UV SPECTRAL PROPERTIES OF PHENYLALANINE POWDER

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ABSTRACT A technique is described which makes it possible to measure the absorption spectrum of phenylalanine powder. Reflectance spectra are used to calculate the amount of light scattered from the samples; thus, the treatment of the data makes it unnecessary to normalize the absorbance to some value known from other work. Further, the unique scattering in the spectral region of an absorption band can be found. The fluorescence quantum yield of phenylalanine powder at 300K is calculated to be 1.0 ± 0.1, a sizeable increase over the value of 0.04 found for aqueous phenylalanine solutions.

INTRODUCTION

Investigations of the interaction of radiation with condensed systems are especially important to radiation biology for two reasons. First, theories based on studies in gaseous systems are not always applicable to condensed biochemical materials. For example, the classical "one-ionization" model does not appear to explain much of the data on inactivation of proteins in the dry state (1). Second, the interaction of excited states should be much greater in condensed systems than in gases.

Light absorption by solids has been studied previously by several techniques. For example:

(*a*) Preiss and Setlow reported the absorption spectra in the near and vacuum UV region for a few amino acids and proteins. The samples were obtained as powder layers by drying saturated solutions on CaF_2 slides (2).

(*b*) Tanaka utilized a microspectrophotometer to measure the UV absorption spectra of small crystals of substituted benzenes (3).

(*c*) Ley and Arends compared the difference in absorption between two glycine crystals of different thicknesses placed on an aperture directly in front of a photomultiplier tube; thus, reflected light from the face of both crystals should have been the same (4).

(*d*) Jacobs *et al.* measured the UV absorption of ethyl chlorophyllide microcrystals in an acetone—H_2O suspension (5).

(*e*) Biochemicals have been sublimed onto quartz slides under conditions of low pressure and high temperature for absorption studies (6).

(*f*) Under certain conditions the absorption spectrum of very fine powders can be inferred from the reflectance spectrum (7).

In addition, the absolute and relative fluorescence quantum yields of organic crystals at ~300K have been measured. For instance, Wright used the lifetimes and emission spectra of fluorescence from very small and very large crystals to determine the quantum yields of anthracence, p-terphenyl, and trans-stilbene (8). Kristianpoller found the quantum yield of solid sodium salicylate without determining the absorption or emission spectra (9). Much earlier work has been reviewed by Pringsheim (10). (See also Hochstrasser, 11).

In some of the above methods the interpretation of the data is complicated by the possible destruction of crystallinity during preparation of the samples (*e.g.*, sublimation), or by changes in scattering owing to non-uniformity of sample surfaces, or by the simultaneous occurrence of "crystal" and "solution" spectra. Usually spectral measurements made from solid material are normalized to some region of the absorption spectrum for the same compound in solution. This is done to correct for the large fraction of the incident light which is reflected by solids. Unfortunately, however, reflectance in the vicinity of an absorption band is frequently difficult to determine at the appropriate scattering angle.

The present paper describes a technique which minimizes many of the above difficulties and makes it possible to measure separately the absorption, reflection, and emission spectra and the quantum yield of powders such as phenylalanine. The only requirement on the powder is that a layer with maximum optical density of about unity can be obtained.

EXPERIMENTAL MATERIALS AND METHODS

Grade A DL-phenylalanine from CalBioChem, Los Angeles, was recrystallized from water to give plate-like crystals prior to being ground to a powder and sifted. Particles of minor dimension 44 to 74 μ were spread onto a water surface; after a brief period of equilibration part of the powder film was quickly "scooped" off the water surface with a quartz microscope slide and dried at 50°C. Successive layers could be deposited if the slide were thoroughly dried between applications and the "dipping" process was completed quickly. We worked only with single layers, however, since subsequent layers are not deposited as uniformly.

Absorption measurements were made with a Cary Model 15 recording spectrophotometer having a diffuse reflectance attachment. The emission measurements were made with an Aminco (American Instrument Co., Silver Spring, Maryland) spectrophosphorimeter equipped with a "front-face" cell (12), where the emission stimulated at the surface of the sample facing the incident radiation is measured almost exclusively.

Absorption and emission from a solution of quinine sulfate (from Matheson, Coleman, and Bell Chemical Co., Cincinnati, Ohio) were measured in a cell made by sandwiching 50 μ Teflon strips (the smallest practical size) between two quartz slides; the edges were

sealed with paraffin. Even though the phenylalanine powder layers were only about 3 μ thick, the difference between this and the 50 μ thick standard solution is negligible in terms of the geometry of the system.

METHOD OF ANALYSIS

Absorption and Emission. Let T be the ratio of light detected by the two photomultipliers "seeing" the sample and blank respectively regardless of the measurements being made (see below).

Further let A_λ = the fraction of light absorbed by the sample at any given wavelength λ; B_λ = the fraction of light scattered in a backward direction from the sample (*i.e.*, 90° to 180° from the path of the incident beam). F_λ = the fraction of light scattered in a forward direction from the sample (*i.e.*, 0° to 90° from the path of the incident beam).[1]

By placing the phenylalanine covered slides at the position normally occupied by the cuvette in the spectrophotometer (see Fig. 1.1) the fractional transmission at any wavelength λ is given by

$$T_{1,\lambda} = 1 - A_\lambda - B_\lambda - F_\lambda \qquad [1]$$

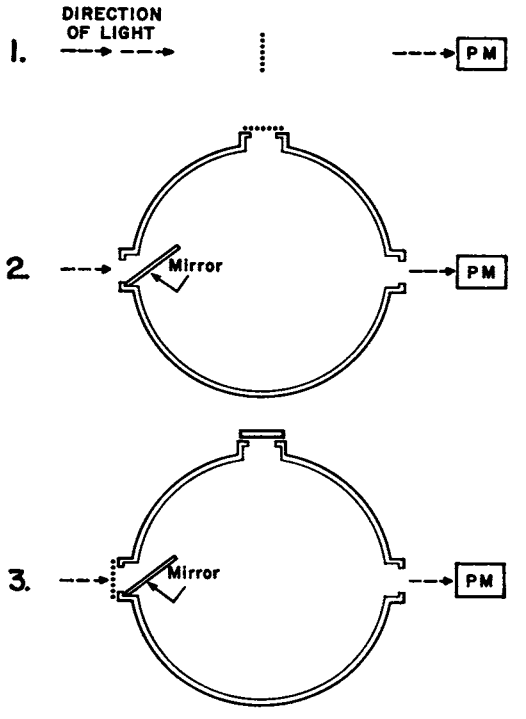

FIGURE 1 Schematic diagram showing arrangement of the sample (. . . .) with respect to the reflectance sphere and the photomultiplier tube (PM).

[1] Since the slit in front of the photomultiplier tube subtends only about 10^{-9} radians of arc, presumably only a small fraction of the light scattered forward is detected by the photomultiplier tube.

With the slide mounted on the sample port of the integrating reflectance sphere (see Fig. 1.2)

$$T_{2,\lambda} = B_\lambda \qquad [2]$$

If the sample port of the integrating reflectance sphere is covered with magnesium oxide (having a reflectivity of essentially unity) and the sample slide is placed directly in front of the integrating sphere (see Fig. 1.3)

$$T_{3,\lambda} = 1 - A_\lambda - B_\lambda \qquad [3]$$

The three linearly independent equations [1] through [3], can be solved at any wavelength for the three unknown quantities A_λ, B_λ, and F_λ since the values of T_1, T_2, and T_3 are measured continuously from $\lambda = 220$ to $\lambda = 300$ mμ. With the values thus obtained for A_λ the extinction coefficient ε_λ for phenylalanine powder can be computed as follows:

$$\epsilon_\lambda = \frac{\text{OD}}{10^3 \cdot \sigma} \qquad [4]$$

where OD is the optical density and σ is the surface density of the powder in moles per cm^2 (it is to be noted that different quantities are usually used for the denominator—*i.e.*, for worw with solutions surface density is usually specified as $(c \cdot d)$ where c is the concentration (moles/liter) and d is the path length (cm) through the solution). The average surface density can be measured by weighing a slide before and after application of the phenylalanine layer and dividing the increment in weight by the area covered.

Fluorescence Yields. Let A_s and A_q be the number of quanta absorbed by the sample and by a standard quinine sulfate solution, respectively, and E_s and E_q be the number of quanta emitted by the sample and by the standard when A_s and A_q are absorbed. Then the respective quantum yields are $Y_s = E_s/A_s$ and $Y_q = E_q/A_q$. If the optical density of the sample and the quinine sulfate standard are equal, then $A_s = A_q$ and

$$\frac{Y_s}{Y_q} = \frac{E_s}{E_q} \qquad [5]$$

The areas under emission curves, plotted on a cm^{-1} scale, were used to compute (E_s/E_q). A value of 0.55 was taken for Y_q, as given by Melhuish (13) for a solution in 0.1 N H_2SO_4.

RESULTS AND DISCUSSION

Absorption and Reflection Spectra. The absorption and reflection spectra of phenylalanine powder are given in Fig. 2; the absorption spectrum is also plotted on a molar extinction coefficient scale in Fig. 3. Since it was possible to solve explicitly for A_λ, it was unnecessary to "normalize" the absorption spectrum of the powder to some region of an absorption spectrum of phenylalanine in solution.

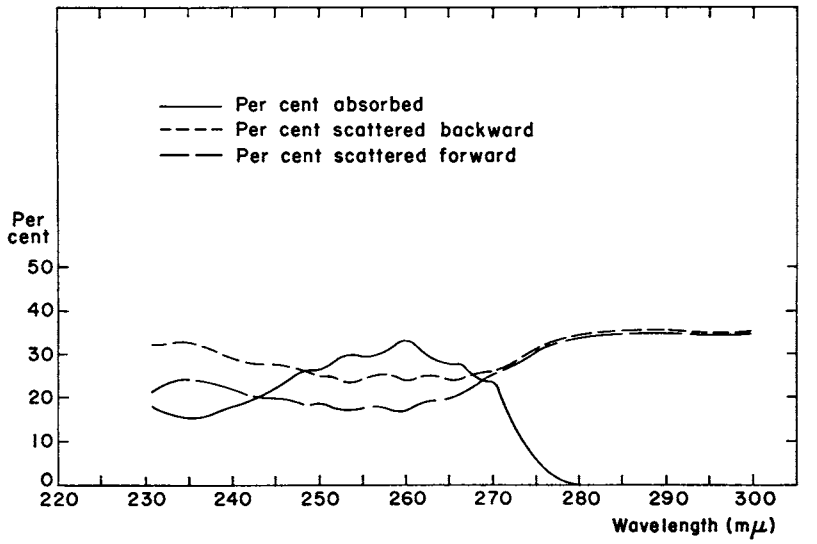

FIGURE 2 Absorption and reflection spectra for phenylalanine powder.

Thus, it is possible to test the accuracy of our method as follows: probably phenylalanine powder should not absorb appreciably in the wavelength region 280

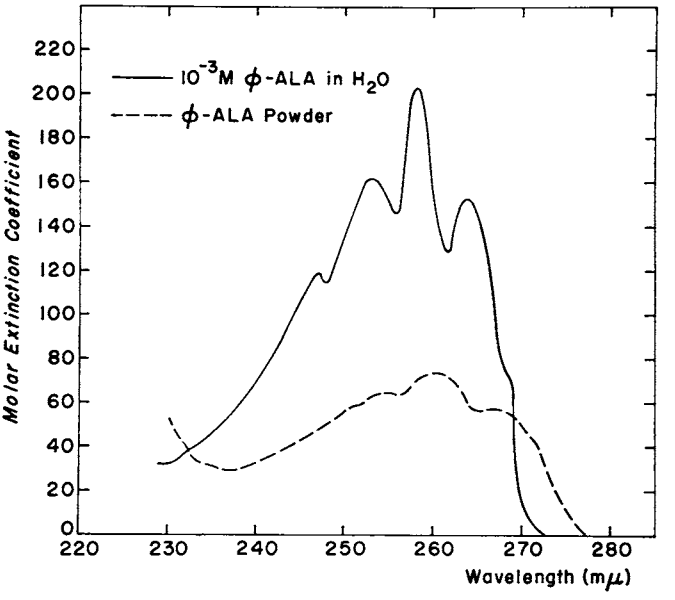

FIGURE 3 Absorbance spectra for phenylalanine powders and for an aqueous phenylalanine solution.

to 300 mμ since phenylalanine solution shows no absorption there. This expectation is borne out, the percentage absorption of phenylalanine powder being within 0.5 per cent of zero in this wavelength region (Fig. 2).

It appears to be important that about 60 per cent of the photons which do not reach the sample phototube are accounted for by scattering, not by absorption (see Fig. 2). Further, the amount of scattering changes by about half in the region of the absorption band from the value at 290 to 300 mμ, and is apparently not the same for all directions of scattering. These data serve to demonstrate the problems inherent in the "normalization" procedures often applied to absorption spectra of solids.

The values for $\varepsilon\lambda$ in Fig. 3 were calculated using an average surface density of 0.38 mg/cm^2 (=2.3 μM/cm^2). Assuming that the density of phenylalanine powder is 1.3 gm/cm^3 the average thickness of powder on the slide is 3 μ. Since the fraction of incident light transmitted through the sample is a logarithmic rather than linear function of the thickness of the powder layer, the possibility of errors induced by variations in film thickness is treated in the Appendix.

The absorbance of phenylalanine powder is compared in Fig. 3 with the corresponding spectrum of phenylalanine in a water solution. Three features of these spectra seem noteworthy:

(*a*) The vibrational structure in the powder spectrum is much less pronounced than in the solution spectrum. This is probably the result of lessened resolution; that is, only low light intensities are available when dealing with reflectance spectra so that larger slits are used.

(*b*) The maximum absorbance of the solid phenylalanine is shifted about 30 A (400 cm^{-1}) toward lower energies relative to the maximum absorbance of phenylalanine in solution. From the shapes of the two spectra, it seems likely that this is the result not of changed absorption in selected vibronic bands but an actual shift of the entire electronic band. Such band shifts are typically noted in solid state spectra (14).

(*c*) The absorbance of phenylalanine powder shows a marked hypochromicity (*i.e.*, decreased oscillator strength of the whole band) compared with the absorbance of phenylalanine in solution. Using the common equation for calculating the intensity of an absorption band (15), and integrating between the absorption minimum near 232 mμ and the long wavelength absorption limit in Fig. 3, the one dimensional oscillator strengths of phenylalanine in powder and in solution are found to be 1.35×10^{-3} and 2.70×10^{-3}, respectively.

The red shift and hypochromicity [features (b) and (c)] probably result from interactions within the crystalline environment. Even though both types of effects can be associated with exciton migration, the 250 mμ absorption band in phenylalanine is of such a nature that other possibilities must be considered. That is, this band corresponds in general shape and energy to a symmetry-forbidden band in

benzene (14, 16), so that the intensity of absorption by phenylalanine could be determined by at least two factors:

(*a*) Vibrations within the benzene ring can induce a small dipole moment by changing the symmetry of the ring.

(*b*) Likewise, the high degree of symmetry of benzene is destroyed by the substitution of a methyl (or other) radical for one of the hydrogen atoms (17).

In the first case the rate of energy transfer will probably be small inasmuch as band *splitting* effects, which lead to exciton migration, involve interaction energies of only 1 to 10 cm^{-1} in vibrationally-induced bands, whereas band *shifting* effects may involve perturbations of 100 to 1000 cm^{-1} but do not lead to energy transfer (14, 16). Nevertheless, McClure has pointed out that a band splitting of only 1 cm^{-1} may result in 1000 energy transfer acts during the lifetime of an excited state (16).

The second factor, substituent induction of an absorption band, appears to account for a somewhat smaller increase in absorption intensity than vibrational induction in the case of toluene (16). Because of the similarity of their chromophores, the situation in toluene is probably applicable to phenylalanine. Even so, it seems likely that energy transfer arising from substituent effects should be more efficient than that depending upon electronic-vibrational interactions. That is, identical symmetry distortions—which may be necessary for transfer—will not occur with high probability in pure benzene, whereas the substituent effect will be present in all phenylalanine molecules in a crystal (16, 17).

Fluorescence Yields. Five measurements of the fluorescence quantum yields gave values of 1.0 ± 0.1 for excitation at 250 mμ. It is probably realistic to attach somewhat larger uncertainty than ±0.1 to these measurements: the band width of the Aminco (using a slit width of 2mm) is about 100 A which is comparable to the width of the 250 mμ absorption band in quinine sulfate. Thus, accidental displacement of the exciting wavelength by only 5 mμ could change the fraction of quanta absorbed by the standard from 73 to 61 per cent.

The value of *ca.* 1.0 obtained from phenylalanine powder is at least an order of magnitude greater than the value of 0.04 for the fluorescence quantum yield of phenylalanine in solution reported by Teale and Weber (18) and duplicated by us. This situation has been observed in several kinds of organic molecules where yields increase markedly—approaching unity—in going from solution to the crystalline state (*e.g.*, 10). Such an enhancement probably depends upon at least three factors:

(*a*) Aggregation should restrict the movement of the individual molecules, and thus probably decrease collisional quenching (see 11).

(*b*) Energy transfer from an excited molecule in a crystal would result in a practically equivalent state, thus avoiding quenching due to transfer of energy to "solvent" molecules.

(*c*) As pointed out by Hochstrasser, intersystem crossing in crystals should be

lower than for solutions because the number of molecular configurations available to molecules in a crystal is limited by the rapid transfer of energy. Thus, the nuclear configurations which are most likely to lead to high intersystem crossing rates (because of the shapes of the singlet and triplet potential surfaces) may not be available to the molecules in a crystal (16). Although this might lead to an expectation of low phosphorescence yields from aggregates, actually about 10 to 20 per cent of the photons emitted by phenylalanine powder at 77K are from the lowest triplet state (19). Thus, the possible deviation of the quantum yield of this material from unity probably reflects the extent of the intersysem crossing.

There are two factors which, in principle, might affect the quantum yield of a powder:

(*a*) *Reabsorption of Fluorescence.* The emission spectrum of phenylalanine powder overlaps only about 10 per cent of the area of the absorption band in question. Light whose wavelength is in this overlap region can penetrate more than 10μ into pure phenylalanine; however, the average thickness of the powders in these experiments was about 3μ. Thus, there should be little reabsorption to interfere with the quantum yield measurements.

(*b*) *Particle Geometry and Orientation.* In the present work, although the powder particles have a major dimension of 44 to 74μ the average thickness of the layers is only 3μ. The original crystals were flat plates; thus it seems likely that the ground up particles are still essentially flat plates. Examination of the slide preparation with a microscope confirms this. Accordingly, the electric vector of the incident light will probably "see" one of the three components of the absorbing dipoles less strongly than the others. Thus, the experimental results could conceivably be biased by this factor. The crystallographic structure of phenylalanine is not available but Gurskaya has published the crystal structure of L-plenylalanine hydrochloride which is likely to be similar to that of the former compound (20): the two kinds of crystals have the same space group, the same number of molecules per unit cell, similar unit cell dimensions, and in each case the molecules are joined in double layers. If Gurskaya's data are pertinent to DL-phenylalanine the position of the benzene ring in the unit cell implies that light propagation along either of the other two axes of the crystal would not increase the measured oscillator strength by more than about 20 per cent. Thus the hypochromicity associated with aggregation as compared to aqueous solutions of phenylalanine probably cannot be associated with orientation of the particles.

APPENDIX

The following is a consideration of the possible errors induced by the assumption of an average surface density for absorption measurements.

Assume that there are N particles on a microscope slide and that each particle consists of cubic layers stacked normally to the face of the slide. Further, assume that the

possible number of layers in the particles are given by Y_i, $i = 0, 1, 2, \ldots \chi$, that there are N_i particles having Y_i layers and that there is a total of X layers, or cubes, divided among the N particles. If the cubic layers are of unit dimensions and unit mass, and the material has unit molecular weight then the average surface density σ is given by

$$\sigma = \frac{X}{N} \qquad [6]$$

also

$$X = \sum_{i=0}^{\chi} N_i Y_i \qquad [7]$$

and

$$N = \sum_{i=0}^{\chi} N_i \qquad [8]$$

I. Assume all particles to be equally thick. The amount of transmitted light I is given by

$$I = N \cdot 10^{-10^3 \epsilon \sigma} = Ne^{-2.3 \cdot 10^3 \epsilon \sigma}$$

for unit incident light intensity. Expressing the exponential factor as a power series gives the result:

$$I = N - 2.3 \cdot 10^3 N \epsilon \sigma + \frac{(2.3 \cdot 10^3)^2 N \epsilon^2 \sigma^2}{2} - \cdots \qquad [9]$$

Substituting from equation [6] gives

$$I = N - 2.3 \cdot 10^3 \epsilon X + \frac{(2.3 \cdot 10^3)^2}{2} \epsilon^2 \frac{X^2}{N} - \cdots \qquad [10]$$

II. In the more general case, where the particles may not have the same thicknesses

$$I = \sum_{i=0}^{\chi} N_i e^{-2.3 \cdot 10^3 \epsilon Y_i}$$

$$= \sum_{i=0}^{\chi} N_i \left[1 - 2.3 \cdot 10^3 \epsilon Y_i + \frac{(2.3 \cdot 10^3)^2 \epsilon^2 Y_i^2}{2} - \cdots \right] \qquad [11]$$

The use of Y_i in equation [11] can be justified by recalling the assumptions of the second paragraph of this Appendix. Substitution of [7] and [8] into [11] gives:

$$I = N - 2.3 \cdot 10^3 \epsilon X + \frac{(2.3 \cdot 10^3)^2}{2} \epsilon^2 \sum_{i=0}^{\chi} N_i Y_i^2 - \cdots \qquad [12]$$

Comparison of the amount of light transmitted past N particles in the case of equal thickness, as given by equation [10], with the more general case, as given by equation [12], shows that to first order in ε the two expressions are identical. The term in ε^2 is different, however, since $X^2/N \neq \Sigma_{i=0}^{\chi} N_i Y_i^2$ or, using equation [7] and [8]:

$$\frac{\left[\sum_{i=0}^{\chi} N_i Y_i \right]^2}{\sum_{i=0}^{\chi} N_i} \neq \sum_{i=0}^{\chi} N_i Y_i^2$$

Examination of equations [10] and [12] shows that the third terms in each are really terms in the square of the optical density. Thus, for optical density of about 10^{-1} both of the series converge rapidly and we find that, for such a case, equations [10] and [12] are essentially identical. Since we have maintained an optical density of about 10^{-1} in all of our measurements we conclude that the assumption of an average surface density does not appreciably affect the results of the present work.

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