CHAPTER 1

Absorption Spectrophotometry

M. Pernarowski

FACULTY OF PHARMACY
UNIVERSITY OF BRITISH COLUMBIA
VANCOUVER, BRITISH COLUMBIA

1.1 Electromagnetic Radiation 3
1.2 Absorption of Radiant Energy 5
A. Absorbing Groups 6
B. Solvent Effects 8
1.3 Quantitative Spectrophotometry 13
A. Beer's Law 13
B. Spectrophotometric Terminology 14
C. Graphical Presentation of Data 15
D. Reliability of the Measurement 17
E. Determination of a Drug in a Dosage Form 18
F. Experiment 1.1: The Determination of Procaine HCl in an Injection 20
1.4 Deviations from Beer's Law 21
A. Real Deviations 21
B. Instrumental Deviations 21
C. Chemical Deviations 23
1.5 Analysis of Mixtures 25
A. Solution of Simultaneous Equations 25
B. Constancy of the Absorbance Ratio Value 27
C. Experiment 1.2: The Identification of Aminosalicylic Acid USP 28
D. Application of Absorbance Ratios to the Analysis of Binary Mixtures 28
E. Experiment 1.3: Simultaneous Analysis of Procaine HCl and Tetracaine HCl 30
F. Experiment 1.4: Simultaneous Analysis of Theobromine and Caffeine 31

1.6 Colorimetry 32
A. Experiment 1.5. Limit Test for Iron in Heavy Magnesium Carbonate BP 33
B. General Requirements for the Colored Substance 34
C. Chemistry of Colorimetry 35
D. Experiment 1.6. Analysis of Diphenhydramine HCl Tablets 38
1.7 Special Methods 39
A. Precision Spectrophotometry 39
B. Differential Analysis 41
C. Experiment 1.7. Analysis of Morphine Sulfate Tablets 42
D. Spectrophotometric Titrations 42
1.8 Spectrophotometers and Colorimeters 45
A. Radiation Sources 45
B. Filters and Monochromators 49
C. Absorption Cell 52
D. Radiation Detectors 52
E. Accuracy of Wavelength and Absorbance Scales 54
Questions 55
References 56

Absorption spectrophotometry is of fundamental importance to the pharmaceutical analyst. Many drugs absorb electromagnetic radiation. By using the proper instruments and techniques, the analyst can, in many instances, determine the amount and nature of a drug in a dosage form, in a reaction vessel, or in a biological system. These determinations are of importance in the assessment of the drugs produced by the industry, in research, and in the clinical evaluation of the effectiveness of many pharmaceuticals. Without spectrophotometry, quality control would be impossible and many of the research programs of the drug industry and the universities would not have yielded the drugs that are now available to the public.

Radiant energy is energy transmitted as electromagnetic radiation. *Absorption spectrophotometry* is the measurement of the absorption of radiant energy by various substances. It includes the measurement of the absorptive capacity for radiant energy in the ultraviolet, visible, and infrared regions of the spectrum. However, the techniques and instrumentation associated with infrared spectrophotometry differ, in some respects, from those used to study the absorption of light and ultraviolet energy. The subject is, therefore, discussed in Chapter 2.

The sun is our most important *source* of radiant energy. Many naturally occurring substances—droplets of water, the leaves on trees, chemicals imbedded in rocks—may act as *dispersing*, *absorbing*, or *reflecting* devices for radiant energy. Man's eyes are the most important *detectors* of radiant energy. The italicized words are, in essence, the component parts of a spectrophotometer or colorimeter. Man, therefore, has utilized the principles of

spectrophotometry from the day that he became aware of his surroundings. Without light, life would be impossible. Without the subtleties of light absorption, reflection, and transmission, life would be a continuous psychedelic experience. Without the differentiating capabilities of the eye, the world would be a study in gray.

The first "analyst" to utilize the principles of spectrophotometry was probably the trader who visually compared the color quality of the materials that he bought and sold. The first historical reference to product control may be found in the writings of Pliny, the Roman encyclopedist.[1] He reported that iron in vinegar could be detected by dipping a piece of papyrus soaked in an aqueous extract of gallnut tannins into the solution being tested. The papyrus turned blue or black if iron was present. However, absorption spectrophotometry was not used extensively by the pharmaceutical analyst until after World War II. Inexpensive but sophisticated instruments became available at that time and contributed to our understanding of the applications of spectrophotometry to the point that many textbooks are devoted solely to the subject matter covered in this chapter. Reviews in scientific journals are published periodically[2,3] and are an important source of information for the novice and the expert in spectrophotometry. The treatment in this chapter may whet the readers' appetite, but can hardly satisfy the student who wishes to learn about the subtleties of the subject. References 4–6 are, therefore, reserved for those who may wish to pursue any of the material covered herein in more detail.

1.1 ELECTROMAGNETIC RADIATION

Electromagnetic radiation may be described in terms of its wavelike properties. The *wavelength* (λ) of a beam of electromagnetic radiation is the linear distance, measured along the line of propagation, between two points which are in phase on adjacent waves. The unit of wavelength is the *angstrom* (Å). It is equal to 1/6438.4696 of the wavelength of the red line of Cd. This is almost, but not exactly, equal to 10^{-8} cm. Therefore, 10 Å are equal to 1 mμ (millimicron). One centimeter is equal to 10,000 μ (microns) or to 10^7 mμ.

The *frequency* (ν) of the beam is the number of cycles occurring per second (H_z). The relationship between wavelength (in centimeters) and frequency is stated mathematically in Eq. (1.1)

$$\nu = \frac{c}{\lambda} \tag{1.1}$$

The velocity (c) of electromagnetic radiation in a vacuum is equal to 3.0×10^{10} cm/sec. This value is used in most calculations, but will vary if the electromagnetic radiation passes through something other than a vacuum.

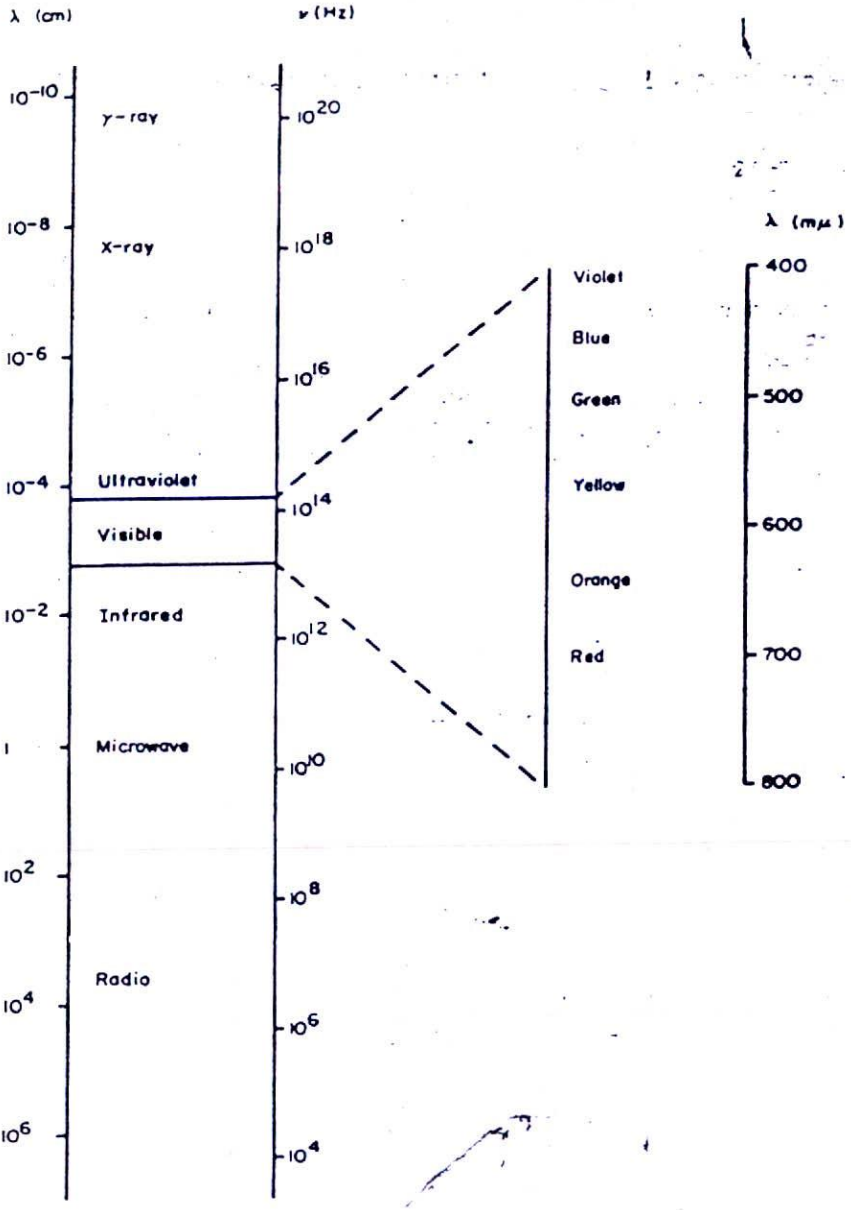


FIGURE 1.1: The electromagnetic spectrum.

The pharmaceutical analyst calculates, but rarely uses, frequency values. The values are large and, therefore, cumbersome to use when presenting spectral data in a graphical form. For example, a wavelength of 300 mμ is equal to a frequency of 1×10^{15} H_z. For this reason, most analysts prefer to define the spectrum in terms of wavelength or *wave number* (σ). Wave number is the number of waves per unit length or the reciprocal of the wavelength (in centimeters). The unit is the reciprocal centimeter.

$$\sigma = \frac{1}{\lambda} \tag{1.2}$$

In some instances, radiant energy behaves as if it were made up of discrete packets of energy or *photons*. The energy (E) of a photon depends on the frequency of the radiation.

$$E = h\nu \tag{1.3}$$

or

$$E = \frac{hc}{\lambda} \tag{1.4}$$

Planck's constant (h) is equal to 6.62×10^{-27} erg sec. Several simple calculations will show that the energy of a photon increases as the wavelength decreases. X-rays, for example, are much more energetic than ultraviolet energy.

Sir Isaac Newton, in 1666, described in some detail the visible region of the electromagnetic spectrum. When a beam of light is passed through a prism, it is dispersed and, by projecting this dispersed radiation onto a screen, the six major colors of the electromagnetic spectrum can be observed. However, this region is but a small part of the entire spectrum. For example, if we attempted to draw it to scale, it would be 100,000,000 ft long if we let 1 μ equal 1 ft and drew the spectrum to 100 m. (The scale in Fig. 1.1 is not linear but logarithmic.) However, the subject matter in this chapter is concerned with only two regions of this spectrum. (~~The ultraviolet region extends from 10 to 380 mμ~~. Special instruments are required for studies in the far or vacuum ultraviolet region (10 to 200 mμ). The analyst, therefore, depends upon ~~visible (380 to 780 mμ)~~ and ultraviolet energy (200 to 380 mμ) for the routine analysis of pharmaceuticals.

1.2 ABSORPTION OF RADIANT ENERGY

Molecules are as energetic as the modern teenager. They rock, roll, twist, and bend and, if the "music" is of the right frequency, the electrons within the molecule move from the ground to an excited state.

The total energy in a molecule is the sum of the energies associated with the translational, rotational, vibrational, and electronic motions of the molecule or the electrons and nuclei in the molecule. This classical explanation of

particle motion and its relationship to energy is limited by the Heisenberg uncertainty principle, which states that the "exact" orbit for the motion of a particle cannot be determined because position and velocity cannot be measured at the same time. With this limitation in mind, the energies that contribute to total molecular energy can be defined.

Translational energy is associated with the motion (velocity) of the molecule as a whole.

Rotational energy is associated with the overall rotation of the molecule.

Vibrational energy is associated with the movement of atoms within the molecule.

In general, these atomic and molecular motions are related to the absorption of infrared energy and are not pertinent to the subject matter in this chapter.

Electronic energy is associated with the motion of electrons around the nuclei.

When a molecule absorbs visible or ultraviolet energy, an electron or electrons will be raised to a higher energy level if the energy requirement for that transition is equal to the energy of the incoming photon. This statement is valid only for certain types of electrons. The electrons in the inner shells of atoms and those that are shared by two adjacent atoms are not affected to the same degree by incoming radiation as those that cannot be localized within the molecule. Electrons of the latter type give rise to spectra in the ultraviolet and visible regions of the electromagnetic spectrum. Such electrons are found in conjugated double bonds. Saturated molecules, therefore, will not absorb ultraviolet energy.

An excited electron returns to the ground state in about 10^{-9} to 10^{-8} sec. Energy must now be released to compensate for the energy absorbed by the system. If the electron returns directly to the ground state, heat is evolved. If it returns to the ground state by way of a second excited state, energy is released in the form of heat and light.

If a large amount of energy is absorbed by certain substances, bonds may be ruptured and new compounds formed. For example,

However, dramatic changes are the exception rather than the rule. Changes are usually minimal and, for this reason, ultraviolet spectrophotometry is considered to be a nondestructive method of analysis.

A. ABSORBING GROUPS

A *chromophore* is a group which, when attached to a saturated hydrocarbon, produces a molecule that absorbs a maximum of visible or ultraviolet energy at some specific wavelength (or wavelengths). A number of simple chromophores are listed in Table 1.1. Although the type of information in Table 1.1 is useful to the research chemist, its value to the

pharmaceutical analyst has decreased since the advent of inexpensive and sophisticated infrared spectrophotometers. Functional groups can be readily identified by subjecting the unknown to infrared energy.

Many molecules contain two or more chromophores. The interaction of radiant energy with the molecule then depends upon the relative positions of the two chromophores in the molecule.

TABLE 1.1: Absorption Bands for Simple Chromophoric Groups*

Chromophore		Example	$\lambda_{max.}$, mμ	ϵ†
Acetylene	—C≡C—	Acetylene	173	6,000
Amide	—$CONH_2$	Acetamide	< 208	—
Azo	—N=N—	Azomethane	347	5
Azomethine	> C=N—	Acetoxime	190	5,000
Carbonyl	RHC=O	Acetaldehyde	293	12
Carbonyl	RR'C=O	Acetone	271	16
Carboxyl	—COOH	Acetic acid	204	60
Ethylene	RCH=CHR	Ethylene	234	0.05
			193	10,000
Nitrate	—ONO_2	Ethyl nitrate	270	12
Nitrile	—C≡N	Acetonitrile	< 160	—
Nitrite	—ON=O	Octyl nitrite	370	55
			230	2,200
Nitro	—NO_2	Nitromethane	271	19
Sulfone	> SO_2	Dimethyl sulfone	< 180	—
Sulfoxide	> S=O	Cyclohexyl methyl sulfoxide	210	1,500
Thiocarbonyl	> C=S	Thiobenzophenone	620	70

* This table is reproduced, with permission, from an application data sheet (No. UV-77-MI) published by Beckman Instruments, Inc., Fullerton, California.
† Molar absorptivity.

a. When two chromophores are separated by more than one carbon atom, total absorption is the sum of the absorption of each of the two chromophores.

b. When two chromophores are adjacent to each other, the absorption maximum shifts to longer wavelengths (*bathochromic shift*) and the intensity of absorption is increased (*hyperchromic effect*). The opposite effects can be produced by changing the structure of the organic substance. A shift to a shorter wavelength is called a *hypsochromic shift*; a reduction of intensity is called a *hypochromic effect*.

c. When two chromophores are attached to the same carbon atom, there is a summation of absorption and a shift toward longer wavelengths, but the degree of change is less than that shown by conjugated chromophores.

There are two kinds of chromophores. The *simple* chromophores (see Table 1.1) give rise to *R bands*. The molar absorptivity value (see Section 1.3 for a definition of ϵ) for this type of band is usually less than 100. There are

two types of *complex* chromophores. The first type is that found in aromatic compounds whose structures contain a benzene ring. These chromophores give rise to *B bands*. The molar absorptivity values of these bands range from 250 to 3000. The second type has the following formula:

$$A{-}CH{=}CH{-}CH{=}B$$

A is equal to H, R, OR, SR, NR_2, O⁻, S⁻, or ⁺NR. B is equal to $CH{=}CHR$, CR_2, NR, O, S, $^+NR_2$, ^+OR, or ^+SR. These chromophores give rise to K bands. The molar absorptivity values for these bands are more than 10,000.

Auxochromes are groups which, when introduced into an absorbing system, cause a bathochromic shift. Auxochromes are either coordinatively saturated (e.g., $-^+NH_3$) or coordinatively unsaturated (e.g., $-NH_2$). The hydroxyl group, amino groups and their substituted derivatives, alkyl groups, and halogens will produce changes in absorption spectra.

B. SOLVENT EFFECTS

The absorption spectrum of a drug depends, in part, on the solvent used to solubilize the substance. A drug may absorb a maximum of radiant energy at one wavelength in one solvent, but will absorb little at the same wavelength in another solvent. These changes in spectrum are due to:

a. The nature of the solvent
b. The nature of the absorption band
c. The nature of the solute

These effects can be correlated with the polarity of the solvent. As the polarity of the solvent increases, R bands are displaced to shorter wavelengths. K bands are displaced to longer wavelengths. Polar solutes are more

TABLE 1.2: Wavelength Ranges for Commonly Used Solvents

Solvent	Wavelength range, mμ
Acetonitrile	200–800
Carbon tetrachloride	265–800
Chloroform	245–500
Cyclohexane	210–800
Dimethylformamide	270–800
Ethanol	210–800
Ether	220–800
n-Heptane	210–800
n-Hexane	210–800
Isooctane	210–800
Isopropanol	210–800
n-Pentane	210–800
Water	200–800

sensitive to solvent change than are nonpolar solutes. Molar absorptivity values will vary with changes in solvent. The absorption spectrum of a drug is, therefore, meaningful to the pharmaceutical analyst only if the solvent that has been used to solubilize the solute is specified.

The most widely used solvents are listed in Table 1.2. Solvents will begin to absorb ultraviolet radiant energy at some specific wavelength. For example, chloroform absorbs ultraviolet energy so strongly below 245 mμ that

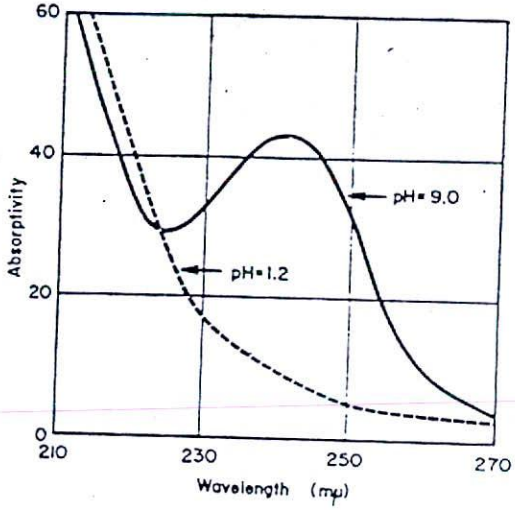


FIGURE 1.2: Absorption spectrum for phenobarbital at pH 1.2 and 9.

it cannot be used as a solvent for spectral studies at that or at a lower wavelength setting. Effective solvent ranges are given in Table 1.2. Moreover, solvents must not contain trace impurities. Many impurities (e.g., the benzene in absolute alcohol prepared by azeotropic distillation) absorb radiant energy and complicate the analysis. Spectral grade solvents are sold by chemical supply houses, but, in many instances, the analyst can readily purify those that he normally uses in the laboratory.

The effect of pH on the absorption spectrum for phenobarbital is shown in Fig. 1.2. These pH effects are of vital importance to the pharmaceutical analyst because they enable him to carry out the analysis of one substance in the presence of another (see Sections 1.3 and 1.7) and to determine the structural characteristics of certain drugs. Like the solvents listed in Table 1.2, buffers must transmit ultraviolet energy if they are to be used for the determination of the spectral characteristics of drugs. Many buffers do not meet this requirement, but those listed in Table 1.3 transmit a maximum of ultraviolet energy at most wavelengths.

TABLE 1.3: Absorbance Values for Buffers[a]

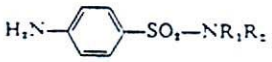
pH	Ml of 0.2 *M* solutions of buffer constituents in 100 ml of buffer	Wavelength, mμ									
		220	230	240	250	260	270	280	290	300	310
1.1	HCl, 47.4 ml KCl, 2.72 ml	0.011	0.007	0.004	0.003	0.003	0.002	0.001	0.000	0.000	0.000
2.0	HCl, 5.95 ml KCl, 44.1 ml	0.006	−0.003	−0.005	−0.002	−0.002	−0.003	−0.003	−0.003	−0.001	0.000
2.8	KH_2PO_4, 25.0 ml HCl, 3.50 ml	0.004	0.003	0.003	0.003	0.005	0.001	0.002	0.001	−0.001	0.000
4.0	KH_2PO_4, 25.0 ml HCl, 0.25 ml	0.004	0.004	0.001	0.001	0.002	0.002	0.001	0.001	0.000	0.000
5.1	KH_2PO_4, 25.0 ml NaOH, 0.25 ml	0.027	0.024	0.023	0.023	0.022	0.015	0.015	0.013	0.011	0.000
5.8	KH_2PO_4, 25.0 ml NaOH, 2.82 ml	0.039	0.029	0.021	0.016	0.015	0.016	0.015	0.011	0.010	0.000
7.0	KH_2PO_4, 25.0 ml NaOH, 14.8 ml	0.042	0.030	0.021	0.018	0.017	0.015	0.014	0.011	0.007	0.000
7.9[b]	H_3BO_3-KCl, 25.0 ml NaOH, 2.00 ml	0.076	0.050	0.032	0.026	0.023	0.023	0.023	0.018	0.013	0.000
9.0[b]	H_3BO_3-KCl, 25.0 ml NaOH, 10.7 ml	0.147	0.105	0.077	0.067	0.063	0.058	0.053	0.035	0.030	0.000
10.0[b]	H_3BO_3-KCl, 25.0 ml NaOH, 22.0 ml	0.128	0.063	0.044	0.036	0.031	0.025	0.023	0.021	0.018	0.000
10.8	Na_2HPO_4, 25.0 ml NaOH, 4.18 ml	0.209	0.101	0.074	0.065	0.057	0.053	0.050	0.045	0.038	0.000
11.8	Na_2HPO_4, 25.0 ml NaOH, 21.6 ml	0.545	0.163	0.104	0.089	0.080	0.070	0.061	0.052	0.045	0.000

[a] This table is reproduced through the courtesy of the Division of the State Racing Commission, Dept. of State of New York, 148-03 Hillside Avenue, Jamaica, N.Y., 11435.

[b] H_3BO_3-KCl solutions are 0.2 *M* with respect to each constituent.

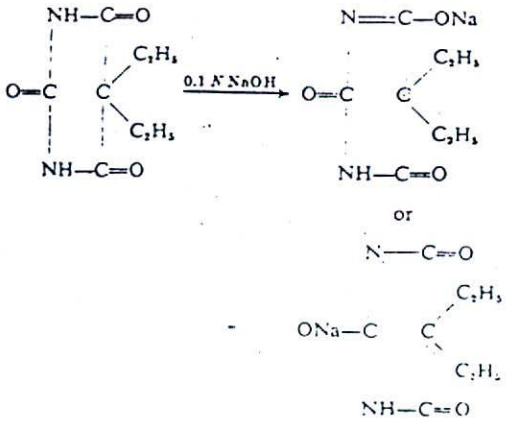
Many researchers have characterized drugs by studying their absorption spectrum in buffered solutions. Vandenbelt and Doub[7] were the first researchers to identify the absorbing groups of the sulfanilamido derivatives by determining their spectral characteristics in acidic and basic solutions. The spectral band associated with the sulfanilamido portion of the molecule is observed at 257–259 mμ; with the thiazole structure in sulfathiazole, at 280–283 mμ and at 258 mμ; with the pyridine in sulfapyridine, at 311 mμ; with the sulfanilyl portion, at 261 mμ; and with the pyrimidine ring, at 241 mμ.

In 1 N hydrochloric acid solution, sulfonamides with the following structure:



absorb little of the radiant energy above 230 mμ. The auxochrome, under these conditions, is $-NH_3$. In 1 N sodium hydroxide solution, a more efficient auxochrome is formed ($-NH_2$), and the substance now exhibits an absorption maximum at 251 mμ.

Under acidic conditions, phenobarbital does not absorb ultraviolet energy, to a significant degree, at 240 mμ. The tautomeric forms of phenobarbital are shown in the following equation:



Under alkaline conditions, the chromophoric system is $-C=N-C=O$. At a pH of 9, a high intensity absorption band may be observed at 240 mμ. However, in 0.1 N sodium hydroxide solution, this band appears at 255 mμ.

Solvents and buffers are, therefore, useful adjuncts in structural determination studies. The literature on the subject is comprehensive. The annual reviews cited earlier and the major textbooks on spectrophotometry provide the levels of information required for advanced study.

Absorption maxima for a selected group of common drugs are given in Table 14. Absorption spectra for many drugs may be found in a recently published manual.*

TABLE 14 Spectrophotometric Data for Common Drugs*

Drug	Solvent	λ_{max}	a
Acetazolamide	0.1 N HCl	265	48
Acetophenetidin	0.5 N NaOH	245	70
Amobarbital sodium	0.5 N NaOH	255	35
Antazoline HCl	Water	242	52
Apomorphine HCl	Water	273	67
Ascorbic acid	0.05 N HCl	243	35
Benzocaine	0.5 N NaOH	278	120
Caffeine	Water	273	51
Chloramphenicol	Water	278	30
Chlorcyclizine HCl	Alcohol	230	44
Chloromethapyrilene citrate	Alcohol	242	41
Chlorpheniramine maleate	Water	262	15
Cortisone acetate	Alcohol	240	40
Cyanocobalamin	Water	273	51
Cycloserine	Water	226	40
Dibucaine HCl	0.05 N HCl	246	59
Dimenhydrinate	0.05 N HCl	276	30
Epinephrine	0.5 N NaOH	296	57
Ergotamine	Alcohol	316	18
Hydrocortisone	Alcohol	240	44
Isoniazid	Dilute HCl	266	38
Levarterenol bitartrate	Dilute HCl	279	82
Methapyrilene HCl	Alcohol	240	63
Methyl Salicylate	0.05 N HCl	237	66
Morphine	1 N NaOH	258	31
Nicotinic acid	0.05 N HCl	260	37
Novobiocin	0.1 N NaOH	307	60
Oxytetracycline HCl	Buffer (pH 2.0)	353	28
Phenylbutazone	Water	255	45
Prednisone	Water	245	44
Procaine HCl	Water	290	66
Promethazine HCl	Water	298	93
Pyridoxine HCl	0.1 N HCl	292	42
Salicylamide	0.05 N HCl	235	59
Sulfamethazine	0.05 N HCl	244	62
Tetracaine HCl	Water	311	75
Tetracycline HCl	0.25 N NaOH	380	37
Thiamine HCl	Water	270	28
Thonzylamine HCl	Alcohol	244	74
Tripelennamine HCl	Dilute HCl	314	27

* The values in this table are illustrative. Exact values should be determined in the laboratory before carrying out an analysis on an unknown solution.

1.3 QUANTITATIVE SPECTROPHOTOMETRY

Electromagnetic radiation is absorbed by gases, liquids, and solids. If the absorption is total, the laws derived in this section are of no value to the pharmaceutical analyst. If, however, the substance transmits a part of the radiant energy that reaches it, the qualitative and quantitative characteristics of that substance can be easily determined.

A. BEER'S LAW

Beer's law is actually a combination of two laws. The first law relates absorptive capacity to the thickness of the absorbing medium. It was first enunciated by Bouguer in 1729 and restated by Lambert in 1768. According to this law, each layer of equal thickness absorbs an equal fraction of the radiant energy which passes through it. Mathematical statements relating the quantities involved may be presented in two different ways. In the first instance (the absorptive capacity varies directly as the logarithm of the thickness.) In the second instance (the intensity of the transmitted energy decreases exponentially as the thickness of the absorbing medium increases arithmetically.

The second law relates absorptive capacity to the concentration of the solute in the solvent. Beer, in 1852, enunciated a law which, in rather obscure language, stated that the absorptive capacity of a system is directly proportional to the concentration of solute in that system. Historical accuracy need not concern us here. However, for those who are interested in a critical examination of Beer's paper, the paper by Pfeiffer and Liebhafsky[9] gives the details of the origin of this law.

The intensity of a beam of monochromatic radiation dP decreases as it passes through each increment of absorbing material db. Therefore:

$$-\frac{dP}{db} \propto P \tag{1.5}$$

where P is the incident radiant power. A proportionality constant may now be introduced into Eq. (1.5).

$$-\frac{dP}{db} = KP \tag{1.6}$$

Rearrange Eq. (1.6).

$$-\frac{dP}{P} = K\,db \tag{1.7}$$

Integrate Eq. (1.7)

$$-\log P = Kb + C \tag{1.8}$$

If P_0 is equal to the incident radiant power reaching a given area per second, b, the thickness, is then equal to zero and C is equal to $-\log P_0$.

$$-\log P = Kb - \log P_0 \tag{1.9}$$

$$\log \frac{P}{P_0} = -Kb \tag{1.10}$$

Bouguer's law [Eq. (1.10)] may also be presented in its exponential form.

$$P = P_0 e^{-Kb} \tag{1.11}$$

Beer's law, which deals with the relationship between absorptive capacity and the concentration c of the solute in the solution, may be derived in the manner illustrated previously.

$$-\frac{dP}{dc} = K'P \tag{1.12}$$

and

$$\log \frac{P}{P_0} = -K'c \tag{1.13}$$

The exponential form is

$$P = P_0 e^{-K'c} \tag{1.14}$$

The constants in Eqs. (1.11) and (1.14) may now be combined and incorporated into an equation which includes the concentration and thickness symbols.

$$P = P_0 e^{-k''bc} \tag{1.15}$$

Rearrange and convert to base-10 logarithms.

$$\log \frac{P_0}{P} = k''bc \tag{1.16}$$

Substitute A for $\log P_0/P$ and a for k''. The Bouguer-Beer law is, therefore, written in the following form:

$$A = abc \tag{1.17}$$

This is the equation of a straight line with an intercept value of zero and a slope value of ab.

B. SPECTROPHOTOMETRIC TERMINOLOGY

The author of this chapter was first exposed to spectrophotometric nomenclature in a course on spectrophotometry given by Dr. M. G. Mellon, Professor of analytical chemistry at Purdue University. The following statement is copied from the notes taken at that time: "The literature in this field is a fine example of writers doing about as they pleased." Attempts have been made to standardize nomenclature, but too many authors still do as they

please. The pharmaceutical analyst depends, however, on the *United States Pharmacopeia* (USP) and the *National Formulary* (NF) for guidance in many areas, and it is only natural that he accepts the nomenclature given in those books. Fortunately, this terminology is acceptable to most scientists.

Transmittance T is the quotient of the radiant power P transmitted by a sample divided by the radiant power P_0 incident upon the sample. The per cent transmittance is equal to $100\,T$.

Absorbance A is the negative logarithm, to the base 10, of the transmittance. Spectrophotometers and colorimeters record either the absorbance or the transmittance of the solution.

Absorptivity a is the quotient of the absorbance divided by the concentration c of the solution (in grams per liter) and the absorption path length b in centimeters. The absorptivity value varies with the wavelength of the incident energy. However, at a specified wavelength, the absorptivity value for a drug is a constant if Beer's law is obeyed.

Molar absorptivity ϵ is the quotient of the absorbance divided by the concentration c of the solution (in moles per liter) and the absorption path length b in centimeters. It is also the product of the absorptivity and the molecular weight M of the substance.

The definitions and symbols given herein are preferable to those listed in the last column of Table 1.5. Such terms are confusing and unacceptable to the editors of most scientific journals.

TABLE 1.5 Spectrophotometric Terminology[a]

Term	Symbol	Definition	Obsolete terms
Absorbance	A	$-\log T$	Absorbancy Extinction Extinctance Optical density
Absorptivity	a	$a = A/bc$ (c = concentration in grams per liter)	Absorbancy index Extinction coefficient Specific absorption coefficient Specific extinction coefficient
Molar absorptivity	ϵ	$\epsilon = aM$ (M, molecular weight)	Molar absorbancy index Molar absorption coefficient Molar extinction coefficient
Path length	b	Length of cell in centimeters	l or d
Transmittance	T	P/P_0	Transmission Transmittancy

[a] USP terminology.

C. GRAPHICAL PRESENTATION OF DATA

An *absorption spectrum* is a graphic representation of A, $\log A$, a, ϵ, $\log \epsilon$, or T plotted against wavelength. Visible and ultraviolet spectra are, in general, plotted on graph paper which is divided along the abscissa into millimicrons. Frequency or wave number plots are rare.

1. Absorbance vs. Wavelength

The absorbance data obtained from a spectrophotometer can be plotted directly on a graph. However, the absorbance varies with the concentration of the solute in the solution. Therefore, the concentration of the solute in the solution must always be included in the caption of such spectrum.

2. Log A vs. Wavelength

Beer's law can be further modified by taking the logarithm of both sides of Eq. (1.17).

$$\log A = \log a + \log bc \tag{1.18}$$

The logarithm of bc is a constant over the wavelengths at which the absorbance is recorded. At each wavelength, $\log A$ is decreased by a fixed quantity. This means that the spectra of solutions containing the same substance but in different quantities will be parallel to one another. If they are not, the system does not obey Beer's law.

A recording spectrophotometer plots absorbance vs. wavelength. However, certain instruments can be modified to plot $\log A$ vs. wavelength. Such modified instruments can be used to determine reaction rates in kinetic studies. If the reaction is first order, a plot of $\log A$ vs. time is a straight line whose slope is equal to the reaction rate constant.

3. Absorptivity vs. Wavelength

This is, in effect, a plot of a constant vs. wavelength. The absorptivity value, at each wavelength, is calculated by dividing A by bc. This type of plot produces one spectrum over the entire concentration range and is illustrated in Fig. 1.2.

4. Molar Absorptivity vs. Wavelength

This is also a plot of a constant versus wavelength. Because the molecular weight of the substance is included in the calculation, substances with similar absorptivity values but different molecular weights will yield different spectra.

5. Log ϵ vs. Wavelength

If a spectrum exhibits two absorption maxima and if one is very weak and the other is very strong, a plot of ϵ vs. wavelength may require a change of scale. The log ϵ plot increases the magnitude of the weaker bands and decreases the magnitude of the stronger bands.

6. Transmittance vs. Wavelength

Transmittance and absorbance values may be obtained from the scales of most spectrophotometers. However, transmittance values are usually converted to absorbance values and plots of T vs. wavelength, are, therefore, rare.

D. RELIABILITY OF THE MEASUREMENT

All spectrophotometric analyses are based on a comparison of the amount of energy transmitted by the solvent with the amount transmitted by the solution.. The T value so determined is then used in subsequent calculations. The transmittance scale of a spectrophotometer is usually divided into 100 equal units. It is, therefore, possible to read per cent transmittance to one decimal place. However, this does not mean that the concentration can be determined with the same precision over the entire scale. The transmittance can be determined with a constant absolute error over much of this range, but the effect of this error on the analysis can be substantial if the T value is more than 0.6 or less than 0.2.

Beer's law states that $-\log T$ is equal to abc. If $-\log T$ is converted to a natural logarithm, the derivative of the equation is

$$-\frac{0.434}{T}\,dT = abdc \tag{1.19}$$

Divide Eq. (1.19) by $-\log T = abc$ and rearrange:

$$\frac{dc}{c} = \frac{0.434}{T \log T}\,dT \tag{1.20}$$

Using finite increments,

$$\frac{\Delta c}{c} = \frac{0.434}{T \log T}\,\Delta T \tag{1.21}$$

For all practical purposes, the relationship between concentration error $\Delta c/c$ and absorbance error is nearly constant between 20 and 60% T. If ΔT is equal to ± 0.005 and T is equal to 0.95, the error in the concentration will be of the order of $\pm 10\%$. Similar errors will result if the solution absorbs most of the radiant energy. However, if T is equal to 0.40, the error in concentration will be less than $\pm 1.4\%$.

If the derivative of Eq. (1.21) is made equal to zero, it can be shown that the per cent error in concentration is a minimum when the absorbance value is equal to 0.434. Solutions should, therefore, be prepared in such a way that the absorbance value is about 0.45. Values of not less than 0.3 and not more than 0.6 are generally acceptable.

Many scientists have studied the accuracy and precision of photoelectric spectrophotometry. The papers by Gridgeman[10,11] and Edisbury[12] cover, in detail, the subject matter presented in this section.

E. DETERMINATION OF A DRUG IN A DOSAGE FORM

Procedures for the spectrophotometric determination of drugs in dosage forms are published in the scientific literature or in the pharmacopeias. The pharmaceutical analyst must, therefore, refer to these sources constantly, but it is possible to generalize and outline a procedure that can be used to determine the amount of active ingredient in many pharmaceuticals.

1. The drug to be analyzed must be available in a relatively pure form. If its purity is not known, it must be characterized and, if necessary, recrystallized or purified in some other way. Reference standards are available for many of the drugs described in the USP[23] and NF.[13]

2. An optically transparent solvent is selected (see Tables 1.2 and 1.3) and, if necessary, its purity is checked. A cell is filled with the solvent, transferred to a spectrophotometer, and the instrument is balanced to read 100% T.

3. The drug is dissolved in the solvent and a portion of the solution is transferred to a spectrophotometric cell. The absorption spectrum for the drug is determined by using either a manually operated or a recording spectrophotometer. As a general rule, the final concentration should be about 10 mg of drug per liter of solution. However, the scientific literature or the pharmacopeias should be consulted if it is known that absorptivity values are published therein. For example, the absorptivity value for phenylbutazone at 265 mμ in pH 8 buffer is about 66. If the cell length is equal to 1 cm, a solution containing 10 mg of drug per liter of solution will give an A value of 0.66 at 265 mμ, the wavelength at which the drug absorbs a maximum of ultraviolet energy. This concentration will, therefore, yield a suitable spectrum.

The spectrum is now examined and the wavelength at which the drug absorbs a maximum of energy is determined. All subsequent determinations are carried out at this wavelength.

4. It is important, at this point, to consider the effect of the solvent on the analysis. For example, the A value for phenylbutazone changes rapidly (see Fig. 1.3) between a pH of 3 and 6. The drug could be determined in a pH 1 or 2 buffer. However, this acidic substance is more soluble in alkaline media. If the pH of the solution is 7 or more, the absorptivity value is

relatively constant at 265 mμ, the wavelength of maximum absorption. Analyses should not be attempted if the pH of the buffer is more than 3 or less than 6.

Solvent selection must also be based on the possible presence of interfering substances in the dosage form. Phenylephrine HCl can be dissolved in a pH 6 buffer and determined at 272 mμ. However, the absorptivity value at this wavelength is low and, more important, methylparaben, which is added to certain aqueous preparations containing the vasoconstrictor, absorbs some of the radiant energy at this wavelength. The absorbance value is, therefore,

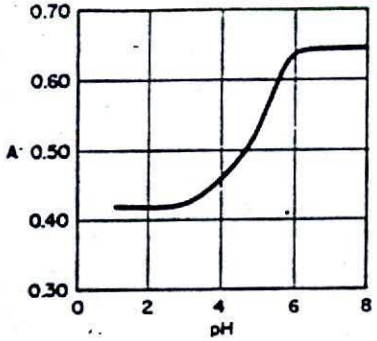


FIGURE 1.3: Effect of pH on the absorbance value for phenylbutazone. (Each liter of buffered solution contains 10 mg of phenylbutazone.)

due to both the preservative and the phenylephrine HCl. Concentrations cannot be calculated under these circumstances. However, by changing the pH to 13, the 272-mμ absorption maximum shifts to higher wavelengths and a new and more intense maximum appears at 237 mμ. Methylparaben absorbs little energy at this wavelength. The concentration of the phenylephrine HCl in the solution can now be determined with considerable accuracy.

5. The absorptivity value for the drug at the wavelength of maximum absorption is determined by measuring the A values of solutions containing different quantities of drug. The results are plotted (A vs. c) and, if Beer's law is obeyed, there will be a linear relationship between absorbance and concentration.

Some analysts will use published absorptivity values. These values, for the most part, have been accurately determined, but, because there are differences between instruments, most researchers prefer to determine their own values. Most of the spectrophotometric tests and assays in the USP and NF call for comparison against the appropriate reference standard.[14] The analyst is, therefore, determining the absorptivity value and the absorbance of the unknown at the same time and under the same circumstances.

6. The drug in the dosage form is now separated from any interfering substances. In the example given in Section 4, the methylparaben was not separated from the phenylephrine HCl, but many dosage forms contain contaminants which absorb energy at all wavelengths and under all circumstances. These must be removed by extraction, chromatography, distillation, or by other means before dissolving the drug in the chosen solvent. The absorbance of this solution is determined and the amount of drug in the dosage form calculated.

Here is an example of a typical calculation. A tablet is purported to contain 100 mg of phenylbutazone. The analyst reduces the tablet to a fine powder, extracts it with ethanol, filters the extracting solvent, and dilutes to 100.0 ml. A 10.0-ml aliquot is then diluted to 1000.0 ml with a pH 8 buffer (see Table 1.3). The absorbance A for the solution at 265 mμ is 0.64. The absorptivity a, under these conditions, is known to be 66. The cell length b is 1 cm.

$$c = \frac{A}{ab} \text{ g/liter}$$

$$\text{Milligrams phenylbutazone in the tablet} = \frac{0.64}{66} \times 10 \times 1000 = 97.0 \text{ mg}$$

$$\text{Per cent of label claim} = \frac{97.0}{100} \times 100 = 97.0\%$$

F. EXPERIMENT 1.1: DETERMINATION OF PROCAINE HCl IN AN INJECTION

It is assumed that the injection contains no interfering substances.

Weigh accurately 100 mg of procaine HCl and dissolve in 250.0 ml of water. Dilute a 25-ml aliquot of this stock solution to 1 liter with water. Mix well and transfer a portion of the solution to a 1-cm sample cell. Fill the reference cell with water. Transfer the cells to the cell compartment of the spectrophotometer (Beckman model DU spectrophotometer, or the equivalent). Record the absorbance of the solution at 220 mμ and at 10-mμ intervals thereafter. The last reading should be taken at 340 mμ. Plot A vs. wavelength. Determine the wavelength at which the drug absorbs a maximum of ultraviolet energy. Check the results by recording the absorbance of the solution at 1-mμ intervals in the vicinity of the wavelength which shows maximum absorption.

Dilute a 25.0-ml aliquot of the stock solution to 1000.0 ml with water. Record the absorbance of this solution at the wavelength at which procaine HCl exhibits maximum absorption. Repeat the procedure using 23.0-, 21.0-, 19.0-, and 17.0-ml aliquots of the stock solution. Calculate absorptivity and molar absorptivity values. Plot A vs. c to check for compliance with Beer's law.

Dilute 10.0 ml of the procaine HCl injection (unknown) to 100.0 ml with water. Dilute a 10.0-ml aliquot of this solution to 1000.0 ml with water. (These dilutions are based on an injection which is purported to contain 1% w/v procaine HCl.) Record the absorbance of the solution at the wavelength at which procaine HCl exhibits maximum absorption. Calculate the concentration of procaine HCl in the injection.

1.4 DEVIATIONS FROM BEER'S LAW

There are no known exceptions to Bouguer's law. If the concentration of a solution is fixed, there is a linear relationship between absorbance and path length. However, deviations from Beer's law are frequently observed by the analyst in the laboratory. A plot of absorbance vs. concentration is not always a straight line or is a straight line only within well-defined limits. Some of these deviations are due to the nature of the solution being examined; others are due to chemical changes in the solution or to the type of radiant energy used in the measurement process.

A. REAL DEVIATIONS

Beer's law describes the absorption process within the solution accurately only if the concentration of solute is kept below that which leads to molecular (or ionic) interaction. The degree of molecular interaction depends upon the concentration. As the concentration increases, the charge distribution on the molecule changes. Each solute molecule in a concentrated solution does not, therefore, absorb radiant energy in the same manner as does the same molecule in a dilute solution. If interaction occurs at higher concentrations, Beer's law will not be obeyed.

Beer's law should take into consideration not only the concentration but also the refractive index (n) of the solution. It is generally assumed that the absorptivity value remains constant as the concentration increases. However, it is known that this constant does change with concentration (that is, Beer's law is not obeyed) and that the value will remain constant[15] only if it is multiplied by $n/(n+2)^2$. This limitation of the law is not noticeable in dilute solutions.

B. INSTRUMENTAL DEVIATIONS

Beer's law assumes that monochromatic radiation is available for the determination. All spectrophotometers isolate, in theory, the wavelengths specified on the scale of the monochromator. However, under actual operating conditions, solutions are exposed to several wavelengths of radiant energy.

The *spectral slit width* is the total range of wavelengths emerging from the exit slit of a spectrophotometer. It is this width that determines the "purity" of the radiant energy used for the absorbance measurement. By definition, the spectral slit width is equal to two times the dispersion in millimicrons per millimeter times the slit width in millimeters. The *effective band width*, however, is the span of wavelengths emerging from the exit slit whose end wavelengths have half of the energy of the central wavelength.

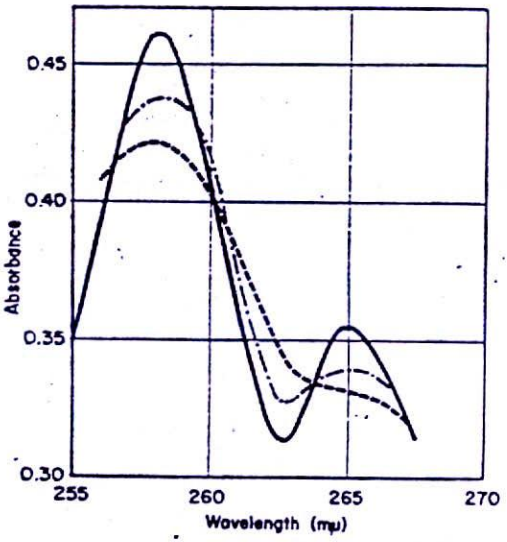


FIGURE 1.4: Absorption spectrum for a 0.08% aqueous solution of procyclidine HCl for slit widths 0.4 mm (———), 1.2 mm (–·–·–), and 2.0 mm (– – – –). Reproduced with permission from *J. Pharm. Pharmacol.* See Ref. 16.

If the slit width is fixed, Beer's law will be obeyed even though the spectrophotometer delivers polychromatic radiation to the solution. The assumption in the latter statement is that the absorptivity value remains relatively constant over the wavelengths isolated by the slit. This situation is true if the absorption maximum is flat, that is, there is little change of absorbance with wavelength around the wavelength of maximum absorption. However, if the absorbance is measured on the slope of an absorption spectrum, absorbance changes rapidly with wavelength. Under these circumstances, deviations from Beer's law may occur.

If the slit width is changed, the energy reaching the solution is not only polychromatic but polychromatic to a different degree. This means that the same solution may absorb more (or less) radiant energy at the second slit setting. This does not usually happen, but certain drugs are sensitive to spectral slit-width changes. There will be no linear relationship between

absorbance and concentration for these drugs unless the slit width is kept constant during the entire operation.

Figure 1.4 illustrates the effect of slit width on absorbance values. The narrower the slit, the greater the absorbance. Both Rogers[16] and Gibson[17] discuss the theory associated with this phenomenon.

C CHEMICAL DEVIATIONS

The solute in the solution may associate, dissociate, or react with the solvent. These processes produce two or more species and, in some instances, these have different absorptivity values at the wavelength of maximum absorption. The analyst is, therefore, being asked to analyze a solution which contains two or more substances. Under these circumstances, there can be no linear relationship between absorbance and concentration.

If a weak acid (e.g., phenylbutazone) is dissolved in a suitable solvent, the following reaction occurs.

$$HX \rightleftharpoons H^+ + X^-$$

Since absorbances are additive,

$$A = a_{HX}bc_{HX} + a_{X^-}bc_{X^-} \tag{1.22}$$

Divide by c:

$$\frac{A}{c} = \frac{a_{HX}bc_{HX}}{c} + \frac{a_{X^-}bc_{X^-}}{c} \tag{1.23}$$

If the pH of the solution is kept constant, the ratio A/c is independent of c. There is, therefore, a linear relationship between absorbance and concentration. If the pH of the solution is changed between determinations, Beer's law will not be obeyed.

(A spectrophotometric determination is not normally carried out at those pH's at which absorbance is changing rapidly with pH)(see Section 1.3E). However, this change of absorbance with pH can be used analytically for the calculation of pK_a values. A weak acid, at or below a certain pH, will be in the molecular form. Similarly, at some higher pH and above, it will be in the ionic form. A solution whose pH is between these two values contains both forms. The amount of acid transformed to its salt form (x) can be calculated from spectrophotometric data.

$$x = \frac{\epsilon^{\prime\prime} - \epsilon^{-}}{\epsilon^{\prime} - \epsilon^{-}} \tag{1.24}$$

The molar absorptivity value for the ionic form is equal to $\epsilon^{\prime}$, for the molecular form, to ϵ^{-}, and for the mixture, to $\epsilon^{\prime\prime}$. By using the appropriate buffers, the absorption spectrum for the various species can be prepared and the molar absorptivity values at the wavelength at which the acid absorbs a maximum of radiant energy can be calculated. The apparent dissociation

constant can be determined graphically (A vs. pH) or can be calculated by using a modified form of the Henderson-Hasselbach equation.

$$pK_a = pH - \log \frac{\alpha}{1 - \alpha} \tag{1.25}$$

The method is described in detail in a paper by Sager et al.[18]

In the example just cited, two species with different absorptivity values at the same wavelength are formed in the solution. Depending on the pH,

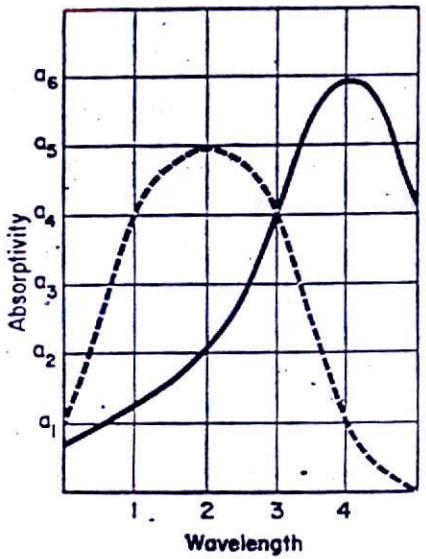


FIGURE 1.5: Hypothetical absorption spectrum for X (———) and Y (– – – –), illustrating spectral characteristics necessary for binary analysis.

aqueous solutions of potassium chromate may contain CrO_4^{-2}, $HCrO_4^-$, and $Cr_2O_7^{-2}$.

$$Cr_2O_7^{-2} + H_2O = 2HCrO_4^- = 2H^+ + 2CrO_4^-$$

Since the molar absorptivity values for the dichromate ion and the two chromate species are different at the wavelength of maximum absorption, chromate solutions, when diluted with water, deviate from Beer's law. However, the absorbance values for solutions containing the chromate (or dichromate) ion are directly proportional to their molar concentrations. If the solution is made alkaline (with 0.05 N potassium hydroxide solution), Beer's law is obeyed. Solutions of this type (that is, potassium chromate in 0.05 N sodium hydroxide solution) not only obey the law but have been so accurately characterized that they are used to check photometric scales for accuracy (see Section 1.8).

1.5 ANALYSIS OF MIXTURES

Absorption spectrum for two hypothetical substances, X and Y, are shown in Fig. 1.5. Solutions containing both X and Y cannot be analyzed by measuring an absorbance value at any one wavelength because this value represents the absorbance due to both of the components in the solution. However, if X does not react with Y and each component follows Beer's law, a binary mixture containing both components can be easily analyzed by measuring absorbances at two wavelengths.

All derivations herein are based on the spectral characteristics of X and Y (see Fig. 1.5). The b symbol in Beer's law has been dropped because it is assumed that the length of the cell is equal to 1 cm. Absorbances are usually additive. However, this additivity should be checked experimentally before any of the equations derived in this section are used to determine the concentrations of the components of a binary mixture.

A. SOLUTION OF SIMULTANEOUS EQUATIONS

At λ_2, the total absorbance (A_2) is equal to the sum of the absorbances due to X and Y. Therefore:

$$A_2 = a_3C_y + a_2C_x \tag{1.26}$$

Similarly, at λ_4,

$$A_4 = a_1C_y + a_4C_x \tag{1.27}$$

Multiply Eq. (1.26) by a_1 and Eq. (1.27) by a_3.

$$a_1A_2 = a_1a_3C_y + a_1a_2C_x \tag{1.28}$$

$$a_3A_4 = a_3a_1C_y + a_3a_4C_x \tag{1.29}$$

Subtract Eq. (1.28) from Eq. (1.29). Therefore:

$$a_3A_4 - a_1A_2 = a_3a_4C_x - a_1a_2C_x \tag{1.30}$$

$$C_x = \frac{a_3A_4 - a_1A_2}{a_3a_4 - a_1a_2} \tag{1.31}$$

Similarly:

$$C_y = \frac{a_4A_2 - a_2A_4}{a_3a_4 - a_1a_2} \tag{1.32}$$

Before a mixture can be analyzed, the analyst must determine the absorptivity values for X and Y at λ_2 and λ_4. These values are substituted into Eqs. (1.31) and (1.32). The absorbance of the mixture at λ_2 and λ_4 is now determined and Eqs. (1.31) and (1.32) solved for C_x and C_y.

The procedure for the determination of salicylic acid in the presence of acetylsalicylic acid[19] is representative of the many methods of analysis in the pharmaceutical literature which are based on Eqs. (1.31) and (1.32).

An accurately weighed sample of acetylsalicylic acid (360 mg) is dissolved in 100.0 ml of chloroform. The absorbance of this solution is determined at 308 mμ, the wavelength at which salicylic acid absorbs a maximum of radiant energy. A 1-ml aliquot of this solution is diluted, with chloroform, to 100.0 ml. The absorbance of this solution is determined at 272 mμ, the absorption maximum for acetylsalicylic acid. Concentrations are calculated by using the following equations:

$$\text{mg acetylsalicylic acid} = 1347A_{278} - 2.3A_{308}$$

$$\text{mg salicylic acid} = 3.58A_{308} - 3.49A_{278}$$

The researchers claim that over a range of 98–100% purity for acetylsalicylic acid the method has an error of less than 0.20%.

Binary mixtures cannot be analyzed unless:

a. Spectral data for the pure components are available.

b. The absorptivity values for the components can be easily and accurately determined.

c. The absorptivity values for the components are sufficiently different at the chosen wavelengths to permit an accurate solution of Eqs. (1.31) and (1.32).

d. The absorbance values for the mixture are accurately determined.

Requirements a and d are more easily met than are b and c. Absorptivity values are difficult to determine if one of the components is a poor absorber of radiant energy at one of the wavelengths chosen for the analysis. Moreover, if the spectral characteristics of the one component approximate those of the other, the absorptivity values at the wavelengths chosen for the analysis will be similar. Under these circumstances, Eqs. (1.31) and (1.32) cannot be accurately solved.

The grouped absorptivity values in Eqs. (1.31) and (1.32) can be determined by measuring the absorbances of solutions containing both X and Y. Nibergall and Mattocks[20] claim that the values so determined are more accurate than those obtained by examining solutions containing only X or Y. Eqs. (1.31) and (1.32) can be modified by combining a values.

$$C_x = K_1A_1 - K_2A_2 \tag{1.33}$$

and

$$C_y = K_3A_2 - K_4A_1 \tag{1.34}$$

If the analyst prepares a series of solutions containing both X and Y and measures the absorbances of these solutions at the chosen wavelengths, the unknowns in Eqs. (1.33) and (1.34) are K_1, K_2, K_3, and K_4. Using the absorbance data obtained for the mixtures, the K values can be calculated by the least squares method. Solutions containing unknown quantities of X and Y can now be quickly analyzed by measuring absorbances and substituting these into Eqs. (1.33) and (1.34).

The multiple regression method is described in detail in the paper by Niebergall and Mattocks.[20] The authors analyzed several solutions containing salicylic acid and salicylamide and concluded that their method was more accurate than that described in this section.

All spectrophotometric methods are based on the assumption that the component (or components) being determined is the only absorber in the solution. However, trace impurities are not always held back by the separatory processes used in the laboratory. Irrelevant absorption is a distinct possibility if these impurities contain chromophoric groups which absorb energy at or near the wavelength chosen for the analysis. Such absorption can produce spurious results and is particularly troublesome when binary mixtures are being analyzed.

Irrelevant absorption in two-component spectrophotometric analysis can be corrected for by using orthogonal functions. The details associated with the use of such functions are presented in a paper by Glenn.[21] Glenn states that harmonic analysis is based on the fact that a given function can be expanded in terms of a set of orthogonal functions of the same variable λ. In other words, the function can be broken down into a set of fundamental shapes, that is, orthogonal functions. An absorption spectrum $f(\lambda)$ can be decomposed into many fundamental shapes (g_0, g_1, g_2, etc.). Each shape is coupled to an appropriate coefficient, which is proportional to concentration. Absorbances are measured at several wavelengths and substituted into the appropriate equations. The author tested the derived equations by analyzing a mixture which contained phenol and epinephrine. The analytical results were not affected by absorbing impurities. The mixture could be analyzed by measuring absorbance values at 270 and 283 mμ, the wavelengths at which the components absorb a maximum of radiant energy, and substituting these into Eqs. (1.31) and (1.32). However, if the solution contains an absorbing impurity which increases the absorbance value at 270 mμ by one-quarter and at 283 mμ by one-third, the per cent recovery of epinephrine and phenol from solutions containing known amounts of the drug and the preservative will be 137.8 and 118.2%, respectively. It is obvious that Glenn's method is superior to that based on Eqs. (1.31) and (1.32). Although the method is complicated, it does deal with a serious spectrophotometric problem. The paper should be read by those who are interested in a more sophisticated approach to the analysis of binary mixtures.

B. CONSTANCY OF THE ABSORBANCE RATIO VALUE

If Beer's law is obeyed at all wavelengths, it may be easily shown that the ratio of two absorbance values determined at two wavelengths is a constant. The spectral characteristics for X (see Fig. 1.5) are used to illustrate the constancy of the absorbance ratio value. At λ_4,

$$A_4 = a_x b C_x \tag{1.35}$$

At λ_3,

$$A_3 = a_4 b C_x \tag{1.36}$$

Divide Eq. (1.35) by Eq. (1.36).

$$\frac{A_4}{A_3} = \frac{a_5 b C_x}{a_4 b C_x} = \frac{a_5}{a_4} = Q{:}4{:}3 \tag{1.37}$$

The ratio of two absorbance values is equal to the ratio of two constants (that is, absorptivity values) and is, therefore, equal to a constant. The symbol for the value is Q. For the purposes of this chapter, Q:290:280 indicates that this particular constant is calculated by dividing the absorbance value at 290 mμ by that at 280 mμ, the solution and the cell being the same in both cases.

The Q value is independent of concentration and thickness of the solution and can, therefore, be used to assess the purity of pharmaceutically important substances. Q values are given in the NF and the USP for many drugs (aminosalicylic acid, noscapine, promazine HCl, etc.), but the use of such values for the identification of pharmacopeial drugs is a relatively recent development. In actual fact, absorbance ratios have been used for this and other purposes since the turn of the century.[22]

C. EXPERIMENT 1.2: IDENTIFICATION OF AMINOSALICYLIC ACID USP[23]

Dissolve 250 mg of aminosalicylic acid in 3 ml of 4% w/v sodium hydroxide solution. Transfer to a 500-ml volumetric flask, dilute to volume, and mix. Transfer a 5-ml aliquot to a 250-ml volumetric flask containing 12.5 ml of pH 7 phosphate buffer (see USP, p. 913), dilute to volume, and mix.

This solution, when compared in a suitable spectrophotometer against a blank of the same buffer in the same concentration, exhibits absorbance maxima at 265 $\pm$ 2 mμ and 299 $\pm$ 2 mμ, and the Q:265:299 value is between 1.50 and 1.56.

D. APPLICATION OF ABSORBANCE RATIOS TO THE ANALYSIS OF BINARY MIXTURES

About 1900, Hüfner[22] showed that a smooth curve resulted when the ratio of the absorbances determined at 540 and 560 mμ were plotted against the per cent reduced hemoglobin in a sample of oxyhemoglobin. The analytical implications of Hüfner's approach to the determination of one component in the presence of another were not immediately apparent. However, the theory associated with this method of analysis is now well understood,[24–26] and several pharmaceutically important mixtures have been analyzed[27,28] by using absorbance ratios rather than absorptivity values.

From Beer's law, the total absorbance at λ_4 (see Fig. 1.5) is equal to the sum of the absorbances due to X and Y. Therefore:

$$A_4 = a_4bC_x + a_1bC_y \tag{1.38}$$

Similarly, at λ_3,

$$A_3 = a_3bC_x + a_1bC_y \tag{1.39}$$

The two wavelengths chosen for the analysis are the isoabsorptive wavelength and the wavelength at which X absorbs a maximum of radiant energy. (An isoabsorptive point is the wavelength at which two substances have similar absorptivity values.) Divide Eq. (1.38) by Eq. (1.39) and each term in the resulting equation by $C_x + C_y$. Substitute Fx for $C_x/(C_x + C_y)$ and Fy for $C_y/(C_x + C_y)$. Fx and Fy are equal to the fraction of the respective components present in the mixture. Therefore:

$$\frac{A_4}{A_3} = \frac{a_4Fx + a_1Fy}{a_3Fx + a_1Fy} \tag{1.40}$$

However:

$$Fy = 1 - Fx \tag{1.41}$$

Therefore:

$$\frac{A_4}{A_3} = Fx\left(\frac{a_4}{a_3} - \frac{a_1}{a_3}\right) + \frac{a_1}{a_3} \tag{1.42}$$

A_4/A_3 is equal to Q_o, the absorbance ratio for the binary mixture. Similarly, a_4/a_3 is equal to Qx, the absorbance ratio for pure X, and a_1/a_3 is equal to Qy, the absorbance ratio for Y. Therefore:

$$Q_o = (Qx - Qy)Fx + Qy \tag{1.43}$$

Equation (1.43) is the equation of a straight line having a slope value of $Qx - Qy$ and an intercept value of Qy. The *relative* analysis of a binary mixture can, therefore, be carried out by determining the absorbance ratio values for the pure substances only. More important, Eq. (1.43) is concentration independent. The analysis of a binary mixture does not, therefore, require careful dilution or accurate weighings. However, the analyst can only determine relative concentrations, that is, the per cent of X or Y in the mixture.

If the analyst must determine C_x and C_y, numbers can be substituted into Eq. (1.46). At λ_3,

$$A_3 = a_3(C_x + C_y) \tag{1.44}$$

and

$$C_x + C_y = A_3/a_3 \tag{1.45}$$

Therefore:

$$C_x = \frac{Q_o - Qy}{Qx - Qy} \times \frac{A_3}{a_3} \tag{1.46}$$

C_y may be determined in a similar manner.

If a wavelength other than an isoabsorptive wavelength is chosen as the second analytical wavelength, a plot of Q_a vs. Fx results in a smooth curve. The analyst must, therefore, prepare a series of solutions containing X and Y, measure absorbances, calculate Q_a values, plot the results, and then use this graph for subsequent analyses. For this reason, an isoabsorptive wavelength should be chosen as the second analytical wavelength.

This method of analysis depends upon the use of an isoabsorptive point. The point must, therefore, be accurately isolated. This can be done by superimposing absorptivity vs. wavelength curves for the components (for example, as in Fig. 1.5) and then determining absorptivity values at and around the wavelength at which the curves cross. The method is tedious. However, the point can be isolated accurately and quickly by preparing two solutions, one containing X and the other Y. The solutions are so prepared that the concentrations of X and Y are the same. A portion of the one solution is placed in the sample cell; the other is placed in the reference cell. The cells are transferred to a spectrophotometer and absorbance values are determined over the range of wavelengths straddling the isoabsorptive point. The wavelength at which the absorbance value is zero represents an isoabsorptive point.

E. EXPERIMENT 1.3: SIMULTANEOUS ANALYSIS OF PROCAINE HCl AND TETRACAINE HCl[25]

Absorption spectra for procaine HCl, tetracaine HCl, and a procaine HCl-tetracaine HCl mixture are shown in Fig. 1.6.

Weigh accurately 160 mg of tetracaine HCl and dissolve in 500.0 ml of water. This is solution A. Weigh accurately 160 mg of procaine HCl and dissolve in 500.0 ml of water. This is solution B. Prepare six solutions in the manner indicated (Table 1.6).

Calculate the per cent tetracaine HCl in each solution. For example, solution 1 contains 100% tetracaine HCl and solution 6 contains 0% tetracaine HCl. Using a recording spectrophotometer, prepare absorption spectra for the six solutions. The curves should intersect at or near 297.5 mμ, the isoabsorptive point. Tetracaine HCl absorbs a maximum of radiant

TABLE 1.6

Solution	Milliliters solution A per liter of water	Milliliters solution B per liter of water
1	25.0	0.0
2	20.0	5.0
3	15.0	10.0
4	10.0	15.0
5	5.0	20.0
6	0.0	25.0

energy at or near 311 mμ. Calculate Q:311:297.5 values. Using the data obtained for each of the six solutions (Q and Fx values for solutions 1–6), calculate the slope and intercept values by the method of least squares.[29] Substitute these values into Eq. (1.43). Calculate the same values from absorbance data obtained for solutions 1 and 6. Do the values agree?

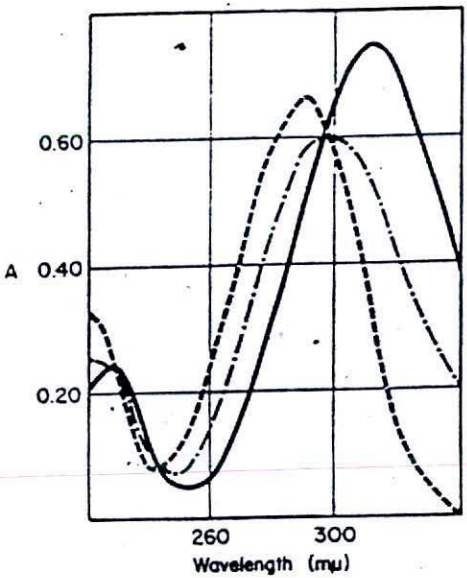


FIGURE 1.6: Absorption spectrum for procaine HCl (– – –), tetracaine HCl (———), and a procaine HCl-tetracaine HCl mixture (– · – · –). (The solutions contain 10 mg of drug per liter of water. The mixture contains 5 mg of procaine HCl and 5 mg of tetracaine HCl per liter of water.)

Using graduated cylinders, dilute a solution containing unknown quantities of procaine HCl and tetracaine HCl to the point that absorbance values at 311 mμ and 297.5 mμ can be accurately measured. Substitute the Q:311:297.5 value for this solution into the numerical form of Eq. (1.43). Calculate the per cent tetracaine HCl (and the per cent procaine HCl) in the solution.

F. EXPERIMENT 1.4: SIMULTANEOUS ANALYSIS OF THEOBROMINE AND CAFFEINE[30]

Weigh accurately 100 mg of caffeine and dissolve in 100.0 ml of water. This is solution A. Weigh accurately 100 mg of theobromine and dissolve in 100.0 ml of water. This is solution B. Prepare six solutions in the manner indicated (Table 1.7).

Using a recording spectrophotometer, prepare absorption spectra for the six solutions. Calculate a_{240} and a_{273} values for caffeine and theobromine and substitute these into Eqs. (1.31) and (1.32).

The binary mixture is analyzed by measuring absorbances at 240 and 273 mμ. Why did Miles and Englis[30] select these wavelengths?

TABLE 1.7

Solution	Milliliters solution A per liter of 0.1 N NaOH	Milliliters solution B per liter of 0.1 N NaOH
1	10.0	—
2	9.0	—
3	8.0	—
4	—	8.0
5	—	9.0
6	—	10.0

Dilute an aliquot of a solution containing unknown quantities of theobromine and caffeine to 1000.0 ml with 0.1 N sodium hydroxide solution. The volume of the aliquot should be such that the absorbance values at 240 and 273 mμ are not less than 0.2 and not more than 0.6. Measure absorbances at 240 and 273 mμ. Substitute these values into the numerical forms of Eqs. (1.31) and (1.32) and solve for C_x and C_y.

1.6 COLORIMETRY

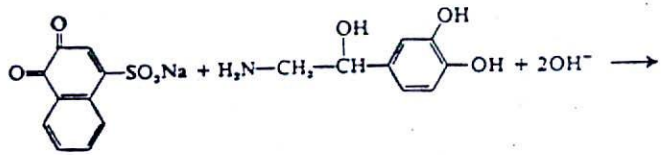
Colorimetry is the determination of the light absorptive capacity of a system. A quantitative determination is, therefore, carried out by subjecting a *colored* solution to those wavelengths of visible energy which are absorbed by that solution. Since such determinations are based on the absorption of energy, the mathematical principles associated with colorimetry are analogous to those described in Sections 1.3 and 1.5.

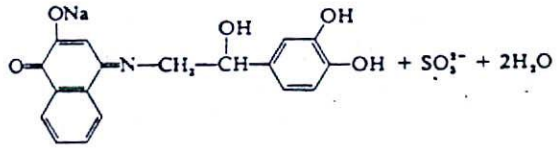
Determinations can also be carried out by comparing a solution containing an unknown amount of a colored substance with a solution containing a known amount of the same substance. This approach to colorimetry is characteristic of the *limit tests* in the pharmacopeias for a number of organic and inorganic contaminants in certain drugs. The method is comparative and is based on the analyst's ability to distinguish between different intensities of the same color. Ideally, the colored substance should transmit a maximum of energy over the 500- to 600-mμ range, because the eye functions best at these wavelengths. However, the pharmaceutical analyst is primarily concerned with the chemistry of color formation. For this reason, limit tests are based on the formation of substances whose colors range from violet to red.

A drug cannot be analyzed colorimetrically unless it falls into one of three categories:

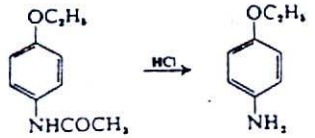
a. The drug must be self-colored. Some pharmaceutically important substances—for example, pyrvinium pamoate, phenolsulfonphthalein, methylene blue, dithiazanine iodide, etc.—are colored. The substance may be dissolved in a suitable solvent and its absorbance determined at the wavelength at which it absorbs a maximum of visible energy.

b. The drug must react with a reagent to produce a substance which is colored. For example, a reddish substance is formed when norepinephrine is reacted with sodium 1,2-naphthaquinone-4-sulfonate in alkaline media.[31]





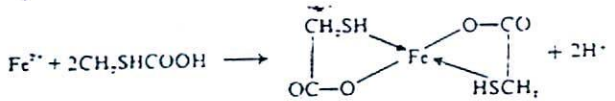
c. The drug must be converted to a derivative which reacts with a reagent to produce a substance which is colored. For example, acetophenetidin can be converted to phenetidin, which reacts with chromic acid to produce a colored substance.[32]



A. EXPERIMENT 1.5: LIMIT TEST FOR IRON IN HEAVY MAGNESIUM CARBONATE BP[33]

The test is based on the reaction between iron and thioglycollic acid in a solution buffered with ammonium citrate.

$$2Fe^{3+} + 2CH_2SHCOOH \rightarrow 2Fe^{2+} + \dot{C}OOH{-}CH_2{-}S{-}S{-}CH_2{-}\dot{C}OOH + 2H^+$$



Apparatus. A Nessler cylinder is made of clear glass with a nominal capacity of 50 ml. The overall height of the cylinder is about 160 mm; the external height to the 50-ml mark is 109 to 124 mm; the thickness of the wall is 1.0 to 1.5 mm; the thickness of the base is 1.0 to 3.0 mm.

Standard Iron Solution. Dissolve 173 mg of ferric ammonium sulfate in 100 ml of water, add 5 ml of dilute hydrochloric acid solution (10% w/w), and dilute to 1000 ml with water.

Test Solution. Dissolve 0.10 g of heavy magnesium carbonate in 5 ml of water and 0.5 ml of hydrochloric acid, add 2 ml of a 20% w/v solution of citric acid, two drops of thioglycollic acid, mix, and make alkaline with ammonia solution (10% w/w). Dilute to 50 ml with water.

Standard Solution. Dilute 2 ml of the standard iron solution with 40 ml of water. Add 2 ml of a 20% w/v solution of citric acid, two drops of thioglycollic acid, mix, and make alkaline with ammonia solution (10% w/w). Dilute to 50 ml with water.

Test. Compare the color of the two solutions in Nessler cylinders after 5 min. The color of the test solution is not more intense than the color of the standard solution.

B. GENERAL REQUIREMENTS FOR THE COLORED SUBSTANCE

Solutions containing a colored substance are not always amenable to colorimetric measurement. Solutions should, therefore, meet certain general requirements. If one or more of these requirements are not met, the conditions of assay must be carefully spelled out. If they are not, the analyst may not be able to attain the accuracy reported in the literature for that method of analysis.

1. The Solutions Should Be Intensely Colored

Color intensity and sensitivity go hand in hand. If absorbance values change rapidly as the concentration of the constituent in the solution increases, the analyst will be able to determine relatively small quantities of drug. However, if concentrations must be changed drastically (e.g., from 1 to 10 mg) to produce a measurable change in absorbance, the method is not sensitive enough for routine use. In other words, the intensity of the color has changed little, even though there has been a tenfold increase in concentration.

2. The Solutions Must Be Unaffected by pH Changes

Acid-base indicators, potassium chromate, and many other substances (see Section 1.4C) are affected by the pH of the solution. Measurements, in such instances, must be carried out on solutions whose pH is such that the

absorbance does not change when the pH of the solution is altered by 1 or 2 units.

3. The Solutions Should Be Stable

When colorimetric methods are developed in the laboratory, it is customary to determine the absorbance value of a solution at various times. If the absorbance value changes with time, the solution must be measured at some specified time or the system must be chemically stabilized.

4. The Constituent Should React Quickly and Quantitatively with the Reagent at Room Temperature

If the colored substance forms slowly, heat may be used to drive the reaction. However, excessive heat may lead to the production of secondary substances. If such substances absorb visible energy at or near the measuring wavelength, Beer's law will not be obeyed.

5. The Solutions Should Obey Beer's Law

Colored solutions will deviate from Beer's law for the reasons given in Section 1.4. One other factor should be considered herein. Filter photometers are often used to determine absorbance values. Depending on the type of filter in the instrument, the band width may be as much as 50 to 60 mμ. Under such circumstances, a plot of absorbance vs. concentration is not always linear. If the deviation from linearity is not too drastic, the graph can be used to determine the concentrations of solutions containing unknown amounts of substance.

To check for compliance with Beer's law, the analyst plots absorbance vs. concentration. However, even if this type of plot is linear, it gives no indication of the accuracy of the method over a certain concentration range. For this reason, some analysts prefer to plot per cent T vs. log c. Such a plot (a *Ringbom* plot) is illustrated in Fig. 1.7. The concentrations at which the analyst can measure absorbance most accurately are defined by the linear portion of the curve. For the example given, best accuracy will be attained for solutions containing from 1 to 6 mg of the substance

C. CHEMISTRY OF COLORIMETRY

Colored substances are formed by reacting the constituent with either an inorganic or an organic reagent. Reaction may, therefore, be classified on the basis of the type of reagent used and on the type of reaction which occurs when the constituent is reacted with the reagent.

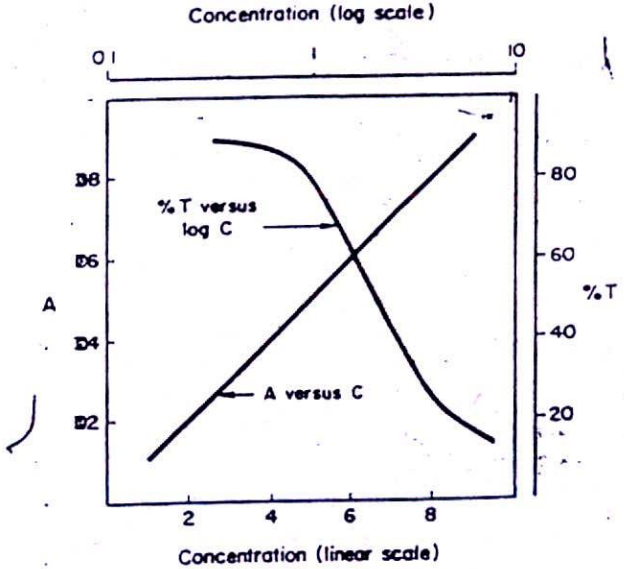


FIGURE 17: A comparison of the A vs. c plot with the Ringbom plot.

1. Inorganic Reagents

The reagent may oxidize, reduce, or complex with the constituent. Several of the more common reactions are outlined here:

a. Identification of Copper[34]

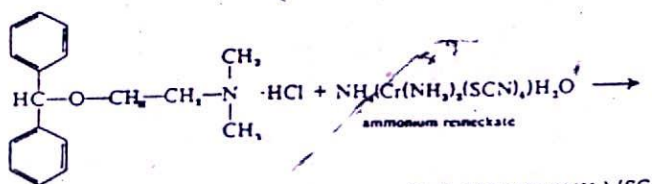
$$\underset{\text{(blue)}}{Cu^{2+} + 4NH_3 \rightarrow Cu(NH_3)_4^{2+}}$$

b. Identification of Iron[35]

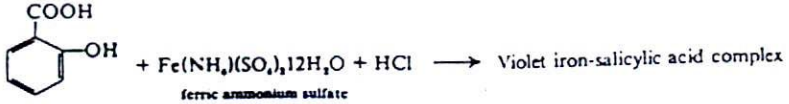
$$\underset{\text{ferrocyanide}}{4Fe^{3+} + 3Fe(CN)_6^{4-}} \rightarrow \underset{\text{prussian blue}}{Fe_4(Fe(CN)_6)_3}$$

This reaction was first reported in the literature in 1841 by Harting.[36]

c. The Determination of Diphenhydramine HCl[37]



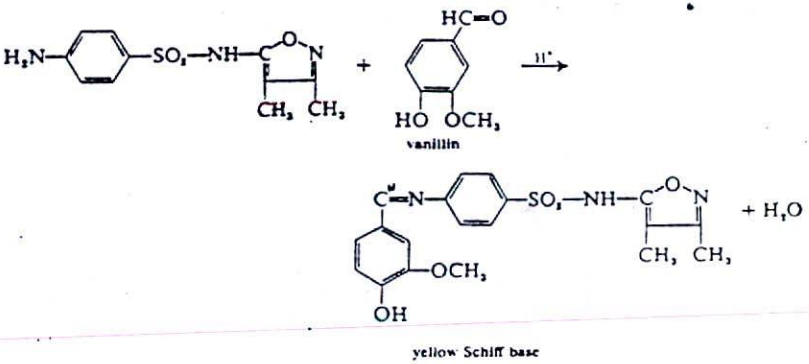
d. Limit Test for Salicylic Acid in APC Tablets[38]



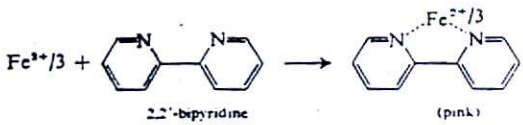
2. Organic Reagents

These reagents form salts, couple, or chelate with the constituent.

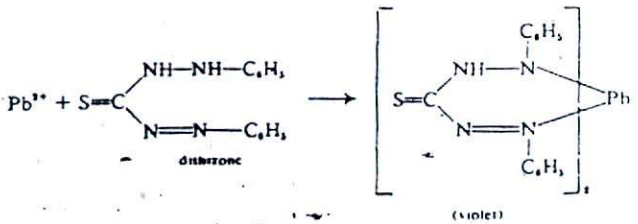
a. The Determination of Sulfisoxazole



b. The Determination of Iron

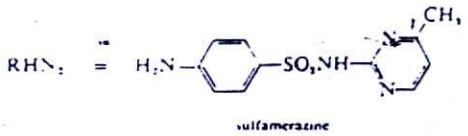


c. Limit Test for Lead[39]

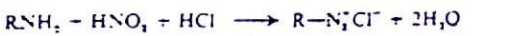


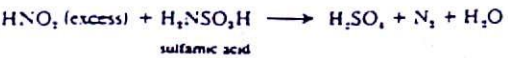
d. The Determination of Sulfonamides.[40,41] This reagent was first used by Bratton and his co-workers and is now known as the Bratton-Marshall reagent. The analysis is carried out by diazotizing the amine group on the

aryl amine, coupling the product to the reagent, and determining the absorbance of the diazo compound at 545 mμ.

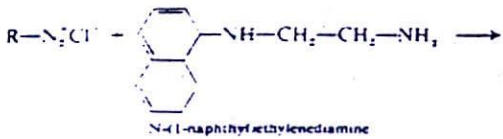


sulfamerazine

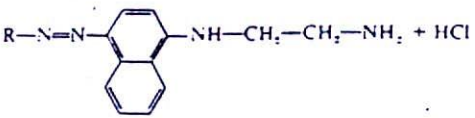




sulfamic acid



N-(1-naphthyl)ethylenediamine



No attempt has been made to either spell out the details associated with a particular analysis or to list all the reagents that are described in the USP or the NF. Analytical details and a list of reagents may be found in either the references cited or in *Remington's Pharmaceutical Sciences*.[42]

Like the product, the reagent must meet certain general requirements, listed here, but few of the many organic and inorganic color-forming reagents can meet all of these requirements. The ideal reagent should (a) be colorless, (b) develop the color rapidly, (c) be involved in a reaction whose mechanism is known, (d) react stoichiometrically with the constituent, (e) produce a colored substance with an absorption maximum at a specific wavelength, and (f) react only with the substance being analyzed.

D. EXPERIMENT 1.6: ANALYSIS OF DIPHENHYDRAMINE HCl TABLETS[37]

Ammonium Reineckate Solution. Shake 1 g of ammonium reineckate with 50 ml of water. Filter.

Preparation of a Calibration Curve. Weigh accurately 100 mg of diphenhydramine HCl and dissolve in 50.0 ml of water. Pipet exactly 3.0, 4.0, 5.0, 6.0, and 7.0 ml of this solution into five 30-ml beakers. Adjust the volume in each beaker to 10 ml with 1% H_2SO_4 solution. Place the beakers

in a shallow ice bath and add to each, dropwise, 5 ml of ammonium reineckate solution. Allow the precipitate to digest in the ice bath for 1 hr. Collect the precipitate in a sintered-glass crucible of medium porosity. Wash the precipitate with two 5-ml portions of cold water (5°C), dissolve in acetone, transfer the solution to a 25-ml volumetric flask, and dilute to 25.0 ml with acetone. Using a Spectronic 20 spectrophotometer, or the equivalent, measure the absorbance of each of the solutions at 525 mμ. Plot absorbance vs. concentration.

The Analysis of Tablets. Weigh accurately 20 tablets and reduce to a fine powder using a mortar and pestle. Accurately weigh a sample of the powder containing the equivalent of 100 mg of diphenhydramine HCl. Transfer to a beaker, add 50 ml of 1% H_2SO_4 solution, and digest on a steam bath for 15 min. Cool and filter into a 100-ml volumetric flask. Add sufficient 1% H_2SO_4 solution to make 100.0 ml of solution. Subject 10.0 ml of this solution to the procedure just described, that is, add, dropwise, 5 ml of ammonium reineckate solution, etc. Determine, from the calibration curve, the quantity of diphenhydramine HCl in the solution. Calculate the number of milligrams of diphenhydramine HCl in a tablet of average weight.

1.7 SPECIAL METHODS

Spectrophotometric methods of analysis (or, for that matter, any method of analysis) should be simple, specific, and precise. Unfortunately, simplicity, lack of specificity, and poor precision go hand in hand. The pharmaceutical analyst must, therefore, use more elaborate methods if he wishes to analyze for a substance in the presence of an impurity or to increase the precision with which the component is determined. Several of the more popular approaches to standard analytical problems are given herein. However, these should be judged on the basis of what they will do under a particular set of circumstances. If the gain in specificity or precision is relatively small, the pharmaceutical analyst will not use these methods because they are time-consuming and not generally applicable to the analysis of drugs in a quality control laboratory.

A. PRECISION SPECTROPHOTOMETRY

If a solution transmits less than 20% or more than 60% of the incident radiant energy, spectrophotometric errors may be excessive. (Section 1.3D should be reviewed at this point.) A concentrated solution can be diluted or a dilute solution can be concentrated. However, several researchers[43–45] have described techniques that yield a high degree of precision even if the solution contains amounts of drug in excess of or less than those usually subjected to spectrophotometric analysis.

The analyst operates most spectrophotometers by manipulating the slit width, sensitivity, and dark-current knobs. (The wavelength, absorbance-transmittance, and shutter controls must also be moved, but these operations are not directly pertinent to this discussion.) The dark-current knob is used to zero the instrument when the phototube is in darkness. The slit width and sensitivity knobs are used to set 100% T with solvent only in the beam of radiant energy. However, these controls can be manipulated under different circumstances and it is these circumstances that form the basis for precision spectrophotometry.

Reilley and Crawford[43] describe four spectrophotometric methods. The first method is that described in Section 1.3E.

1. Ordinary Method

The instrument is set to read zero with the phototube in darkness, that is, the shutter is closed, and to read 100 when exposed to energy which has passed through pure solvent.

2. Transmittance-Ratio Method

This method differs from the ordinary method in that the 100% T setting is made with a reference solution somewhat more dilute than the solution being analyzed.

3. Trace Analysis Method

The instrument is set to read 100 when exposed to energy which has passed through pure solvent and to read zero when exposed to energy which has passed through a reference solution somewhat more concentrated than the solution being analyzed.

4. General Method

The instrument is set to read zero and 100 using reference solutions.

In essence, therefore, the absorbance-transmittance scale on the spectrophotometer can be expanded at will. This results in a gain in precision, but the methods do suffer from a number of disadvantages. Beer's law is not always obeyed and, in some cases, absorption is such that a solution cannot be zeroed or set to 100 by using the dark current or slit-width knobs. On the plus side, Reilley and Crawford show in their paper that a limiting reference transmittance of 78% by method 3 yields a 4.5-fold increase in precision over method 1.

If methods 3 and 4 are to be used, the following procedure should be followed: (a) the range of concentrations over which the measurements are to be made is determined and the extreme points of the range are selected as reference solutions; (b) a series of solutions of known concentration lying between and including the reference solutions is prepared; (c) the instrument

is set to its highest operating sensitivity; (d) the reference solution of highest concentration is used to zero the instrument and the reference solution of lowest concentration is used to set the instrument to 100, using dark-current and slit-width knobs alone; (e) the remaining solutions are measured and the data is used to plot a calibration curve; (f) the solution containing an unknown quantity of drug is measured and the concentration is determined graphically.

A colorimetric method of analysis for fluoride is based on one of the methods just described.[48] The researcher analyzed 10 samples, each containing 400 μg of fluoride, and found that the coefficient of variation was only 0.2%.

B. DIFFERENTIAL ANALYSIS

This method of analysis may be used whenever the absorptive properties of a given chromophore can be modified selectively in the presence of a mixture of chromophores. A differential spectrum is obtained by subtracting the spectrum of the starting material from the spectrum of the product. The absorbances of any unmodified chromophores are thereby cancelled out. The method can be used, therefore, to determine the quantity of drug in a mixture of absorbing substances without separating the one from the others. Structurally related substances (e.g., the barbiturates) can also be determined in the presence of each other by using this method of analysis.[49]

A drug can be quickly and easily analyzed by the differential method. For example, Demetrius and Sinsheimer[50] have used this method for the determination of eugenol in pharmaceuticals. All absorbance measurements were carried out at 296 mμ. (Solutions containing known quantities of eugenol were investigated and, on this basis, all subsequent measurements were carried out in solutions having pH values of 3.0 and 12.0. See Section 1.2B. Similarly, the characteristics of the differential spectrum dictate the wavelength at which absorbance is measured.) The concentration of eugenol in pharmaceuticals can be determined by using the following equation.

$$\text{g/liter} = (\Delta A \times 164.2)/\Delta\epsilon$$

$\Delta\epsilon$ is determined by comparing spectrophotometrically two solutions containing identical quantities of eugenol. The pH of the first solution is 3.0. A portion of this solution is transferred to a cell and placed in the reference beam of the spectrophotometer. (In a single beam instrument, this solution acts as the "blank.") The pH of the second solution is 12.0. This solution is transferred to the sample side of the spectrophotometer. The absorbance is measured and then calculated to a molar basis. This constant, according to Demetrius and Sinsheimer, is equal to 3886. ΔA is equal to the observed absorbance at 296 mμ of a given concentration of eugenol in basic solution less the absorbance of the same concentration of eugenol in acid medium. The molecular weight of eugenol is equal to 164.2.

It should be obvious, therefore, that differential analysis is based on the transmittance ratio method (see previous section) and on a change in chromophoric characteristics with a change in solution pH. The method does assume that the chromophoric groups in the contaminating substances are not affected by the change in solution pH.

C. EXPERIMENT 1.7: ANALYSIS OF MORPHINE SULFATE TABLETS[31]

A differential spectrum for morphine, that is, the curve obtained by subtracting the absorbances of the ultraviolet spectrum in acid solution from those of the ultraviolet spectrum in alkaline solution, shows absorption maxima at 256 and 298 mμ. All absorbances are measured at 298 mμ.

Determination of the $\Delta\epsilon$ Value for Morphine. Weigh accurately 80 mg of morphine and dissolve in 100.0 ml of alcohol. Dilute a 10.0-ml aliquot of this solution and 1.0 ml of 1.0 *N* sodium hydroxide solution to 100.0 ml with water. Dilute a second 10.0 ml-aliquot and 1.0 ml of 0.10 *N* sulfuric acid solution to 1000 ml with water. Measure the absorbance of the alkaline solution at 298 mμ relative to the acid solution in the reference cell. Calculate $\Delta\epsilon$.

The $\Delta\epsilon$ value should be equal to approximately 2346.

Assay of Morphine Sulfate Tablets. Weigh accurately 20 ¼-gr tablets and reduce to a fine powder using a mortar and pestle. Accurately weigh a sample of the powder containing the equivalent of 90 mg of morphine sulfate. Dissolve the drug in water, filter, and dilute the filtrate to 100.0 ml with water. Dilute two 10.0-ml aliquots as described under the determination of the $\Delta\epsilon$ value for morphine. Measure the relative absorbance of the alkaline solution at 298 mμ. Calculate the amount of morphine sulfate pentahydrate in the aliquot by using the following formula.

$$\text{g/100 ml} = 379.4A/(10\Delta\epsilon)$$

This method of analysis can be used to determine morphine in tincture of opium and in camphorated tincture of opium. However, the assay of such preparations requires preliminary separation because narceine, narcotine, papaverine, and some of the nonalkaloidal constituents of opium show absorbance changes with changes in the pH of the solution. The separatory procedure is described in the paper cited.

D. SPECTROPHOTOMETRIC TITRATIONS

A titration, in its simplest form, involves the reaction of the constituent with a titrant to form a product:

$$X + Y \rightarrow Z$$

If an indicator is used to locate the end point, a slight excess of Y, the titrant, will produce a color change. In a spectrophotometric titration, the absorbance of the solution at a specified wavelength is measured after each addition of titrant. The results are plotted (A vs. milliliters of titrant), and the end point is determined graphically. The point at which the two straight lines intersect is the end point of the titration. As in conductometric titrations, the graph is constructed on the basis of data obtained well before and well after the end point. The addition of small increments of titrant at and around the end point is, therefore, unnecessary. However, absorbance values must be corrected because the volume changes as titrant is added to the solution. Instruments for carrying out such titrations automatically are now available.

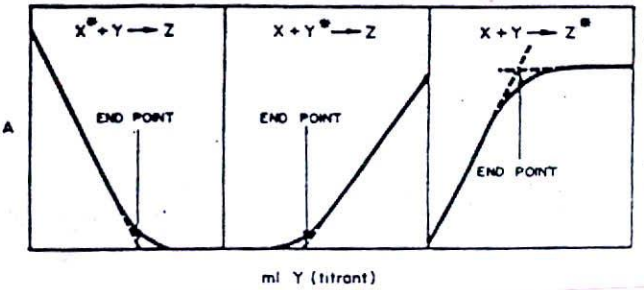


FIGURE 1.8: Spectrophotometric titration curves. (The asterisk indicates the species which absorbs radiant energy.)

If X, the substance being analyzed, absorbs radiant energy at a specified wavelength and its absorptivity value is known, an analysis can be carried out directly. A spectrophotometric titration serves no useful purpose. However, if the constituent is contaminated with other absorbing substances, a spectrophotometric titration can be carried out if a titrant can be found which reacts selectively with X. If X and the contaminants are the only species that absorb radiant energy, the absorbance will decrease as titrant is added. The absorbance will remain constant after the end point, that is, after X has been completely converted to Z, a nonabsorbing substance. Typical plots are shown in Fig. 1.8.

If Y is the only species in solution which absorbs radiant energy, the solution will not absorb radiant energy until the end point is reached. At that point, the absorbance values increase as the concentration of Y increases.

If Z is the only species in solution which absorbs radiant energy, the absorbance will increase as product is formed. The absorbance will remain constant after the end point because the amount of Z in solution is constant.

The plots presented in Fig. 1.8 are for hypothetical substances. The papers

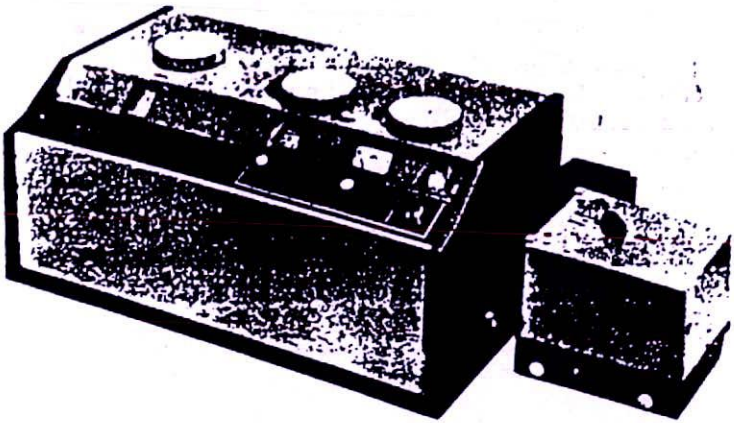


FIGURE 1.9: A Beckman model DU-2 spectrophotometer. Courtesy of Beckman Instruments, Inc., Fullerton, Calif.

by Goddu and Hume[52] and by Higuchi et al.[53] give specific examples of spectrophotometric titrations and discuss, in detail, the advantages and disadvantages of the technique. The method can be used when dilute solutions (10^{-4} M or less) are being analyzed, when binary mixtures of weak acids or bases are being titrated, and when the color change at the end point is poor.

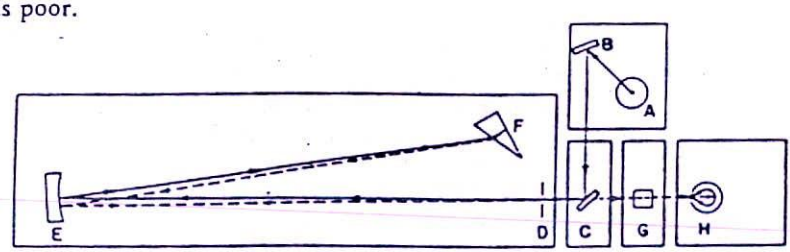


FIGURE 1.10: Schematic diagram of the Beckman model DU spectrophotometer. Light from the source (A) is focused on the condensing mirror (B) and directed in a beam to the 45° slit-entrance mirror (C). The slit-entrance mirror deflects the beam through the slit (D) and into the monochromator to the collimator mirror (E). Light falling on the collimator mirror is rendered parallel and reflected to the prism (F), where it undergoes refraction. The back surface of the prism is aluminized so that light refracted at the first surface is reflected back through the prism, undergoing further refraction as it emerges. The desired wavelength of light is selected by rotating the wavelength selector on top of the monochromator case. This control adjusts the position of the prism. The spectrum from the prism is directed back to the collimating mirror which centers the chosen wavelength of light on the slit and the sample (G). Light passing through the sample strikes the phototube (H), causing a voltage to appear across a load resistor. Voltage is amplified and registered on the null meter. Courtesy of Beckman Instruments, Inc., Fullerton, Calif.

1.8 SPECTROPHOTOMETERS AND COLORIMETERS

A spectrophotometer is an instrument which is capable of isolating "monochromatic" radiation. The desired wavelength is isolated by using a prism or grating and auxiliary mirrors and slits which, collectively, form the monochromator of the instrument. The wavelength dial on a spectrophotometer is set to a specific value, but the radiation leaving the exit slit is rarely monochromatic. However, some of the more sophisticated instruments can isolate, at the exit slit, a band of energy which is 1 mμ or less in width.

A colorimeter or filter photometer isolates several wavelengths of radiant energy by using a filter. Three or four filters are supplied with each instrument and each filter will pass a maximum of light at certain specified wavelengths. These filters isolate "polychromatic" radiant energy. However, both terms—that is, "monochromatic" and "polychromatic"—have real meaning only when the characteristics of the dispersing device are clearly stated. For example, a colorimeter equipped with an interferometric filter will isolate a bandwidth of 10 to 20 mμ. The Spectronic 20, a grating-type instrument with fixed slits, produces a band pass of 20 mμ.

All instruments must be equipped with a radiation source, a device for isolating the desired wavelength, a container or cell for the solution to be examined, and a detector of radiant energy. Diagrams of the optical systems of several of the more common instruments are shown in Figs. 1.10, 1.12, 1.13, and 1.14.

A. RADIATION SOURCES

The radiation source must meet three requirements:

a. The radiation should be continuous, that is, its spectrum should include all of the wavelengths required for the analysis.

b. The power of the beam should be such that the solution will transmit, under normal circumstances, some or all of the radiant energy at all wavelengths.

c. The source should be stable. It should be obvious that the power of the beam must remain constant throughout the measurement. If the solvent is subjected to more radiant energy (P_0 at the detector) than the solution (P at detector), the absorbance value has little meaning. Most instruments are now equipped with regulators to prevent fluctuations in beam intensity, and some instruments are so designed that P_0 and P are measured simultaneously.

Shorter wavelengths of radiant energy are emitted as the temperature of the radiation source is increased. Ultraviolet radiant energy can be obtained from a tungsten lamp by increasing voltage. However, the lamp would burn out quickly and, for this reason, the better spectrophotometers are equipped with dual sources.

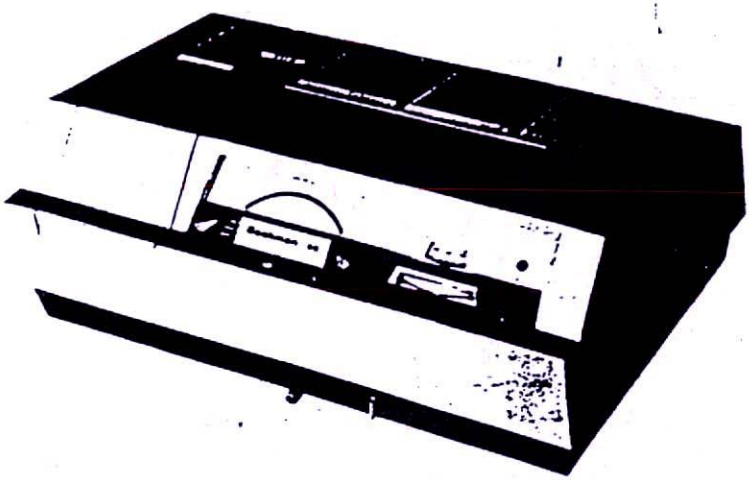


FIGURE 1.11: A Beckman model DB spectrophotometer. Courtesy of Beckman Instruments, Inc., Fullerton, Calif.

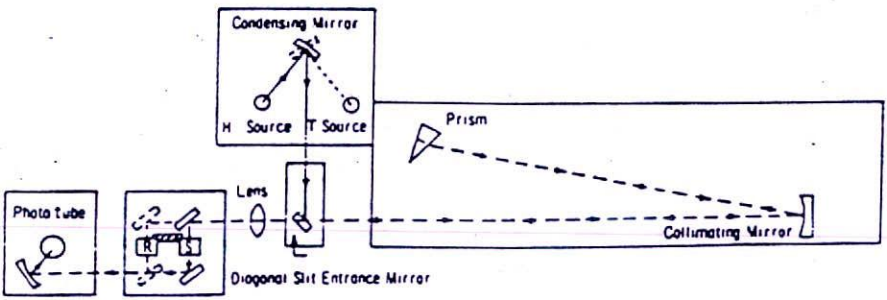


FIGURE 1.12: Schematic diagram of the Beckman model DB spectrophotometer. Light from the source is focused by the condensing mirror and directed to the monochromator diagonal mirror. This mirror directs the light through the entrance slit into the monochromator to the collimating mirror. Light falling on the collimating mirror is collimated and reflected to the prism, where it undergoes refraction. The back surface of the prism is aluminized so that light refracted at the first surface is reflected back through the prism, undergoing further refraction as it emerges from the prism. The desired wavelength is selected by rotating the wavelength control. The spectrum is directed back to the collimator, which centers the chosen wavelength on the exit slit. This light is directed alternately through the sample and reference path by the vibrating mirror. Courtesy of Beckman Instruments, Inc., Fullerton, Calif.

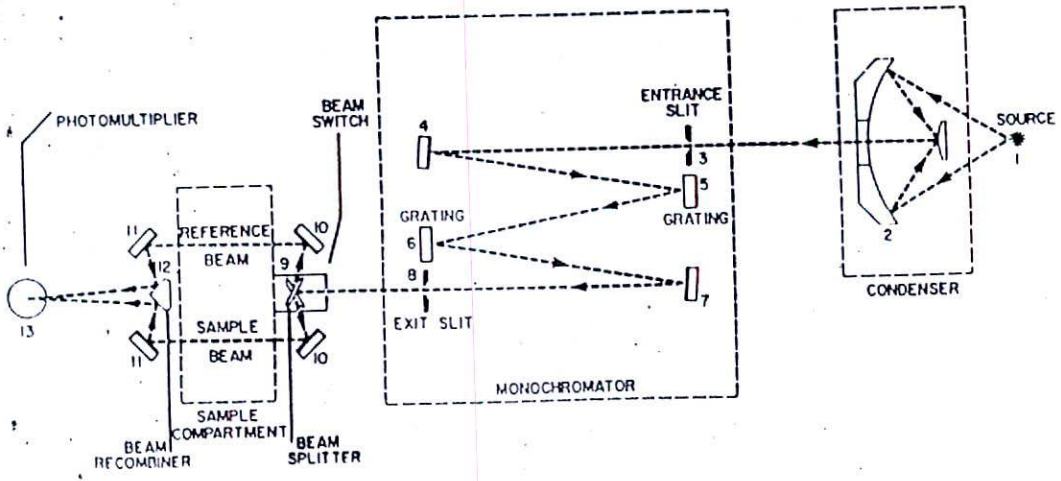


FIGURE 1.13: Schematic Diagram of the Spectronic 505. The beam from the source (1) enters the condenser (2), which focuses a ten-times-enlarged, achromatic image of the lamp filament on the entrance slit (3) of the double grating monochromator. The beam passes through the slit to a collimating mirror (4), is reflected to the first reflectance grating (5) where it is dispersed, then to a second reflectance grating (6) where it is further dispersed. The twice-dispersed beam is reflected from the second collimating mirror (7), passes through the monochromator exit slit (8), and strikes the beam splitter (9) where it is divided into reference beam and sample beam. The two beams are alternately chopped by the rotating beam switch and reflected by mirrors (10) through the sample and reference cells. Leaving the cell compartment, the unabsorbed light from the two beams is reflected by mirrors (11) to the beam recombiner (12) and brought back into coincidence on the photomultiplier (13). The alternate sample and reference beams give electrical pulses proportional to the intensity of the beams. Courtesy of Bausch & Lomb Inc., Rochester, N.Y.

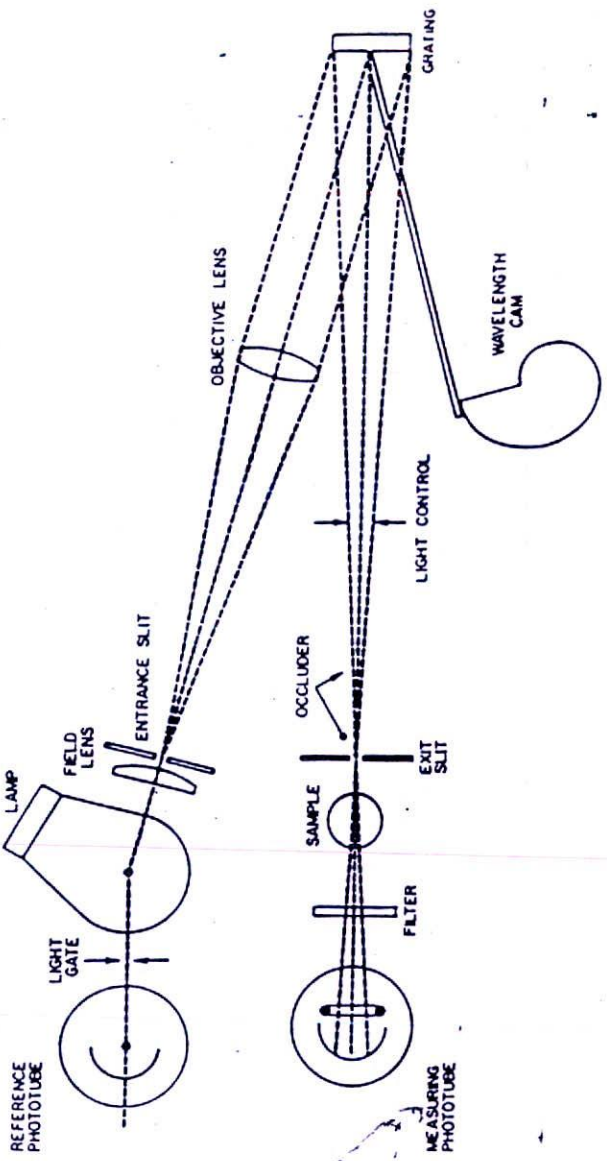


FIGURE 1.14: Schematic Diagram of the Spectronic 20. Courtesy of Bausch & Lomb, Inc., Rochester, N.Y.

Sources of Visible Radiation. In the visible region of the spectrum, the source is an electric light bulb with an incandescent filament. The lamp may be operated from a storage battery or by a 110-V source. If a 110-V source is used, a constant voltage transformer should be used to stabilize lamp output. The tungsten lamp emits radiant energy in the region between 350 and 2500 mμ. Its relative intensity decreases as the wavelength decreases.

Sources of Ultraviolet Radiation. A hydrogen discharge lamp consists of a pair of electrodes in a glass tube with a quartz window. The lamp contains hydrogen gas at a reduced pressure. When a voltage is applied to the electrodes, the hydrogen molecules are excited and produce radiant energy between 185 and 365 mμ.

Both the Beckman DB and DU-2 spectrophotometers are equipped with both hydrogen and tungsten lamps. The Spectronic 505, however, is equipped with a combination light source. This contains a tungsten lamp, a mercury lamp (for wavelength calibration), and a deuterium lamp. The latter lamp contains deuterium gas and is approximately three times brighter than a hydrogen lamp of comparable design and wattage. The Spectronic 505 is a grating type instrument with fixed slits. The bandwidth is equal to 5 Å. In an instrument with variable slits, the slit width is increased if there is not enough energy at the detector to produce an instrument reading. In the Spectronic 505, the output of the source must be increased in order to accomplish the same purpose.

B. FILTERS AND MONOCHROMATORS

The desired wavelength can be isolated by using either a filter or a monochrometer.

Filters. Filters may be made of glass, gelatin, or of two thin semitransparent metallic films separated by a thin film of cryolite or other dielectric material.

Glass Filters. Colored glass filters will transmit certain wavelengths of light, that is, each filter has a characteristic absorption spectrum. The filter is made by incorporating the oxides of certain metals into the glass. For example, cobalt produces a blue filter, manganese, a purple filter, and iron, a green filter.

The filter must be matched to the solution being analyzed. For example, if the colored solution absorbs a maximum of energy at 550 mμ, the filter must transmit a maximum of energy at that wavelength. It is difficult, if not impossible to match transmittance maxima with absorption maxima. The effective bandwidth varies from filter to filter, but some glass filters will transmit a band whose width approaches 150 mμ. Some filters have bandwidths of 25 to 50 mμ, but these transmit no more than 25% of the radiant

energy emitted by the source. Little energy reaches the solution (and less, the detector) and, for this reason, some analyses cannot be carried out by using filter photometers.

Gelatin Filters. A gelatin filter is made of two pieces of glass separated by a thin sheet of dye suspended in gelatin. The bandwidth of the filter may be as much as 50 mμ. Gelatin filters are not as stable as glass filters.

Interferometric Filters. Narrow bandwidths (10 to 20 mμ) can be obtained by using an interferometric filter. The filter consists of two thin semi-transparent metallic films separated by a thin film of dielectric material. When light strikes the filter, it is partially reflected by the first metallic layer. The portion that is transmitted passes through the transparent dielectric material and is then partially reflected by the second metallic surface. If this reflected portion is of the right wavelength, it will be partially reflected from the first surface in phase with light of the same wavelength that is entering the surface at this point. That particular wavelength is, therefore, reinforced. All other wavelengths interfere with each other.

The wavelength at which an interference filter passes light can be calculated from the following equation.

$$n\lambda = 2t \tag{1.47}$$

The thickness of the dielectric material is equal to t, and n is an integer. For example, if the thickness of the transparent material is equal to 500 mμ and n is equal to 1, λ is 1000 mμ. If n is equal to 2, λ is 500 mμ.

Monochromators. A monochromator resolves polychromatic radiation into its component wavelengths and focuses several of these wavelengths onto the solution in the cell. Radiation enters the monochromator through an entrance slit, is collimated with a lens or mirror, dispersed by a prism (or grating), returned to a lens or mirror, and focused upon an exit slit. The entrance and exit slits may be one and the same thing or may be separate entities.

The effective bandwidth striking the solution depends upon the nature of the dispersing device, the wavelength, and on the widths of the entrance and exit slits. In a prism spectrophotometer, the dispersion is nonlinear with wavelength. This means that any given slit width will pass a much wider band of wavelengths in the higher than in the lower wavelength regions. In a grating instrument, dispersion is very nearly a linear function of wavelength. Any given slit width will, therefore, pass nearly the same band of wavelengths in all regions.

The Prism. Polychromatic light can be dispersed with a prism. The velocity of light in a medium depends upon the refractive index of that medium.

$$v = c/n \tag{1.48}$$

where v is the velocity of light in the medium, n is the refractive index, and

c is the velocity of light in vacuo. For example, the velocity of light in glass ($n = 1.5$) is $\frac{2}{3}$ the velocity in vacuo. Because its velocity depends upon the medium, light will be refracted and reflected when it passes from one isotropic medium to another isotropic medium. However, refraction occurs only when the refractive indices of the two media are different.

Snell's law equates the angle of incidence θ to the angle of refraction θ'.

$$n \sin \theta = n' \sin \theta' \tag{1.49}$$

The angle of refraction depends, therefore, upon the angle of incidence, the refractive indices of the two media (n and n'), and, last, upon the wavelength of the radiant energy. This means that the shorter wavelengths are bent to a greater extent than are the wavelengths in the upper regions of the spectrum. With white light, this dispersion results in the usual visible spectrum.

Many spectrophotometers are equipped with a 30° Littrow prism with a reflecting back. Light enters and emerges from the same face and, in effect, produces the same dispersion as that which occurs with a 60° Cornu prism. Moreover, any birefringence within the prism is cancelled out since light passes in both directions. The lens in a monochromator of this type serves as the collimator and the objective lens. The instrument is so designed that the entering and refracted rays travel the same path except that one lies above the other.

Glass prisms absorb ultraviolet radiant energy. Ultraviolet spectrophotometers are, therefore, equipped with quartz prisms. The useful range of the latter prism is from 180 to 4000 mμ; of the former, from 350 to 2000 mμ.

The Grating. Polychromatic light can also be dispersed with a transmission or a diffraction grating. Grating spectrophotometers are usually equipped with diffraction gratings.

A diffraction grating consists of a large number of parallel, equidistant lines ruled on a polished surface. Each inch of grating may have as few as 5000 and as many as 50,000 parallel grooves. For example, the Spectronic 20 is equipped with a grating which has 600 grooves/mm. The Spectronic 505, however, is equipped with two gratings with 1200 grooves/mm.

A grating may be visualized as a plane or concave surface with a large number of parallel equidistant slits. Each illuminated slit acts as a source. At any angle θ', and at some distance greater than x, the distance between the centers of two adjacent slits, the path difference between rays coming from these slits is equal to $x \sin \theta'$. If the path difference is equal to an integral number of wavelengths, reinforcement of the radiation will occur. At any other angle, destructive interference occurs. The latter statements assume parallel perpendicular incident radiation and an infinite number of slits. The mathematical relationship between the quantities involved is given in Eq. (1.50).

$$x(\sin \theta' + \sin \theta) = \pm m\lambda \tag{1.50}$$

θ' is the angle of diffraction, θ is the angle of incidence, and m is an integer. Spectra with m equal to $\pm 1, \pm 2, \ldots$, are called first-, second-, . . . , order spectra. Each value of m gives rise, therefore, to a spectrum. If m is equal to zero, white light will be undispersed. However, with each succeeding value of m, dispersion occurs. The grooves can be shaped in such a way that as much as 80 to 90% of the diffracted radiant energy is concentrated into a specified order.

This brief description of gratings may be supplemented by the information in Meehan's treatise on optical methods.[4] A grating monochromator has certain advantages over those that disperse radiation with prisms. Dispersion is linear, and construction of recording spectrophotometers is simplified because prism instruments require complex cam arrangements. However, prism instruments give slightly better dispersion in the 200- to 250-mμ range.

C. ABSORPTION CELL

The solution which is subjected to radiant energy is held in a transparent cell or cuvette. This cell is an integral part of the instrument's optical system and must be kept scrupulously clean at all times. Cells are usually washed with distilled water or a mild sulfonic detergent solution. If a stronger cleaning agent is required, the cells may be soaked in a 50:50 solution of 3 N HCl and alcohol.

The optical windows of cuvettes are made from silica (for measurements in the ultraviolet and visible regions) or pyrex (for measurements in the visible region). Cuvettes are available in all shapes and sizes. However, the most common size is the 1-cm cell which holds between 5 and 6 ml of solution. Standard cells are usually sold in pairs. Most analysts mark one of the cells of the set and reserve it for solvent only. The other cell is filled with the solution being analyzed. Cells are usually matched to within 2% T. The manufacturers' specifications are acceptable for most purposes, but it may be necessary, at times, to correct for differences in cell length.

D. RADIATION DETECTORS

Phototubes, photomultiplier tubes, and barrier layer (photovoltaic) cells may be used to detect and measure radiant energy. Radiant energy must be converted into electric energy, and the signal produced by the detector must be directly proportional to the amount of radiant energy striking it.

The Phototube. A phototube consists of a concave photoemissive surface (the cathode) and a wire anode. The cathode and the anode are enclosed in an evacuated tube. When radiant energy strikes a cathode coated with potassium, cesium, or an alkaline-earth oxide, electrons are emitted and accelerated toward the anode. However, a potential must be applied (90 V

for most phototubes) in order to achieve maximum collection of electrons at the anode. If the applied potential is sufficiently high, the current produced is proportional to the amount of energy reaching the detector. The current produced by the phototube must be amplified before the actual measurement is made. Phototubes emit electrons even if they are in the dark (thermal emission of electrons) and a small current will flow. This is compensated for in most instruments by a "dark current" control.

Most spectrophotometers are equipped with two phototubes. The blue-sensitive phototube responds to ultraviolet and visible energy (to about 600 mμ) and the red-sensitive phototube detects energy of longer wavelengths.

The Photomultiplier Tube. A photomultiplier tube consists of a photosensitive cathode, an anode, and a number of other electrodes called "dynodes." Each dynode functions both as a cathode and an anode—a cathode for the dynode ahead of it and an anode for the dynode behind it. This is brought about by maintaining each dynode at a potential somewhat more positive than that of the preceding electrode.

When the electrons from the cathode strike the first dynode, several additional electrons are liberated. These, in turn, strike the next dynode and again liberate additional electrons. This process produces a cascade of 10^6 or more electrons at the anode.

Standard spectrophotometers are not normally equipped with photomultiplier tubes, but they can be readily installed if the analyst finds it necessary to detect and measure minimal amounts of radiant energy.

The Barrier Layer Cell. A barrier layer cell consists of an iron or copper plate upon which is deposited a layer of selenium or cuprous oxide. This semiconducting material is covered with a transparent film of gold, lead, or silver. The metallic film serves as the collector electrode. The plate, the semiconducting material, and the metallic film are enclosed in a transparent envelope.

The electrons in selenium or cuprous oxide are not mobile under normal circumstances. However, when light strikes the cell, electrons are liberated, penetrate the interface or barrier between the semiconducting material and the metallic film, and are, in this way, transferred to the collector electrode. If the metallic film is connected by way of an external circuit to the plate, a current, which is proportional to the amount of light striking the cell, will flow. This current (10 to 100 mA) can be measured with a galvanometer or microammeter.

The barrier layer cell is used to detect and measure visible radiation. The sensitivity of the cell is similar to that of the human eye, that is, it detects radiation best in the 500- to 600-mμ region. However, its sensitivity is poor at low levels of illumination and, if used for prolonged periods of time, it exhibits fatigue. Under such circumstances, the transmittance reading will decrease with time. This can be rectified by keeping the cell in the dark.

The cell is cheap, rugged, and requires no external source of electric energy. The inexpensive colorimeters are usually equipped with detectors of this type.

E. ACCURACY OF WAVELENGTH AND ABSORBANCE SCALES

The absorbance-transmittance and the wavelength scales of the better spectrophotometers are reasonably accurate. However, the wise analyst calibrates the instrument before carrying out crucial measurements.

The Wavelength Scale. This scale may be checked by inserting a holmium oxide filter into the sample beam, recording absorbance values at various

TABLE 1.8: Absorbance and Transmittance Values for Standard Potassium Chromate Solution[a]

Wavelength, mμ	T	A	Wavelength, mμ	T	A
210	0.000	—	330	0.715	0.145
215	0.037	1.432	335	0.605	0.218
220	0.350	0.456	340	0.485	0.314
225	0.600	0.222	345	0.380	0.420
230	0.680	0.168	350	0.280	0.553
235	0.620	0.208	355	0.202	0.695
240	0.509	0.293	360	0.148	0.830
245	0.408	0.389	365	0.115	0.939
250	0.319	0.496	370	0.102	0.991
255	0.268	0.572	375	0.103	0.987
260	0.232	0.635	380	0.118	0.928
265	0.201	0.697	385	0.152	0.818
270	0.180	0.745	390	0.207	0.684
275	0.173	0.762	395	0.300	0.523
280	0.189	0.724	400	0.410	0.387
285	0.254	0.595	404.7	0.520	0.284
290	0.372	0.430	410	0.635	0.197
295	0.527	0.278	420	0.748	0.126
300	0.705	0.152	430	0.824	0.084
305	0.830	0.081	435.8	0.861	0.065
310	0.900	0.046	440	0.884	0.054
315	0.905	0.043	450	0.928	0.033
320	0.867	0.062	460	0.961	0.017
325	0.810	0.092	470	0.981	0.008
			480	0.992	0.004
			490	0.998	0.001
			500	1.000	0.000

[a] Dissolve 0.0400 g of potassium chromate in sufficient 0.05 N potassium hydroxide to make 1 liter of solution. The solution is measured in a 1-cm cell. (Exact absorbance values, that is, to four decimal places, may be found in Refs. 17 and 54. This table is reproduced through the courtesy of the National Bureau of Standards, Washington, D.C.)

wavelengths, and comparing the position of absorption bands with standard values. The holmium oxide filter exhibits absorption maxima at 279.3, 287.6, 333.8, 360.8, 418.5, 536.4, and 637.5 mμ.

The best single source of visible and ultraviolet energy for wavelength calibration is the quartz-mercury arc. The lines at 253.7, 302.25, 313.16, 334.15, 365.48, 404.66, and 435.83 mμ may be used to check the wavelength scale. The 486.13- and the 656.28-mμ lines of the hydrogen lamp may be used for the same purpose.

The Absorbance Scale. The spectral characteristics of copper sulfate, cobalt ammonium sulfate, and potassium chromate solutions have been determined by the National Bureau of Standards.[17] These solutions can be used to check the absorbance (or transmittance) scale of the spectrophotometer. Absorbance and transmittance values, at various wavelengths, are given for potassium chromate in Table 1.8.

QUESTIONS

Q1.1. If the absorbance value, at 275 mμ, for the standard potassium chromate solution (see Section 1.8E) is 0.762, what is the transmittance value for a solution containing 0.03 g potassium chromate per liter of solution?

Q1.2. Prove mathematically that

$$\alpha = \frac{\epsilon' - \epsilon''}{\epsilon' - \epsilon''}$$

See Eq. (1.24). *Hint:* Divide an absorption cell into two compartments. The first compartment contains the ionic form and the second the molecular form of the acid. If the incident radiant power is P_1, the intensity of the beam entering the second compartment is P_2 and P_3 is the intensity of the beam leaving the cell. The total transmittance of the cell is equal to P_3/P_1. For the first compartment,

$$P_2 = P_1 e^{-\epsilon' bc\alpha}$$

or

$$-\log P_2/P_1 = \epsilon' bc\alpha$$

where ϵ' is the molar absorptivity of the ionic form and α is the fraction of acid that has ionized. Continue.

Q1.3. If the absorbance value for a solution in a 1-cm cell is 0.210, what is the transmittance value for the same solution in a 2-cm cell? What is the transmittance value in a 4-cm cell?

Q1.4. Absorptivity values for drugs X and Y at two different wavelengths are:

	$a_{340\ m\mu}$	$a_{375\ m\mu}$
X	60	0
Y	20	40

A mixture of X and Y contains twice as much X as Y. Suggest a method of analysis for the mixture and give the necessary equations.

Q1.5. A substance absorbs a maximum of radiant energy at 350 mμ. What are the frequency and wave number values at this wavelength?

Q1.6. How would you determine barbiturates spectrophotometrically? Can barbiturates be resolved spectrophotometrically? If so, how? (See Section 1.7B.)

Q1.7. An analyst is given a solution which is purported to contain drugs X and Y. He prepares a solution of X and one of Y and measures absorbances at 250 mμ, the absorption maximum for X, at 265 mμ, an isoabsorptive point, and at 290 mμ, the absorption maximum for Y.

Solution	$A_{250\ m\mu}$	$A_{265\ m\mu}$	$A_{290\ m\mu}$
10 mg X/liter	0.720	0.400	0.150
9 mg Y/liter	0.090	0.360	0.468
Unknown	0.612	0.340	0.128

Calculate the amounts of X and Y in the unknown solution.

Q1.8. Criticize the simultaneous equation method for the analysis of binary mixtures.

Q1.9. Criticize the absorbance ratio method for the analysis of binary mixtures.

Q1.10. What type of source would you recommend for a recording spectrophotometer with narrow fixed slits? Why?

Q1.11. Look up Refs. 40 and 41 and describe in detail the procedure for the determination of sulfonamides.

Q1.12. An analyst is asked to analyze phenylbutazone tablets NF. The tablets are purported to contain 100 mg of drug per tablet. The analyst weighs 20 tablets (4.2010 g) and reduces them to a fine powder. A 202.6-mg portion of the powdered tablets is extracted with alcohol, the solution is filtered, and the filtrate is made to 100.0 ml with alcohol. A 10.0-ml aliquot of this solution is diluted to 1 liter with 0.1 *N* sodium hydroxide solution. The absorbance value for the solution is 0.622. The analyst searches the literature and finds that Pernarowski has reported an absorptivity value of 66 for this drug. All measurements were carried out in a 1-cm cell.

a. Calculate the number of milligrams of drug in a tablet of average weight.

b. Does the brand comply with NF limits for phenylbutazone tablets?

c. Criticize the analyst's procedure.

REFERENCES

1. J. H. Yoe, *Anal. Chem.*, 29, 1246 (1957).
2. D. F. Boltz and M. G. Mellon, *Anal. Chem.*, 38, 317R (1966).
3. W. Crummett, *Anal. Chem.*, 38, 404R (1966).
4. E. J. Meehan, *Optical Methods of Analysis*, Wiley, New York, 1964.
5. A. Weissberger, (ed.), *Physical Methods of Organic Analysis*, Part III, Wiley (Interscience), New York, 1960.
6. M. G. Mellon, *Analytical Absorption Spectroscopy*, Wiley, New York, 1950.
7. L. M. Vandenbelt and L. Doub, *J. Am. Chem. Soc.*, 69, 2714 (1947).

8. I. Sunshine and S. R. Gerber, *Spectrophotometric Analysis of Drugs*, Thomas, Springfield, Ill., 1963.
9. H. G. Pfeiffer and H. A. Liebhafsky, *J. Chem. Educ.*, **28**, 123 (1951).
10. N. T. Gridgeman, *Photoelec. Spectrometry Group Bull.*, **4**, 67 (1951).
11. N. T. Gridgeman, *Anal. Chem.*, **24**, 445 (1952).
12. J. R. Edisbury, *Photoelec. Spectrometry Group Bull.*, **5**, 109 (1952).
13. *The National Formulary, Twelfth Edition*, Mack Printing, Easton, Pa., 1965, p. 490.
14. *The National Formulary, Twelfth Edition*, Mack Printing, Easton, Pa., 1965, p. 495.
15. G. Kortum and M. Seiler, *Angew. Chem.*, **52**, 687 (1939).
16. A. R. Rogers, *J. Pharm. Pharmacol.*, **11**, 291 (1959).
17. United States Department of Commerce, *Nat. Bur. Std., LC484*, 40 (1949).
18. E. E. Sager, M. R. Schooley, A. S. Carr, and S. F. Acree, *J. Res. Natl. Bur. Std.*, **35**, 521 (1945).
19. R. B. Tinker and A. J. McBay, *J. Am. Pharm. Assoc. Sci. Ed.*, **43**, 315 (1954).
20. P. J. Neibergall and A. M. Mattocks, *Drug Std.*, **28**, 61 (1960).
21. A. L. Glenn, *J. Pharm. Pharmacol.*, **15**, S123 (1963).
22. L. Heilmeyer, *Spectrophotometry in Medicine*, Adam Hilger Limited, London, 1943, p. 65.
23. *United States Pharmacopeia*, Mack Printing, Easton, Pa., 17th rev. ed., 1965.
24. M. Pernarowski, A. M. Knevel, and J. E. Christian, *J. Pharm. Sci.*, **50**, 943 (1961).
25. M. Pernarowski, A. M. Knevel, and J. E. Christian, *J. Pharm. Sci.*, **51**, 688 (1962).
26. A. L. Glenn, *J. Pharm. Pharmacol.*, **12**, 595 (1960).
27. M. Pernarowski, A. M. Knevel, and J. E. Christian, *J. Pharm. Sci.*, **50**, 946 (1961).
28. F. Yokoyama and M. Pernarowski, *J. Pharm. Sci.*, **50**, 953 (1961).
29. W. A. Wallis and H. V. Roberts, *Statistics: A New Approach*, Free Press, New York, 1956, p. 252.
30. J. W. Miles and D. T. Englis, *J. Am. Pharm. Assoc. Sci. Ed.*, **43**, 589 (1954).
31. *British Pharmacopoeia 1963*, Pharmaceutical Press, London, 1968, p. 20.
32. E. F. Degner and L. T. Johnson, *Anal. Chem.*, **19**, 330 (1947).
33. *British Pharmacopoeia 1963*, Pharmaceutical Press, London, 1968, p. 560.
34. *The National Formulary, Twelfth Edition*, Mack Printing, Easton, Pa., 1965, p. 458.
35. *United States Pharmacopeia*, 17th rev. ed., Mack Printing, Easton, Pa., 1965, p. 879.
36. M. G. Mellon, *Anal. Chem.*, **24**, 924 (1952).
37. F. J. Bandelin, E. D. Slifer, and R. E. Pankratz, *J. Am. Pharm. Assoc. Sci. Ed.*, 39, 277 (1950).
38. *The National Formulary, Twelfth Edition*, Mack Printing, Easton, Pa., 1965. p. 42.
39. *The National Formulary, Twelfth Edition*, Mack Printing, Easton, Pa., 1965, p. 462.
40. *The National Formulary, Twelfth Edition*, Mack Printing, Easton, Pa., 1965, p. 500.
41. K. A. Connors, *Am. J. Pharm. Ed.*, **29**, 29 (1965).
42. E. W. Martin (ed.), *Remington's Pharmaceutical Sciences*, Mack Printing, Easton, Pa., 1965, p. 1578.
43. C. N. Reilley and C. M. Crawford, *Anal. Chem.*, **27**, 716 (1955).
44. R. Bastian, R. Weberling, and F. Palilla, *Anal. Chem.*, **22**, 160 (1950).
45. C. F. Hiskey, *Anal. Chem.*, **21**, 1440 (1949).
46. C. F. Hiskey, J. Rabinowitz, and I. G. Young, *Anal. Chem.*, **22**, 1464 (1950).
47. C. F. Hiskey and I. G. Young, *Anal. Chem.*, **23**, 1196 (1951).
48. J. J. Lothe, *Anal. Chem.*, **28**, 949 (1956).
49. L. A. Williams and B. Zak, *Clin. Chim. Acta*, **4**, 170 (1959).
50. J. C. Demetrius and J. E. Sinsheimer, *J. Am. Pharm. Assoc. Sci. Ed.*, **49**, 523 (1960).
51. J. L. Casinelli and J. E. Sinsheimer, *J. Pharm. Sci.*, **51**, 336 (1962).
52. R. F. Goddu and D. N. Hume, *Anal. Chem.*, **26**, 1679 (1954).
53. T. Higuchi, C. Rehm, and C. Barnstein, *Anal. Chem.*, **28**, 1506 (1956).
54. United States Department of Commerce, *Natl. Bur. Std., LC1017* (1955).