

A CONVENIENT METHOD OF ANALYSIS OF HUMIC ACID IN FRESH WATER¹

Humic Acid Analysis

Dean F. Martin and Richard H. Pierce, Jr. *

Department of Chemistry and Marine Science Institute
University of South Florida, Tampa, Florida 33620

Humic acids are generally defined operationally as those decomposition products of plants and animals that comprise the base-soluble, acid- and alcohol-insoluble fractions of the soil.² These materials are of interest because of their geological significance, possible nutritive properties, involvement of biological processes, and the challenge afforded by their chemical and structural complexities.³ Our particular interest in humic acid distribution in fresh water derives from an interest in the evident involvement of humic acids in red-tide outbreaks.⁴⁻⁷ This note describes a convenient method for determining the humic acid content of natural waters.

EXPERIMENTAL

Standard Humic Acid Samples. These were isolated from bog soil (adjacent to the Hillsborough River) using the method described by Overstreet⁸ and others.³ The humic acid samples, obtained as dark brown solids, had

* Present address: Department of Agricultural Chemistry, University of Rhode Island, Kingston, R.I.

solution was determined at 520 mμ. The concentration of humic acid in the 100-ml sample was determined from the standard curve prepared from the absorbance of standard humic acid solutions extracted by the same procedure. The sample concentration may also be determined from the linear relationship

$$\text{Humic acid concentration (ppm)} = \text{Absorbance} \times 1/\underline{m}$$

Here, \underline{m} is the slope of the linear calibration plot, absorbance as a function of concentration, expressed as ppm humic acid.

Those water samples not analyzed immediately after collection were kept frozen to inhibit any biological or chemical change which might alter the humic acid concentration. Studies show a slight increase in the humic acid concentration of sample frozen for periods up to two months, but this increase was less than the precision of the analysis procedure and is, therefore, considered insignificant.

DISCUSSION

The extraction procedure follows the operational definition of humic acid and is applicable to routine analysis for humic acid. The method was tested for precision or reproducibility by analyzing ten different standard solutions made from standard humic acid extracted from river soil and collected at two different times from the same location.¹¹ For these standards, the slope of the standard curve, \underline{m} , was found to be 0.02 with a standard deviation of 0.002. It should be noted that there is an instrumental fluctuation of ± 0.005 . (This absorption range corresponds to a humic acid concentration of about 0.5 ppm.) The procedure has been tested over the concentration range of 0.5 ppm to 40 ppm which is a useful range. Most rivers on the west coast of Florida, for example, have humic acid concentrations of 1 to 10 ppm.¹¹ The region of the visible spectrum which gives a large scale deflection

characteristic infrared absorption bands (KCl pellet) at 2.95 μ , 3.42 μ , 5.84 μ , and 6.10 μ , which correspond to the reported values.^{9,10} Humic acid standard solutions were prepared by dissolving 40.0 mg of dried humic acid in 10 ml of 0.5 N NaOH solution. The alkaline solution was diluted with distilled water to 1 liter to give a stock solution (40 ppm; pH = 11); other concentrations were prepared by diluting aliquot portions with distilled water.

Analysis of Natural Water Samples. Three steps were involved in the analysis. First, a 100-ml water sample, contained in a 250-ml separatory funnel, was treated with 5 ml of glacial acetic acid, shaken vigorously, and 15 ml of isoamyl alcohol was added. After additional shaking, the mixture was allowed to stand until the two layers separated and the humic acid was precipitated at the interface. A minimum of five hours was allowed for complete separation.

Second, the contents of the separatory funnel were filtered through a medium porosity sintered-glass filter funnel under vacuum. The humic acid precipitate was collected on the sintered-glass, washed with distilled water and 95% ethanol to remove any humatomelanic acid present, and air dried. The precipitate was removed from the funnel by dissolving in 5.0 ml of 0.5 N NaOH solution. The NaOH solution was added in two aliquot portions to insure the complete dissolution and removal of humic acid. The alkaline solution was transferred from the filter funnel by vacuum filtration into a 5.0-ml calibrated spectrophotometric test tube contained in the filter flask. Since the vacuum often vaporized some of the NaOH solution, this was replaced by the dropwise addition of 0.5 N NaOH solution, through the filter funnel.

Finally, the test tube containing the alkaline solution was transferred to a Bausch and Lomb Spectronic 20, where the adsorption of the for these concentrations is 520 m μ .

Preliminary studies indicate that the extraction procedure is applicable to sea water samples, though a slightly different value of \bar{m} is observed.

ACKNOWLEDGMENTS

This research was supported by the Florida Department of Natural Resources. One of us (DFM) acknowledges with gratitude the support of a Career Development Award (1 KO4GM 42569-01) from the National Institute of General Medical Sciences, U.S. Public Health Service.

REFERENCES

1. Contribution No. 28, University of South Florida, Marine Science Institute.
Contribution No. 146, Marine Research Laboratory, Florida Department of Natural Resources.
2. G. Tollin and C. Steelink, Biochim. Biophys. Acta, 112, 377 (1966).
3. A. P. Black and R. F. Christman, J. Amer. Water Works Ass., 55, 753 (1963).
4. R. M. Ingle and J. Williams, Fla. Bd. Conserv. Prof. Papers Ser., 8, 1 (1966).
5. R. M. Ingle, U. S. Dept. of Interior Spec. Sci. Rep.-Fish, 521, 3 (1965).
6. D. F. Martin, Adv. Chem. Ser., 67, 255 (1967).
7. A. Prakash and M. A. Rashid, Limnol. Oceanogr., 13, 598 (1968).
8. R. A. Overstreet, Fla. Bd. Conserv. Leaflet Ser., VI, (3) No. 1, (1966).
9. B. K. G. Theng, J. R. H. Wake, and A. M. Posner, Soil Sci., 102, 70 (1966).
10. R. B. Midwood and G. T. Felbeck, Jr., J. Amer. Water Works Ass., 60, 357 (1968).
11. D. F. Martin, M. T. Doig, III, and R. H. Pierce, Jr., Fla. Dept. Nat. Resources, Prof. Papers Ser., 12, In press.