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Complex methodological approach to the studies of natural microlayers at the air/water interface

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Abstract

The investigations of sea-surface microlayers are crucial for better understanding of the mechanisms and physico-chemical processes at natural phase boundaries which play an important role in the marine environmental protection and global climate change. Sea-surface microlayers were studied in an original sample without any pretreatment and as ex-situ reconstructed films after previous extraction of the sample by organic solvents of different polarity. For physico-chemical characterization of natural and ex-situ reconstructed sea-surface microlayer samples a complex methodological approach was applied. Monolayer techniques, Brewster angle microscopy, reflection spectroscopy and electrochemical methods were used. Samples were collected from a Middle Adriatic eutrophicated sea-lake. Results were compared and discussed with respect to previously reported results obtained by analysing the samples from the same area and from the Northern Adriatic.

Keywords: Sea-surface microlayer; Monolayer techniques; Spectroscopic techniques; Electrochemical methods

1. Introduction

Most bio-geo-chemical processes in natural waters take place at different phase discontinuities, among which the biggest one is the atmosphere–ocean boundary. Physical, chemical and optical properties of this interface is strongly influenced by the presence of sea-surface microlayers which are derived from multiple sources and composed of different natural and anthrophogenic hydrophobic compounds. It is defined as the top of 1–1000 μm of the ocean surface. Natural organic material that forms microlayers is mostly the result of biological activity and comprises very different groups of organic compounds, from lipids to the more complexed glycopeptido–lipido–oligosaccharide complexes [1–4]. Sea-surface microlayers are also enriched in anthrophogenic organic and metal pollutants, specially in coastal regions [5–7].

The importance of surface microlayers at the sea water/air interface has been discussed years ago from different points of view. The exchange of gases across the sea surface is of global biogeo-

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chemical importance and is strongly influenced by sea-surface microlayers [8,9]. Sea-surface microlayers play a special role in the transfer of mass, heat and momentum across the air-sea interface and is also a site of adverse biological activities. They are known to affect the surface wave field, what includes the inhibition of wave growth, enhancement of wave damping and reduced exchange of energy between waves of different frequencies. [10–13].

It is identified as a key interface to study in order to better understand the fate and effects of airborne contaminant and particulate inputs into natural waters, having thus an important role in aquatic environmental protection and global climate change.

Among major interests of sea-surface microlayer investigations are estimation of natural film morphology, relationship between the concentration and type of substances and the properties of films regarding the communication between the film and subsurface, toxic effects in the surface microlayers, photochemical processes and their role in radiation energy transfer [2].

One of the important problem in microlayer studies is the influence of sampling methodology using samplers of various type and material, what results in different type and thickness of collected microlayer material [2,14–16].

Important information on geophysical and chemical parameters like surface temperature, salinity, wind, waves etc. can be also obtained using remote sensing methods from satellites and aircraft [17–21]. Some of methods of ocean sensing like infrared and microwave radiometry, ocean colour sensors, radar scatterometer, imaging radar, altimetry, etc. are recently reviewed in detail [22].

Sea-surface microlayers can be studied in an original sample without any pretreatment, or investigated as ex-situ reconstructed films after previous extraction by organic solvent. In this work the advantages of complex methodological approach are demonstrated by applying different techniques in the study of sea-surface microlayers. Monolayer techniques, reflection spectroscopy, Brewster angle microscopy and electrochemical methods have been used. To our knowledge two of these techniques, namely reflection spectroscopy and capacity measurements of microlayer films transferred from the air/water interface to the mercury electrode have been used for the first time in microlayer studies.

2. Experimental

2.1. Collection and pretreatment of samples

Samples L_1, L_2, L_3 and L_4 have been collected from the small Middle Adriatic eutrophicated sea-lake Rogoznica. Samples L_1, L_2, L_3 taken during summer and sample L_4 in autumn. The lake is protected from the effects of the winds due to the relatively high banks, the sea surface was calm and the samples have been taken in the sunny afternoon. A Garrett 16-mesh stainless steel screen, which collects top 100–400 μm layer [23] has been used for microlayer sampling.

The content of surface active substances in surface microlayer sample was determined on the basis of the capacity current measurements using ac voltammetry, as described in detail in previous papers [24,25]. Surfactant activity is expressed in terms of the equivalent of the nonionic surfactant tetra-octylphenolethoxylate (Triton-X 100; in mg l^{-1}) and equals to 0.33, 0.85, 0.65 and 0.75 mg l^{-1} for samples L_1, L_2, L_3 and L_4, respectively. Surfactant activity of subphase (water collected at 20 cm depth) was 0.13, 0.13, 0.06 and 0.05 mg l^{-1}. Enrichment factors for samples L_1, L_2, L_3 and L_4 were 2.54, 6.64, 10.16 and 14.42, respectively, indicating that those samples were very rich in organic material. For sample L_4 the concentration of dissolved organic carbon (DOC) was estimated as well and was 4.74 mg l^{-1}, which is almost three times higher than the mean DOC value of 1.75 mg l^{-1} recently reported for Rogoznica Lake samples [26].

Surface microlayer samples (500 ml) have also been extracted by one of the following organic solvents: chloroform, *n*-hexane and/or dichloromethane. After repeated extractions, combined extracts were evaporated in a rotary evaporator. The dried extracts have been dissolved again in the 25 ml of corresponding organic solvent and analysed as reconstructed microlayers.

2.2. Measurements at air/water interface

Surface pressure–area (π–A) and surface potential–area (ΔV–A) isotherms were measured in a Teflon trough enclosed in a tight box and thermostated. A Wilhelmy balance (15 mm wide filter paper) was used to measure the surface pressure, and the surface potential was measured using a vibrating plate condenser. Compression velocity was 60 $cm^2\ min^{-1}$.

Reflection spectroscopic measurements were performed with the reflection spectrometer for measurements under normal incidence of light [27]. The reflection was measured and expressed as the difference in reflectivity betwen the surface covered with monolayer and the monolayer-free solution surface.

The morphology of monolayers was investigated by using a Brewster angle microscope BAM 2 manufactured by NFT (Nanofilm Technologie GmbH), Göttingen, Germany. Details of the principle and design of the Brewster angle microscope have been described elsewhere [28–30].

All measurements of ex-situ reconstructed films were made on aqueous 0.55 mol l^{-1} NaCl as subphase at room temperature.

2.3. Electrochemical measurements

The surfactant activity of the original samples of microlayer was determined by capacity current measurement using ac voltammetry (*out-of-phase* signal).

Reconstructed films were formed by spreading the microlayer extract in organic solvent onto the electrolyte solution. Films were then transferred to the mercury surface by vertically dipping the electrode through the film. The technique of transferring the lipid film from the air/water interface to the mercury surface is described in more detail [31,32]. Afterwards, the capacitance of the film was estimated by ac voltammetry (*out-of-phase* signal), and the influence of the film on the redox processes of cadmium was studied by ac voltammetry (*in-phase* signal).

Measurements were performed with a digital multimode polarograph microAutolab-type II (Eco Chemie B.V., Uttrecht, The Netherlands) connected with GPES 4.6 software. A standard polarographic Metrohm cell of 100 cm^3 equipped with a three-electrode system was used. A hanging mercury drop electrode (HMDE, Metrohm, Switzerland) of the surface area $A = 0.01245 cm^2$ was used as a working electrode, Ag | AgCl | 3 M KCl as the reference electrode and a platinum wire as the auxiliary electrode.

Pure nitrogen was used for deaeration of the solutions. Measurements were made in original samples or in reconstructed films where 0.55 mol l^{-1} NaCl was used as subphase.

2.4. Chemicals

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

Sodium chloride (NaCl) from Kemika, Croatia was heated at 450 °C for 5 h and purified with charcoal.

Deionized water from a Milli-Q system (Millipore Corp.) was used to prepare the subphase.

3. Results and discussion

3.1. Monolayer techniques

The π–A isotherms of the microlayer samples are shown in Figs. 1–3. π–A isotherms are given using the area in square centimetres as abscissae instead of area per molecule as it is usual in monolayer studies. This is due to the complexity and unknown composition of microlayer samples. However, scaling of π–A isotherms using chemical attributes was proposed by Frew and Nelson [33]. They developed solid phase extraction methodology for isolating microlayer surfactants for surface physical and chemical characterization introducing thus the possibility to express π–A isotherms on a specific area basis [34]. Nevertheless, π–A isotherms of microlayer samples, even if not scaled on a specific area basis are useful to compare the physical state of different

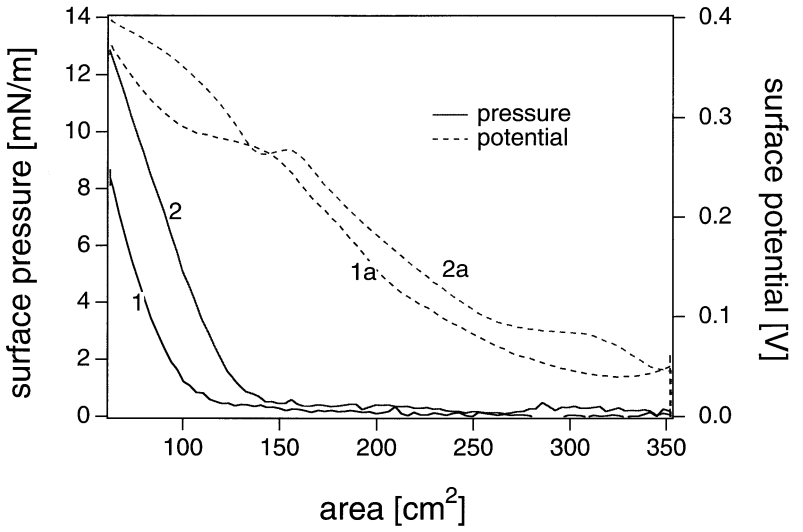

Fig. 1. Surface pressure–area ($\pi-A$) and surface potential–area ($\Delta V-A$) isotherms of the reconstructed lake surface microlayer L_1. Curves 1 and 1a are *n*-hexane extracts and curves 2 and 2a are chloroform extracts.

microlayers if the experimental conditions are kept constant.

In Fig. 1 the $\pi-A$ and $\Delta V-A$ isotherms of the reconstructed lake-surface microlayer L_1 are shown. The $\pi-A$ and $\Delta V-A$ isotherms for *n*-hexane extracts are denoted with the curves 1 and 1a and those for chloroform extracts with the curves 2 and 2a. Both $\pi-A$ isotherms are of liquid expanded type without phase transition. The increasing parts of both isotherms are parallel to each other although the difference in the area at which the rising part of $\pi-A$ isotherm starts is 25 cm^2, i.e. film forming material in chloroform extract shows an isotherm which undergoes a transition from gaseous to liquid expanded phase at a larger area and at very low surface pressure. The compression/expansion isotherms for sample L_2 (chloroform extract) and sample L_3 (*n*-hexane extract) are shown in Fig. 2. Measurement was done also with the sample L_1 but data are not presented here. Samples L_1 (both extracts) and sample L_2 (Fig. 2a) gave no significant compression/expansion hysteresis, indicating the formation of very stable and rapidly relaxing films. The same was observed with the chloroform extract of the sample L_3. In contrast, the *n*-hexane extract of sample L_3 showed a pronounced hysteresis (Fig. 2b). The appearance of a hysteresis during compression/expansion cycles is often observed with lipid monolayers at the air/water interface, especially in the case of lipid mixtures. The phenomenon is attributed to a slow response of the composite monolayers to changes of the surface pressure and reflects the influence of relaxation processes like molecular reorganization. It was reported earlier by Bock and Frew [35] that the isotherms of natural films often exhibit pronounced hystereses upon compression and expansion. Such behaviour was attributed to the fact that natural films are derived from complex mixtures of compounds present in sea water and varying proportions of components with different $\pi-A$ characteristics. However, the observed hysteresis in isotherm of *n*-hexane extract of sample L_3 was unusual, showing higher pressure-area paths during expansion with respect to those during compression. Such unusual hysteresis was recently observed in the isotherms of distearoylphosphatidylcholine–fatty acid mixtures at the air/water interface [36].

Collapse of the investigated films occurred at lower surface pressures when compared to the collapse of model lipids such as phospholipids [37]. The collapse pressures for chloroform extracts of samples L_1 and L_3 were 13 and 14 m mN^{-1}, and those for *n*-hexane extracts of the same samples 9

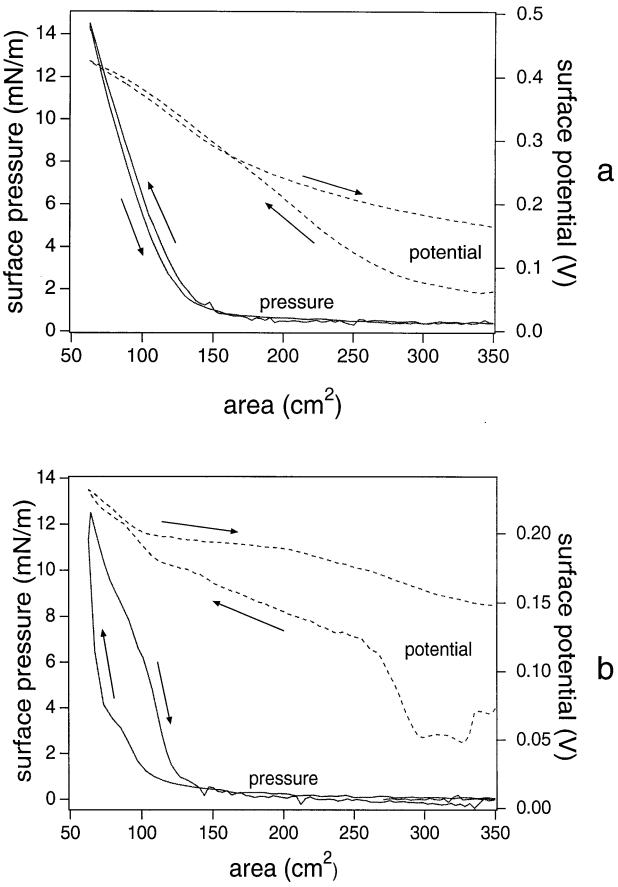

Fig. 2. (a) Compression/expansion hysteresis of surface pressure–area ($\pi-A$) and surface potential–area ($\Delta V-A$) isotherms of the *n*-hexane extracts of the sample L_2. (b) Compression/expansion hysteresis of surface pressure–area ($\pi-A$) and surface potential–area ($\Delta V-A$) isotherms of the *n*-hexane extracts of the sample L_3.

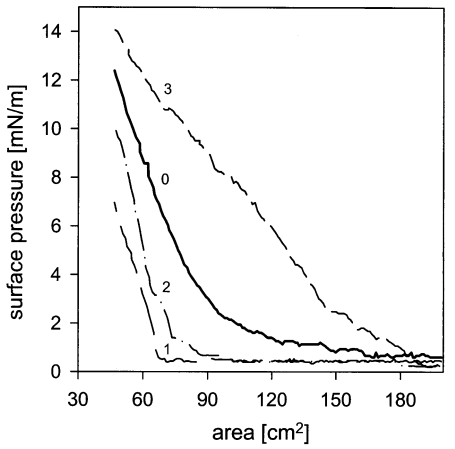

Fig. 3. Surface pressure–area ($\pi-A$) isotherms of the sample L_4: original sample, curve (0); *n*-hexane extract, curve (1); chloroform extract, curve (2); and dichloromethane extract, curve (3).

and 12 mN m^{-1}, respectively. The $\pi-A$ isotherm of sample L_2 (Fig. 2a) is also of the liquid expanded type without detectable phase transition and showing a collapse pressure at $\pi \approx 15$ mN m^{-1}. The $\pi-A$ isotherms of the original microlayer sample and of the ex-situ reconstructed films of the sample L_4 are shown in Fig. 3. The isotherms of the reconstructed films from *n*-hexane and chloroform extracts are less expanded when compared to the original sample and the dichloromethane extract. Since experimental conditions (temperature, spreading volume, compression speed) were the same, such a result could be explained by the presence of different film forming material in different extracts of the same microlayer sample. The three organic solvents used show different physico-chemical properties with dielectric constants 1.9, 4.8 and 8.9 for *n*-hexane, chloroform and dichloromethane, respectively [38]. According to the polarity of the organic solvents we can only assume that in these samples film forming material contained water soluble substances combined with hydrophobic matrix in different proportions. Chloroform and *n*-hexane extracts contain more hydrophobic lipid material, which is known to give more condensed isotherms, while dichloromethane, being the most polar solvent among the three solvents used, can also extract hydrophilic polar material bound to the hydrophobic matrix. Although it is to be expected that in sea-surface microlayers hydrophobic fraction of organic material is predominant, different results were reported, too [39]. A more detailed study of the samples collected from distant areas, in different seasons and using solvents in a wide range of polarity, from polar to completely unpolar, can be of great importance in distinguishing the organic matter present in natural samples with regard to hydrophobicity.

From the $\pi-A$ isotherms, thermodynamic parameter describing viscoelastic properties of mono-

layers (compressional modulus C_s^{-1}) was calculated according to the relation $C_s^{-1} = -A(d\pi/dA)$ [40]. C_s^{-1} was then recorded at limiting specific area A_0, which is obtained by extrapolating the linear part of the surface pressure-area curve to zero surface pressure. Higher C_s^{-1} means a more condensed (less compressible) monolayer. The inverse value of compressional modulus is compressibility, and higher values are obtained from more compressible and elastic films.

The compressibility values for samples L_1, L_2, L_3 and L_4 are given in Table 1. Samples denoted by 'a' are extracted with chloroform, those denoted by 'b' are *n*-hexane and those by 'c' dichloromethane extracts. The values of compressibility presented in Table 1 are in the range of previously reported values for microlayer samples [41]. Values are higher than those for single organic compounds. For comparison, the compressibilities of the monolayers of some pure compounds are as follows: cholesterol and stearic acid 0.0012 and 0.0019 m mN^{-1}, respectively; some proteins between 0.015 and 0.03 m mN^{-1}; sedimentary humic acid in different molecular weight range between 0.024 and 0.036 [42]; chlorophyll 0.021 m mN^{-1}; vitamin A 0.019 m mN^{-1}, and sodium dodecylbenzene sulfonate (NaDBS) 0.017 m mN^{-1} [41]. It is obvious that natural films, being a complex mixture of different organic substances with different properties, are less condensed, i.e. more expanded than monolayers of simple compounds. This is also the reason that the compressibility of a real microlayer sample does not represent a true thermodynamic parameter. It is known that for the determination of wave damping ratios taking into account the theory of Marangoni waves [43] the dilational moduli obtained under dynamic conditions by the channel method are more suitable than the compressibility values obtained under quasi-static conditions in a Langmuir trough [44,45]. Although these values are higher if compared with dilational moduli, they can be a useful parameter for qualitative estimation and comparison of different microlayers elasticity.

In Table 1 values for surface potential of samples L_1, L_2 and L_3 at the pressures corresponding to full compression are also given. The values of compressibility and surface potential of different films reported previously are given for comparison and specially marked. The surface potential values are positive in the whole area region without significant fluctuation in ΔV–A isotherm. They increase by decreasing the area, which can be seen from the Table 1 and Figs. 1 and 2. Such behaviour indicates that only molecular density and not the surface dipole moment was changed by compressing the monolayer.

Table 1
Values of compressibility and surface potential for different lake (L)- and sea (S)-surface microlayers

Sample		Compressibility (m mN^{-1})	ΔV (V)
L_1	a	0.064	0.40 ($\pi = 13$ m mN^{-1})
L_1	b	0.079	0.38 ($\pi = 9$ m mN^{-1})
L_2	a	0.068	0.42 ($\pi = 15$ m mN^{-1})
L_3	a	0.058	0.40 ($\pi = 15$ m mN^{-1})
L_3	b	0.061	0.23 ($\pi = 12$ m mN^{-1})
L_4	a	0.051	
L_4	b	0.059	
L_4	c	0.083	
L_5[a]	a	0.057	0.25 ($\pi = 15$ m mN^{-1})
S_1[b]	a	0.046	0.29 ($\pi = 23$ m mN^{-1})
S_2[b]	a	0.077	0.27 ($\pi = 23$ m mN^{-1})
S_3[b]	a	0.076	0.31 ($\pi = 13$ m mN^{-1})
S_4[a]	a	0.098	0.27 ($\pi = 20$ m mN^{-1})
S_5[a]	a	0.064	0.30 ($\pi = 15$ m mN^{-1})

Samples denoted by a are extracted with chloroform, those denoted by b are *n*-hexane extracts and those by c dichloromethane extracts.

[a] Ref. [64].

[b] Ref. [62].

3.2. Spectroscopic techniques

3.2.1. Reflection spectroscopy

Regarding optical properties of marine samples, the enrichment in light absorbing material at 280 nm was found in the sea-surface microlayer [46] and was ascribed to the dissolved phenolic material. This material could originate from exudation of polyphenolic compounds from marine macroalgae or from plant and algal decays in the case of humic substances. This material, known as chromophoric dissolved organic matter (CDOM), represents the predominant light absorbing part of dissolved organic material, which is chemically

complex and not very well defined mixture of anionic organic oligoelectrolytes containing phenolic surface active moieties [47,42]. Most of light absorbing substances absorb only in the UV ($\lambda <$ 400 nm) region, but some of unidentified chromophores in CDOM absorb also in the visible ($\lambda <$ 700 nm) region. The enrichment in light absorbing material in the microlayers has significant effect on transformation processes at the interface and on the properties of interface itself [2]. Several spectroscopic methods like infrared reflection absorption spectroscopy (IRRAS) [48–50], Raman spectroscopy [51] and laser spectroscopy [52] was shown to be useful for characterization of CDOM in microlayers. In studies of ex-situ reconstructed surface microlayers we applied reflection spectroscopy method which is based on the fact that the reflection is enhanced due to the presence of chromophores at the air/water interface within the spectral range of chromophore absorption [27]. Reflection is dependent on the chromophore density and orientation at the interface while molecules dissolved in the aqueous subphase do not contribute to the signal. The method was developed by Möbius and coworkers and till now was used for the studies of interactions of water soluble species with insoluble lipid monolayers and in studies of lipid–protein interactions [53–58]. The reflection spectra ΔR, measured at normal incidence of light from the ex-situ reconstructed surface microlayer L_3 (*n*-hexane and chloroform extracts) with maxima at 370 and 350 nm, respectively, are shown in Fig. 4a and b. Reflection spectra were recorded at different initial surface pressures. The results show that the reflection ΔR increases with increasing the initial surface pressure. From Fig. 4a and b it can be seen that the value of ΔR at the pressure of 15 mN m^{-1} for the reconstructed film of sample L_3 is 0.35% for *n*-hexane extract and 0.10% for chloroform extract. The same ratio was observed with sample L_1, where at the pressure of 15 mN m^{-1}, ΔR value for *n*-hexane extract was 0.25% and the value for chloroform extract was 0.095 (data not shown here). Since the pressure is correlated to the concentration of surface active material and the magnitude of reflection signal is dependent on the density of chromophores at the interface we can conclude that more organic matter with an absorption in the range from 300 to 600 nm was present in *n*-hexane extract than in chloroform extract of the same microlayer sample. Taking into account polar properties of the used solvents, one can assume that CDOM in the particular sample was of predominantly hydrophobic character. However, to be able to draw more certain conclusions about the character of CDOM from the reflection measurements, further measurements are needed. These preliminary reflection spectroscopic results of ex-situ reconstructed sea surface microlayers open new possibilities to study CDOM present in microlayers and its interaction with different solutes from the bulk solution which can be either of natural or anthropogenic origin.

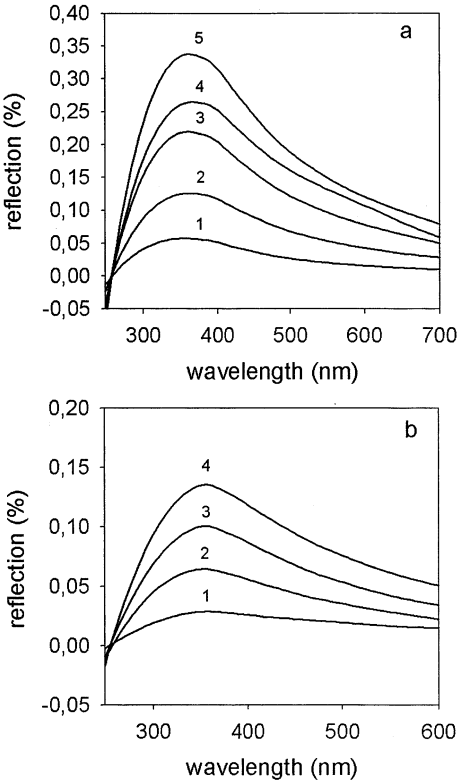

Fig. 4. (a) Reflection spectra obtained from *n*-hexane extracts of the surface microlayer L_3 spread on the sodium chloride solution taken at different surface pressures: (1) 2; (2) 5; (3) 7.5; (4) 10 and (5) 15 mN m^{-1}. (b). Reflection spectra obtained from chloroform extracts of the surface microlayer L_3 spread on the sodium chloride solution taken at different surface pressures: (1) 2; (2) 10; (3) 15 and (4) 17 mN m^{-1}.

3.2.2. Brewster angle microscopy

Brewster angle microscopy (BAM) is based on lateral changes of the refractive index and/or the thickness of the film. p-Polarized light is focused on the air/water interface at the Brewster angle which is about 53° when using visible light. In the case of clean aqueous surface there is no reflection. If the angle is kept constant, the optical situation changes in the presence of a monolayer and reflection can be observed. BAM was developed as a powerful method for optical characterization of monolayers at the air/water interface, providing information about the homogeneity of the film, existence and formation of domains, phase transition and adsorption of material from the aqueous phase [28,29,59,60]. We applied this technique to the studies of natural aquatic microlayers and phytoplankton culture samples a few years ago [61–64]. BAM was shown to be a very efficient analytical tool for optical characterization and visualization of natural microlayers.

BAM images of ex-situ reconstructed surface films obtained from samples L_1 and L_2 have been recorded under increasing surface pressure and are shown in Figs. 5 and 6. These images have been taken by using high quality BAM 2 instrument with high resolution. Both samples showed liquid condensed domains (small bright areas) in liquid expanded phase and/or in gas analog phase (dark background). At the beginning, domains are small and they grow rapidly surrounded by coexisting liquid phase during compression. In sample L_1 domains appeared immediately after spreading, and upon compression they were pushed together making liquid condensed phase. The appearance of a liquid condensed phase at low surface pressure indicates strong attractive lateral interactions as has been observed, e.g. with monolayers of long-chain fatty acids [65] and recently with monolayers of crown ethers [66]. BAM images of sample L_2 did not show formation of domains at surface pressure ≤ 1 mN m^{-1} (Fig. 6a), however the entire monolayer area was covered with LC domains after the compression to $\pi = 6.1$ mN m^{-1} (Fig. 6b). The morphology of the reconstructed microlayer samples L_1 (both extracts) and L_2 (chloroform extracts) is very similar and characteristic features as in images of previously reported sea-surface microlayers S_1 [62] and S_5 and L_5 [64] can be identified. In those previous measurements homogeneous films in which no features were detected during the compression to high lateral pressures have been observed as well [62]. In those cases it was assumed that only one monolayer phase was present, leading to the increased film brighteness of the image, without formation of any structure upon compression. However, if the domains smaller than 1 μm diameter are formed, the phase transition may not be detected with our instrument. If two phases are present in a monolayer, the phase separation

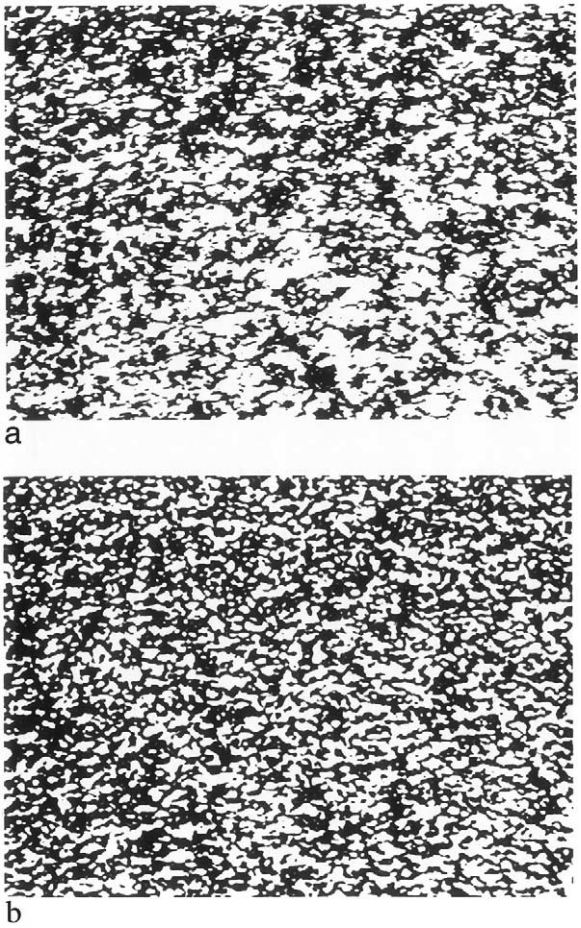

Fig. 5. (a) BAM video image of the ex-situ reconstructed surface microlayer sample L_1 (chloroform extract), $\pi = 0.5$ mN m^{-1}. (b). BAM video image of the ex-situ reconstructed surface microlayer sample L_1 (*n*-hexane extract), $\pi = 0.5$ mN m^{-1}.

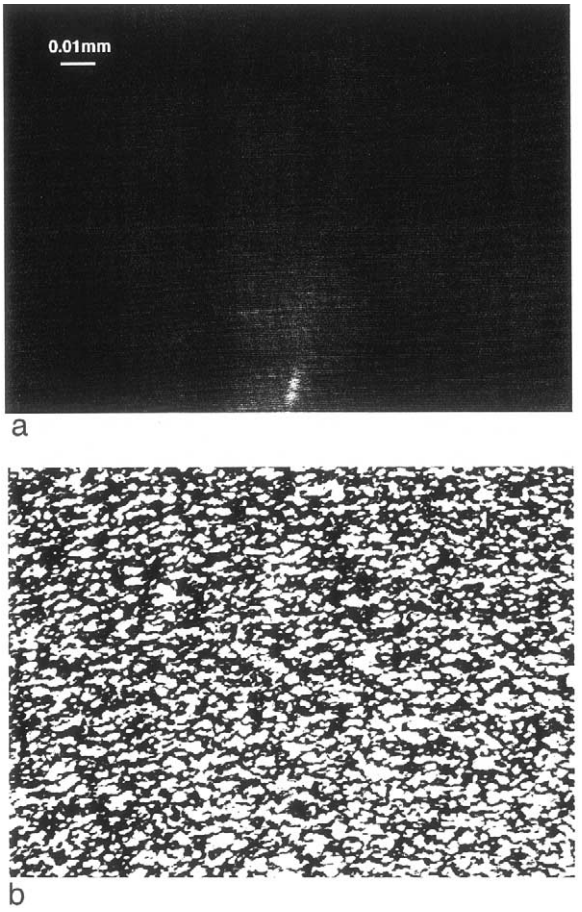

Fig. 6. (a) BAM video image of the ex-situ reconstructed surface microlayer sample L_3 (chloroform extract), $\pi = 0.2$ mN m^{-1}. (b). BAM video image of the ex-situ reconstructed surface microlayer sample L_3 (chloroform extract), $\pi = 6.1$ mN m^{-1}.

can easily be seen by BAM if optical properties of the two materials are sufficiently different. When comparing BAM results with surface pressure measurements for the same samples, which showed no phase transition of the films L_1, L_2 and L_3 (chloroform extracts), it is obvious that π–A isotherms are insufficient for film characterization. BAM contributes considerably to a better characterization of the microlayer samples and enables optical visualization of the film morphology.

In previous papers we already demonstrated that BAM can be a very efficient analytical tool for characterization and visualization of natural films at the air/water interface. However, all BAM experiments, including those presented here, have been done so far with ex-situ reconstructed natural microlayers on aqueous subphases. The next step in our investigations will be the attempt to take BAM images directly from the air/water interface, i.e. to do in-situ measurements. Preliminary investigations have been done by using portable Brewster angle microscope MiniBAM at the marine station of the Ruđer Bošković Institute in Šibenik, Middle Adriatic. The MiniBAM was mounted on a small platform lowered to the water surface and was connected to an automobile battery as a power supply. The images were recorded with the portable PC. The surface microlayer could be observed; however, it was practically impossible to take images due to rapid motion of the water surface and too slow capturing of the pictures by the existing PC imaging system program. Nevertheless, a direct observation of the microlayer at the water surface using MiniBAM is possible, but it needs technical improvements what will be done in the near future. This will enable us to study the dynamics of its formation and of temporal changes in field experiments.

3.3. Electrochemical techniques

For many years the capacity measurements have been used for determination of surface active substances in natural aquatic samples [67]. A comparison of the capacitance–potential or current–potential curves of the real samples with those of selected model substances served as a tool for rough characterization of natural surface active substances.

Here we applied modified electrochemical method to study the ex-situ reconstructed natural films. The method is modified in the sense that the organic solvent extract of natural surface film was spread onto the electrolyte solution and then transferred from the air/electrolyte interface to the mercury surface by vertical dipping the electrode through the film. Afterwards, the capacitance of the film was determined by using phase sensitive alternating current voltammetry (out-of-phase signal). The method was originally designed and used for the studies of model lipid monolayers

and their interactions with various species in the bulk solution and as a very sophisticated system for the study of the structure and functioning of biological membranes [31,32,68–73]. To our knowledge it has not been used for the characterization of natural aquatic films until present. The capacity–potential curves of the original sea-surface microlayer sample L_4 and the subphase taken 20 cm below are shown in Fig. 7a (curves 2 and 1). The enrichment in surface active material in the surface microlayer sample related to the subphase is obvious from the lower capacitance value for the microlayer (at $E = -0.6$V, Fig. 7a). Capacity potential curves for ex-situ reconstructed films of *n-hexane*, chloroform and dichloromethane extracts of the microlayer sample L_4 are shown in Fig. 7b. When comparing capacity-potential curves for the original sample (Fig. 7a) and those for the reconstructed films (Fig. 7b) the differences in the shape of the curves, namely the position of desorption waves can be seen. Desorption peak of the original film (Fig. 7a) appears at a very negative potential indicating that very polar organic material is predominant in the sample. Through extracting the organic material with solvents of different polarities and by comparing the shape of capacitance curves and the position of desorption peaks of three different extracts, it can be seen that desorption peaks shift towards more positive potential with decreasing the extracting solvent polarity (Fig. 7b). The obtained values of capacitance minima (at −0.6 V) for ex-situ reconstructed films L_1, L_2 and L_3 are 3.5, 2.3 and 1.6 μF cm^{-2}, respectively. Capacitance values of *n*-hexane, chloroform and dichloromethane extracts of the sample L_4 are 4.14, 3.75 and 3.33 μF cm^{-2}, respectively (Fig. 7b). For comparison, the values for model lipid monolayers are for lecithins around 2 μF cm^{-2} [31] and 3.0 μF cm^{-2} [72], for dimyristoylphospatidic acid (DMPA) 2.9 μF cm^{-2} [72] and for dimethyloctadecyl ammonium bromide (DOMA) 1.78 μF cm^{-2} [74]. In principle, low capacitance value corresponds to adsorption with the hydrocarbon chains oriented towards mercury. The theoretical calculated value for a 2.5-nm-long carbon chain perpendicular to the mercury and for a dielectric constant of ~2 is 0.7 μF cm^{-2} [68]. The values for ex-situ reconstructed films L_1, L_2, L_3 and L_4 are close or equal to the minimum values for model lipid monolayers. We can assume that reconstructed films are in a condensed phase. Discrepancy from theoretical capacitance value for perpendicular orientation both for the model lipids and the reconstructed films is probably due to folding and overlapping of the hydrocarbon chains on the mercury surface.

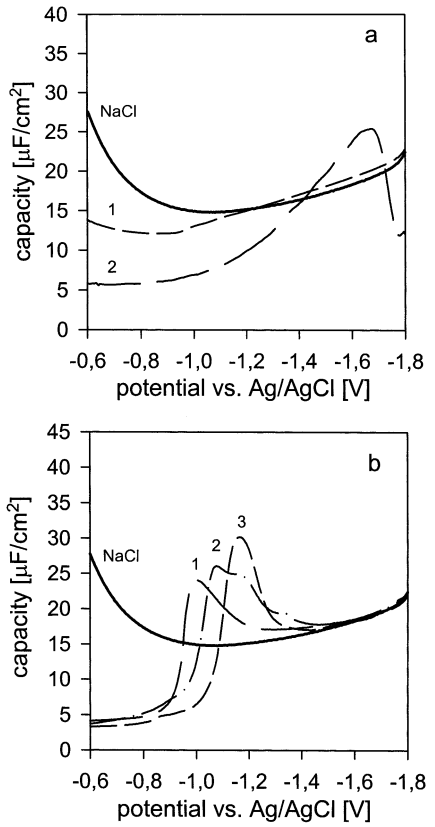

Fig. 7. (a) Capacity–potential curves of 0.55 mol l^{-1} NaCl, water from the depth of 20 cm (subphase) (curve 1) and the original microlayer sample L_4 (curve 2). (b). Capacity–potential curves of 0.55 mol l^{-1} NaCl and ex-situ reconstructed microlayer sample L_4: *n*-hexane extract (curve 1); chloroform extract (curve 2) and dichloromethane extract (curve 3).

Additional characterization of the structure of sea-surface microlayers has been done by using an electrochemical probe. Redox processes of cadmium were chosen as indicator of the permeability of different films adsorbed at mercury electrode for cadmium ions. Mass and charge transfer

processes at the covered electrode surface depend on the porosity of the adsorbed layer, as well as on the mechanisms of exchange reactions at the interfaces. The cathodic (reduction) and anodic (oxidation) waves of cadmium in the presence of the reconstructed films of the microlayer sample L_4 transferred from the sodium chloride solution to the mercury surface are shown in Fig. 8. The difference between these two processes is the following. The reduction of cadmium is occuring at the electrode surface previously modified by adsorbed organic layer, i.e. the ion must pass from the solution to the mercury surface across the organic coating on the electrode. Oxidation process is taking place also at the electrode modified by the film, but cadmium is first accumulated in mercury at the potential more negative than standard redox potential, and then, by applying the potential scan in the positive direction, cadmium is passing from the mercury across the hydrophobic layer to the aqueous solution. Both processes are strongly affected by the presence of transferred films which is shown in Fig. 8 and Table 2.

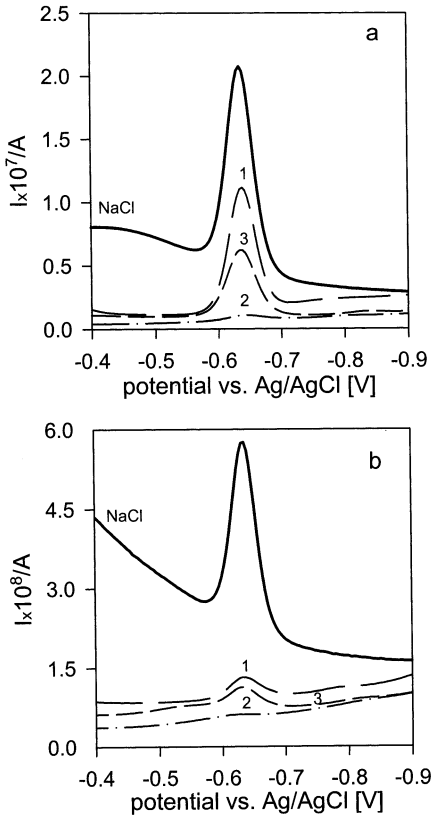

Fig. 8. (a) Ac voltammetric cathodic waves (in-phase signal) of 10^{-4} mol dm^{-3} cadmium in 0.55 mol l^{-1} NaCl (curve 0) and in the presence of ex-situ reconstructed films of microlayer sample L_4. *n*-Hexane extract (curve 1), chloroform extract (curve 2) and dichloromethane extract (curve 3). (b). Ac voltammetric anodic waves (in-phase signal) of 10^{-5} mol l^{-1} cadmium in 0.55 mol l^{-1} NaCl (curve 0) and in the presence of ex-situ reconstructed films of microlayer sample L_4, *n*-hexane extract (curve 1), chloroform extract (curve 2) and dichloromethane extract (curve 3).

It is known that the effect of adsorbed layer on the reduction of cadmium can differ from the effect on oxidation process, depending on the type of organic coating which can act either as a barrier for the transport or can complex the metal [75]. In our case, substances present in the original sample and in all three extracts represent a barrier for both processes. Stronger inhibition was observed with the extracts than with the original sample, as expected. However, if we assume that by *n*-hexane mostly lipid material is extracted, we could expect strongest inhibition of cadmium reduction with *n*-hexane extract since it is known that lipid material causes higher degree of inhibition than the substances such as proteins, polysaccharides and humic substances [32,76,77] whose extraction with the solvents of higher polarity should be more efficient. The reflection measurements indicated that in *n*-hexane extract more CDOM,

Table 2
Influence of the original sample L_4 and of the reconstructed films from *n*-hexane, chloroform and dichloromethane extracts on the redox processes of cadmium

Sample L_4	I/I_0 (cathodic wave)	I/I_0 (anodic wave)
Original sample	0.73	0.71
n-Hexane extract	0.52	0.11
Chloroform extract	0.03	0.02
Dichloromethane extract	0.32	0.11

I denotes the current for electrode process of cadmium in presence of the original or the reconstructed microlayer sample. I_0 denotes the current for electrode process of cadmium in absence of microlayer sample.

which is mostly ascribed to the dissolved phenolic material, was present than in chloroform extracts. Taking into account that polyaromatic substances like pyrene do not inhibit electrode reduction of cadmium [78] we could suppose that such substances combined with lipids and extracted by *n*-hexane could cause smaller inhibition than lipids alone.

4. Conclusion

1. Complex methodological approach was applied to the investigation of sea-surface microlayers enabling better insight into the morphology of natural films, relationship between the concentration and type of substances and the properties of films regarding the exchange between the film and subsurface. Studies of the original and reconstructed films were performed.

2. Advantages of using complex investigations are obvious. Comparing results of monolayer studies and BAM measurements we realized that in measurements of $\pi-A$ isotherms for samples L_1, L_2 and L_3 (chloroform extracts) no phase transition was observed, while BAM images of same samples showed liquid condensed domains in liquid expanded phase, meaning that $\pi-A$ isotherms were insufficient for film characterization. On the other hand the elastic properties of films were very well characterized by $\pi-A$ isotherms.

3. The fact that organic matter in sea-surface microlayer contains a complex mixture of different substances of biological and anthropogenic origin represents an important reason to fractionate the original sample by extraction with organic solvents of different properties which can be then successfully used for the studies of reconstructed films either directly on the air/water interface using reflection spectroscopy or electrochemically by transferring them to the mercury electrode. This offers a new analytical possibility to investigate the interactions in films, i.e. the effect of potential organic pollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated byphenyls (PCBs), metals, etc. present as solutes in the aqueous phase on the structure and morphology of the reconstructed layers.

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