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Marine Chemistry 96 (2005) 99–113

MARINE CHEMISTRY

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Reflection of light from the air/water interface covered with sea-surface microlayers

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Received 24 June 2004; received in revised form 19 November 2004; accepted 7 December 2004
Available online 2 February 2005

Abstract

The sea-surface microlayer as a boundary between the ocean and the atmosphere, where important physicochemical, biological, and photochemical processes take place, plays a major role in the exchange processes of gases, materials, and energy.

However, its chemical composition, structure, and involved interactions are very complex and yet not fully understood. Here, we have applied optical/spectroscopic methods and fractal analysis to gain better insight and information regarding composition, structure, and processes in sea-surface microlayer. Regarding the optical properties of sea surface, the material known as chromophoric dissolved organic matter (CDOM) represents the predominant light-absorbing part of dissolved organic material. The enrichment of light-absorbing material in the microlayers has significant effect on transformation processes at the interface and on the properties of interface itself. Original and reconstructed sea-surface microlayer samples have been characterized and visualized by reflection spectroscopy and Brewster angle microscopy (BAM). Due to the presence of chromophores at the air/water interface, the spectrum of reflected light is changed within the spectral range of chromophore absorption, depending on the chromophore density and orientation at the interface. On the other hand, BAM provides information about the homogeneity of the film, existence and formation of domains, phase transitions, and adsorption of material from the aqueous phase to the interface. In this study we have analyzed original and *ex-situ* reconstructed sea-surface microlayer samples from the middle Adriatic (salt lake Rogoznica) and from Norwegian fjords in the vicinity of Tromso. The reflection spectra and BAM images of microlayers have been taken at different surface pressures. Also, the film formation on initially clean surface was monitored by BAM. It was found that the mechanisms responsible for initial film formation and its later development are diffusion from the bulk and adsorption on the interface followed by subsequent aggregation. Fit of experimental data with theoretical results indicates that the adsorption obeys Frumkin-type isotherm with attractive lateral interaction. Fractal analysis applied to the BAM images detected and identified a second-order phase transition during

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0304-4203/$ - see front matter
doi:10.1016/j.marchem.2004.12.003

reconstructed layer compression and provided information on mechanisms involved in spontaneous formation and structural changes of the sea-surface microlayer sample.

Keywords: Sea-surface microlayer; Optical properties; Reflection spectroscopy; Brewster angle microscopy; Fractal analysis

1. Introduction

Natural organic matter plays a significant role in photophysical and photochemical processes in natural water providing a main pathway for the absorbance of light. Chromophoric dissolved organic matter (CDOM) is the predominant light-absorbing fraction of the dissolved organic matter (DOM). Spectral characteristics of CDOM can be very useful in differentiation of samples from different environments. Most natural water samples exhibit decrease in absorbance from UV to the visible part of the spectrum (Helz et al., 1994). The optical properties of CDOM are also very interesting and important in remote sensing application (Hoge and Lyon, 2002).

CDOM absorbing at 280 nm within the sea-surface microlayer was observed (Carlson and Mayer, 1980), indicating that dissolved phenolic material was predominant in CDOM. This material was found to be chemically complex. It is a not very well defined mixture of anionic organic oligoelectrolytes containing phenolic surface active moieties. Although most of light-absorbing substances absorbs only in the UV region (λ<400 nm), some of unidentified chromophores in CDOM absorb also in the visible (400 nm<λ<700 nm) region. The importance of CDOM is enhanced by its significant enrichment in the surface microlayer in respect to its content in subsurface water column (bulk). This enrichment of light-absorbing material in the microlayers, a process whose sources and mechanisms are yet not fully understood, has a significant effect on transformation processes at the interface and on the properties of interface itself.

CDOM is usually characterized by fluorescence and UV/VIS absorption spectroscopy (Green and Blough, 1994; Kowalczuk et al., 2003). Some other spectroscopic methods like infrared reflection absorption spectroscopy (IRRAS), Raman spectroscopy, and laser spectroscopy were also shown to be useful for characterization of CDOM in microlayers (Liss and Duce, 1997). Another useful approach is based on techniques that use reflection of electromagnetic radiation (light) in the visible range of spectrum. These techniques provide means not only for characterization of investigated material but also its visualization on microscopic level.

Reflection spectroscopy and Brewster angle microscopy (BAM) are two of numerous techniques based on reflection of the light from the air/water interface. Usefulness of reflection spectroscopy is based on the fact that the presence of chromophores at the air/water interface modifies the reflected light within the characteristic spectral range of chromophore absorption. The reflection depends on the chromophore density and orientation at the interface. The reflection spectra of light from the air/water interface $\Delta R(\lambda)$ can be measured at normal or oblique incidence (Grüniger et al., 1983, Orrit et al., 1986). In Brewster angle microscopy (BAM), p-polarized light is incident on the air/water interface at the Brewster angle (53° with respect to the surface normal for a clean air/water interface). Then, theoretically, in the case of a clean water surface, there is no reflection of the incident light. However, in the presence of a thin layer on the surface, the reflection can be observed. In that context BAM has proved to be very efficient for on-line control and visualization of topographical film properties such as homogeneity, stability, and domain structures (Hénon and Meunier, 1991, Hönig and Möbius, 1991).

If the considered surface structure is self-similar on certain measurement/observation scale ranges (i.e., looks the same at different magnification), it is a physical realization (within certain upper and lower cutoff limits) of a mathematical fractal that looks the same on any scale and can be described with fractal dimension that is different from the dimension of the embedding (Euclidean) space. In short, such structure exhibits fractal characteristics. As it turned out that the occurrence of fractal structures in nature is rather

common, it is not surprising that fractal analysis has found its applications in diverse scientific fields ranging from physics and chemistry, to biology and cosmology (Mandelbrot, 1982).

Since adsorbed films are structures that often exhibit fractal characteristics, we expect that valuable information can be gained through fractal analysis of surface microlayers. Fractal analysis not only enhances the understanding of the topography of a considered structure, but also enables one to study its changes, interactions, and internal dynamics. Such an analysis often provides information on mechanisms responsible for structure generation and its growth.

The aim of this work was to investigate and characterize the sea-surface microlayers as well as films reconstructed from extracts by using reflection spectroscopy and to visualize these films by Brewster angle microscopy. Fractal analysis of BAM images of selected microlayer samples was applied in order to estimate a fractal dimension of microlayer and its change during compression or spontaneous time evolution of layer.

2. Experimental

2.1. Collection and pretreatment of samples

Samples have been collected from the small Middle Adriatic eutrophicated sea-lake Rogoznica, which is protected from the effects of the winds due to the relatively high banks (samples R1, R2, and R3) and in Tromso, Norway (samples N1, N2, N3, N4, N5, N6, N7, and N8). Samples R1, R2, R3 and R4 were taken in October 2001, February 2002, June 2002 and October 2003, respectively. The Norwegian samples were taken during field experiments organized in June 2001 (samples N1, N2, and N3) and in August 2002 (samples N4, N5, N6, N7, and N8). A Garrett 16-mesh stainless steel screen, which collects the top 100–400 μm layer (Garrett, 1965), has been used for microlayer sampling.

Sea-surface microlayers have been studied as original samples without any pretreatment, or investigated as *ex-situ* reconstructed films after previous extraction by organic solvents of different physicochemical properties. A volume of 500 ml of surface microlayer sample has been extracted several times by small aliquots of solvent and, after repeated extractions, the combined extracts were evaporated in a rotary evaporator. The dried extracts have been redissolved in 25 ml of the corresponding organic solvent and analysed as *ex-situ* reconstructed microlayers. The solvents that were used in this study are: *n*-hexane, chloroform, and dichloromethane.

Very reactive fraction of organic matter in the sea is surface active, participating in different types of interactions in water column and at phase boundaries. The content of surface active substances (SAS) in surface microlayer samples and corresponding subsurface water (20 cm depth) was determined by capacitive current measurements using alternating current voltammetry and is expressed in terms of the equivalent amounts (mg/l) of an arbitrary model nonionic surfactant Triton X-100. This method has been used for years to determine SAS in natural waters (Ćosović and Vojvodić, 1987). Concentrations of SAS in the investigated samples, as well as enrichment factors (EF) of constituents in the microlayer with respect to the subjacent water, are presented in Table 1.

2.2. Chemicals

Chloroform (HPLC) (Kemika, Croatia) and p.a. grade from Baker Chemicals (Holland), and *n*-hexane and dichloromethane p.a. grade from Kemika (Croatia) were used as extracting and spreading solvents.

Table 1
Content of surface active substances (SAS) in microlayers and corresponding subsurface and enrichment factor of constituents in the microlayer with respect to subsurface water

Sample and date of sampling	SAS in microlayer (mg/l) eqv. T X-100	SAS in sublayer (mg/l) eqv. T X-100	Enrichment factor (EF)
R1 (16/10/2001)	0.710	0.110	6.4
R2 (20/02/2002)	0.600	0.170	3.5
R3 (24/06/2002)	0.320	0.300	1.1
N1 (June 2001)	0.123	0.075	1.6
N2 (June 2001)	0.200	0.130	1.5
N3 (June 2001)	0.152	0.125	1.2
N4 (August 2002)	0.140	0.058	2.4
N5 (August 2002)	0.130	0.045	2.9
N6 (August 2002)	0,173	0.054	3.2
N7 (August 2002)	0.215	0.074	2.9
N8 (August 2002)	0.198	0.039	5.0

Sodium chloride (NaCl) from Kemika, Croatia (heated at 450°C for 5 h and purified with charcoal) and suprapur from Merck, Darmstad, Germany (without pretreatment) and deionized water from a Milli-Q system (Millipore) were used to prepare the subphase.

2.3. Procedures

Reflection spectroscopic measurements were performed with a reflection spectrometer for measurements under normal incidence of light (Grüniger et al., 1983). The reflection was measured and expressed as the relative difference in reflectivities between the surface covered with monolayer (R_c) and the monolayer-free solution surface (R_0). In the following, ΔR is given as percentage($\Delta R=((R_c-R_0)/R_0)\times 100$) (e.g., $\Delta R=0.5\%$ has to be read as $\Delta R=0.005$.) The reflection spectrum is complement to the absorption spectrum in solution. The signal ΔR depends on the average orientation of the transitions moments at the interface. In the cases of a spatially statistical orientation and random orientation in the layer plane, the reflectivity is enhanced as compared to the clean water surface in the spectral range of light absorption by the transition moments (i.e., $\Delta R>0$). At small surface densities, the reflection ΔR is proportional to the surface density of the chromophores.

The morphology of monolayers was investigated by using a Brewster angle microscope miniBAM manufactured by NFT (Nanofilm Technologie GmbH) Göttingen, Germany. Details of the principle and design of the Brewster angle microscope have been described elsewhere (Hönig and Möbius, 1991). The size of the field of view in BAM images is 6.50×4.84 mm (768×572 pixels).

BAM experiments have been performed either with *ex-situ* reconstructed microlayers in laboratory under surface pressure controlled conditions, or directly on the air/water interface both in the laboratory and field experiment. The procedures of these experiments were as follows.

The direct measurement on air/water interface was performed in the Ruđer Bošković Institute's marine station situated in Šibenik, Middle Adriatic. It was *semi-field* experiment. Water taken from the sea in front of the marine station was filled in 3-l glass container. A portable Brewster angle microscope MiniBAM was lowered to the water surface and the Brewster angle was adjusted. Several BAM images of the water surface have been taken during 24 h. The water has been filtered using Millipore filter system, and therefore, no particles larger than 0.45 μm may be adsorbed at the surface.

The attempt was also done to take BAM images directly from the sea surface in front of marine station (*in-situ*). The miniBAM was mounted on a small platform lowered to the water surface. A black tube was placed underneath the microscope. In order to reduce the influence of waves, the platform with BAM was placed within a section of the water surface that was separated using a large metal cylinder of about 50 cm diameter and 1.5 m length placed vertically into the sea and protruding some 20–30 cm above sea surface. The microscope mechanism for fine adjustment of height that was under water was protected by a plastic bag. The miniBAM was connected to an automobile battery as a power supply and the images were captured with the portable PC (laptop).

All measurements of *ex-situ* reconstructed films were made at room temperature on aqueous 0.55 mol/l NaCl solution as the subphase.

Fractal analysis of BAM images of particular microlayer was done using box counting method (Russell et al., 1980).

3. Results and discussion

3.1. Reflection spectroscopy

Reflection measurements have been performed with *ex-situ* reconstructed microlayers, which were prepared by spreading different extracts of the microlayer samples on the sodium chloride solutions. Natural organic matter in sea-surface microlayer, as well as in sea water, is a complex mixture of substances like polysaccharides, proteins, peptides, lipids, and humic substances with different polarity, hydrophobicity, and molecular weights. Except naturally occurring substances, the compounds of anthropogenic origin are also present. Different separation techniques (ultrafiltration techniques, different resins, etc.) were applied for fractionation and characterization of organic matter in natural waters. These

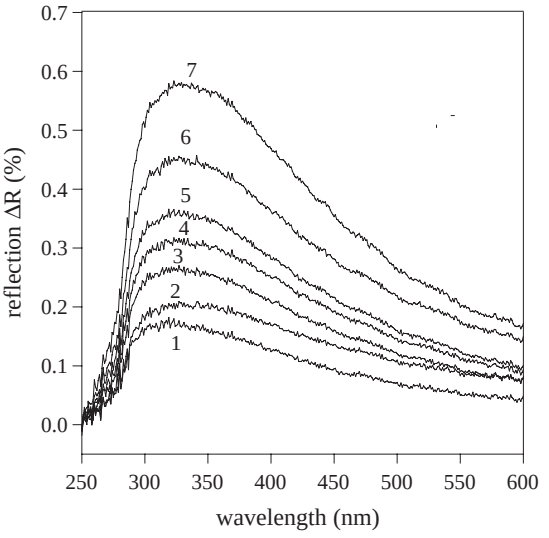

Fig. 1. Reflection spectra obtained from *n*-hexane extracts of the surface microlayer R1 spread on the sodium chloride solution taken at different surface pressures: (1) 2; (2) 5; (3) 10; (4) 15; 85) 20; (6) 30; (7) 35 mN/m.

techniques are based on different physico-chemical characteristics such as molecular weight/size (Guo et al., 1995), hydrophobic/hydrophilic properties (Vojvodić *et al., 1994*), and/or acido-basic properties (Afcharian et al., 1997). In our work the organic matter present in samples of sea-surface microlayers was fractionated by extraction with solvents of different polarity. These were *n*-hexane, chloroform, and dichloromethane with dielectric constants 1.9, 4.8, and 8.9, respectively (Smith, 1994). The selection of a material in re-spread films is governed primarily by the applied solvent. As it is well known, hexane and chloroform extraction selects hydrophobic and dominantly hydrophobic material, respectively, while dichloromethane extraction leads to hydrophilic material. Furthermore their different spreading kinetics influences the morphology of the monolayer: a more rapid spreading is expected to result in a more homogenous distribution of material. Also it may be expected that slowly evaporating spreading solvent like hexane will cause larger domains in the monolayer than chloroform. More thorough discussion on solvent influence on the properties of monolayer at air/water interface can be found in the literature (Gericke et al., 1993, Hönig et al., 1992).

Reflection spectroscopy was recently applied to characterize sea-surface microlayers, and it has been observed that the reflection ΔR of the film depends on the surface pressure (Kozarac et al., 2003). The results presented in this work confirm this observation. The reflection spectra ΔR, measured at normal incidence of light from the *ex-situ* reconstructed sample R1 (hexane extract) with maxima at 350 nm taken at different surface pressures, are presented on Fig. 1. In previous work (Kozarac et al., 2003) it was noticed that the reflection signals for the reconstructed films of the same samples were higher for hexane extracts than for chloroform ones. This was an indication that

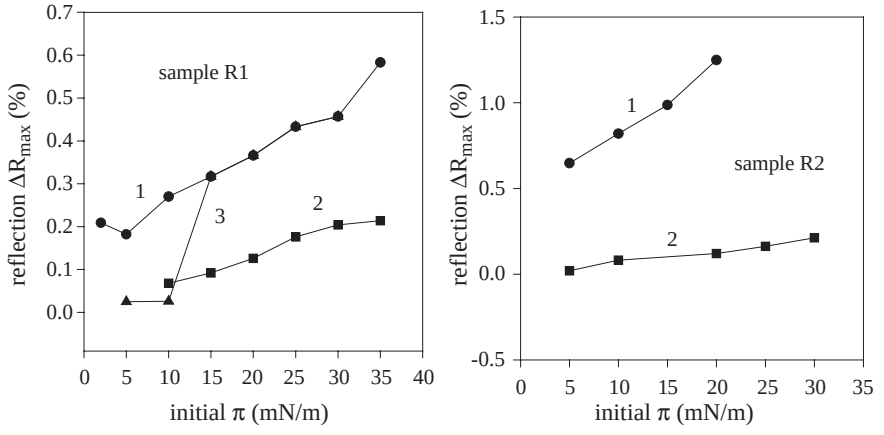

Fig. 2. Relation between maximum reflection ΔR_{max} (%) and initial surface pressure for different extracts of (a) microlayer sample R1 and (b) microlayer sample R2. Curves 1(●), 2(■), and 3(▲) correspond to the *n*-hexane, dichloromethane, and chloroform extracts, respectively. Symbols denote experimental data, and curves linear-segment fit.

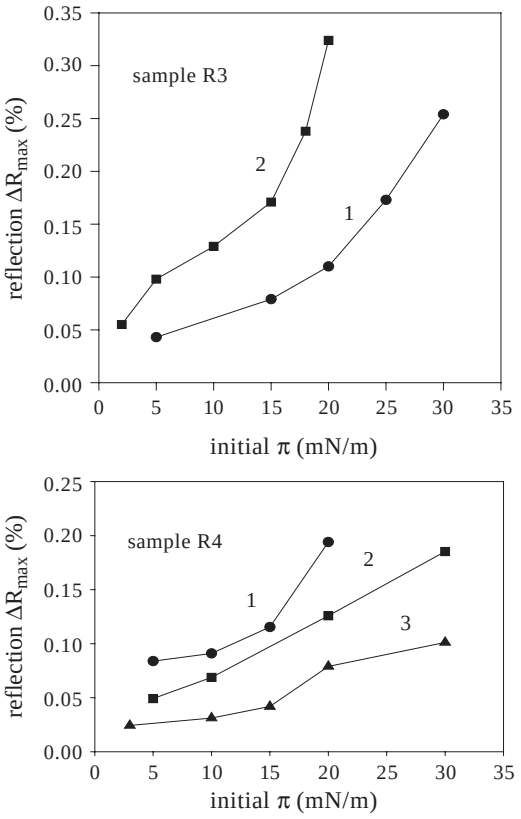

Fig. 3. Relation between maximum reflection ΔR_{max} (%) and initial surface pressure for different extracts of microlayer samples R3 and R4. Curve 1: *n*-hexane extract; curve 2: dichloromethane extract; curve 3: chloroform extract.

CDOM in that particular sample was predominantly hydrophobic. The results presented in Fig. 2 show the relation between reflection of the film and the surface pressure for samples R1 and R2. The film area A_f decreases with increasing surface pressure π, as evident from measurement of the surface pressure–area isotherm (not shown here). Consequently, the surface density of chromophores increases with increasing π. Fig. 2 shows indeed an increase of the reflection signal at the maximum, ΔR_{max}, of the films with increasing surface pressure. The reflection ΔR_{max} in the case of the hexane extract is larger than for the chloroform extract. The reflection values of the film formed from the dichloromethane extract of sample R1 are smaller than the values for hexane extracts at low surface pressures and approach those at surface pressures higher than 15 mN/m. This increase may be due reorientation of the chromophores at higher surface pressure. A different behavior was observed in sample R3 (Fig. 3). Dichloromethane extracts of sample R3 showed much higher reflection signal as compared to the signal of hexane extract in the whole range of film density. Such result indicates that CDOM in sample R3 was dominantly hydrophilic. According to hydrophobic properties of selected model substances observed by fractionation of surface active substances on XAD-8 resin (Vojvodić et al., 1994; Gašparović et al., 1997), the following main groups can be distinguished: (1) very hydrophobic lipids, (2) hydrophilic polysaccharides, and (3) amphiphilic humic and fulvic material, which is slightly more hydrophobic than proteins.

Reflection measurements have also been performed on Norwegian samples. Dichloromethane extracts of all samples and hexane extracts of only two samples were tested. Dichloromethane extracts of samples N2 and N5 showed the highest reflection values among the investigated samples (Figs. 4 and 5a and b), while the dichloromethane extract of sample N1 had lower concentrations of CDOM in comparison to samples N2 and N5, but still the reflection signal approaches a value of ΔR=0.15% at higher surface pressures (Fig. 5a), indicating the hydrophilic charac-

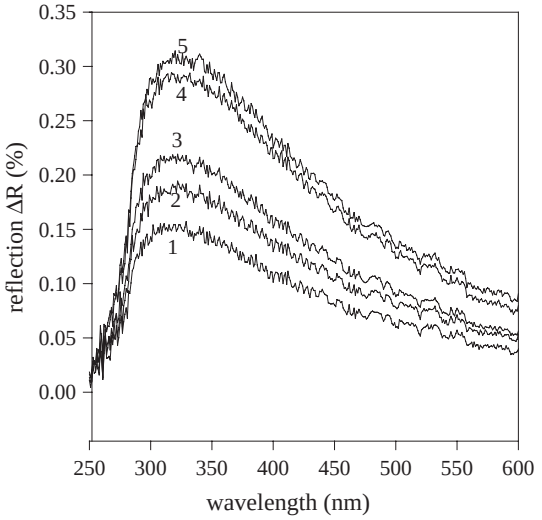

Fig. 4. Reflection spectra obtained from dichloromethane extracts of the surface microlayer N2 spread on the sodium chloride solution taken at different surface pressures: (1) 2; (2) 5; (3) 10; (4) 20; (5) 25 mN/m.

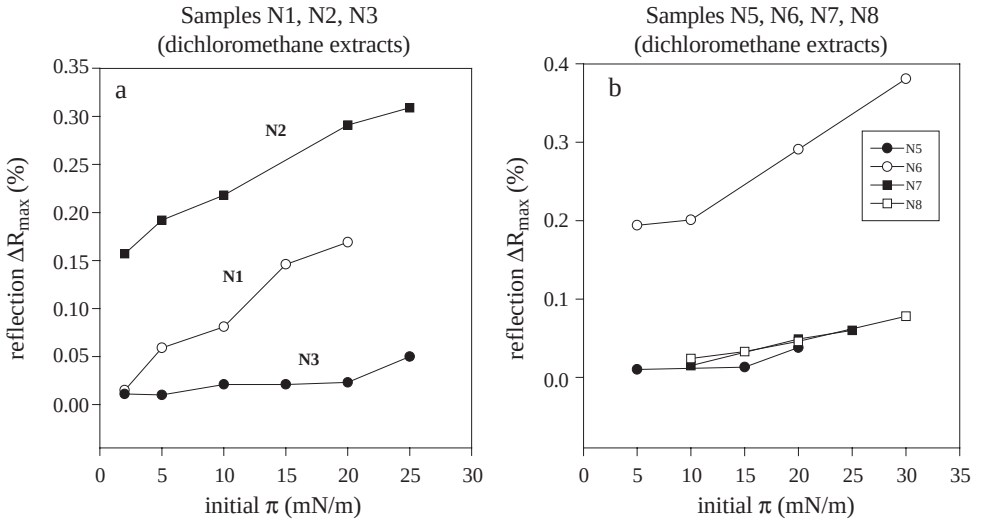

Fig. 5. Relation between maximum reflection ΔR_{max} (%) and initial surface pressure for dichloromethane extracts of (a) samples N1, N2, and N3 and (b) N5, N6, N7, and N8.

ter of CDOM in these samples. This is in agreement with data for SAS and DOC obtained by Gašparović and collaborators (Gašparović, private communications). From the SAS/DOC ratio, it is possible to evaluate the hydrophobic/hydrophilic characteristics of organic matter in natural water samples (Gašparović and Ćosović, 2003). Detected low SAS/DOC values for microlayer samples N1, N2, and N5 correspond to the adsorption characteristics of more hydrophilic material.

Dichloromethane extracts of samples N3, N6, N7, and N8 did not produce any significant reflection (ΔR<0.1%) at all surface film densities (Fig. 5a and b) and no reflection at all was obtained from the dichloromethane extract of sample N4. That CDOM was not present in samples N6 and N8 was proved by hexane extracts, which did not show any reflection even at high surface pressures.

In Norwegian samples whose dichloromethane extracts did not give any reflection (N4) or reflection signal was <0.1% (N3 and N7), CDOM could be either of hydrophobic character or was not present at all.

3.2. Brewster angle microscopy (BAM)

It is known that BAM provides information about the homogeneity of the film, existence and formation of domains, phase transitions, and adsorption of material from the aqueous phase. We have previously applied this technique to investigate phytoplankton culture samples and *ex-situ* reconstructed sea-surface microlayers (Kozarac et al., 1998; Gašparović et al., 1998; Kozarac et al., 2000). Also, BAM can significantly contribute to the characterization and visualization of the film topography of sea-surface microlayers. In most BAM experiments presented here, *ex-situ* reconstructed microlayers have been used. However, the BAM images presented in Fig. 6 have been taken directly from the air/water interface, showing the formation and development of microlayer during 24 h. The film is formed by adsorption of amphiphilic material of a different chemical nature, which is accumulating at the surface. In image (b), the dark areas are also covered by some film, since in BAM images even at low surface pressure, liquid condensed domains can be seen as small bright areas embedded in the liquid expanded phase, which is perceived as the dark background. The bright spots may be flocks or aggregates with thickness smaller than a few microns since we do not see interference fringes like in the case of dust particles. It is worthwhile to mention again that the water has been filtered using the Millipore filter system and, therefore, no particles larger than 0.45 μm may be adsorbed at the surface. The patterns visible in

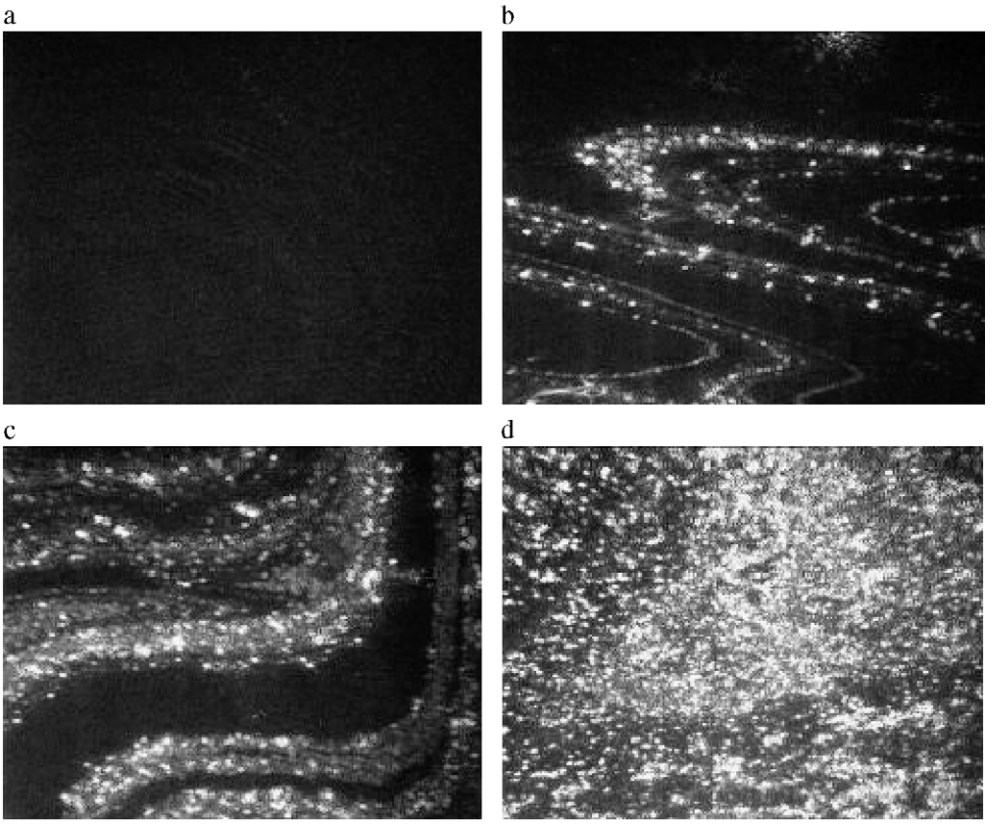

Fig. 6. BAM images taken from sea water sample after (a) 0; (b) 2; (c) 10; (d) 24 h.

images (b) and (c) are probably due to convection in the subphase, since we did not compress the film. The growth rate of the bright domains was determined by evaluating the area fraction of domains (f_{domain}) with brightness level exceeding a given threshold value. The measured time development of the area fraction f_{domain} of bright domains in the film is depicted in Fig. 7 (curve 1). The process is rather slow, and the time scale involved rather long, but eventually bright domains should cover the whole surface. We suppose that the responsible mechanisms of bright domains formation are diffusion of molecules from the bulk and adsorption at the interface, followed by subsequent aggregation. First, we consider the solution of adsorption process due to semi-infinite diffusion to a planar interface, including lateral interaction between the molecules (i.e., Frumkin-type of adsorption isotherm). The solution of diffusion

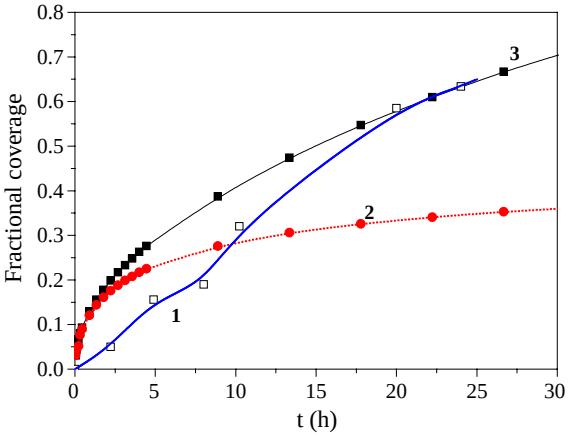

Fig. 7. Area fraction of bright domains determined from BAM images as function of time: symbols denote experimental points and curve 1 fit with B-spline. Curves 2 and 3 correspond to time evolution of surface coverage calculated from Eq. (1) for Ψ=1 and a=0 and a=2, respectively.

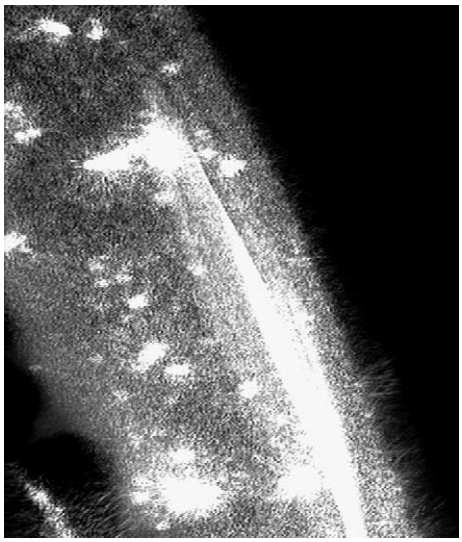

Fig. 8. BAM images taken directly from the sea surface (*in-situ* measurement).

equation including Frumkin adsorption isotherm and appropriate boundary conditions is given by:

$$\theta(z) = \sqrt{z} - \frac{1}{2BC^*}\int_0^z \frac{\theta/(1-\theta)}{\sqrt{z-\eta}} e^{-2a\theta} \mathrm{d}\eta, \qquad (1)$$

where θ is relative coverage at the interface, B is adsorption coefficient, C^* is bulk concentration, and a is coefficient of lateral interaction ($a>0$ implies attractive interaction). z and η are non-dimensional parameters defined by: $z=\Lambda t$ and $\eta=\Lambda\tau$, where $\Lambda=4C^{*2}D/\Gamma_m^2\pi$ (here, D is diffusion coefficient and Γ_m=maximal possible surface concentration) (Rampazzo, 1969; Gašparović et al., 2004).

This is a Voltera's integral equation of second kind for $\theta(z)$ (Morse and Feshbach, 1953). It can be solved numerically for different values of parameters $\Psi=BC^*$ and a, providing calculation of time development of surface coverage assuming hydrophobic material that does not return back to the bulk.

Eq. (1) has been solved for two cases: adsorption without lateral interaction (hence Langmuir adsorption), and assuming attractive lateral interaction between adsorbed molecules.

The fit of measured time development of bright area fraction with solutions of Eq. (1) assuming no interaction and lateral attraction between molecules is presented in Fig. 7 (curves 2 and 3, respectively). It can be seen that in early times the observed fractional surface coverage with bright domains is significantly lower that calculated, irrespectively of the model used in calculation. This is due to the fact that at early stage, the aggregate sizes are below resolution limit of BAM system (about 8 μm) and hence invisible in the image. As the average aggregate size becomes greater with time they became visible, and the observed coverage rapidly approaches that predicted by theoretical domain growth curve with attractive interaction. Hence we conclude that the growth of bright domains is indeed a consequence of diffusion of dominantly hydrophobic material from the bulk that interacts with lateral attraction. Subsequent aggregation of material was subjected to fractal analysis as described later.

The image of a sea-surface microlayer recorded *in-situ*, directly at sea surface, is shown in Fig. 8. The surface microlayer could be observed showing a topography very similar to that of the water samples in the laboratory. However, the *in-situ* images could be taken only from a calm sea surface because of

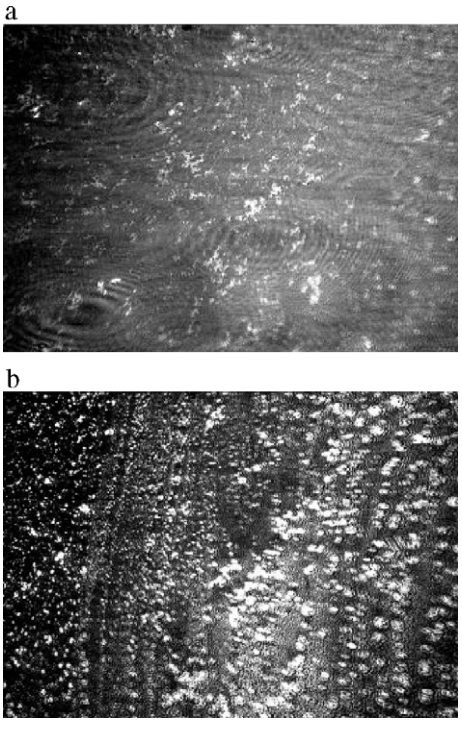

Fig. 9. BAM images of hexane extracts of the microlayer samples: (a) R2 (π=4.1 mN/m) and (b) R3 (π=5.9 mN/m).

vertical motion of the water level inside the metal cylinder and black tube protecting BAM. Nevertheless, it has been demonstrated that a direct observation of the microlayer at the water surface (*in-situ* measurements) using the miniBAM is possible and will give us better insight in its formation and long-term changes.

BAM images from different extracts of microlayer samples R2 and R3 are shown in Figs. 9, 10, and 11. The film of hexane extracts, especially that of sample R3 (Fig. 9), shows some similarity to the sample taken in front of Marine station (Fig. 6d), but is different in appearance from the chloroform and dichloromethane extracts, which are rather similar to each other. The topography looks like a foam structure with gas-analog phase surrounding liquid-phase film. The images show apparently two phases in contrast to the heterogeneous film of the hexane extract with a whole range of domains with different brightness.

In reflection measurements, the influence of the solvent used for extraction was manifested as the difference in observed reflection signals for the hexane, chloroform, and dichloromethane extracts, corresponding to the predominantly hydrophobic or hydrophilic character of CDOM. BAM images of three extracts of the same samples also show a different topography due to a different nature of the compounds extracted by selected solvents and differences in involved interactions (Figs. 9a, 10a, and 11a; Figs. 9b, 10b, and 11b.

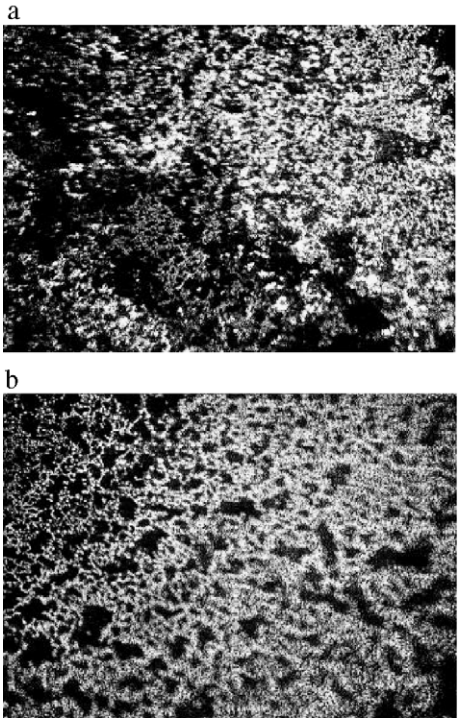

Fig. 10. BAM images of chloroform extracts of the microlayer samples: (a) R2 (π=9.8 mN/m) and (b) R3 (π=11.6 mN/m).

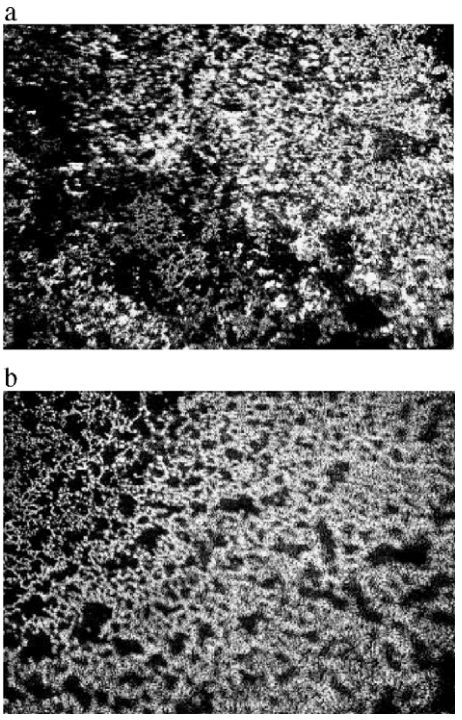

Fig. 11. BAM images of dichloromethane extracts of the microlayer samples: (a) R2 (π=7.9 mN/m) and (b) R3 (π=9.1 mN/m).

BAM images of Norwegian sample N8 taken at surface pressures of 10.3, 8.6, 10.2, and 10.2 mN/m for original sample, hexane, chloroform, and dichloromethane extracts are shown on Fig. 12a–d. The initial microlayer (Fig. 12a) is very homogeneous with very few bright spots. By compressing the film, a surface pressure of 22.5 mN/m (not shown here) was achieved, proving the presence of a film although no structure was observed. Again, a strong similarity of the topography between chloroform and dichloromethane extracts (Fig. 12c and d) and the clear difference to the hexane extract (Fig. 12b) films was observed. At this surface pressure, chloroform and dichloromethane extracts yield net-like structures while hexane extracts show larger domains of different brightness which grow with increase of pressure.

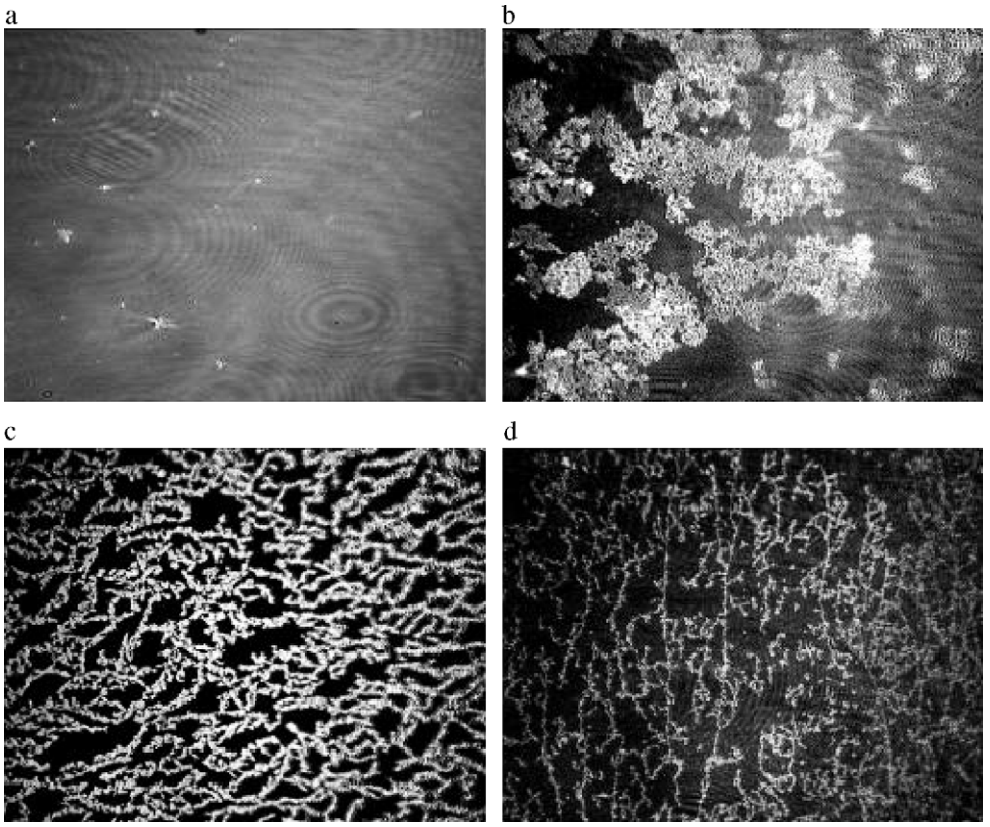

Fig. 12. BAM images of: (a) original microlayer sample N8; (b) hexane extract; (c) chloroform extract; and (d) dichloromethane extract of the sample N8. Surface pressure: (a) 10.3; (b) 8.6; (c) 10.2; and (d) 10.2 mN/m.

BAM images of the chloroform extract of the sample N8 (*ex-situ* reconstructed microlayer) taken at different surface pressures that result in change from gas-analog to condensed film phase are shown in Fig. 13. The formation of dense-packed layer from net structure at low surface pressures can be seen. The corresponding surface pressure–area ($\pi-A$) isotherm is given, too. The usefulness of surface pressure concept lies in the fact that the surface pressure–area ($\pi-A$) isotherms can provide significant information about surface microlayer characteristics including, but not limited to: mean molecular mass, modulus of elasticity, specific area, first and/or second order phase transitions, etc.—all due to the fact that chemical composition of natural films respond to physical forcing factors and that the response is reflected in $\pi-A$ isotherms (Berger and Means, 1985, Frew and Nelson, 1992, Bock and Frew, 1993). Furthermore, in cases of multicomponent film the degree of mixing and formation of layered structures may be inferred from corresponding isotherm data and apparent chemical and structural properties of SAS in microlayer samples can be related to the respective physical measurements (Pogorzelski and Kogut, 2001, Pogorzelski, 2001).

The isotherm shown in Fig. 13 is of the expanded type without obvious phase transitions. However, the occurrence of different phases in the film is evident from the BAM images, showing again that $\pi-A$ isotherms alone are insufficient for detection and characterization of higher order phase transitions corresponding to complex structural changes (Kozarac et al., 2000; Kozarac et al., 2003). Very high surface pressure of 65.7 mN/m in comparison with surfactants in natural marine waters is obtained probably due to the fact that it is an extract of a microlayer. The maximum surface pressure obtained for the original sample had significantly lower value of 22.5 mN/m.

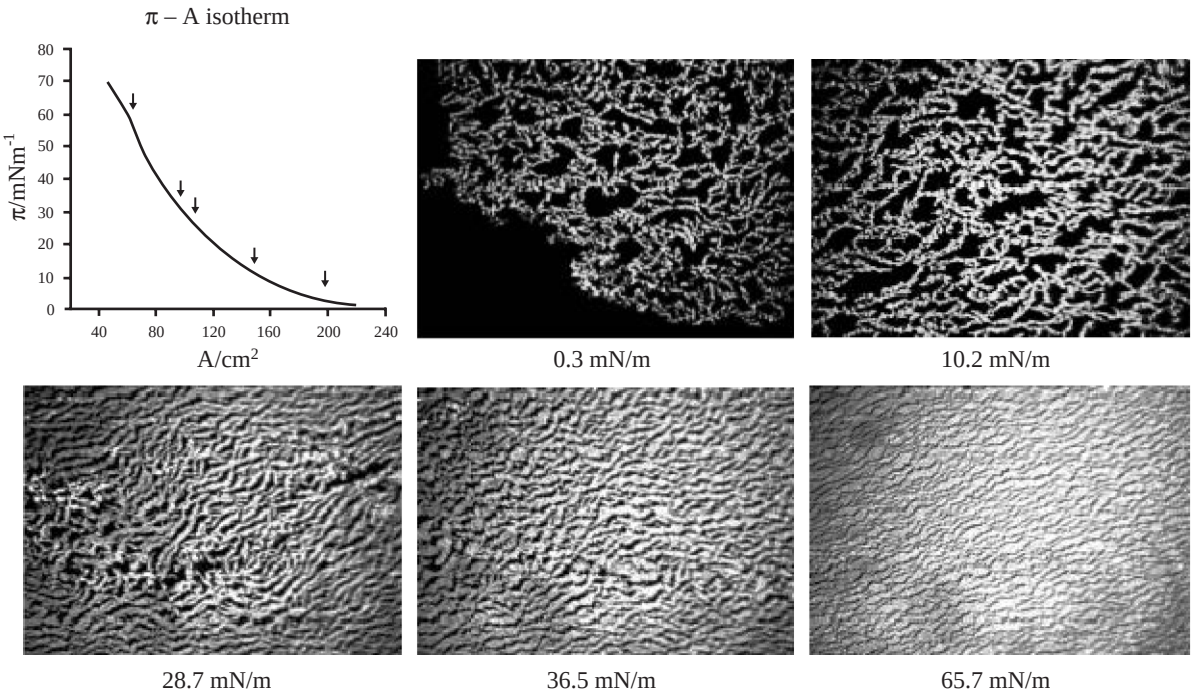

Fig. 13. BAM images of microlayer sample N8 (chloroform extract) at different surface pressures and corresponding $\pi - A$ isotherm.

3.3. Fractal analysis

Fractal dimension is pertinent not only as operative measure of chemical surface/interface irregularity but also a tool for study of surface structure and adsorbate–adsorbent as well as adsorbate–adsorbate interaction. (Pfeifer and Avnir, 1983). It affects global, statistical mechanical behavior of adsorbed layers, as the cooperative phenomena are strongly dimension-dependant. Hence, the BAM images of considered microlayer were also subjected to fractal analysis in order to obtain a better insight into the involved structural changes. To estimate the fractal dimension of the microlayer and its change during compression or time evolution, we have applied the Box counting method (Russell et al., 1980). This method provides efficient means for estimation of fractal dimension of general two-dimensional objects and as such is long known and widespread. The fractal dimension was calculated after applying the median filter to the recorded image.

First, fractal analysis was applied to the series of BAM images corresponding to the time development of bright domains in the microlayer due to diffusion of hydrophobic material to the interface that was considered before. The fractal dimension of bright domains vs. time is depicted in Fig. 14. It can be observed that the fractal dimension grows from 1.36 to 1.88. In the earliest phase the low fractal dimension (1.36) reflects the string-like aggregation of very small clusters along

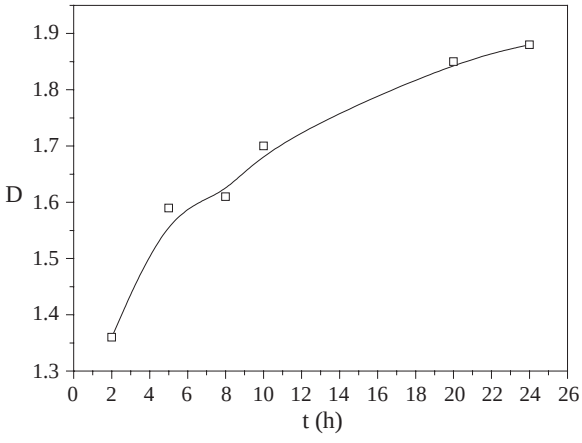

Fig. 14. The fractal dimension of bright domains vs. time. Symbols denote experimental points corresponding to that of Fig. 7, while curve represents B-spline fit.

subphase convective flows (cf. Fig. 6b). Subsequently in the formation of aggregates at the surface, the crucial factor is the competition between two-dimensional diffusers (at interface) and "three-dimensional" adsorbers (coming from the bulk). In the beginning (low surface coverage) the odds of a particle adsorbing from solution inside an aggregate perimeter are negligible (new particles are added to existing cluster mostly through diffusion on the surface, and not from the bulk), so the aggregate takes on the highly branched structure and low fractal dimension characteristic of two-dimensional DLA ($D \leq 1.6$). As the coverage increases, probability that the particle from the bulk gets adsorbed within the cluster perimeter increases, and growth continues through adsorption-limited growth that results in more dense aggregates with higher fractal dimension. The crossover from diffusive growth to adsorption limited growth depends simply on aggregate size. (Schwartz et al., 1992). Hence, initial growth is fast, linear, while in later times it has adsorption-limited kinetics. Fractal dimension grows from 1.61 to 1.88—these values correspond to reaction limited polydisperse cluster–cluster and particle–cluster aggregation in $2d$, respectively.

Second, the change of fractal dimension of chloroform extract of sample N8 in dependence on applied pressure was studied. The result is depicted in Fig. 15. We have established that the considered microlayer is indeed a fractal structure whose dimension changes with surface pressure. The gradual increase of fractal dimension proportional to compression is observed (Fig. 15a). However, a slight plateau can be observed in the pressure range ~10–16 mN/m. This plateau can be unambiguously identified from the corresponding derivative depicted in Fig. 15b. We relate the occurrence of this plateau to a second order phase transition–percolation. If we denote by p_c the percolation threshold, and by p fraction of sites that are occupied than for $p<p_c$, no phase transition can occur since the system breaks up into isolated finite clusters that cannot sustain long-range order. The behavior of diverging parameters, such as correlation length and number of occupied sites belonging to a cluster, near the percolation threshold is characterized by "critical exponents." These exponents are "universal" in the sense that they do not depend on details of local structure geometry but only on dimensionality of the consid-

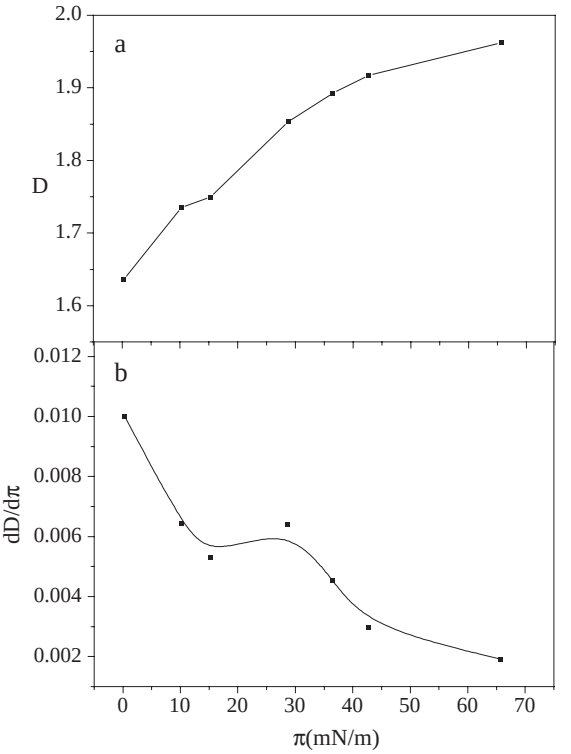

Fig. 15. (a) Change of fractal dimension of a reconstructed microlayer with pressure (chloroform extract of sample N8). Respective pressures in images (a–f) are 0.3, 10.2, 15.3, 28.7, 36.5, 42.7, and 65.7 mN/m. (b) Corresponding derivative of the fractal dimension.

ered system. The critical exponents γ_p and υ_p of percolation theory are related to fractal dimension of percolation cluster at critical point through:

$$D = \gamma_p/\upsilon_p$$

Dunn et al. (1975) have estimated the values of critical exponents γ_p and υ_p to be 2.38 ± 0.02 and 1.34 ± 0.02, respectively. Hence, the corresponding fractal dimension $D=1.78\pm0.04$. This value is in good agreement with estimated fractal dimension $D=1.75$ of the "plateau." Also the corresponding average fractional coverage in the plateau region of 0.62 ± 0.04 compares favorably with theoretical prediction $p_c=0.652$ and $p_c=0.592$ of bond/honeycomb and site/square in $d=2$, respectively. After the percolation cluster is formed, the process continues with compaction of the layer that is accompanied by increase of fractal dimension approaching $D=2$. At higher pres-

sures ($\pi \geq 42$ mN/m), the formation of long and narrow domains (similar to nematic ordering on molecular level) corresponding to long range order can be observed. The boundaries of the domains are fractal with $D_b \approx 1.3$ and 1.14 at π=42 and 65 mN/m, respectively. This indicates that the monolayer on a length scale intermediate between the correlation length of positional order (hundreds of angstroms) and the optical domain size (tens of microns) can be treated as a well oriented texture.

4. Conclusions

We have used a combination of optical/spectroscopic techniques, namely Brewster angle microscopy and reflection spectroscopy, and fractal analysis to study processes and structures at interface.

It has been shown that processes at natural phase boundaries which influence global environmental problems can be efficiently studied by these techniques. New insight into the formation, temporal variations, and properties of the sea-surface microlayers as well as in processes occurring in this film can be gained by using BAM and reflection spectroscopy. BAM provides information about the homogeneity of the film, existence and formation of domains, phase transitions, and adsorption of material from the aqueous phase to the interface.

Application of fractal analysis to BAM images provides additional complementary means for characterization of materials, structures, and dynamical processes at the interface providing information on (second order) phase transitions, interactions, and aggregation mechanisms at the interface and structural changes under applied pressure.

In the future we want to extend our investigations to *in-situ* studies of formation, change, and behavior of microlayers by using BAM, and to investigate the growth and structural changes of films by fractal analysis.

Acknowledgements

Financial support of the Croatian Ministry of Science, Education, and Sport, and the Norwegian Research Council, CPSSE programme, project no. 149583/730 are gratefully acknowledged. Financial support for Z.K. from the Alexander von Humboldt Foundation and for D.M. from the Fonds der Chemischen Industrie, Germany, is gratefully acknowledged.

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