Monolayer States of a Cyanine Dye Studied by a Spectroscopic Technique

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(Z. Naturforsch. 28 c, 144–148 [1973]; received November 29, 1972)

Monolayer states, absorption spectroscopy, cyanine dyes, dye dimers

A single beam, single reflectance photometer for investigation of monolayers at the air water interface is described. A cyanine dye monolayer is studied. The molecular arrangements in different monolayer states are derived from the observation of the monomer-dimer equilibrium of the dye chromophores.

1. Introduction

The usual techniques for the investigation of lipid monolayers at the air-water interface involve measurements of macroscopic variables of the interface. Conclusions about the molecular arrangement can be derived only rather indirectly. The most powerful tools to obtain direct information on the molecular scene such as X-ray diffraction, IR-spectroscopy and electron-microscopy are applicable only with monolayers transferred onto a solid support. However in the special case of a surface active dye absorption spectroscopy in the visible range can be applied to the study of the dye monolayer at the air-water interface. Here from the absorption spectra direct inferences on the state of the chromophores *i. e.* on the molecular arrangement within the film may be drawn. In this paper measurements with a monomethin cyanine dye are reported. Due to two paraffin chains attached to the five-membered rings this dye has lipid properties. The overall shape of this molecule is not too much different from that of usual lipids. Measurements are performed with a single beam, single reflectance spectrophotometer. This experimental design is more versatile and somewhat simpler than several other experimental set-ups described in the literature[1–5].

2. Experimental Technique

The working principle of the photometer used is shown in Fig. 1. A light beam is focussed onto a mirror below the water surface of a Langmuir trough and reflected back onto a ligth sensitive device. The monolayer under investigation is enclosed between two barriers and can be moved into the light beam. The

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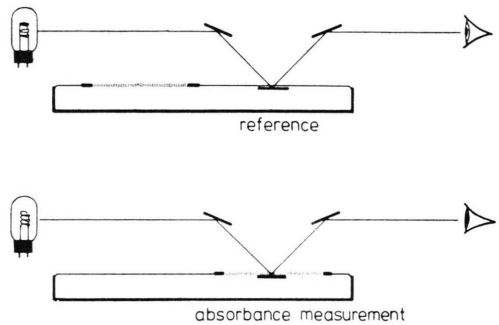

Fig. 1. Scheme of a single beam monolayer photometer. The monolayer is enclosed between two barriers and can be moved along the Langmuir trough. A light beam is focussed into the trough and reflected back into a light sensitive device. The trough is equipped with a Wilhelmy balance, an area registrating device, motor driven barriers, and a constant pressure device. The two barriers can be moved independently or may be coupled. The photometer equipment and the different types of measurement are described in the text.

trough[6] is circular and milled from teflon. The two barriers rest on the edges of the trough and are motor-driven by two concentric axles. The barriers may be moved independently or coupled in order to compress or to transport the enclosed film. The film area between the barriers is measured by means of a pontentiometer, the film pressure is measured by a Wilhelmy balance attached to one of the movable barriers. Surface pressure and area may be registered on an X-Y recorder (Hewlett Packard 7001 AM). A constant pressure device is also included in the electronic control unit.

A tungsten filament lamp (Osram 100 W, 12 V) in combination with a highly stabilized power supply (Metronic 102 CC) is used as a light source. The filament is focused onto the entrance slit of a monochromator (Jarrel, Ash 82-410). By several lenses and a 60°-prism the exit slit is focused onto a mirror placed 0,5 cm below the water surface (microoptical bench set, Spindler & Hoyer). The light beam is then focused onto the photocathode of a photomultiplier (EMI 9558 QB) by a prism and several lenses.

A stabilized high voltage supply (Nucletron NU 1250 B) is used for the multiplier. The anode current is dropped at a 100 kΩ resistance. After passing through an impedance transformer the signal voltage is observed directly in a digital voltmeter (Fluke 8300 A) or registered on a line recorder (Rohde & Schwarz ZSG 1), the reference signal being compensated by a stabilized power supply (Hewlett & Packard 6205 B). Absorbance measurements are reproducible within $5 \cdot 10^{-4}$ absorbance units.

Besides recording surface pressure — surface area isotherms, this monolayer photometer may be applied to several types of measurement:

a. Monolayer scanning: The reference is taken at a given wavelength. The monolayer enclosed between the two barriers is slowly moved across the light beam which has a diameter of about 1 mm². The signal is recorded continually. In this manner the homogeneity of the film can be controlled.

b. Surface absorbance — surface area — isotherm: The reference is taken at a given wavelength. The monolayer enclosed between the two barriers is shifted into the light beam. The monolayer is compressed slowly. The absorbance signal is registered on the Y axis of an X-Y recorder (Hewlett, Packard 7001 AM), whereas the area is registered on the X-axis.

c. Surface absorbance spectrum: The wavelenght is scanned stepwise. After each step the monolayer is shifted out of the beam, the reference is taken, the monolayer is shiftet back into the beam to take the absorbance. In the reference position the multiplier high voltage is adjusted automatically to give the same reference signal at all wavelengths. In this manner a calibrated spectrum is obtained. While scanning the film back and forward it is always stopped automatically in the same positions for reference and measurement. A disadvantage of this single beam procedure is the small film loss occuring at the edges upon frequent shifting of the monolayer. This decrease in surface concentration however can be compensated by the constant pressure device.

3. Measurements

a. Substance

The different types of measurement mentioned above are used in addition to the surface pressure — surface area — isotherm for characterization of the monolayer behaviour of a surface active monomethin cyanine dye. This dye (see Fig. 2) forms a stable monolayer at the air water interface[7]. The synthesis of the dye is described in ref[8]. A 17,5 μM solution in freshly distilled chloroform is used for spreading. Different amounts of the solution are distributed on a surface of highly purified water (distilled twice, once on alkaline permanganate). The measurements are performed at room temperature (approx. 25 °C).

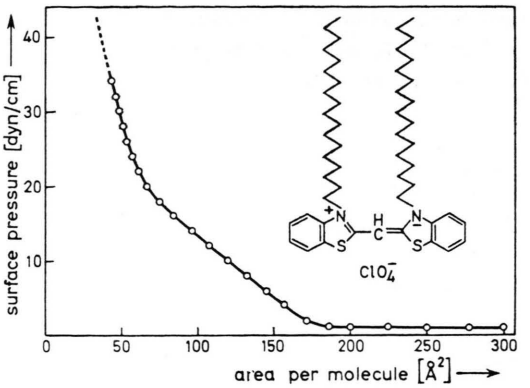

Fig. 2. Surface pressure versus area per molecule of a monolayer of the cyanine dye, which is depicted in the figure. The concentration of the chloroform solution for spreading is 17,5 μM. The area values have been obtained after keeping the surface pressure constant for five minutes at each point.

b. Surface pressure — surface area — isotherm

1 ml of a 17,5 μM solution of the dye in chloroform is spread on 350 cm² of a clean water surface. (A larger initial area per molecule does not change the compression isotherm).

The surface pressure at a certain area depends on the compression rate. In order to obtain equilibrium values the compression is interrupted after each increase of surface pressure by two dyn/cm and the pressure is kept constant for ten minutes. The resulting surface pressure — surface area isotherm is shown in Fig. 2. Above 180 Å²/molecule the pressure is nearly constant at 1 dyn/cm. At 180 Å²/molecule the pressure starts to increase linearly until an area of about 60 Å²/molecule is reached. Then the rise in surface pressure becomes very steep. A definite collapse area cannot be determined, since, because of its stiffness, the film collapses earlier in front of the compressing barrier than near the balance.

c. Monolayer scanning

In order to control the homogeneity of a dye monolayer the film is scanned at various surface concentrations as described. The absorbance is recorded at 430 nm, the absorption maximum of the dye. It is found that at surface areas larger than about 170 Å²/molecule the absorbance fluctuates strongly during scanning. At areas smaller than 170 Å²/molecule the

absorbance is practically constant all throughout the film. Two examples of scanning records are shown in Fig. 3, one at an area above, one at an area below 170 Å²/molecule.

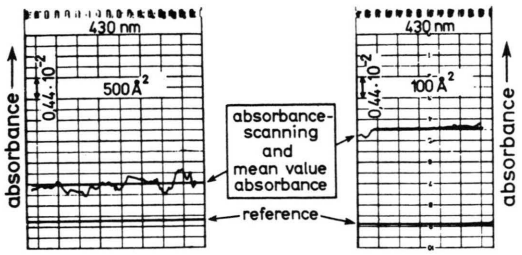

Fig. 3. Surface absorbance at 430 nm (absorption maximum of the dye monomer (see Fig. 2) *versus* time during scanning the monolayer across the light beam of the photometer. One scan is taken at 500 Å²/molecule showing fluctuations typical for films at areas larger than about 170 Å²/molecule. The other scan is taken at 100 Å²/molecule and is typical for areas smaller than 170 Å²/molecule. The straight line at the bottom of the graphs is the zero line, the straight line across the absorbance line indicates the mean absorbance of the film.

d. Surface absorbance — surface area — isotherm

The absorbance at 430 nm is registered as a function of the film area. (In the inhomogeneous region the film is scanned back and forth at different film areas to obtain a mean value). The results are plotted in Fig. 4. It is shown that below $60 \cdot 10^{12}$ molecules/

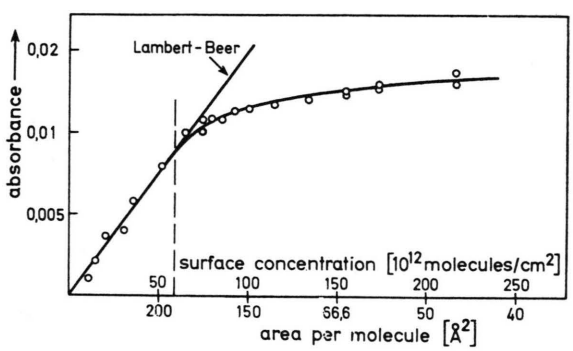

Fig. 4. Surface absorbance at 430 nm (absorption maximum of the dye monomer) *versus* surface concentration of a monolayer of the cyanine dye (see Fig. 2). The points were obtained in two experiments. The dotted line indicates the border between homogeneous films (on the right) and fluctuating films (on the left).

cm² *i. e.* above about 170 Å²/molecule the absorbance is proportional to the surface concentration. In this region the Lambert-Beer law is valid. Below 170 Å²/molecule the absorbance at 430 nm increases far less than it should according to the Lambert-Beer law.

e. Surface absorbance spectrum

The complete absorption spectrum of the dye is recorded at different surface areas as described. Fig. 5

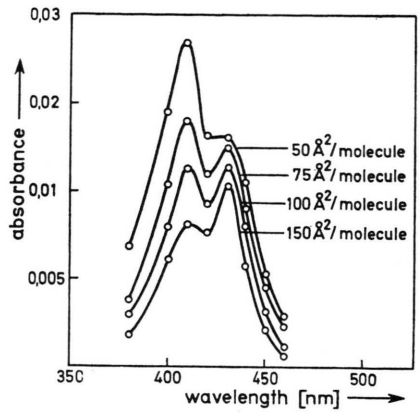

Fig. 5. Surface absorbance *versus* wavelength of a monolayer of the cyanine dye (see Fig. 2) at different areas per molecule. All points depicted in the figure are taken from the same film. It was shown by many control measurements that the full spectrum may be drawn as done in the figure.

shows that at an area of 150 Å²/molecule an absorption maximum at 430 nm is predominant. At lower surface areas a band at 410 nm starts to increase and becomes relatively more intense than the original band at 430 nm.

4. Discussion

The measurements described in the previous section are explained by the following molecular scheme:

If the surface area is larger than about 170 Å²/molecule, the molecules floating around on the water surface form islands. The islands consist of dye molecules whose hydrophobic chains are parallel to the surface with its charged chromophores tilted into the water. The chains form the two dimensional solvent for their own chromophores. In this region the phase transition takes place from the "gaseous" state (which is not investigated) to a "liquid" state which is reached at 170 Å²/molecule. At this area all islands coalesce and a homogeneous film is formed. The arrangement of the dye molecules in the "liquid" film immediately after coalescence is the same as in the islands. By further compression however the solvent,

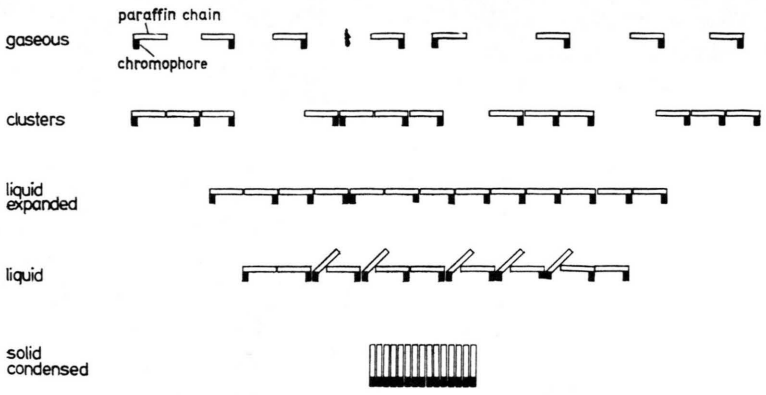

Fig. 6. Schematic drawing of the molecular arrangements of the cyanine dye in different monolayer states. Details are mentioned in the text.

i. e. the paraffin chains, is squeezed out and the chromophore "solution" is concentrated. The film changes from a "liquid expanded" state into a "condensed solid" state. At an area of about 50 Å²/molecule all chains are in a vertical position. The film consists of a closly packed layer of dye chromophores with a loosely packed layer of paraffin chains above it (Fig. 6).

This concept may be supported by the experiments in the following manner: By playing around with CPK models a conformation of the dye molecule can be obtained where the two paraffin chains are in a horizontal position with the chromophore tilted into an edge-on position. In this conformation the molecule occupies an area of about 170 Å². The scanning experiments (Fig. 3) show that at 170 Å²/molecule the film becomes homogeneous upon compression indicating that the molecules are in close contact with each other. The molecular conformation mentioned is the only one which may be correllated reasonably well with this experimental molecular area. This area concides with the point where the surface compressibility decreases abruptly (Fig. 2) the film entering the socalled liquid domain of the surface pressure — surface area isotherm[9]). Thus the liquid state at its largest area is assumed to consist of a monolayer of paraffin chains lying in the plane, the charged chromophores being somewhat below in a more aqueous environment. The homogeneous film dissociating into islands at larger areas is shown directly by the scanning experiments at 430 nm (Fig. 3).

The absorption band at 430 nm is due to a π-π^x-transition of the monomeric chromophore. Dimers are formed by two dye molecules in close contact with each other the two chromophore planes being parallel. The absorption maximum of the dimers is shifted to a shorter wavelength (410 nm) due to the coupling of the two π-π^x-transitions of the monomers[10,11]. The spectrum at 150 Å²/molecule (Fig. 5) shows that just after the coalescence of the islands to an expanded liquid film mainly monomers are present. This is in accordance with the picture just mentioned where the chromophores are separated from each other by the paraffin chains.

At larger areas on the one hand the film breaks off into islands (Fig. 3), on the other hand the absorbance at 430 nm decreases according to the Lambert-Beer law (Fig. 4). This indicates that the molecular arrangement in the islands and in the most expanded liquid film is identical.

The absorbance increase at 430 nm and areas smaller than 170 Å²/molecule is far less than it should be according to the Lambert-Beer law (Fig. 4). This indicates a decreasing content of dye monomers. Figure 5 shows the correlated strong increase of the absorbance at 410 nm, indicating the formation of dimers. This is caused by a concentration of dye chromophores within the liquid film. It is attained by squeezing out the paraffin chains separating the chromophores into the space above the chromophores.

A dye molecule with both paraffin chains perpendicular to the surface, *i. e.* in the plane of the chromophore rings, occupies about 45 Å² as seen with CPK models. The absorption spectrum shows that at 50 Å²/molecule practically only dimers are present (Fig. 5). Thus at this point all "solvent chains" are completely squeezed out and forced into a vertical position. The

very low compressibility below this area (Fig. 2) confirms the closely packed character of this film.*

The argumentation as proposed demonstrates that a detailed description of the monolayer states can be obtained looking at the behaviour of the chromophores. The picture derived from the spectroscopic measurements is fully consistent with the qualitative features of the surface pressure — surface area isotherm.

The monolayer states mentioned (islands, liquid, condensed) are discussed in the literature from many different points of view (see[9]). In the special case described here it is possible to relate rather definite molecular arrangements to these monolayer states.

* The spectra described were obtained only when the dye was spread from a highly diluted solution (17,5 μM). When a higher concentration was used (100 μM) a narrow absorption band of high intensity at 460 nm was observed at all surface areas. Scanning at this wavelength showed high fluctuations of absorbance in the liquid domain also. This band is due to J-aggregates[10–13]. Apparently islands of aggregates are formed during spreading, as the chloroform evaporates and the dye solution becomes highly concentrated. After compression these islands are mixed with film areas of low aggregate content. J-aggregates were also obtained when spreading a dilute dye solution (17,5 μM) and compressing at a very high rate. The J-aggregate content at 15 dyn/cm increased to a certain extent with increasing rate of compression.

The author wishes to thank H. Meyer for advice in electronic problems, Dr. Jutta Peters for help in preparing the manuscript and Prof. H. Kuhn for his continuous interest in this work.

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