Trace Analysis

Spectroscopic Methods for Molecules

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PREFACE

As recently as 1979 (1), the field of organic trace analysis was heralded as a "new frontier." Its development as an analytical subdiscipline has been driven by the demands of applied scientists such as toxicologists and environmentalists for increasingly sensitive and selective elucidation of tiny amounts of species (pesticides, toxins, pollutants) in complex matrices (soil, blood, plants). In turn, increasing capabilities in instrumentation have led to new discoveries concerning the importance of organic trace materials and consequent demands for even greater sensitivity and selectivity. This upward spiral seems likely to continue until the ultimate capability is reached of detecting and characterizing one molecule, even if it is the only one of its kind, present at some arbitrary location in the volume of the known universe!

A quantitative definition of trace analysis will not be given in this preface, because its meaning in the past has varied widely with the background, interest, and intent of various authors. The definition must take into consideration the context of the sample, the analytical technique, and the particular analysis. Nevertheless, it is widely agreed that the term trace signifies a constituent which is present in minor concentration in another material referred to as the matrix. It is important to not confuse trace methods with micromethods. The latter refers to the processing and analysis of very small (microscopic) amounts of matter. Frequently, trace analysis is performed on microsamples using micromethods. Also, a trace analytical procedure may involve a concentration step followed by a micromethod of determination.

Spectroscopic instruments are an important analytical tool in trace analysis because they not only provide the means for quantitative determination, but they also provide a spectrum which serves as a "fingerprint" for confirmation of the identity of the trace constituent. However, in a complex matrix where the spectra of major and minor components are similar, the presence of the trace component may be unrecognized or even lost in the "noise" of the major constituents. This has led to the development and widespread use of hyphenated instruments in which a chromatographic technique is used to separate the components of a mixture and present them, one at a time, to a spectroscopic instrument which then provides their fingerprints. An excellent appraisal of the possibilities of these hyphenated techniques is given by Hirschfeld (2).

In this book we have chosen to concentrate on four spectroscopic techniques and their hyphenated combinations for trace analysis. Many readers will be

surprised that we have not included the workhorses of trace analysis—mass spectrometry and its hyphenated combinations. We felt that a proper treatment of this instrument should be carried out in a separate monograph. In contrast, UV–vis absorption, fluorescence, infrared, and nuclear magnetic resonance (NMR) have recently seen vast improvements in their sensitivity and versatility to the point where they deserve consideration as trace analytical tools, especially with rapid development of related hyphenated methods for each. Accordingly we are pleased to have found five experts to review the use of these four instruments for trace analysis.

Chapter 1, by Professor Ratzlaff of the University of Kansas, deals with advances in ultraviolet–visible absorption spectrophotometry. The applicability of this technique to trace analysis, like the others treated in this book, has been broadened by dramatic advances in technology which have led to improved sensitivity. As Ratzlaff points out, the current scanning spectrophotometers are virtually photoelectron noise-limited down to absorbances of 10^{-5}. Gathering of more light requires further advances in light sources and high throughput monochromators. However, his review of novel laser based methods of absorbance detection shows that absorbance differences of 10^{-7} are attainable. This is especially true now that a method for reducing flicker noise in gas lasers has been discovered. (3).

Luminescence spectroscopy is described in Chapter 2 by Professor Hurtubise of the University of Wyoming. In some ways, this technique represents the ultimate in sensitivity and selectivity. With regard to the former, individual molecules (4) and atoms (5) have been detected and even imaged (6). With regard to the latter, low temperature spectroscopies have allowed determination of specific polyaromatic hydrocarbons in complex matrices with almost no sample preparation. Recently the technique of supersonic jet spectroscopy has been developed as a method for obtaining high resolution fluorescence spectra. The interfacing of this type of spectroscopy to a capillary GC provides an unusually powerful hyphenated method (7). Even for liquid samples at room temperature, impressive work has been done taking advantage of temporal gating (8) to reduce Raman interference. Also, multidimensional analysis holds promise as a technique to deconvolute overlapping spectra (9).

The advent of the Fourier transform spectrometer has made infrared spectroscopy a viable technique for trace analysis. Commercial instruments are available which can attain baseline noise levels of less than 10^{-4} absorbance units (peak to peak) after averaging 100 scans. As Dr. Smith of Dow Corning, the author of Chapter 3, points out, of equal importance to signal-to-noise ratio is the capability to measure diverse samples through various types of accessories. For example, Griffiths showed some years ago that diffuse reflectance provided a very convenient way to measure solid samples with many advantages over the conventional approach of pelleting or mulling. Most recently, the introduction

of the circle cell has greatly simplified the handling and measurement of aqueous solutions (10). A final development of importance concerns the possibility that Raman spectroscopy can be done using the FT-IR's interferometer to measure the emission spectrum stimulated by a near infrared laser (11).

The final chapter in this book concerns the use of NMR spectroscopy as a trace analysis tool. As Professors Rabenstein and Nakashima explain, major advances in magnet technology (superconducting solenoids yielding field strengths of up to 11.75 tesla) and the advent of the Fourier-transform technique have combined to allow the routine determination of submicrogram quantities of small molecules. With such sensitivity, it may prove feasible to measure the proton NMR spectrum of liquid chromatographic effluents on the fly. The selectivity of NMR has been increasing lately as new pulse sequences have been developed. The two dimensional NMR spectroscopies NOESY and COSY greatly improve the resolving power of NMR and increase its structural information content as well.

Finally, we want to emphasize the key role that proper analysis of the data from these sophisticated instruments can play. The rapidly emerging field of chemometrics should be kept in mind (12). Excellent progress is being made on methods for solving the challenging problems of (a) the quantitation of a limited number of known components in a variable background of unknowns (13), and (b) the application of multivariate statistics and pattern recognition of spectroscopic data to properties of a sample other than chemical composition, for example, the cancer causing potential of a specific fraction of a petroleum sample.

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CONTENTS

CHAPTER

1

TRACE ANALYSIS BY ULTRAVIOLATE-VISIBLE SPECTROPHOTOMETRY

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1. INTRODUCTION

A variety of techniques for trace analysis exist, and this text addresses several of the more important ones. Some of the fundamental definitions of trace analysis are discussed in Chapter 3 (Section 1): limits of detection (LOD), limits of quantitation (LOQ), and the problems of sampling and contamination that apply to all trace analyses.

Spectrophotometry in the visible region of the spectrum is one of the oldest analytical techniques, and it is still the most commonly used. The concept is simple: When a solution exhibits a color, the color results from the selective absorbance of certain wavelengths of light, and the concentration of analyte in that solution can be related to the amount of light absorbed. At one time, that light intensity was measured by visual comparison with standards, but now absorbance is measured by sophisticated instruments which provide greatly increased *sensitivity* and *selectivity*, the keys to trace analysis.

In this chapter, we begin by defining fundamental physical and instrumental parameters which are part of the measurement of absorbance in condensed-phase measurements (Section 1). Then methods for increasing sample signal and reducing interferent signals are discussed (Section 2). The chapter concludes (Section 3) with promising methods involving UV and visible absorption measurements which have not yet become standard.

1.1. The Origin of Ultraviolet–Visible Spectra

The energy of a photon in the UV–visible range of the electromagnetic spectrum lies between about 1.6 and 8 eV. This energy range corresponds roughly to the energy of outer-electron transitions, that is, a change of one in the electronic quantum number n. For a photon striking the molecule to be absorbed, it first

must have the same energy as the energy level transition; then, the probability of its absorption is determined by quantum mechanical selection rules. Therefore, the electronic transition energy determines the region of the spectrum, and the probability determines the intensity of the absorption.

The energy of the transition depends on the type of bond and the environment of the bond. For example, the wavelength of maximum absorbance, λ_{max}, for a variety of $\pi \rightarrow \pi^*$ transitions in organic molecules depends on the molecule; the transitions of C═C and C≡C bonds have higher energy, and the absorbance occurs well into the UV region, at about 170 nm, compared with the absorbance of a C═N bond, which occurs at about 190 nm. Conjugated bonds absorb at significantly longer wavelengths.

If the transitions excited by ultraviolet and visible photons involved electronic energy levels exclusively, the spectrum would appear the same as atomic absorption spectra, a series of lines in the plot of absorbance versus wavelength corresponding to the changes in n. However, the total energy of a system depends on electronic, vibrational, and rotational energy. (We can neglect the translational energy E_{tran}, which is very small.) Following the Born–Oppenheimer approximation, they may be considered independently so that the total energy, E_T, is simply

$$E_T = E_{el} + E_{vib} + E_{rot} \tag{1}$$

where E_{el}, E_{vib}, and E_{rot} are the electronic, vibrational, and rotational energies of the system. The energy of an absorbed photon must correspond to a change in E_T, ΔE_T, which is the sum

$$\Delta E_T = \Delta E_{el} + \Delta E_{vib} + \Delta E_{rot} \tag{2}$$

Typical values of ΔE_{vib} are an order of magnitude smaller than ΔE_{el}, and the energy of a rotational transition is smaller by an additional one to two orders of magnitude.

These changes in energy are illustrated in Fig. 1 (1). At the time that a change occurs in the electronic energy level (quantum number n), an increase or decrease in vibrational quantum number v and/or rotational quantum number J may also take place. From that result, we would expect a series of lines with a symmetrical envelope: a line corresponding to the energy of the electronic transition alone ($\Delta n = 1$, $\Delta v = 0$, $\Delta J = 0$), and a symmetrical set of lines corresponding to the electronic transition plus or minus the energy change of some vibrational transitions; each of those lines would then be divided into more lines resulting from changes in rotational levels, $\Delta J = \pm 1$.

However, this fine structure is not normally observable. Except in low-pressure

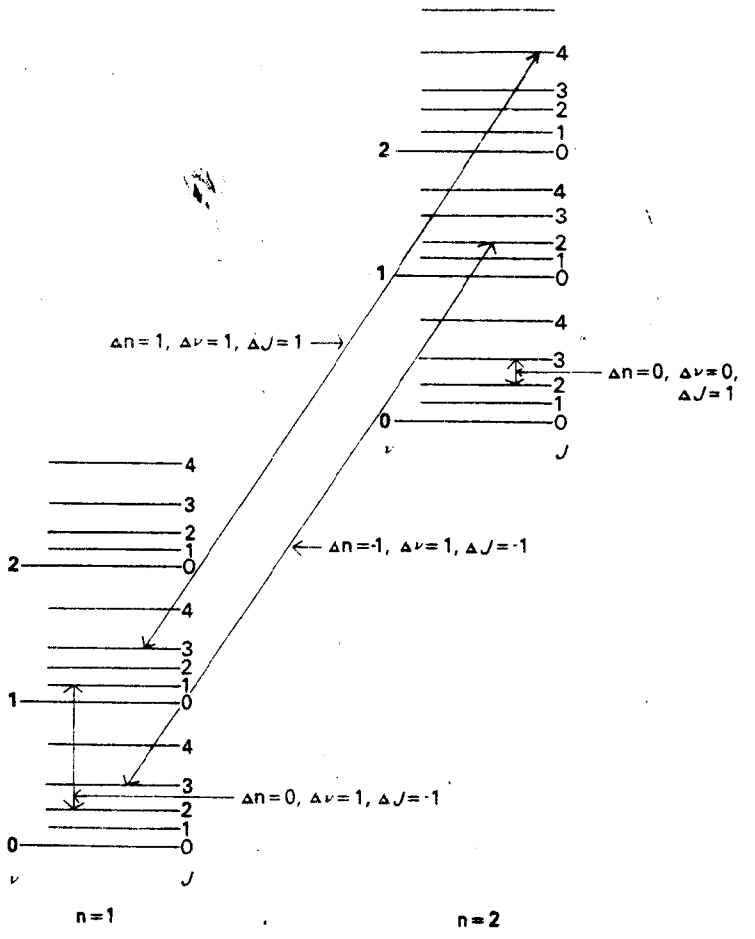

Fig. 1. Partial energy-level diagram for a typical molecule showing some allowed transitions. Transitions in which $\Delta n = 1$ result in absorbance in the UV–visible range.

vapors, the rotational structure is obliterated by the effects of the molecule's environment (the solvent or neighboring molecules), so that free rotation is obstructed, and the rotational energies are randomly perturbed. The consequence is the nearly total loss of rotational structure in the spectrum under normal analytical circumstances.

The vibrational structure suffers a similar fate. The example shown in Fig. 2 (2) shows the effect of solvent on the vibrational/rotational structure of *sym-*

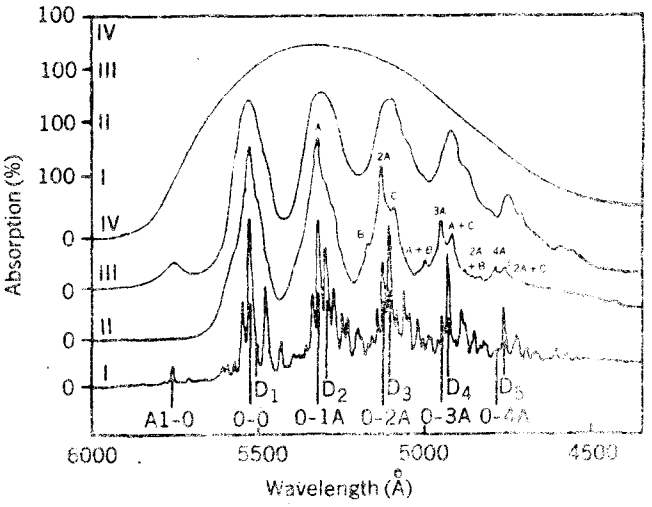

Fig. 2. Absorption spectrum of *sym*-tetrazine. Curve I, vapor at room temperature; curve II, at 77 K in a glass; curve III, in cyclohexane at room temperature; and curve IV, in aqueous solution at room temperature. (Reprinted from reference 2 by permission of The Chemical Society.)

tetrazine. The vibrational transitions are clearly visible in the vapor, and rotational transitions can also be detected; when placed in cyclohexane, the rotational structure is obliterated. A polar solvent, water, smooths even the remaining vibrational bands as a result of the stronger solvent interactions.

1.2. Beer's Law

As shown in Fig. 3, the radiant power of the incident beam may be diminished by absorption, scattering, and reflective losses (3). The diminution in the beam power due to scattering and reflective losses can contribute to errors, which will be considered later. In order to be quantitative, the measurement should respond only to absorption of radiation, and that response is described by a relationship variously attributed to Beer, Bouguer, Bunsen, and Lambert. The most useful component is the relationship of absorption to concentration, which was pointed out by Beer.

1.2.1. Quantitative Relationship

If an absorbing medium is uniform and nonscattering, the power after passing through thickness *db* will be decreased so that the ratio of the change in radiant power $d\phi$ to the radiant power incident on *db*, ϕ, is a constant. That is,

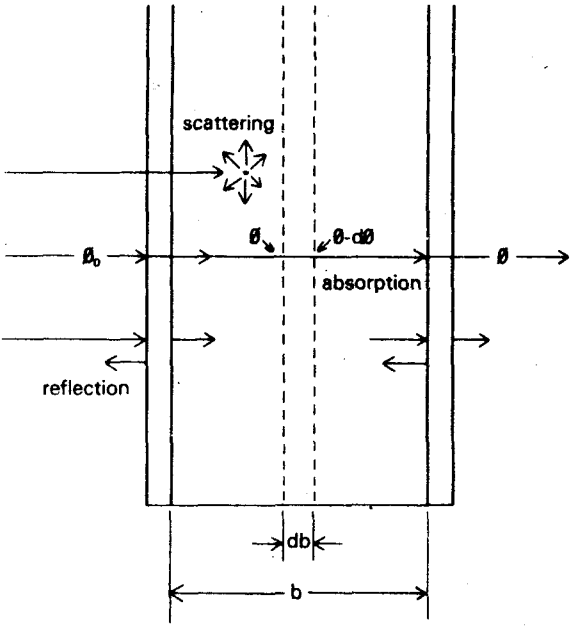

Fig. 3. Illustration of the phenomena that diminish the intensity of a transmitted light beam. In addition to absorption, scattering and reflection also take place.

$$\frac{d\phi}{\phi} = -k\, db \tag{3}$$

If an integration is performed over the length of the cell, from 0 to b, the attenuation in the incident radiant power ϕ_0 resulting in the transmitted radiant power ϕ is

$$\ln \frac{\phi}{\phi_0} = -kb \tag{4}$$

The transmittance T is defined as that quotient;

$$T = \frac{\phi}{\phi_0} \tag{5}$$

The relationship between the path length and attenuation in radiant power is

sometimes known as Lambert's law, although it was first formulated by Bouguer in 1729 (1).

Beer, in 1852, stated the relationship between the attenuation in incident power and the number of molecules present. When a photon passes through a sample containing molecules capable of absorbing the photon, the photon may be absorbed. First, it must strike the molecule. Then, absorption requires both that the energy of the photon match the energy of a transition, and that the transition be allowed. The extent to which this may occur is measured as the molar absorptivity, ϵ. It can be expressed in terms of the area of the molecule, a, which helps determine the probability of a photon striking the molecule, and P, the probability of a transition determined by quantum mechanical selection rules. The result is

$$\epsilon \cong 9 \times 10^5 \, P a_m \tag{6}$$

where a_m is expressed in square nanometers (4). The area of a typical molecule is about 0.01 to 0.1 nm^2; therefore, if the transition is allowed so that the probability is high ($P = 0.1$ to 1), ϵ may be as high as 10^5, resulting in a very intense absorption. Weak absorptions result from forbidden transitions where $P \leqq 0.01$.

The constant in the equation is chosen so that $\epsilon = 2.303k$ when b is expressed in centimeters and c is expressed in moles per liter. The consequence is commonly called Beer's law:

$$-\log \frac{\phi}{\phi_0} = \epsilon bc \tag{7}$$

The value $-\log(\phi/\phi_0)$ is defined as absorbance and is assigned the symbol A so that $A = -\log T = \epsilon bc$.

1.2.2. Nomenclature

Although other terms enjoy common usage, the term absorbance is used by analytical chemists, the recommendation of the American Society for Testing Materials (5,6), having been endorsed by the journal *Analytical Chemistry*. It is preferred to the terms optical density or extinction, which are used in some quarters. Similarly, the ratio T (Eq. (5)) is transmittance, not transmission or transmittancy. The term molar absorptivity for ϵ is preferred to the term "extinction coefficient." When the concentration is expressed in grams per liter, the "absorptivity" is used in Beer's law with the symbol a; that is, $A = abc$. The

symbol b, not l or d, is used for the path length. Finally, the term spectrophotometry is reserved for the measure of the *ratio*, or function thereof, of radiant power.

1.2.3. *Deviations from Beer's Law*

The deviations from Beer's law are generally grouped into two groups, real and apparent. Those real exceptions, where the true absorbance/actual concentration relationship is not linear, are found primarily at extremely high concentrations and are seldom important in trace analysis. Details can be found in standard analytical spectroscopy texts (e.g., reference 1).

Apparent deviations are produced when the experimental requirements are not met; for example, Beer's law is defined for the condition in which the incident radiation is monochromatic and the path length through the sample is constant for the entire beam cross section. These requirements are only met approximately. Consequently, a brief review of the effects of these approximations is useful. The factors which contribute to apparent deviations, considered separately below, include multiple internal reflections, multiple internal scattering, stray light, bandpass errors, and chemical errors.

1.2.4. *Multiple Internal Reflections*

As illustrated in Fig. 4, the transmitted beam suffers diminution due to reflection before entering and after leaving the sample solution. These losses can be determined from the Fresnel equation. Whenever light passes from one medium of refractive index n_1 to a second medium of refactive index n_2, some of the light is reflected. If the light is incident normally, the fraction reflected, f, is determined by the refractive indices of the media:

$$f = \{(n_1 - n_2)/(n_1 + n_2)\}^2 \tag{8}$$

Considering the possible interfaces through which the beam travels in a cell containing water or a common solvent, only the glass–air interface is significant. If $n_{air} = 1.00$ and $n_{glass} = 1.5$, then $f = 0.04$.

By following the path of the beam through the cell, as in Fig. 4, the effect of reflection at the glass–air interface on the transmitted power ϕ can be obtained. First, after the light has passed through the solution once, about 4% of the light (f_1, the reflection at the solution–glass interface at the exit face of the cell) is reflected back through the solution. If the absorbance of the solution is A, the light beam upon reaching the exit face of the cell is attenuated by the factor 10^{-A}. After the 4% (f_1) is reflected back through the solution, 4% of that power

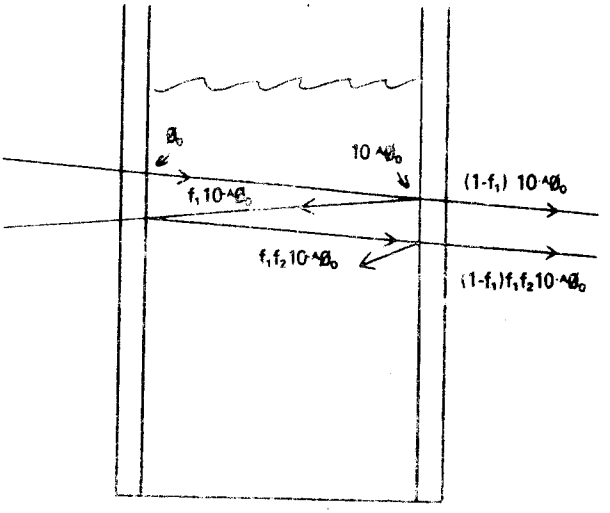

Fig. 4. Light path of the energy reflected within the cell.

(f_2) is reflected back through the solution a third time. Neglecting any further reflections, the power transmittance is

$$\Phi = (1-f_1)\,[10^{-A} + f_1 f_2 10^{-3A}]\,\Phi_0 \tag{9}$$

The value of ϕ_0 in Eq. (7) is usually determined from a reference (blank) solution, which experiences the same reflections except that $A = 0$. Therefore, the measured value of the absorbance, A', is related to the real absorbance, A by the equation

$$\begin{aligned} A' &= -\log \frac{(1-f_1)\,[10^{-A} + f_1 f_2 10^{-3A}]\phi_0}{(1-f_1)\,[1 + f_1 f_2]\,\phi_0} \\ &= -\log \frac{[10^{-A} + f_1 f_2 10^{-A}]}{[1 + f_1 f_2]} \end{aligned} \tag{10}$$

Typical values of f_1 and f_2 are 0.04. The relative error is largest at trace concentrations, but at a maximum of less than 0.3%, it is normally insignificant for most trace determinations. However, it should not be ignored; much higher f values have been suggested when reflections take place off other optics, apertures, and slits (7,8).