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ORIGINAL CONTRIBUTION

P. Degen
H. Rehage
F. -G. Klärner
J. Polkowska

Characterization of Langmuir-monolayers of molecular clips by means of Brewster-angle-microscopy

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P. Degen (✉) · H. Rehage
Lehrstuhl für Physikalische Chemie II,
University of Dortmund,
Otto-Hahn-Str. 6,
44227 Dortmund,
Germany
E-mail: patrick.degen@uni-dortmund.de
Tel.: +49-231-7553913
Fax: +49-231-7555367

F. -G. Klärner · J. Polkowska
Institut für Organische Chemie,
University of Duisburg-Essen,
Universitätsstr. 3-5, 45141 Essen,
Germany

Abstract Molecular clips are able to selectively bind electron deficient aromatic and aliphatic substrates, and these processes are usually investigated in dilute solutions of organic solvents. Caused by discrepancies between polar and hydrophobic groups, molecular clips are surface-active compounds and, in analogy to surfactants, they can form monomolecular films at the water surface. In this publication, we systematically investigated the self-association process and the phase-behaviour of three different molecular clips with the polar head groups -OCH_2COOH (a), -OCH_2COOEt (b), and -OCONHPh (c) by means of surface-pressure-area-isotherms and Brewster-angle-microscopy (BAM). We observed marked differences for all investigated surface-active compounds. The molecular surface areas of the three clips, determined from pressure-area-isotherms, could be traced back to the molecular diameters of the amphiphilic compounds. In several experiments we investigated the influence of diverse film compression and expansion steps. Hysteresis effects could be explained by different film morphologies. In a series of experiments we could show that the aromatic guest molecule 1,2,4,5-tetracyanobenzene (TCNB), which strongly binds to molecular clips, did not influence the phase diagrams and film structures.

Keywords Molecular clips · Langmuir films · Brewster angle microscopy

Introduction

In recent years, amphiphilic monolayers which often represent two-dimensional model systems, have been the subject of extensive studies. The main interest has been focused on phase transitions and ordering processes in two dimensions [1–4]. The monolayer technique is a useful instrument for model studies, because surface pressure/area (π–A) isotherms reflect the intermolecular forces operating in 2D arrangements of molecules and these data provide information on the molecular packing [5]. Depending on temperature, there are characteristic differences in the transitions between the different monolayer states with increasing molecular density. Usually the single-phase regions are described as gaseous (G), liquid expanded (LE), liquid condensed (LC) and solid condensed (SC) state. By extrapolation of the isotherms from solid state to zero pressure, the size of the amphiphilic molecules can be estimated assuming a dense packing of these molecules [6]. In many cases, the isotherm has a marked plateau regime. Normally, these plateaus are representing a first order phase transition between a LE and a LC phase. Besides these 2D phase transitions, there are numerous examples like supersaturating of the LE or gaseous phase, hysteresis or 2D/3D transition witch can also lead to the onset of plateau regimes [4, 7, 8]. Microscopic observations of the textures of Langmuir-monolayers became possible one and a half decade ago, first by the means of fluorescence microscopy and more recently caused by new techniques

of ellipsomeric microscopy and Brewster-angle-microscopy (BAM). Extensive description of BAM is already summarized in several publications [9–12]. The main advantage of BAM compared to fluorescence microscopy is that the morphology of monolayers at the air/water interface can be visualized on a microscopic scale without adding new compounds as fluorescent probe molecules. At present, BAM is one of the most informative methods for the analysis of monolayers structures [2]. BAM uses the zero reflectance of an air/water surface for parallel (p) polarized light at the Brewster angle of incidence. The condensed phase of a monolayer leads to a measurable change in reflectivity, thus, allowing the visualization of the monolayer morphology. Molecular clips are interesting model substances, on the grounds of their supermolecular properties like self-assembling and molecular recognition [13]. Specific interactions between different molecules are important in many areas of biological and supramolecular chemistry, e.g. in antigen–antibody recognition or enzyme–substrate binding [14]. Self-assembling might be used for designing new materials, on the basis of molecular sensors. Specific interactions between molecules depend on specific, mostly non-covalent, interactions of arenes with other aromatic units. These π–π or arene–arene interactions seems to be particularly important for the design of efficient synthetic receptors. These specific host-guest-relationships were often studied in dilute organic or aqueous solutions by Klärner et al. [13, 15, 16]. Because of the discrepancy between polar and hydrophobic groups, molecular clips have striking surface active properties and, in analogy to surfactants, they can form monomolecular films at the water surface. This phenomenon offers the opportunity to investigate molecular recognition processes in the vicinity of fluid surfaces. Details of the film structures of molecular clips were already discussed in two recent publications [14, 15]. In this article, we present systematical studies of the surface-active properties of three different molecular clips, which were spread at the water surface. In a series of experiments we investigated the influence of three polar head groups (-OCH_2COOH, -OCH_2CO_2Et and -OCONHPh) on the film structure (Fig. 1). We also explored specific interactions with the aromatic guest molecules 1,2,4,5-tetracyanobenzene (TCNB). The results of these measurements give new insight into fundamental processes of self-association, and they might also be useful for developing new chemical sensors on the basis of Langmuir–Blodgett films.

Materials and experimental techniques

The molecular clips were spread on the pure water surface from a 10^{-3} M solution in Chloroform (p.a. Merck). In a similar way, we also spread 1:1 mixtures of molecular clips and TCNB. The water was obtained from a pure water system (Seralpur PRO 90 CN). Synthesis and molecular properties of the molecular clips are described elsewhere [12, 15, 16]. A schematic drawing of the molecular clip with three different substituents is shown in Fig. 1.

The film structures of these molecular clips were investigated by means of pressure-area isotherms and BAM. The π–A isotherms were recorded using a Langmuir trough (001BAM) and a continuous Wilhelmy-type measuring system. These instruments were constructed by NIMA Technology (Coventry, England). BAM-images were performed with a BAM-2 apparatus, which was developed by the Nanofilm Technology Company in Göttingen, Germany. Extensive description of BAM-technique is summarized in recent publications [9–11]. The lateral resolution of BAM is limited by the wavelengths of the incident laser beam (690 nm). The BAM images were recorded in a CCD camera, and a video-system was used for image storage and analysis.

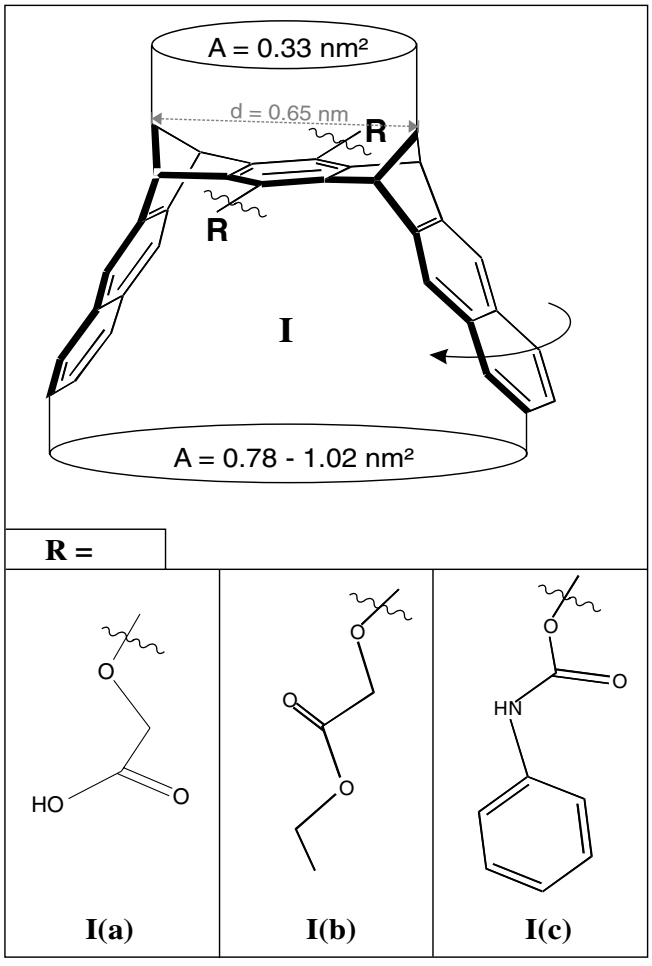

Fig. 1 Structure of investigated molecular clips (I) synthesized by Klärner et al. [12, 16]

The distortion due to the incidence at the Brewster angle was corrected by digital image processing software. The experimental system was additionally equipped with a temperature control, Level-O-matic and vibration isolation system (mod-2).

Variation of the time interval (5 min–2 h) between the spreading process and the beginning of the compression did not influence the data. Therefore, in all our $\pi-A$ isotherm measurements, we started the compression 15 min after spreading. The compression and the expansion velocities were adjusted to 0.2 nm^2 $molecule^{-1}$ min^{-1}. This was the slowest possible velocity of our trough for continuous compression experiments. Slower compression rates were discontinuous and did not have any influence on the slope and shape of the isotherms. Each $\pi-A$ curve was confirmed by at least two repeated, independent measurements. The temperature was always fixed at 20°C.

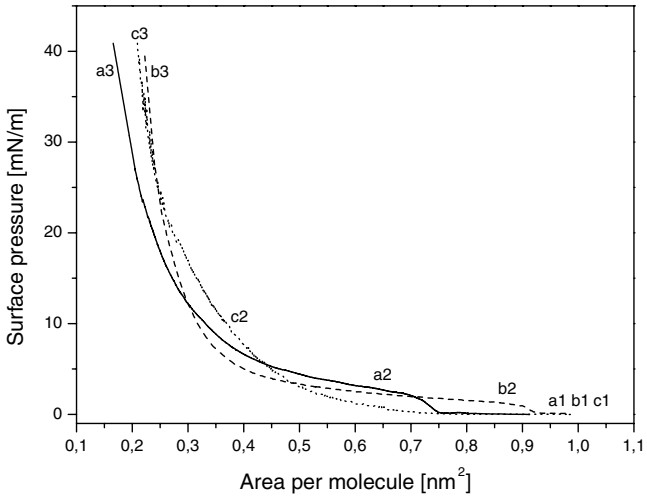

Fig. 2 π/A-Isotherms of three different molecular clips at the air-water interface: *continuous line* I(a), *dotted line* I(b), *dashed line* I(c)

Results

Influence of three different substituents on the phase-behaviour of a molecular clip during film compression

Figure 2 describes the surface pressure-area isotherm for the monolayers of three different clips.

Relevant changes of the film properties are indicated by marked points. The corresponding BAM-images are summarized in Fig. 3. As indicated by the surface pressure-area isotherms, the three clips form Langmuir-monolayers at the air–water surface. The occurrence of multilayers usually lead to a sharp decline of the surface pressure and bright BAM-images. The isotherms of I(a) and I(b) show plateau regimes at about 1.2 and 2.5 mN/m. Such plateaus often describe the transition between the LE and the LC phase [12]. Other reasons for the formation of regimes of constant surface pressure are supersaturating of the LE or gaseous phase, hysteresis effects or 2D/3D transitions [7, 8].

For I(a) and I(b) the film forms a homogenous gaseous phase in the regime of zero-surface pressure immediately after spreading. The first bright areas, showing the presence of domain structures, became visible at points a2 and b2 in Fig. 2. This corresponds to

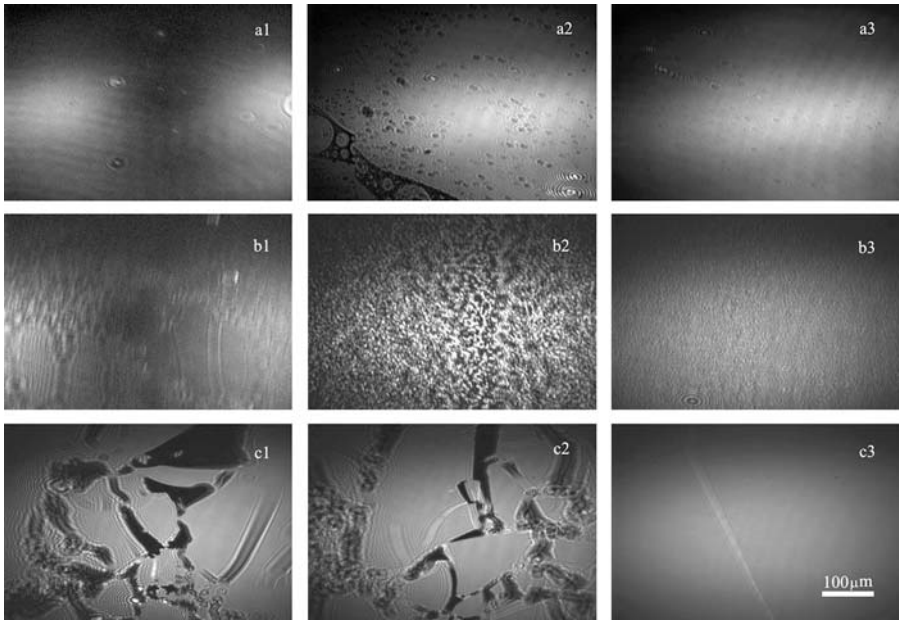

Fig. 3 Brewster-angle-microscopy-images of the monolayers of three different molecular clips during compression. Parameters correspond to the points indicated in Fig. 2

the onset of the plateau regime. Unfortunately, the BAM images presented in Fig. 3 did not show the formation of well-defined phase domains. We observed, instead, rather unstructured bright areas in addition to a darker phase. If domains or aggregates were formed, these structures were small and below the limit of optical resolution (690 nm). Finally, at high surface pressure of $\pi > 15$ mN m^{-1}, a continuous phase without microscopic defects was obtained.

At the present state we cannot explain the appearance of the plateau regimes on the basis of BAM images alone. Only a systematic change of the plateau in the π-A isotherms towards higher pressures as the temperature increases can provide doubt-free evidence for a LE/LC transition. Unfortunately, we did not have enough material for a large number of measurements at different temperatures.

The $\pi-A$ isotherm of I(c) differs from the others, because we did not detect any plateau regime. Consequently, we may state that a distinct two-phase region did not occur. In contrast the BAM-images showed that already for zero-surface pressure and large areas the clip I(c) formed large aggregates and dense zones up to 300 μm (c_1 in Fig. 3). Thus the BAM-images showed that I(c) did not spread homogenously. This phenomenon was evidently induced by strong attractive interactions between the molecular clips. When the linear portion of the solid-condensed phase was extrapolated to zero-surface pressure, the interception gave molecular areas of approximately 0.3 nm^2/molecule. Assuming that the molecules can rotate around their principal axis, this corresponds to an effective diameter of 0.68 nm. By semi-empirical computation (CS MOPAC Pro), this distance approximately corresponds to the diameter of the bridging carbon atoms (see Fig. 1). The smallest possible distance between the bridging carbons atoms obtained from molecular models is not smaller than 0.58 nm, corresponding to an effective area of 0.26 nm^2. Thus, it turns out that under compression the arene groups of the molecular clips were oriented perpendicular to the water surface (Fig. 4) [14].

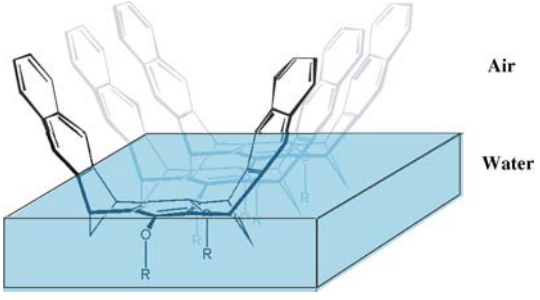

Fig. 4 Schematic drawing of the molecular orientation of the polar clips at the water surface

Even in the compressed state, the polar head-groups of the molecular clips did not have a marked influence on the required surface areas (Table 1).

Surprisingly, the measured surface areas did not depend on the chemical composition of the polar head groups. This phenomenon was probably induced by the flexibility and different molecular conformations of the polar groups. In organic solvents like Chloroform at least one group was orientated in direction of the cavity of the clip molecules [13, 15]. In the vicinity of the water surface, however, we might assume that both polar groups are oriented in the direction of the polar solvent. On the other hand, very small molecular diameters can also point to the existence of 2D/3D transitions, although we did not observe bright structures during the compression up to surface pressures of about 45 mN/m.

Influence of compression on the phase behaviour of three molecular clips

For the three molecular clips various compression–expansion cycles led to hysteresis effects of the $\pi-A$ isotherms. Typical results are represented in Fig. 5 for the clip I(a). We also observed similar properties for clip I(b) and I(c).

The surface pressure of the expansion curves was lower than the corresponding data of the compression isotherms. If the expansion or compression processes are too fast for molecular diffusion, there is not enough time for amphiphiles to attain true equilibrium structures. This structural re-organization process is often denoted as a relaxation process. In our measurements, the second compression was always different from the first one, even after waiting a period of 12 h. So the observed hysteresis was not caused by relaxation processes. The solubility of the molecular clips is very low (< 0.05 mg/100 ml water at 20°C) so that diffusion into the water sub-phase cannot explain the observed hysteresis. In a series of experiments, we did not see any evidence for relaxation processes of the surface pressure, which were caused by diffusion into the water sub-phase. The aggregates formed by the first compression process were very stable (like a two-dimensional crystal), and they did not decay into separated clip monomers (this holds, at least for 12 h and at a temperature of 20°C). Another possibility to explain the shape of the $\pi-A$ curves is the

Table 1 Influence of the polar head groups on the required surface area of molecular clips in the solid condensed phase

Molecular clip	Required space (nm^2)
I(a)	0.29 ± 0,08
I(b)	0.28 ± 0,01
I(c)	0.27 ± 0,02

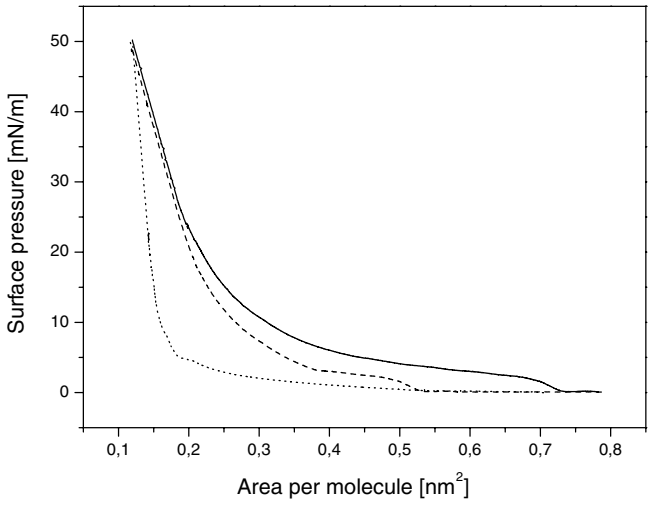

Fig. 5 Reversibility studies of the π/A-isotherms of monolayers of the clip I(a) at the air–water interface: *continuous line* first compression; *dotted line* expansion; *dashed line* second compression after 30 min

occurrence of 2D/3D transitions. In analogy to the π–A isotherms, the BAM images showed the irreversible processes of structure formation (Fig. 6).

The left column of Fig. 6 provides information of the first compression process of I(b). During the first compression, we did not observe bright structures in BAM-images. This suggests that no 2D/3D film formation took place. The bright irregular structures in the first pictures of the right column were observed during the second compression. These images show metastabile aggregates, which were formed during the first surface concentration process. During the film expansion after the first compression (last picture of the left column without bright structures) the film breaks into large aggregates. These fragments, showing irregular structures, were observed in the second compression experiment (first picture of the right column). The striking effect of luminance of the structures in the first picture of the right column is caused by the high contrast with respect to the pure water surface (black area between the structures) (page 11, line 9–18).

In the regime of high surface pressures the aggregates started to overlap, and they finally formed a homogeneous, dense film of solid state. Due to the fact that many molecular clips were already aggregated in the condensed phase, the first increase in pressure was shifted towards smaller areas per molecule (Fig. 5). At higher pressures the isotherms of the first and the second compression processes were almost identical.

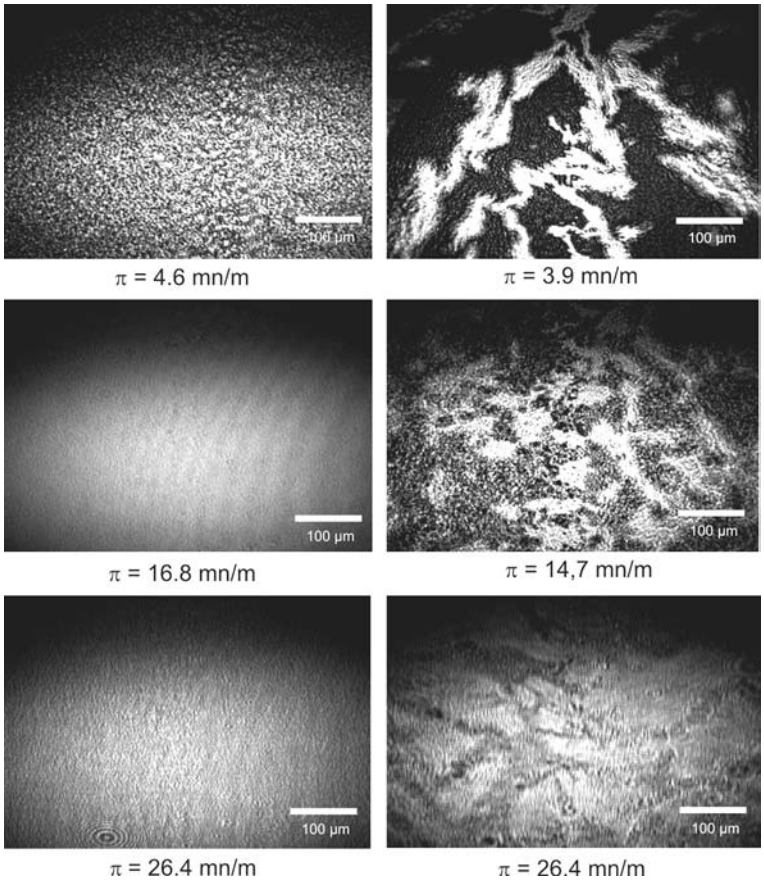

Fig. 6 Brewster-angle-microscopy-images of monolayers of the molecular clip I(b) during the first (*left column*) and second compression (*right column*)

For the three clips hysteresis was only observed between the first and the second compression step. The $\pi-A$ isotherms and the BAM-images of the third compression cycle were always similar to the second one. This typical behaviour is summarized in Fig. 7.

The observed phenomena can also be explained by the formation of three-dimensional aggregates. Such crystal-like structures were also formed at high concentrations in solutions.

Influence of molecular guest (TCNB) on the phase behaviour of the molecular clips

It has recently been shown by Klärner et al. [13, 15, 16] that the investigated molecular clips are able to selectively complex electron deficient aromatic and aliphatic substrates in dilute solutions of organic solvents. In a series of experiments, we investigated the host–guest relationship between the three molecular clips and the aromatic substrate TCNB, which is not soluble in water. Relevant results are summarized in Fig. 8.

Both $\pi-A$ isotherms are almost identical. Similar results were also obtained for the two other clips. There is evidently no significant influence of the guest molecule TCNB on the surface properties of the clips. In addition to the $\pi-A$ isotherms, the BAM-images did not show significant differences between the pure clips and their corresponding complexes with TCNB. In solution, the three clips can bind TCNB inside their cavity [13]. This process was already investigated by H-NMR measurements for complexes between TCNB and I(a) [13, 15].

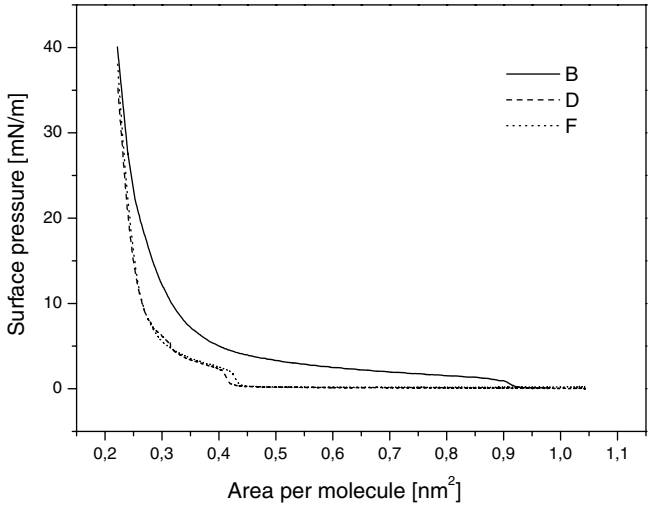

Fig. 7 Reversibility studies of the π/A-isotherms of monolayers of the molecular clip I(a) at the air–water interface: *continuous line* first compression; *dotted line* second compression; *dashed line* third compression. Between the three compressions processes the film was expanded for 30 min

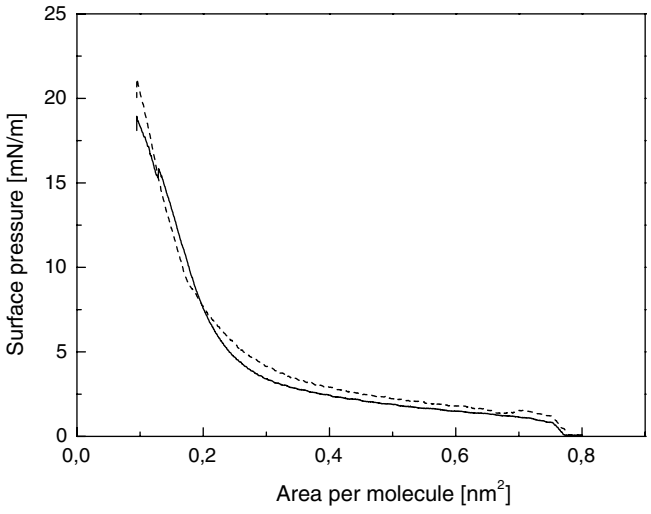

Fig. 8 Influence of TCNB on the π/A-isotherm of I(b): *continuous line*: addition of TCNB; *dotted line*: without guest molecule TCNB

In the crystalline state, however, TCNB was located in the gap between two naphthalene-units. The cavity was empty in this case [13]. In this co-crystal the distance between the side arms was of the order of 1 nm, and this result did coincide with semi-empirical AM1 and DFT pBP/DN (vide infra) calculations of the empty clip I(a) [13]. The packing of the pure clip I(a), investigated by single-crystal structure analysis, was characterized by introducing one methyl group of each molecule into the neighbouring cavity. These intermolecular contacts expanded the clip by 0.14 nm compared to the empty compound. In our experiments, the clips were evidently oriented perpendicular to the water surface. The polar groups should be oriented towards the solvent phase, and in such molecular conformations, the methyl groups cannot fill the cavity of neighbouring clips. This could be one reason why we did not find any differences in the phase behaviour between the clips and the correspondent "complexes", because in both cases the distance between the side arms was approximately 1 nm. This could be shown schematically in molecular models (Fig. 9), where the distance between the side arms was reduced from 1.2 nm to 1.0 nm.

From these experiments, we may state that either there is no complex formation between TCNB and the investigated clips (like in the crystalline state), or these effects are too small to observe relevant changes at the air–water surface.

Conclusion

In a series of experiments, we used $\pi-A$ isotherms and BAM-images to investigate monolayers of three molecular clips. The data are consistent with the assumption,

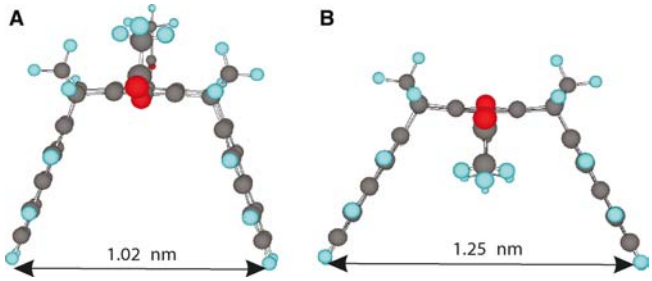

Fig. 9 Simplified molecular model of I(a) in two different orientations of the polar groups. At the water surface the groups can turn out of the cavity (case A) and under these conditions the distance between the side arms is reduced from 1.25 nm to 1.02 nm

that the molecular clips were oriented perpendicular to the water surface. We observed that the different polar head groups -OCH_2COOH, -OCH_2COOEt, and -OCONHPh had striking effects on the phase behaviour. Surprisingly, the measured surface areas did not depend on the chemical composition of the polar head groups. This phenomenon was probably induced by the flexibility and different molecular conformations of the polar groups. The clip I(c) did not spread homogenously at the water surface, but formed stable aggregates, and the phase behaviour was different from the characteristic properties of the other clips. We could show that the compression cycle had a marked influence on the phase structure of the three molecular clips. The formation of condensed aggregates during the compression was at least partially irreversible within the observed timescales. The stability of the condensed aggregates was most significant for molecular clip I(c). These results point to the existence of strong attractive interaction between the clip molecules I(c). Compared to the measurements in dilute organic solvents, we could not detect a marked influence of guest molecules (TCNB) on the film properties of the three molecular clips. This agrees with results obtained in the crystalline state [13].

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