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Investigation of the Self-Organization of Lutein and Lutein Diacetate by Electronic Absorption, Circular Dichroism Spectroscopy, and Atomic Force Microscopy

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Spontaneously forming H- and J-type chiral self-assemblies of lutein and lutein diacetate were studied by means of ultraviolet–visible and circular dichroism spectroscopy (CD). Spectroscopic properties of their thin films has been determined for the first time. Existence of chiral superstructures in both solution and solid phases with opposite and same handedness was demonstrated. The molecular exciton theory and the exciton chirality rule were applied to define both qualitatively and quantitatively the spectral shifts, band shape changes, and the highly intense but quite different excitonic CD activity in the two forms of aggregates. Atomic force microscopy images revealed definite threadlike morphology in lutein diacetate films referring to the formation of a nematic liquid crystal phase which probably exists in the aqueous solution either. On this basis, the vibronically coupled CD bands of lutein diacetate are attributed to an excitonic interaction arising from the twisted nematic layers.

Introduction

Carotenes and carotenoids are a widely distributed group of naturally occurring dyes, essential to plants for photosynthesis, acting in light-harvesting and, especially, in protection against destructive photooxidation.¹ They are known to be a main dietary source of vitamin A in humans. Protective effects of carotenoids against serious disorders such as cancer, heart disease and degenerative eye disease have been recognized, and have stimulated intensive research into the role of carotenoids as antioxidants and as regulators of the immune system.² They are used extensively as safe, natural colorants for food, feed, and cosmetics.

Molecular aggregates have attracted considerable attention bridging the gap between the physics of a single molecule and structurally ordered crystals. Moreover, molecular self-assembly in biological structures is highly specific and fundamental to the correct functioning of an organism.³ It was known long ago that carotenoids, similarly to some special dye molecules, form under certain conditions molecular aggregates which have quite fascinating optical properties, such as a large blue shift in the ultraviolet–visible (UV/vis) spectrum and an unusually strong exciton type optical activity.⁴ These fascinating properties originate from strongly delocalized wave functions of the excited states and can be interpreted by the exciton theory⁵ which is widely applied to explain spectral characteristics of molecular aggregates,⁶ thin films,⁷ biopolymers,⁸ and composite molecules.⁹ In aggregates, formed by carotenoids, the molecules are weakly and reversibly bound by various types of intermolecular forces (i.e., van der Waals, H-bond) assumed to be weak in comparison with intramolecular forces of the electronic system.

Classified as card-pack (H-type) or head-to-tail (J-type) associates, aggregated forms of carotenes and carotenoids have been subject to several studies, including UV/vis,¹⁰ fluorescence,¹¹ resonance Raman,¹² and chiroptical spectroscopic methods.¹³

Until now, however, there is no detailed picture about the nature and molecular organization of these aggregates and we are far from the complete understanding of mechanisms which govern the formation of their chiral superstructures. Moreover, there are only very limited data about the supramolecular assemblies built up by esterified xanthophylls although they are one of the naturally occurring forms of carotenoids having hydroxyl function.¹⁴

Lutein, containing three centers of chirality, is the main xanthophyll found in the major light-harvesting pigment–protein complex of higher plants. It is involved in energy transfer mechanisms during photosynthesis.¹⁵ Together with zeaxanthin, lutein is selectively concentrated in the macular region of the human eye presumably in aggregated form and assumed to protect the retina by absorption of harmful blue light.^{10f,16} In fatty-acid ester form, lutein is widely distributed in fruits, flowers and yellow autumn leaves as well.

Lutein and its diacetate (Figure 1) were chosen in order to characterize their supramolecular self-assemblies in detail by means of UV/vis and circular dichroism (CD) spectroscopy. To investigate whether the chiral superstructures exist in solid state, thin films and KBr disks of lutein and lutein diacetate were prepared and examined spectroscopically. Thin films also were subjected to measurements by atomic force microscopy (AFM) to obtain visual information on the nature of aggregates. By the help of molecular modeling studies, the results were interpreted in the light of molecular exciton theory.

Experimental Section

UV/Vis and CD Measurements of Solutions and Thin Films. Samples for spectrometric analyses were obtained from

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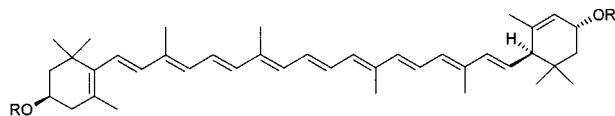


Figure 1. The chemical structure of *all-trans*-lutein ((*all-E,3R,3'R,6'R*)- β,ϵ -carotene-3,3'-diol) and lutein diacetate ($R = -H$ for the lutein and $-COCH_3$ for the diacetate).

freshly prepared 10^{-4} M stock solutions of lutein and lutein diacetate in acetone. Fifty microliters of a 10^{-4} M stock solution was diluted either by 950 μ L acetone or by 200 μ L acetone and 750 μ L doubly distilled water, resulting in identical, 5×10^{-6} M concentrations for spectroscopic samples in both media. After aqueous dilution, the aggregation of lutein was characterized by a loss of visual color, while the lutein diacetate solution exhibited color deepening. All samples remained clear with no sign of opalescence or precipitation. UV/vis and CD spectra were taken immediately and after 16 h for the aqueous dilutions, on a Jasco J-715/150S spectropolarimeter at 25 °C, in a rectangular cuvette with 0.5 cm path length. Temperature control was provided by a Peltier thermostat. All solutions and thin films gave the same CD spectra when they were recorded by performing a 180° rotation of the cuvette around the incident light. Until the repeated measurements, the aqueous solutions were stored at room temperature in a dark place and showed no apparent changes.

Fabrication of Thin Films for UV/Vis and CD Measurements. Fifty microliters of an acetonic stock solution (10^{-4} M) was spread on the clean surface of the wall of a 0.5 cm quartz cuvette with an automatic pipet at room temperature. The solvent evaporated within 30 s by the aid of a gentle current air.

Preparation of KBr Disks. To prepare the lutein KBr disk, a mixture of 0.087 mg lutein and 200 mg KBr was ground and pressed into a disk with a thickness of 0.6 mm and a radius of 13 mm. The disk was rotated around the optical axis and the CD recordings were made for several positions in order to check reproducibility of the spectra.

To prepare the lutein diacetate KBr disk, a mixture of 0.046 mg lutein diacetate and 130 mg KBr was ground and pressed into a disk with a thickness of 0.45 mm and a radius of 13 mm. Because the CD spectrum showed sign inversion with the sample rotation its scientific value is doubtful and not reported.

Materials. Lutein was isolated from green paprika (*Capsicum annum*) according to the method described elsewhere.^{17a} Lutein diacetate was obtained by acetylation of lutein.^{17b} Crystallization from benzene–hexane yielded red crystals with a purity of 98% (HPLC).

All solvent used were of spectroscopic grade. Kalium bromide for spectroscopy (Merck) was utilized to prepare the disks.

Atomic Force Microscopy. Microscopic analysis was carried out by using a commercial atomic force microscope (Nanoscope III, Digital Instruments, Santa Barbara, CA). AFM images were obtained in air at room temperature by using contact mode imaging. The spring constant of the NT-MDT tip was 0.12 N/m.

Thin films of lutein and lutein diacetate were prepared on quartz and on highly oriented pyrolytic graphite (HOPG) surfaces. For the thin film preparation procedure, 30 μ L from the freshly prepared acetonic solution of the carotenoids (4×10^{-4} M) was spread on the surface with an automatic pipet at room temperature. Solvent evaporation was facilitated by the aid of a gentle current air.

Calculation of Spectroscopic Properties of Lutein and Lutein Diacetate from their UV/Vis Spectra. The oscillator strength f was evaluated from the integration of the visible absorption band:¹⁸

$$f = 4.319 \times 10^{-9} \int_{\sigma_2}^{\sigma_1} \epsilon(\sigma) d\sigma$$

The electronic transition moment is $M = \sqrt{D}$ where D is the experimental dipole strength calculated by the equation¹⁹

$$D = 9.184 \times 10^{-39} \int_{\sigma_2}^{\sigma_1} (\epsilon(\sigma)/\sigma) d\sigma$$

Mean wavelengths of the visible absorption bands were obtained by the following equation:

$$\bar{\sigma} = \frac{\int_{\sigma_2}^{\sigma_1} \epsilon \sigma d\sigma}{\int_{\sigma_2}^{\sigma_1} \epsilon d\sigma}$$

where ϵ is the molar extinction coefficient in $M^{-1} \text{ cm}^{-1}$ and σ is wavenumber in cm^{-1} : $\sigma_1 = 28\,571.4 \text{ cm}^{-1}$ (350 nm) and $\sigma_2 = 19\,047.6 \text{ cm}^{-1}$ (525 nm), except for the aqueous solution where $\sigma_2 = 18\,181.8 \text{ cm}^{-1}$ (550 nm). For lutein (in acetone),

$$\bar{\sigma} = 22\,513.8 \text{ cm}^{-1} \quad (444.2 \text{ nm})$$

For lutein diacetate (in acetone),

$$\bar{\sigma} = 22\,515.1 \text{ cm}^{-1} \quad (444.1 \text{ nm})$$

For lutein diacetate (in aqueous solution),

$$\bar{\sigma} = 22\,504 \text{ cm}^{-1} \quad (444.4 \text{ nm})$$

Molecular Modeling Calculations. For molecular model calculations, the initial coordinates of lutein atoms were modeled using the Sybyl 6.6 program (Tripos Inc., St. Louis, MO) on a Silicon Graphics Octane workstation under Irix 6.5 operation system. Structures of monomers and dimers were minimized using the Powell Conjugate Gradient method and MMFF94 force field until the convergence was less than 0.001 kcal/(molÅ). The Connolly program was applied to calculate the solvent accessible surface of the lutein molecule.

Results and Discussion

UV/Vis and CD Spectra of Lutein and Lutein Diacetate in Acetone Solution. Polyenes possessing C_{2h} symmetry have a strong, electronic dipole allowed $S_0 \rightarrow S_2$ ($^1A_g \rightarrow ^1B_u$) excitation in the visible region; its transition moment M is polarized along the long axis of the all-trans polyene chain.²⁰ The electronic transition between S_0 and the first excited S_1 state is forbidden because both S_0 and S_1 have the same A_g symmetry.

In acetone, the mentioned $\pi-\pi^*$ type excitation of lutein and lutein diacetate appears between 350 and 550 nm with a rich vibrational fine structure (Figure 2). Calculated from the measured UV/vis spectra (see experimental), the values of the oscillator strength f and the transition moment M match well the “allowedness” of this transition (Table 1). The longest wavelength vibronic subband represents approximately the 0–0 transition in a vibronic progression, whereas the pattern of subsidiary maximum and shoulders at shorter wavelength is set by the 0–1, 0–2, etc. transitions of a dominant vibrational mode of frequency.²¹ The second derivative spectrum of lutein (Figure 2, Table 1) clearly shows the progression of this vibration ($\sim 1480 \text{ cm}^{-1}$) which can be attributed mainly to the C=C ($1500\text{--}1600 \text{ cm}^{-1}$) and partly to the C–C (ca. 1200 cm^{-1}) stretching modes.²¹

In organic solution, at room temperature, chiral carotenoids have no definite CD activity in the visible region despite the

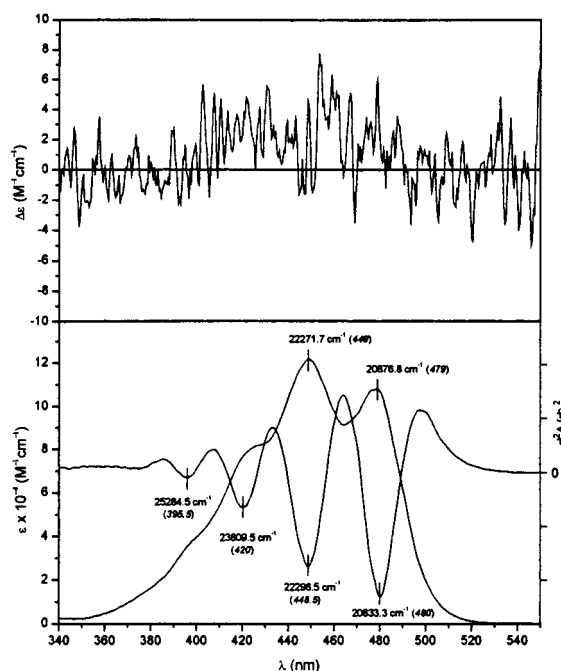


Figure 2. UV/vis and CD spectra of lutein in acetone. The second derivative curve of the absorption spectrum indicates the vibrational peaks positions. The spectra of lutein diacetate, which is very close to that of lutein, are not shown for clarity.

TABLE 1: UV/Vis Spectral Properties of Lutein and Lutein Diacetate.

	lutein	lutein diacetate
molar extinction coeff (ϵ), $\text{M}^{-1}\text{cm}^{-1}$	121600 (449 nm)	124000 (448.5 nm)
transition moment (M), cgs unit	1.40×10^{-17}	1.38×10^{-17}
transition length (r), Å	2.92	2.88
oscillator strength (f)	2.11	2.04
vibrational positions (σ), cm^{-1}		
0-0	20833.3 (480 nm)	20855.1 (479.5 nm)
0-1	22296.5 (448.5 nm)	22321.4 (448 nm)
0-2	23809.5 (420 nm)	23809.5 (420 nm)
0-3	25284.5 (395.5 nm)	25316.5 (395 nm)

strong light absorption (Figure 2). The linear polyene chain is nearly planar having no intrinsic chirality and the chiral end groups cause only weak asymmetric perturbation on the $^1A_g \rightarrow ^1B_u$ transitions. Relevant CD bands, originating from the molecular chirality, are located below 350 nm.²² Because the esterification has only a minimal influence on the electronic excitation of the polyene chain, the UV/vis and CD spectra of lutein diacetate is similar to that of lutein (Table 1).

UV/Vis and CD Spectra of Lutein in Aqueous Acetone Solution. By virtue of their hydrophobic character and extended π -system, carotenoids are highly susceptible to aggregation. Water addition alters dramatically the UV/vis spectrum of lutein, causing a large blue shift and bandwidth narrowing (Figure 3). The new absorption band at 371 nm has no vibrational fine structure, but it is asymmetric, showing a long absorption tail toward the visible range which carries weak traces of vibronic features. These spectral changes were permanent after 16 h except for a modest hypsochromic shift of the maximum to 368.5 nm.

In an aggregate, the molecules are noncovalently bound and close to each other and this situation favors evolving of excitonic resonance interaction between them. The behavior of the visible band of lutein upon water addition is typical of the formation

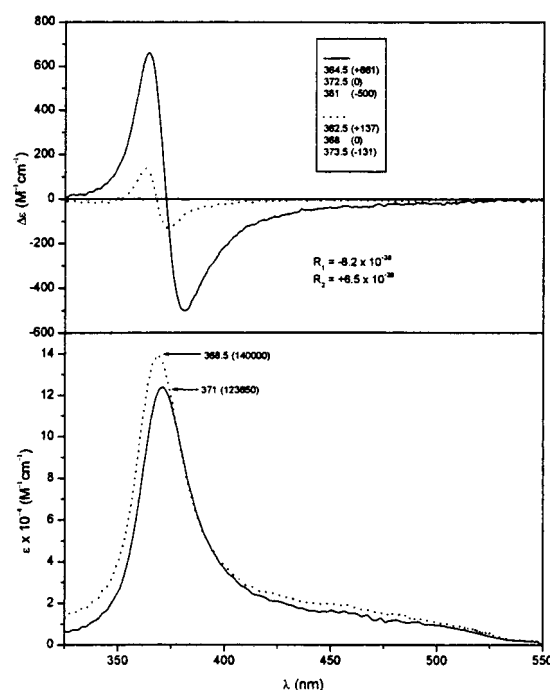


Figure 3. UV/vis and CD spectra of lutein in aqueous acetone. Solid line, after mixing; dotted line, after 16 h.

of stacked dimers and higher aggregates and the resulting exciton states. The simplest case is the parallel, so-called H-type dimer (where "H" means hypsochromic), in which the excited-state levels of the monomer split in two upon dimerization (Figure 4). This splitting is a result of the two possible orientations of the electronic transition moments of the polyene chains. Because of Coulombic repulsion, the in-phase oscillation means the existence of the higher energy member of the excited states. The out-of-phase combination which would lead to a red-shifted lower energy state, however, is forbidden because it has no net electronic transition moment, so is absent from the spectrum. In this case, therefore, only a blue-shifted peak can be observed in the absorption spectrum indicating the H-type geometry, in which the transition dipoles of the interacting lutein molecules are parallel to each other and orthogonal to the line connecting the long axes of molecules (the absorption spectrum was measured carefully between 550 and 750 nm, but no detectable band was found).

According to the point-dipole approximation, the interaction energy (V_{12}) between the neighboring transition dipoles depends on their value M , the intermolecular distance R , and the mutual geometries α and Θ in the following way:²³

$$2V_{12} = 4 \left(\frac{N-1}{N} \right) \frac{1}{4\pi\epsilon_0} \left(\frac{M^2}{R^3} \right) (\cos \alpha - 3 \cos \Theta_1 \cos \Theta_2) \quad (1)$$

where $2V_{12}$ is the exciton bandwidth in joule (see Figure 4); N , the aggregation number of interacting chromophores; M , electronic transition moment of the monomer in Coulomb meter; R , the point-dipole—point-dipole distance in meter, taken to be the distance between the centers of mass of the composite monomers; α , the angle between the transition dipoles; Θ_1 and Θ_2 , the angles between the transition dipoles and the line between the chromophore centers (tilt angle); and ϵ_0 , permittivity of free space ($8.8542 \times 10^{-12} \text{ C}^2 \text{ N}^{-1} \text{ m}^{-2}$).

The interaction energy ($V_{12} = 6121.1 \text{ cm}^{-1}$) was obtained as the difference between the 0-0 band energies of the lutein in monomer state and in aggregated form (see Table 1 and Figure

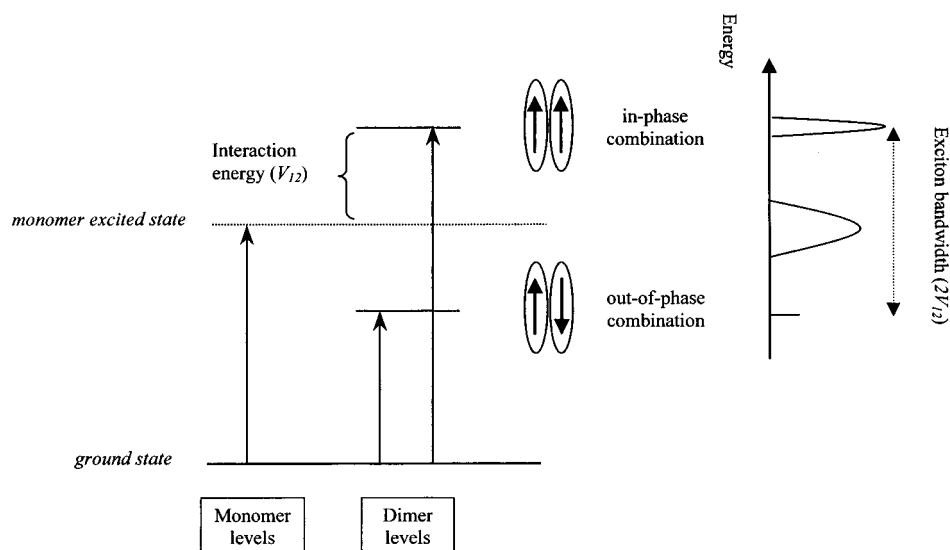
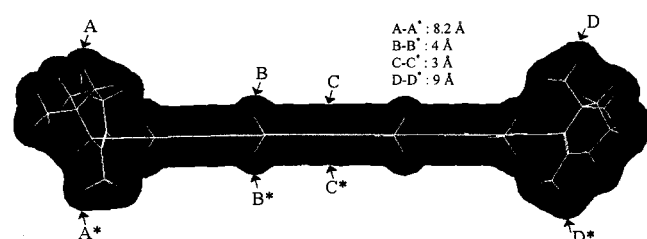


Figure 4. Schematic exciton band energy diagram showing selection rules of a parallel (card pack) dimer compared to a monomer (on the right: spectral effects of the exciton coupling).



<i>R</i> (Å)	3.50	4.50	5.00	5.50	6.00	6.50
<i>α</i> (degree)	36	35	23	18	17	15

Figure 5. Connolly surface of the geometry optimized lutein molecule (side view) and some characteristic geometrical parameters. Insetted table: see text.

3). At first approximation, we assumed that $\alpha = 0^\circ$, $\Theta_1 = \Theta_2 = 90^\circ$, and $N = 2$; so we obtained 5.45 Å for the intermolecular distance in the dimer. This value means the distance between the planes of polyene chains. However, the presence of the bulky end groups must be taken into account. In Figure 5, the Connolly surface of the lutein molecule shows approximately the spatial extension of the six-membered rings. To decrease unfavorable steric interactions between them, the alignment of molecules should slightly twist ($\alpha \neq 0^\circ$).

With the aid of the molecular modeling method, the angle between the long axes of the two dimerized lutein molecules were measured at given distances (see the insetted table in Figure 5). It can be concluded that in the dimer $\alpha = 20^\circ$, if $R > 5$ Å. Because the value of $\cos 20^\circ$ is 0.94, it does not modify strongly the result obtained by eq 1. Likely, intermolecular H-bonds between the hydroxyl groups play important roles in the formation of dimers as was evidenced by IR spectroscopy of the violaxanthin aggregates.²⁴ Further computational studies are in progress to characterize more exactly the stereochemistry of such molecular associates.

The large blue shift and the vibrationless quality of the new absorption band of lutein strongly resemble the so-called strong exciton coupling case of Simpson and Peterson.²⁵ In this model, criteria has been developed for two limiting cases, namely, strong and weak coupling, in terms of the strength of the intermolecular interaction relative to the width of the absorption

band for the individual molecule. In strong coupling, splitting between the excited energy levels $2V_{12}$ is considered to be significantly greater than the Franck–Condon bandwidth $\Delta\epsilon$ of the corresponding electronic transition level in the monomer:²⁶

$$2V_{12}/\Delta\epsilon \gg 1 \quad (2)$$

The Franck–Condon bandwidth is defined as the difference between the 0–0 band position (σ_{0-0} , see Table 1) and the weighted mean wavenumber of the monomer absorption band ($\bar{\sigma}$, see the Experimental Section). According to this, $\Delta\epsilon = 1680.7$ and $12242.2 \text{ cm}^{-1}/1680.7 \text{ cm}^{-1} = 7.28$

The structural background of the lacking vibrational structure is the tightly organized assembly, in which the single molecules loose their individual wave function including the electronic, vibrational, and rotational parts and behave as a supermolecule with a single electronic and vibrational wave function. So, the rate of the excited-state interactions between molecules becomes comparable to the time needed for the electronic transition in a single lutein molecule (10^{-14} – 10^{-15} s). The time required for the resonance interaction between excited states of the neighboring lutein molecules (so-called resonance lifetime) can be determined as follows:²⁶

$$t = \frac{h}{2V_{12}} = 2.76 \times 10^{-15} \text{ s} \quad (3)$$

where h is Planck's constant. The energy transfer rate t^{-1} is $3.64 \times 10^{14} \text{ s}^{-1}$. Consequently, the resulting excited state can no longer be associated with a single molecule, but must be associated with a whole assembly of interacting molecules.

The long tail on the lower energy side of the blue-shifted peak indicates that not all lutein molecules are organized in H-type geometry. The red-shifted spectral part probably originates from the contribution of a minor fraction of head-to-tail assembly (see lutein diacetate). Such coexistence of these distinct assemblies with different populations was reported for carotene^{12b,10d} and carotenoid aggregates,^{10b-c,12a} respectively. Furthermore, we reported a time-dependent conversion of a head-to-tail type assembly to an H-type one.²⁷ Some authors^{10c,12b} assigned the weak red-shifted band above 500 nm to the forbidden exciton pair of the strong blue-shifted peak. In our

opinion, however, this band indicates another molecular organization, discussed in detail at lutein diacetate, rather than the lower energy exciton component. It should be noted that the sign of interaction energy (eq 1) depends on the product of the $\cos \Theta_1 \times \cos \Theta_2$. Accordingly, if for a small fraction of the dimers $\Theta_1 \neq \Theta_2$, then different combinations of the angles may give rise to different signs of the interaction energy thus contributing to the observed spectral red shift.

Abrupt changes in the lutein absorption are accompanied by the appearance of a very strong bisignate CD band in the region of the blue-shifted absorption (Figure 3), exhibiting a first negative Cotton effect at 381 nm and a second positive one at 361.5 nm with negative A value ($\Delta\epsilon_{\text{first}} - \Delta\epsilon_{\text{second}} = -1160$). The zero crossover point (372.5 nm) nearly matches the absorption maximum.

The anisotropy factor, defined as $g = \Delta\epsilon/\epsilon$, reaches its maximum values of $+7 \times 10^{-3}$ at 361 nm and -6.2×10^{-3} at 386.5 nm, respectively. The rotational strengths of these observed Cotton effects (in cgs unit) were obtained by integration of peak areas:¹⁹

$$R = 2.296 \times 10^{-39} \int (\epsilon(\sigma)/\sigma) d\sigma \quad (4)$$

Having nearly equal absolute values (Figure 3), they are opposite in sign and seem to satisfy the so-called "regional sum rule", which is one of the requirements of the exciton coupling arising between interacting chromophores.²⁸ To obtain such an exciton-type CD couplet, the polyene chains must be in a chiral arrangement in the aggregated state. In other words, the interacting lutein molecules have to accommodate a given angle. The helical twisting of the conjugated π -system as a potential source of the chiral exciton splitting can be neglected because such distortion would produce only one, positive or negative, CD band. Because the measured chirality originates from the intermolecular interactions of the electric-dipole transitions of the polyene chains, the exciton chirality rule²⁹ can be applied. This reveals to dominate negative dihedral angle between the interacting chirally arranged chromophores within the assemblies. Obviously, the molecular chirality of the end groups is responsible for the chiral arrangement (achiral molecules, e.g., β -carotene, have no CD activity in the aggregated form). At first sight, it would be tempting to assign the exciton chirality to the twisted dimers. Taking into consideration, however, the closely parallel arrangement of lutein molecules indicated by the behavior of the absorption spectrum, the presence of such strong CD bands is rather surprising.³⁰ Furthermore, as found earlier in the case of capsanthol derivatives, the CD intensity greatly depends on the concentration and reaches extraordinary values, while the blue-shifted UV band does not.³¹

Additionally, amphotericin B, containing seven conjugated double bonds, gives rise to closely similar UV and CD spectra in aqueous solution and shows very large A value at certain concentrations.³²

Therefore, we propose the following:

- the formation of small units, e.g. stacked dimers, in which the molecules are closely packed are responsible for the large blue shift of the absorption maximum;
- excitonic interaction arising between the chirally arranged small units causes the intense CD couplet.

Naturally, the CD activity arising from dimers may contribute to the optical activity, but not in a decisive manner.

It should be noted that the CD spectrum showed prominent time dependency (Figure 3). After 16 h, the degree of the left-handed chiral organization was reduced by ca. 80% according

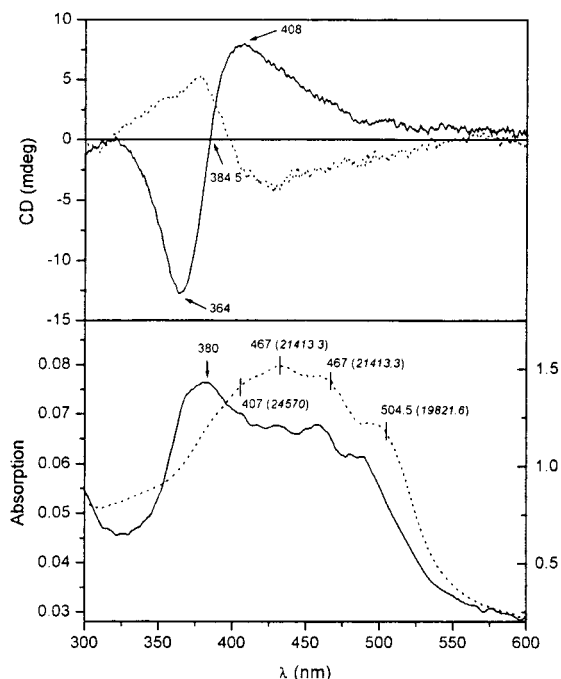


Figure 6. UV/vis and CD spectra of lutein film on a quartz slide (solid line) and lutein crystals in a KBr disk (dotted line, right axis). Vibrational peak positions obtained by the second derivative (the corresponding wavenumber values are in parentheses).

to the new g_{max} values ($+1.2 \times 10^{-3}$ at 362 nm and -1.1×10^{-3} at 374.5 nm). Presumably, the higher order chiral organization is destroyed in time but the short-range parallel packing prevails, as shown by the uniform absorption spectrum.

UV/Vis and CD Spectra of Lutein Thin Film and Crystals in a KBr Disk. Surprisingly, the lutein film shows a definite *right-handed* chiral exciton couplet corresponding to the blue-shifted absorption band at ca. 380 nm, proving that the molecules are in a chiral arrangement within the film with the opposite sense as observed in the aqueous solution (Figure 6). The g values calculated for the film, -5.6×10^{-3} at 363 nm and $+3.4 \times 10^{-3}$ at 409.5 nm, are comparable with those found in the aqueous solution showing the degree of chiral organization to be nearly equal in both cases. The fact that an achiral manipulation can cause sign inversion in the supramolecular chirality is known, but is not a usual phenomenon.³³

This suggests little energy difference between the "diastereomeric" structures, on the other hand, draws the attention to the potential role of water molecules in the aggregate organization. It is important to note that a significant fraction of the molecules is organized into different states as proved by the red-shifted long-wavelength side of the electronic spectrum analogously to that measured in the KBr disk. In the latter, the spectrum broadens on the short-wavelength side either, because around 380 nm a shoulder is present. Subtracting the normalized absorption spectrum of the lutein diacetate (in KBr, see Figure 9) from the that of the lutein, we obtain a definite peak at 380 nm suggesting the existence of a card-packed (H-type) molecular ordering in the crystal. In this region, the CD spectrum shows a weak, left-handed exciton couplet which is approximately in mirror image relation with one found in the thin film (Figure 6.). This points out that in the crystal only a minor portion of the molecules preserves the chirality found in the aggregates.

Atomic Force Microscopy of Lutein Thin Film. The AFM images showed unordered, rather amorphous aggregate formation all over the quartz surface (Figure 7).

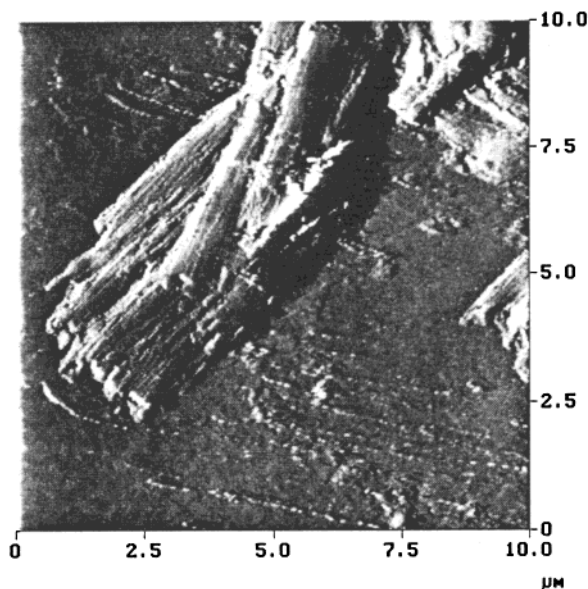


Figure 7. AFM image of lutein film on a quartz slide.

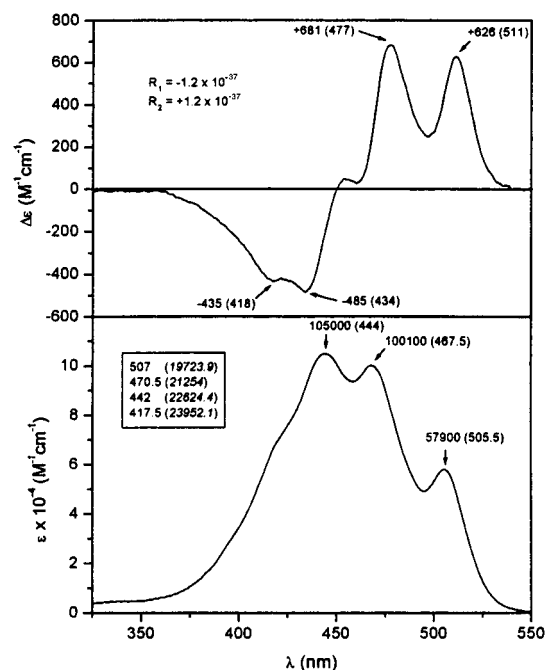


Figure 8. UV/vis and CD spectra of lutein diacetate in aqueous acetone. Inset shows the vibrational peak positions obtained by the second derivative (the corresponding wavenumber values are in parentheses).

UV/Vis and CD Spectra of Lutein Diacetate in Aqueous Acetone Solution. Contrary to lutein, water addition affects quite differently the UV/vis spectrum of lutein diacetate by widening and shifting the main absorption band toward longer wavelengths (Figure 8). The spectrum was unchanged after 16 h (not shown). By means of the second derivatives, the positions of vibronic bands were located and compared to those found in acetone (Figure 8, Table 1).

As can be seen, all vibrational peaks are red shifted by ~ 1200 cm^{-1} and the spacings between neighboring peaks are reduced. The 0–0 peak can be found above 500 nm. The Franck–Condon bandwidth of the absorption band measured in acetone is 1660 cm^{-1} (see the Experimental Section) while the same value for the aqueous solution is 2780.1 cm^{-1} proving a significant band broadening. It is well-known that the absorption

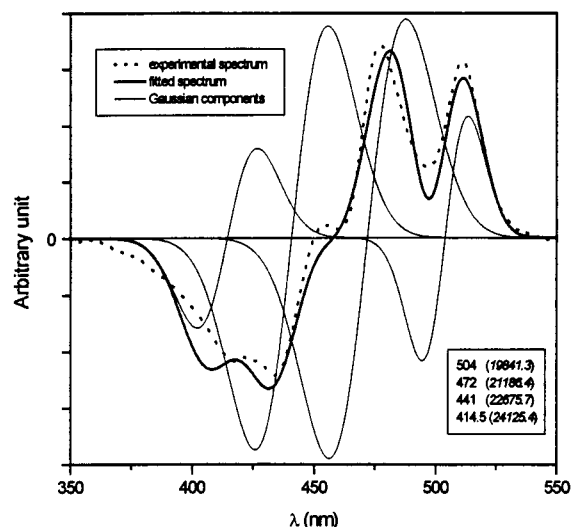


Figure 9. Reconstruction of the CD spectrum of aqueous lutein diacetate solution by four Gaussian exciton components. The inset shows the zero-line intersections of the positive–negative Gaussian couplets.

maximum of a carotenoid depends mainly on the solvent polarizability $[(n^2 - 1)/(n^2 + 2)]$ where n is the refractive index of the solvent.³⁴ In a solvent of larger n , carotenoids have a longer absorption maxima. Within the aggregate, lutein diacetate molecules are surrounded by themselves and this situation can be treated as if the molecules would be solved in a solvent with enhanced polarizability (obviously, the polarizability of polyene chains is greater than that of acetone). Consequently, the excitation energy is decreased and the spectrum is red shifted. However, this effect should not alter the overall shape of the spectrum; therefore, the observed spectral changes can be accounted: the increasing dispersion forces and the weak vibronic coupling between the polyene chromophores causes band broadening and lowering of the vibronic spacing.

It must be emphasized that analogous spectral changes were reported in the electronic absorption spectrum of all-trans- β -carotene in spin-coated (SC) and the Langmuir–Blodgett films.³⁵ It was concluded that the short-range order similar to that of the single-crystal exists in the films and the β -carotene molecules is weakly aggregated. These results reinforce our conclusions because lutein diacetate behaves quite similarly in aqueous solution and in thin film. Unfortunately, eq 1 cannot be applied because V_{12} obtained from the spectral shifts contains the dispersive interaction term as well.

These spectral characteristics indicate that the interacting molecules are no longer in such close contact as those in the lutein assembly and that they preserve their own wave functions significantly. As a consequence, the resonance energy transfer is slower and vibrationally coupled.

Furthermore, the red shift may indicate a sort of head-to-tail arrangement of the esterified molecules described in the J-aggregates. The structural basis of the difference between the lutein and diester aggregates is the lack of the free hydroxyl functions and the presence of the bulky acetyl groups in the latter. In the absence of intermolecular H-bonds, the molecules are not forced to a closely packed parallel arrangement and their long axes may shift side-by-side resulting a bathochromic shift.

These moderate changes of the visible absorption band is accompanied by, however, dramatic transformation of the CD spectrum. High and broad, positive–negative Cotton effects evolve having pronounced vibrational fine structure and showing no time dependency (Figure 8). The positive and negative CD

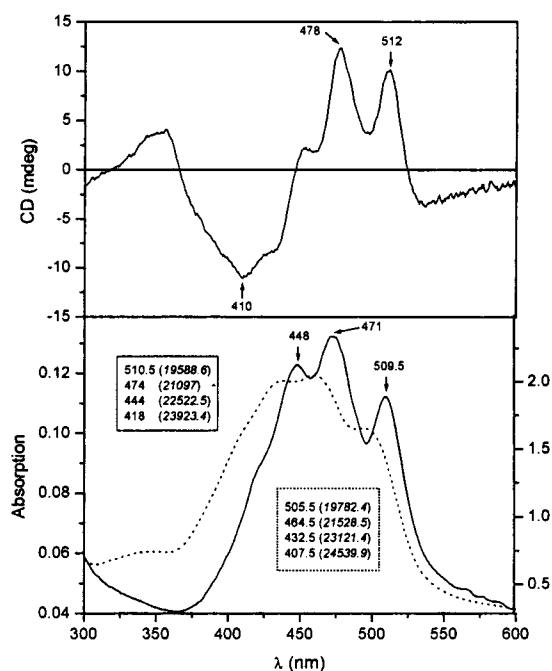


Figure 10. UV/vis and CD spectra of lutein diacetate film on a quartz slide (solid line) and lutein diacetate crystals in a KBr disk (dotted line, right axis). Insets show the vibrational peak positions obtained by the second derivative (the corresponding wavenumber values are in parentheses).

maxima do not coincide with the maxima of the vibrational components in the absorption spectrum. The positive A value ($\Delta\epsilon_{477} - \Delta\epsilon_{434} = +1166$) suggests right-handed chirality between the coupled chromophores. The maximum g values were measured at 517 nm ($+1.4 \times 10^{-2}$) and 408 nm (-7.3×10^{-3}), respectively. The rotational strengths of positive and negative areas of the Cotton effects are very similar to each other ($\pm 1.2 \times 10^{-37}$ cgs unit). Therefore, it appears that chiral exciton coupling between the $^1A_g \rightarrow ^1B_u$ transition moments results in these split Cotton effects. This coupling, however, greatly differs from that observed in lutein aggregate. The vibronic components split individually and, in an ideal case, the resulting exciton couplets would have zero-line intersections at the absorption maxima of the corresponding vibronic bands.¹⁹ However, coalescence of the couplets leads to a CD curve measured by us and also reported for cis isomers of astaxanthin diacetate at -180°C .^{14b} An attempt has been made to resolve the CD spectrum of lutein diacetate and to show how the splitting occurs. On Figure 9, the experimental spectrum is tentatively reconstructed from four Gaussian CD couplets each of which having plus-minus order of the sign from the longer wavelength side.

Their zero crossover points fit the vibrational peak positions obtained from the second derivative (cf. Figure 8). Such vibrational exciton coupling was observed between the 1L_a transitions of the anthracene chromophores locked in a chiral position in the composite molecule¹⁹ and in molecular associates of vitamin D₃ derivatives at low temperature.³⁶ Furthermore, it is worth mentioning that, with a high anisotropy factor, a very similar intense vibronic splitting was described for several cases in the field of optically active polymers, e.g., chiral polythiophenes, having extended π -system.³⁷

UV/Vis and CD Spectra of Lutein Diacetate Thin Film and Crystals in a KBr Disk. The CD spectrum of a lutein diacetate film shows the presence of an analogous chiral superstructure with the same handedness as observed in aqueous

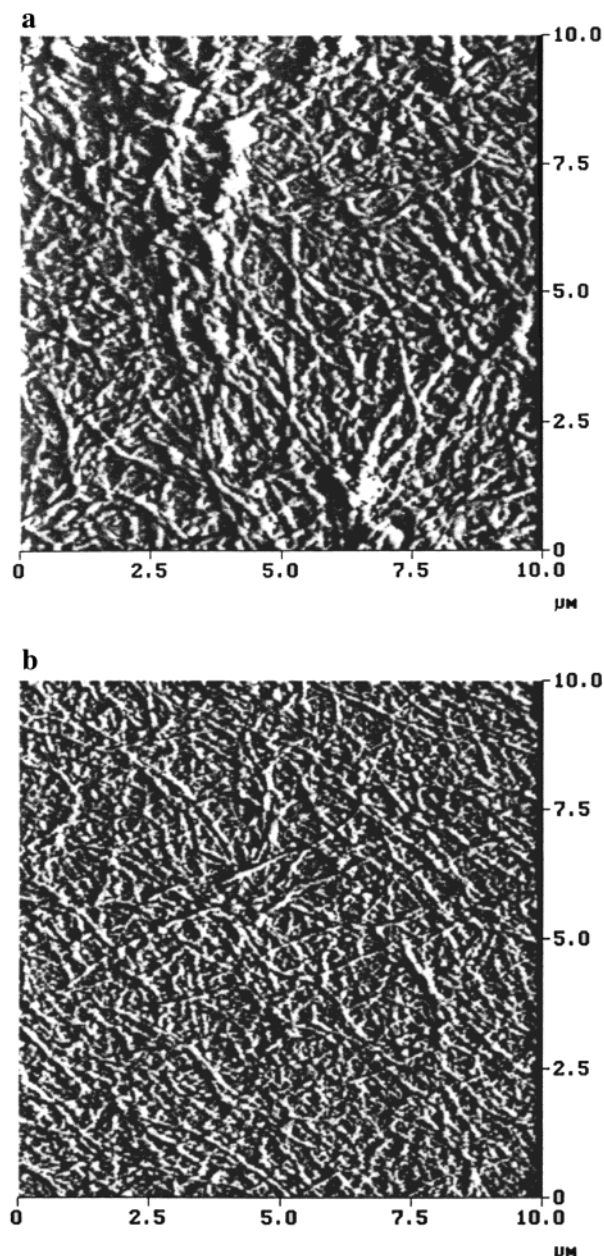


Figure 11. (a) AFM image of lutein diacetate film on a quartz slide. (b) AFM image of lutein diacetate film on a graphite surface.

solution (Figure 10). The maxima of the visible absorption band of the film show further modest red shifts (~ 4 nm) relative to the aqueous solution. An additional peak appeared about 356 nm having no corresponding absorption band. Presumably, rapid evaporation of the solvent initiates analogous aggregation as aqueous dilution. The absorption spectrum of the KBr disk is also red shifted but broader than the observed one in aqueous solution or thin film. This suggests the enhancement of intermolecular interactions in the crystal relative to the aggregated phase.³⁵ The vibrational peak positions well match to those found in lutein crystals and the two band contours are similar except for the short wavelength side. This means that the major fraction of lutein and lutein diacetates are packed in the crystal by analogous, head-to-tail manner lacking supramolecular chirality.

Atomic Force Microscopy of the Thin Film Composed by Lutein Diacetate. Figure 11a shows the formation of a pronounced fiberlike structure with junctions at certain places.

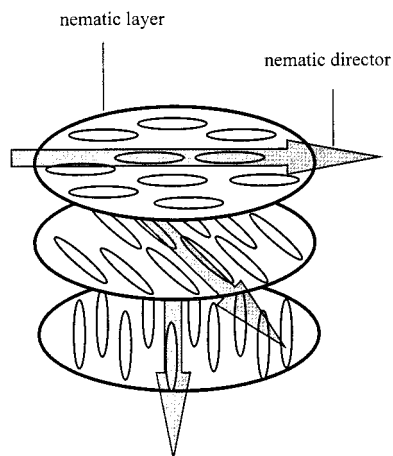


Figure 12. Representation of chirally twisted nematic layers composed by lutein diacetate molecules.

The formation of these “knots” is presumably due to the inhomogeneity and the hydrophilic character of the quartz surface. The formation of thin film on the hydrophobic surface of HOPG shows the same threadlike structure, but the layer is uniform and no junctions can be observed (Figure 11b). Diameters of threads are ranged between 60 and 120 nm on the HOPG and 120–180 nm on the quartz slide, respectively. This beautiful threadlike morphology is highly characteristic of liquid crystals, a more exact replica of the so-called nematic mesophases in which the constituent molecules tend to remain oriented along a specific direction, named “nematic director”. The degree of orientational order present in a nematic liquid crystal is not nearly as perfect as in a crystal, but still represents an amount of order absent in an ordinary liquid. Within a nematic layer, lutein diacetate molecules are parallel but they can freely move along the nematic director and do not form stacked dimers or oligomers found in lutein aggregates. This molecular arrangement resembles rather to a head-to-tail formation which may contribute to the bathochromic shift of the absorption spectrum.

On the other hand, because lutein diacetate is optically active it forms chiral nematic phase which means that the layers twist with respect to those above and below (Figure 12). The twisting direction between the layers are the same, governed by the molecular chirality through weak, noncovalent intermolecular interactions. Therefore, it seems plausible that a right-handed twisting between the layers may be responsible for the observed strong exciton chirality in the visible region. The fact that a similar CD spectrum was obtained in the aqueous solution suggests that lutein diacetate is able to form lyotropic mesophase either.³⁸

Our findings are in full harmony with the early observation of Sitte³⁹ who described very similar diacylluteine strands and clusters showing pronounced optical anisotropy.

It is worth mentioning that liquid crystalline type aggregates of cyanine dyes were also reported.⁴⁰ Because liquid crystals also seem to be intimately involved in the functions of biological structures,⁴¹ these unique properties of carotenoids deserve further investigations in the future.

Conclusions

Two requirements must be satisfied to obtain chiral carotenoid assemblies: the existence of configurational chirality and suitable conditions for the aggregation process. However, our results and literature data point out that the presence of water is not the only means to generate aggregation. Also, other circumstances, e.g., low temperature or rapid evaporation of

solvent forcing carotenoid molecules to approach each other, may produce this phenomenon.

In solution and thin film, lutein forms a typical H-aggregate in which the molecules are closely packed and oriented nearly parallel. Simple exciton calculation, based on the point-dipole approximation, showed that in a hypothetical lutein dimer the molecules are separated by ca. 5.5 Å and aligned by sharp angle ($<20^\circ$) because the steric hindrance of the end groups. We propose that such small units (dimers and/or oligomers) held by intermolecular H-bonds are responsible for the large blue shift and loss of vibrational structure in the electronic absorption spectrum. On the other hand, the chiral arrangement of these units leads the observed strong excitonic CD activity with opposite handedness in solution and thin film. Lacking the opportunity to form intermolecular H-bonds, lutein diacetate aggregates with weakly interacting monomers displaying slow, vibronically coupled energy transfer. The moderate bathochromic shift of the absorption spectrum is explained by the enhancement of the refractive index inside the aggregates and by the existence of a head-to-tail type molecular arrangement. The band shape changes can be attributed to the vibronic coupling of molecules. On the AFM images, lutein diacetate exhibited beautiful threadlike morphology suggesting the existence of a nematic liquid crystalline mesophase. Because the CD spectra of the film and the solution was very similar, it is highly probable that the same mesophase exists in both circumstances.

This means the ability of lutein diacetate to form a lyotropic mesophase in aqueous solution. Supposedly, because the centers of asymmetry, the nematic layers composed by lutein diacetate twisted to each other allowing a chiral exciton interaction between them manifested by intense, vibronically coupled CD bands.

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