

Polarization Spectroscopic Studies of Ordered Samples

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Contents

Linear Polarization	371
Transition Moment Directions	373
Aligned samples	374
LD Spectroscopy	376
Mathematical descriptions of aligned samples	376
Recording and Analyzing LD Spectra	377
Reduced spectra – symmetrical molecules	379
Low-symmetry molecules	379
Conclusion and Perspectives	382
References	382

Linear Polarization

In linearly polarized light all electric field vectors have the same direction, called the polarization direction. It is always perpendicular to the direction of the light beam. Light in nature, in particular scattered light, is often partially linearly polarized. Light from the blue sky is scattered light and is thus also partially linearly polarized in a direction that is perpendicular to the plane defined by the point on the blue sky that is being observed, the observer, and the sun (however, a heavy layer of clouds will often eliminate the light polarization from the blue sky).

This remarkable phenomenon of light polarization from the blue sky has been exploited by mankind for centuries. When the Vikings traveled from Scandinavia to North America almost exactly 500 years before Columbus, the Icelandic sagas report that they used “sunstones” as navigational instruments. Only after having received comments from amateur archaeologists it was realized that sunstones probably were naturally occurring linear polarizers which allow a determination of the polarization of the light from the sky and thus the position of the sun, even when it cannot be seen. The technology was later forgotten, but was reinvented in the 20th century and was now called a Twilight Compass or

Biographical Sketches



Erik W. Thulstrup, born in Haderslev, Denmark in 1941, graduated with an M.Sc. in Chemistry and Physics at Aarhus University in 1967, and obtained his Ph.D. there in 1970. He was Associate Professor of Chemistry at Aarhus University between 1972 and 1981, and Chairman of the Chemistry Department from 1975. Between 1981 and 1989 he was Professor of Chemistry at the Danish Pedagogical University, Copenhagen, between 1989 and 1998 he was Senior S&T Specialist in the World Bank, Washington DC, and he is presently Professor of Chemistry at Roskilde University, Denmark. He has held visiting positions, among other, at the University of Florida, University of Utah, University of California at Berkeley, and the University of Bologna. He is a fellow of TWAS, the Academy of Sciences for the Developing World, and of the Norwegian Academy of Sciences.

a Kollsman's Compass.¹ It was used for flights in Polar areas where magnetic compasses do not work.

It is remarkable that the light polarization from the blue sky is also crucial for the navigation by insects such as bees or ants, the eyes of which are impressive optical systems, with the ability to detect and analyze light polarization as one of the abilities. For a fascinating study of the navigational skills of desert ants, see reference 2.

Light from the blue sky is blue because it is scattered in the atmosphere and only the blue rays reach us (except when the sun is very low, at sunrise and sunset). Such scattered light is linearly polarized. Many other kinds of light around us is also scattered, and thus linearly polarized. Much of it is scattered by horizontal surfaces (lakes, roads, etc.) and is therefore linearly polarized in the horizontal direction. This light is responsible for most of the unpleasant glare we observe.

Light polarization may also help determine directional properties within molecules. In the 19th century, Ambronn studied dyed, aligned membranes with polarized light and found that the light absorption changed when the polarization direction of the light beam was changed.³ The Linear Dichroism (LD) of a sample is defined as the change in absorbance that occurs when the electric vector of the light beam is rotated 90°. LD spectroscopy may be used for samples that are not isotropic. Many other samples than liquid solutions are non-isotropic and dichroic. For these samples (e.g. biological samples) spectroscopic experiments performed with linearly polarized light may provide two linearly independent spectra instead of just one. This often provides new information.^{1,4,5}

Let us look at an example: the IR spectrum of anthraquinone in Figure 1. On top is shown a normal IR spectrum, but below are given the two LD spectra of anthraquinone aligned in a stretched polyethylene sheet. The spectrum in the center (E_V) has been recorded with light linearly polarized perpendicular to the stretching direction of the sheet, while the bottom spectrum (E_U) has been recorded with light linearly

polarized along the stretching direction of the sheet. It is easy to see that the two spectra are quite different; peaks marked x are much stronger in E_V than in E_U , while peaks marked z are much stronger in E_U than in E_V . Peaks marked y are only slightly stronger in E_V than in E_U . The reason is that vibrational transitions have a directional property, some are said to be polarized along molecular axis x, other along y and some along z. Clearly the LD spectra make it possible to distinguish between these three kinds of transitions.

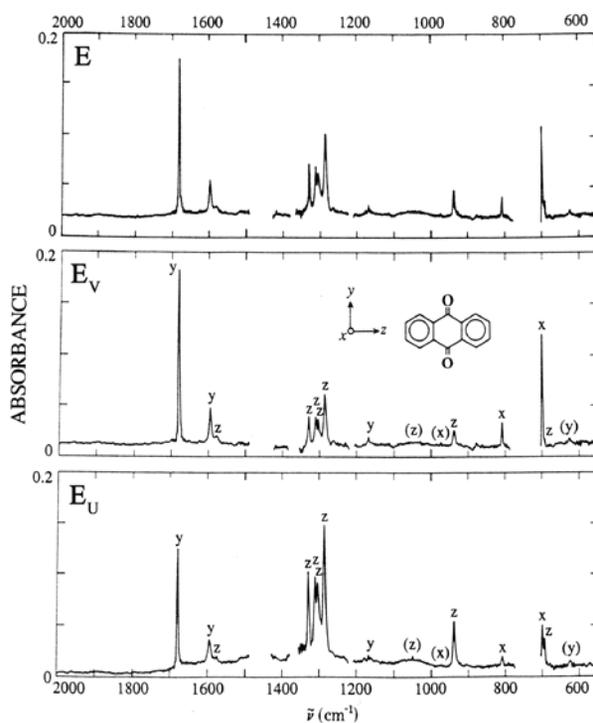


Figure 1. Infrared (IR) spectra of 9,10-anthraquinone.^{4,6} Top: Ordinary IR absorption; center and bottom: linear dichroism spectra in stretched polyethylene. Transition moment directions are given in accordance with the coordinate system. Reprinted from *Spectrochimica Acta*, Vol number 43A, Authors: J. Spanget-Larsen, D. H. Christensen, E. W. Thulstrup, Title of article: Symmetry assignments of vibrations in 9,10-anthraquinone aligned in stretched polyethylene, Pages No. 331–335, Copyright (1987), with permission from Elsevier (Reference 6).

Biographical Sketches



Peter W. Thulstrup, born in Aarhus, Denmark in 1973, graduated with an M. Sc. in Chemistry and Life Sciences from Roskilde University, Denmark in 1999. Subsequently, he studied bioinorganic chemistry and circular dichroism spectroscopy at the Royal Veterinary and Agricultural University, Denmark, where he finished his Ph.D. in 2003. In 2004 he was a post doc. researcher at the Quantum Protein Centre at the Technical University of Denmark, working with vibrational circular dichroism spectroscopy. Currently, he holds a position as Assistant Professor of Bioinorganic Chemistry at the Department of Natural Sciences at the Royal Veterinary and Agricultural University, Denmark, where he provides chemical education and works on research in the fields of spectroscopy and applied theoretical bioinorganic chemistry.

In order to understand the difference between these types of transitions better we have to take a closer look at molecular transition moments.

Transition Moment Directions

In optical spectroscopy in the infrared (IR), visible, or ultraviolet (UV) regions (the following discussion covers all these wavelength regions) light absorption is caused by molecular transitions that are either vibrational, electronic, or a combination of these (vibronic transitions). These transitions are characterized by transition energy, transition intensity, and their directions within the molecular framework. The direction of a transition is determined by the transition moment between the ground state $|0\rangle$ and the excited state $|i\rangle$

$$\mathbf{M}_i = \langle i | \mathbf{M}_{\text{op}} | 0 \rangle$$

\mathbf{M}_{op} is the electric dipole operator, simply a sum over all charged particles that are present in the molecule of their position vector times their charge. It is a vector operator and this makes also \mathbf{M}_i a vector, with a specific direction in the molecule. It is unfortunate that this wonderful property is so often overlooked in spectroscopic research. The direction of \mathbf{M}_i in the molecule is called the transition moment direction for the transition between states $|0\rangle$ and $|i\rangle$. The probability for absorption of linear polarized light polarized along a direction $\boldsymbol{\epsilon}$ is proportional to $(\mathbf{M}_i \cdot \boldsymbol{\epsilon})^2$. Thus, when the transition moment is perpendicular to $\boldsymbol{\epsilon}$ the molecule, it will not be able to absorb light, but when \mathbf{M}_i and $\boldsymbol{\epsilon}$ are parallel, light absorption will reach a maximum. In general, the transition probability is proportional to $\cos^2(\mathbf{M}_i, \boldsymbol{\epsilon})$, where $(\mathbf{M}_i, \boldsymbol{\epsilon})$ is the angle between \mathbf{M}_i and $\boldsymbol{\epsilon}$. In a sample with many differently aligned molecules, the absorption will be proportional to $\langle \cos^2(\mathbf{M}_i, \boldsymbol{\epsilon}) \rangle$, where $\langle \rangle$ indicates averaging over all sample molecules.

We can now see why Polaroid sunglasses have become so popular. The lenses in these sunglasses are linear polarizers that are able to remove the horizontally polarized electric vectors, e.g. the often very unpleasant glare from horizontal surfaces such as snow, water, even roads. However, the lenses do not absorb vertically polarized components of the light, for which $\cos^2(\mathbf{M}_i, \boldsymbol{\epsilon}) = 0$, and are thus still transparent. The lenses are made of stretched (extruded) poly(vinylalcohol) sheets that contain iodine. The iodine atoms form long chains in the direction of the extrusion and these absorb all horizontally polarized light, including unpleasant glare.⁴ For an account of the Polaroid story, see.⁷

The transition moment directions in a molecule, that possesses some kind symmetry, are determined

by the symmetries of the states the transition takes place between; these are usually the ground state and an excited state. In a molecule of C_{2v} symmetry (e.g. H_2O) with a totally symmetric ground state (A_1) only three different kinds of excited states may be reached through absorption of a photon. This has to do with the fact that \mathbf{M}_{op} , the electric dipole operator, has only three components, corresponding to the three dimensions of space. We may choose these so that they correspond to the direction of the H_2O symmetry axis (z), the direction perpendicular to it in the molecular plane (y), or the direction perpendicular to the molecular plane (x).

In order for the transition moment to be nonzero, the transition moment integral $\langle i | \mathbf{M}_{\text{op}} | 0 \rangle$ must have a totally symmetric integrand (non-totally symmetric integrands do not contribute, since contributions from different parts of space will cancel). Thus, only excited states of A_1 , B_1 and B_2 symmetry may be reached through absorption of a single photon, while transitions to states of A_2 symmetry are symmetry forbidden. Electric vectors along z are able to excite the molecule to states of A_1 symmetry, electric vectors along x will excite states of B_1 symmetry, and electric vectors along y will excite states of B_2 symmetry. This is true both for electronic and vibrational (as well as vibronic) transitions. A similar situation exists for molecules of D_{2h} symmetry, like 9, 10-anthraquinone from the example in Figure 1 and for molecules of D_2 symmetry.

Figure 2 shows the situation for a molecule of D_{2h} symmetry and the three symmetry classes that can be reached by light absorption through symmetry allowed transitions.

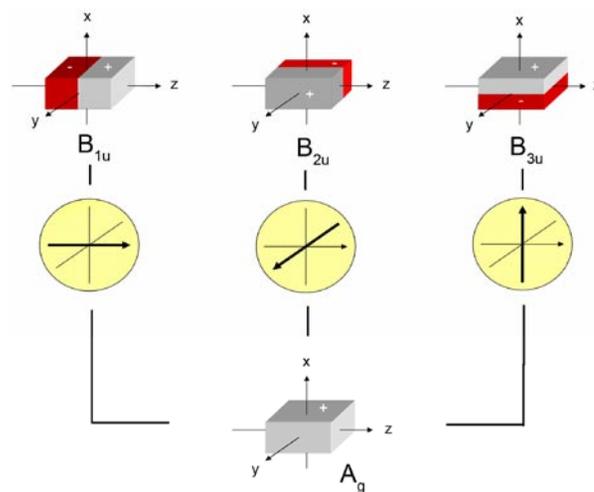


Figure 2. A molecule of D_{2h} symmetry like 9,10-anthraquinone with three symmetry planes: xy, xz, and yz. Excited states of B_{1u} , B_{2u} , and B_{3u} symmetry may be reached from a totally symmetric ground state (A_g) by absorption of a linearly polarized photon, polarized along one of the three molecular axes.¹ Redrawn from reference 1 with permission from VCH Publishers.

Table 1. Overview of two-photon events and the corresponding physical processes.¹

Photon 1	Annihilated	Annihilated	Annihilated	Annihilated
Photon 2	Annihilated	Annihilated	Created	Created
Timing	Simultaneous	Successive	Simultaneous	Successive
Physical Process	2-photon Absorption	Photoinduced Dichroism	Raman Scattering	Photo-luminescence

We are now able to understand the difference between the two LD spectra in Figure 1. Transitions with moments along the long molecular axis (z) are on the average best aligned with the stretching direction U of the polyethylene film. Thus, they dominate when the electric vector of the light is along U . On the other hand, especially transitions with moments along the shortest molecular dimension, the out-of-plane direction x , dominate when the electric vector is perpendicular to U (along V). Now we know that if $E_U \gg E_V$ for a transition the transition must be z polarized. Similarly, if $E_V \gg E_U$, it is x polarized, and if $E_U \approx E_V$ it is y polarized. This leads directly to one of the classic applications of LD spectroscopy: The use for spectral assignments. For molecules with symmetry elements the transition moment direction often is a strong criterion (that is, one that says either yes or no) which directly specifies the excited state symmetry. The information contained in Figure 1 thus immediately tells us which of the three kinds of excited states a given transition goes to, either $B_{1u}(z)$, $B_{2u}(y)$, or $B_{3u}(x)$.

An equally important application of LD spectroscopy may be the determination of sample or molecular structures. To a large extent, the sample structure determines the result of an LD experiment; and this is what makes it possible to extract structural information from an LD experiment. A typical example is the following: 1) the direction of a transition moment in a molecule is known (e.g. from another LD experiment), and 2) the direction of the transition moment in the sample (laboratory) system is determined, then the combined information may be used to make conclusions about the sample or molecular structure. One may, for example, identify the orientation of the molecule in the sample or the orientation of a group within the sample molecule relative to a sample axis. An example of the latter is the determination of the pyramidalization angle of the NH_2 group in aniline from LD spectra of aniline aligned in polyethylene at 12 K.⁸

In this connection it is important to stress that an LD experiment cannot by itself determine the complete orientation distribution function (except in the simple case of a perfect alignment). The information that may be obtained from LD spectroscopy is usually only average values (over all the sample molecules) of cosine squares of the angles between transition moments in molecules and the sample axes. Usually, any set of such averages will correspond to an infinite number of possible orientation

distribution functions for the sample molecules.

Other kinds of spectroscopy may also be performed on aligned samples with linearly polarized light, including two photon spectroscopy, such as Raman spectroscopy and luminescence spectroscopy (fluorescence and phosphorescence), where one photon is absorbed and another one created. Two-photon experiments in which both photons are absorbed, are two-photon absorption and photoinduced dichroism as shown in Table 1.

Two-photon experiments contain information on both the sample structure and on the optical properties of individual molecules, e.g. the Raman polarizability tensor. The information on sample structure is similar to that obtained in LD spectroscopy, except that it not only includes orientational averages of the second powers of the directional cosines, but also the fourth powers. One may say that while LD absorption spectroscopy provides information on the average molecular alignment, two-photon spectroscopy may provide information about the spread of the molecular alignment around the average alignments. The underlying expressions are quite complicated, although for molecules with some symmetry such as C_{2v} they are simpler, but they are not derived or given here. However, the possibilities of two-photon polarization spectroscopy are highly interesting.^{1,9}

Aligned samples

Many naturally occurring samples are aligned. This is true for many biological samples, although this alignment is often only partial, not perfect. Also industrial products are frequently aligned, for example plastic (polyethylene) bags, which have two layers of extruded (and aligned) polyethylene that together make the bag very strong.

Although one might expect that crystals would be ideal samples for LD spectroscopy, this is not the case, although both single and mixed crystals have been used for polarization spectroscopy.¹ However, these experiments are often difficult, for a number of reasons. For example, often very thin slices of single crystals are required, and these are often difficult to produce. For mixed crystals, in which the molecules are diluted, this is not a major problem, but the interpretation of the spectra is often difficult. However, a number of impressive studies on single crystals have been performed, for example the work on heme-protein complexes by Eaton and Hochstrasser.^{10,11}

Aligned subsets of a molecular sample may be selected by a photon; this is called photo-orientation. If a sample absorbs linearly polarized light with its electric vector along axis U, the sub-set that is selected by the absorption, will have a symmetrical orientation distribution around axis U. Similarly, the set of remaining molecules will also be aligned. Also selection with natural (unpolarized) light will produce aligned samples (the electric vectors of natural light are all perpendicular to the direction of the light beam). If the absorbing molecules undergo a chemical reaction an aligned sample is produced, at least if the molecules do not rotate after the light absorption. Thus samples for photo-orientation are usually fixed, for example in a rare gas matrix at low temperatures.

Other alignment techniques include alignment in electric or magnetic fields. Molecules with a permanent electric dipole or an anisotropic electric polarizability may be aligned in strong electric fields. Similarly, molecules with a permanent magnetic dipole or an anisotropic electric susceptibility may be aligned in magnetic fields, although this effect is quite weak.¹²

The most useful alignment technique is alignment in partially aligning solvents, in particular stretched polymers, such as polyethylene or poly(vinylalcohol), but nematic liquid crystals have also been used.¹³ For macromolecular polymers, such as DNA, the flow-LD technique has proven extremely useful.¹⁴ In flow-LD, a laminar flow is created in an isotropic solution held between a static and a rotating cylinder (a so-called Couette cell). The induced flow gradient affects orientation only in sample molecules of sufficient length and rigidity, for example DNA molecules.^{2,14} Small molecules in general remain in isotropic solution, but are oriented if they bind to DNA, allowing for determination of information, not only about DNA itself but also about its small molecule complexes. The flow-LD method has been extended to the study of lipid micelles.¹⁵ For the study of many biological samples the use of membranes as orienting media is highly attractive, see reference 16.

In general in order to be useful aligning solvents must have:

- a low baseline absorption or scattering
- an ability to align the solutes of interest
- an ability to dissolve the wanted solutes, sometimes in large quantities (especially for IR spectroscopy)

In the examples in this limited review the focus is on stretched polymers, but the expressions that are derived apply to any uniaxial sample. The common polymers polyethylene and poly(vinylalcohol) used as

aligning solvents in LD spectroscopy are transparent in the visible and UV regions, down to 210 nm. Furthermore, polyethylene, best suited as a solvent for non-polar molecules, is fairly transparent in most of the IR region, with only narrow absorbing regions around 720 cm^{-1} , 1450 cm^{-1} , and 2900 cm^{-1} .^{1,4} Poly(vinylalcohol), required for larger amounts of polar solutes, has a much more complicated IR spectrum than polyethylene, and the baseline problems are considerable in IR spectroscopy. Therefore poly(vinylalcohol) is rarely used as an aligning solvent in IR LD spectroscopy, although a few examples exist.¹⁷ As a result, relatively few polar molecules aligned in stretched polymers have been studied by IR LD spectroscopy.

In order to achieve an oriented sample, it is necessary apply mechanical stretching to the polymer. Usually, the polymers are used as films, ranging from ca. 0.1 to 2 mm in thickness. Depending on the film thickness the force necessary to acquire an adequate degree of stretching varies, but can be achieved through a simple hand-powered stretching device.⁴ The degree of stretching affects the degree of orientation induced in the polymer; usually polymers are stretched to ca. 5–10 times the original length. Solute molecules can be introduced into the polymers in different ways.^{1,4} For poly(vinylalcohol) it is often advantageous to introduce the solute molecules into the melted (liquid) polymer and subsequently casting and drying a film. For polyethylene it is often adequate to soak a polymer film in a chloroform solution of the sample molecules.

The extent of the solute alignment in polyethylene and poly(vinylalcohol) also depends on the molecular shape; however, the two solvents are able to align essentially all non-spherical solutes sufficiently. The nature of the alignment of molecules in polyethylene has been studied more extensively than alignment in other solvents.^{18–22} Polyethylene consists of crystalline and amorphous parts; it is believed that solute molecules in stretched polyethylene are located on the surface of crystalline regions.^{20–22} It has also been shown that high crystallinity improves the aligning power, but reduces the ability to dissolve large quantities of the solute. In pure polyethylene sheets the amorphous regions have no alignment, while the crystalline regions are well aligned.²¹

It is usually assumed that samples aligned in stretched polymers are uniaxial, i.e. that one sample axis (U, the stretching direction for the polymer) is unique and all directions perpendicular to U (laboratory axes V and W) are equivalent, which simplifies the interpretation of the spectra. Fortunately, there is considerable evidence for this assumption, at least for polyethylene. Already many years ago, X-ray diffraction studies of a thin, stretched polyethylene sheet^{18,19} showed that the sheet itself was uniaxial, and later

IR LD studies of solutes in stretched polyethylene²¹ confirmed the uniaxiality of the solute alignment.

LD Spectroscopy

LD absorption spectroscopy is usually performed in the ultraviolet, visible, or infrared regions on ordinary instruments equipped with a linear polarizer. The polarization may be either static or modulated. For the UV-visible region often calcite Glan prisms are used as polarizers, although even Polaroid sunglass lenses or polarizers taken from discarded liquid crystal displays can be used in the visible region. In the infrared region aluminum or gold grid polarizers or even less expensive sheet polarizers may be used. Usually several polarizers are required to cover the whole IR region. Today, most high-end UV-VIS and FT-IR instruments have a built-in, but usually optional, capability for the use of polarized light. It may be added that many circular dichroism instruments can also be used for linear dichroism measurements, taking advantage of the enhanced sensitivity from the detection of a modulated signal.⁵

The two LD spectra may be obtained, simply by rotating the polarizer 90°. It may be placed in the light beam, either before or after the sample, but should not be separated from the sample by any other optical elements. Both depolarization and scattering by the sample is quite common in the UV region (less so in the IR). The so-called baseline absorption by a pure stretched polymer is usually not primarily absorption, but scattering. Aligned samples are often birefringent and the light polarization may change through the sample unless the beam is polarized either along or perpendicular to the sample axis U. Therefore all spectra should be recorded with light polarized only along U, V, or W.

Mathematical descriptions of aligned samples

Fortunately, an interpretation of LD spectra does not require knowledge of the orientation distribution function of the sample molecules, although many have believed so.¹ Let us look at a molecule absorbing light polarized along ϵ due to a transition with transition moment \mathbf{M}_i . Then the transition probability is proportional to $\cos^2(\mathbf{M}_i, \epsilon)$, where (\mathbf{M}_i, ϵ) is the angle between \mathbf{M}_i and ϵ . This quantity must be averaged over all sample molecules and our transition probability becomes proportional to $\langle \cos^2(\mathbf{M}_i, \epsilon) \rangle$, which contains all the orientational information required to describe the directional properties of the light absorption and we will simply use the name K_i for this average related to a transition along \mathbf{M}_i :

$$\langle \cos^2(\mathbf{M}_i, U) \rangle = K_i$$

The corresponding averages along the three laboratory axes satisfy the relation:

$$\langle \cos^2(\mathbf{M}_i, U) \rangle + \langle \cos^2(\mathbf{M}_i, V) \rangle + \langle \cos^2(\mathbf{M}_i, W) \rangle = 1$$

In a uniaxial sample the absorption probability for light polarized along V and W will be the same, and we have:

$$\langle \cos^2(\mathbf{M}_i, V) \rangle = \langle \cos^2(\mathbf{M}_i, W) \rangle = (1 - K_i)/2$$

Now we can see that the K_i s are very useful quantities. We already know K_i values for some samples: If, for example, the sample is isotropic, all K_i s will be equal to 1/3, and if the alignment is perfect, K for a transition along U will be 1, while those for transitions perpendicular to U will be 0. The K_i s do not define the orientation distribution function, but contain no more and no less than those properties that are needed to describe the orientational effects of the absorption of a single photon of polarized light.

If the molecule has some symmetry, for example D_{2h} like anthraquinone, or just C_{2v} like water, we have seen that only three different transition moment directions may be observed (x, y, and z). This means that at most three different K values are observed; we call them K_x , K_y , and K_z . In the IR LD spectra of anthraquinone in Figure 1 (K_x , K_y , K_z) turned out to be (0.10, 0.30, 0.57), close to the theoretical value of 1 for $K_x + K_y + K_z$. The K values are consistent with the molecular shape, as it is usually the case for alignment in polyethylene. Figure 3 shows the “orientation triangle” in which the larger K value, here called K_z is plotted against the “middle” K value, here called K_y , for a large number of symmetrical molecules aligned in stretched polyethylene.

The corners of the triangle are:

$$(K_y, K_z) = (0, 1), (1/3, 1/3), \text{ and } (1/2, 1/2).$$

The value of the third K , K_x , is given by $K_x = 1 - K_y - K_z$, which for each of the three corners is 0, 1/3 and 0, respectively.

Similarly, the left side of the triangle ($K_y = K_x$) corresponds (not surprisingly) to the alignment of “rod-shaped” molecules, while the bottom side ($K_y = K_z$) represents the alignment of “disk-shaped” molecules. Note that only the top corner corresponds to a single orientation distribution; this is so, since the samples are assumed to be uniaxial. All other points in the triangle represent an infinite number of molecular orientation distributions.

One note of caution: The literature has many examples of descriptions of sample alignment that makes specific, but not always simple or well-defined, assumptions about the complete orientation distribution, e.g. the Tanizaki Model, the Fraser-Beer Model, and others.¹ However, these descriptions are usually much more complicated than the one based on the K s and since the assumptions often are incorrect, they tend to fail in an unpredictable way.¹

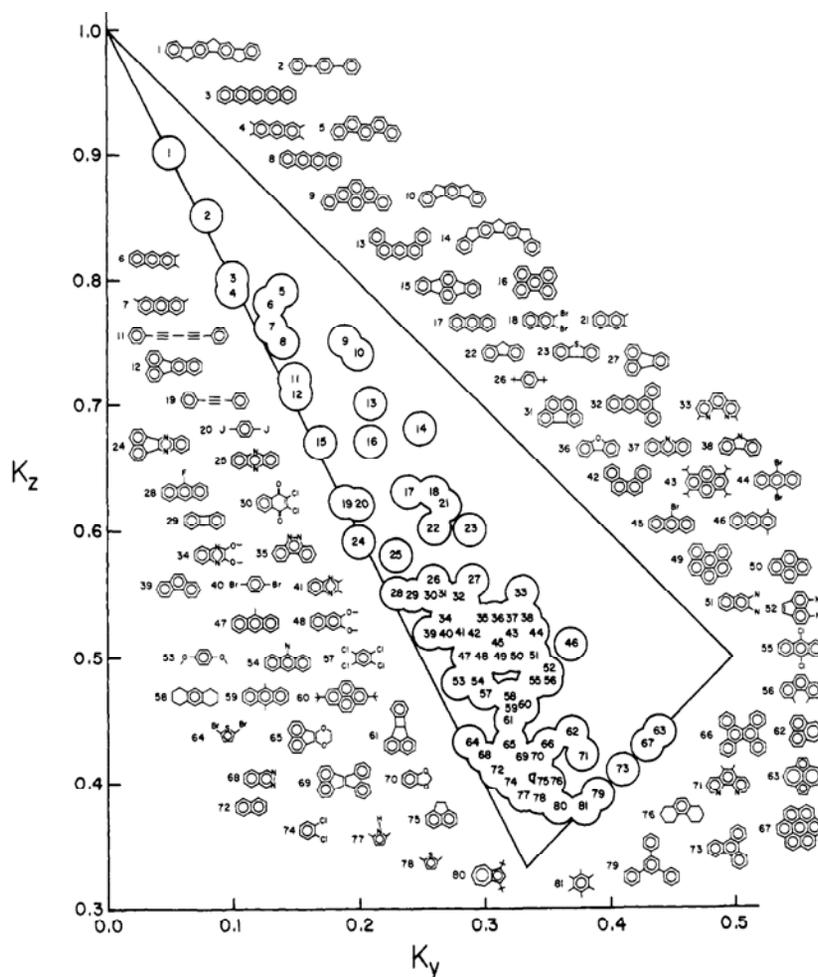


Figure 3. The orientation aromatic molecules in stretched polyethylene at room temperature. Reprinted with permission from reference 23. Copyright (1982) American Chemical Society.

Recording and Analyzing LD Spectra

The solute absorbance recorded with light polarized along the sample axis U is:[#]

$$E_U(\tilde{\nu}) = \sum \langle \cos^2(\mathbf{M}_i, U) \rangle A_i(\tilde{\nu}) = \sum K_i A_i(\tilde{\nu}) \quad (1)$$

The sum is over all transitions to states i ; $A_i(\tilde{\nu})$ is simply the contribution to the absorbance from transition i , the contribution that would be observed if \mathbf{M}_i were perfectly aligned with ϵ . This is equal to three times the contribution observed in an isotropic sample (e.g. in liquid solution) where all K s are $1/3$.

The absorbance observed with ϵ perpendicularly to U (that is, along V or W that are identical in a uniaxial sample) would similarly be:

$$E_V(\tilde{\nu}) = E_W(\tilde{\nu}) = \sum \frac{1}{2}(1-K_i)A_i(\tilde{\nu}) \quad (2)$$

It is now easy to determine K_i values for spectral regions without overlapping transitions. In such cases the K values are determined directly from the dichroic ratios $d(\tilde{\nu})$ at the wavenumber of the transition. The dichroic ratio is defined as

$$d(\tilde{\nu}) = E_U(\tilde{\nu})/E_V(\tilde{\nu}) \quad (3)$$

and we have from (1), (2), and (3) for a non-overlapping transition j located at $\tilde{\nu}_j$:

$$K_j = d(\tilde{\nu}_j)/[2 + d(\tilde{\nu}_j)]$$

Regions with no overlap between transitions are quite common in IR spectroscopy, but rare in UV spectroscopy, where it usually only occurs for the first, lowest energy (singlet-singlet, singlet-triplet transitions are not considered, since they tend to be far too weak to be observed) transition. Fortunately, also the more common case in UV spectroscopy, of overlapping transitions, may be treated, as long as the transition to be studied appears with a recognizable spectral feature (a peak or a shoulder). The TEM method^{1,4} simply forms

[#] The LD spectra in the following are assumed to be corrected for baseline effects by subtraction of the absorbance of an empty polymer sheet.

linear combinations of the observed spectra $E_U(\tilde{\nu})$ and $E_V(\tilde{\nu})$:

$$E_U(\tilde{\nu}) - xE_V(\tilde{\nu})$$

If the spectral feature of interest due to transition j , disappears in the linear combination for a given $x = x_j$, we know, according to (1) and (2), that:

$$K_j = x_j(1-K_j)/2 \text{ or } K_j = x_j/(2+x_j)$$

The highly efficient TEM technique makes it possible to determine K_j , even in the case of multiple spectral overlap.

Reduced spectra – symmetrical molecules

We have seen that the situation is particularly simple for molecules of C_{2v} and D_{2h} symmetry (“symmetrical molecules”). For these molecules, all transition all moments were aligned along one of the symmetry-determined molecular axes x , y , and z and only three different K_j s could be observed, namely K_x , K_y , and K_z . The group of molecules with such properties also includes molecules of the less common D_2 symmetry. Note that the three K s are not independent, but add up to one by definition:^{1,4}

$$K_x + K_y + K_z = 1$$

In some cases the observed absorbance (consisting of contributions of different transition moment directions) may be separated into components corresponding to a specific transition moment direction. This may, for example, help reveal transitions hidden due to overlap with stronger transitions, and it provides a useful overview of the spectrum. The most important case is the one in which only intensity polarized along two molecular axes is present; this is the case for example for π - π^* transitions in a planar molecule like anthraquinone, as discussed below. If only y - and z -polarized intensity is present, and $A_x(\tilde{\nu}) = 0$ in a spectral region, we have for the sums of y and z polarized spectral intensity, respectively:^{1,4}

$$A_z(\tilde{\nu}) = [(1-K_y)E_U(\tilde{\nu}) - 2K_yE_V(\tilde{\nu})] / (K_z - K_y) \quad (4)$$

$$A_y(\tilde{\nu}) = [2K_zE_V(\tilde{\nu}) - (1 - K_z)E_U(\tilde{\nu})] / (K_z - K_y) \quad (5)$$

Thus we are able to plot the sum of the contributions to the absorbance from transitions polarized along either molecular axis y , or axis z .

Let us now look at the UV spectrum of anthraquinone in Figure 4, with plenty of spectral overlap. We see that with the help of equations (4) and

(5) we have been able to construct $A_z(\tilde{\nu})$ and $A_y(\tilde{\nu})$ from the observed spectra. Since $A_x(\tilde{\nu}) = 0$ in the spectral region, the sum of $A_y(\tilde{\nu})$ and $A_z(\tilde{\nu})$ is equal to 3 times $E(\tilde{\nu})$, the spectrum that would be observed in a normal measurement on a similar, but isotropic (unaligned) sample.

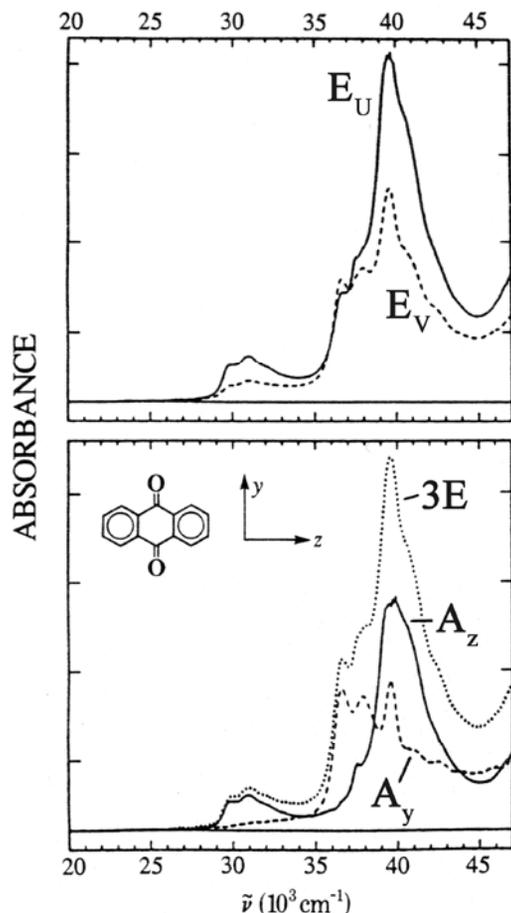


Figure 4. Top: Polarized UV spectra of 9,10-anthraquinone in stretched polyethylene. Bottom: Reduced spectra and the isotropic spectrum $3E = E_U + 2E_V = A_y + A_z$. Note: the vertical scales on the top and bottom are different. Reprinted from Chemical Physics, Vol number 104, Authors: B. O. Myrvold, J. Spanget-Larsen, E. W. Thulstrup, Title of article: Transition-moment directions in 9,10-anthraquinones - symmetrical cases, Pages No. 305–313, Copyright (1986), with permission from Elsevier (Reference 24).

A look at the spectrum which would be obtained in normal spectroscopy, $E(\tilde{\nu})$, shows that 3 UV transitions may be clearly recognized. The first has an onset around $30,000 \text{ cm}^{-1}$, the second one (almost a shoulder) appears near $36,000 \text{ cm}^{-1}$, and the third, strong transition has its peak around $40,000 \text{ cm}^{-1}$. Measurements on a more concentrated solution would also reveal what is really the first singlet-singlet transition (responsible for the tail below $30,000 \text{ cm}^{-1}$ in Figure 4), but from the present spectrum only three transitions are clearly recognizable.

In contrast, the reduced LD spectra $A_z(\tilde{\nu})$ and $A_y(\tilde{\nu})$ provides much more information. $A_z(\tilde{\nu})$ has distinct peaks around 30,000 and 40,000 cm^{-1} and a shoulder at 38,000 cm^{-1} , while $A_y(\tilde{\nu})$ has regular peaks, that seem to be independent transitions, at 36,000 and 39,000 cm^{-1} . In addition, a large amount of vibrational structure has been revealed.

The separation of the spectral intensity into $A_x(\tilde{\nu})$ and $A_z(\tilde{\nu})$ was only possible because it could be assumed that $A_x(\tilde{\nu})$ was zero in the region. Such regions with only two different transition moment directions are common for symmetrical, polycyclic hydrocarbons in regions where only (in-plane polarized) π - π^* transitions are observed. The benefit of a separation of the spectral intensity in regions of much band overlap is obvious.

We may now return to Figure 1. Although a separation is not possible because the spectrum has contributions from both $A_x(\tilde{\nu})$, $A_y(\tilde{\nu})$, and $A_z(\tilde{\nu})$, the limited amount of spectral overlap makes an analysis simple.⁶ This IR LD spectrum of anthraquinone was at first measured in order to compare with the K-values in the UV spectrum (Figure 4). However, it turned out that it made a number of new assignments of vibrational transitions possible, including that of the intense quartet around 1300 cm^{-1} , which was earlier wrongly assigned. Also, the weak z-polarized fundamental near 700 cm^{-1} had never been correctly assigned before. Due to the relatively strong appearance of z polarized transitions in $E_U(\tilde{\nu})$ it is clearly visible in this spectrum – but (of course) much less so in $E_V(\tilde{\nu})$ or in the normal spectrum $E(\tilde{\nu})$.

Low-symmetry molecules

In a general molecule there are no or only a few symmetry elements and no or few restrictions on the possible directions of transition moments. While the information obtained in LD spectra of such molecules is still useful, it will usually have a more qualitative nature. However, there are some important cases of molecules with a minimum of symmetry in which quantitative information may be extracted. The most important case is that of a molecule with a plane of symmetry.

In molecules with a plane of symmetry, including planar molecules, the transition moments must be located in the symmetry plane (the x, y plane) or perpendicular to it (along z). This places some limits on the possible K values: For the out-of-plane polarized transitions the situation is simple. We have:

$$K = K_z$$

Thus the identification of z-polarized transitions (out-of-plane polarized transitions in planar molecules) is usually simple. For the in-plane polarized transitions the situation is more complex. Let us assume that the

in-plane axes are chosen so that the x-axis corresponds to the smallest possible in-plane K value, $K_x = \langle \cos^2(x, U) \rangle$, and the y-axis so that it corresponds to the largest in-plane K value, $K_y = \langle \cos^2(y, U) \rangle$. Note that determining or even estimating these directions in the molecular framework is not always simple, as discussed below. However, it is reassuring to know that the two directions, x and y, determined this way, will always be perpendicular.¹ The direction in the molecule corresponding to the largest K-value is called the molecular orientation axis. Thus, if the orientation axis is located in the symmetry plane, it will be equal to axis y. If we are able to determine two of the three values K_x , K_y , and K_z , then the measured value of K_i for an in-plane polarized transition will immediately provide us with the numerical value of the angle between \mathbf{M}_i and the orientation (y) axis, ϕ_i . We have for ϕ_i :¹

$$\tan^2 \phi_i = (K_y - K_i)/(K_i - K_x) \quad (6)$$

How can K_x and K_y be determined? We know that for any in-plane polarized transition i, we have

$$K_x \leq K_i \leq K_y$$

The picture that is usually obtained from the determination of K values for a planar molecule is shown in Figure 5.



Figure 5. K values in an IR LD spectrum of a planar molecule. A single out-of-plane K_z value and many K values between K_x and K_y are observed.

We also know that

$$K_x + K_y = 1 - K_z$$

The restrictions in the possible values for (K_x , K_y) are in most cases very helpful, especially when it is possible to determine K_z and to treat the z-polarized transitions separately. Comparison with symmetrical molecules of similar shape may also help determine (K_x , K_y), and this has surprisingly rarely been a serious problem. It is also necessary to determine the position in the molecule of the y-axis, typically the molecular orientation axis, when it is not given by symmetry. Again, molecular shape considerations are often very useful; it is usually assumed that the orientation axis is perpendicular to the smallest cross-section of the molecule. But also in other kinds of information may be useful, e.g. transitions with known transition moments.

Finally, it must be kept in mind that the determination of $\tan^2 \phi_i$ only provides information of the numerical value of the angle with the (orientation axis

y ; φ_i may be either positive or negative. In other words, \mathbf{M}_i may be located on either side of y in the molecular framework. It has often been possible to determine the sign of φ_i by using information from fluorescence polarization spectroscopy.¹ This technique provides information about the angles between transition moments for the absorbing transitions and the transition moment direction of the emitting transition. The example below illustrates many of the typical problems and opportunities for low symmetry molecules.

In a fairly complete study,²⁵ several kinds of important spectroscopic information were obtained, namely from:

- UV-visible and IR linear dichroism spectroscopy (using stretched polyethylene as an anisotropic solvent)
- UV-visible magnetic circular dichroism (MCD) spectroscopy
- fluorescence polarization spectroscopy performed on isotropic samples, as well as
- quantum mechanical calculations

Based on this, the electronic transitions in the near-UV and visible regions were convincingly assigned for the pericondensed benzenoid hydrocarbon anthanthrene (dibenzo[def,mno]chrysene or dibenzo[cd,jk]pyrene, see Figure 6.

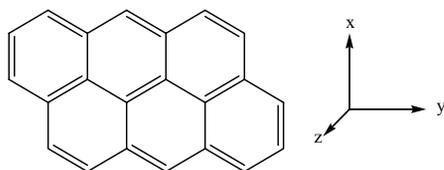


Figure 6. The anthanthrene molecule (dibenzo[def,mno]chrysene) and the chosen coordinate system.

As usual, especially the determination of polarization directions from LD spectra turned out to be very useful. The assignments included the very weak L_b transition (the Clar α -band), which is a very difficult case due to its overlap with the much stronger L_a transition (the Clar p -band) at higher energy. The resulting assignment differs from earlier assignments, but it was further confirmed by studies of solvent and temperature shifts.

The molecular point-group symmetry of anthanthrene is C_{2h} ; this means that transition moment directions for dipole allowed transitions are restricted to the direction perpendicular to the molecular plane (the plane of symmetry) or along any direction in this plane. It was assumed that all spectral intensity in the visible and near-UV region was of π - π^* type and therefore polarized in the molecular plane. Thus the K -values for the individual spectral features (determined by the TEM

method) may be used to predict the angles φ_i between the transition moments of the observed spectral features i and a well-defined axis in the molecular plane (the orientation axis) according to the above expression (6). By inspection, the orientation axis the molecular y -axis, was chosen parallel to the long axes of the anthracene moieties of anthanthrene (see Figure 6, which also shows the other two axes, of which only the out-of-plane axis z is determined by symmetry).

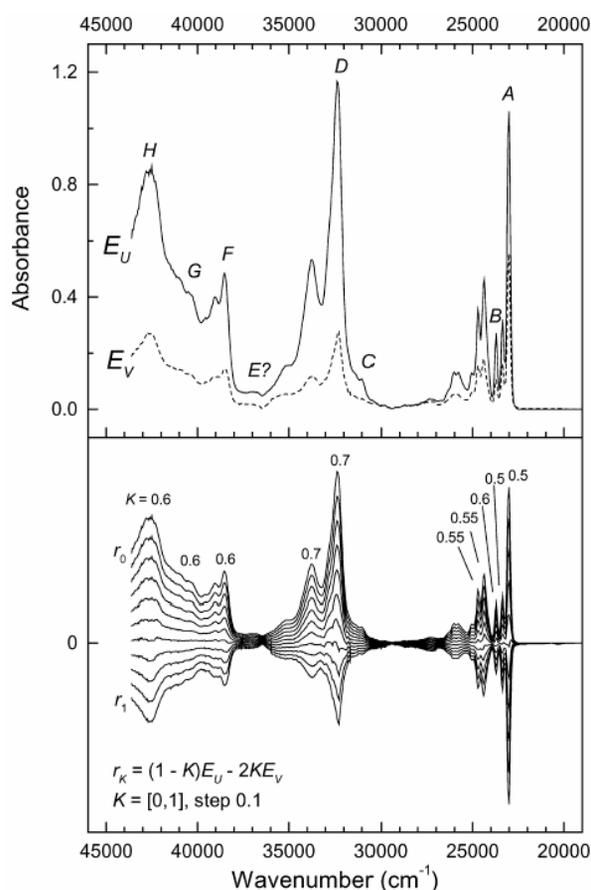


Figure 7. Top: linear dichroic absorption spectra E_U and E_V for anthanthrene in stretched polyethylene at 90 K. Bottom: a series of reduced absorbance curves r_K for $K = 0$ to 1 in steps of 0.1. Reproduced by permission from reference 25. Copyright 2005 From Electronic States of Anthanthrene. Linear and Magnetic Circular Dichroism, Fluorescence Anisotropy, and Quantum Mechanical Calculations" by C. J. Johannessen, A. Gorski, J. Waluk, J. Spanget-Larsen. Reproduced by permission of Taylor & Francis Group, LLC., <http://www.taylorandfrancis.com>.

The UV VIS LD spectra are shown in Figure 7. According to Equation 6 the numerical value of the angle φ_i between the transition moment \mathbf{M}_i and the orientation axis y may be determined from the observed K_i value, K_x and K_y . The value of K_x is provided by the IR LD spectrum, in which at least 5 transitions have K_i values equal to 0.10 ± 0.02 and thus must be out-of-plane (z) polarized. Therefore $K_x + K_y = 0.90$. The

value of K_y is known to be larger than or equal to any observed K_i value. The largest observed K_i value is 0.70; thus the lower limit of K_y is 0.70. The upper limit is given by $1 - 2K_z = 0.80$ (the case for a rod-like alignment). Thus we have:

$$0.70 \leq K_x \leq 0.80$$

The authors argue that it is unlikely that K_x is close to the upper limit, $K_x = 0.80$, since the molecule is far from rod-like. Fortunately, studies of the fluorescence excitation polarization spectra, providing information on relative angles between the transition moments for the emitting transition and the absorbing ones, turn out to be very useful and lead to the conclusion that $K_x = 0.74$. Thus the authors obtain:

$$(K_x, K_y, K_z) = (0.74, 0.16, 0.10)$$

Now Equation (6) may be used to calculate φ_i values for specific spectral features, specifically the transitions L_a , L_b , B_b , and B_a , with observed K_i values 0.5, 0.6, 0.7, and 0.6, respectively (Figure 7). This leads to the following numerical values for the angles the transition moments form with the y-axis: 40° , 29° , 15° , and 29° . From the fluorescence polarization information on relative angles the authors conclude that the angles must be either:

$$\begin{aligned} &(+40^\circ, +29^\circ, -15^\circ, \text{ and } +29^\circ), \text{ or} \\ &(-40^\circ, -29^\circ, +15^\circ, \text{ and } -29^\circ) \end{aligned}$$

both these results are fully compatible with the experimental evidence, and only a comparison with the calculated angles can solve the dilemma. The results for the transition moment angles from the quantum mechanical calculations are:

$$(+42^\circ, +34^\circ, -21^\circ, \text{ and } +36^\circ)$$

which clearly are consistent only with the first of the two possibilities. The similarity of the calculated and experimental results is an indication of the accuracy of both experiment and the relatively simple theoretical model used, see Figure 8.

It may be added that the paper provides a considerable amount of additional data, which will not be discussed here.²⁵ Further accounts of transition moment directions for other polyaromatic compounds can be found in the literature. For references to numerous examples, see references 1, 23 and 26.

Similar UV-visible studies, using stretched polyethylene as an orienting solvent, have made it possible to determine transition moment directions for electronic transitions in *trans*-stilbene and *trans*-azobenzene,²⁷ see figures 9 and 10.

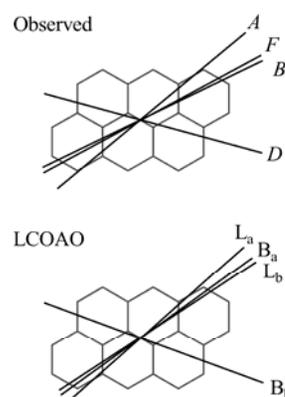


Figure 8. Experimental polarization directions for transitions *A*, *B*, *D* and *F* (see Figure 7), and moment directions for the *L* and *B* type π - π^* transitions from an LCOAO calculations. Reproduced by permission from 25. Copyright 2005 From Electronic States of Anthanthrene. Linear and Magnetic Circular Dichroism, Fluorescence Anisotropy, and Quantum Mechanical Calculations” by C. J. Johannessen, A. Gorski, J. Waluk, J. Spanget-Larsen. Reproduced by permission of Taylor & Francis Group, LLC., <http://www.taylorandfrancis.com>.

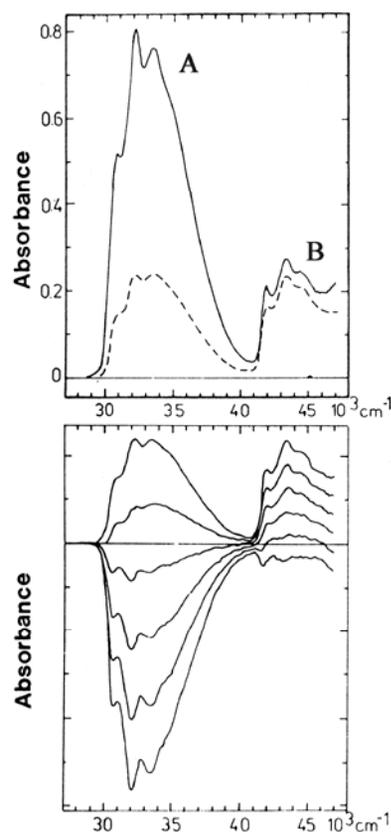


Figure 9. UV-VIS LD spectra of *trans*-stilbene in stretched polyethylene. Top: E_U (full curve) and E_V (broken curve). Bottom: Use of the TEM method; linear combinations $E_V - xE_U$, with $0 \leq x \leq 1$. Reprinted from Spectrochimica Acta Part A: Molecular Spectroscopy, Vol. number 46. Authors: P. Uznanski, M. Kryszewski, and E. W. Thulstrup, Title of article: Polarized absorption spectroscopy of *trans*-azobenzene and *trans*-stilbene in stretched polyethylene films, Pages No. 23–27, Copyright (1990), with permission from Elsevier (reference 27).

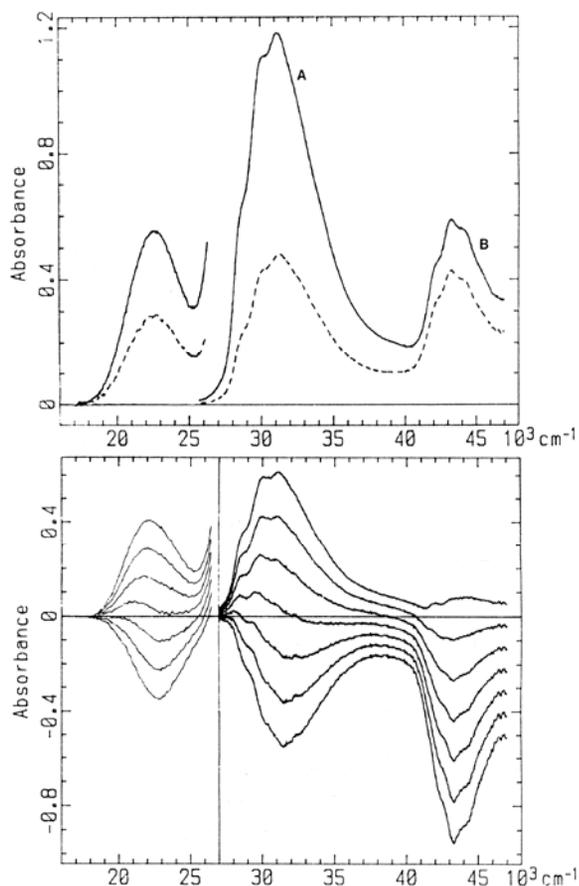


Figure 10. UV-VIS LD spectra of *trans*-azobenzene aligned in stretched polyethylene. Top: E_U (full curve) and E_V (broken curve). Bottom: Use of the TEM method; linear combinations $E_U - xE_V$, with $1.2 \leq x \leq 3.6$ ($1.5 \leq x \leq 2.1$ for the low-energy band). Reprinted from *Spectrochimica Acta Part A: Molecular Spectroscopy*, Vol. number 46. Authors: P. Uznanski, M. Kryszewski, and E. W. Thulstrup, Title of article: Polarized absorption spectroscopy of *trans*-azobenzene and *trans*-stilbene in stretched polyethylene films, Pages No. 23–27, Copyright (1990), with permission from Elsevier (reference 27).

Based on these results it is concluded that the two molecules have very different geometries, at least in the polymer matrix. Not surprisingly, *trans*-stilbene, $(K_x, K_y, K_z) = (0.70, 0.17, 0.13)$, has a planar configuration of C_{2h} symmetry, while *trans*-azobenzene, $(K_x, K_y, K_z) = (0.64, 0.18, 0.18)$, is nearly rodshaped with C_2 symmetry. It is concluded that the phenyl groups are twisted considerably around the C-N bonds. Later, a very simple example of photochemistry on an aligned sample was performed in a study of the *trans-cis* photo-isomerization of azobenzene aligned in a stretched polyethylene film.²⁸ UV-visible LD spectra of the initial sample of *trans*-azobenzene and of the mixture of *cis*- and *trans*-azobenzene that resulted after irradiation with natural (unpolarized) light at 337 nm, and the isomerization process was studied in detail. Among other, it was shown that it occurs

according to an inversion mechanism. It is interesting that *cis*-azobenzene in the polymer attains a globular geometry with the phenyl rings twisted perpendicular to the C-N-N-C plane. A number of other studies have been performed on related compounds including certain *s*-tetrazines,²⁹ and azapyrenes.^{30,31}

Conclusion and Perspectives

LD spectroscopy and its extensions to two-photon experiments (in particular luminescence and Raman spectroscopy) are only of interest for non-isotropic samples. However, numerous important samples are aligned, and have important properties determined by the alignment, from liquid crystals (e.g. in liquid crystal displays) over nano-technological devices to biological samples. Polarization (LD) studies of such samples are often able to provide additional, new information, for example on the sample structure, which is one of the properties often sought. New optical spectroscopic applications are entering medicine, where especially IR and Raman spectroscopy seem to become important, and many of the samples studied, e.g. from the human body, are aligned. Even the new, fast growing analytical technique, Near Infrared spectroscopy, NIR - today widely used in food technology and elsewhere - shows clear polarization effects for aligned samples. These effects may become useful, but this possibility has so far not been investigated much. Also in NIR spectra, polarization studies provide no additional information for isotropic samples, but many of the important samples that are routinely analyzed by NIR spectroscopy are actually aligned. This is also the case for some ordinary food items. For example, while the NIR LD spectrum of a hamburger is of little added interest, the NIR LD spectrum of a pork chop is filled with exciting structural information!

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