Technical Publication

Title: The Use of Multiple Wavelength Ultraviolet-Visible Absorbance
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ASA Publication Number: 62

Presented at: WEF Specialty Conference
Automating to Improve Water Quality
Minneapolis, MN
June 25-28, 1995

THE USE OF MULTIPLE WAVELENGTH ULTRAVIOLET-VISIBLE ABSORBANCE SPECTROMETRY FOR ON-LINE ANALYSIS OF WASTEWATER PROCESS SAMPLES

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ABSTRACT: Absorbance spectrometry in the ultraviolet-visible wavelength range is a well established technology for laboratory analysis and is now being used in a new generation of on-line process instruments. Unlike instruments used to detect a specific chemical parameter by measuring relative intensity at a single wavelength, this new generation of instruments can simultaneously detect numerous points of information across a range of wavelengths. This allows detection of an absorbance signature from a process sample which can be processed to obtain information about the chemical content of the sample using pattern recognition software. This allows the instrument to identify one or more chemical substances in the sample. Chemicals that have strong natural absorbance spectra can be detected based on the spectral contribution of the chemical to the overall absorbance signature of the solution. Chemicals without strong natural absorbance in the wavelength range detected by the instrument may require addition of a reagent prior to analysis.

INTRODUCTION

The detection of a color change in a sample following the addition of a reagent is fundamental to the field of chemical analysis. Such tests typically rely on reactions between the reagent and the target chemical to form a new compound with unique light absorbance characteristics proportionate to the concentration of the target chemical. Accordingly, there are numerous analytical procedures and instruments in everyday use that apply fundamental absorbance spectrometry techniques.

Fundamental concepts. All forms of absorption spectroscopy are based on the measurement of changes in energy. When a molecule absorbs radiation, its energy increases. The increase in energy is reflected in the form of changes in electronic, vibrational or rotational energy of the molecule [RAO 67]. Quantum mechanics tells us that only certain discrete energy levels are possible in atoms and molecules and that the atoms or molecules must exist at all times in one or another of these "allowed" energy states. The lowest energy level is called the "ground state" and higher levels are referred to as "excited states" (see Figure 1). Under normal conditions, an atom or molecule occupies

the ground state, but if energy is acquired from an external source, the energy level can jump from the ground state to one of the permitted excited states [THO 74].

The transitions that take place as a result of energy absorbed in the ultraviolet region occur in the valence electrons of the molecule [SIL 81]. Since the interaction between electrons to form chemical bonds are also due to the valence electrons and the orbitals comprising the partially filled outer shells of atoms, there is a relationship between bonding characteristics and ultraviolet absorption spectra for atoms and molecules. Thus, it can be said that it is the valence electrons that are responsible for the electronic spectra of molecules in the visible and ultraviolet regions of the spectrum as well as for the chemical reactivity of these molecules [SCH 77].
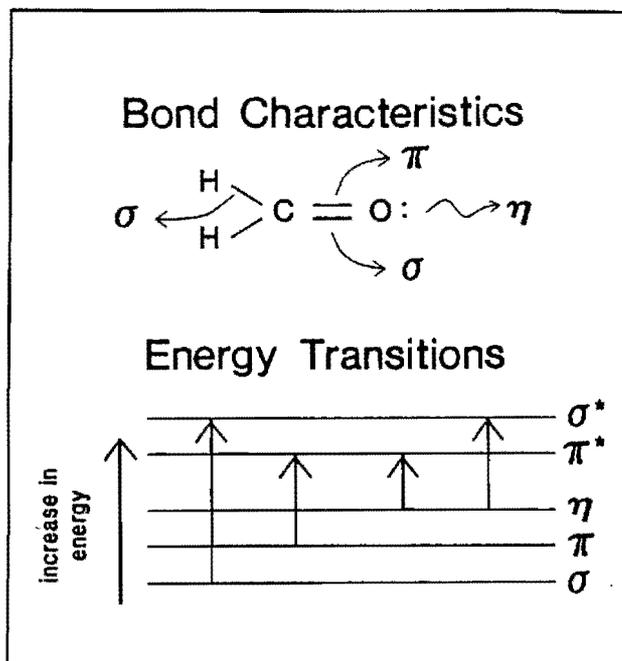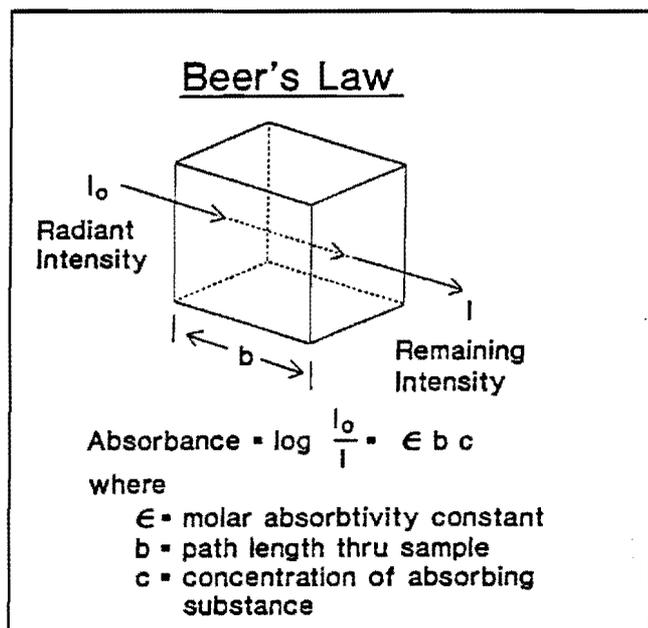
Figure 1. Energy Transitions.

Beer's law. It is typical for analytical procedures using spectrometry to require a measurement of absorbance intensity at a single significant (usually peak) wavelength. The identification of a specific wavelength for analysis in a given procedure is based on two primary criteria. First, there should be an absence of interferences from other chemicals in the sample so that the light intensity measured is influenced only by the target compound. Second, light absorbance at the selected wavelength must conform to Beer's law for a given concentration range of the target compound. This law, illustrated in Figure 2, relates the change in light absorbance through a fixed amount of sample to the concentration of an absorbing compound in the sample. If Beer's law is followed, the change in light absorbance will be proportionate to the concentration of the absorbing compound.

$$\text{Absorbance} = \log \frac{I_o}{I} = \epsilon b c$$

where

ϵ = molar absorbtivity constant
b = path length thru sample
c = concentration of absorbing substance

Figure 2. Beer's Law.

Multiple wavelength instruments. As a result of the familiar photometric procedures routinely performed for chemical analysis, we have become accustomed to thinking of spectrometry in terms of a device which is intended to be tuned to only one specific wavelength. Although many forms of ultraviolet, visible and near-infrared absorbance spectrometry perform analysis by detecting only a single wavelength, absorbance spectra for many target chemicals or compounds are available over a RANGE of wavelengths (the absorption band) and can be detected with the proper instrumentation. This is frequently done in an attempt to characterize the composition of an unknown through analysis

of its absorbance spectrum or comparison with the spectra of known substances.

There are several laboratory instruments in wide use whose purpose is to detect and record absorbance spectra within a specific region of the spectrum. These instruments are limited to the analysis of static (non-moving) samples and require a controlled environment for the analyzer due to the sensitivity of the optical components. Detection is performed in a stepwise manner from one wavelength to the next with adjustments performed immediately before or immediately after transmission of light through the 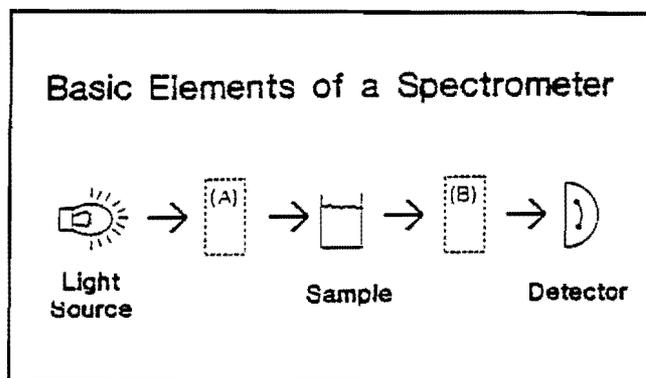sample. (See Figure 3) These instruments often use special monochromators (prisms or diffraction gratings) which are mechanically adjusted to permit the analyzer to step through a series of wavelengths in discrete intervals. Some instruments use a light source that is specially filtered to step through a series of wavelength intervals. Such instruments are relatively slow and can be very sensitive to temperature variation and vibration, making them unsuitable for use in many factory or field environments.

Figure 3. Spectrometer Elements.

REAL TIME ANALYSIS

If absorption spectrometry is to be used for real time analysis of multiple component chemical solutions, several limitations inherent in the current laboratory technology needed to be addressed.

Static vs. real time samples. Laboratory photometric instruments are designed to analyze a sample that has been physically extracted from a location. Considerable time may transpire between the extraction of the sample and the actual processing of the sample in the laboratory. In a laboratory setting the sample can, if necessary, be physically or chemically altered prior to analysis. If laboratory analysis is performed on an instrument that steps through a series of wavelengths, the sample can be held in the instrument for whatever time is required to complete the analysis.

Real time chemical analysis applications, such as automatic process control or quality monitoring, require an analysis that is not remote in time or place. The detection of a target chemical can be performed directly in the solution being analyzed (in-situ analysis) or at least in a fractional sample that is pumped from the solution to an automatic analyzer (on-line analysis). This implies close proximity between the solution and the analyzer and reasonably short intervals between each analytical event. Of course, the same techniques used in a laboratory can sometimes be performed at the point of sample extraction using portable apparatus (on-site analysis), but the direct intervention of an operator is required.

If on-line analysis is not instantaneous and continuous or if sample lines are very long, the fraction of the sample being analyzed may no longer reflect the actual conditions in the original solution at the time of processing. Such forms of analysis could continue to be termed "on-line" analysis but could

only be considered "near real time" analysis. In solutions that change slowly this may be a distinction without a difference. But if the solution is unstable, this distinction may be significant.

Multicomponent chemical analysis. If a solution to be analyzed contains more than one absorbing component, the absorption spectra detected will be a 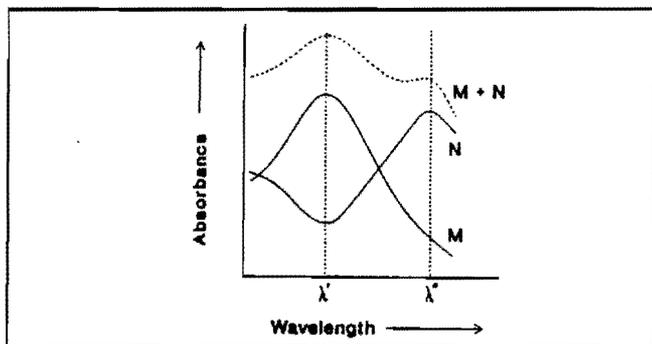function of all absorbing components. In liquid media, the overlap of absorption spectra for the individual components will result in smooth combined spectra as illustrated in Figure 4. If absorbance spectrometry is to be performed on-line, hardware and software techniques that are capable of rapidly detecting and interpreting these overlapped spectral signatures is required. This also implies that the analysis system has access to information concerning the shape and positions of spectra for each absorbing component over a range of concentrations, so that combination effects can be derived mathematically.

Figure 4. Two component absorbance signatures.

APPARATUS FOR ON-LINE SPECTROMETRY

The apparatus required to perform on-line spectrometry can be classified into three functional areas: methods for transmittance of light through a fixed distance of sample, methods to detect light absorbance at multiple wavelengths and methods to process multiple wavelength information.

Light transmittance through a sample. A number of approaches are possible. Fiber optics can be used to achieve some degree of separation between the sample and a light source, or the light source can be positioned to radiate light directly into the sample. Various optical components can be used to filter and/or collimate the light prior to the point of entry into the sample. Finally the sample itself can be contained within a chamber where the walls are optical components of fixed path length. In this case, means must be designed into the system to deal with fouling of optical surfaces in contact with the sample. Alternatively, the sample can be self contained in a free falling stream with light penetration into the sample through a small volume of air. In this case fouling is reduced, but means must be designed into the system to avoid bubbles in the sample, produce a constant sample cross section and prevent fogging of optical surfaces within the sample chamber.
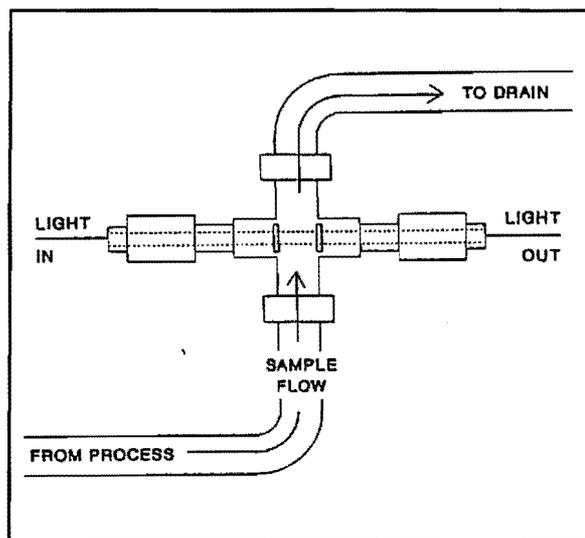

Figure 5. Flow Through Optical Cell.

An example of a flow-through optical probe is illustrated in Figure 5. This design allows a either

continuous or intermittent flow of a sample through the cell. Light can be transmitted through the sample continuously or at intervals governed by the instrument design.

Light detection following transmittance. Given the need to detect information at multiple wavelengths for on-line analysis, several methods are available. If only a few wavelengths are to be detected, one approach is to separate the light and use individual diode detectors for each desired wavelength. Another approach is to use a single photodiode detector in conjunction with optical filters that pass only the wavelengths desired for analysis. When many individual wavelengths are desired, a variation of this approach uses an optical grating to separate the light into individual wavelengths, while projecting a sequence of individual wavelengths onto a detector.

The development of photodiode detector arrays has made it possible to simultaneously detect an entire wavelength range with high resolution and thus read spectral signatures on-line, even in a flowing sample. These detectors eliminate the need to mechanically index through wavelength intervals, eliminating movable gratings and mirrors and thus eliminating the moving parts that would otherwise compromise use of the instrument in field or factory environments. Since array detectors are available in as many as 1024 increments a broadband source of light can be used without filters to detect highly resolved absorbance signatures, although a fixed grating is required to separate the light into individual wavelengths prior to projection onto the array for detection, as illustrated in Figure 6.
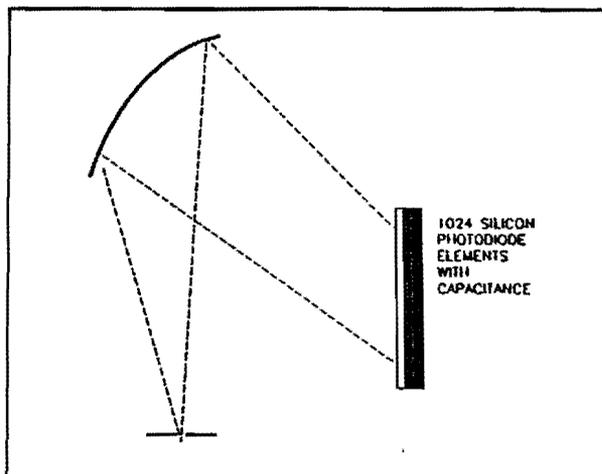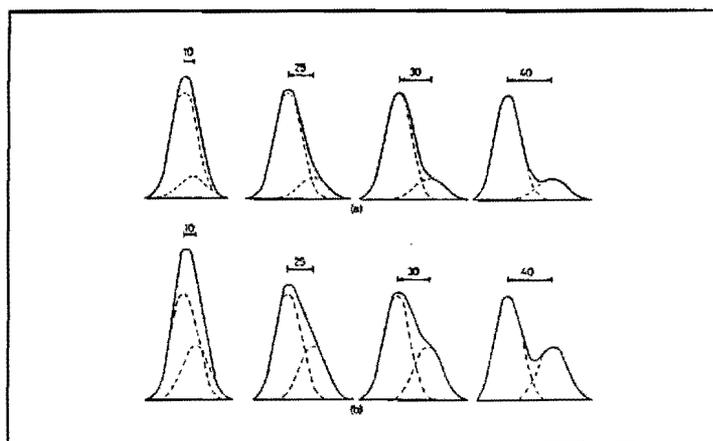
Figure 6. Photodiode Detector Array.

Figure 7. Component effect on absorbance spectra.

The detection system can be located adjacent to the sample or the detection system can be separated by some distance from the sample, with optical fibers acting as a light pipe. In either case, some method must be used to obtain an initial light intensity measurement at each detected wavelength in order to measure the intensity drop through the sample. This can be done by measuring the light output by the system through a non absorbing media.

Pattern recognition. On-line analysis typically involves solutions that contain numerous components, resulting in overlapping or closely grouped spectra. The overall absorbance spectra for a liquid is a smooth pattern that results from the combined light absorbance effects of individual components. This is illustrated in Figure 7, which shows the changing absorbance signature for a solution with two absorbing components. As the

relative concentration of the components change, the absorbance signature is also changed. If the components have absorbance spectra that change with concentration in a predictable manner, there would be only one combination of individual absorbance spectra that could "explain" a combined absorbance signature detected for this solution. That combination of individual absorbance spectra can also be used as a measurement of concentration for the components. We call this process "pattern recognition".

Multivariate characterization. The pattern recognition process includes an initial analysis of standards and spiked samples. This process is often called "calibration" but, to avoid confusion with the single wavelength measurement of high and low laboratory standards, the procedure should be called "multivariate characterization". This is performed to obtain a file of absorbance signatures for different concentrations of a target compound in pure media and in typical sample media, using the same instrument that will be used for on-line analysis. The multivariate characterization process provides a set of light intensity values needed to produce a pattern recognition algorithm which can ultimately be used for on-line analysis.

In a simple solution, such as one containing a single component in transparent media, the number of wavelengths used for analysis is not significant provided they are within a range that obeys Beer's law for the absorbing component. A virtually identical correlation can be obtained for calculated concentration values with one, two or three wavelengths as with thirty or fifty. If a second component is added which contributes overlapping spectra, wavelength selection and number become significant. At least two wavelengths are required if both components are to be independently analyzed, and this is only possible if a wavelength can be selected which is significantly affected by only one component. As the solution becomes more complex, a larger number of properly selected wavelengths improves the correlation between a calculated concentration for a target compound and the actual concentration of the compound. This can be seen in Table I.

Table I. Nitrate correlation, calculated vs. actual concentration.

Number of Wavelengths (excluding reference)	Simple Matrix (deionized water)	Complex Matrix (wastewater)
1	.99	---
2	.99	.82
3	.99	.88
5	.99	.92
10	.99	.96
20	.99	.98
30	.99	.99
40	.99	.99
50	.99	.99

On-line analysis steps. There are three basic steps involved in the process of using multiple wavelength absorbance spectra for on-line chemical analysis. These steps are illustrated in Figure 8. **Quantification** involves converting detected spectra for multivariate characterization solutions and unknowns into numerical values that can be processed using mathematical and statistical procedures. **Preprocessing** of raw data reduces the effects of instrument noise and transforms absorption information into forms that permit more efficient analysis. **Analysis** of absorbance values uses

Figure 8. Spectral Analysis Steps.

pattern recognition techniques to identify individual components and calculate their concentrations in a sample.

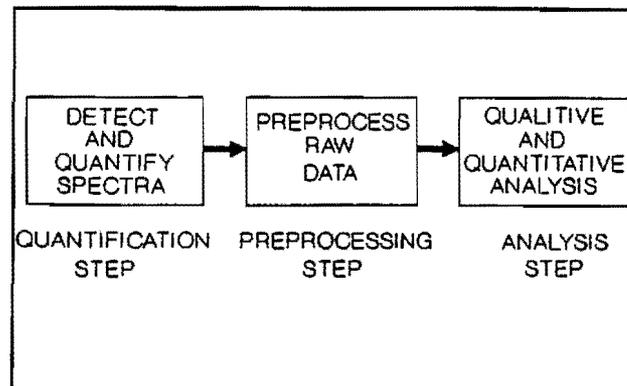**Primary vs secondary analysis.** Although numerous chemicals have natural absorbance spectra in the ultraviolet-visible range (including many nutrients, heavy metals, organics and aromatic compounds), many other chemicals do not. When on-line UV-VIS spectrometry is used for direct detection of a natural absorbance spectra, the term "primary" analysis is used. When chemical reagents must be employed to induce a detectable absorbance spectra, the techniques are termed "secondary" analysis.
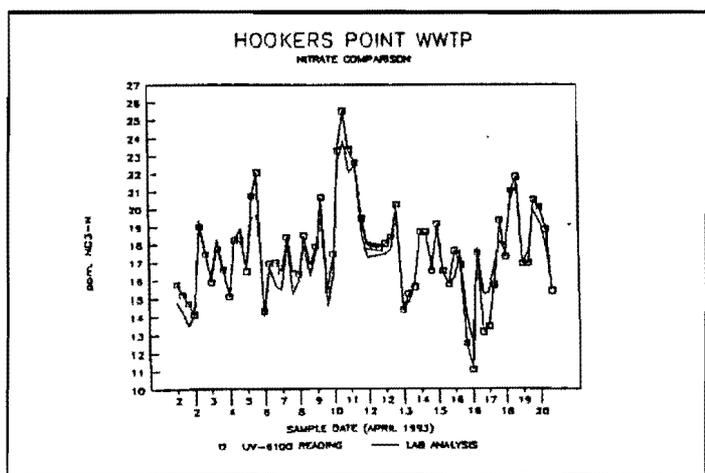
MAJOR APPLICATIONS AND RESULTS

Figure 9. Nitrate Analysis, On-line vs. Laboratory.

On-line absorbance spectrometry is currently in use for primary analysis of nitrate in municipal well water and for primary nitrate analysis in nitrified wastewater. Figure 9 illustrates results from an large municipal wastewater treatment plant in Florida. The figure shows the degree of agreement between on-line nitrate results using a thirty wavelength pattern recognition algorithm compared to the results from laboratory analysis of grab samples. At another wastewater facility, a single instrument is being used for individual analysis of nitrate and nitrite, with secondary analysis of ammonia, all in a common sample line. The technology has also been successfully used for on-line primary analysis of metals (iron, copper) in water and wastewater, primary analysis of natural organic material (NOM) in surface water and on-line secondary analysis of phosphate in water and wastewater.

CONCLUSION

New technology is converting ultraviolet-visible absorption spectrometry from a laboratory technology to one that can successfully be used in field and factory applications for on-line chemical analysis of complex solutions. These technology improvements include photodiode detector arrays and pattern recognition analysis techniques to permit analysis of samples using multiple wavelengths.

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