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by Richard D. Johnson

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Moffett Field, Calif.

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A CRITICAL STUDY OF THE ABSORPTION OF POLYPEPTIDES IN THE FAR-ULTRAVIOLET AS A MEANS OF EXTRATERRESTRIAL LIFE DETECTION

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SUMMARY

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It recently has been proposed and concluded feasible that the far-ultraviolet spectrum of polypeptides can be successfully used in a life detection device. A critical review of that work shows there can be serious errors in both the position and intensity of the absorption maxima in this spectral region under the conditions proposed. Results of critical experiments are presented to clearly illustrate these errors and show them to be attributable to stray light. In addition, a survey of materials other than polypeptides which also absorb in the same spectral region indicates that interference from nonpeptides can be substantial. This interference coupled with stray light errors precludes confidence in far-ultraviolet absorption as an approach to extraterrestrial life detection.

author

INTRODUCTION

It has been proposed (refs. 1,2) and concluded feasible (refs. 2,3) to detect extraterrestrial life by means of the ultraviolet absorption of polypeptides in the far ultraviolet (uv) spectral region, 1850-2200 Å.

Briefly, the method (ref. 2) proposed to analyze aqueous extracts of soils with a double-beam, broad-band spectrophotometer operating in the 1850-2200 Å region. The extract was to have been treated with hydrochloric acid, divided into two aliquots, and placed in both beams of the instrument. The reduction in absorption produced by heating one of the samples was then to have been construed as evidence that the soil contained polypeptides.

This proposed method was based upon experiments in which a conventional single-monochromator laboratory instrument in the double-beam mode was used to detect the decrease in absorption produced by heating a series of samples with acid. The resulting hydrolysis of the peptides was used for distinguishing such material from other substances with absorption in the same region. The hydrochloric acid was necessary not only to promote hydrolysis but also to protonate any free carboxylate ions which also absorb in the same spectral region.

The present paper provides experimental evidence of sources of error in the proposed method and evaluates its feasibility.

EQUIPMENT AND MATERIALS

Three commercial scanning uv spectrophotometers were used. Two contained double monochromators (used in ref. 1) while the third (used in refs. 2,4) contained a single monochromator. All were operable to below 1850 Å.

The cells were high-grade fused silica of 10.0 and 1.0 mm path lengths. Demountable cells were also tried, but found to be extremely difficult to compensate in regions of high solvent absorption.

The chemicals and solvents used were either analytical or spectrograde.

PROCEDURES

All instruments were purged with nitrogen before use until no oxygen bands could be detected above 1850 Å.

Stray light was measured by the method used by Doty (ref. 5): a 10.0 mm cell filled with isopropyl alcohol was placed in the sample beam, and the solvent and cell actually used in the analysis were placed in the reference beam. The alcohol cuts off any transmission below 2050 Å so that any energy indicated by the detector below this point constitutes stray radiation of wavelength greater than 2050 Å.

RESULTS AND DISCUSSION

When attempts were made to duplicate the measurements of reference 2, it was found that double monochromator spectrophotometers apparently lacked the energy necessary to keep the slits from fully opening. As shown in figure 1, the solvent, 0.1 N hydrochloric acid, cuts off transmission below 1950 Å even with the 1.0 mm cell. In contrast, the authors of reference 2 were able to operate well below this point with narrow slits and low noise levels; they noted this incongruity when they changed spectrophotometers in the course of their work. For their work a single monochromator instrument was finally chosen since Doty (ref. 5) had used such an instrument in previous successful uv studies of polypeptides. Although the life detection approach to polypeptide analysis was closely patterned after Doty's work it did not consider Doty's evaluation of the possible errors.

There are two contributions to the higher apparent energy levels in the single monochromator instrument. First and most obvious is the fact that the energy is truly higher since there are fewer optical surfaces. Given equivalent lamps and detectors and assuming 90-percent efficiency for each additional mirror and the extra monochromator, it can be estimated that a double monochromator spectrophotometer will have about 50 percent of the energy of a single monochromator instrument. On the other hand, the double monochromator instruments have much lower stray light levels. Thus, of the energy reaching the detector, the single monochromator version contains a higher percentage of light other than the primary wavelength. This stray radiation hitting the detector thus satisfies the slit servo system to the point that the slits do not fully open as they should, even when there is essentially no primary radiation left.

In actuality, when used in a normal manner, both single and double monochromator instruments possess stray light levels below 1 percent in the region below 2000 Å. Since stray radiation is expressed as a percentage of the total radiation incident upon the detector, when an absorbing substance is placed in the beam, the primary radiation is absorbed, leaving a greater percentage of stray radiation by a reduction in the total energy. Even with water as a reference, stray light levels are essentially undetectable without scale expansion in both types of instruments.

With 0.1 N hydrochloric acid in a 1.0 mm cell in the reference beam of the single monochromator instrument the stray radiation rises rapidly at 1950 Å and reaches approximately 40 percent at 1750 Å, as shown in figure 2. In double monochromator instruments, the slits promptly open when the energy falls off at the point of solvent absorption.

References to the errors resulting from stray light are numerous (refs. 5-17). To summarize briefly, given equation (1) for the Beer-Lambert laws, the measured absorbance is given in equation (2) after correction for stray light.

$$A = -\log \frac{I}{I_o} = cd\epsilon \tag{1}$$

$$A \text{ measured} = -\log \frac{(I + I \text{ stray})}{(I_o + I \text{ stray})} = c_{meas} d\epsilon \tag{2}$$

where A is absorbance, c is concentration, d is path length, ϵ is the extinction coefficient, and I and I_o are the intensity of the sample and reference beams, respectively, incident upon the detector. The errors are the greatest at the spectral limits of the instrument (such as the far uv) where the detector sensitivity is lower to the primary radiation than it is to stray light, and where the source intensity is lower than it is at other wavelengths. The errors are also more serious for high absorbance values.

To vividly demonstrate these errors, an experiment was devised to test the effect of hydrochloric acid upon the spectrum in a single monochromator instrument without any complications due to pH. The sample and appropriate reference in 10.0 mm cells were placed in tandem with separate 1.0 mm cells containing either water or hydrochloric acid. With no sample, the 100-percent line was run to show balanced beams. The spectrum with water in the short path cell showed no change from that of the sample alone. However, 0.1 N hydrochloric acid drastically altered the spectrum due to the higher percentage of stray light. Figure 3 shows that the spectrum of acetone vapor in nitrogen, versus nitrogen is the same as that with the 1.0 mm cells with water added to each beam; when the water is replaced by the hydrochloric acid, the spectrum is altered so that the peaks below 1950 Å are entirely absent.

Figure 4 illustrates the same effect with phenylalanine versus water. In this example, not only is the absorbance greatly in error, but the actual peak maximum shifts from 1880 to 1940 Å.

The results pertaining to peptide detection in a Potomac River sand extract were reported (ref. 2) to produce a shift from 1800 to 1930 Å, and to reduce absorbance upon adjustment of the pH. Exactly the same result would have been obtained had the acid been placed in a separate cell in each beam. The actual changes observed by further heating of the acidified samples are quite small and could easily be attributed to loss of hydrochloric acid upon heating.

From the above examples, the results previously attributed to the presence of polypeptides can be optical artifacts possessing no relation to the sample itself. In this case the errors of shift in absorption peak position and intensity have been shown. Other authors (refs. 7,9,16) have shown that false maxima can result from some cases of high stray light. Errors of a similar sort, but due to partial molar volume effects have been reported (refs. 18,19) and might be expected to also play a role in this case. They have not been evaluated, however.

The problems arising from stray light could be reduced or eliminated by the use of sulfuric rather than hydrochloric acid, and a matched pair of filters which pass light only below 2200 Å.

INTERFERENCE FROM NONPEPTIDES

Reference 2 lists 23 compounds which are said to interfere with the analysis. This list is by no means comprehensive. The data from volume I of the Sadtler uv collection[1] show that at 2000 Å 213, or 70 percent of the 308 entries, have specific extinction coefficients greater than the average of the 23 compounds reported to interfere. This estimation, however, crude, suggests the level of interference which might be expected in view of the thousands of organic compounds that may be present in a soil sample. With trace amounts of many interfering substances, while the individual interference levels may not be significant, the sum of all the absorbing species could be expected to interfere seriously.

A list of organic compounds which absorb in this spectral region includes olefins, acetylenes, aldehydes, ketones, carboxylic acids, acid chlorides, amides, alkyl nitrates, alkyl halides, ethers, alcohols, amines, and aromatics (refs. 20,21), not to mention numerous inorganic ions (ref. 18). Certainly any sample thought to contain polypeptides would have a liberal quantity of the above species.

The suggestion that acid hydrolysis be used to distinguish peptides from other substances is not really a safeguard against interference since many of

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[1]Sadtler Research Laboratories, Philadelphia, Pa.

the interfering substances also react with acid and could give a similar change in absorption. Extensive fractionation of the sample is necessary to minimize interference.

CONCLUSION

The contribution of stray light to the measured absorption of materials in the far uv has been shown to be substantial in the case of highly absorbing solvents.

While the spectra of genuine polypeptides are in error when run in hydrochloric acid below 1950 Å, the absorption is real. However, in soil samples the stray light errors may easily account for the spectral features attributed to polypeptides. It has been shown that interference from nonpeptides can be substantial. Without complete fractionation of the sample, interference cannot be avoided.

The combination of the effects of stray light and interference precludes confidence in this experimental approach to extraterrestrial life detection.

Ames Research Center
National Aeronautics and Space Administration
Moffett Field, Calif., May 6, 1965

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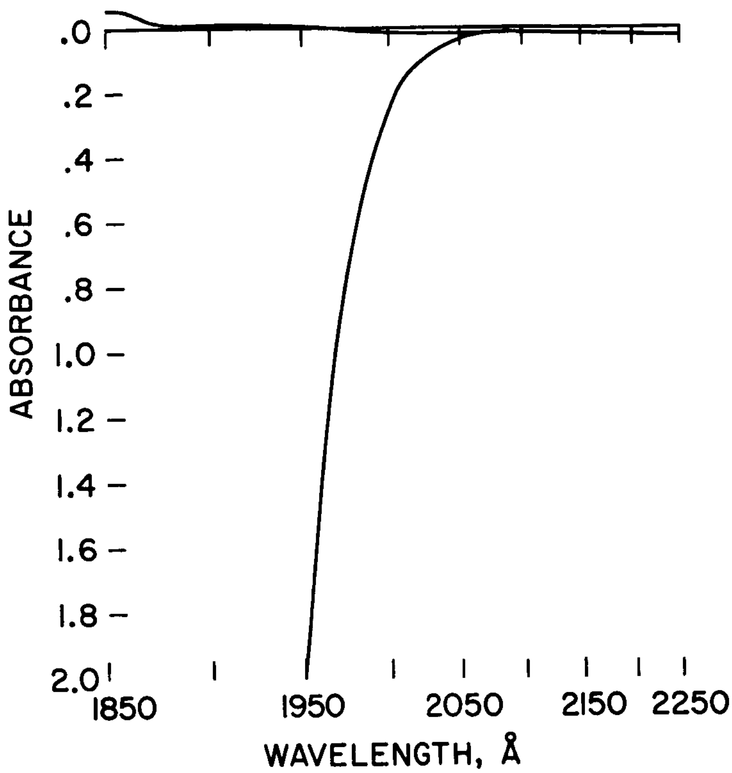

Figure 1.- Ultraviolet spectrum of 0.1 N HCl versus water (1.0 mm).

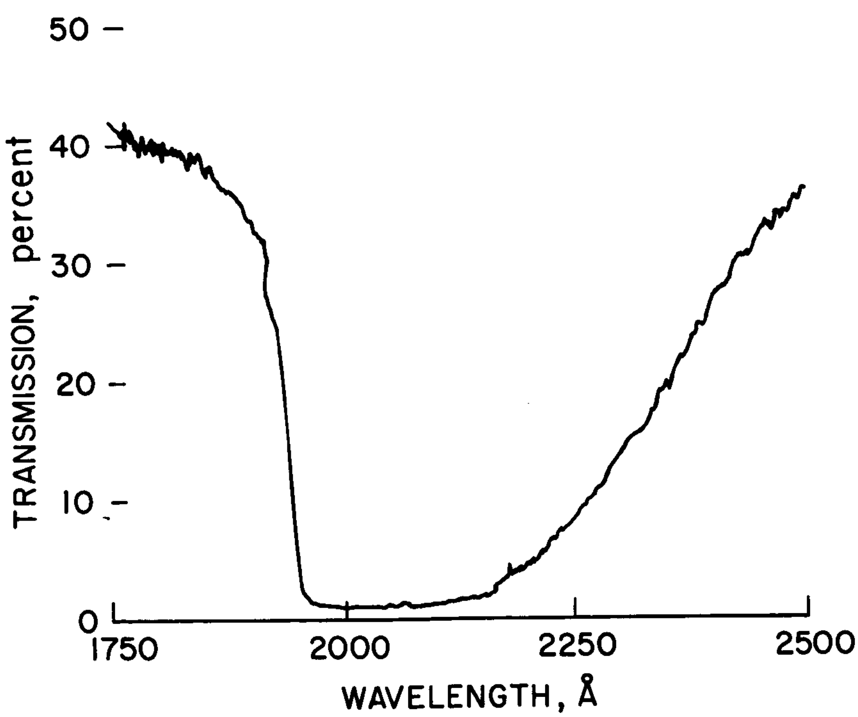

Figure 2.- Ultraviolet stray light test; isopropyl alcohol (10.0 mm) versus 0.1 N HCl (1.0 mm).

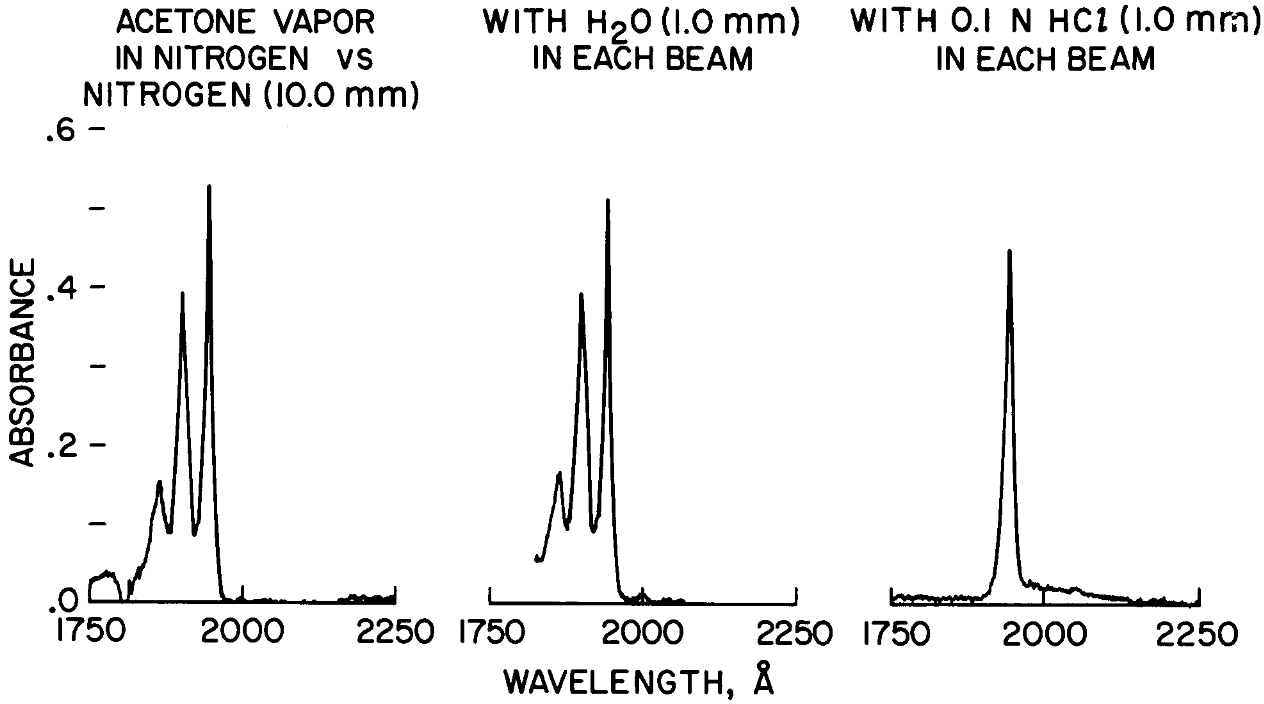

Figure 3.- Ultraviolet spectrum of acetone vapor in nitrogen, versus nitrogen.

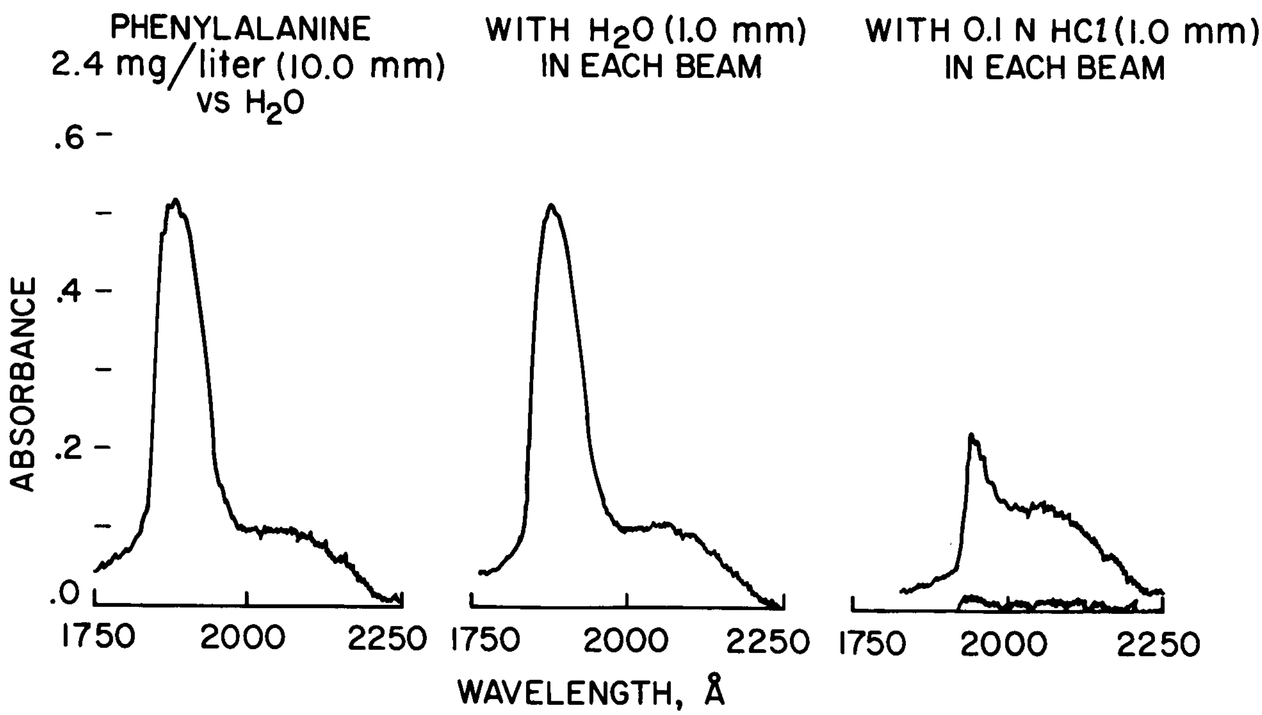

Figure 4.- Ultraviolet spectrum of phenylalanine versus water.

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