Optical anisotropy of uniformly aligned planar surfactant lyotropic nematic doped with hemoglobin

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Abstract

The system under study is a nematic phase of lyotropic surfactant liquid crystal, cetylpyridinium chloride/hexanol/brine. Optical birefringence measured for well-aligned surfactant nematic (surfonematic) is weakly dependent on the light wavelength within the studied spectral region (500–700 nm) and its absolute value is about 6×10^{-4}. Doping of the surfonematic by hemoglobin (1.5% by weight) does not affect the birefringence value, suggesting that the hemoglobin does not produce its own birefringence and does not affect the scalar orientational order parameter of the surfonematic matrix. The absorption coefficients of hemoglobin in the surfonematic matrix exhibit a broad band shifted several tens of nanometres towards longer wavelengths, when compare to the spectrum of hemoglobin dissolved in water. The latter indicates that the hemoglobin residues form molecular complexes with the surfactant molecules. Weak ($\sim2\times10^{-2}$) linear dichroism of hemoglobin at the wavelengths corresponding to light absorption by oxygen bonds gives the estimation of the orientational scalar order parameter of hemoglobin molecules in well-aligned surfonematic matrix ($\sim10^{-2}$).

Keywords: birefringence, dichroism, hemoglobin, surfactant nematic

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1. Introduction

Success in decoding of genomes for several living organisms has demonstrated a power of molecular biology equipped with physical and chemical characterization techniques. High efforts of biologists are now focused on the studies of protein. A new field of biology, proteomics, is signified by large-scale protein analysis and needs high-throughput approaches. Progress in the proteomics studies is largely dependent on availability and functionalities of samples with sufficient amount of proteins. In majority of cases, the studies are performed using single crystals composed of protein molecules. A single biocrystal represents a convenient possibility for structural characterization of biological molecules. In contrast to a solvent or a powdered amorphous sample, where the

probed molecules are disordered, the signal detected from a single crystal is significantly amplified due to periodical and orientational molecular arrangement. However, the growth of single biocrystals is still an art rather than experimental techniques. Only tiny crystals have been obtained, which are usually not larger than a few hundreds of micrometers, randomly dispersed on the substrate surface or in bulk in the environment of mother solution. In many cases, the crystal specimens are overlapped or shadowed by other solid inclusions and thus are not applicable for characterization, especially by optical methods. There is a great need in development of new techniques providing protein samples compatible with the modern characterization techniques, in particular optical ones. Another possibility for preparation of anisotropic protein samples is using a lyotropic liquid crystal (LLC) as an aligning matrix, in which protein molecules are dissolved. This approach has been employed in the NMR studies [1–7]. However, there is a difficulty: in contrast to thermotropic analogues, the uniform alignment in the LLC is hard to achieve. So, the alignment in the NMR experiments is induced using high magnetic fields. The orientation provided with magnetic fields is weak and very slow, requiring hours for stabilization. This possibility is not applicable for the optical investigations. The latter fact might explain the absence of literature results on optical characterization of biological macromolecules dispersed in the LLC matrices. Recently the alignment techniques for preparation of uniformly oriented planar [8] lyotropic chromonematic cells have been developed. Uniform planar chromonematic cells have been used for optical characterization of a number of chromonematics [9]. Chromonematics are the phases analogous to conventional thermotropic nematics. They belong to one of the two distinctive classes of LLCs, namely to lyotropic chromonic liquid crystals (LCLCs) [10]. LCLCs are formed by plank-like aromatic molecules, which aggregate in water solution, stacking face-to-face onto each other and forming rod-like aggregates. In the ground state of chromonematic phase, the aggregates are parallel, with a scalar orientational order parameter relatively high even at the transition to isotropic phase (compare the value 0.42 typical for thermotropic nematics to 0.7 or higher for chromonematics [9]). Another distinctive class of LLCs covers liquid crystal phases formed by surfactants in a liquid solvent. It is called as lyotropic surfactant liquid crystals (LSLCs). Surfactant nematics are analogues of thermotropic and lyotropic chromonic nematics. In surfactant nematics (referred to as *surfonematics* further on), amphiphilic rod-like molecules form cylindrical or disc-like aggregates, such that their hydrophobic chains are pointed inside the aggregate and their hydrophilic heads form its surface. In contrast to chromonematics, there are no techniques to align surfonematics. In Section 2 of this paper, we report on successful and reliable planar alignment of surfonematic of a lyotropic system cetylpiridinium chloride/hexanol/brine, comprised in a flat cell between mechanically buffed glass substrates. In Section 3 we use these planar samples for measurements of optical birefringence and its dispersion. Having a reliable technique for preparation of uniform planar surfonematics at our disposal, we then test this anisotropic medium as an aligning matrix for hemoglobin molecules. For this aim, we measure the

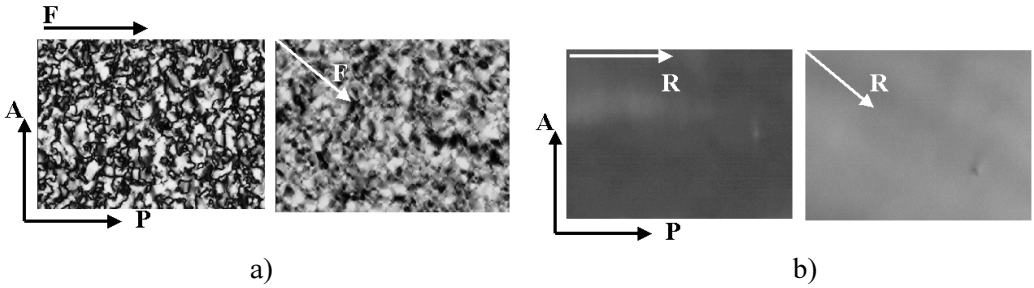
absorption spectra of surfonematic doped with the hemoglobin and check whether these well-aligned cells have dichroism, from which the orientational scalar order parameter of hemoglobin molecules in the surfonematic matrix could be estimated (Section 4). Finally, we draw conclusions in Section 5.

2. Preparation of uniform planarly aligned surfonematic cells

2.1. Materials. The system under study is brine solution of cetylpyridinium chloride (CPCl, surfactant) and hexanol (co-surfactant). CPCl, hexanol and hemoglobin have been purchased from Sigma and the brine for injections (pharmaceutical water solution of NaCl, 0.9% by weight) has been produced by Human Godollo, Budapest, Hungary.

The phase diagram of the system in its brine-rich side (75–98% of brine by weight) has been studied in details in [11–13]. Three phases separated by biphasic regions are present on the phase diagram drawn in the coordinates $1-\phi$ vs. h/c, with ϕ being the brine concentration and *h/c* being the co-surfactant-to-surfactant amount ratio. At lower brine concentrations (starting from about 65% by weight) and for low *h/c*, spherical micelles transform into cylindrical worm-like aggregates. According to [14], nematic phase is located in a rounded closed region of the phase diagram ($63 < \phi < 65\%$; $0.11 < h/c < 0.19$). At lower ϕ and higher *h/c* one finds a hexagonal columnar phase. We have prepared a solution with the composition $\phi = 64\%$, $h/c = 0.11$ that corresponds to the nematic phase, according to [14]. To check that we really deal with the nematic phase, we have inspected its textures under the optical polarization microscope (see below).

2.2. Cell preparation. Glass substrates washed in distilled water and isopropanol, separated by $75\,\mu m$ thick Mylar strip spacers, are fixed together with 5-min epoxy applied on both sides along the spacers. Surfonematic solution fills the cell through one of the open sides due to lowered pressure from a vacuum cleaner connected by a rubber pipe to the opposite open side of the cell. After filling the residual solution is removed and the open sides are sealed again with 5-min epoxy. After glue solidification, the cell is covered from the sides by 1-hour epoxy in order to close possible crack in the first epoxy layer,



a) b)

Fig. 1. Textures of surfonematic between the crossed polarizers **P** and **A** placed between: (a) untreated glass substrates (the filling direction **F** is parallel to the polarizer, left, and makes the angle $45°$, right); (b) rubbed glass substrates (the rubbing direction **R** is parallel to the polarizer, left, and makes the angle $45°$, right).

which could appear on its solidification. In one hour the cell is placed on a hot stage (HS-2 from Instec) under the optical polarization microscope (Leitz) for textural inspection. Fig. 1a demonstrates that the alignment of the surfonematic induced by pressure gradient is poor (the cell filling direction **F** is parallel to the polarizer direction **P** on the left photo and it is rotated by 45^{o} with respect to **P** on the right one). To improve the alignment, we have tested several different substrates usually used for planar alignment of thermotropic nematics: rubbed polyimid 2555 (Nissan) and LARC CP1 [15]. We have found that a uniform director alignment might be hardly achieved when following literally the alignment procedures. According to our experience on the alignment of chromonematics, planar orientation in these liquids crystals is obtained with substrates covered by rubbed polymers, which produce homeotropic alignment for conventional thermotropic nematics without rubbing. We have indeed found that the surfonematic under study can be uniformly aligned in a planar fashion with the substrates covered by the rubbed polymer JALS-204. It is important to stress that a good alignment for surfonematics needs rubbing the polymer layer more intensively than for the usual case of thermotropic nematics: one has to rub it 50–60 times, while 1–2 times are enough for thermotropic nematics. It is worth noticing that heating of the cell to the isotropic phase helps to eliminate misalignments appearing during the cell filling. For the composition $\varphi = 64\%$ and $h/c = 0.11$ we find the nematic-isotropic phase transition at the temperature $T = 30°C$. This is important for the preparation of samples doped with hemoglobin, in order to prevent hemoglobin denaturation, which takes place above $40°C$. We have also tested clean glass substrates unidirectionally rubbed 50 times and obtained a uniform director orientation. Polarization microscope photographs (see Fig. 1b) demonstrate the textures of surfonematic between the rubbed glass substrates placed between the crossed polarizers. The rubbing direction **R** is parallel to one of the crossed polarizers (**P** or **A**) in Fig. 1b (left) and occupies a $45°$-azimuthal position in Fig. 1b (right). If one compares Fig.1a and Fig.1b, good quality of the alignment becomes evident. The texture in Fig. 1a represents the so-called Shlieren texture [16], a characteristic nematic texture, which differs strictly from characteristic textures for the smectics (a Focal Conic Domain texture) and the columnar phase (a texture of Developable Domains). The textural analysis ensures that we indeed deal with the nematic phase.

Our experiments show that clean rubbed glass substrates orient surfonematic planarly. This can be expected if one takes into account that cleaned glass carries negative electrostatic charge while the ionic CPCl residues are electrically positive. Electrostatic adsorption of the CPCl residues normally to the glass substrate surface favours the planar orientation of cylindrical aggregates at the substrate surface. Additional negative electrostatic surface charging is expected after rubbing the glass.

Surfonematic cell with the clean rubbed glass substrates manifest a good optical quality and so it has been used for the measurements of dispersion of optical birefringence.

3. Birefringence dispersion of a surfonematic

The measurements were performed using a two-channel Hitachi spectrophotometer. The cell was placed normally to the light beam. The polarization efficiency of our dichroic film polarizers, overlapping both channels, was constant (99.9%) in the spectral region $500 - 700$ nm under studies.

Polarization microscopic observations indicate clearly that the phase retardation of the cell is much lower than 2π. Gray colour of the cell placed in diagonal position between the crossed polarizers reveals that the optical path difference between the ordinary and extraordinary rays for the $75\ \mu$m thick planar cell is smaller than 50 nm, thus corresponding to the phase retardation of about $\pi/15$. This latter allows us to determine the birefringence of surfonematic as a function of light wavelength from the transmittance T_{45}, measured under the condition of diagonal position of the cell director with respect to the parallel polarizers:

$$\Delta n(\lambda) = \frac{\lambda}{\pi d} \arccos \sqrt{T_{45}(\lambda)}\,. \qquad (1)$$

The transmittance T_{45} is calculated as the ratio of spectrophotometer signals measured for $45°$- and $0°$-azimuthal positions of the nematic director. The results are shown in Fig. 2 by solid circles. We have checked this result, while measuring the birefringence with the Senarmont technique (the He-Ne laser light wavelength $\lambda = 632.8$ nm). The corresponding result ($|\Delta n| = 6 \times 10^{-4}$) shown as open triangle in Fig. 2 agrees well with the data alluded to above.

It follows from these results that the birefringence of surfonematic under test is low, at least two orders of magnitude lower than that typical for conventional thermotropic nematics. Such a low magnitude could be expected owing to dilution of the system. It can also be a manifestation of small scalar order parameter: the aggregates have a shape of flexible worm-like micelles, which significantly bend at fluctuations. In the studied spectral region the birefringence is practically constant within the experimental accuracy, which is about 5%. The authors [17] claim that there might be at least two mechanisms for the birefringence in lyotropic liquid crystals. The first is concerned with the anisotropy of molecules, while the second one (i.e., the form birefringence) is caused by anisotropy of the aggregates. Indeed, one can expect a positive birefringence for a lamella composed of rod-like surfactant molecules, oriented perpendicularly to the lamella, while an assembly of parallel lamellae made of isotropic material and dispersed in the isotropic solvent should produce a negative birefringence [18]. Interplay between the positive and negative contributions from the molecular anisotropy and the form-birefringence has been reported for different materials forming surfactant lamellar L_α phase [17,19]: at some dilution, non-oriented samples appear uniformly dark or slightly coloured (actually blue in most of the reported experiments), whereas at lower volume fractions of the solvent, usual bright colours typical for strongly birefringent materials are observed. It has been

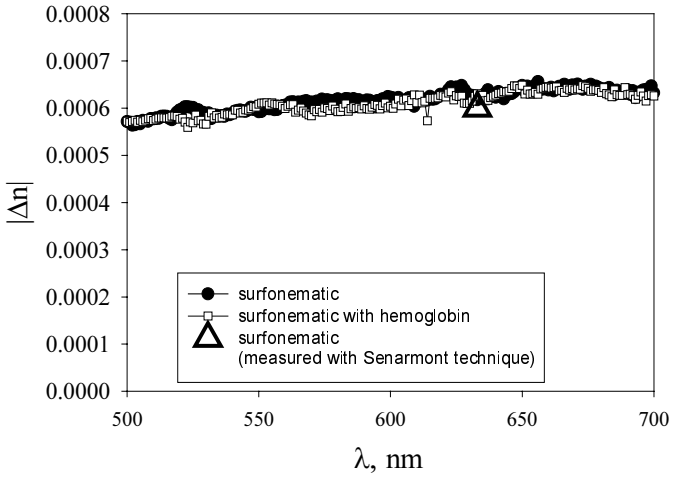


Fig. 2. Birefringence dispersion for the pure surfonematic (solid circles) and that doped with hemoglobin (1.5% by weight) measured with the spectral technique; open triangle stands for the value obtained for the surfonematic without hemoglobin measured with the Senarmont technique.

shown in [17] that the anomalous birefringence of diluted lyotropic smectics can be simply understood as vanishing of the birefringence at some particular dilution, for which the negative form birefringence of the stack of lamellae matches exactly the positive birefringence of each lamella. The exact cancellation only occurs at a particular wavelength, which would depend on the composition.

In our case we have just the opposite situation: the rod-like molecules staked into rods are expected to produce a negative optical sign and the form birefringence from the long rods made of isotropic material and dispersed in the solvent would produce positive birefringence [18]. We have not observed any change in the birefringence sign and therefore conclude that the compensation of the birefringence contributions due to these two mechanisms does not take place for the studied surfonematic and/or in the studied spectral region.

4. Optical anisotropy of surfonematic doped with hemoglobin

The sample was prepared by adding CPCl and hexanol to the brine solution (0.9% NaCl) of hemoglobin (1.5% by weight) in the amounts giving $\phi = 64\%$ and $h/c \simeq 0.11$. Polarized spectra of planar cell measured at the director azimuths of 0°, 45° and 90° with respect to the parallel polarizers are shown in Fig. 3. Optical transmittance T for anisotropic light-absorbing uniaxial media of the thickness d and with its optical axis being at an angle α with respect to the parallel polarizers can be written as [21]

$$T = \frac{e^{-\xi}}{2}\left[\left(1+\cos^2 2\alpha\right)ch\delta - 2sh\delta\cos 2\alpha + \cos\Delta\varphi\sin^2 2\alpha\right], \qquad (2)$$

where

$$\delta = \frac{2\pi\left(k_0 - k_{90}\right)d}{\lambda};\ \xi = 2\pi\left(k_0 + k_{90}\right)d/\lambda;\ k_{90} = -\frac{\lambda}{4\pi d}\ln T_{90};$$

$$k_0 = -\frac{\lambda}{4\pi d}\ln T_0;\ \Delta\varphi = \frac{2\pi}{\lambda}\Delta nd.$$

Spectral dependences of the absorbing indices $k_0(\lambda)$ and $k_{90}(\lambda)$ are shown in Fig.3 by thick and thin lines, respectively. As seen from Fig. 3, the spectra of hemoglobin dissolved in water (the $k(\lambda)$ dependence shown by a dotted line) and in the surfonematic (the dependences $k_0(\lambda)$ and $k_{90}(\lambda)$ shown by thin and thick lines, respectively) differ essentially. For the hemoglobin in water we find two characteristic peaks of its oxy–hemoglobin form: at $\lambda_1 = 538$ nm and $\lambda_2 = 576$ nm. The first peak is hard to find in the spectra of hemoglobin in the surfonematic, while the second one is clearly visible in $k_{90}(\lambda)$ but absent in $k_0(\lambda)$. The spectra for hemoglobin dispersed in the surfonematic matrix exhibit an intensive wide absorption band in the region of $540 - 640$ nm. Comparison of the spectra observed for the hemoglobin in water and that in surfonematic indicates unambiguously that the hemoglobin residues create molecular complexes with positively charged surfactant ionic residues. In addition, the spectra $k_0(\lambda)$ and $k_{90}(\lambda)$ display dichroism.

Dispersion of the dichroism parameter calculated as

$$\eta(\lambda) = \frac{k_0(\lambda) - k_{90}(\lambda)}{k_0(\lambda) + k_{90}(\lambda)} \tag{3}$$

is shown in Fig. 4. A nonzero dichroism value would indicate that the hemoglobin molecules are aligned in the surfactant matrix.

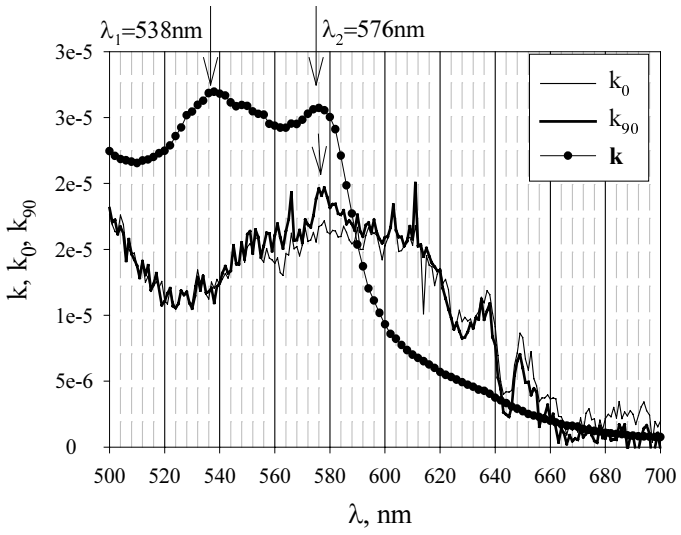


Fig. 3. Dispersion of the absorption coefficients for hemoglobin dissolved in water (*k*, dot line) and that placed in the aligned surfonematic matrix ($k_0(\lambda)$ and $k_{90}(\lambda)$, shown as thin and thick lines, respectively)

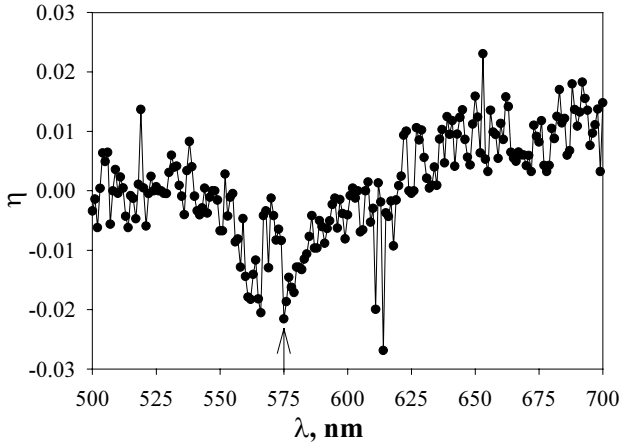


Fig. 4. Dispersion of dichroism for the hemoglobin placed in the aligned surfonematic matrix (calculated from the data $k_0(\lambda)$ and $k_{90}(\lambda)$ shown in Fig. 3).

Using the spectral data for the absorbing indices $k_0(\lambda)$ and $k_{90}(\lambda)$ and the known angle between the molecular dipole moment and the director and following the approach for orientationally ordered, light-absorbing anisotropic medium [20], one can calculate the orientational scalar order parameter for hemoglobin molecules. It is seen from Fig. 5 that the dichroism of hemoglobin-doped cell has a broad extremum ($\eta \approx -0.02$) in the region from 560 to 580 nm. It is worth noticing that the mentioned spectral region is characteristic for distinction between the oxy- and desoxy-hemoglobin forms and it corresponds to light absorption by the oxygen chemical bonds in hemoglobin. A negative dichroism value suggests that the absorption dipole moment is significantly tilted with respect to the director, being rather perpendicular than parallel to the director. Therefore, we conclude that the hemoglobin molecules in the surfonematic matrix are aligned such that the oxygen bonds are roughly perpendicular to the director. In such a case one can estimate the scalar order parameter as

$$S = \frac{1-N}{1+\frac{1}{2}N}, \tag{4}$$

where $N = \frac{k_0}{k_{90}}$ is the dichroic ratio. Then we obtain the estimation $S \sim 10^{-2}$ for the light wavelength corresponding to the dichroic peak $\eta \approx -0.02$. The obtained value of the order parameter is low, if compared with the maximally possible value equal to 1. This indicates that the ordering of hemoglobin molecules in the aligned surfactant matrix is rather weak, though it is about one order of magnitude higher than that reported in the NMR experiments [2], where the surfactant liquid crystal is aligned with high magnetic field. We thus are led to conclude that the orientation order of hemoglobin molecules in the surfonematic matrix aligned by rubbed glass substrates is much stronger than it is usually produced by magnetic fields in thick samples without special anchoring.

Considering that the phase retardation of the cell under study is much smaller than 2π, we can calculate the birefringence according to the analysis performed for optically uniaxial light-absorbing media [21]:

$$\Delta n = \frac{\lambda}{2\pi d} arc\cos\left(\frac{4T_{45} - (T_0 + T_{90})}{2\sqrt{T_0 T_{90}}}\right). \quad (5)$$

Dispersion of the birefringence derived in this manner for the surfonematic doped with hemoglobin is shown in Fig. 2 by open squares. It is seen from Fig. 2 that, within the experimental accuracy, the birefringence values for the hemoglobin-doped surfonematic coincide with those obtained for the surfonematic without hemoglobin. Two important conclusions follow from this result: first, the hemoglobin molecules do not exhibit their own birefringence and, second, they do not affect the orientational scalar order parameter of the surfonematic.

5. Conclusions

As far as we know, we report for the first time a successful alignment of a surfonematic between the rubbed glass substrates and the corresponding measurements of its birefringence. The absolute birefringence value has proven to be low enough (6×10^{-4}). Although in the most cases of industrial applications of liquid crystals a high birefringence is sought for, there are situations when low birefringences are of some interest, too. One of the examples is Focal Conic Polarization Microscopy, where high birefringence of liquid crystals worsens resolution of the technique. Another application of low-birefringent LLCs could be related to studies for dispersion properties of birefringent biological objects introduced in surfactant matrices. Here high birefringence background of the LLC matrix would only shadow the effect of the dispersed particles.

Hemoglobin dissolved in the well-aligned surfonematic matrix displays polarized absorption spectra that differ from the corresponding spectra typical for the aqueous solution of hemoglobin: (1) a wide absorption band located in the spectral region of 540–640 nm and peculiar for the hemoglobin in surfonematic is different from that for its aqueous solutions and (2) the polarized spectra of hemoglobin in the aligned surfonematic display the effect of dichroism. The dichroism of hemoglobin molecules in the surfonematic matrix is maximal in the spectral region that corresponds to the light absorption by oxygen bond. The first oxygen absorption band seen in the spectrum of aqueous solution of hemoglobin at $\lambda_1 = 538$ nm is hard to find in the spectra of hemoglobin dispersed in the surfonematic matrix. The second characteristic oxygen absorption band located at $\lambda_2 = 576$ nm is present in $k_{90}(\lambda)$ though absent in $k_0(\lambda)$. These features indicate that the hemoglobin residues form anisotropic complexes with positively charged surfactant residues. These complexes are aligned by the surfonematic matrix but are not birefringent and do not affect the orientational order of the surfonematic matrix. The orientational order parameter of hemoglobin molecules in the

well-aligned surfonematic matrix is about 10^{-2}, which is one order of magnitude higher than that achieved when using high magnetic fields as an alignment action. The maximal dichroism of hemoglobin molecules aligned in the surfactant matrix is observed for the spectral region, which is characteristic for the oxygen band. In our opinion, the latter can be used for characterization of the degree of hemoglobin oxygenation and might be also useful when searching for optical markers of the diseases accompanying with oxygen transportation disturbances.

Acknowledgement

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