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Fractionation of Organic Matter in Natural Waters on Sephadex Columns

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■ Sephadex columns have been used to fractionate organic matter in natural water into apparent molecular weight groups. Moderately colored creek water was fractionated into 10 fractions. The ratios of dichromate-oxidizable organic matter, color, and organic nitrogen were different for each fraction. Various lakes, streams, and leachable organic matter from lake sediments showed different organic carbon elution patterns. Gel permeation chromatography shows promise as a tool for fractionation of natural water organic matter and should aid in its characterization.

The study of the composition of organic matter present in natural waters may proceed via two routes: testing for a specific compound with a specific analytical technique or attempting to isolate and identify the principal compounds in a concentrate of natural water samples. Vallentyne (1957) and others (Lee, 1966) have reviewed the specific organic compounds found in natural waters. It is evident from these studies that the organic matter in natural waters is composed of many thousands of compounds, a large number of which have not been identified, since the total mass of specific compounds found does not approach the milligrams per liter of nonfilterable organic carbon found in most natural waters. Because of the large discrepancy between typical concentrations of known compounds and "dissolved" organic carbon found in natural waters, studies that attempt to isolate and identify the major components concentrated from natural waters are needed. Such a study has been initiated in this laboratory. The first phase of this project was devoted to an evaluation of Sephadex columns to fractionate concentrates of natural water organic matter into groups of compounds. This paper reports on this phase of study and is an expansion of a similar study reported by Gjessing (1965).

Characteristics of Sephadex Chromatography

In recent years, the use of column chromatography to separate complex mixtures of compounds has received considerable attention as a result of the development of substrates that separate compounds on the basis of the molecular exclusion principle. One of the more popular substrates is Sephadex, a modified dextran obtained by crosslinking linear macromolecules, consisting of a three-dimensional network of polysaccharide chains (Flodin, 1963). Sephadex is neutral and contains few ionic sites. Its pores are determined by the degree of crosslinkage of the polymer. Seven grades of Sephadex are available, each with a different pore size (Table I).

Separation on Sephadex columns (gel filtration) is achieved by a type of molecular sieving. A small volume of the concentrate (less than 3% of the column volume) to be separated is placed on the top of the column. Small molecules move with the elutant both within and outside the Sephadex particles. Molecules larger than the pore size of the gel particles cannot penetrate the particles and, therefore, move rapidly down the column with the elutant. The smaller molecules penetrate the gel particles to a varying extent, depending on their shape and

Table I. Sephadex Types and Fractionation Range

Type	Approximate Limit for Complete Exclusion, MW	Fractionation Range, MW
G–10	700	0–700
G–15	1,500	0–1,500
G–25	5,000	100–5,000
G–50	10,000	500–10,000
G–75	50,000	1,000–50,000
G–100	100,000	5,000–100,000
G–150	150,000	5,000–150,000
G–200	200,000	5,000–200,000

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size. Some compounds, such as protein, aromatics, and heterocyclics, are adsorbed by or interact with the gel particles. Therefore, separations achieved by Sephadex columns are dependent on size, shape and, to some extent, the functional groups present in the molecule.

Experimental Procedure

Concentration of Water Samples. Water was concentrated by evaporation under reduced pressure in a Precision Scientific Laboratory evaporator at 35° to 40° C. Using this apparatus, 20 liters of water were concentrated to approximately 2 liters in about 4 hours, then concentrated to 50 ml. using a Büchi Rotovapor concentrator. All water samples were filtered through glass fiber filters (Whatman GF/A) prior to concentration.

Water samples used were derived from Black Earth and Six Mile Creeks and Cranberry and Mendota Lakes. Both creeks originate in marshes and are located in Dane County near Madison, Wis. Black Earth Creek, at Water Chemistry Station 0, has moderate color in the spring runoff derived from snow melt and rains. Water Chemistry Station 0, on Six Mile Creek, is just downstream from a large marsh and the water is moderately colored throughout the year. The color in both creeks is derived from decaying vegetation in the marshes and surrounding farmlands. Lake Mendota, a hard water, eutrophic lake located at Madison, Wis., has a dissolved organic carbon of 10 mg. per liter and a color of 5 to 10 units. Cranberry Lake, located in Price County, Wis., is moderately colored. It is used as a source and disposal area for waters used in a neighboring cranberry marsh. At the time of sampling (early spring) no water had been used for this purpose for at least 6 months. Additional characteristics of these waters and the other samples used in this investigation are presented in Table II, with the results from the concentration of the different waters. All samples listed were concentrated by the evaporation procedure described, except for the mud, Lake Mendota 24/8, Lake Mendota 24/8–^{14}C, and algae.

The mud sample was a concentrated water extract of surface sediment taken from University Bay of Lake Mendota at a depth of 17 meters on May 6, 1965 (stored at +4° C. for 2 months). Three hundred grams (dry weight) of the sediments were extracted four times with 2.5 liters of distilled water. The combined extracts (10 liters) were filtered (Whatman GF/A) and evaporated to 10 ml.

Lake Mendota 24/8 was sampled Aug. 24, 1965, and stored until Nov. 20, 1965, at +4° C.

Lake Mendota 24/8–^{14}C was sampled Aug. 24, 1965. To this water were added 1 mg. per liter of PO_4 as $NaHPO_4$, 20 mg. per liter of N as $NaNO_3$, 1 mg. per liter of Fe as $FeCl_3$,

Table II. Composition of Water Samples and Concentrates

			Filtered Water				Precipitate		Filtered Concn. of Water Sample				pH		
	Date of collection 1965	Concentration Factor	COD, mg./l.	Color, mg. Pt/l.	Organic nitrogen, mg. N/l.	Conductivity, µmhos/cm. 20° C.	% COD total	Dry weight, mg./l.	% COD	% COD after storage	% Color	% Nitrogen	Water	Concentrate	% COD in Distillate
Black Earth Creek St. 0 (BEC)	Mar. 9	400	42.1	80	2.2	360	18[a]	61	74	75	...	...	7.2	9.0	2
Six Mile Creek St. 0 (SMC)	Mar. 24	400	25.8	25	...	380	...	...	87	...	...	...	...	7.4	0
Cranberry Lake (N-Wis)	Apr. 14	300	15.6	25	...	130	...	...	58	55	...	...	6.3	...	7
Lake Mendota (lake)	Feb. 24	400	15.2	5	0.5	300	16[b]	68	77	...	...	...	...	...	...
Lake Mendota (lake)	Mar. 4	400	15.5	5	0.5	300	11[c]	98	78	56	...	...	8.3	8.3	10
Lake Mendota (lake)	Aug. 24	390	16.3	8	0.3	800	...	...	77	...	48	99	...	...	9
Lake Mendota-^{14}C (lake ^{14}C)	Aug. 24	390	12.6	9	0.3	775	...	...	75	...	50	96	...	...	7
Lake Mendota (lake)	Nov. 23	400	14.3	16	0.4	370	...	...	94	...	67	86	...	...	2
Algae, *Chlorella*/ Gorham (algae)	Nov. 24	300	24.9	42[d]	0.7	825	...	...	87	...	...	...	...	...	3
Mud University Bay (mud)	May 6	1000	38.0	...	...	...	...	...	...	...	...	...	...	...	..

[a] = 48% organic matter in precipitate dissolved in 10% H_2SO_4.
[b] = 88% organic matter in precipitate dissolved in 10% H_2SO_4.
[c] = 65% organic matter in precipitate dissolved in 10% H_2SO_4.
[d] = Sample turbid, indicating poor removal of algae by filters.

and 2.5 mg. per liter of standard Na_2CO_3–^{14}C (2.58×10^6 d.p.m.). Filtered air was bubbled through 20 liters of this mixture and it was irradiated with artificial light. After 30 days under these conditions, the mixture was stoppered and stored for 2 months at room temperature in the dark.

Algae (Table II) is a concentrate of a filtered unialgal culture of *Chlorella*, grown for 10 days in Gorham's (Hughes, Gorham, *et al.*, 1958) medium.

In all cases, the concentration resulted in a solid and a liquid phase. From Table II, it may be concluded that 60 to 95% (mean 78%) of the organic matter in filtered natural waters remains in solution during the concentration process, and that a substantial part of the organic matter in the precipitates dissolves in 10% sulfuric acid. There is evidence that the color of the samples decreases when concentrated.

It is possible that the concentration and handling techniques used in this study may have changed the composition of the organics from that present initially in the sample. No attempt has been made to determine the extent of alteration.

The nonfilterable concentrate from the vacuum evaporation of the sample, usually 3 to 5 ml., was placed on top of the Sephadex column. Distilled water was passed through the column to elute the concentrate at a rate of approximately 0.5 ml. per minute. The columns consisted of a glass tube 30 mm. in diameter and 70 to 100 cm. long. Fractions (3 to 15 ml.) from the effluent of the column were collected in test tubes by a Gilson volumetric fraction collector. The chemical oxygen demand (COD), color, and, on some samples, organic nitrogen were determined on each fraction using the Technicon AutoAnalyzer (Technicon Controls, undated). The color of the fraction was determined at 420 mμ with standards based on the chloroplatinate scale (American Public Health Association, 1965). The COD is a measure of a heated dichromate-oxidizable organic matter under the conditions of the test and is merely indicative of the location of organic matter in the fractions. The Standard Methods COD (American Public Health Association, 1965) was used to determine the initial COD in the water samples and in the concentrates prior to fractionation, such as those listed in Table II. A study was conducted to determine the relationship between AutoAnalyzer COD and Standard Methods COD on selected fractions from the Sephadex column. It was found (Table VI) that 50 to 70% of Standard Methods COD was measured by the AutoAnalyzer COD procedure. The value was dependent on the particular fraction tested and showed that the AutoAnalyzer procedure used has less rigorous oxidation conditions than the Standard Methods procedure.

The specific conductivity of each fraction was determined with an Industrial Instruments conductivity bridge Model RC 16B2, using a pipet-type conductivity cell.

The Sephadex was obtained from Pharmacia Fine Chemicals, Inc., Piscataway, N. J.

Results

The results obtained for the samples listed in Table II are presented in Figures 1 to 7. In all except Figure 2, the concentration of measured parameters is plotted against a distribution coefficient, K_D (Flodin, 1963).

The areas under the different curve fractions illustrated in the figures are listed in Tables III, IV, and V, as per cent of the

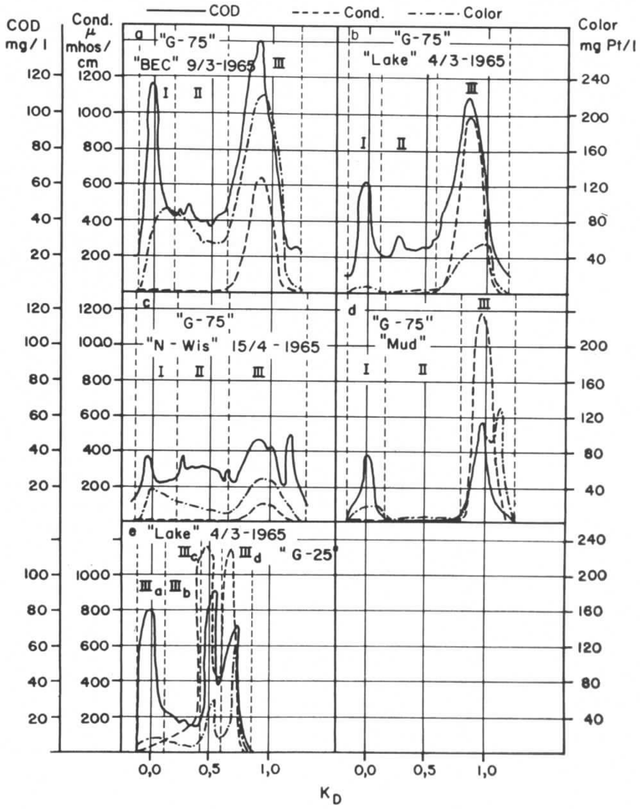

Figure 1. Separation of organic matter in water on Sephadex columns

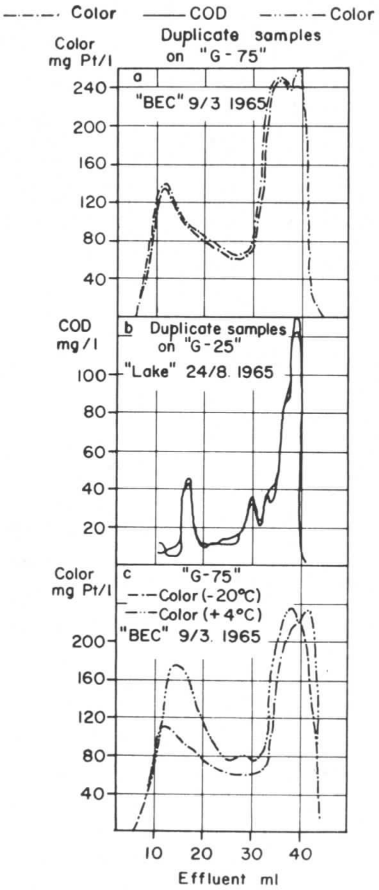

Figure 2. Reproducibility of separation of organic matter in water, from Black Earth Creek, on Sephadex columns

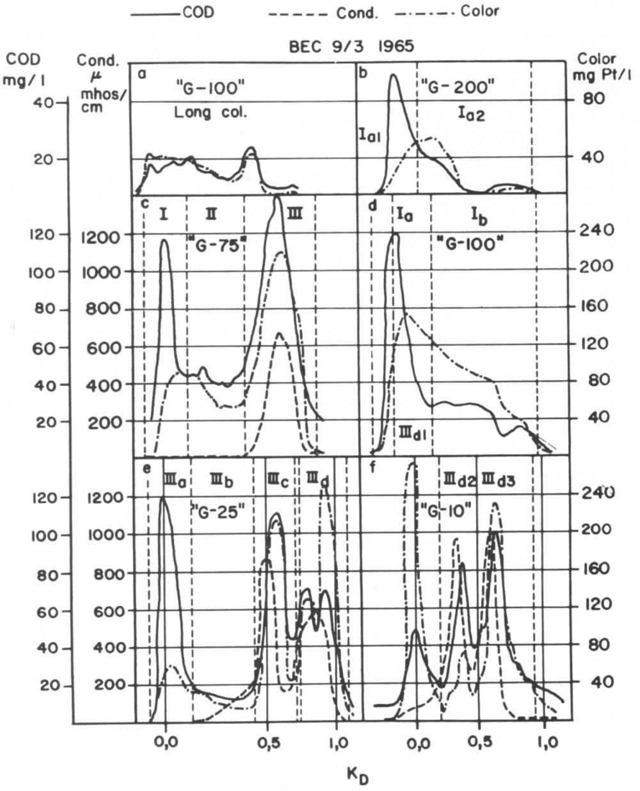

Figure 3. Separation of organic matter in water on Sephadex columns

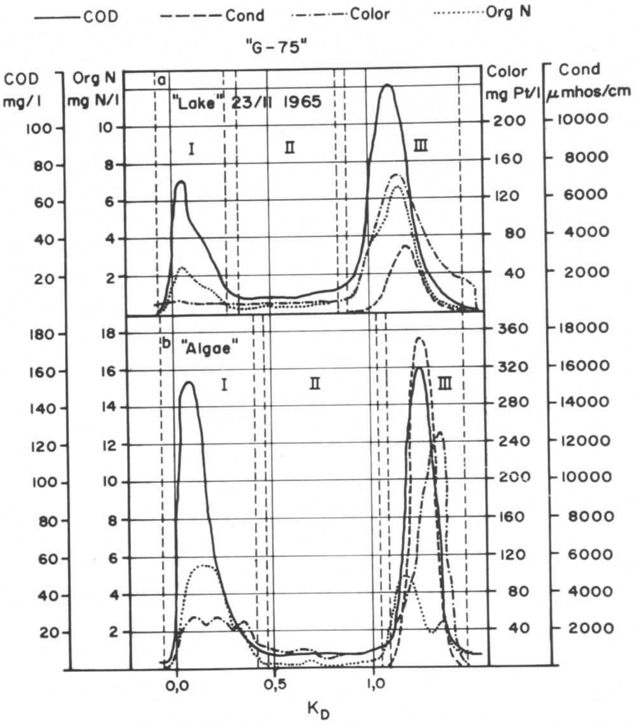

Figure 5. Separation of organic matter in water on Sephadex columns

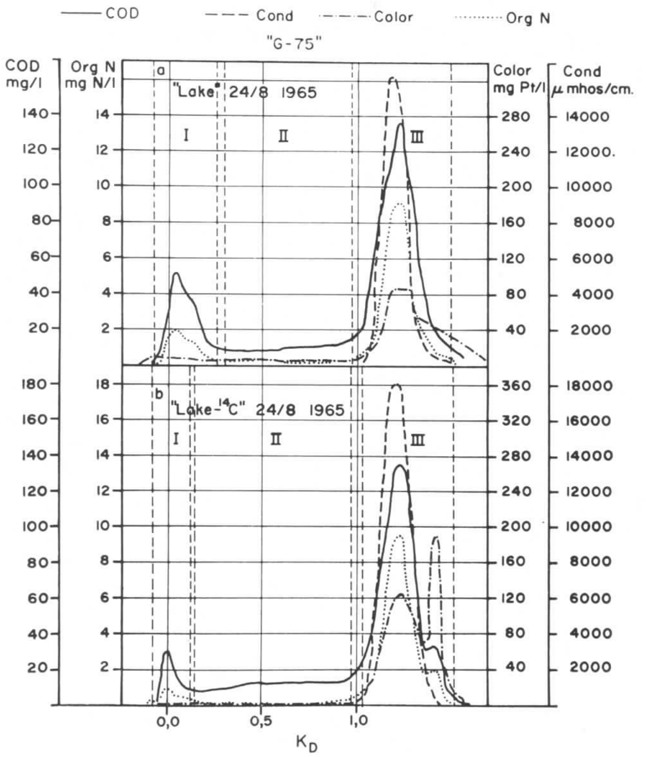

Figure 4. Separation of organic matter in water on Sephadex columns

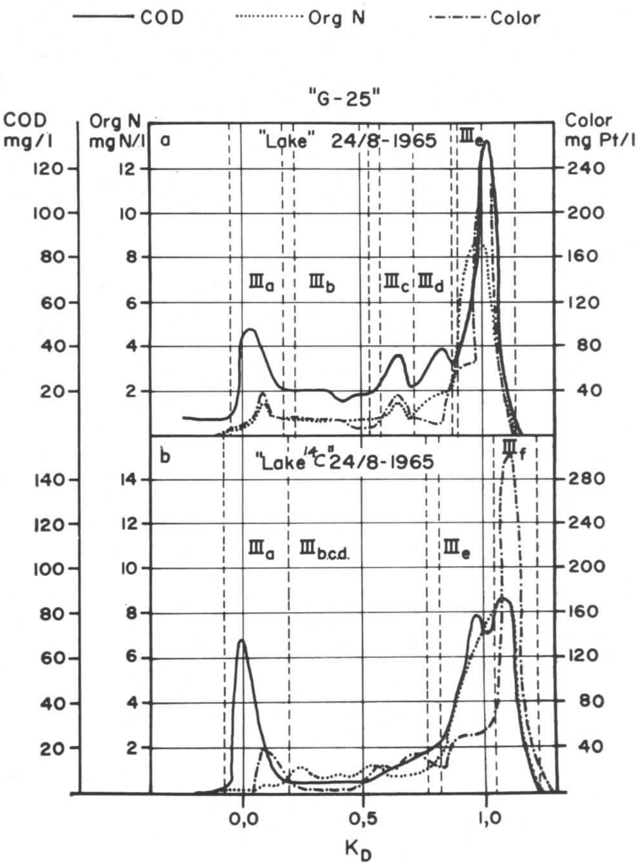

Figure 6. Separation of organic matter in water on Sephadex columns

total area. Tables III and IV also include the ratio of the areas for color and COD, and for organic nitrogen and COD. The fractions representing the peaks of the organic matter (Figures 5, 6, and 7) were divided into three fraction sets: *a* is the top fraction representing the maximum of the COD; *b* and *c* are the corresponding fractions to the left and right of the maximum.

Discussion

Reproducibility. Figure 2, *a* and *b*, illustrates the reproducibility obtained using the gel filtration technique on natural water organics in duplicate runs performed in succession on the same column. There was little difference in the results on changing from one column to another or for the first or later runs on the same column. The slight differences in replicate results can be accounted for by the analytical error.

Recovery. The degree to which the organic matter was recovered, using Sephadex columns, differed according to the grade of Sephadex used. Grade G-75 gave a recovery between 87 and 95%, with a mean of 92%. The recovery with other grades of Sephadex was 98 to 100%.

In all experiments, grade G-75 was used for the original concentrate, whereas the other grades always have been employed for concentrates of fractions obtained from previous runs on G-75.

Effect of Storage. Figure 2 *c* illustrates the results from two ways of storing the concentrated water organics for 2½ months, one at −20° C., the other at +4° C. It is obvious that storage results in a change of composition, even though Figure 2 illustrates only the change of color.

Variation between Samples. Figure 1, *a*, *b*, and *c*, illustrates the difference between the organic matter in the samples of

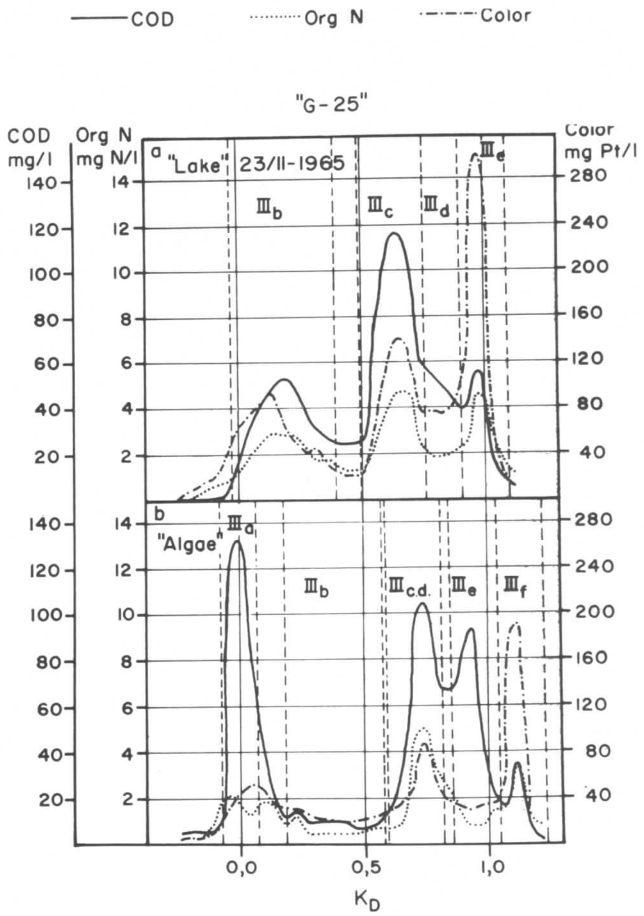

Figure 7. Separation of organic matter in water on Sephadex columns

Table III. Fractionation on Sephadex G-75

	Fraction I					Fraction II					Fraction III				
	% COD	% Color	% N	Color/COD	N/COD	% COD	% Color	% N	Color/COD	N/COD	% COD	% Color	% N	Color/COD	N/COD
BEC	25	16	. . .	0.7	. . .	16	22	. . .	1.5	. . .	59	62	. . .	1.0	. . .
N-Wis	16	24	. . .	0.9	. . .	31	22	. . .	0.4	. . .	53	54	. . .	0.6	. . .
Mud	35	11	. . .	0.4	. . .	0	0	. . .	. . .	. . .	65	89	. . .	1.6	. . .
Lake 4/3	17	5	. . .	0.0	. . .	14	6	. . .	0.1	. . .	69	89	. . .	0.6	. . .
Lake 24/8	18	0	15	0.0	0.4	12	0	6	0.0	0.2	70	100	79	0.5	0.6
Lake 24/8–^{14}C	6	0	6	0.0	0.4	19	0	6	0.0	0.1	75	100	88	0.7	0.6
Lake 23/11	26	0	20	0.0	0.4	11	0	8	0.0	0.4	63	100	72	0.8	0.5
Algae	51	26	53	0.3	0.5	2	12	4	0.0	0.0	47	62	43	0.9	0.5

Table IV. Fractionation on Sephadex G-25

	% COD						% Color						% N					
	IIIa	IIIb	IIIc	IIId	IIIe	IIIf	IIIa	IIIb	IIIc	IIId	IIIe	IIIf	IIIa	IIIb	IIIc	IIId	IIIe	IIIf
BEC 9/3/65	31	5	33	31	...	9	12	8	33	47	...	...	...	...	...	...	...	...
Lake 24/8/65	18	12	11	13	46	...	9	10	9	7	65	...	7	11	7	13	62	...
Lake 24/8 ^{14}C–65	22	←16→			32	30	6	←19→			18	57	2	←22→			37	39
Lake 23/11/65	...	32	43	15	10	...	...	39	31	1	19	...	...	38	30	13	19	...
Algae	35	4	←31→		25	5	19	16	←23→		15	27	23	12	←34→		15	16

	Color / COD						N / COD					
	IIIa	IIIb	IIIc	IIId	IIIe	IIIf	IIIa	IIIb	IIIc	IIId	IIIe	IIIf
BEC 9/3/65	0.3	1.5	0.9	1.4	...	...	...	...	...	...	...	...
Lake 24/8/65	0.4	0.6	0.4	0.4	1.0	...	0.3	0.6	0.5	0.7	1.0	...
Lake 24/8 ^{14}C–65	0.2	←1.0→			0.5	1.6	0.1	←1.2→			0.9	1.1
Lake 23/11/65	...	1.0	0.6	1.2	1.0	...	...	0.7	0.4	0.6	1.3	...
Algae	0.3	2.5	←0.4→		0.4	3.9	0.3	1.3	←0.5→		0.2	1.6

natural water studied and that the gel filtration method may be useful in classification of lakes and characterization of organic matter in water.

The percentage of organic matter represented by Fraction-Set I on Sephadex G-75 varies between 6 and 35% in the samples studied (Figure 1 and Table III). This has, according to the literature, a molecular weight above 50,000 to 70,000. Lake Mendota 24/8–^{14}C has the low value of 6% and the mud extract the high value of 35% for the fraction.

The percentage distribution of the organic matter in Fraction-Set II (Table III) varies between samples, particularly when mud extract is compared with the sample from Lake Cranberry (N-Wis). The organics in this Fraction-Set from Lake Mendota samples were colorless.

Similarly, Table IV and Figures 6 and 7 clearly suggest that the compositions of the organic solutes in the different water samples vary. It is even difficult to point out similarities between some samples.

Molecular Distribution. Figure 3 illustrates the results obtained from five types of Sephadex; G-200, G-100, G-75, G-25, and G-10. From the curve presenting the COD values in Figure 3, it is seen that the soluble organic matter in a 400-fold concentrate from Black Earth Creek (BEC) may be separated into at least 10 fractions, according to molecular size. The measured area under the peaks is presented in Table VI. The values in the per cent COD column of Table VI are given as a percentage of the total COD in filtered water. Although the generality of the findings may be questionable, it is reasonable to conclude that the main part of the organics in natural waters are fractionable and probably do not consist of a continuous distribution of all sizes of molecules. In this table, an estimate of the molecular weights and molecular sizes of the organics is recorded, based on the distribution coefficients obtained and calculated by equations reported in the literature for the estimation of protein molecular weight (Andrews, 1964).

These values must be considered as a very rough estimate of molecular weight and molecular size, since none of the values have been confirmed through conventional methods.

Heterogeneity of Peak Values. Considering Figures 2 and 3, comparing the appropriate maxima of the COD and color curves, it is seen that the two maxima are not always in phase. The discrepancy is particularly noticeable for the lower molecular weight fractions, which again suggests that correlation is less marked between the organic matter and the color for these fractions. The maxima of the conductivity curves, in particular those from Sephadex G-25 and G-10, have different positions than in the color and COD curves.

Seasonal Variations. Figures 1, *b*, 4, *a*, and 5, *a*, and Table III present the separation of concentrated water from Lake Mendota with G-75 on March 4, October 24, and November 23, respectively. Examination of these data shows that it is impossible to point out any obvious difference in the separation patterns. On the other hand, the curves representing the separation on G-25, of the same samples (Figures 1, 3, 6*a*, and 7*a*, and Table IV) clearly illustrate the seasonal difference in the composition of the organic compounds in Lake Mendota. This is further confirmed by Table VII.

The results of the study on ^{14}C-labeled organics (Lake Mendota 24/8–^{14}C) were not completely satisfactory. However, peak I from Sephadex G-75 was found to contain significantly less organic carbon in the ^{14}C sample than is normally found in Lake Mendota water. The COD/N ratio is the same in both, but the ratios of the two COD values differ. Corresponding differences were found on the fraction of Sephadex G-25. Table II indicates that the addition of nutrient salts

Table V. Fractionation on Sephadex G-10

	% COD			% Color			$\frac{\text{Color}}{\text{COD}}$		
	IIId$_1$	IIId$_2$	IIId$_3$	IIId$_1$	IIId$_2$	IIId$_3$	IIId$_1$	IIId$_2$	IIId$_3$
BEC 9/3/65	19	30	51	41	11	48	2.9	0.5	1.3

Table VI. Fractionation of Black Earth Creek Sample

Peak	Grade	Molecular Weight Range	% COD	Molecular Size Range, Radius, Cm. × 10^{-8}
I a$_1$	G–200	>200,000	6	100
I a$_2$	G–200	100,000–200,000	4	10–50
I b	G–100	50,000–100,000	6	5–30
II	G–75	20,000–30,000	11	10–25
IIIa	G–25	3,000–5,000	12	20
IIIb	G–25	....	2	5–15
IIIc$_1$	G–25	100–5,000	13	4
IIId$_1$	G–10	....	2	...
IIId$_2$	G–10	....	4	0–4
IIId$_3$	G–10	<700	6	...
Ppt. not sol. in H_2SO_4			9	
Ppt. sol. in H_2SO_4			9	
Loss on G–75			10	
Distillate			2	
Total			96	

Table VII. Composition of Fractions from the Sephadex Separation

	$\frac{\text{COD (S.M.)}^{a}}{\text{COD (A.A.)}^{b}}$				$\frac{\text{Color}}{\text{COD (S.M.)}}$				$\frac{\text{N}}{\text{COD (S.M.)}}$			
	24/8	24/8–^{14}C	23/11	Algae	24/8	24/8–^{14}C	23/11	Algae	24/8	24/8–^{14}C	23/11	Algae
Water	1.4	1.5	1.8	1.9	0.5	0.7	1.1	Turb.	1.8	2.4	2.8	2.9
concn. (1:250)	1.9	1.9	1.9	2.1	0.5	0.7	1.2	1.0	3.6	5.3	4.1	3.7
IB	1.2	1.4	1.1	1.3	0.1	0.5	0.3	0.2	1.9	2.6	3.4	1.7
IA	1.2	3.5	1.4	1.7	0.1	0.1	0.2	Turb.	2.1	0.7	2.5	2.0
IC	1.2	1.6	1.0	1.2	0.2	0.5	0.4	0.3	2.5	2.3	3.2	5.0
II	2.0	1.2	2.0	2.3	0.3	0.2	0.7	0.4	1.8	0.8	2.2	0.6
IIIa	0.8	1.8	...	1.0	1.6	0.9	...	0.9	5.6	3.3	...	1.7
IIIb	2.4	...	1.4	1.0	0.1	...	0.8	...	1.7	...	6.6	4.8
IIIc	1.8	...	...	...	...	...	...	...	...	...	...	...
IIId	1.7	...	...	...	0.3	...	...	...	1.7	...	...	...
IIIe	...	...	5.1	...	...	...	0.9	...	...	...	2.1	...
IIIf	...	...	...	1.1	...	1.0	...	4.2	...	3.7	...	4.7
IIIcB	...	...	1.2	...	...	...	0.2	...	...	...	...	...
IIIcA	...	...	1.2	...	...	...	1.1	...	...	...	...	...
IIIcC	...	...	0.7	...	...	...	0.9	...	...	...	...	...
IIIcdB	...	...	...	0.6	...	...	...	1.2	...	...	...	...
IIIcdA	...	...	...	0.7	...	...	...	1.4	...	...	...	...
IIIcdC	...	...	...	0.5	...	...	...	1.7	...	...	...	...
IIIeB	...	0.7	...	...	...	0.3	...	...	...	5.0	...	...
IIIeA	...	0.7	...	...	...	0.2	...	1.8	...	...	...	3.9
IIIeC	...	...	...	...	...	0.2	...	3.0	...	...	...	5.0

[a] S.M. = Standard Methods.
[b] A.A. = AutoAnalyzer

to Lake Mendota water, and bubbling air through it, result in a loss of approximately 20% of the organic matter.

Organics from a *Chlorella* Culture. Figures 5*b* and 7*b* present data for the concentrated extract of a *Chlorella* culture grown in Gorham's medium and separated on G-75 and G-25, respectively. More than 50% of the organics in this concentrate probably have a molecular weight above 50,000. The results listed in Tables II, III, IV, and VI do not give a basis for emphasizing any differences between this sample and the other water samples. However, the separation pattern of this algal concentrate on G-25, is, in many respects, different from the other samples.

Determination of Organic Carbon. Since certain compounds are known to be resistant to oxidation by the Standard Methods COD procedure, a brief study was conducted to determine the relationship between AutoAnalyzer COD and organic carbon content of certain fractions from the Sephadex columns. The organic carbon, on selected fraction, was determined by high temperature, dry combustion in a Leco low-carbon analyzer. Two milliliters of the sample were evaporated in a Vycor liner at 50° to 70° C. in the presence of a few drops of $1N$ H_2SO_4 to remove carbonates. When the sample was dry, the Vycor liner was placed in a quartz-covered crucible and carried through the normal operating procedure of the instrument (Table VIII).

Since the theoretical ratio of COD to organic carbon is 2.5, it is evident that approximately 30 to 40% of the organic carbon present in these samples is measured by the AutoAnalyzer COD procedure used.

Conclusions

Sephadex columns can fractionate natural water organic matter into a number of distinct fractions. Using different grades of Sephadex, it was possible to separate the Black Earth Creek sample into 10 fractions. This fractionation should greatly aid the study of the organic matter present in natural waters. One of the advantages of the technique is that the high molecular weight fractions are desalted by passage through the column. It is evident from this and previous studies (Gjessing, 1965; Povoledo, 1964) that the organic matter from different waters have different elution patterns. Based on these results, it is possible to classify lakes according to their organic matter elution patterns from different grades of Sephadex and other gels. The ratios of color, organic nitrogen, and organic carbon vary between fractions. These ranges may also aid in classification and study of composition.

One of the most significant results of the study is that the color in natural waters is composed of several groups of compounds, a significant number having molecular weight above 50,000. On the basis of separations achieved on G-75, it appears that low colored waters have most of their color in the low molecular weight fraction; in moderately or highly colored waters, more color is found in the high molecular weight fraction. Even though it is impossible to assign accurate molecular weights to each fraction because of possible sorption and other side effects, it is clear that Shapiro's (1957) average molecular weight of 456, for natural water color, is of questionable value.

Table VIII. Relationship of Organic Carbon to AutoAnalyzer COD for Natural Water Organics

Organic Carbon, Mg./L.	COD (A.A.), Mg./L.
40	48
41	36
60	50
128	130
265	250

Although not investigated in this study, subsequent studies in this and other laboratories have shown the pH of the eluent to affect markedly the organic carbon elution pattern on Sephadex columns.

Finally, the results obtained may, to some degree, be due to methods used in sample concentration, storage, and elution. It is possible that large, loosely bound molecules, present in the water at the time of sampling, are broken into smaller units in sample pretreatment. Hopefully, polymerization reactions were minimized by never taking the organics to dryness and by working only with aqueous systems—i.e., no organic solvents. There is a good possibility that some organic matter present in the concentrates was colloidal and not in true solution, since a 1-micron pore size filter was used. Further study will be necessary to determine the influence of methods of sample preparation on elution patterns.

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