF LIME TREATMENT TO DOMESTIC WASTEWATER

NAME	BUZZELL & SAWYER, 1967	BISHOP et al 1972	SCHMID & McKINNEY, 1969	STAMBERG et al, 1970	ALBERTSON & SHERWOOD, 1969
pH	11		9.5	11.5	9.5 - 10.5
TOTAL TOC REMOVAL, % RESIDUAL TOC, mg/l		80		80 20	
TOTAL BOD REMOVAL, %	50 - 70	80	60		60 - 70
SS REMOVAL, %		91	90		>80
P REMOVAL, % RESIDUAL, mg/l	80-90 (TOTAL)	98 0.15	80	<0.3 (TOTAL)	>80
NOTABLE POINTS	Low BOD may cause problems in activated sludge.		Sludge production 1.5 x normal.	Residual turbidity <2 JTU	Solids recycle claimed to reduce lime dosage.

Zuckerman's work has been criticized strongly by Weber (1970) who points out a number of flaws in Zuckerman's carbon adsorption work. Weber was unable to demonstrate any improved adsorption by high pH treatment and indicates that larger molecules frequently adsorb more effectively on activated carbon. Zuckerman's claim also contradicts some established biochemical methods which require fairly drastic conditions to break down high molecular weight carbohydrates and proteins. Notable also is the fact that carbohydrates, a major sewage component, are very stable in alkali.

Lime treatment has been used as well for color removal from kraft mill effluents (Gould, 1971; Oswalt, 1970; Davis, 1969). The effluent is contacted with a high dose of CaO or $Ca(OH)_2$ and the sludge is recycled back to the kiln for recalcination to CaO. An 80% immediate removal of color is claimed. Apparently, very little additional color removal is obtained by dosing beyond the saturation point of lime. The exact mechanism of color removal is not well understood but believed to be precipitation of dissolved lignins (Gellman, 1970).

2.7 Sorption of Organics on Inorganic Surfaces

A brief description of the mechanism of adsorption will be given; more extensive descriptions are given in books by Hair (1971), Faust and Hunter (1971), and Stumm and Morgan (1970).

Adsorption on inorganic surfaces can be caused by electrostatic interactions and/or specific interactions such as covalent bonds, hydrogen bonding, and hydrophobic and hydrophilic bonding (Hair, 1971).

The extent of electrostatic interaction depends on the pH and ionic strength of the medium because these parameters control the charge and electrical double layer of the adsorbate and adsorbent. Every ionic

solid has a point of zero charge (PZC) and carries a net negative surface charge at pH's above and a net positive surface charge at pH's below the PZC. Thus, cations would be expected to adsorb above the PZC, anions below and minimum electrostatic interaction would be expected at the PZC.

The extent of specific interaction depends on the nature of the inorganic surface and organic molecule. Specific adsorption can affect the nature of the surface and affect electrostatic interaction by causing charge reversal.

A few investigators have looked at adsorption of organics on inorganics in natural water and wastewater systems. Suess (1970) found that the TOC in seawater samples was reduced by 10 to 14% following exposure to calcite. Surface active compounds such as fatty acids, proteins, lipids, fatty alcohols and fatty esters were present in sufficient quantity in seawater to form one or more layers on carbonate. Using both model systems and seawater, he concluded that calcite adsorbs selectively by a chemisorption mechanism. Lipoid compounds and amino acid containing substances were the major constituents adsorbed. No mention was made of the pH at which he was working or any effort to control pH.

Brownstein and Murphy (1972) in concentrating wastewater samples noted that approximately two-thirds of the organic material was lost by coprecipitation with carbonates. They noted a much lower TOC loss (17%) on hydroxylapatite.

In Hair's book (1971), it is stated that proteins adsorb to a large variety of surfaces. The mechanism is believed to involve ion exchange as well as physical adsorption.

2.8 Chemical Modification of Biological Material

Because of the pure compound studies carried out in this project a brief description of polysaccharides, proteins, ribonucleic acid, and humic acid is provided.

Carbohydrates have the general formula $(C.H_2O)_n$ where n is ≥ 3 and fall into three main categories:

1. Monosaccharides consisting of 3 to 9 carbon molecules.
2. Oligosaccharides consisting of monomers linked by formation of the glycosidic bond and containing 2 to 10 monomer units.
3. Polysaccharides consisting of more than 10 monomer units. Dextrans and starch fall into this category.

Polysaccharides represent the bulk of the carbohydrates in nature. Most polysaccharides are polydisperse i.e. consist of molecules of various molecular weights in contrast to proteins which are monodisperse (White, Handler and Smith, 1964). All polysaccharides and oligosaccharides are the result of linkage by the glycosidic bond:

$$G_1 - OR + H - OG_z \rightleftharpoons G_1 - OG_z + ROH$$

Hydrolysis is the reverse of the above reaction in which the ROH group would be water HOH. With the exception of phenolic and enolic glycosides, glycosides are stable to alkali (Aspinall, 1970).

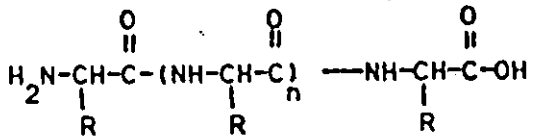
Polysaccharides are hydrolysed under acid conditions. Because the glycosidic bonds have varying acid lability, one cannot specify an overall adequate procedure for their complete acid hydrolysis (Pigman and Horton, 1970). Table 7 presents a summary of the conditions normally employed for acid hydrolysis with some polysaccharides.

TABLE 7 - REACTION CONDITIONS FOR HYDROLYSIS OF POLYSACCHARIDES TO OLIGOSACCHARIDES

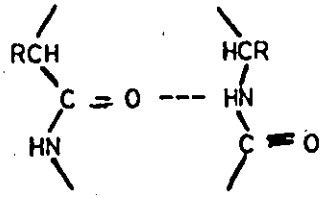
(AFTER PIGMAN AND HORTON, 1970)

POLYMER	TYPE OF OLIGOSACCHARIDE PRODUCED	ACID CONCENTRATION	TEMPERATURE (°C) AND TIME (hr.)
AMYLOSE	MALTO	0.33N H_2SO_4	100°, 2
	MALTO	0.1N HCl	100°, 4
DEXTRAN	ISOMALTO	0.33N H_2SO_4	100°, 10
INULIN	INULO	0.01N HCl	70°, 0.5
(1→2)-β-D - GLUCAN	SOPHORO	1N H_2SO_4	70°, 18
GLUCOMANNAN	GLUCO-MANNO	30% HCOOH	100°, 4
CAPSULAR POLYSACCHARIDES	URONIC ACID	2N H_2SO_4	100°, 3

Proteins consist of ∝ amino acids joined by peptide linkages:



Proteins follow a folded and ordered structure called the ∝-helix as the result of hydrogen bonding:



Other bonds providing stability to the helix are the C-N bond and hydrophobic bonding. Proteins are dipolar ions at the isoelectric point:

$$(H_3\overset{+}{N})_m - R - (COO^-)_n$$

At pH's below the isoelectric point the protein carries a net positive charge and at pH's above the isoelectric point it carries a net negative charge. Proteins also show a strong UV absorbance with a maximum at 280 mμ.

Water soluble proteins are globular and many are spherical or nearly spherical in shape. Minimum solubility occurs at the isoelectric point. Proteins can be precipitated from solution by high concentrations of neutral salts possibly due to dehydration (White, Handler and Smith, 1964). Chemical agents or pH extremes can cause reversible or irreversible modification in the helix structure. Denaturation is considered to be an irreversible unfolding and can result in insolubility or the exposure of amino acid residues (Means and Feeney, 1971).

Hydrolysis of proteins occurs by splitting the peptide linkage

through the addition of water. Agents used for hydrolysis are acids, bases and enzymes. Under acid conditions, the protein is refluxed with 6N HCl for 18 to 24 hrs. Alkaline hydrolysis is of limited use because it can destroy many amino acids. The protein is refluxed with 5N NaOH or 5N $Ba(OH)_2$ for 50 to 70 hours in evacuated tubes. Enzymes are used in hydrolysis to attack specific linkages (Blackburn, 1968).

White, Handler and Smith (1964) state that nucleic acids are cell components and can be naturally bound to proteins. They are classed into two groups; ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), according to the type of sugar they contain. They have a strong UV absorbance with a maximum near 260 mμ. Both components have a range of high molecular weights (20,000 to several million for RNA). Unlike DNA, RNA is hydrolysed by weak alkali (0.3 to 1N NaOH or KOH for 16 hours at room temperature (Colowick and Kaplan, 1957)).

Humic acid consists of a mixture of compounds synthesised during the natural breakdown of plant and animal substances. Humic acid has been known to precipitate out under various conditions of added electrolyte (Rebhun and Manka, 1971; Shapiro, 1966; Brogden, 1971). The breakdown of humic acid under alkaline conditions was investigated by Swift and Posner (1972) who treated humic acid samples for 30 days with 1N NaOH under either nitrogen or oxygen. The greatest change in molecular weight occurred under oxidative conditions. Gel filtration results indicated that the high molecular weight fractions ($> 1.5 \times 10^6$ molecular weight) were reduced to zero and a large reduction in the molecular weight range of 1.0×10^5 to 1.5×10^6 occurred.

Hydrolysis has been used to advantage in several phases of waste

treatment. In the Porteous Process (Porteous, 1969), cellulose in waste is broken down to simple sugars using HCl and elevated temperature. The sugars formed can be fermented to commercial ethanol with yeast. Yang and Gaudy (1973) applied the "hydrolytic assist" to a pilot extended aeration process. Excess sludge was acidified to pH 1 with 0.35N H_2SO_4 and autoclaved at 121°C for 5 hours. This provided at least 75% solubilization of the biomass and the neutralized hydrolyzate was recycled into the aeration tank as a readily usable substrate. Aly and El-Dib (1971) studied the persistence of some pesticides in the aquatic environment by looking at their hydrolysis under various pH and temperature conditions. The four insecticides were low molecular weight carbamate esters. All were found to be stable in acid but varied widely in stability in alkali. At pH 10, Sevin was 99% hydrolysed in 100 minutes; Baygon in 1.18 days; while Pyrolan and Dimetilan were unaffected.

In summary, the following effects can be expected when treating wastewater to high pH with an excess of lime:

Surface active compounds, particularly lipoid and amino-containing substances, can be adsorbed to precipitates. The amount of adsorption would depend on a number of factors including type and amount of precipitate, and the pH and ionic strength of the medium. Carbohydrate and protein bonds would be stable at high pH with proteins possibly undergoing denaturation and precipitation. Compounds related to those causing color in water, humic acids and lignins, would likely be precipitated out. Labile low molecular weight compounds related to esters would show varying stability at high pH. RNA would probably be hydrolysed.

CHAPTER 3

3. EXPERIMENTAL METHODS

3.1 Materials

Domestic Wastewater

Samples were taken from the Dundas treatment plant after the barminutor. All samples were taken during weekday periods between the hours of 10 A.M. and 4 P.M. Excess solids were filtered out with glass wool and lighter solids were allowed to settle in the lab for one to two hours. The decant was then subjected to further filtration or chemical treatment. All samples were stored at 4°C to minimize bacterial action.

Three different samples were collected on July 11, Aug. 10 and Aug. 23, 1973, and are numbered chronologically.

Pure Compounds

As both high molecular weight proteins and carbohydrates were claimed to be hydrolysed under conditions of high pH, several representative compounds from these two classes were chosen for study (Table 8). Dextran T-40 and inulin were chosen because they represent extremes in stability. As was noted (Table 7, in Chapter 2) dextran requires rather strong conditions for cleavage while inulin is broken down under fairly mild acid treatment. Both compounds have been previously fractionated on GPC and well characterized. Chymotrypsinogen-A and ribonuclease-A were chosen because they are fractionable with both Sephadex G-75 and the 75Å glass gel and have been characterized for GPC analysis. Urea and humic acid were chosen because of their probable relation to wastewater components and have also been analysed previously by GPC. Ribonuc-

TABLE 8-PROTEINS AND POLYSACCHARIDES

SUBJECTED TO CHEMICAL TREATMENT

CLASS	COMPOUND	AVG.MOLEC. WT.	MANUFACTURER
POLYSACCHARIDE	INULIN	5,200	FISHER SCIENTIFIC
POLYSACCHARIDE	DEXTRAN T-40	20,500	PHARMACIA
PROTEIN	CHYMOTRYP-SINOGEN-A	25,000	PHARMACIA & WORTHINGTON BIOCHEMICAL
PROTEIN	RIBONUCLEASE-A	13,700	PHARMACIA & WORTHINGTON BIOCHEMICAL

TABLE 9 - PURE COMPOUNDS USED FOR GPC CALIBRATION

CLASS	COMPOUND	AVG.MOLEC. WT.	MANUFACTURER
POLYSACCHARIDE	DEXTRAN T-70	35,000	PHARMACIA
POLYSACCHARIDE	DEXTRAN T-40	20,500	PHARMACIA
POLYSACCHARIDE	DEXTRAN T-10	6,670	PHARMACIA
POLYSACCHARIDE	INULIN	5,200	FISHER SCIENTIFIC
OLIGOSACCHARIDE	RAFFINOSE	505	EASTMAN
OLIGOSACCHARIDE	SUCROSE	342	J.T. BAKER
MONOSACCHARIDE	GLUCOSE	180	FISHER SCIENTIFIC
ALCOHOL	ETHYLENE GLYCOL	60	FISHER SCIENTIFIC
PROTEIN	OVALBUMIN	45,000	PHARMACIA
PROTEIN	CHYMOTRYP-SINOGEN-A	25,000	PHARMACIA & WORTHINGTON BIOCHEMICAL
PROTEIN	RIBONUCLEASE-A	13,700	PHARMACIA & WORTHINGTON BIOCHEMICAL
PROTEIN	INSULIN	5,000	McMASTER BIOCHEMISTRY DEPT.

leic acid (RNA) was chosen because it is a component of all cell matter and is susceptible to mild alkaline hydrolysis.

The pure compounds chosen for calibration of the GPC columns, (Table 9) represent homologous, well-defined series of compounds commonly used for this purpose. With the polysaccharides, the molecular weights used were the peak values in the molecular weight distributions.

For calibration of the total carbon analysers, urea was used as an organic standard because it is less liable to bacterial degredation than other organics. For inorganic carbon calibration, a mixture of carbonate and biocarbonate was used as recommended in the Beckman manual.

Chromatographic Gels

Glass beads of controlled pore size manufactured by Corning Glass, and Sephadex, a cross-linked dextran, manufactured by Pharmacia were used in this study. The sizes and approximate separation ranges of these gels are presented in Table 10. The Sephadex gels were allowed to swell in distilled water before use.

3.2 Equipment

Gel Filtration Apparatus

Three different chromatographic columns were used in this study:

1. Pharmacia K15/90 column packed with Sephadex G-75,
2. Pharmacia K26/70 water jacketed column packed with Sephadex G-15, and
3. a 3/8 inch outer diameter, 316 stainless steel column consisting of four sections, each 4 feet in length with sintered stainless end fittings and packed with Corning Glass gel.

The K15 and K26 columns were operated downflow and fed by gravity

TABLE 10

GEL TYPES USED AND FRACTIONATION RANGES

GEL TYPE	FRACTIONATION BASED ON PEPTIDES AND PROTEINS	RANGE BASED ON CARBOHYDRATES
CORNING CONTROLLED PORE GLASS, 75A		600 - 30,000
SEPHADEX G-75	3,000 - 70,000	1,000 - 50,000
SEPHADEX G-15	- 1,500	- 1,500

from a 2½ liter "mariotte container". The stainless steel column was a pumped horizontal flow system using an ALC 202 positive displacement pump supplied by Waters Associates. All tubing used in the chromatographic work was 1/16 inch stainless steel or polyethylene.

Effluents from the chromatographic columns were monitored continuously by a differential refractometer and differential UV monitor in series. The refractometer was a Waters model R403 designed for flowing systems and equipped with a static reference and heat exchanger. 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 or flowing reference unit equipped with 254 mμ UV source.

The UV and RI outputs were recorded using combinations of a number of different recorders, namely Honeywell Electronic Model 19, Philips Model PM 8220, Philips Model PM 8100, and Philips Model PM 8010. Discrete samples of chromatographic column effluents were collected after passing through the UV and RI detectors, using a Braun Simplex fraction collector.

Figure 6 is a schematic representation of the process.

Total Carbon Analysers

Total carbon analyses were done using a Beckman 915B carbon analyser and a Technicon autoanalyser system. The Beckman analyser was equipped with a total carbon (TC) channel and an inorganic carbon (IC) channel. Total organic carbon (TOC) was found by difference. The low level TOC fractions from the chromatography columns were normally analysed at high gain (8 to 10) using 20 μl sample injections.

The effective range of detection for the autoanalyser is 1 to 30 mg/l of TC. As samples consisting of roughly 200 mg/l TC are diluted 6

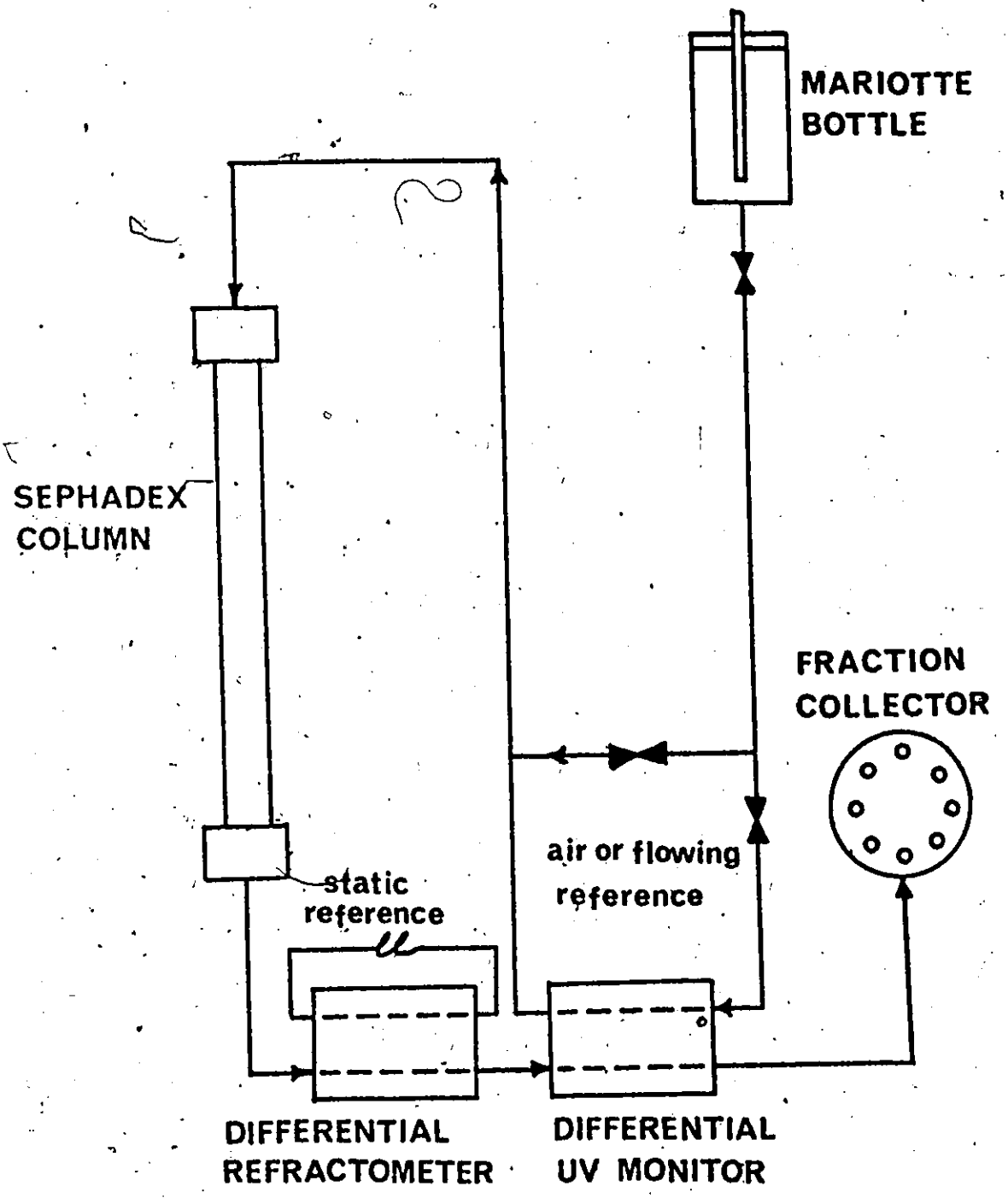


FIGURE 5. FLOW SCHEMATIC OF GEL CHROMATOGRAPHY ANALYSIS

to 10 times in passing through the chromatographic columns, this analyser is well suited to column effluent analysis. All wastewater samples were stripped of inorganic carbon so that TC analysis is representative of organic content. Operation of the autoanalyser has been described elsewhere (Addie and Murphy, 1973). Briefly the system mixes the sample with potassium persulfate, a powerful oxidizing agent; oxidizes the carbon to CO_2 with ultraviolet radiation; absorbs the CO_2 formed in dilute caustic and measures the change in conductivity of the dilute caustic solution. Addie and Murphy (1973) found good agreement between TC analysis by autoanalyser and by Beckman.

Flash Evaporator

Wastewater samples were concentrated to the range of 200 to 300 mg/l of TOC using a Buchler rotary flash evaporator. Using a water aspirator to draw vacuum, samples could be concentrated at low temperature (38°C), so as to eliminate volatilization and damage to organics (Brownstein and Murphy, 1972). Temperature was maintained by a thermostatically controlled temperature bath during the concentration.

Jar Tester

The coagulation studies were done with a Phipps and Bird six-paddle jar tester. Speed range for the unit was 0 to 100 RPM. The jars were square, open and of one liter capacity; the paddles were 3 x 1 inch in size.

Ultrafiltration Unit

Ultrafiltration of pure compounds and wastewater was done using an Amicon 10-PA ultrafiltration cell equipped with freon propellant and diaflow membranes UM05, UM2 and UM10 having molecular weight cutoffs of

500, 1000 and 10,000 respectively. Molecular weight cutoff indicates the lowest molecular weight which is retained by the membrane.

Filter Papers

Filter papers used were Sartorius glass fiber prefilters, and 0.45μ and 0.1μ pore size membranes. All membranes were prewashed with 250 ml. of distilled water before use.

3.3 Procedures

GPC Column Preparation and Operation

The glass gel column, packed and ready for use, did require coating with Carbowax 20 M (polyethylene glycol) to neutralize active sites on the glass. To coat the glass gel one liter of 1% carbowax solution was pumped through the column, followed by a distilled water rinse for at least 24 hours to wash out excess carbowax.

The Sephadex columns were packed according to the procedure outlined in "Gel Filtration in Theory and Practice" (Pharmacia, 1971).

Samples were adjusted to room temperature before being applied to the GPC columns. Application to the glass gel column was done using a measured volume loop (2 ml.) connected to the column by a four-way valve. For application to the K15 and K26 Sephadex columns the flow was stopped; the eluant above the gel was removed and a measured amount of sample was layered on top of the gel bed and allowed to drain into the gel. To ensure that all of the sample would remain in the gel, about ½ ml. of eluant was layered on top of the bed and allowed to drain into the gel. The top of the column was refilled with eluant, closed and flow was resumed. Eluant flow rate was checked periodically by timed volume measurement. Fractions were collected at a rate of 5 or 10 per hour, depending on the eluant flow

rate, and analysed for organic carbon using either the Beckman carbon analyser or autoanalyser. Fractions not immediately analysed were sealed and stored in the refrigerator at 4°C.

Gel column calibration and operating conditions are described in Chapter 4. While a number of different eluants were tried with the gravity-fed K15 and K26 Sephadex columns, only distilled water eluant was used with the glass gel column to avoid damaging the pumps.

Chemical Treatment of Pure Compounds

The pure compounds were dissolved in 200 ml. of distilled water and contacted with $Ca(OH)_2$ or NaOH in 250 ml. erlenmeyer flasks. The flasks were sealed with parafilm and the contents were agitated using epoxy-coated magnetic stirring bars. Samples were withdrawn at time periods ranging from one hour to one week and neutralized with HCL.

To study the effect of pH12+ on these compounds and their GPC behavior, both $Ca(OH)_2$, a slurry at the desired concentration, or NaOH, which is totally soluble, were employed. Initially dextran T-40, inulin, chymotrypsinogen-A, ribonuclease-A and later urea were contacted with 0.04M NaOH. The solution pH was above the measurement limit of the pH meter, but was calculated to be 12.6 pH units (Appendix B).

To ascertain where hydrolysis products would elute from the chromatographic column if the compounds were fully or partially broken down and to what degree they could be broken down under stronger conditions; the compounds dextran T-40, inulin, and chymotrypsinogen-A were subjected to stronger conditions of acid and base hydrolysis (Table II). Acid hydrolysis conditions are modelled after those previously summarized in Chapter 2. For comparison purposes, analogous conditions were chosen for base

TABLE II - STRONG ACID AND BASE HYDROLYSIS CONDITIONS FOR PURE COMPOUNDS

COMPOUND	ACID HYDROLYSIS CONDITIONS	BASE HYDROLYSIS CONDITIONS
INULIN	.02N HCl 3 hours, 77°C	.02N NaOH 3 hours, 77°C
DEXTRAN T-40	.5N H_2SO_4 9 hour reflux	.5N NaOH 9 hour reflux
CHYMOTRYPSINOGEN-A	6N HCl 24 hour reflux	

hydrolysis with NaOH being substituted for the acid.

Because ribonucleic acid components have been reported to interact with Sephadex (Gelotte, 1960; De Bersaques, 1967) methods outlined in "Methods in Enzymology" (1957) were used to assess the degree of hydrolysis of RNA by lime. RNA was contacted with lime at pH 10 and pH 12 + (1500 mg/l $Ca(OH)_2$) for one hour and twenty-four hours. Any unhydrolysed RNA was precipitated from solution by cooling the sample to 4°C and adding trichloroacitic acid (TCA) to 5% concentration. After five to ten minutes were allowed for precipitation, the sample was filtered through a 0.1 μ membrane and the optical density of the filtrate was measured at 260 nm using a Bausch and Lomb Spectronic 600 ultraviolet analyser. The degree of hydrolysis was evaluated by comparison to undrohydrolysed and completely hydrolysed samples. Complete hydrolysis of RNA was obtained by contacting with 0.5 M KOH for sixteen hours at 35°C (Colowick and Kaplan, 1957).

Humic material in natural water has been reported to precipitate out under strong electrolyte conditions or as the calcium salt (Shapiro, 1966; Brogden, 1971; Rebhun and Manka, 1971). To investigate this property, dried humic acid (K&K Laboratories) was dissolved in dilute NaOH and 250 mg/l of alkalinity as $CaCO_3$ was added using $NaHCO_3$ to simulate natural conditions. The humic acid solution was neutralized to pH 7.5 using HCl; part of the solution was used as a control and the remainder was treated with increasing doses of lime up to 2000 mg/l using the jar tester. The jar testing procedure consisted of six minutes of "rapid mix" at 100 RPM; thirty minutes of "slow mix" at 30 RPM, and a final settling period. Treated and untreated samples were filtered through 0.45 μ membranes and analysed for color or TOC.

Wastewater Preparation

The raw wastewater normally contained about 30 mg/l of "soluble" TOC, soluble organics being defined as those remaining in solution after filtering through a 0.1 μ membrane. The optimum TOC range for gel filtration analysis is 200 to 300 mg/l so that filtered samples required concentration by seven to ten times. A schematic of the wastewater preparation procedure is illustrated in Figure 7.

Lime Treatment of Wastewater

The objective was to compare the efficiency of lime treatment at various pH levels. Of particular interest was the effect of high pH (12+) compared with low pH (9.5 to 10) on the "soluble" organics in wastewater. To accomplish this, six one-liter jars of sewage were set up; two were maintained at low pH; two at intermediate pH, and two at high pH. One set of samples was removed after one hour of treatment and the other set

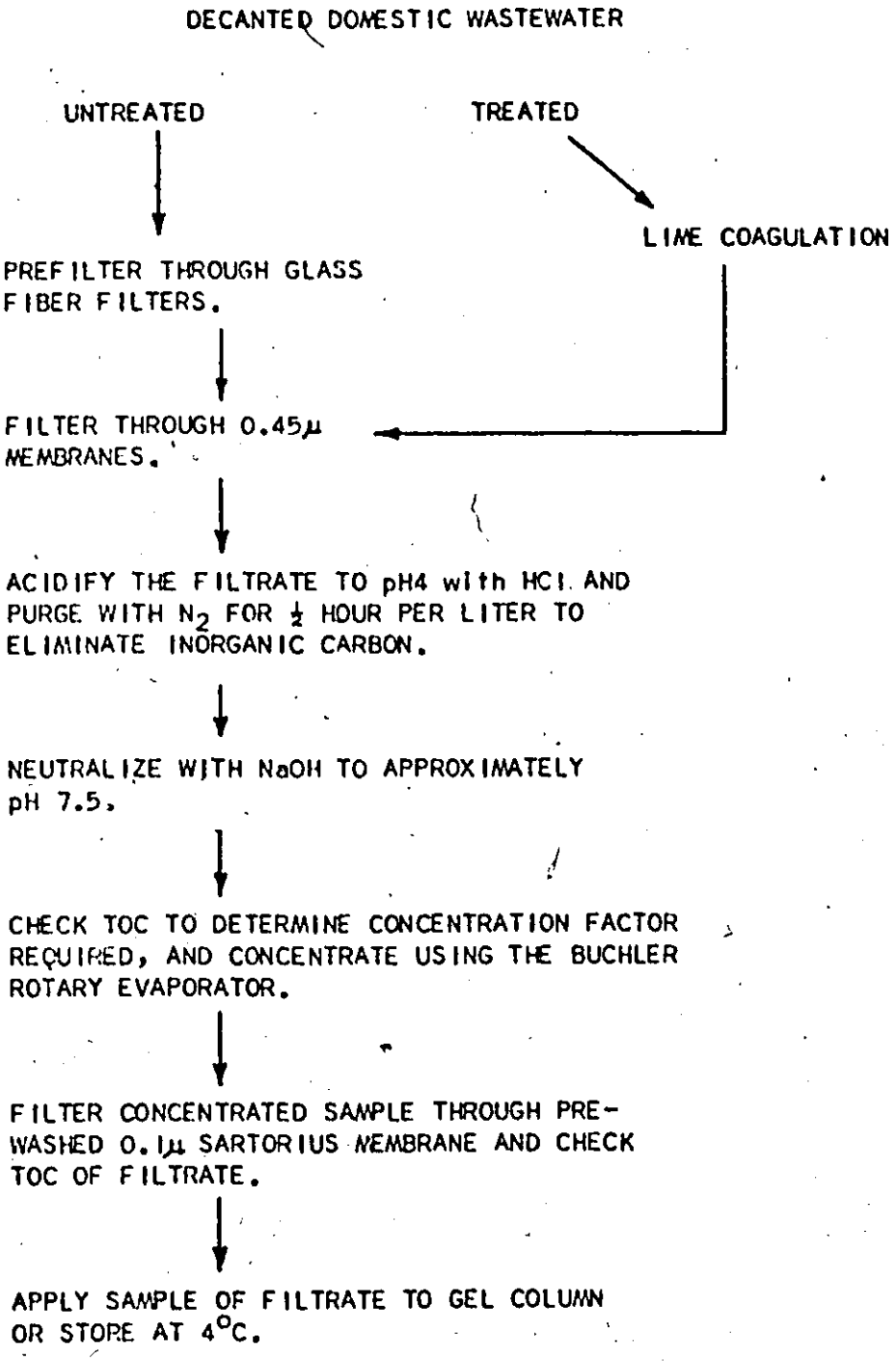


FIGURE 7 - WASTEWATER PREPARATION PROCEDURE

removed after twenty-four hours. Decanted domestic wastewater was used in all the tests. Before the coagulation runs, lime dosage requirements and the alkalinity of the wastewater were determined. Alkalinity was estimated according to "Standard Methods" (1971). Lime dosage requirements were taken from a standard curve constructed by measuring the pH of the wastewater sample with increasing additions of lime. The procedure adopted in the coagulation studies is as follows.

1. All samples were "rapid mixed" at 100 RPM for six minutes. The one hour samples were "slow mixed" at 30 RPM for thirty minutes, then allowed to settle for twenty-five minutes. The one day samples were "slow mixed" for twenty-four hours before settling.

2. After settling, 25 ml. samples of the supernatant were taken with a pipette for total phosphate analysis. Samples were immediately acidified to about pH 2 using 0.2 ml. of concentrated HCl and filtered through 0.45 μ membranes. Total phosphate analysis was done by Technicon autoanalyser using standard Technicon techniques.

3. Uncoagulated waste and the supernatant from each coagulation jar was analysed for turbidity using the Hellige turbidimeter.

4. The pH of each jar at the beginning and end of the coagulation test was monitored using a Fisher Accumet, Model 230 pH meter and a Beckman pH meter, model N.

5. Samples were prepared for TOC and gel filtration analysis using the procedure outlined in Figure 7. All samples were concentrated to roughly the same TOC value.

6. Conductivity of the concentrated samples was measured at 20°C using a Bach-Simpson conductivity meter.

7. Color of the concentrated samples was measured at a wavelength of 480 nm using a Bausch and Lomb Spectronic 20 colorimeter.

Microbial activity at the low pH level was checked by treating two samples of the same wastewater with lime and inhibiting microbial growth in one sample with 200 mg/l of $HgCl_2$. This mercuric chloride dose is based on recent values in the literature (Ghosh and Zugger, 1973). Plate counts to determine the number of viable organisms in the supernatant of each sample were done using procedures outlined in "Standard Methods" (1971). After filtering through 0.1μ membranes and stripping to remove inorganic carbon, the TOC of both samples were compared.

CHAPTER 4

4. EFFECTS OF ELUANT AND SAMPLE COMPOSITION ON GPC ELUTION BEHAVIOR

4.1 Analysis of Pure Compounds with Porous Glass Gel

As outlined in Chapter 2, the rigid porous glass gel has a number of advantages over softer compressible gels such as Sephadex. However, it was decided not to use this gel for any further pure compound or wastewater studies because of its adsorptive tendencies and ion exchange properties. The presence of these non-steric interferences was demonstrated by the following series of experiments.

1. A sample of the protein, chymotrypsinogen-A, was completely retained by the column. The glass gel then was coated with polyethylene glycol, as suggested by the manufacturer, to neutralize adsorptive sites. The protein sample now yielded a small, broad peak at the exclusion volume. With protein samples that had been treated with lime or sodium hydroxide a sharp but also weak second UV peak was obtained at the lower limit of the column. As this second peak was present even with initial samples, it was presumed to be an artifact of the column rather than the result of hydrolysis product.

2. The chymotrypsinogen-A sample that had been treated for one week with 0.04M NaOH was eluted through the column and fractions of the eluant were collected and analysed for TOC. Only about 12% TOC recovery was obtained, indicating that a significant amount of protein still was being retained by the column.

3. As the polysacchadides used did not absorb in the UV and their elution position on RI was masked by inorganics, the fractions were analysed for

TOC to establish elution position (Table 12). The TOC recoveries were generally 80 to 90%, indicating that very little if any carbohydrate material was retained by the column. There was some concern over desorption from the column when a small UV peak was observed which was the result of non-carbohydrate material. This UV peak, which eluted at an elution volume equivalent to that of the inorganic salt in the sample, increased in size with samples that had been subjected to stronger base conditions (1N NaOH), leading to the suspicion that desorption was caused by inorganics in the sample. This possibility was checked by passing a 1% NaCl solution through the column. Suspicions were confirmed when a large amount of TOC was eluted from the column when the NaCl front broke through.

Sephadex is an alternate gel material which should not be subject to these interferences. As with the glass gel, it was necessary to define any anomalies which could exist. Choice of eluant composition and the effect of sample inorganic content on the elution of organics warranted preliminary investigation.

4.2 Analysis of Pure Compounds with Sephadex

Effect of Eluant Composition and Sample Inorganic Content on Elution

The presence of sufficient ionic background has been shown to eliminate electrostatic solute-gel interactions. However, it can also increase adsorption of solute on the gel by making more of the gel available to the solute and can alter the size of flexible molecules.

The fractionation of inorganics in a wastewater sample, for example, may result in variations in ionic strength during the fractionation yielding possible combinations of the above effects. Another consideration,

TABLE 12 - ANALYSIS OF POLYSACCHARIDES USING GLASS GEL

SAMPLE	TREATMENT CONDITIONS	TOC IN	TOC OUT				TOC RECOVERY
			ELUTION POSITION	TOC	ELUTION POSITION	TOC	
		µg	MLS	µg	MLS	µg	%
INULIN	0.04M NaOH, zero time	336*	137	230			68.5
INULIN	0.04M NaOH, 4 hours	568	138	480			84.5
INULIN	0.04M NaOH, 28 hours	555	138	383	168	16	72
INULIN	0.04M NaOH, 1 week	549	136	488			89
DEXTRAN T40	0.04M NaOH, 1 week	570	95	469			82.3
DEXTRAN T40	1M NaOH, 25 hours	550	100	463	147**	142	110

* Sample not completely soluble.

** Anomalous UV peak also observed at this position.

when dealing with a mixture of organics as found in wastewater, is that operating conditions that are beneficial to one group of compounds may be detrimental to another group.

The results of the elution of standard proteins and polysaccharides with NaCl, Na_2SO_4 and distilled water eluant and with NaCl or Na_2SO_4 added to the sample is presented in Table 13. The upper limit of separation or void volume of the Sephadex G75 column is defined by blue dextran 2000 and corresponds to a Kav of 0;the approximate lower limit of separation is defined by the elution position of NaCl and corresponds to a Kav of 0.91. On examining Table 13, it is evident that proteins are drastically affected by the inorganic content of the sample and the eluant, while carbohydrates are relatively unaffected. The reason for the adsorptive and ionic effects of the protein with the gel material is, no doubt, due to the molecule being highly charged and possessing both hydrophobic and hydrophilic characteristics. Carbohydrates are neutral, hydrophilic molecules and are eluted without much interference.

Elution of the protein, ribonuclease-A, with distilled water resulted in a poorly resoluted peak near the exclusion volume. This seems to be the result of exclusion of part or all of the protein sample due to the small amount of negative charge on the gel and the negatively charged protein molecule. When NaCl was added to the sample and eluted with distilled water good resolution was obtained, but the protein now was eluted near the lower limit of separation. Adding NaCl or Na_2SO_4 to the eluant resulted in consistent elution positions and good resolution of the protein at an intermediate Kav value.

TABLE 13 - SEPHADEX G75 ELUTION POSITIONS OF PROTEINS AND POLYSACCHARIDES UNDER VARIOUS CONDITIONS OF ADDED ELECTROLYTE

SAMPLE	ELECTROLYTE ADDED TO SAMPLE	ELUANT	ELUTION VOLUME mls.		K_{av}
			UV TRACE	RI TRACE	
BLUE DEXTRAN 2000		.04M NaCl	53.2	51.8	0
NaCl		Distilled Water & .04M NaCl		140	0.91
RIBONUCLEASE-A		Distilled Water		56	0
RIBONUCLEASE-A	NaCl	Distilled Water		128	0.78
RIBONUCLEASE-A		.02M NaCl	102	100	0.50
RIBONUCLEASE-A	NaCl	.04M NaCl		92	0.42
RIBONUCLEASE-A		.01M Na_2SO_4	98.5	96.5	0.46
RIBONUCLEASE-A	NaCl	.01M Na_2SO_4	90.3	88	0.37
RIBONUCLEASE-A + INSULIN		Distilled Water		135	.86
				144	.94
RIBONUCLEASE-A + INSULIN		.02M NaCl	99.8	97.4	.47
			150	149	1.0
CHYMOTRYPSINOGEN-A	NaCl	.04M NaCl		76.8	0.26
OVALBUMIN		.01M Na_2SO_4	66.5	65.0	0.13
INSULIN		.01M Na_2SO_4		143	0.94
DEXTRAN T70	NaCl	Distilled Water		53.5	0.02
DEXTRAN T40		Distilled Water		53.3	0.01
DEXTRAN T40		.02M NaCl		53.7	0.02
DEXTRAN T40		.01M Na_2SO_4		52	0
DEXTRAN T40	Na_2SO_4	.04M NaCl		51	0

TABLE 13 CONT'D.

SAMPLE	ELECTROLYTE ADDED TO SAMPLE	ELUANT	ELUTION VOLUME, mls.		K_{av}
			UV TRACE	RI TRACE	
DEXTRAN T10		Distilled Water		82.7	0.32
DEXTRAN T10	NaCl	Distilled Water		84	0.33
DEXTRAN T10		.02M NaCl		83.2	0.32
INULIN		.01M Na_2SO_4		110	0.60
INULIN	Na_2SO_4	.04M NaCl		112	0.62
RAFFINOSE		.01M Na_2SO_4		136	0.87
RAFFINOSE	Na_2SO_4	.04M NaCl		141	0.92

This anomalous behavior is difficult to explain by a simple mechanism. It is doubtful that changes in elution position are the result of changes in molecular size since the protein is globular and compact. Either of the following two mechanisms are possible:

1. adsorption in regions of high ionic strength and desorption in regions of low ionic strength, or
2. ion exchange.

With the first mechanism, the presence of NaCl in the sample would enable the protein to enter the gel increasing the chances for adsorption. As the NaCl front is eluted away from the protein, the protein is desorbed. As soon as the protein catches up with the NaCl front the process is repeated again so that the protein follows the NaCl down the column. If this mechanism predominated, poor resolution would be expected when using an ionic eluant. In the second mechanism, the presence of an inorganic salt would decrease ionic interaction between the protein and the gel. Thus, the protein would tend to follow the inorganic front down the column and the presence of an ionic eluant would saturate ionic sites so that the protein could elute freely through the column. It should be noted that although ionic interactions can be eliminated, more specific hydrophobic or hydrophilic interactions can still exist and, in fact, the presence of inorganic salt may enhance these interactions as discussed by Eaker and Forath (1967).

While more experimentation would be required to fully establish the nature of the mechanism, the important point is that the presence of ionic background provides consistent elution behavior independent of the inorganic content of the sample.

Calibration of GPC Columns

With GPC the molecular sizes of unknown compounds are inferred by comparison to an homologous series of proteins or dextrans. Data for calibration of the Sephadex G75 column is taken from Table 13. Replicates appearing in this table were averaged and protein elution positions are those using ionic eluants. Both homologous series of protein and polysaccharides yielded straight line calibrations (Figure 8). The calibration line for polysaccharides is below that for proteins which is in accordance with Andrew's (1970) contention that carbohydrates have a more expanded structure than proteins of the same molecular weight. Inulin, a starch, does not fall on the same line since it is not part of this homologous series.

The K26 column containing Sephadex G-15 was calibrated with an homologous series of carbohydrates using .04M NaCl eluant (Figure 9). This curve applies to both the original and repacked columns. The lower limit of separation as measured by NaCl corresponds to a Kav of 0.52 or a molecular weight in the range of 150. This limiting Kav value is much lower than that for the G-75 column, indicating that a smaller fraction of the internal gel voids is available, possibly due to a higher degree of gel crosslinking.

These calibrations have been used to give an indication of the molecular size of "soluble" organics in wastewater. For a mixture such as this, interpretation can only be approximate since different compounds of similar weight can differ considerably in elution behavior.

4.3 Analysis of Concentrated Wastewater Samples with Sephadex

Effect of Different Eluants

Distilled water as well as four inorganic solutions 0.04M NaCl,

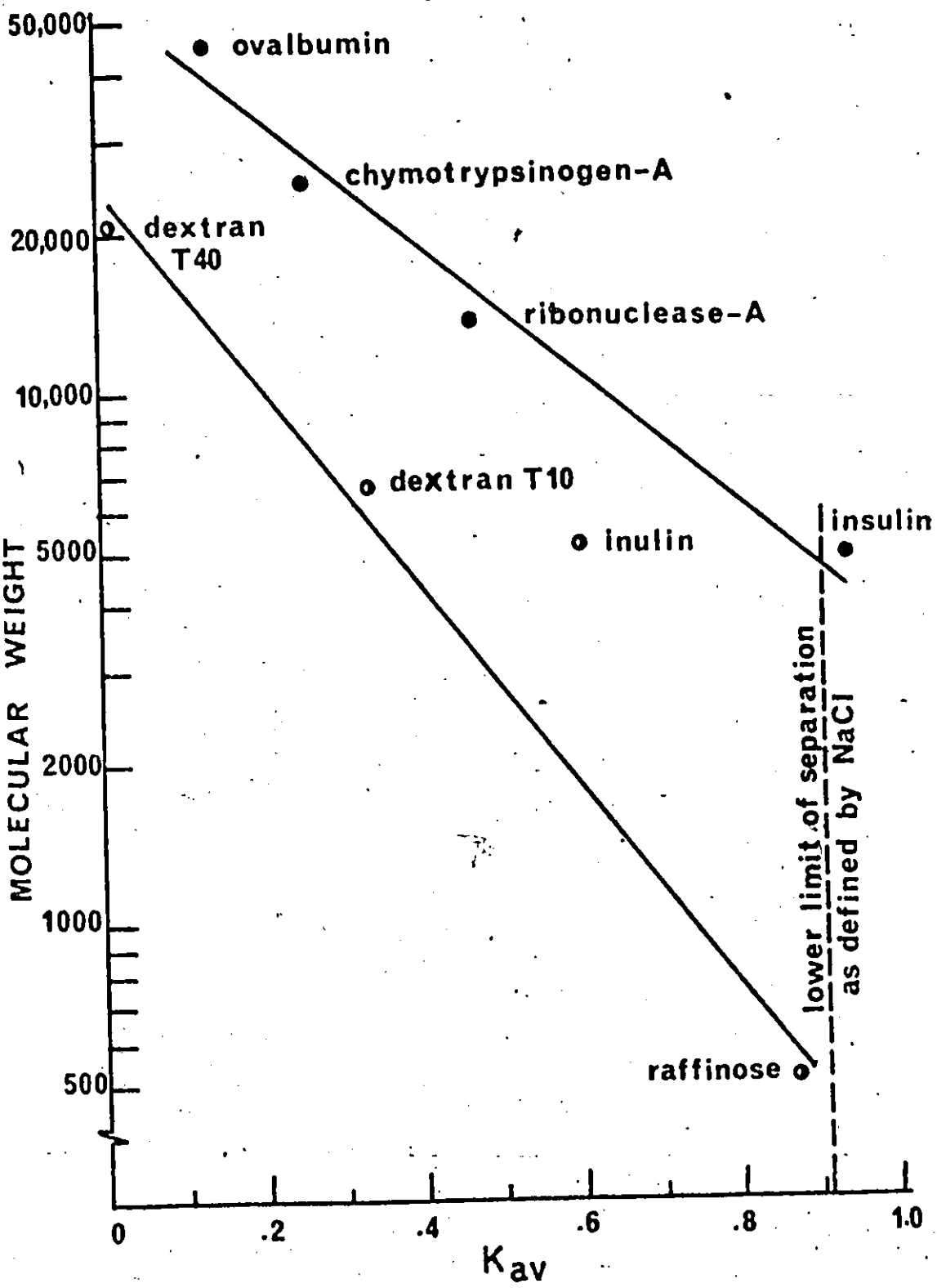


FIGURE 8. CALIBRATION OF THE SEPHADEX G75 COLUMN

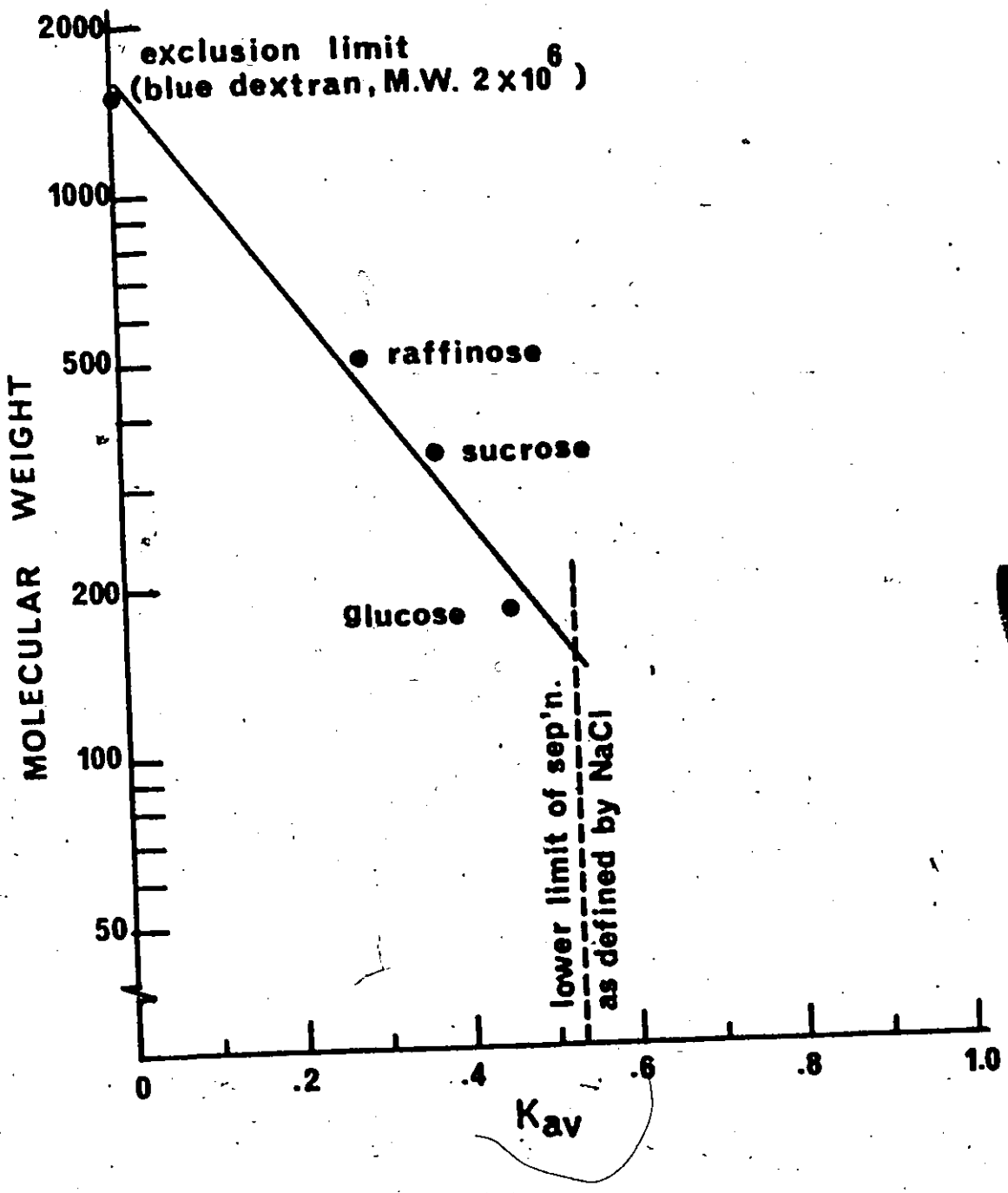
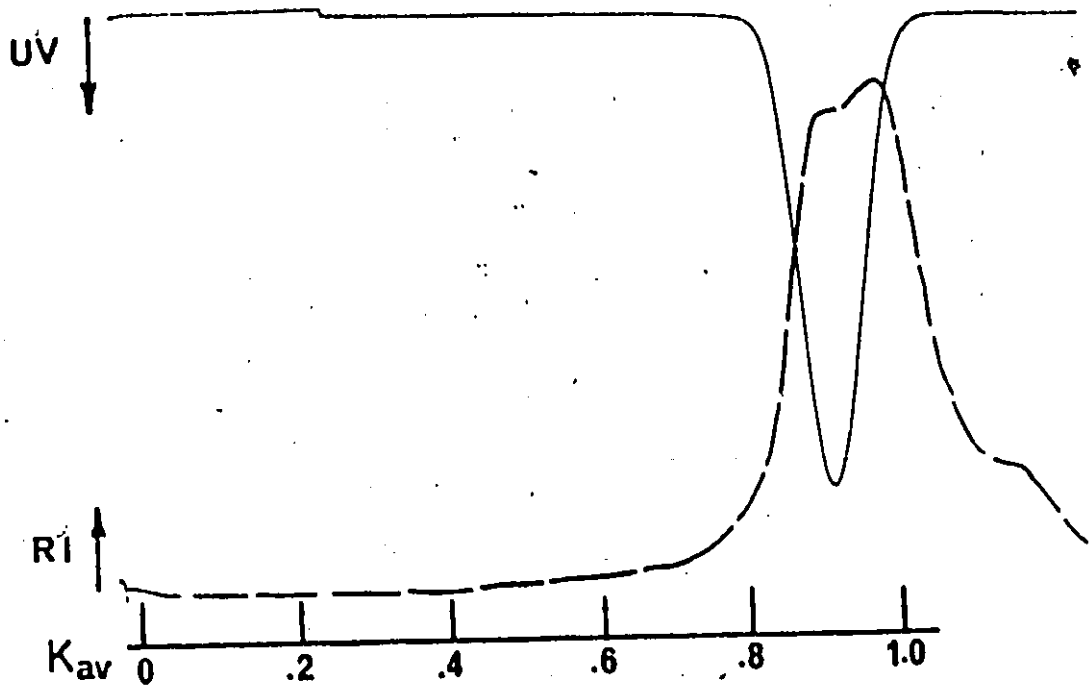


FIGURE 9. CALIBRATION OF THE SEPHADEX G15 COLUMN

0.1M NaCl, 0.034M Na_2SO_4, 0.025M KH_2PO_4 + 0.025M Na_2PO_4 were used as eluants. The final three solutions have an ionic strength of 0.1 (Appendix B). The wastewater sample first was eluted with 0.04M NaCl through the Sephadex G75 column. All of the organics were recovered in a broad peak at the lower limit of the column (Figure 10). Since this was indicative of molecular weights less than roughly 3,000, the Sephadex G15 column was used for further classification of wastewater organics. Figures 11 to 15 present the UV, RI and TOC profiles of the concentrated sample I eluted with these five eluants through the Sephadex G15 column. Table 14 summarizes the positions of major organic peaks and the TOC recovery from the G15 column. The main points to note are:

1. Elution with distilled water through the Sephadex G15 column resulted in three distinct, well-separated peaks of organic carbon. The three TOC peaks occurred at Kav values of 0, 0.15 and 0.45 and corresponded with peak positions on the UV trace. Based on the column calibration with an homologous series of carbohydrates (Figure 9), these elution positions correspond to apparent molecular weights of 1500 +, 1000 and 200 respectively. The TOC profile reveals that the majority of the organic material falls into the two lower molecular weight distributions. The sharp leading edge and protracted falling edge of the two peaks on the RI trace is characteristic of inorganics (Neddermeyer and Rogers, 1968). Looking at the UV and RI traces, one can see that the two major organic peaks occur at the same position as the two inorganic peaks. This raises the question of whether the elution of organics is, in fact, dominated by inorganics in the sample by mechanisms similar to those postulated for proteins in the previous section.



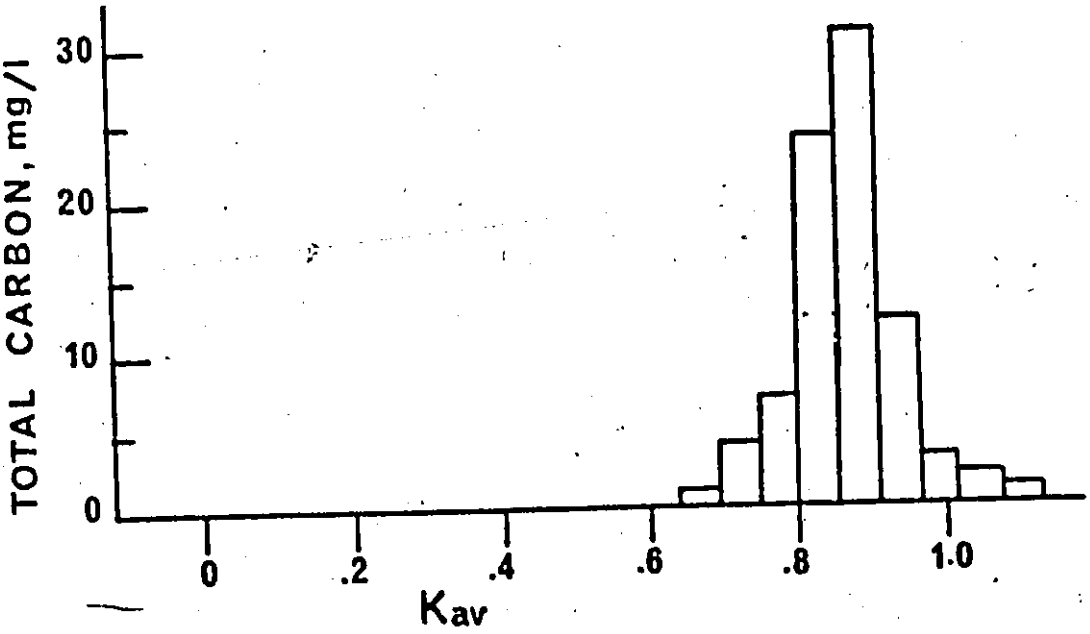
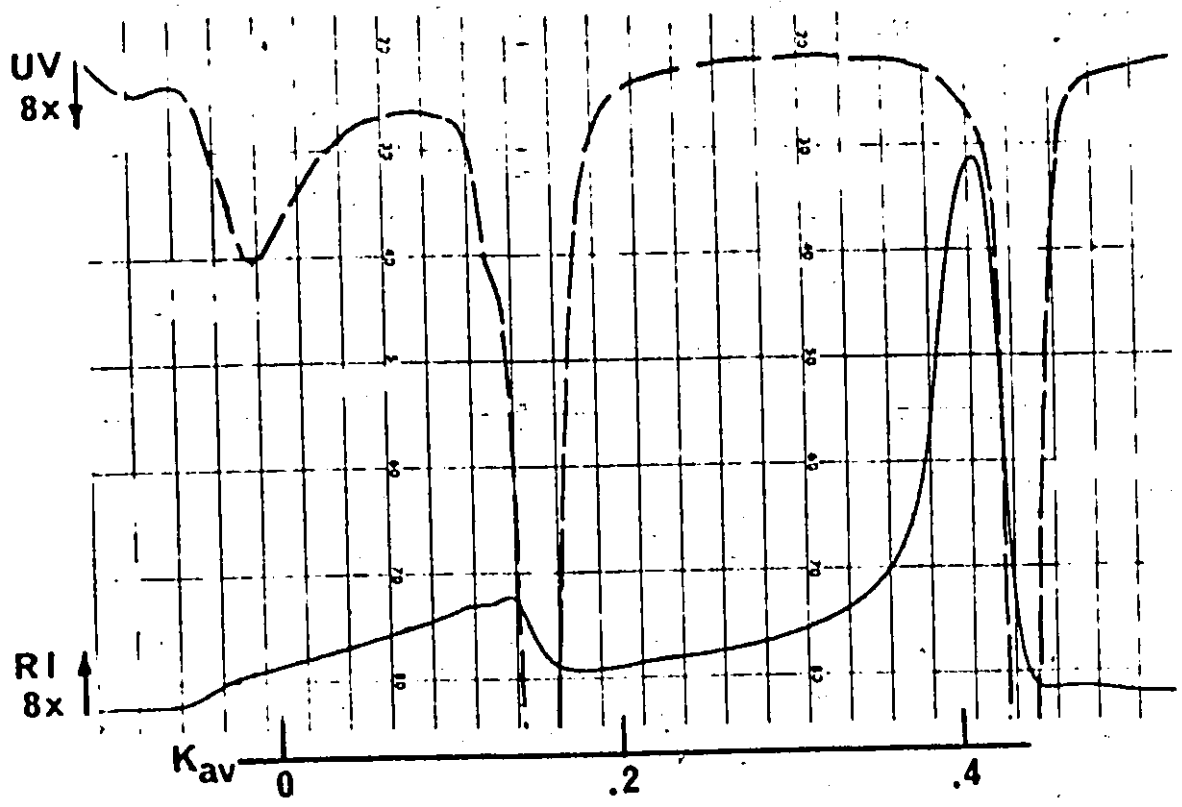


FIGURE 10. SEPHADEX G75 PROFILE OF CONCENTRATED SAMPLE I ELUTED WITH .04M NaCl



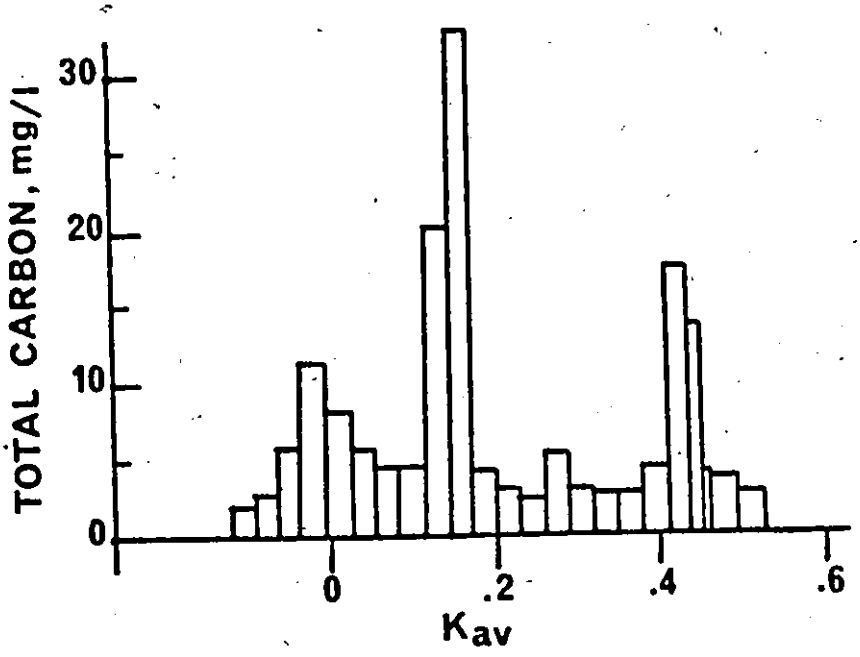
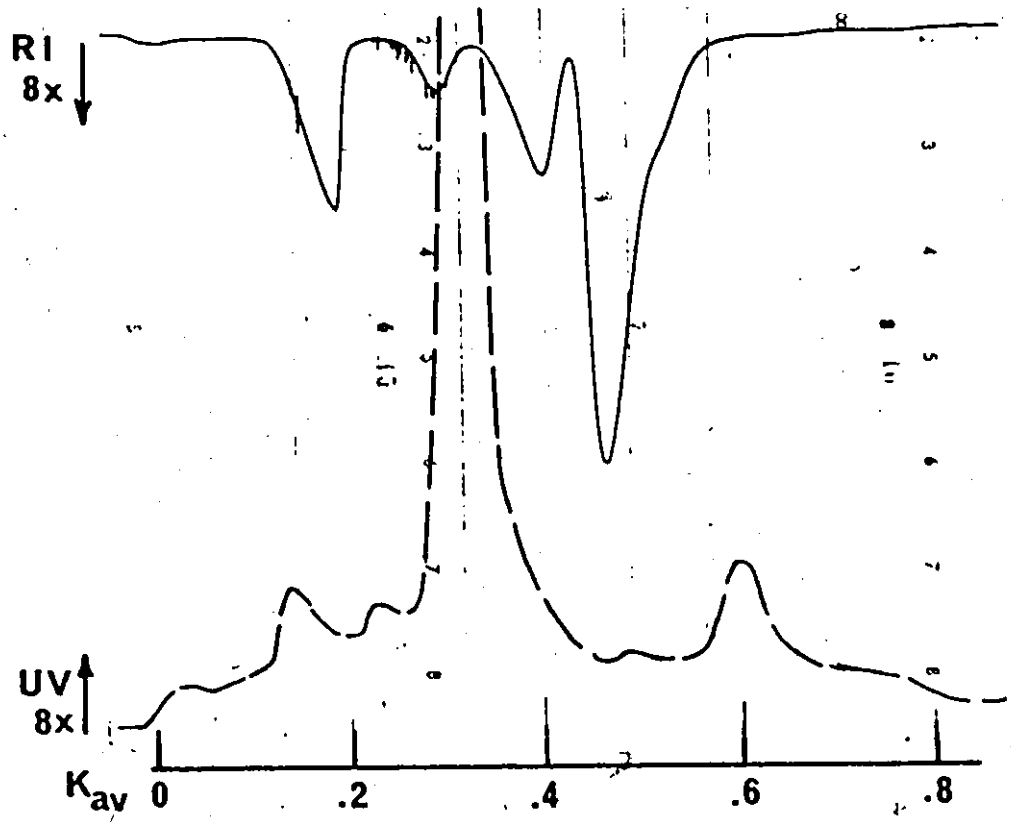


FIGURE 11. SEPHADEX G15 PROFILE OF CONCENTRATED SAMPLE 1 ELUTED WITH DISTILLED WATER



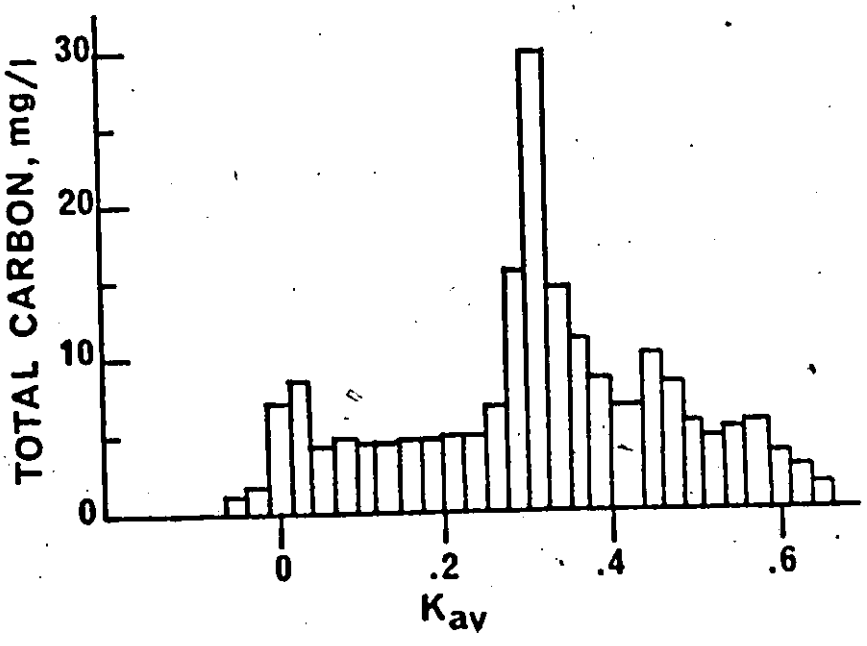
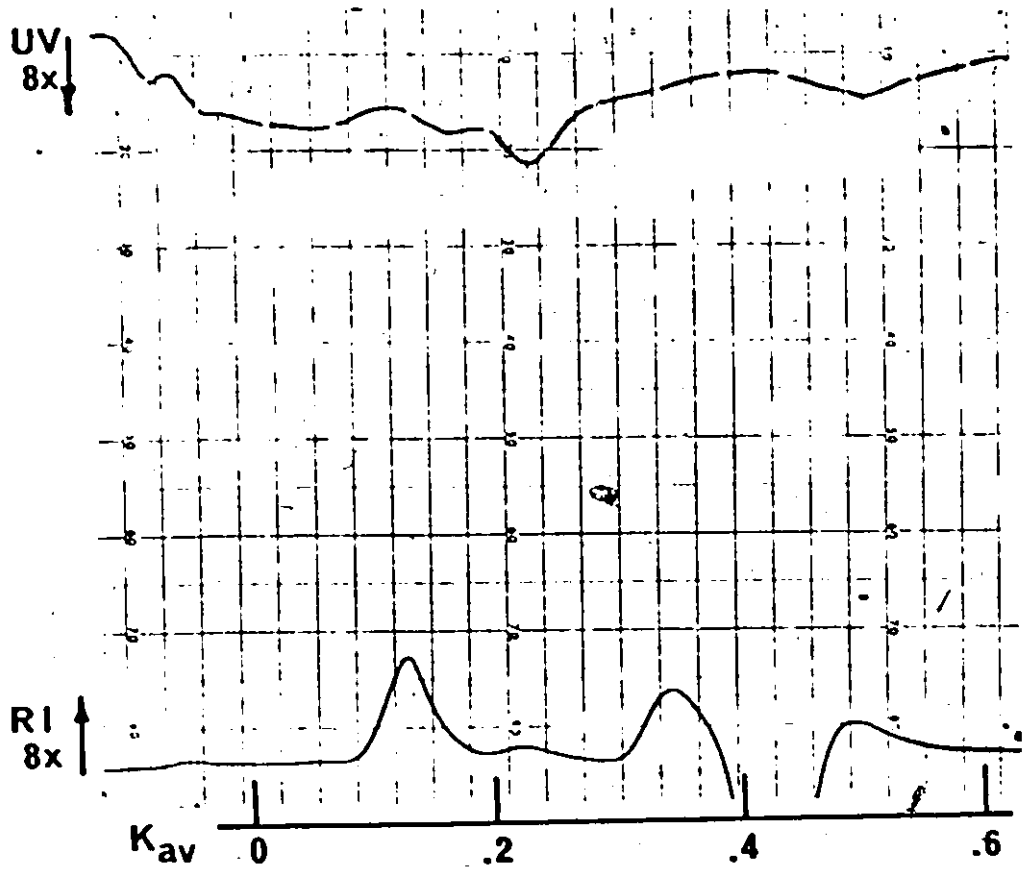


FIGURE 12. SEPHADEX G15 PROFILE OF CONCENTRATED SAMPLE 1 ELUTED WITH 0.04M NaCl (0.04 IONIC STRENGTH)



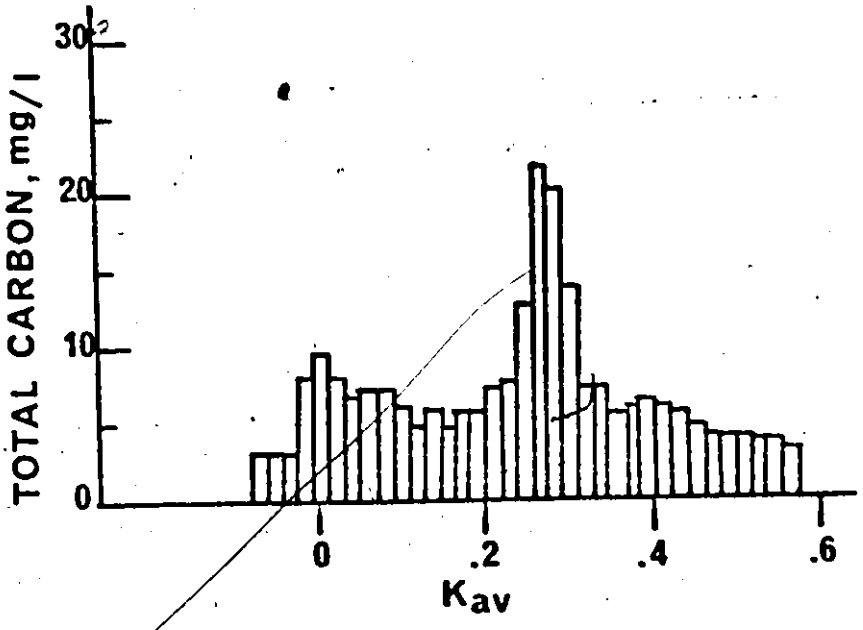


FIGURE 13. SEPHADEX G15 PROFILE OF CONCENTRATED SAMPLE 1 ELUTED WITH 0.1M NaCl (0.1 IONIC STRENGTH)

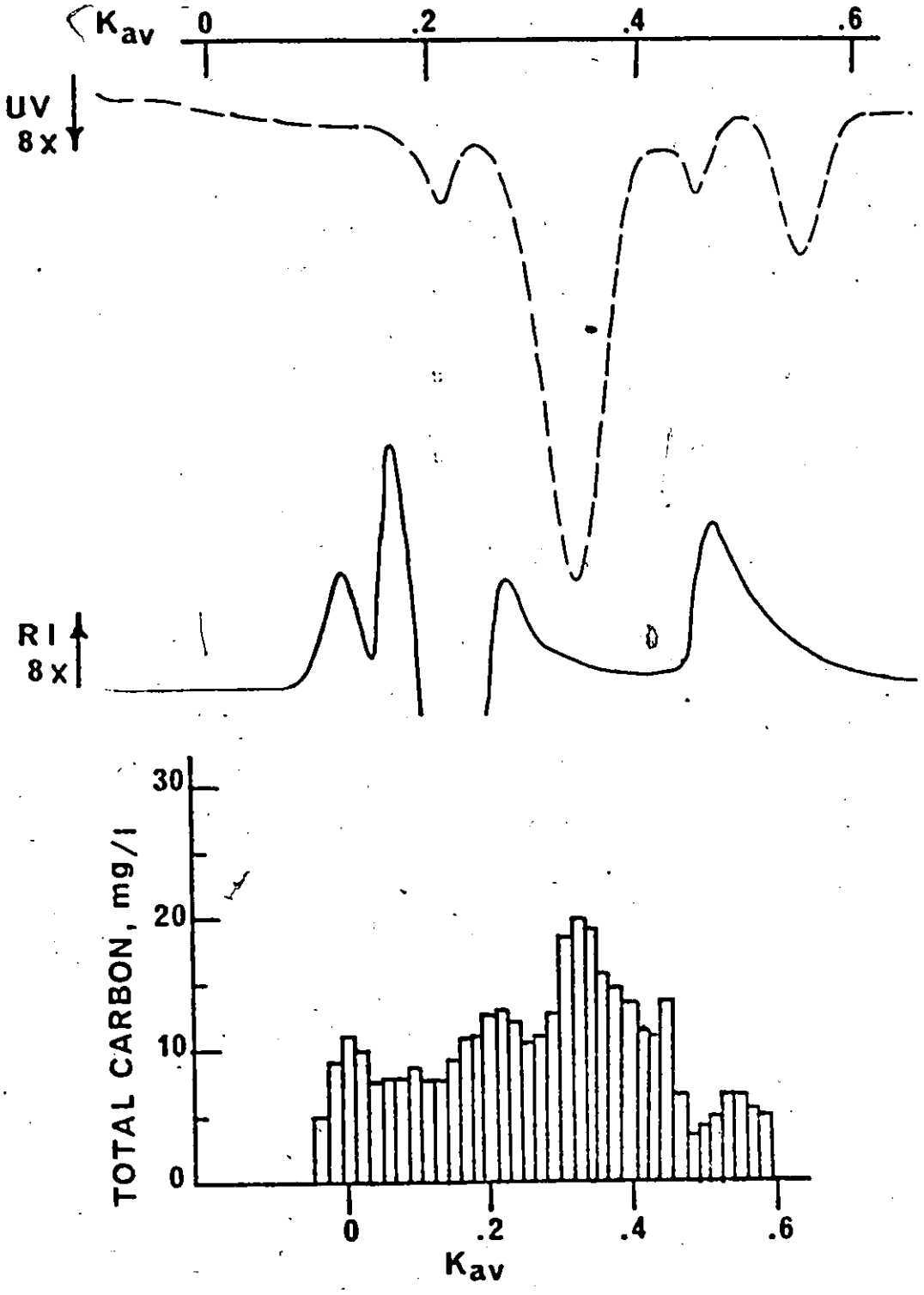


FIGURE 14. SEPHADEX G15 PROFILE OF CONCENTRATED SAMPLE 1 ELUTED WITH 0.03M Na_2SO_4 10.1 IONIC STRENGTH

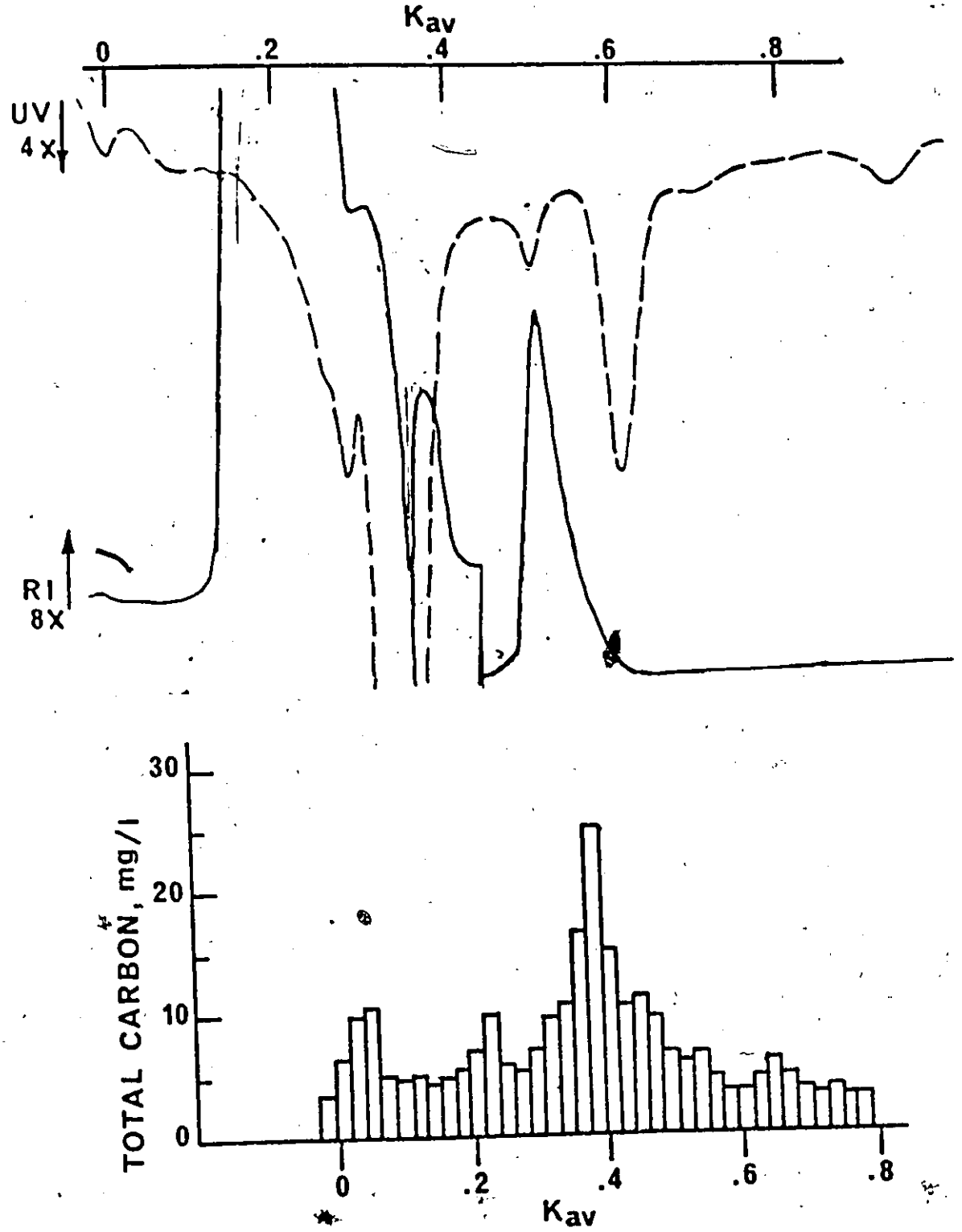


FIGURE 15. SEPHADEX G15 PROFILE OF CONCENTRATED SAMPLE 1 ELUTED WITH .025M KH_2PO_4 + .025M Na_2HPO_4 (0.1 IONIC STRENGTH)

TABLE 14 - COMPARISON OF THE SEPHADEX G15 ELUTION BEHAVIOR OF WASTEWATER SAMPLE 1 WITH DIFFERENT ELUANTS

ELUANT COMPOSITION	IONIC STRENGTH	ELUTION POSITION OF MAJOR ORGANIC PEAKS Kav	COMMENTS	ORGANIC RECOVERY, %
DISTILLED WATER	0	0, 0.15, 0.45		67
.04M NaCl	0.04	0, 0.3	2 smaller peaks between a Kav of 0.4 and 0.6.	63
0.1M NaCl	0.1	0, 0.3	Broad distribution of carbon before and after main peak. Poor correspondence between TOC and UV profiles.	47
0.025M KH_2PO_4 + .025M Na_2HPO_4	0.1	0, 0.4	A number of smaller peaks present.	66.5
0.035M Na_2SO_4	0.1	0, 0.2, 0.32	Broad distribution of TOC.	75

2. Peak resolutions and separation was not as good with inorganic eluants as with distilled water. Instead of two well-separated peaks at Kav's of 0.15 and 0.45 a single TOC peak at a Kav of 0.3 to 0.4 was obtained, along with a broad distribution of carbon either before or after this peak. Changes in the ionic composition of the eluant resulted in some differences in the TOC distribution. Increasing the concentration of NaCl in the eluant from 0.04M to 0.1M had only a slight effect on the TOC profile, but a major effect on the UV profile. With 0.1M NaCl eluant, UV peaks were indistinguishable and the UV trace did not correspond with the TOC profile. With the other eluants, the UV traces were different from each other, but peaks were distinguishable and corresponded quite well with the TOC profile. RI traces with each of the ionic eluants was basically non-informative.

It is doubtful that these changes in elution behavior are due to changes in molecular size, since these molecules should be relatively small and non-flexible. More likely, they are due to solute-gel interactions since the work with pure compounds revealed that neutral, non-interacting polysaccharides were unaffected by changes in eluant or sample compositions.

3. Recovery of organics with all eluants was considerably less than 100%, implying that there is irreversible adsorption of some organics to the gel or that a consistent measurement error exists. Retention of organics by the column seems probable, as with an accountable measurement error of roughly ± 5% (Appendix C), one would expect recoveries to be distributed between, 90 and 110%. Recoveries with all eluants were 63 to 75% with the exception of 0.1M NaCl which was 47%.

Lindqvist (1967) eluted a humic acid sample through Sephadex with 0.05M NaCl and found a strong adsorbance of low and high molecular weight material. He was able to elute some of the adsorbed material from the column by switching from the ionic eluant to distilled water. This procedure was tried with the wastewater sample that had been eluted with 0.1M NaCl. A UV peak of organic material was obtained when the water front broke through the column. It was equivalent to 200 µg of organic carbon or roughly 17% of a typical sample input. A similar UV peak was obtained when the same procedure was applied to a wastewater sample eluted with 0.035M Na_2SO_4. This is further evidence that ionic gradients can play an important part in the interaction of wastewater components with Sephadex.

Thus, contrary to the preliminary investigation with pure compounds, distilled water appears to be the best eluant to use with wastewater samples. While it is not known whether distilled water provides better estimation of the apparent molecular weight of the organics than an ionic background, it results in better peak resolution and separation. There is evidence that considerable solute-gel interactions can occur between Sephadex and some of the components in wastewater, and there is some question when using distilled water whether the elution of organics is influenced by inorganics in the sample.

Effect of Sample Inorganic Content

Samples of concentrated wastewater 3 were spiked with 2000 mg/l of bicarbonate, 500 mg/l of phosphate, 1000 mg/l of sodium chloride and 1000 mg/l of sulfate. These represent the major inorganic components in the wastewater (Table 3), and are expected to be the ones most affected by lime treatment and neutralization. The elution profiles of the original sample

and spiked samples are presented in Figures 16 to 19. A comparison reveals that UV(peaks closest to the inorganic peaks are most affected. With bicarbonate, the third UV peak is dispersed and broken up. The first two peaks are relatively unaffected. Phosphate, which elutes at an intermediate Kav value, caused the second UV peak to break up and part of the organics to elute at the same position as the phosphate peak. Less change in the UV elution profile was observed with sodium chloride and sulfate additions, perhaps because these inorganics elute at the same position as existing inorganic peaks or because the effect is very ion specific.

The results indicate that inorganics in the sample can strongly influence the elution positions of wastewater organics that can enter the gel. The mechanism is probably quite similar to either of those postulated to explain the anomolous behavior of proteins. The question that is raised is whether a true elution profile of organics can be obtained in the presence of inorganics. This could be confirmed by cross-checking the molecular weight distributions with alternate measurement techniques.

An attempt was made to cross-check molecular size ranges using ultrafiltration. The technique first was tested using the polysaccharide, inulin, and a UM05 membrane having a molecular weight cutoff of 500. The molecular weight distribution of inulin has been determined by Granath and Kvist (1967). Their distribution yielded a peak molecular weight of roughly 6000, with none of the molecular sizes extending below 3000 molecular weight. Despite this, 72% of the inulin sample passed through the membrane. For this reason, the method was thought to be too unreliable

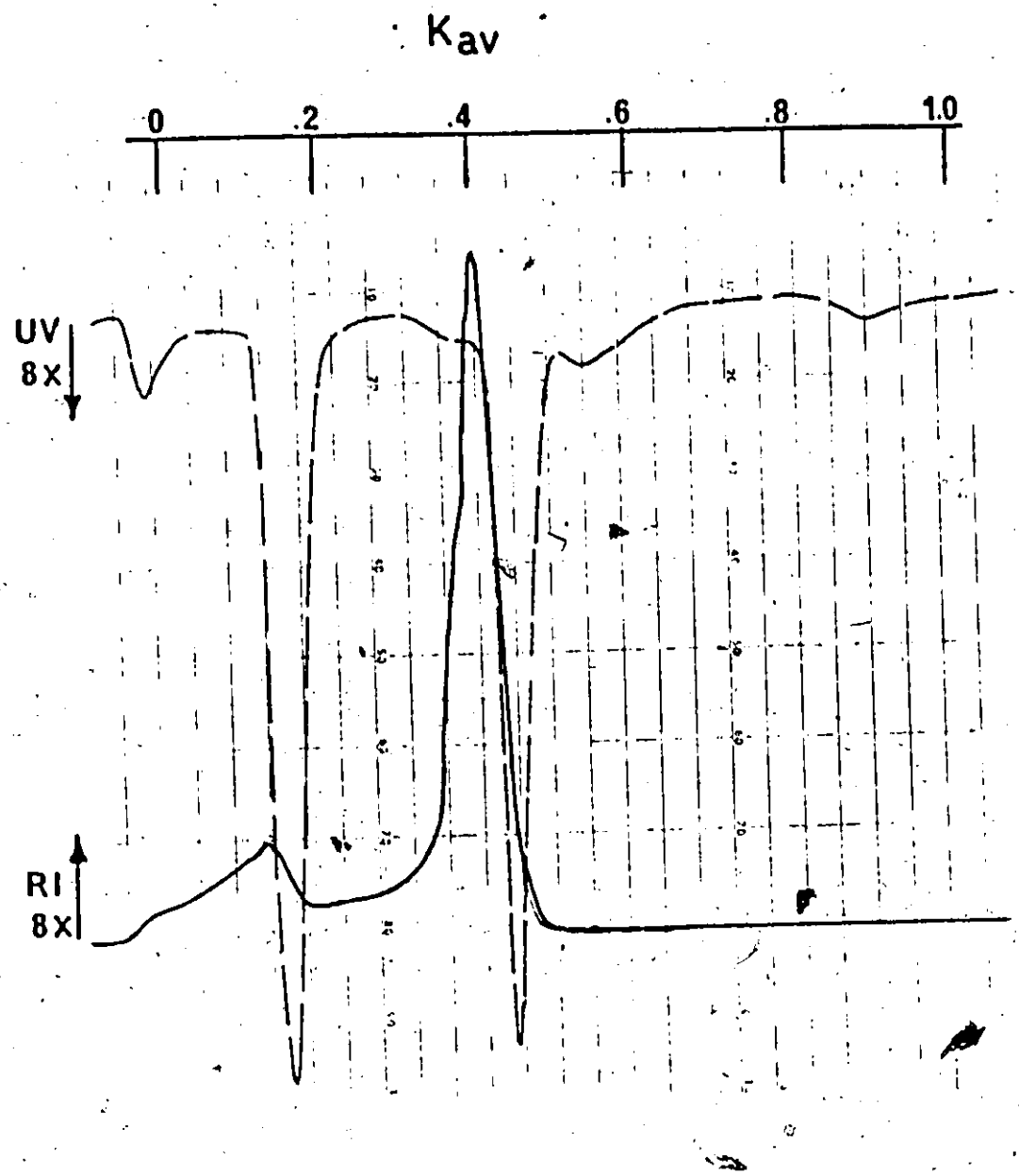


FIGURE 16. SEPHADEX G15 PROFILE OF CONCENTRATED SAMPLE 5 ELUTED WITH DISTILLED WATER

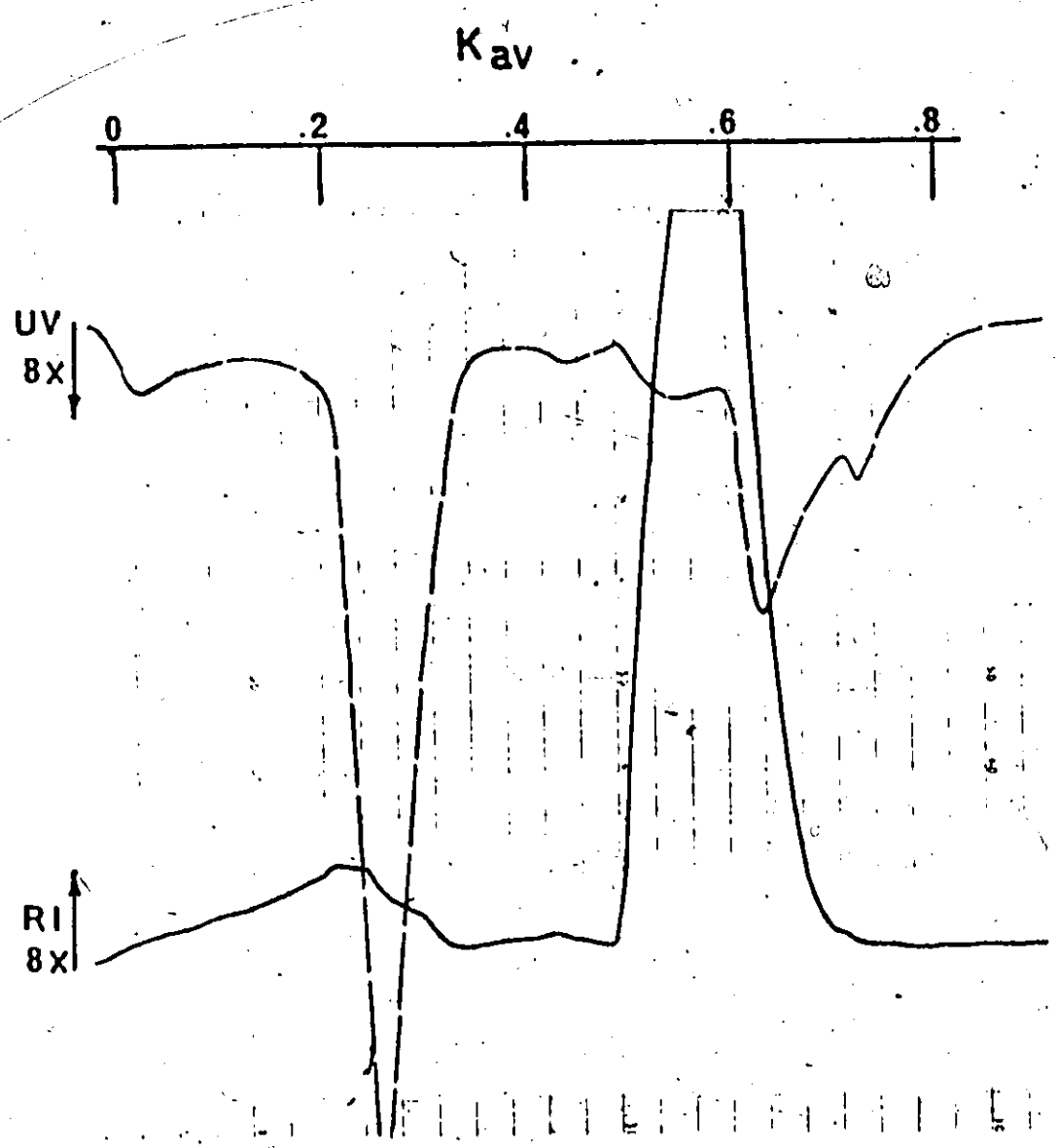


FIGURE 17. SEPHADEX G25 PROFILE OF CONCENTRATED SAMPLE A SPIKED WITH 2500 ppm NaHCO$_3$, (DISTILLED WATER ELUANT)

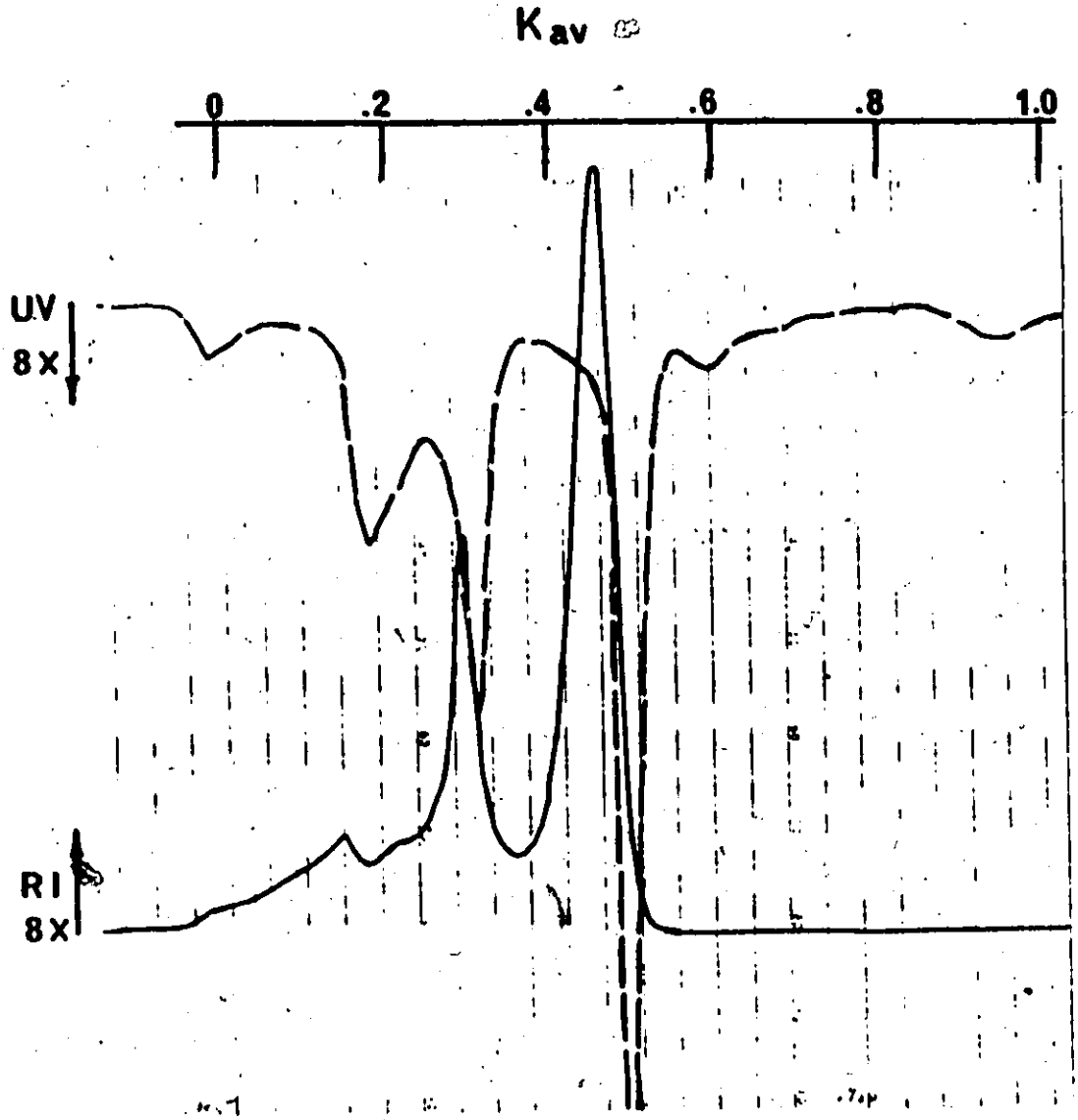


FIGURE 18. SEPHADEX G15 PROFILE OF CONCENTRATED SAMPLE 3 SPIKED WITH 2200 ppm KH_2PO_4 (DISTILLED WATER ELUANT)

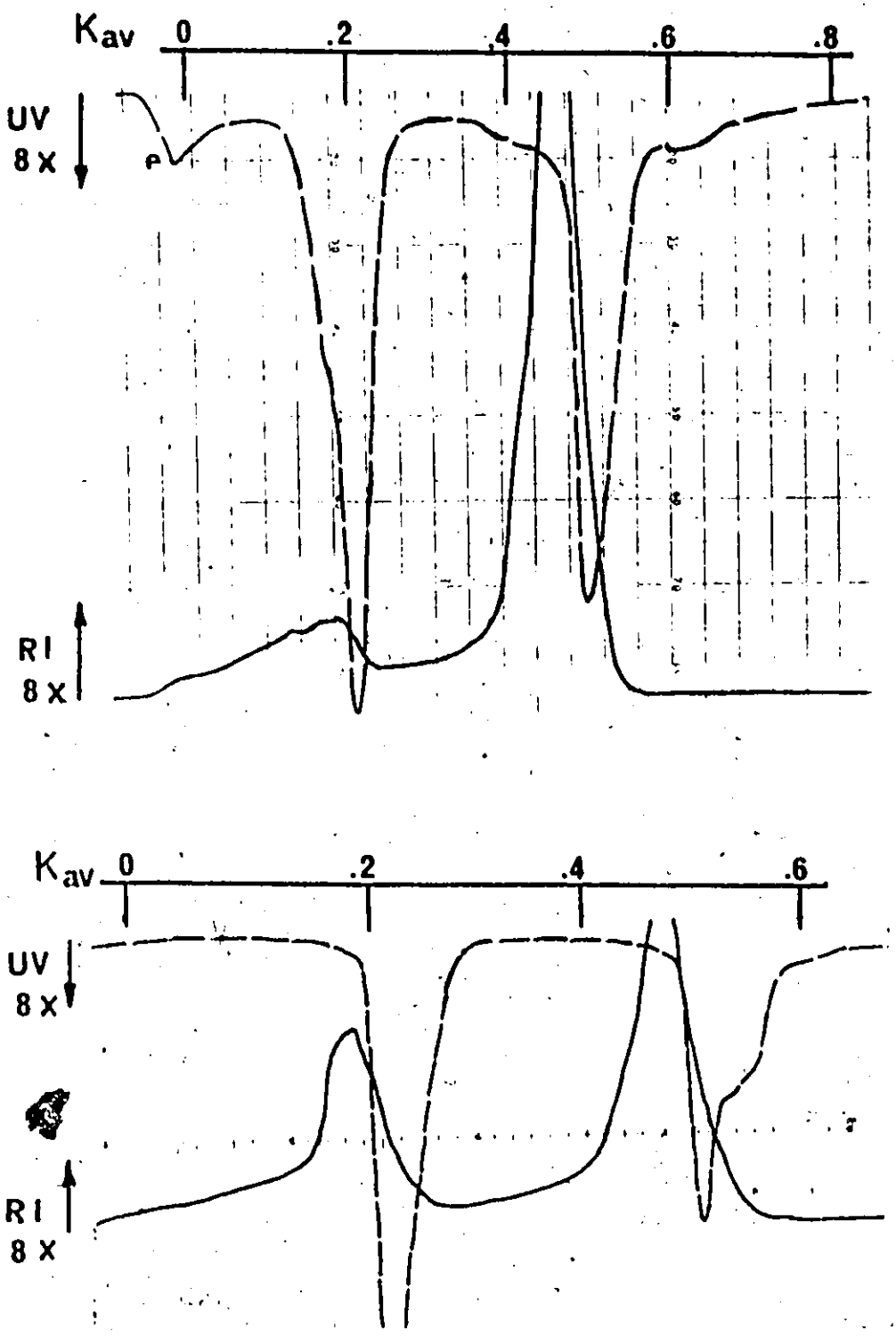


FIGURE 10. SEPHADEX G15 PROFILE OF CONCENTRATED SAMPLE 3 SPIKED WITH 1000 ppm NaCl (ABOVE), AND 1500 ppm Na_2SO_4 (BELOW) USING DISTILLED WATER ELUANT

to be applied to wastewater samples.

In summary, it can be stated that although a number of non-steric mechanisms appear to influence the elution of wastewater organics, a useful separation is obtained where one can ascertain the degree to which any of the organic peaks are affected by chemical treatment.

CHAPTER 5

5. RESULTS AND DISCUSSION

5.1 Chemical Treatment of Pure Compounds

Chemical treatment of some representative pure compounds was done for two reasons:

1. to observe any hydrolysis, precipitation or adsorption effects with sodium hydroxide or lime, and
2. to evaluate their GPC behavior before and after treatment and observe any changes in resolution, peak position or recovery.

Polysaccharides

Samples of dextran T40 and inulin were treated with dilute caustic at pH 12.6 and with strong acid and base. Samples that were eluted through the glass gel column were listed in Table 12 and samples eluted through the Sephadex G-75 column are listed in Table 15. Although the glass gel was found to be unsatisfactory for proteins or polysaccharides treated under stronger conditions, satisfactory results were obtained with polysaccharides treated under milder conditions. Recoveries with both gels were in the range of 70 to 90%.

Both dextran and inulin undergo complete hydrolysis under strong acid conditions. This is verified by complete recovery of hydrolysed material at the lower limit of gel separation. Under analogous base conditions both compounds are stable with elution results indicating that dextran remains completely intact and only a small percentage of inulin is broken down. Both compounds were unaffected by exposure to

TABLE 15. SEPHADEX G75 ELUTION BEHAVIOR OF CHEMICALLY TREATED POLYSACCHARIDES AND PROTEINS ELUTED WITH 0.04M NaCl

SAMPLE	TREATMENT CONDITIONS	TOC IN	TOC PEAK				TOC RECOVERY
		µg	Kav	µg	Kav	µg	%
DEXTRAN T40	Untreated	896	0.05	726			81
DEXTRAN T40	0.04M NaOH, 1 week	570	0.03	440			77
DEXTRAN T40	0.5N NaOH, 9 hour reflux	801	0.03	615			77
DEXTRAN T40	0.5N H_2SO_4, 9 hour reflux	819	0.91	706			86
INULIN	0.04M NaOH, 0 time	504	0.59	445			89
INULIN	0.02N NaOH, 3 hours, 77°C	988	0.59	632	0.95	216	86
INULIN	0.02N HCl, 3 hours, 77°C	921	0.91	800			87
CHYMOTRYPSINOGEN	NaCl added	765	0.32	650			85
CHYMOTRYPSINOGEN	0.04M NaOH, 0 time	341	0.33	250			73
CHYMOTRYPSINOGEN	0.04M NaOH, 1 week	488	0.23	191			37
CHYMOTRYPSINOGEN	N HCl, 24 hours reflux	525	0.97	467			89
RIBONUCLEASE	NaCl added	690	0.46	410			60
RIBONUCLEASE	0.04M NaOH, 1 week	606	*	350			58

*Kav broad and ill-defined.

pH12+ for one week.

Proteins

Chymotrypsinogen-A was subjected to strong acid treatment and dilute caustic treatment at pH 12.6 for one week. Ribonuclease-A was also subjected to dilute caustic treatment for one week. Sodium chloride was added to untreated samples as a control. The results are presented in Table 15.

With the chymotrypsinogen sample that had been acid hydrolysed, complete recovery of hydrolysed material at the lower limit of separation was obtained. Compared to the control samples, the samples that had been treated for one week with dilute caustic demonstrated either a much lower recovery (chymotrypsinogen), or broader peak distribution (ribonuclease). One would not expect the proteins to be hydrolysed under these conditions, but denaturation could easily occur. Denaturation was visibly evident with chymotrypsinogen when some of this protein precipitated out of solution (20% TOC reduction after filtering through a 0.45μ membrane). Denaturation could, no doubt, change the molecular size or result in the unfolding of buried groups which may be more susceptible to solute-gel interactions.

Urea

Urea was treated with dilute caustic for twenty-four hours. Results of the elution of treated and untreated samples with Sephadex G15 are listed in Table 16.

TABLE 16 - SEPHADEX G15 ELUTION BEHAVIOR OF TREATED AND UNTREATED UREA SAMPLES

TREATMENT CONDITIONS	ELUTION POSITION K_{AV}	RECOVERY, %
None	0.63	95.3
.04M NaOH, 24 hours	0.68	102

Both samples yielded single peaks on the RI trace with good TOC recovery. However, elution volumes were greater than the lower limit of separation as defined by NaCl indicating reversible adsorption of urea on the gel. Baker and Porath (1967) also found that urea was reversibly adsorbed on Sephadex. As a result, one cannot form any definite conclusion on the effect of high pH on urea from GPC analysis.

Humic Acid

Profiles of humic acid eluted through the Sephadex G-75 and G-15 columns are presented in Figures 20 and 21. Both profiles show a single peak at the exclusion and another at the lower limit. Similar profiles were obtained by Posner (1963) and Gjessing (1965) in the elution of humic acid with distilled water. The peaks obtained are not representative of the actual molecular weight distribution of humic acid because the excellent separation and resolution is primarily due to ion exclusion and adsorption rather than steric mechanisms (Chapter 2). Posner (1963) showed that when humic acid is eluted with an ionic background resolution is poor and a broad distribution of organic material is obtained. In this respect, there is some analogy between the GPC behavior of humic acid and that of wastewater organics (Chapter 4).

When lime as $Ca(OH)_2$ was added to a dilute humic acid solution, humic acid was removed presumably as a precipitated calcium salt. This presumption is based on Rebhun and Manka's (1971) findings that a large proportion of the humic material in secondary effluent was precipitated from solution as the calcium salt when the sample was concentrated. At low lime dosage (pH 10.2), very little color change was observed. At an intermediate lime dosage (pH 11.7), good removal was obtained as the

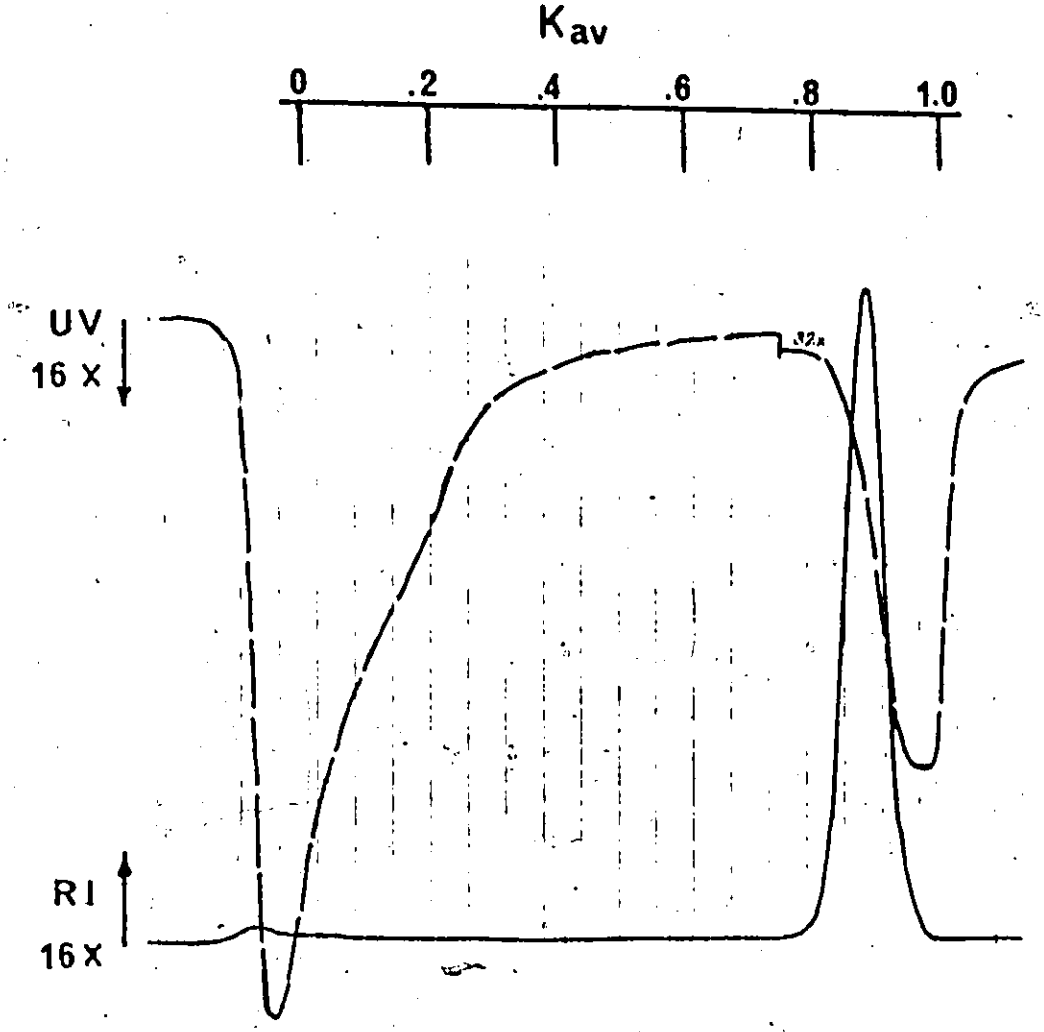


FIGURE 20. SEPHADEX G75 PROFILE OF HUMIC ACID ELUTED WITH DISTILLED WATER

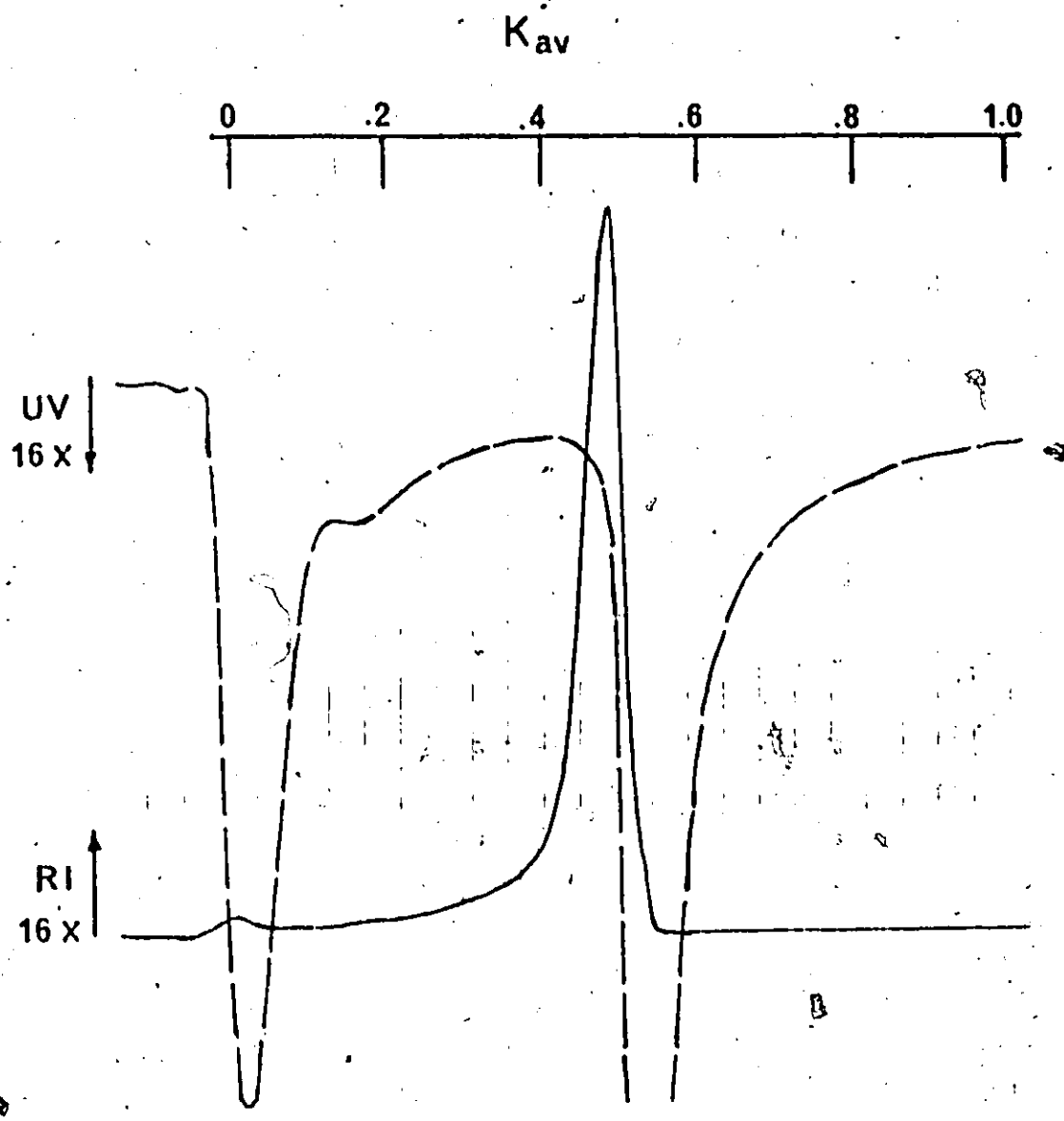


FIGURE 21. SEPHADEX G15 PROFILE OF UMIC ACID ELUTED WITH DISTILLED WATER

color changed from dark brown to light yellow. With a lime dosage of 2000 mg/l (pH 12+), all of the humic acid was precipitated as the solution went from dark brown to clear and the TOC dropped from 29 mg/l to 2.5 mg/l.

Ribonucleic Acid (RNA)

The results of the exposure of yeast RNA to lime at pH10 and pH12+ are summarized in Table 17. The degree of hydrolysis was determined by a comparison of treated RNA samples to unhydrolysed and completely hydrolysed samples. It was assumed that at these dilute concentrations (initial concentration of RNA was 135mg/l) the solution obeyed Beer's law so that concentration is directly proportional to UV absorbance. Since any unhydrolysed RNA was precipitated out using TCA, adsorbance figures were corrected for TCA absorbance.

The results indicate that RNA was completely hydrolysed at the higher lime dosage even after one hour of treatment. At the lower dosage only 38% had been hydrolysed after twenty-four hours.

Summary

Results of the treatment of pure compounds with caustic or lime indicate that:

1. Polysaccharides are extremely stable to alkali conditions and treatment with base does not alter their elution behavior.
2. Proteins are stable in dilute caustic (pH 12+) at room temperature but can undergo denaturation. This can result in precipitation or alteration of their elution behavior.
3. Urea is reversibly adsorbed to Sephadex so that treatment

TABLE 17. TREATMENT OF RNA WITH LIME

TREATMENT CONDITIONS	MEASUREMENT DILUTION	ABSORBANCE AT 260 nm.	ABSORBANCE CORRECTED FOR TCA	HYDROLYSIS %
UNHYDROLYSED	1:1	0.64	0	0
COMPLETELY HYDROLYSED *	10:1	0.37	0.29	100
pH 10, 1 hour	1:1	0.70	0	0
pH 10, 24 hours	10:1	0.19	0.11	38
pH 12-(1500 mg/l $Ca(OH)_2$), 1 hour	10:1	0.37	0.29	100
pH 12- (1500 mg/l $Ca(OH)_2$), 24 hours	10:1	0.40	0.32	100
5% TCA	1:1	0.8		
5% TCA	10:1	0.08		

* By exposure of RNA to 0.5N KOH for 16 hours at 35°C.

effects are inconclusive.

4. At higher lime doses (2000 mg/l), humic acid can be completely precipitated from solution, but is only slightly affected at lower doses (pH 10).
5. At higher lime doses (1500 mg/l), RNA is completely hydrolysed after one hour. Lower pH conditions require much longer time periods for any significant breakdown to occur.

5.2 Lime Treatment of Domestic Wastewater

The major interest in this study was the effect of lime treatment on the "soluble" organics in the wastewater; however, other parameters such as phosphate and turbidity removal were investigated since these would generally dictate the practical level of treatment. Other measurements taken were wastewater alkalinity, as alkalinity has been correlated with lime dosage requirements by other investigators, and the conductivity of the concentrated samples as this parameter may have some bearing on GPC behavior.

To determine the lime requirements for different pH levels, standard curves were made up (Figures 22 and 23), and approximate dosages were taken from these curves. As the curve leveled off sharply after pH 11.5, large doses were required to reach a pH level of 12+. As pH levels much above 12 cannot be measured, a lime dosage of 2000 mg/l was used to assure that this high pH level would be attained. Lime doses at other pH levels were mainly a function of the alkalinity, or buffering capacity, of the wastewater. The pH at the lower doses dropped somewhat over the twenty-four hour test period, probably due to CO_2 absorbed from the air.

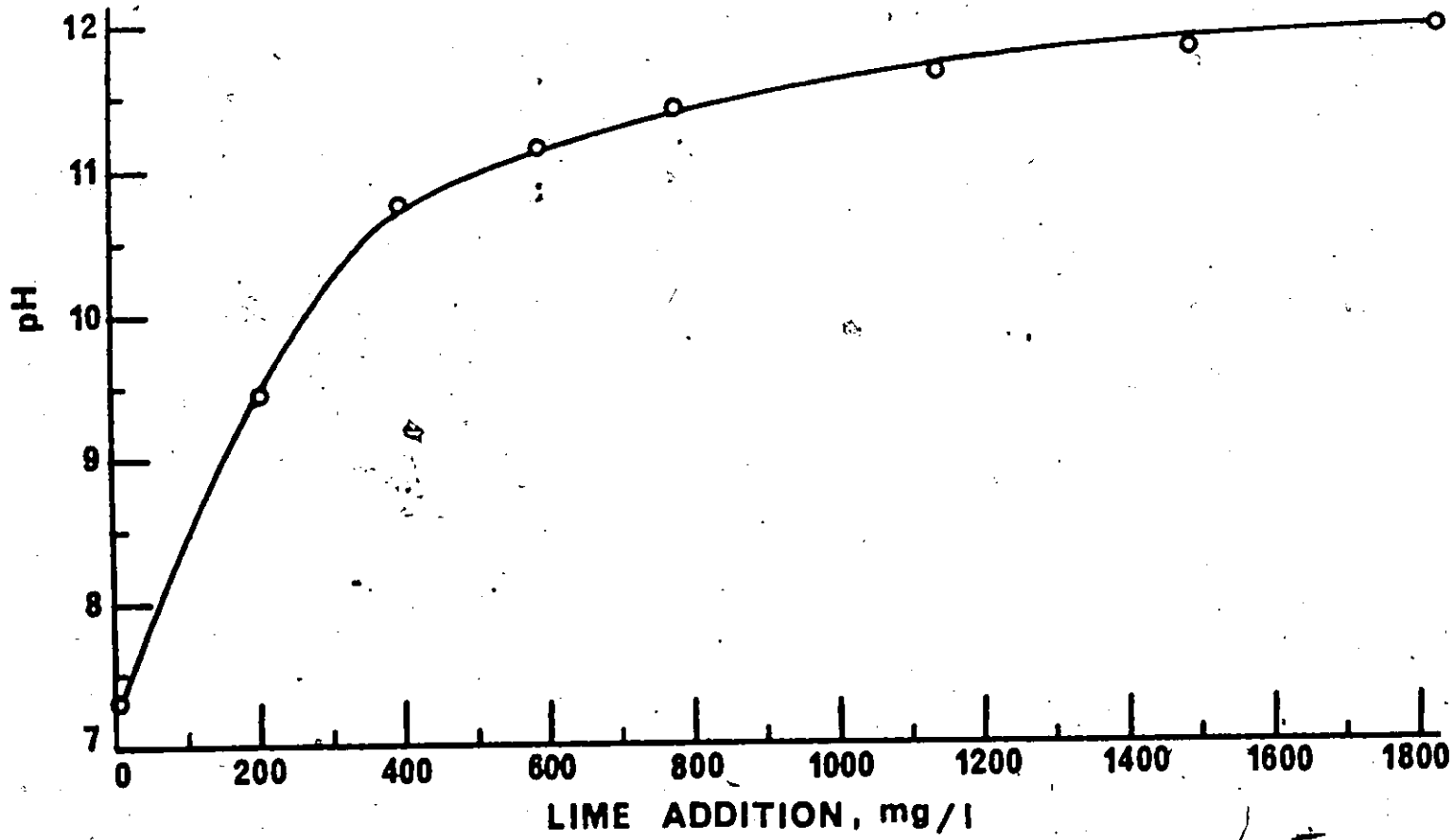


FIGURE 22. PLOT OF pH AGAINST LIME ADDITION FOR WASTEWATER SAMPLE 2

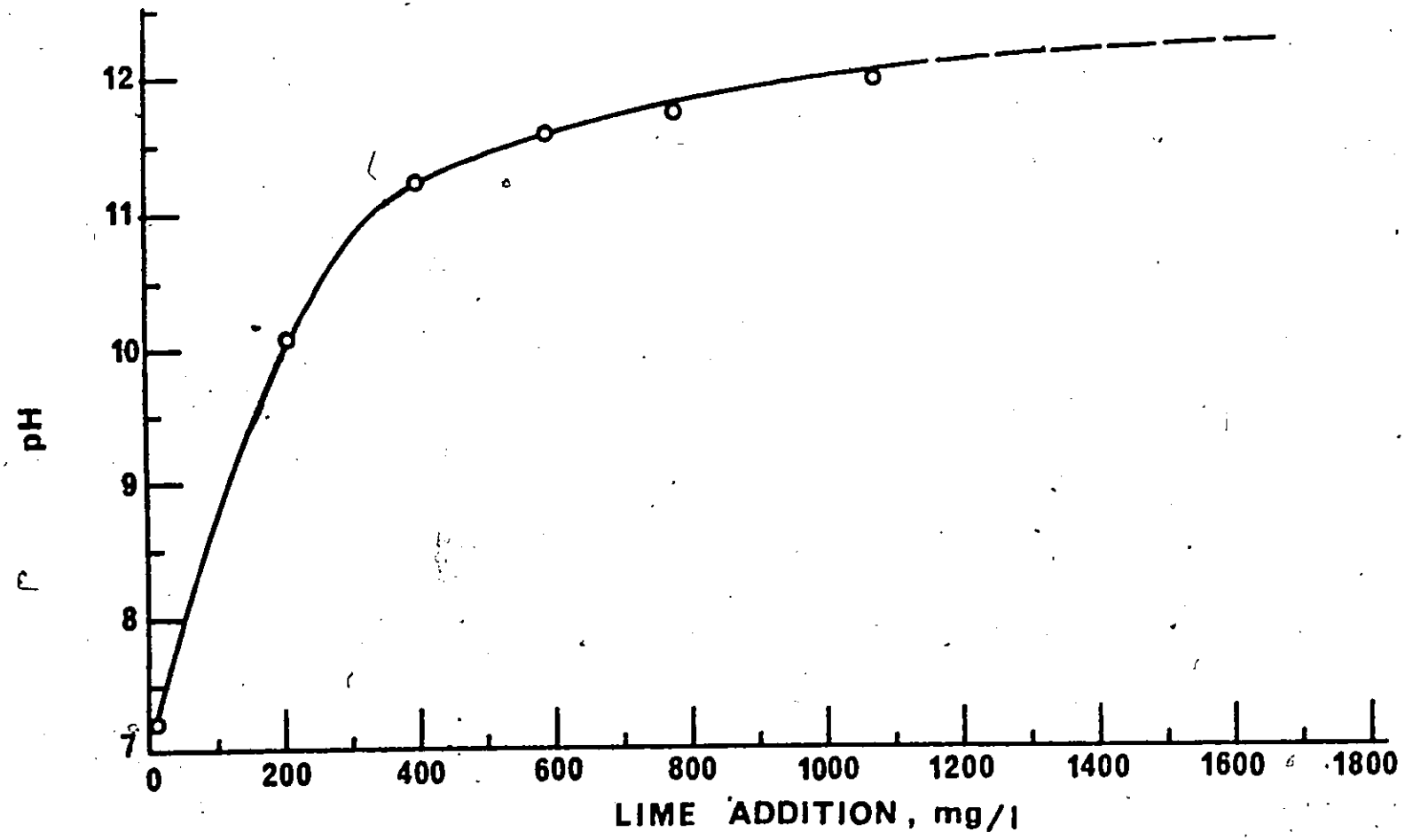


FIGURE 23. PLOT OF pH AGAINST LIME ADDITION FOR WASTEWATER SAMPLE 3

Results of the coagulation of waste samples 2 and 3 are summarized in Tables 18 and 19. There are notable differences in some of the removals obtained with sample 2 as compared with sample 3; but the trends are similar.

Some general comments on the lime coagulation of wastewater are:

1. sludge filterability was excellent at the higher pH's and poor at the low pH,
2. the majority of the floc in all the jars settled within five to ten minutes, and
3. color decreased with increasing lime dosage.

Turbidity Removal

This provided a qualitative assessment of suspended solids removal. With samples treated for one hour, turbidity removal varied from 70% to 95%. With sample 2, maximum turbidity was removed at the medium pH; with sample 3, maximum turbidity was removed at the high pH.

Phosphate Removal

With sample 2 treated for one hour, excellent removals were obtained at all pH levels. With sample 3 treated for one hour, maximum removal was obtained at the medium pH.

Both turbidity and phosphate removal figures agree favorably with those obtained by other investigators (Table 6). The results provide little incentive for increasing the pH above 11 or the detention time above one hour.

Organic Carbon Removal

With sample 2 the organic level in the untreated sample was lower than the one treated for one hour at low pH. This was believed to be due

TABLE 14. LIME TREATMENT OF WASTEWATER SAMPLE 2

	UNTREATED RAW	1 HOUR TREATMENT			24 HOUR TREATMENT		
		LOW pH	MED.pH	HIGH pH	LOW pH	MED.pH	HIGH pH
LIME DOSE, mg/l		280	560	2000	280	560	2000
INITIAL pH	7.3	9.6	10.9	12+	9.6	10.9	12+
FINAL pH		9.6	10.9	12+	8.6	10.7	12+
TC BEFORE CONCENTRATION, mg/l	27.6	30.0	25.2	22.8	18.8	29	22.8
TC ALLOWING FOR DILUTION, mg/l	27.6	30.5	25.9	25.4	19.1	29.8	25.5
TC REMOVAL, %*		0	13.7	15.3	36.4	0	15
TC AFTER CONCENTRATION, mg/l	232	240		235	226		246
CONCENTRATION FACTOR	8.42	7.87		9.08	11.8		9.65
RESIDUAL TOTAL PHOSPHATE, mg/l	4.7	0.4	0.4	0.3	1.7	0.4	0.2
PHOSPHATE REMOVAL, %		91.5	91.5	93.5	63.8	91.5	95.7
RESIDUAL TURBIDITY mg/l SiO_2	84	12	4	23	27	19	16
TURBIDITY REMOVAL, %		85.8	95.3	72.7	67.7	77.4	80.8
CONDUCTIVITY AFTER CONCENTRATION, mMHO	12.8	9.7	13.0	35.5	15.3	12.0	32.0

Alkalinity of wastewater = 262 mg/l as $CaCO_3$.

* Based on initial TC of 30 mg/l.

TABLE 19. LIME TREATMENT OF WASTEWATER SAMPLE 3

	UNTREATED RAW	1 HOUR TREATMENT			24 HOUR TREATMENT		
		LOW pH	MED.pH	HIGH pH	LOW pH	MED.pH	HIGH pH
LIME DOSE, mg/l		200	390	2000	200	390	2000
INITIAL pH		9.85	11.25	12+	9.85	11.25	12+
FINAL pH		9.85	11.25	12+	8.9	10.45	12+
TC BEFORE CONCENTRATION, mg/l	32	29.5	18.7	22.5	23.5	30.5	23.5
TC ALLOWING FOR DILUTION, mg/l	32	29.8	19.2	24.9	23.8	31.1	26
TC REMOVAL, %		6.9	40	22.2	25.6	2.8	18.8
TC AFTER CONCENTRATION, mg/l	208	210		214	214		246
CONCENTRATION FACTOR	6.5	7.04		8.60	9.0		9.45
RESIDUAL TOTAL PHOSPHATE, mg/l	5.15	1.7	0.3	0.9	1.05	0.38	0.22
PHOSPHATE REMOVAL, %		67	94	82.5	79.5	92.8	95.7
RESIDUAL TURBIDITY mg/l SiO_2	98	28.5	15	5	22	5	1
TURBIDITY REMOVAL, %		70.5	84.7	95	77.5	95	99
CONDUCTIVITY AFTER CONCENTRATION, mMHO	6.0	5.8	9.0	32	8.0	5.0	26.5
pH AFTER CONCENTRATION	5.85	5.85	5.85	5.85	5.90	6.15	5.95

Alkalinity of wastewater = 202 mg/l as $CaCO_3$

to microbial degredation in the untreated sample as the time for filtration extended for two to three hours after the initiation of lime treatment. Since results with sample 3 indicated that very little of the organics are removed after one hour of treatment at low pH, the initial TC level was assumed to be 30 mg/l.

Organic removals showed a strong dependence on pH and detention time. Removal figures varied from 0 to 40% and are intermediate to figures obtained by Suess (1970) and by Brownstein and Murphy (1972). Maximum removal with sample 2 was obtained at low pH after twenty-four hours of treatment; and with sample 3 at medium pH after one hour of treatment. In general, removals increased with detention time at low pH; decreased with detention time at medium pH and stayed relatively constant at high pH.

To explain this behavior, we must examine the underlying mechanisms of removal; namely, the adsorption onto surfaces of the precipitate formed; and the direct precipitation and coprecipitation of the organics with the inorganic precipitates. Quite possibly, the removals observed are the result of a combination of these two mechanisms. The predominant mechanism would be expected to be pH and time dependent for the following reasons.

1. The type and amount of precipitate in this system is very pH dependent, so that the quantity and nature of the available surfaces for adsorption or coprecipitation would differ at each pH level.
2. The density and sign of the surface charge is highly pH dependent. The PZC values of some of the precipitates formed are listed in Table 20. Note that surfaces are positively charged at pH's below their PZC and

negatively charged at pH's above their PZC. Since the PZC values varied from 6.9 for hyroxyapatite up to 12.9 for calcium hydroxide, a variable amount of both positive and negative surface would be encountered at all three pH levels.

3. Work with pure compounds has indicated that precipitation of organics as insoluble salts or by denaturation can be very pH dependent.

4. Destabilization and coagulation, charge reversal due to specific adsorption, hydration, and incomplete precipitation would be responsible for changes in the nature, quantity and charge of available surfaces with time.

TABLE 20. POINT OF ZERO CHARGE (PZC) OF SOME IONIC SOLIDS (AFTER PARKS, 1967)

OXIDE	PZC
Hydroxyapatite (Hydrous)	6.9
Calcite	8.9
Hydrous CaO	12.9
Hydrous MgO	12.5

There was concern over the possibility of biological growth in the low pH sample after twenty-four hours. Comparison of results obtained with the non-inhibited sample and that inhibited with $HgCl_2$ are presented in Table 21.

TABLE 21. COMPARISON OF INHIBITED AND NON-INHIBITED LIME-TREATED WASTEWATER SAMPLES.

	Non-Inhibited Sample	Sample Inhibited with 200 mg/l $HgCl_2$
Lime added, mg/l	220	260
Initial pH	9.75	9.75
pH after 24 hours	8.60	9.20
TOC after 24 hours, mg/l	43.5	43.5
Viable cells as determined by plate count	3.4×10^7	2.8×10^3

Pike and Carrington (1972) found that the number of viable cells in settled domestic wastewater averaged 6.3×10^6 cells per ml. Thus, it is evident that although a pH level of 9 to 10 may inhibit bacterial activity, it is not sufficient to kill viable cells, since the number is roughly the same as in untreated wastewater. While the pH of the non-inhibited sample dropped more than the inhibited sample, identical TOC's verified the absence of bacterial degredation.

Gel Filtration Analysis of Lime-Treated Wastewater Samples

The UV, RI and TOC profiles resulting from the elution of untreated and lime-treated samples 2 and 3 are presented in Appendix D. As with previous wastewater fractionations, three organic peaks at Kav elution positions of 0, 0.15 and 0.45 were obtained, corresponding to apparent molecular weights of 1500 +, 1000 and 200 respectively. To determine if any of these peaks were selectively affected, it was neces-

sary to compare the organic content of each peak on the same basis. This was done in the following way:

The TOC profile was divided into three regions. The first region included the first peak and extended to a Kav of 0.105; the second region included the second peak and extended from a Kav of 0.105 to 0.290; the third region included the third peak and extended beyond a Kav of 0.290. The organic content in each region was calculated and samples were adjusted to the same concentration factor and TOC recovery as the untreated samples (Appendix E).

The results of this analysis are summarized in Table 22. Sample 3 showed little change after treatment for one hour at low pH. With sample 2, the difference between the untreated sample and the sample treated for one hour at low pH is likely due to biological degredation in the untreated sample as discussed previously. Treatment at low pH for twenty-four hours selectively decreased the amount of carbon in region 3, while treatment at high pH for one hour or twenty-four hours eliminated organic material in region 1 and lowered the amount of carbon in region 2. The amount of carbon in region 3 remained at the same level. These changes could not be accounted for simply by increased $CaCl_2$ content as adjustment of sample 2 conductivity to the level of the high pH samples using $CaCl_2$ did not alter the organic carbon distribution.

In considering the significance of these results, it is important also to look at the mechanisms which can alter the distribution of organics, namely:

1. removal of organics by selective adsorption,
2. selective removal of organics by precipitation or coprecipitation,

TABLE 22. EFFECT OF LIME TREATMENT ON THE ORGANIC CARBON PROFILE OF WASTEWATER SAMPLES 2 AND 3

SAMPLE	µg ORGANIC CARBON			TOC Recovery,%	ADJUSTED µg ORGANIC CARBON*		
	REGION 1	REGION 2	REGION 3		REGION 1	REGION 2	REGION 3
UNTREATED RAW 2	143	398	401	67.7	143	398	401
RAW 2-$CaCl_2$	115	327	446	64.0	122	346	472
LOW 1 HOUR	113	333	592	72.0	113	333	592
LOW 24 HOUR	102	438	314	63.0	78	337	241
HIGH 1 HOUR	0	288	510	58.5	0	320	565
HIGH 24 HOUR	0	242	520	51.7	0	277	593
UNTREATED RAW 3	68	251	403	57.8	68	251	403
LOW 1 HOUR	35	230	366	50.0	37	246	392
LOW 24 HOUR	76	383	415	68.0	47	235	255
HIGH 1 HOUR	0	275	694	75.5	0	159	401
HIGH 24 HOUR	0	224	625	57.5	0	154	430

* Adjusted to same concentration factor and TOC recovery.

3. hydrolysis or breakdown of organics from high to low molecular weight, and
4. shifts in organic distribution as a result of inorganics added to, or removed from the sample.

It is difficult to separate out these effects, but some do seem more probable than others. Shifts caused by inorganics had little effect on excluded organics. Also, from the work with pure compounds, hydrolysis does not seem probable. The most likely cause for changes in organic distribution is selective adsorption and precipitation.

Although the level of organics in region 3 remained the same after high pH treatment, the possibility of hydrolysis cannot be completely ruled out. For example, the high molecular weight material could have been selectively precipitated or adsorbed, and the low molecular weight material unaffected. On the other hand, some of the low molecular weight material could have been selectively precipitated or adsorbed and replaced by higher molecular weight material that was hydrolysed or shifted. Similar considerations apply to other samples.

To separate out these different effects, the following procedures could be tried.

1. To determine how much organic carbon was removed by direct adsorption, precipitates such as calcite, hydroxyapatite and magnesium hydroxide could be added, and TOC removal measured at different pH levels.
2. The change in the inorganic content of wastewater samples after lime treatment could be measured, and the effect on the elution behavior of organics assessed using a range of pure compounds.
3. To determine if there were shifts from high to low molecular weight

as a result of hydrolysis or changes in inorganic content, the untreated sample could be fractionated and the two higher molecular weight peaks recovered. This organic material then could be reconcentrated to the original level and treated with lime. Other possibilities are acidifying to recover organics that had been removed by precipitation or adsorption before GPC analysis; and the use of a clean system such as sodium hydroxide to prevent adsorption.

Summary

From the viewpoint of turbidity or phosphate removal, there is no incentive to increasing pH above 11 or detention time above one hour. Operation at lower pH's would be less efficient and result in a sludge which is difficult to dewater.

The effect of lime treatment on the "soluble" organics is selective. Maximum organic removal was obtained at the medium pH level (11.2) after one hour of treatment and at the low pH level after twenty-four hours of treatment. Since high molecular weight organics (1500+) represent only a small percentage of the total (10 to 15%) their selective removal or hydrolysis at high pH would not seem to justify the much greater sludge disposal and neutralization problems which would be created. Moreover, the benefits of hydrolysis, even if it is realized, are questionable. In activated carbon treatment, as pointed out by Weber (1970), the higher molecular weight material should be more hydrophobic and, therefore, have a greater tendency for adsorption on activated carbon. This may, in fact, be one of the reasons for its selective removal in lime treatment. With ultrafiltration or reverse osmosis higher molecular weight material also would be easier to remove. In biological treatment, smaller molecular

weight material may be more easily assimilated, but this would depend very much on the compound structure. The much higher inorganic content as a result of high pH treatment and neutralization could have an inhibiting effect on the microorganisms.

Since organic removal appears to be more sensitive to pH than turbidity or phosphate removal, the possibility exists that the pH level may be chosen to optimize carbon removal. Before optimizing, more should be known about the underlying precipitation or adsorption mechanisms.

CHAPTER 6

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

1. Zuckerman and Molof's contention that high molecular weight organics in domestic wastewater such as proteins and polysaccharides are hydrolysed by lime treatment at 12.2 pH is incorrect. Work with pure compounds has indicated that polysaccharides are extremely stable to alkali treatment, and that proteins also are stable, but can be precipitated or altered in shape as a result of denaturation. Humic acid is completely precipitated from solution at higher lime doses. One of the few high molecular weight compounds which would be susceptible to hydrolysis under these conditions is ribonucleic acid (RNA).

Even if hydrolysis by high pH lime treatment were realized, its contribution to advanced treatment processes or biological treatment processes is of questionable value.

2. A more probable explanation for the absence of high molecular weight material in domestic wastewater after high pH lime treatment is selective adsorption of the organics onto inorganic precipitates or precipitation of organics as a result of denaturation or salting out.

3. As much as 40% of the "soluble" organics in wastewater was removed by lime treatment. Removal is highly pH and time dependent.

4. Elution of concentrated wastewater samples with distilled

water through the Sephadex G-15 column yielded three organic carbon peaks corresponding to apparent molecular weights of 1500+, 1000 and 200. There was a good correspondence between the UV and TOC elution profiles. Organic material of 1500+ represented 10 to 15% of the total. When the wastewater was treated with lime at high pH for one or twenty-four hours, organic material in the highest molecular weight peak dropped to zero, decreased in the second peak and remained at the same level in lowest molecular weight peak. In contrast, treatment at low pH resulted in no change after one hour and a drop in the organic content of the lowest molecular weight peak after twenty-four hours.

5. Jar tests indicate that lime treatment at pH 11 with one hour detention can provide greater than 80% turbidity removal and greater than 90% phosphate removal. Lower doses would result in less efficient removal and a sludge which is difficult to dewater. Higher doses would result in about the same removal at higher cost.

6. Porous glass gel, even after coating with polyethylene glycol can exhibit adsorptive and ion exchange properties.

7. Even with Sephadex gel, many organics exhibit non-steric effects. Behavior of a protein sample eluted with distilled water was found to be highly dependent on the inorganic content of the sample. Consistent behavior and good resolution was obtained with an eluant of low ionic strength. In contrast, best resolution and elution behavior of wastewater samples was obtained with distilled water eluant. With wastewater, non-steric effects such as reten-

tion of organics by the column and dependence on sample inorganic content were observed.

6.2. Recommendations

1. The degree to which the fractionation of wastewater organics is influenced by solute-gel interactions should be ascertained. This can be done by checking the molecular weight distributions using measurement techniques such as ultracentrifugation or light scattering. When using these techniques, reconcentration and refractionation as with humic acid may be required.

Eaker and Porath's (1967) technique for eliminating the cation exchange capacity of Sephadex by washing the gel with pyridine should also be tried and its effect on organic elution evaluated.

2. A variety of pure compounds should be used to confirm the mechanism, and assess the impact of changes in sample inorganic content on the elution of organics. The effect on the elution behavior of altering the inorganic composition of the wastewater sample using ion exchange should also be investigated.

3. A preliminary investigation into alternate gel materials and methods for characterizing wastewater organics should be conducted. Methods which seem promising and have been used by other investigators for characterizing wastewater or natural water organics are ion exchange chromatography (Katz, 1972), and pyrolytic gas chromatography (Nelson and Lysyj, 1969).

4. The mechanisms involved in altering the molecular weight distributions of wastewater organics by lime treatment should be isolated using techniques outlined in Chapter 5. In particular, the nature

of organic removal should be established so that maximum reduction in organic content can be achieved.

5. To evaluate the representativeness of batch-scale studies such as this, samples should be compared to those collected from plant-scale runs.

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APPENDIX A

ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

AMW	apparent molecular weight
TOC	total organic carbon
BOD	biological oxygen demand
SS	suspended solids
TC	total carbon
IC	inorganic carbon
UV	ultraviolet
RI	refractive index
ml	milliliter
mg/l	milligrams per liter
O.D.	optical density
mMho	millimhos
TCA	trichloroacetic acid
ppm	parts per million

SYMBOLS

Å	angstrom
μ	micron
mu	millimicron
%	percent
>	greater than
<	less than
≃	approximately equal to
=	equal to

APPENDIX B

SAMPLE CALCULATIONS

Sample Calculation of Solution pH

Consider the dissolution of NaOH in distilled water so that the concentration is 0.04M in NaOH:

Assuming complete dissociation, $[Na+] = 0.04$

Charge balance yields, $[Na+] + [H+] = [OH-]$

For water, $[H+][OH-] = 10^{-14}$

$$0.04 + [H+] = \frac{10^{-14}}{[H+]}$$

Assuming that $[H+]$ is small in comparison to 0.04,

$$\therefore [H+] \quad 2.5 \times 10^{-13}$$

and, $pH = -\log [H+] = 12.6$

Sample Calculation of Eluant Ionic Strength

Consider the eluant that consists of 0.035M $NaSO_4$ in distilled water.

From Butler (1964),

Ionic Strength $= I = 1/2 \sum CiZi^2$

where Ci = concentration of each ion

Zi = ionic charge

Therefore, $I = 1/2\ [Na+]\ (1)^2 + (SO^4]\ (2)^2$

$= 1/2\ (0.070)\ (1) + (0.035)\ (4) = 0.10$

APPENDIX C

SAMPLE CARBON BALANCE AND ERROR ESTIMATION

Table C-1 presents the analyses of fractions collected from the elution of wastewater sample Raw 2 through the Sephadex G15 column.

Errors were estimated using procedures outlined in Barry (1964):

For the multiplication of two quantities,

$(A \pm Ea) \times (B \pm Eb)$

$$Eprod = \pm\sqrt{(EaB)^2 + (EbA)^2}$$

where, A = TOC, mg/l

B = fraction volume, ml

AB = TOC, µg.

Ea is assumed to be $\pm$ 1 mg/l for TOC values less than 10 mg/l and $\pm$2 mg/l for TOC values greater than 10 mg/l.

Eb is assumed to be $\pm$0.2 mls.

For addition of errors,

$$E\ sum = \pm\sqrt{E_1^2 + E_2^2 \ldots + En^2}$$

For the difference of 2 numbers,

$$E\ diff = \pm\sqrt{E_1^2 + E_2^2}$$

For division, $(A \pm Ea) \div (B \pm Eb)$

$$E\ quotient = \frac{1}{B}2\sqrt{(EaB)^2 + (EbA)^2}$$

TOC Out

TOC out = (1005.4 $\pm$ E sum) µg - µg Base TOC

E sum = $\sqrt{1404}$ = 37.5

Base TOC = 0.5 $\frac{mg}{l}$ x 6.1 x 10^{-3} l x 25 samples

= 76.3 µg

TABLE C-1. ANALYSIS OF FRACTIONS COLLECTED FROM THE G15 ELUTION OF WASTEWATER SAMPLE 2

FRACTION NUMBER	VOLUME mls.	TOC mg/l	TOC µg	(Eprod.)^2
19	6.0	1.2	7.2	36.0
20	6.0	5.1	30.6	37.0
21	6.0	9.6	57.6	39.6
22	6.0	6.0	36.0	37.4
23	6.1	3.0	18.3	37.6
24	6.1	2.4	14.6	37.5
25	6.1	3.0	18.3	37.6
26	6.1	3.3	20.1	37.7
27	6.1	4.2	25.6	37.8
28	6.1	31.6	192.3	190.0
29	6.0	17.0	102.0	155.6
30	6.0	3.6	21.6	36.5
31	6.0	2.8	16.8	36.4
32	6.0	2.4	14.4	36.3
33	6.1	3.2	19.5	37.6
34	6.15	3.9	24.0	39.1
35	6.1	4.2	25.6	37.8
36	6.1	5.7	34.8	38.4
37	6.2	5.7	35.3	39.7
38	6.2	8.3	51.5	41.4
39	6.2	27.6	171.1	184.2
40	6.2	6.0	37.2	39.9
41	6.2	1.4	8.7	38.5
42	6.2	1.1	6.8	38.5
43	6.2	1.4	8.7	38.6
44	6.1	1.1	6.8	37.2
			1005.4	1403.9

APPENDIX C Cont'd.

Error = $\pm\sqrt{37.2 \times 25} = \pm 30.5$

Therefore, TOC out = (1005.4 $\pm$ 37.5) µg - (76.3 $\pm$ 30.5) µg

E diff = $\pm\sqrt{1404 + 930} = \pm 48.3$

Therefore, TOC out = 929.1 $\pm$ 48.3 ug.

TOC IN

A = TOC of concentrated wastewater sample = 232 mg/l

B = sample volume applied = 6 ml.

Ea is assumed to be $\pm$ 10 mg/l and Eb is assumed to be $\pm$ 0.2 ml.

Therefore, TOC in = $1392 \pm \sqrt{(60)^2 \pm (46.4)^2}$

$= 1392 \pm 77.0$ ug

Fraction recovered = $\frac{92.9 \pm 48.3 \text{ ug}}{1392 \pm 77.0 \text{ ug}}$

Equotient = $\frac{1}{(1392)^2}\sqrt{(7.16 \times 10^4)^2 + (6.73 \times 10^4)^2}$

$= .051$

Therefore, fraction recovered = 0.668 ± 0.051

APPENDIX D

SEPHADEX G15 ELUTION PROFILES OF WASTEWATER SAMPLES 2 AND 3 TREATED WITH LIME

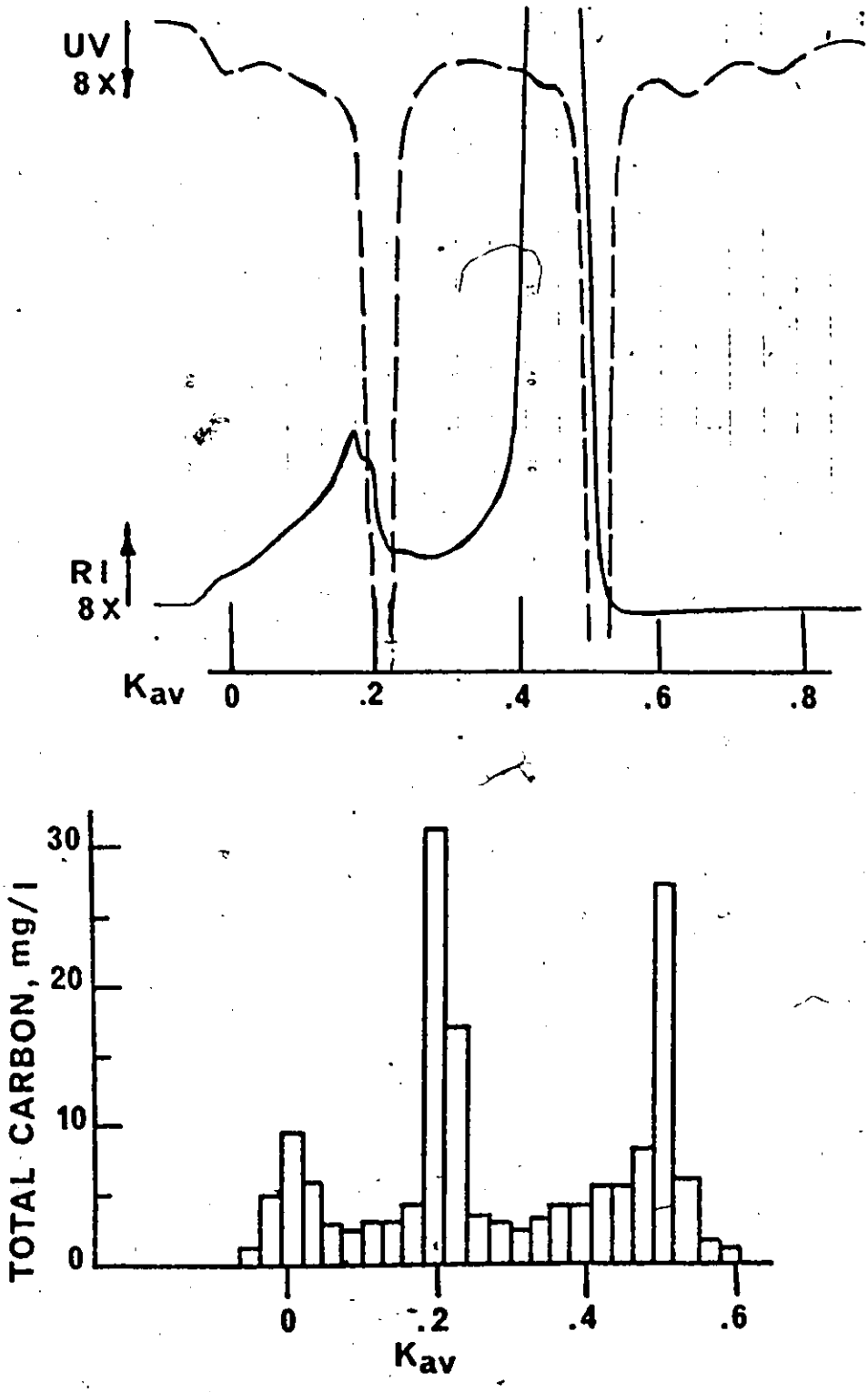
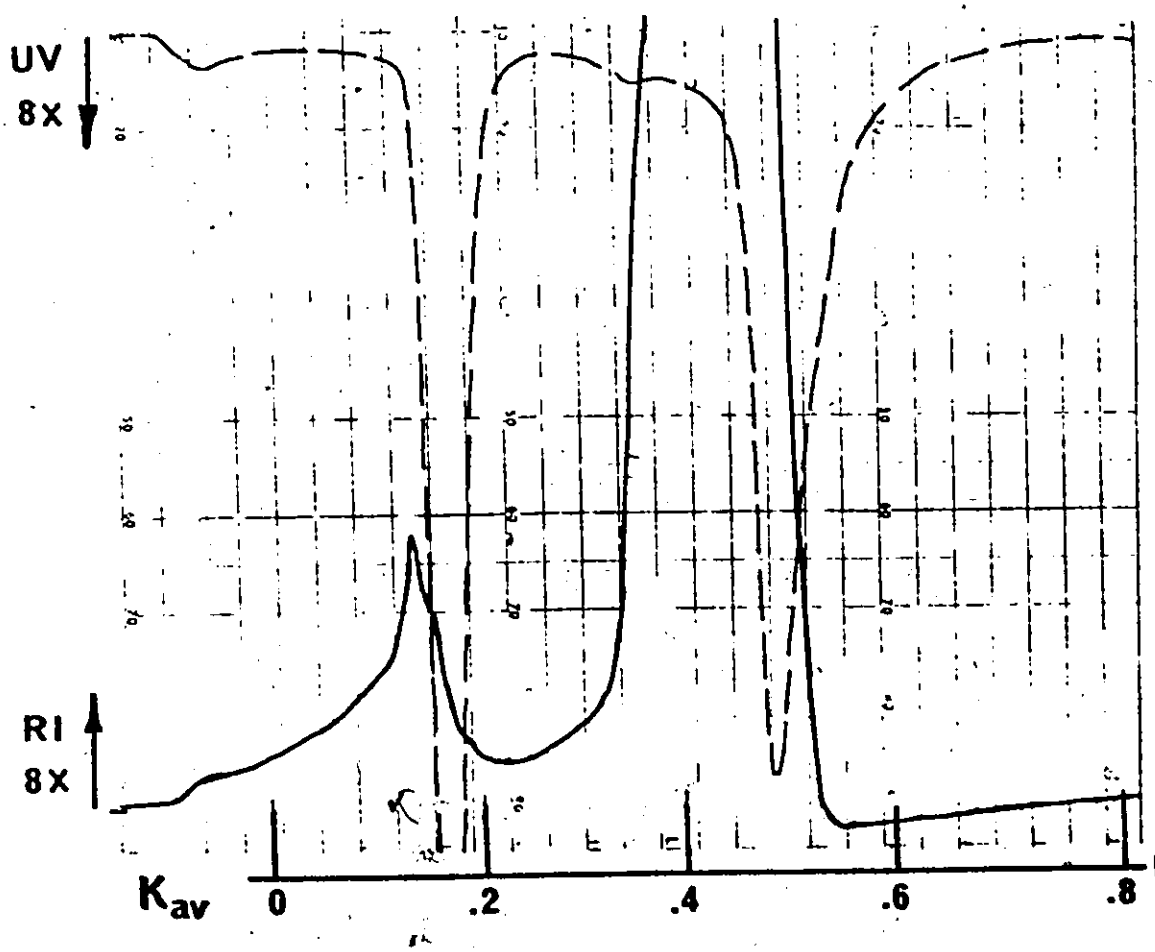


FIGURE D1. SEPHADEX G15 PROFILE OF CONCENTRATED SAMPLE 2 ELUTED WITH DISTILLED WATER



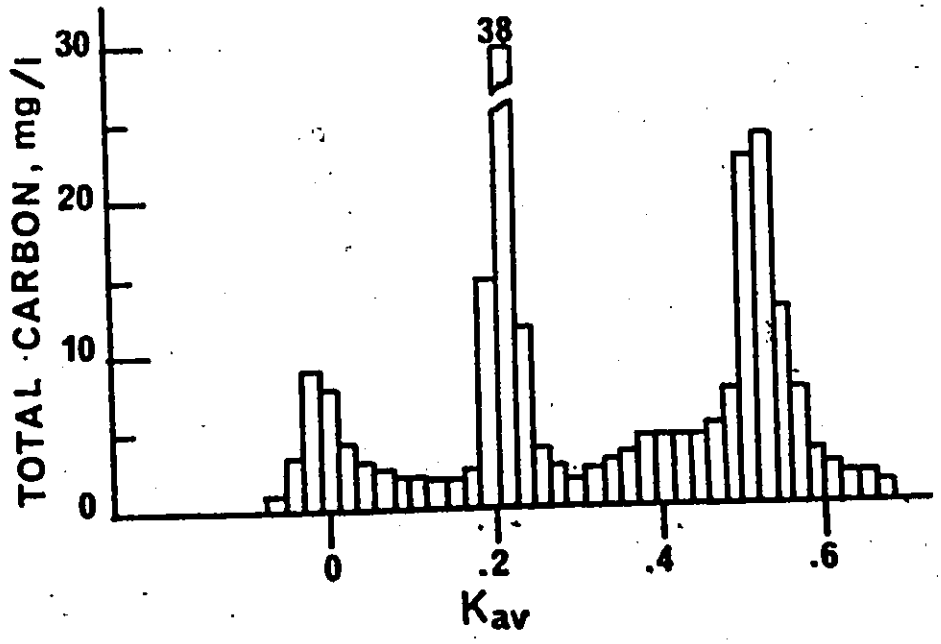


FIGURE D2. SEPHADEX G15 ELUTION PROFILE OF CONCENTRATED SAMPLE 2 + $CaCl_2$ ELUTED WITH DISTILLED WATER

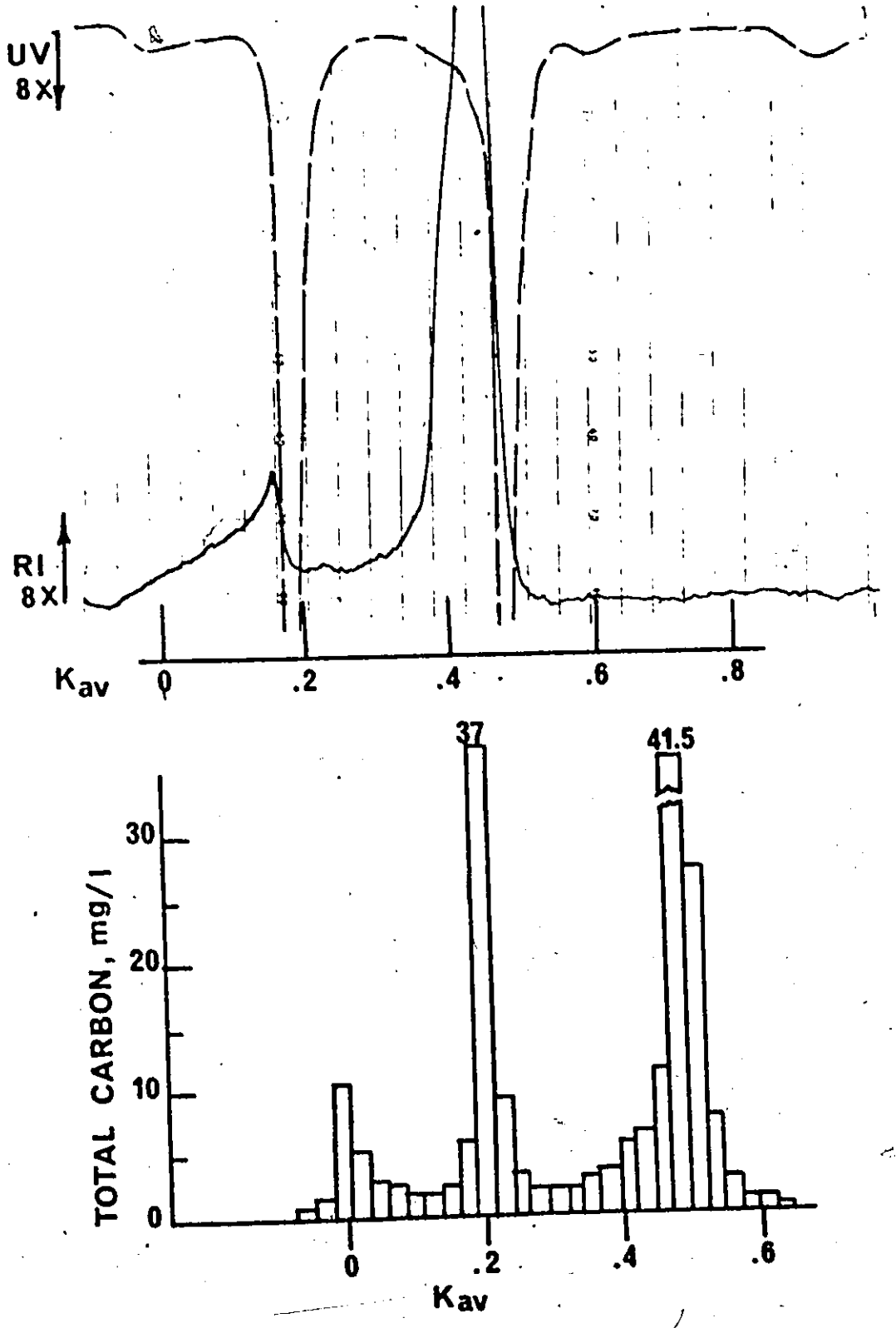
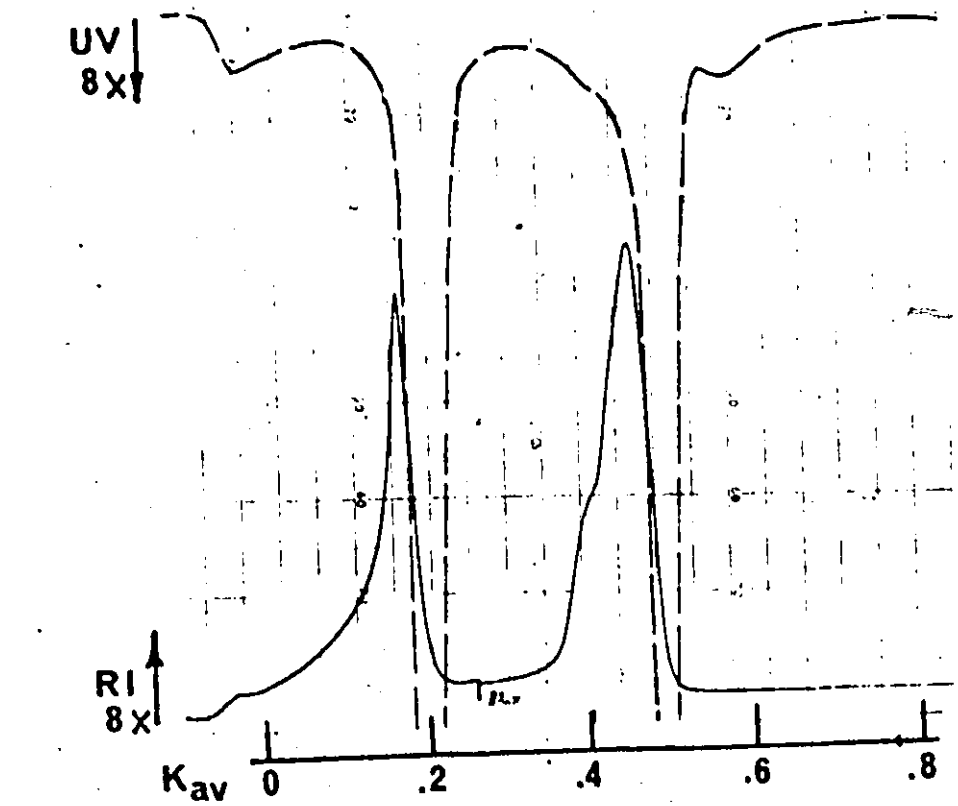


FIGURE D3. SEPHADEX G15 PROFILE OF CONCENTRATED SAMPLE 2, LIME-TREATED FOR 1 HOUR AT LOW pH (DISTILLED WATER ELUANT)



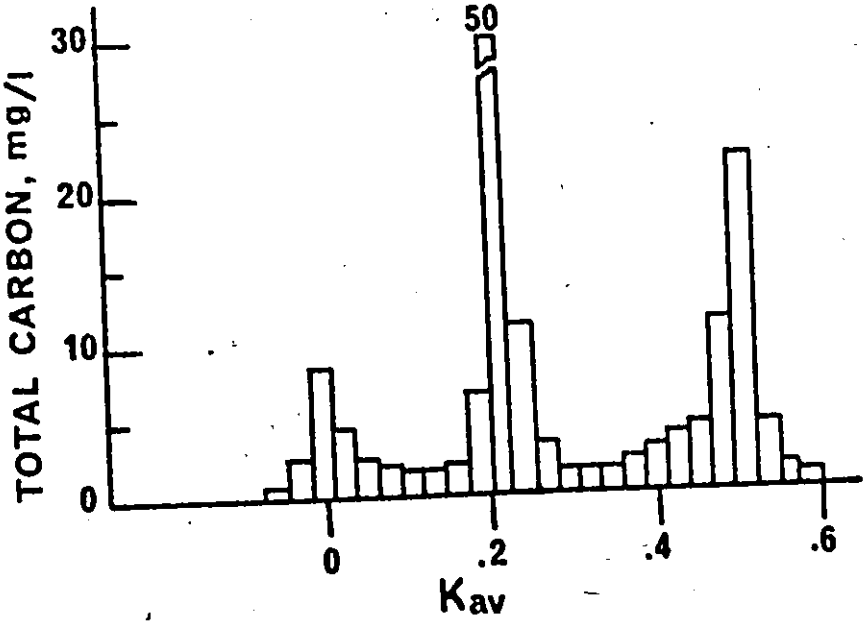


FIGURE D4. SEPHADEX G15 PROFILE OF CONCENTRATED SAMPLE 2, LIME-TREATED FOR 24 HOURS AT LOW pH (DISTILLED WATER ELUANT)

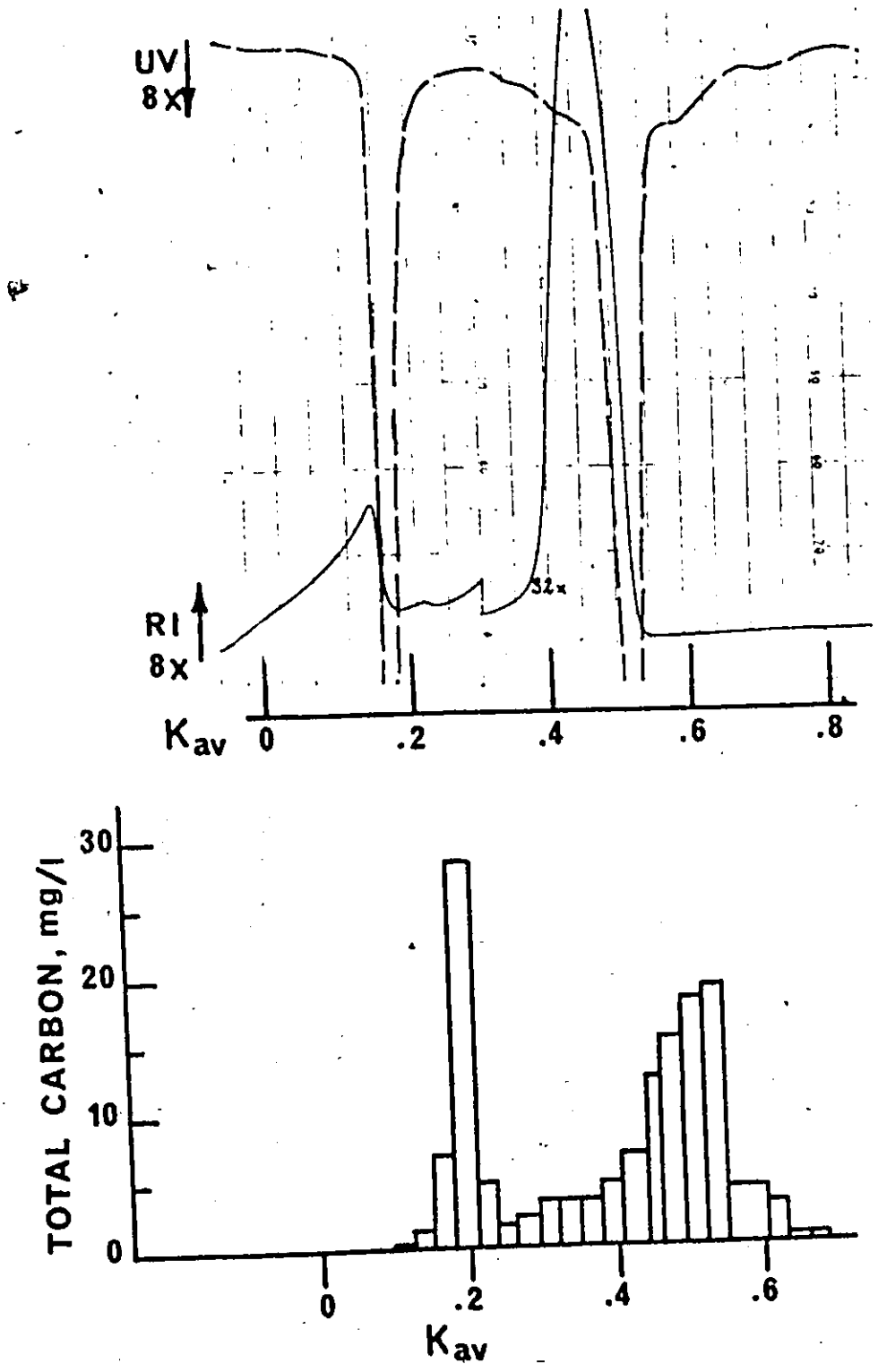


FIGURE D5. SEPHADEX G15 PROFILE OF CONCENTRATED SAMPLE 2, LIME-TREATED FOR 1 HOUR AT HIGH pH (DISTILLED WATER ELUANT)

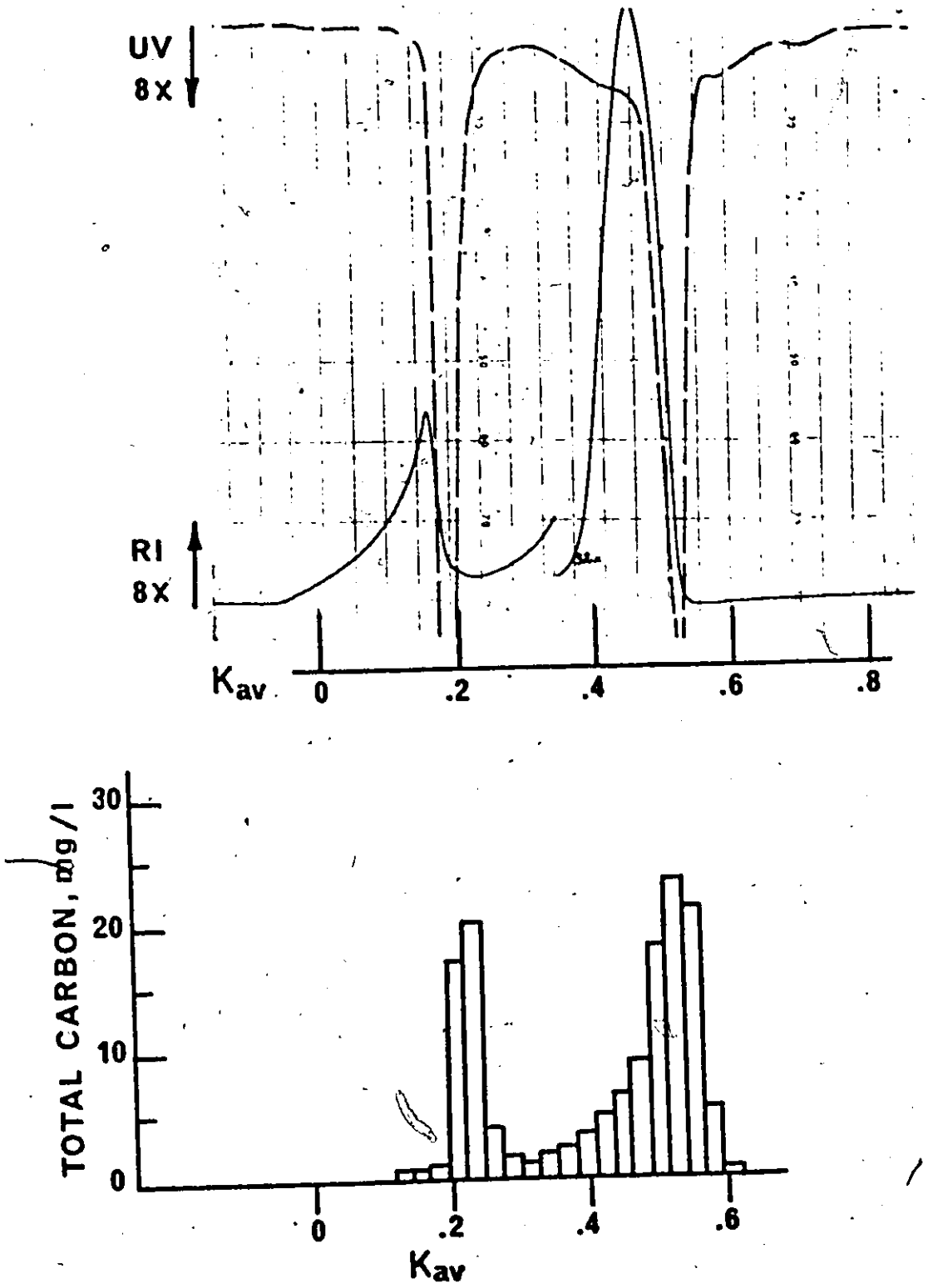
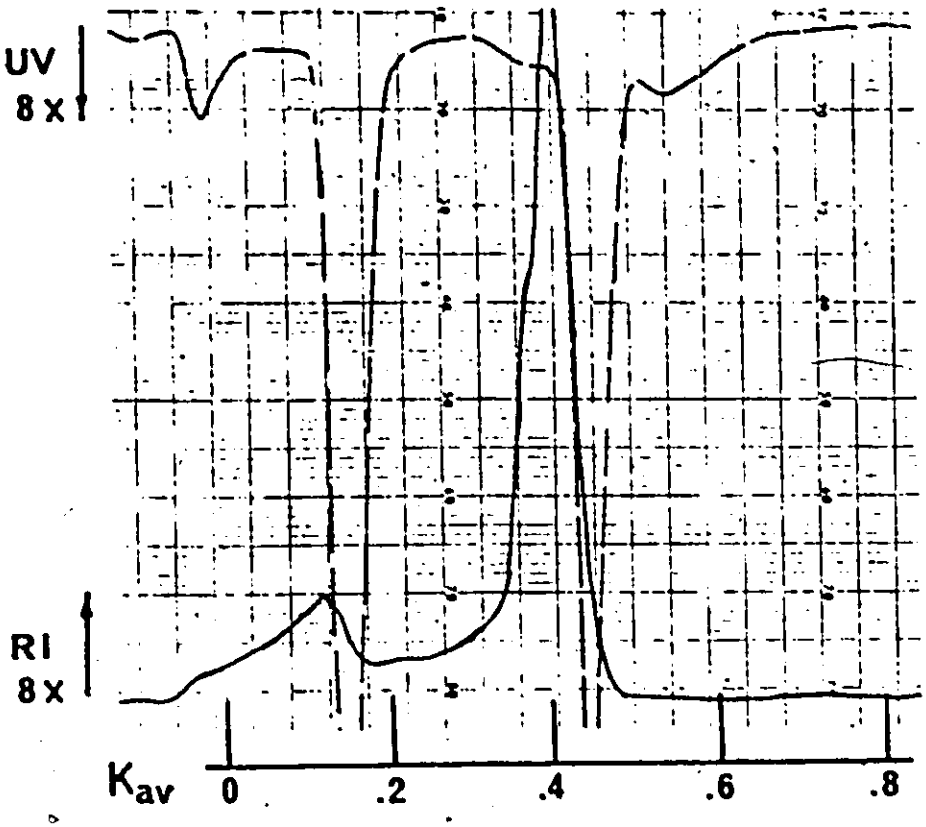


FIGURE D6. SEPHADEX G15 PROFILE OF CONCENTRATED SAMPLE 2, LIME-TREATED FOR 24 HOURS AT HIGH pH (DISTILLED WATER ELUANT)



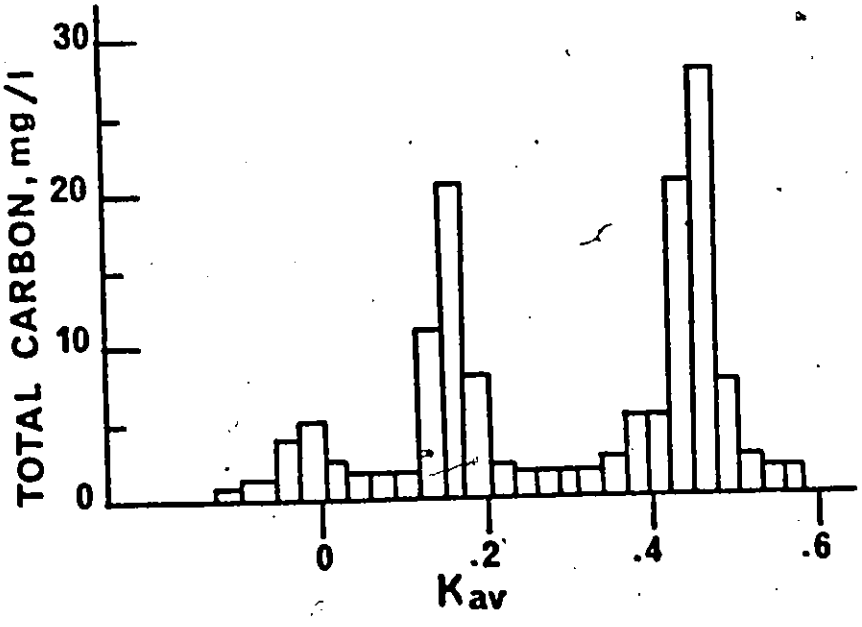
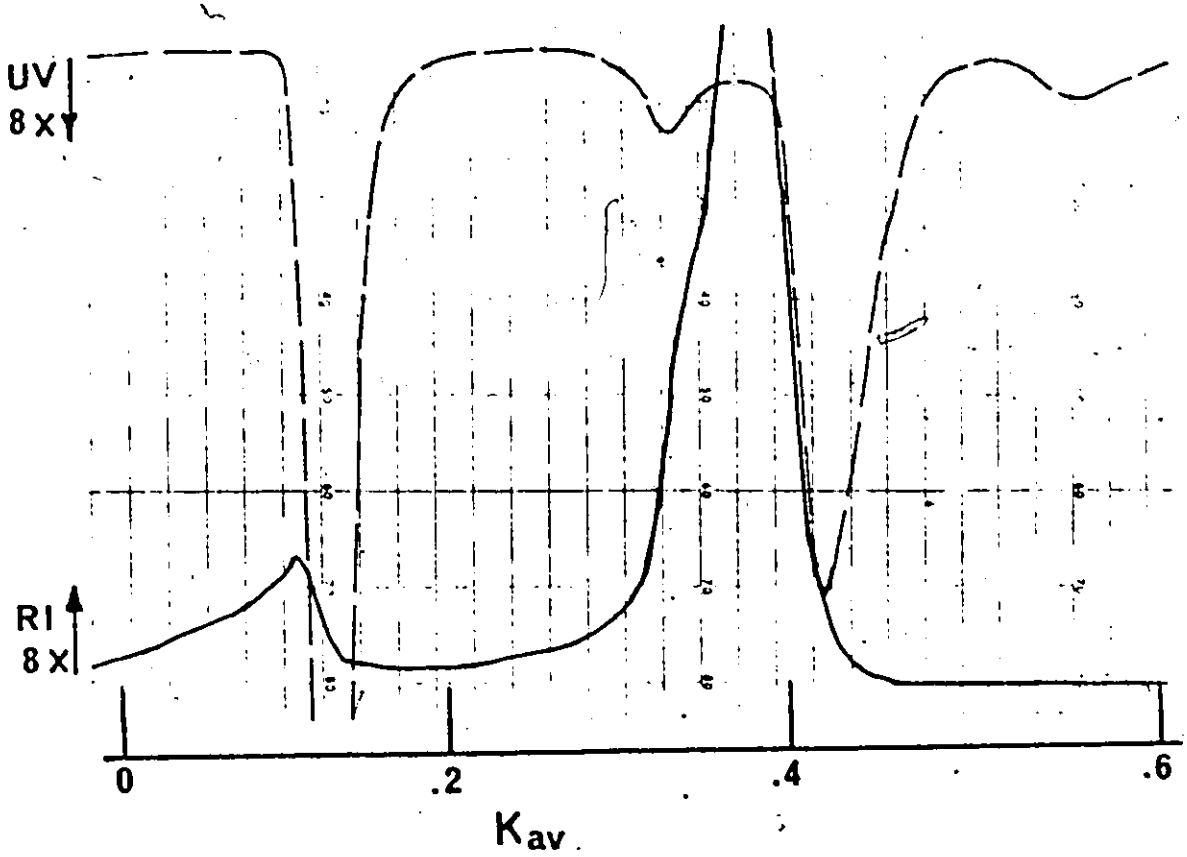


FIGURE 07. SEPHADEX G15 PROFILE OF CONCENTRATED SAMPLE 3 ELUTED WITH DISTILLED WATER ELUANT.



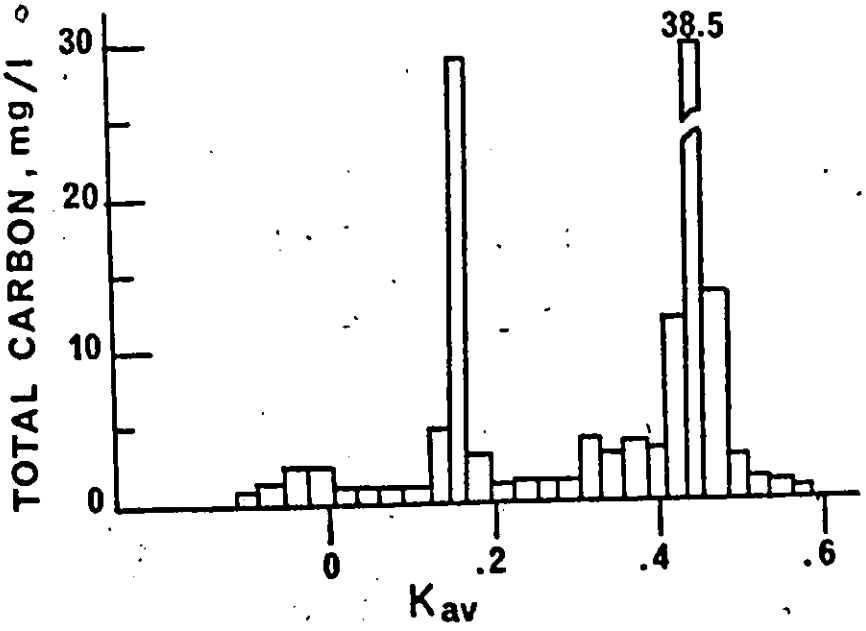


FIGURE D8. SEPHADEX G15 PROFILE OF CONCENTRATED SAMPLE 3, LIME TREATED FOR 1 HOUR AT LOW pH (DISTILLED WATER ELUANT).

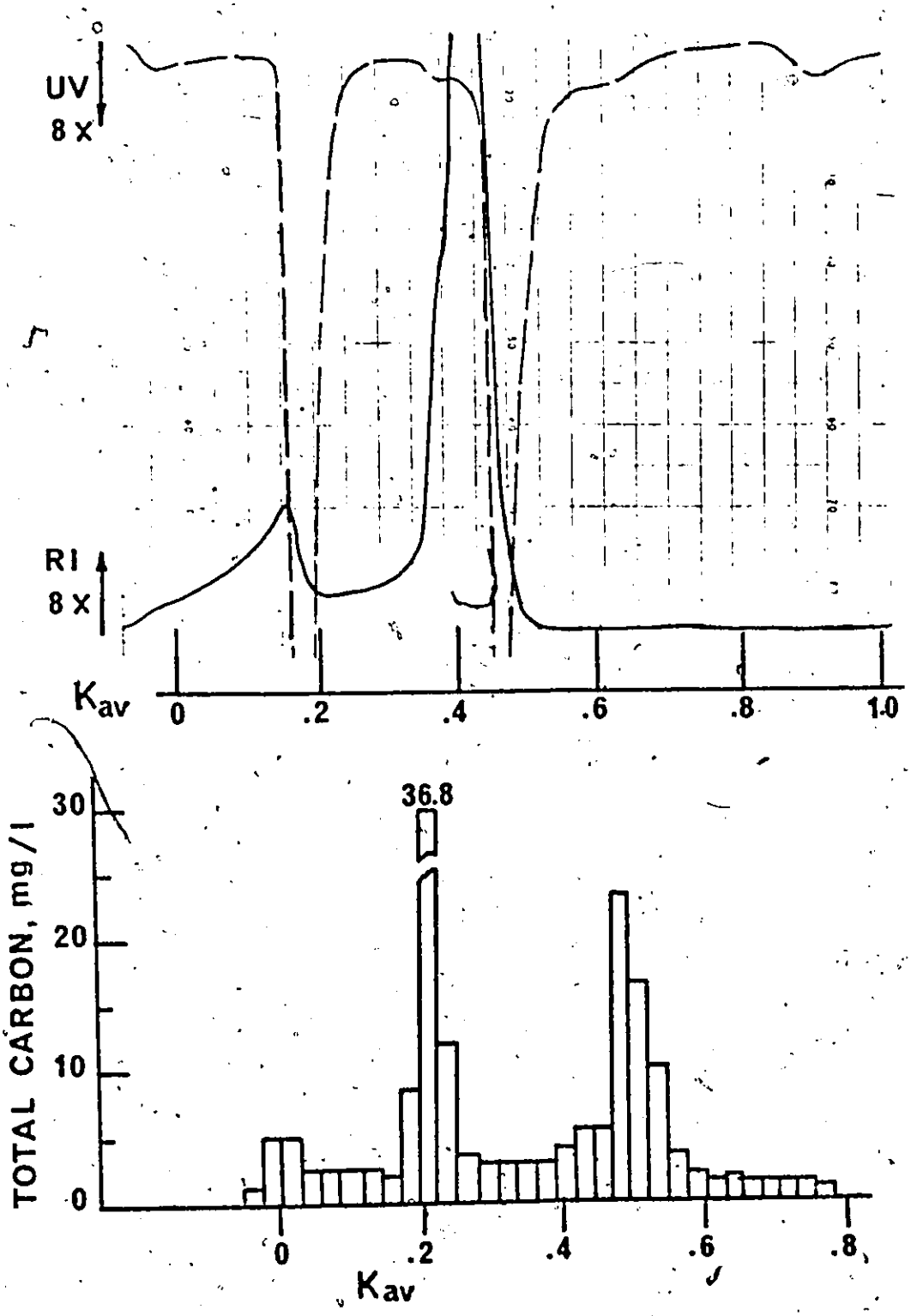


FIGURE D9. SEPHADEX G15 PROFILE OF CONCENTRATED SAMPLE 3, LIME-TREATED FOR 24 HOURS AT LOW pH (DISTILLED WATER ELUANT)

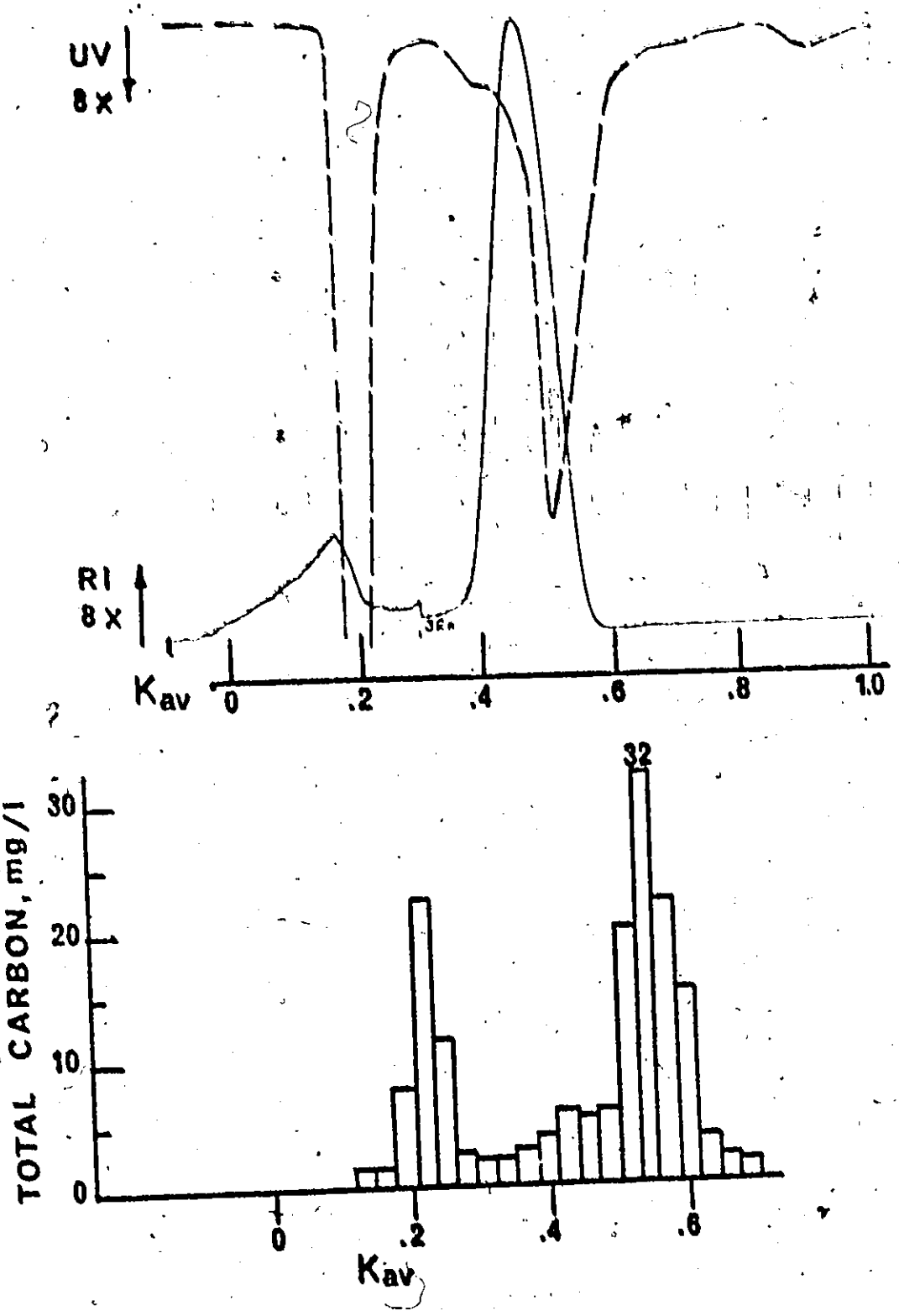


FIGURE D10. SEPHADEX G15 PROFILE OF CONCENTRATED SAMPLE 3a LIME-TREATED FOR 1 HOUR AT HIGH pH (DISTILLED WATER ELUANT)

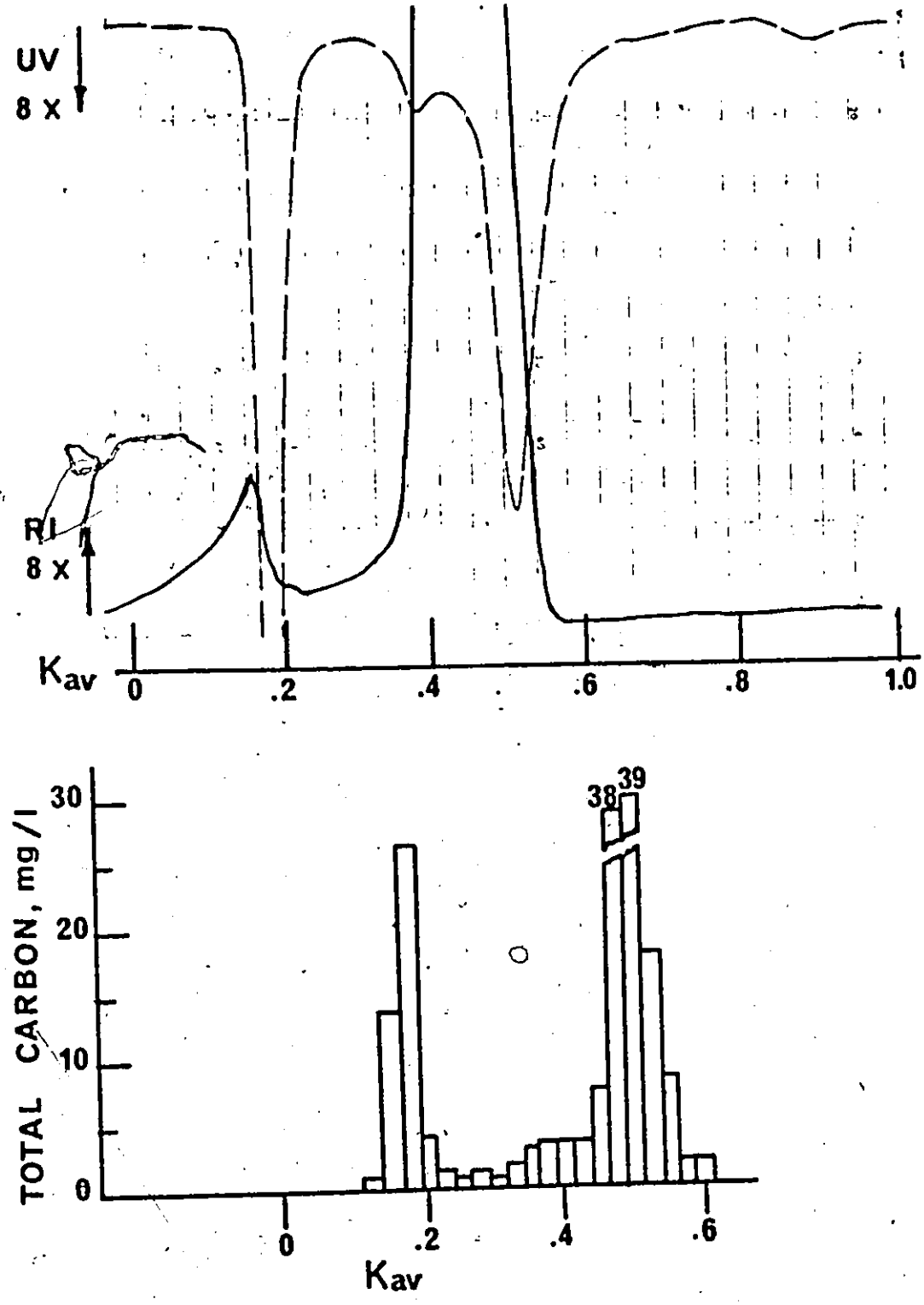


FIGURE D11. SEPHADEX G15 PROFILE OF CONCENTRATED SAMPLE 3, LIME-TREATED FOR 24 HOURS AT HIGH pH (DISTILLED WATER ELUENT)

APPENDIX E

ADJUSTMENT OF WASTEWATER SAMPLES TO SAME CONCENTRATION FACTOR AND RECOVERY.

Consider sample 2, lime-treated at low pH for twenty-four hours;

TOC recovery = 63.0%

Concentration factor = 11.8

For the untreated sample;

TOC recovery = 67.7%

Concentration factor = 8.42

Assuming that variations in TOC recovery are due to random error;

$$\text{Adjustment Factor} = \frac{67.7}{63.0} \times \frac{8.42}{11.8} = 0.767$$

Thus, the organic carbon in each of the three regions of the elution profile was adjusted to the same basis as the untreated sample by multiplying by the above adjustment factor. A similar procedure was followed with other samples.