

# COMPARISON OF TWO METHODS OF N-ETHYLCARBAZOLE CARBOHYDRATE ANALYSIS <sup>1</sup>

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Two currently used N-ethylcarbazole (NEC) methods for determining the amount of dissolved substances in sea water which respond to the NEC reagent and which will be referred to here as "carbohydrates," are the original one described by Erdman and Little<sup>2</sup> and the modification described by Zein-Eldin and May (1958). It has been assumed by many investigators that those methods give comparable results when used to estimate the amount of carbohydrates in water. Recently, however, data obtained through both methods at the Bureau of Commercial Fisheries Biological Laboratory in Galveston, Tex., suggested that their corresponding estimates differ significantly. This difference prompted us to determine experimentally if the two methods yield the same results when used to analyze the carbohydrate content of aliquots from the same sample.

The original method described by Erdman and Little consists of adding the NEC reagent (1 g./l. of 36 N H<sub>2</sub>SO<sub>4</sub>) to the sample in one step, warming the mixture in a water bath, and then comparing color density with that of similarly treated arabinose (D-(-)-Arabinose) standards. This procedure will be called the "one-step" method. The method described by Zein-Eldin and May is the same as the above except that the NEC reagent is added in two steps. We will therefore refer to this procedure as the "two-step" method. Results of both procedures are based on the density of color

development when NEC reagent reacts with carbohydrates. Units are expressed in mg. equivalents of arabinose.

We compared the two methods by analyzing carbohydrate determinations obtained routinely from many samples with each procedure. The one-step method yielded significantly higher results (table 1).

TABLE 1.—Concentration of carbohydrates in samples of water collected from Galveston Island's East Lagoon during the period February–August, 1963

[Units are mg./l. of arabinose equivalents]

| Date, 1963   | Method   |          | Date, 1963 | Method   |          |
|--------------|----------|----------|------------|----------|----------|
|              | One-step | Two-step |            | One-step | Two-step |
|              | Mg./l.   | Mg./l.   |            | Mg./l.   | Mg./l.   |
| Feb. 16..... | 4.15     | 1.06     | 6-14       | 3.80     | 2.27     |
| Feb. 19..... | 4.00     | .61      | 6-18       | 4.71     | 3.76     |
| Mar. 1.....  | 3.80     | 1.98     | 6-21       | 3.92     | 2.32     |
| Mar. 12..... | 4.55     | 1.71     | 6-25       | 3.66     | 1.78     |
| Mar. 15..... | 4.35     | 2.61     | 6-28       | 4.64     | 3.25     |
| Mar. 19..... | 3.65     | .78      | 7-2        | 5.69     | 3.90     |
| Mar. 22..... | 3.50     | 1.15     | 7-5        | 5.85     | 3.70     |
| Mar. 26..... | 3.20     | .85      | 7-9        | 7.21     | 3.18     |
| Mar. 29..... | 2.55     | .73      | 7-12       | 5.07     | 3.24     |
| Apr. 2.....  | 3.11     | 1.86     | 7-16       | 4.28     | 3.03     |
| Apr. 9.....  | 5.14     | 3.01     | 7-19       | 5.36     | 4.45     |
| Apr. 23..... | 3.17     | 2.33     | 7-23       | 4.28     | 2.58     |
| May 8.....   | 4.06     | 1.98     | 7-26       | 6.50     | 3.48     |
| May 10.....  | 3.43     | 2.15     | 7-30       | 4.71     | 3.15     |
| May 14.....  | 3.91     | 2.36     | 8-2        | 5.07     | 2.94     |
| May 17.....  | 3.94     | 2.02     | 8-7        | 5.07     | 2.18     |
| May 21.....  | 3.28     | 2.13     | 8-9        | 7.85     | 3.03     |
| May 24.....  | 2.31     | 2.24     | 8-13       | 4.57     | 2.67     |
| May 28.....  | 3.03     | 1.71     | 8-16       | 5.00     | 3.51     |
| May 31.....  | 3.60     | 2.25     | 8-20       | 4.07     | 2.70     |
| June 4.....  | 4.13     | 2.70     | 8-21       | 2.55     | 2.47     |
| June 7.....  | 3.33     | 1.99     | 8-22       | 3.60     | 2.36     |
| June 11..... | 4.58     | 2.63     |            |          |          |

Upon investigating the reason for the differences noted between estimates secured by the two methods, we found that the color density resulting from interacting NEC and carbohydrates in natural waters varied only slightly with the method used. However, when the NEC reagent was added to standard or calibration samples that consisted of

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<sup>2</sup> Erdman, J. G. and A. B. Little. 1950. Analysis of marine coastal and estuarine waters, 50 pp. Multiple fellowship of Gulf Research and Development Co., Mellon Institute, Pittsburgh, Pa. (Unpublished.)

known concentrates of arabinose in distilled or saline water, the resulting color density varied with the method used. The variation was such

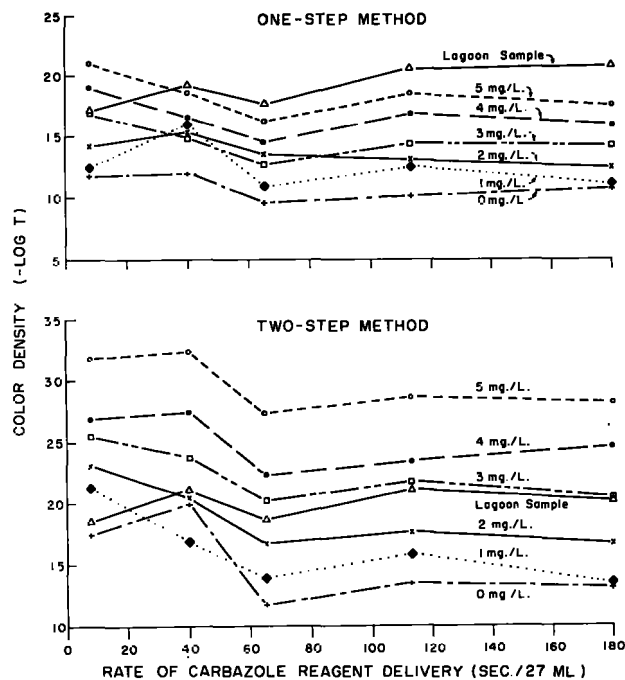


FIGURE 1.—Color density values obtained for six standard water samples and for one sample taken from Galveston Island's East Lagoon using two methods of carbohydrate analysis at five rates of reagent addition.

that estimates by the one-step method were higher than those obtained by the two-step method. We also found that the precision of estimates by either method is reduced if the reagent is added at a rate faster than about 27 ml.—the amount required by each procedure—per 65 seconds.

These conclusions become apparent from figure 1, which shows the raw data used to estimate the carbohydrate concentration in a water sample by both methods at various rates of reagent addition. If we consider only those portions of the curves from the 65-second addition rate and beyond, it can be seen that response of the carbohydrate in the sample to the NEC reagent corresponds to that of 6 to 7 mg./l. of arabinose by the one-step method, and 2.5 to 3 mg./l. by the two-step method. If the entire curves are considered, the sample estimates of carbohydrate concentration vary from about 0.3 to 7.0 mg./l. depending on the method used and also on the rate of reagent addition. The erratic results recorded when the reagent delivery rate was less than about 27 ml. per 65 seconds seem to be characteristic of both methods.

#### LITERATURE CITED

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