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CARBOHYDRATE IN SEA WATER[1]

By

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ABSTRACT

Substances which respond to analytical tests for carbohydrate (with anthrone or N-ethyl carbazole) were found in Pacific Coast water in quantities of from 0.1–0.4 mg/L. In coastal lagoons, however, concentrations as high as 8 mg/L were observed. Normally, most of this is in a dissolved rather than suspended state.

We have little real information about the nature and amount of dissolved organic matter in the sea. Therefore, when it was reported some time ago by Collier, et al. (1950, 1953) that substances having the general chemical properties of carbohydrates had been detected in the Gulf of Mexico in amounts up to 50 mg/L, we began an investigation to find out whether the same thing occurs in or near our area of the Pacific Coast. The earlier work of Krogh (1934), which has impressed us as one of the most important and reliable contributions to this problem, indicated a fairly constant level of dissolved organic matter, at about 5 mg/L. This conclusion, however, was intended to be applicable to the open sea, and it is entirely possible that local conditions, especially near shore, vary widely from such a norm. Indeed, the recent report of Kay (1954) on the distribution of dissolved organic carbon in the shallow waters of the Kielerbucht shows this to be true, although the maximum which he found was only about twice that which Krogh found in open water. Kay's method of of analysis was a modification of Krogh's.

Following the initial report concerning the Gulf of Mexico, already mentioned, Wangersky (1952) endeavored to identify the substances concerned and reported the isolation of a compound having the general absorption spectrum of dehydro-ascorbic acid; he also reported a substance which "gives some indication of being a rhamnoside" and which was said to be present in inshore waters in concentrations up to 0.1 g/L.

REAGENTS AND METHODS

Anthrone. Dissolve one gram of anthrone reagent[2] (recrystallized from benzene and petroleum ether) in one liter of concentrated sulfuric

[1] Contribution of the Scripps Institution of Oceanography, New Series No. 781.

[2] The reagent used was obtained from the Jasonols Chemical Corporation, 1085 Myrtle Ave., Brooklyn 6, New York.

acid. Store in a dark glass-stoppered bottle and keep refrigerated when not in use. Allow the freshly prepared solution to stand four hours before using. It is stable for at least one week.

N-ethyl Carbazole. Dissolve one gram of recrystallized (from ethanol and water) N-ethyl carbazole in one liter of 90% sulfuric acid which has been cooled in an ice bath. Store the solution in a dark glass-stoppered bottle and keep refrigerated when not in use. Avoid exposure to sunlight and air as much as possible. The reagent is stable for at least two days.

Standard Sucrose Solution. Dissolve 500 mg of reagent-grade sucrose in one liter of doubly distilled water and dilute 10.00 ml of this to one liter for a working standard in the range from 0 to 5 mg/L. Add a few drops of saturated mercuric chloride solution as a preservative and store in a refrigerator.

Apparatus. A 70° C (±0.2) water bath is required to develop color with N-ethyl carbazole. AMINCO absorption cells, 100 mm in light path and 16 mm outside diameter, contain about 16 ml and are suitable for the sample volumes involved. Simple adaptors can be made to permit their use in a Beckman DU spectrophotometer. Glass-stoppered Pyrex bottles of 60 ml capacity serve well as reaction vessels.

Anthrone Method. Transfer 7.5 ml of filtered sea water to a 60-ml bottle and slowly add 15.0 ml of anthrone reagent with as little mixing as possible so as to form two discrete layers. Then mix thoroughly and immediately place the stoppered bottle in a pan of water which is maintained near room temperature. Between 15 and 25 minutes later, transfer the sample to a 100-mm absorption cell and read the optical density at 627 mμ. Determine standard curves daily, diluting with doubly distilled water. The range from 0 to 5 mg/L is suitable for a 100-mm cell. Determine reagent blanks with 7.5 ml of doubly distilled water.

N-ethyl Carbazole Method. Measure a 2.5-ml sample of filtered sea water and transfer it to a 60-ml bottle. Add 22.5 ml of N-ethyl carbazole and mix thoroughly. Immediately place the sample in a 70° C (±0.2) water bath and leave for exactly 30 minutes. Transfer the sample bottle from the water bath to a refrigerator for 15 to 20 minutes and then allow it to come to room temperature. Avoid exposure to light at all times. Between 30 to 60 minutes after removal from the water bath, determine the optical density at 562 mμ. Determine standard curves and reagent blanks daily with doubly distilled water.

Discussion. Consistent results with anthrone are largely dependent upon the purity of the reagent and on the careful control of temperature immediately after the reagent and sample are mixed. The purity of the anthrone is essential in maintaining constant calibration curves. If the reagent is not recrystallized, changes as great as 25% may occur in one day; with a recrystallized reagent, less than 5% change was observed in a week. The heat of dilution resulting from mixture of concentrated sulfuric acid with the aqueous sample is sufficiently reproducible to limit the error to less than 5%.

For a given sucrose concentration, the color intensity is 20% greater in sea water of 33 ‰ salinity than in distilled water. This effect is apparently due to the chloride ion. A correction factor must therefore be used when calculating results based on standard curves and reagent blanks determined with distilled water solutions.

In general, the N-ethyl carbazole method is considerably less satisfactory. The reddish-violet reaction product is destroyed easily by sunlight and oxidizing agents, with a resultant dark green coloration. Although there is no salt error with the reagent, the procedure is more laborious and exacting because of the rigid temperature requirements.

The reactions of the reagents with a large number of carbohydrates were studied in some detail. Both have about the same sensitivity with different carbohydrates (within a factor of 2), excepting xylose and arabinose, to which anthrone is much less sensitive. The two behave about equally with sucrose, the standard which was chosen. Further details of the two methods will be reported elsewhere at a later date.

EXPERIMENTAL

Unless otherwise indicated, all samples (to be described later) were filtered through a type-HA "millipore" filter having a porosity of 0.5 μ (Goldberg, et. al., 1952). It was established that passage through these filters does not introduce extraneous carbohydrate. The material in this filtrate will be called "dissolved carbohydrate" with the full realization that it may contain some colloidal forms as well as other interfering substances which may show the same color reactions.

A number of samples of surface sea water were collected from the Scripps Pier and from the kelp beds off La Jolla, California, in May and June 1952 and in August 1954; these were analyzed for dissolved carbohydrate at various times after collection. The methods with both reagents were used on several of these; the results are given in Table I in terms of sucrose concentrations. Each value represents the average of from 2 to 11 determinations made on both untreated aliquots and

TABLE I. Carbohydrates in filtered sea-water samples. Concentrations in mg/L using a sucrose standard.

Sample	Method		Location	Date	
	Anthrone	N-Ethyl Carbazole		Collected	Analyzed
A	—	0.14	Pier	6- 9-52	6-11-52
B	—	0.22	Pier	6-11-52	6-12-52
C	0.38	0.22	Pier	6-17-52	6-18-52
D	0.29	—	Pier	8- 9-54	8- 9-54
D	0.26	—	Pier	8- 9-54	8-10-54
E	0.25	—	Pier	8-10-54	8-10-54
F	0.43 (not filtered)	—	Kelp Beds	5- 1-52	5- 6-52
G	0.30	—	Kelp Beds	4-29-52	5-14-52
G	0.37	—	Kelp Beds	4-29-52	5-18-52
G	0.22	—	Kelp Beds	4-29-52	5-21-52
H	0.30	0.11	Kelp Beds	6-18-52	6-19-52
H	0.21	0.15	Kelp Beds	6-18-52	6-20-52
H	0.45	0.38	Kelp Beds	6-18-52	6-24-52
I	—	0.35	Kelp Beds	6-18-52	6-19-52
I	0.16	0.11	Kelp Beds	6-18-52	6-20-52
I	0.20	0.25	Kelp Beds	6-18-52	6-24-52
Average	0.30	0.21			
Std. Dev.	0.09	0.08			

on portions which were evaporated to concentrate the carbohydrate material. Standard deviations within these groups of multiple analyses were 0.09 and 0.08 mg/L for anthrone and N-ethyl carbazole, respectively. Subsequently the anthrone reagent, because of its greater convenience, was used for most but not all determinations.

Table II shows carbohydrate content as a function of depth for four shallow-water stations at half-mile intervals off Scripps Pier; these samples, taken two days after the peak of a heavy phytoplankton bloom, were not filtered, and the values therefore include any

TABLE II. Carbohydrate content at shallow inshore stations off Scripps Pier on Sept. 3, 1952, following a heavy phytoplankton bloom. Concentrations in mg/L in terms of a sucrose standard. Unfiltered samples.

Depth	Station			
(m)	1	2	3	4
0	0.36	0.26	0.32	0.26
3	0.31	0.26	0.29	0.29
7	0.24			
10		0.31	0.27	0.19
20		0.24	0.22	0.15

Standard deviation of duplicate analyses of each sample : 0.06.

particulate matter hydrolyzed upon addition of anthrone reagent in concentrated sulfuric acid. Duplicate analyses were made on all samples, and the standard deviation between duplicates was 0.06 mg/L.

A number of unfiltered samples, taken from various depths in the general area 1000 to 2000 miles off the coasts of Central America and Mexico were analyzed by the anthrone method; similarly, samples from 100 to 200 miles off the coast of southern California were analyzed with N-ethyl carbazole. These results, not tabulated in detail, fell within the range below 0.7 mg/L of dissolved carbohydrate, with a slight tendency to diminution with depth.

TABLE III. Carbohydrate in coastal lagoons of the San Diego area. Concentrations in mg/L in terms of a sucrose standard.

Lagoon	Carbohydrate	Chlorinity (°/₀₀)
San Luis Rey	3.9	12.2
Loma Alta Creek	3.4	16.0
Buena Vista (Upper)	2.9	4.0
Buena Vista (Lower)	2.2	4.7
Encinas	0.3	18.7
San Marcos	3.4	26.8
San Elijo	5.3	22.2
Del Mar	7.9	3.4
Sorrento (Upper)	1.6	19.7
Sorrento (Lower)	0.5	19.3
San Miguel	0.3–0.9	—

Determinations with the anthrone reagent were made on filtered samples from nine coastal lagoons in the vicinity of San Diego. Results are given in Table III together with chlorinity values as an indication of the influence of fresh-water drainage, sea-water flushing during the tidal cycle, and evaporation. Encinas, Sorrento, and San Miguel lagoons are open at the mouth, thus permitting various degrees of flushing during the tidal cycle; the six other lagoons are separated from the ocean by sand bars or cobble berms. In San Miguel lagoon the indicated values gradually increased from 0.3 mg/L at the mouth to 0.9 at the upper end of an arm 300 yards inland. This lagoon was sufficiently shallow to be completely drained with each low tide. In general, there was a direct correlation between the carbohydrate content and the occurrence of visible suspended organic matter in each lagoon.

When appraising the results reported here, note that the lower end of the sensitivity range of both analytical reagents was used in most cases. The reproducibility of results, as indicated by the standard deviation among multiple analyses of the same sample, is slightly less

than 0.1 mg/L. Indicated concentrations in many of the samples were not more than three or four times this amount, thus making any conclusions dubious. The mere absence or presence of two or more different carbohydrate constituents or of other substances to which the reagents may be sensitive could produce the differences observed in such cases. However, it seems definitely established that, in the open sea at least, there is in our part of the Pacific Ocean no such concentration of "carbohydrate" as has been reported in the Gulf of Mexico.

On the other hand, a small amount of such material does seem to be present in our closed lagoons as a result of land drainage or other causes, but this amount is not more than one-tenth of that in the Gulf region. And, if we may assume that particulate carbohydrate material is readily hydrolyzed by strong acids, the similarity of results from filtered and unfiltered samples indicates that the relative amount of such particulate material is small.

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