

Technical Publication

Title: On-Line UV/VIS Absorbance Spectroscopy and Fluorescence Spectroscopy for Detection of Organic and Aromatic Compounds in Water

ASA Publication Number: 37

Presented at: Biotronics Technologies, Inc.
White Paper

Note: This document was originally published by Biotronics Technologies, Inc. in conjunction with the ChemScan Process Analyzer technology base, now owned by Applied Spectrometry Associates, Inc. Please direct all inquiries and correspondence to:

Applied Spectrometry Associates, Inc.
W226 N555G Eastmound Drive
Waukesha, WI 53186

Phone: (414) 650-2280
FAX: (414) 650-2285

On-Line UV/VIS Absorbance Spectroscopy and Fluorescence Spectroscopy

for

Detection of Organic and Aromatic Compounds in Water

by:

**Bernard J. Beemster
Biotronics Technologies, Inc.
W226 N555B Eastmound Drive
Waukesha, WI 53186
(414) 896-2650 x128**

**WHITE PAPER
March 1994**

Introduction

Molecular spectroscopy based upon ultraviolet, visible and infrared radiation is widely used for the identification and determination of myriad inorganic and organic species. Infrared absorption spectroscopy, for example, is one of the most powerful tools available to the chemist for determining the structure of both inorganic and organic compounds. In addition, it is now assuming an important role in quantitative analysis, particularly for determining environmental pollutants. Unfortunately, infrared absorption is not particularly sensitive for detection of low (ppm) to trace (ppb) concentrations of most compounds.

Molecular ultraviolet/visible absorption spectroscopy is employed primarily for quantitative analysis and is probably more widely used in chemical and clinical laboratories throughout the world than any other single procedure. It is more sensitive than infrared spectroscopy but is unable to detect most saturated compounds.

Molecular fluorescence methods, while less generally applicable than absorption methods, are of considerable importance because of their high selectivity and extraordinary sensitivity. These methods have proved particularly useful for the quantitative determination of molecules of biological and biochemical interest.

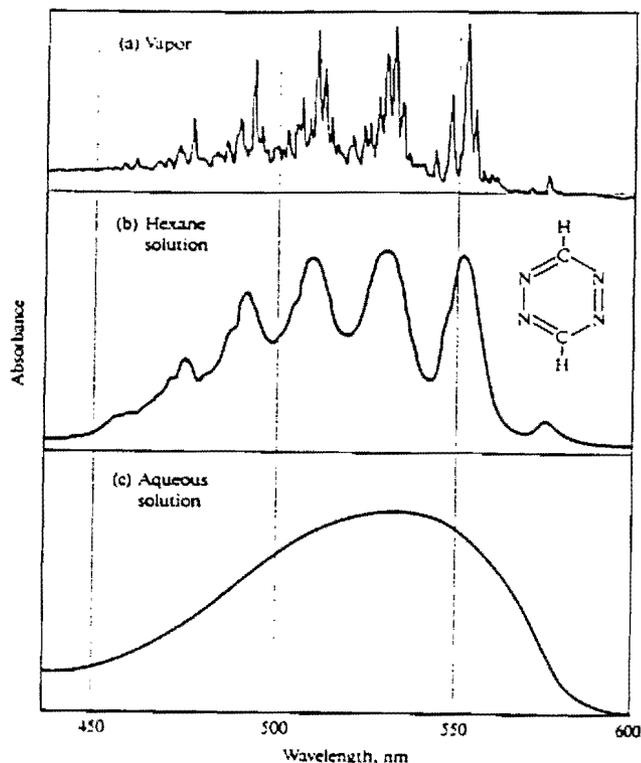
Biotronics Technologies, Inc., has employed ultraviolet/visible absorbance spectroscopy in a family of on-line process analyzer products designed to detect a wide variety of chemical compounds in water, including organic and aromatic compounds. These products are marketed under the ChemScan™ brand name.

Biotronics has also developed several types of on-line fluorescence spectroscopy instruments for detection and analysis of very low to trace concentrations of naturally fluorescent compounds.

Ultraviolet/Visible Absorbance Spectroscopy

Absorption of ultraviolet and visible radiation by molecules generally occurs in one or more electronic absorption bands, each of which is made up of numerous closely packed but discrete lines. Each line arises from the transition of an electron from the ground state to one of the many vibrational and rotational energy states associated with each excited electronic energy state. Because there are so many of these vibrational and rotational states and because their energies differ only slightly, the number of lines contained in the typical band is large and their displacement from one another minute.

Figure 1a, which is part of the visible absorption spectrum for 1,2,4,5-tetrazine vapor, shows the fine structure that is due to the numerous rotational and vibrational levels associated with the excited electronic states of this aromatic molecule. In the gaseous state, the individual tetrazine molecules are sufficiently separated from one another to vibrate and rotate freely, and the many individual absorption lines resulting from the multitude of vibrational/rotational energy states are clearly evident. In the condensed state or in solution, however, freedom to rotate is largely lost, and lines due to differences in rotational energy levels are obliterated. Furthermore, in the presence of solvent molecules, energies of the various vibrational levels are modified in an irregular way. Thus, the energy of a given state in an assemblage of molecules takes on a Gaussian distribution; line broadening is the result. This effect is more pronounced in polar solvents, such as water, than in nonpolar hydrocarbon media. This solvent effect is illustrated in Figures 1b and 1c.



Absorption by Organic Compounds

Two types of electrons are responsible for the absorption of ultraviolet and visible radiation of organic molecules: (1) shared electrons that participate directly in bond formation and are thus associated with more than one atom and (2) unshared outer electrons that are largely localized about such atoms as oxygen, the halogens, sulfur and nitrogen.

The wavelengths at which an organic molecule absorbs depend upon how tightly its various electrons are bound. Thus, the shared electrons in single bonds such as carbon/carbon or carbon/hydrogen are so firmly held that their excitation requires energies corresponding to wavelengths in the vacuum ultraviolet region (below 180 nm). Single-bond spectra have not been widely exploited for analytical purposes because of the experimental difficulties of working in this region. These difficulties are attributable to the fact that both quartz and atmospheric components absorb radiation below 180 nm, a circumstance that requires the use of evacuated spectrophotometers equipped with lithium fluoride optics.

Organic compounds containing double or triple bonds generally exhibit useful absorption peaks in the readily accessible ultraviolet region because the electrons in unsaturated bonds are relatively loosely held and thus easily excited. Unsaturated organic functional groups that absorb in the ultraviolet and visible regions are termed chromophores. Common chromophores include alkene, conjugated alkene, alkyne, carbonyl, carboxyl, amido, azo, nitro, nitroso, nitrate and aromatic groups.

Organic compounds containing sulfur, bromine and iodine also absorb in the ultraviolet region because these elements contain loosely bound unshared electrons that are more easily excited than

the shared electrons of a saturated bond and are thus more readily excited by the absorption of photons.

Fluorescent Species

Fluorescence is one of several mechanisms by which a molecule returns to the ground state after it has been excited by absorption of radiation. Thus, all absorbing molecules have the potential to fluoresce. Most do not, however, because their structure provides radiationless pathways by which relaxation can occur faster than fluorescent emission.

The quantum yield of molecular fluorescence is simply the ratio of the number of molecules that fluoresce to the total number of excited molecules (or the ratio of photons emitted to photons absorbed). Highly fluorescent molecules, such as fluorescein, have quantum efficiencies that approach unity under some conditions. Nonfluorescent species have efficiencies that are essentially zero.

Compounds containing aromatic rings give the most intense and most useful molecular fluorescent emission. While certain aliphatic and alicyclic carbonyl compounds as well as highly conjugated double-bonded structures also fluoresce, their numbers are small in comparison with the number of fluorescent compounds that incorporate aromatic systems.

Most unsubstituted aromatic hydrocarbons fluoresce in solution, with the quantum efficiency increasing with the number of rings and their degree of condensation. The simplest heterocyclics, such as pyridine, furan, thiophene and pyrrole, do not exhibit molecular fluorescence, but fused-ring structures containing these rings often do.

Substitution on an aromatic ring causes shifts in the wavelength of absorption maxima and corresponding changes in the fluorescence peaks. In addition, substitution frequently affects the fluorescence efficiency.

Fluorescence methods are generally one to three orders of magnitude more sensitive than methods based upon absorption because the sensitivity of the former can be enhanced either by increasing the power of the excitation beam or amplifying the detector signal.

Methods for Organic and Biochemical Species

The number of applications of fluorometric methods to organic problems is impressive. Weissler and White have summarized the most important of these in several tables¹. More than 100 entries are found under the heading Organic and General Biochemical Substances, including such diverse compounds as adenine, anthranilic acid, aromatic polycyclic hydrocarbons, cysteine, guanidine, indole, naphthols, certain nerve gases, proteins, salicylic acid, skatole, tryptophan, uric acid and warfarin.

References

1. A. Weissler and C. E. White, in Handbook of Analytical Chemistry, L. Meites, ed., pp. 6-182 to 6-196, New York, McGraw-Hill, 1963.