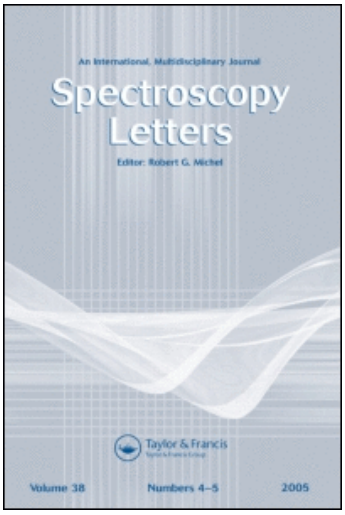

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Comparison of Various Films Used in Biophysical Investigations as Anisotropic Matrices

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COMPARISON OF VARIOUS FILMS USED IN BIOPHYSICAL INVESTIGATIONS AS ANISOTROPIC MATRICES

KEY WORDS: Polyvinyl alcohol films, cellophane, gelatin, phycobiliproteins, synthetic pigments, polarized components of absorption spectra

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INTRODUCTION

Polarized spectra of biological molecules oriented in artificial systems can give useful information of structural and spectroscopical properties of these molecules[1, 2]. Such investigations have been carried out recently for several biological systems: chlorophylls[3, 4, 5, 6, 7], carotenoids[8], biliproteins[9, 10], bacterial reaction centers[11, 12], bacteriochlorophylls[13] etc.

The orientation of the solute molecules can be achieved by the application of an electric or a magnetic field[14, 15], by flow or by mechanical deformation of polymer films[10, 11, 13]. In the last method several different media have been used. The aim of this paper is to provide information facilitating the choice of an anisotropic medium for a given biological sample. For that purpose a comparison of properties of three widely used films from gelatin, cellophane and polyvinyl alcohol (PVA) is presented.

A proper combination of anisotropic medium-solute has to fulfil as many as possible of the following conditions:

1. the solute has to be uniformly distributed in the whole volume of the matrix,
2. the chemical properties of the solute, as well as the film-solute interactions should be independent of the degree of deformation of the medium,
3. the deformation of the matrix should give an efficient orientation of the investigated molecule,
4. before deformation, the investigated species should be randomly orientated,
5. in many cases[2] it is further convenient if uniaxial orientation of molecules can be achieved,
6. any aggregation of the solute molecules should be independent on film deformation.

The behaviour of the investigated films is here tested by using two synthetic dyes: Congo red (C-red) and Solantine red (S-red), which are both molecules of elongated shape, see FIGs 2 and 3. Further, two pigment-protein complexes from red and blue-green algae: phycoerythrin (PE) and phycocyanin (PC) as well as the chromophoric group from PC - phycocyanobilin (PCB).

EXPERIMENTAL

Congo red and Solantine red were of chemical grade purity. C-red was considered an indicator of the film orientation, on the basis of an x-ray examination showing correlation with the linear dichroism of this dye in the same film[16]. Also S-red is known to be very efficiently oriented by anisotropic media[17]. PE and PC were obtained from Ceramium rubrum and Anacistis nidulans, respectively. Methods of extraction and purification of PE and PC are described elsewhere[18]. PCB was obtained by method of O'hEocha[19].

The pigment-protein complexes PE and PC have both four pyrrol chromophoric groups (FIG. 1), which are bonded to proteins of a molecular weight (in the natural hexamer form) about 290.000 and 273.000 daltons, respectively[20]. PCB can occur in several conformations

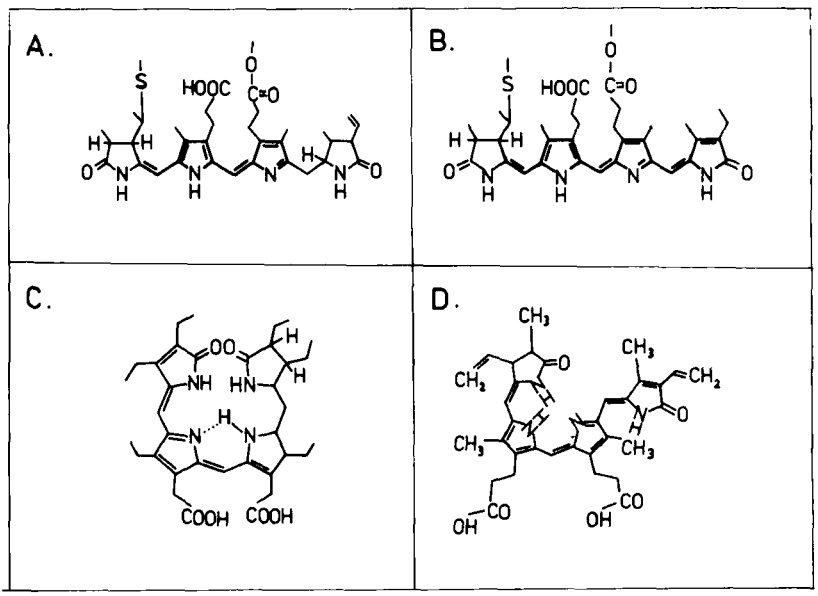

FIG. 1 Structural formulas of phycobilins: phycoerythrobilin (A), phycocyanobilin (B) and a proposed conformation of phycocyanobilin (C)[21] and biliverdin (D)[22].

- linear or more coiled ones [21, 22, 23], depending on the medium-solute interaction[24].

The pigments were introduced in PVA and gelatin films in the following way. The pigment was dissolved in PVA-water or gelatin-water (10% w/v) solution. A film was obtained by pouring such a solution on the glass plate and drying until the film contained about 20% of water. For details about the film prepartion, see Refs 25 (PVA) and 26 (gelatin).

The anisotropy of these films was characterized by their mechanical deformation: a degree of elongation is evaluated as $E = \frac{\Delta l}{l_o} \times 100\%$.

Commercial cellophane film is anisotropic because of the method of production, therefore it can be used as orienting medium without stretching[27]. Cellophane film was immersed in a water solution of dye for 6 to 8 hours, and subsequently rinsed with plenty of distillated water, in purpose of washing away any pigment from the surface, and thereafter dryed. In this way coloured film of C-red and S-red were easily prepared with PCB, however, the pigment has first to be dissolved in methanol and this solution then diluted by water (1:1 v/v) and the cellophane be thereafter soaked in such a mixture, coloured, rinsed and dryed. PE and PC, on the other hand, cannot be introduced into cellophane by this method.

Absorption and linear dichroism spectra were obtained with a Carl Zeiss Jena Specord UV-VIS spectrophotometer equipped with polaroid sheets. The absorption of light at two orthogonal orientations of the film was measured: $A_{\parallel}$ with the electric vector parallel, and $A_{\perp}$ with the electric vector perpendicular to the direction of film stretching; therefore, it was not necessary to introduce the Azumi-McGlynn correction[28].

Table 1. Linear dichroism $\left(S = \frac{A_{\parallel} - A_{\perp}}{A_{\parallel} + 2A_{\perp}} \right)$ of colored films $\left(\Delta S = \pm 0.005 \right)$.

In brackets - values of λ corresponding to measured S.

Pigment / Matrix	$\frac{\Delta I}{I_o} \times 100\%$	Congo red (C-red)	Solantine red (S-red)	Phycoerythrin (PE) 10^{-3}	Phycoerythrin (PE) 10^{-5}	Phycocyanin (PC) 10^{-4}	Phycocyanin (PC) 10^{-3}	Phycocyanobilin (PCB)
gelatin	0%	0.000	0.027	-	S [566] = =0.028	-	0.000	-
	150%	0.008	0.023	-	S [566] = =0.032		0.014	-
cellophane		0.212	0.274	-		-		0.032
polyvinyl alcohol PVA	0%	0.000	0.000	S [495] = =0.000 S [555] = =0.000	S [500] = =0.005 S [555] = =0.008	0.001	0.000	0.000
	300% 200%	0.670	0.318	S [495] = =0.032 S [555] = =0.019	S [500] = =0.036 S [566] = = 0.026	0.041	0.000	0.123

RESULTS AND DISCUSSION

Table 1 collects values of S, defined as $S = \frac{A_{\parallel} - A_{\perp}}{A_{\parallel} + 2A_{\perp}}$, which is a measure of the degree of orientation of the sample and the anisotropy of its absorption. Because of technical difficulties with solubilities only some combinations of solutes and anisotropic matrices could be evaluated.

The anisotropy of absorption of both the synthetic dyes C-red and S-red is practically constant in the whole absorption region, however, with the complexes, the function S was dependent on the wavelength; therefore in this case the λ at which S was calculated is given in bracket in Table 1.

As seen from Table 1 and FIGs 2, 3, C-red and S-red are more efficiently oriented in PVA and cellophane than in gelatin.

The anisotropy of absorption of the PE and PC complexes is of the same order of magnitude in all three matrices (FIGs 4, 5).

As seen from FIG 2 the absorption of C-red in all three films exhibits a red shift with respect to the absorption of this dye in water solution. In a dry PVA film, the monomer (maximum at 512 nm) and dimer (538 nm) absorption bands are well resolved. The shape of the absorption spectrum in cellophane suggests also some contribution from dye aggregates in the long wavelength

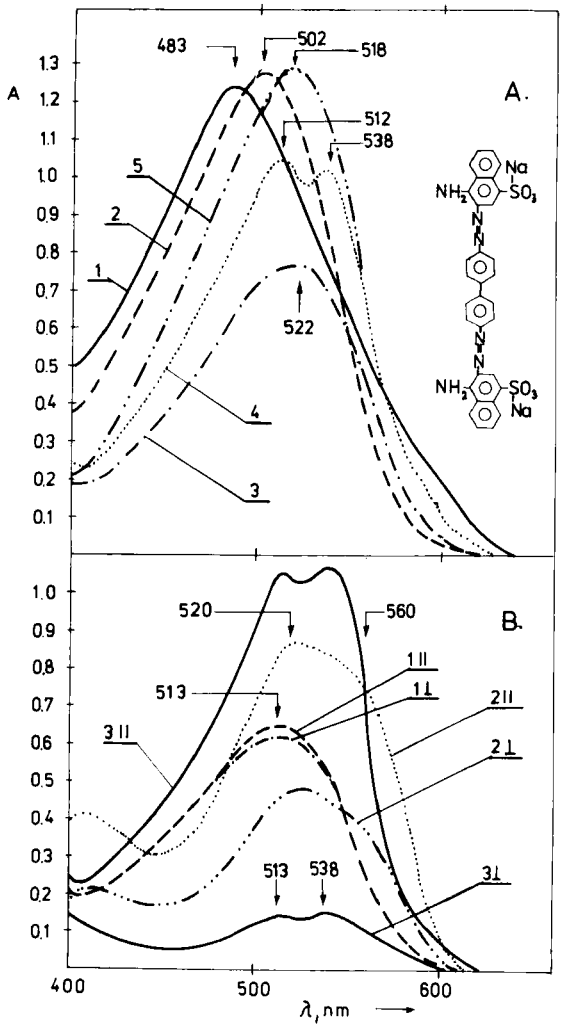

FIG. 2 A. Congo red absorption spectra, 1 - in water, 2 - in methanol, 3 - in PVA-water solution, 4 - in dry PVA film and 5 - in gelatin-water solution = in dry gelatin film.
B. Polarized components of absorption spectra of Congo red, 1 - in gelatin film elongated till 150%, 2 - in cellophane and 3 - in PVA film stretched till 300%.

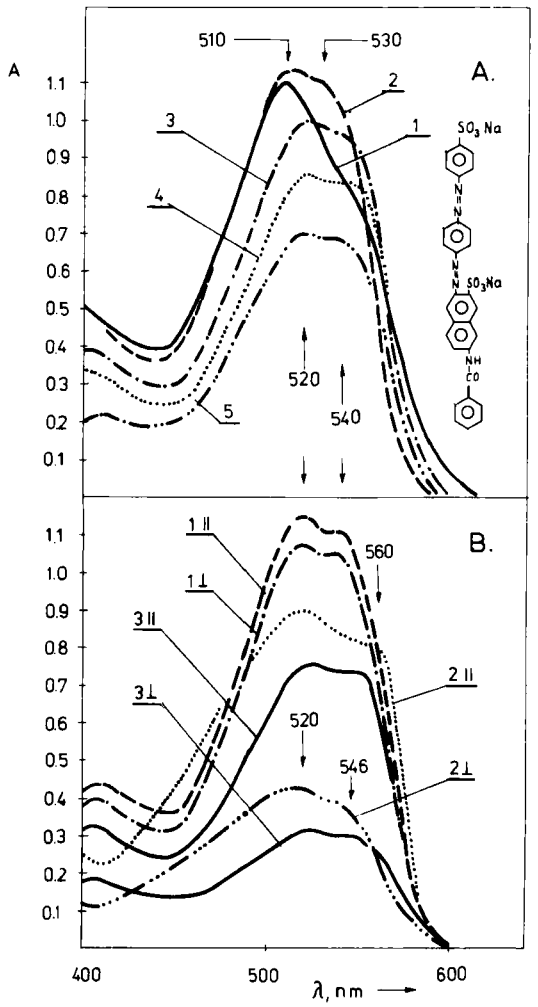

FIG. 3 A. Solantine red absorption spectra (1 - in water, 2 - in methanol, 3 - in PVA-water solution, 4 - in dry PVA film and 5 - in gelatin-water solution = in dry gelatin film).
B. Polarized components of absorption spectra of Solantine red (1 - in gelatin film elongated till 150%, 2 - in cellophane and 3 - in PVA film stretched till 300%).

region (about 560 nm). In gelatin the C-red molecule is almost unoriented, whereas in both the other films it is oriented to a high degree (Table 1 and FIG. 2). In PVA and cellophane the shapes of the parallel and perpendicular absorptions are similar indicating that the monomer and the aggregates are oriented in a similar manner.

Upon comparison of the spectra of S-red (FIG. 3) with those of C-red (FIG. 2) one can see the following small differences

1. S-red in unstretched gelatin exhibits a small positive anisotropy of absorption ($A_{\parallel} > A_{\perp}$), whereas C-red is isotropically distributed. Stretching of the film does not affect the small anisotropy of S-red and it is probable that it can be related to some anisotropy produced in the process of film preparation.
2. PVA orients C-red more efficiently than S-red, whereas the degree of orientation of S-red is a little higher than that of C-red in cellophane.

FIG. 4 shows spectra of PE in different media. In both, gelatin and dry PVA films shape of spectrum is deformed with respect to that in the buffer and in the solutions of PVA and gelatin. In films, the maximum at 548 nm decreases and that at 566 nm increases. The shape of the spectrum, as well as the spectral dependence of the absorption anisotropy, is a function of the pig-

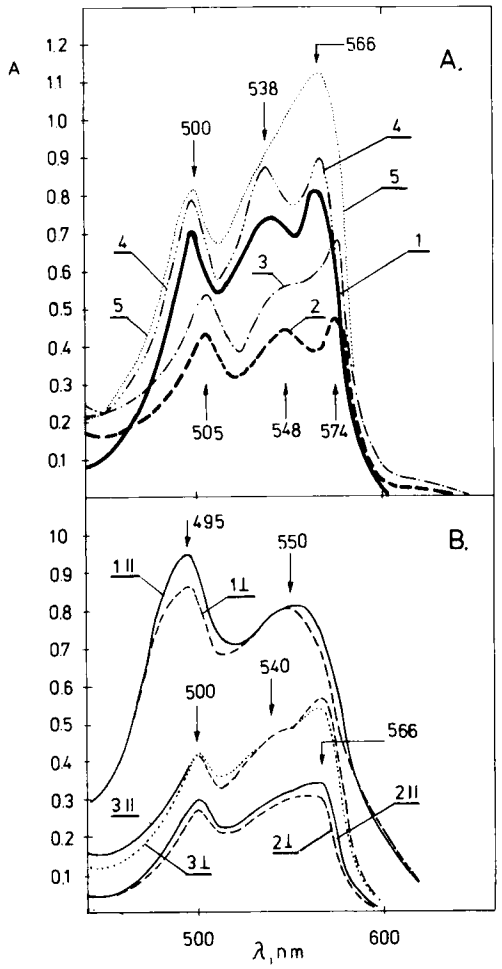

FIG. 4 A. Absorption spectra of phycoerythrin (PE). Curve 1 - in phosfate buffer, 2 - in liquid gelatin (10% gel in water), 3 - in dry gelatin film, 4 - in PVA-water solution and 5 - in dry PVA film.

B. Polarized components of absorption spectra of PE, "high" (10^{-3} M) concentration (curves 1) and "low" (10^{-5} M), concentration (curves 2 and 3); curves 1 and 2 - in PVA film (stretched 300%)and curves 3 - in stretched gelatin film (150%).

ment concentration and the type of the anisotropic matrix. For concentrated PE samples (10^{-3} M) in PVA, the signs of S at long and short wavelengths are different. The transition moments responsible for the absorption at 500 nm (phycourobilin) are well oriented parallel to the direction of stretching in PVA, but almost unoriented in gelatin.

A corresponding set of spectra for PC is presented in FIG. 5. The change in shape of the spectrum as a result of film drying is smaller for PC than for PE. An anisotropy of absorption of PC in PVA is only observed for high pigment concentration, at low, as it was reported previously[9], $A_{\parallel}$ is equal $A_{\perp}$ (FIG. 5). Even at average PC concentration in gelatin, anisotropy of absorption is well measurable.

FIG. 6 shows spectra of PCB and the chromophoric groups of PC. The PCB orientation in all matrices is low in comparison with that of C-red and S-red in the same films (Table 1), which suggests that these chromophores occur in both films, not in elongated conformations, but in semicoiled or coiled forms (FIG. 1)[23, 24]. In PVA film PCB shows large differences in S values between the long (660 nm) and short (608 nm) wavelength regions. This effect, which is not observed for PCB in cellophane film (FIG. 6), can be explained by the two--phase character of PVA[24, 29, 30]. The PVA matrix is com-

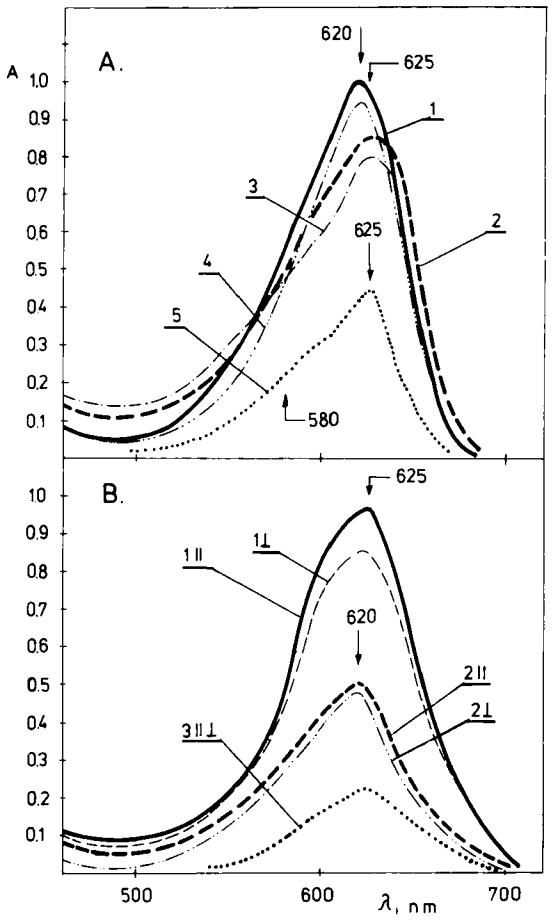

FIG. 5 A. Absorption spectra of phycocyanin (PC). Curve 1 - in phosfate buffer, 2 - in liquid gelatin, 3 - in dry gelatin film, 4 - in PVA-water solution, and 5 - in dry PVA film. B. Polarized components of absorption spectra of PC "high" (10^{-4} M) concentation (curve 1 and 2) and "low", 10^{-5} M, concentration (curves 3); curves 1 and 3 - in PVA sheets stretched (300%) and curves 2 - in stretched (150%) gelatin films.

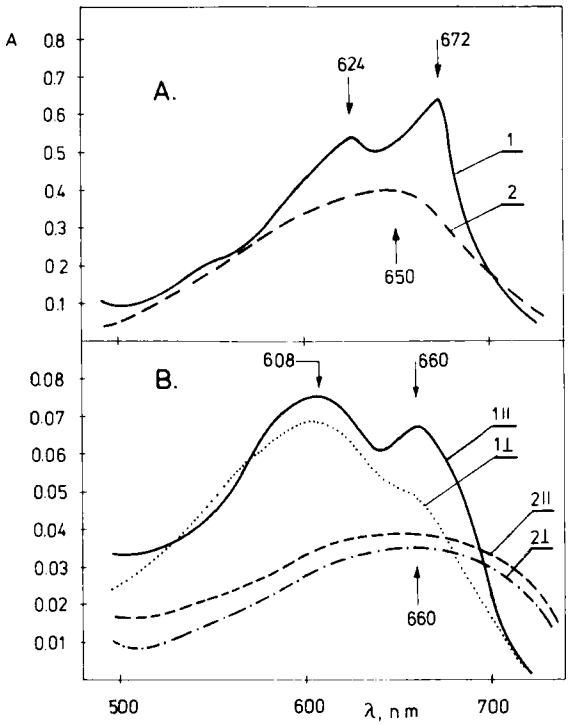

FIG. 6 A. Absorption spectra of phycocyanobilin (PCB) in: 1 - chloroform and 2 - methanol.
B. Polarized components of absorption spectra of PCB: 1 - in stretched (200%) PVA and 2 - in cellophane.

posed of crystallites embedded in an almost isotropic medium. The crystalline region differs in acidity from the rest of matrix, thus the basic form[19] of the pigment (absorbing at 608 nm) occurs predominantly in the amorphous phase, whereas the acidic form (660 nm) is seen in the crystallites. As a result the acidic form is oriented to a higher degree than the basic one.

From the presented result one may draw the following conclusions:

1. PVA and cellophane are more efficient in orienting pigment molecules than gelatin, but the absorption anisotropy of the pigment-protein complexes in PVA and gelatin are similar.
2. The complexes are not only oriented as a whole in the anisotropic films, but may also to some extent be deformed as a result of film stretching, as indicated by different degrees of orientation of various chromophores.
3. The degree of aggregation of the synthetic dyes is independent of the film deformation, but the problem is more complicated in a case of pigment-protein complexes[9, 10].
4. PVA can not always be treated as a uniphasic medium - such a treatment is especially dangerous with PVA for pigments with spectra sensitive to the medium acidity. In other cases it seems to be an excellent matrix, and it should be noted that it does not change the spectral properties of biological pigments more than gelatin (which is often recommended as a more "natural" medium)[26]. It has further the advantage that it can be used both on the complexes and their chromophoric groups, which thus enables comparison of the orientations at similar conditions.

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