First published in:

Progr Colloid Polym Sci (2008) 134: 80–89
DOI 10.1007/2882_2008_090

Published online: 19 August 2008

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Influence of the monolayers composition on bilayer formation during oblique drop impact on liquids

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Abstract We study the dynamics of two phospholipid monolayers brought into contact by oblique drop impact on a liquid surface and bilayer/multilayer formation. Drop impact without monolayers shows that for low impact angles ($\alpha < 23°$) and low drop velocities the drop spreads as a thin sheet on the target liquid without immersion and mixing of the two liquids. When drop and target liquid surface are covered with monolayers, bilayer/multilayer formation is expected. The composition and mechanical properties of the monolayers can strongly influence the pattern of drop impact and bilayer/multilayer formation. Monolayers with either pure saturated or unsaturated phospholipids, and their mixtures with cholesterol were used. We show that under all conditions studied bilayer/multilayer synthesis takes place. Asymmetric bilayers can be produced by the coupling of drop and target monolayers. For some lipid mixtures the drop and target monolayer collapses during drop impact and symmetric bilayers/multilayers are formed.

Keywords Bilayer formation · Oblique drop impact · Phospholipid-monolayers

Introduction

Liquid interfaces with monolayers play an important role in many diverse industrial processes, creating colloidal dispersions such as emulsions, micelles or liposomes. When two monolayers come into contact, bilayers can be synthesized. Phospholipid bilayers form liposomes, which are used as vehicles for drug delivery, in cosmetics and in gene therapy [1].

In the present research, two monolayers are brought into contact by oblique drop impact on a liquid surface. The results presented extend a previous study on oblique drop impact without monolayers, focusing on the surface dynamics and mixing motions [2]. The latter shows that for low impact angles ($\alpha < 23°$) and low drop velocities (Weber number We < 140) the drop spreads as a thin sheet on the target liquid without immersion and mixing of the two liquids.

When the drop and target liquid are covered with monolayers, the behaviour of these monolayers apart from equilibrium and the role of their mechanical properties can be studied.

The surface rheological proprieties can be controlled by using monolayers of different lipid compositions. We used saturated and unsaturated lipids and their mixtures with different amounts of cholesterol. At the experimental temperature and film pressure the saturated lipids were in the liquid condensed or solid phase whereas the unsaturated lipids in the liquid expanded phase [3]. The mechanical properties of the monolayers can be tuned with addition of different amounts of cholesterol. The results are used for the proof of bilayer or multilayer synthesis and the conditions of their occurrence.

Materials and Methods

Materials

The target liquid consists of a glycerol/water mixture with 61% glycerol (w/w) and the density of 1158.6 kg/m^3. The drop bulk liquid also consists of a glycerol/water mixture, but with a higher glycerol content of 62.2% (w/w) and has a slightly higher density of 1161.2 kg/m^3. This density difference was chosen to make the drop bulk liquid to sink after the drop impact into the target liquid due to the density difference to visualize possible phospholipids in the contact zone of drop and target liquid. The surface tension of the glycerol/water mixture is 69 mN/m and the bulk viscosity 11.68 mPa s [4]. The glycerol/water mixture was chosen to simulate the impact of smaller water drops in the same Weber- and Reynolds-number range ($310 \leq We \leq 420$; $250 \leq Re \leq 490$) according to the laws of hydrodynamics similarity.

Monolayers with film pressures $\Pi = 30 \pm 1$ mN/m were formed with either pure 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC) or its mixture with 60 mol % cholesterol and with pure 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) or its mixture with 33 and 60 mol % cholesterol. These concentrations were chosen to avoid phase separation of phospholipids and cholesterol, see [3]. *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt (NBD-PE) labelled on the head group, was used as fluorophore marker of the monolayers. At 3 mol % the brightest intensity of target liquid monolayer was found. To simulate the mechanical properties of NBD-PE, 3 mol % of 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) was used for the monolayers without fluorophore. Calcein, a water-soluble fluorophore, was used for the visualization of the drop bulk liquid. Only one fluorophore was used at a time. The monolayers were deposited using a chloroform solution with a lipid concentration of 0.2 μmol/ml.

DOPC, DPPC and DPPE were obtained from Lipoid (Ludwigshafen, Germany), cholesterol and calcein from Sigma (Taufkirchen, Germany), chloroform > 99% and glycerol > 98% from Roth (Karlsruhe, Germany) and NBD-PE from Invitrogen (Karlsruhe, Germany). All substances were used without further purification. Bidistilled water with the quality for injectable drugs was used for the glycerol/water mixture.

Method

A schematic drawing of the experimental setup is presented in Fig. 1. The drop with a volume of 4 μl (corresponding to a drop diameter D of ~ 2 mm) was produced at the end of a capillary with a pendent drop tensiometer PAT1 from Sinterface (Germany) [5]. To form the monolayer, approximately 0.2 μl of the chloroform phospholipid solution was injected at the drop surface with a μl-syringe as described in [6]. The tensiometer measures the drop volume and area, surface tension σ, film pressure Π, surface elasticity ε and surface dilational viscosity η of the drop monolayer. The target liquid was placed in a cuvette. Here, the monolayer was applied with a ml-syringe by releasing drops of the chloroform/phospholipid solution on different places of the liquid surface. The film pressure of this monolayer was measured with a Wilhelmy tensiometer.

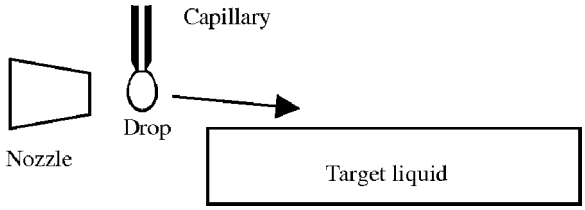

Fig. 1 Simplified sketch of the experimental setup

Once the properties of the drop and target liquid monolayers were determined, the drop was accelerated with a short pulse of compressed air from a nozzle.

The drop impact was recorded simultaneously in both vertical and lateral perspectives with two cameras: first, a high-speed rotating drum camera, which recorded the two perspectives of the drop impact on a 35 mm b/w film. This camera records the first 66 ms of the drop impact with a frame rate of 1666 Hz. The lateral perspective pictures give information about the impact angle $\alpha = 12 \pm 1°$ and the impact velocity $u = 2.3 \pm 0.1$ m/s, from which the Weber number $We = \rho D u^2/\sigma = 380 \pm 20$ was deduced, where: ρ is the drop liquid density, D the drop diameter, u the drop impact velocity, and σ is the surface tension.

Second, a CCD camera connected to computer was used, which records 14 frames/s. This camera is equipped with a light filter and records only fluorophore emission, which is excited at 470 nm (maximum) by a xenon flashlight. The CCD camera detects either monolayer or drop bulk liquid distribution during and after the drop impact. Its lateral perspective shows possible submersions in the target bulk liquid.

All experiments were performed at 21.4 ± 0.4 °C.

Results

Spreading of the Impacting Drop on the Target Liquid Surface

The spreading of the impacting drop is schematically presented in Fig. 2 as proved by Leneweit et al. [2], which studied the oblique drop impact without monolayers. For low impact angles ($\alpha < 23°$) and low drop velocities (Weber number $We < 140$) the drop spreads as a thin sheet on the target liquid without immersion and mixing of the two liquids [2].

Fig. 2 Schematic drawing in lateral perspective of drop spreading on the target liquid surface

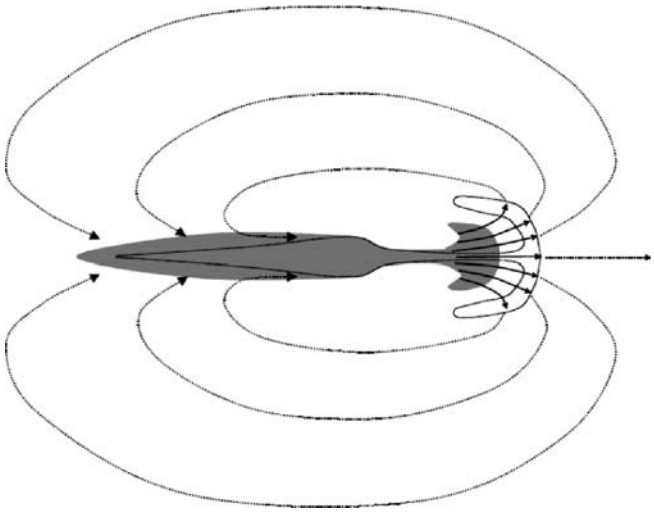

Fig. 3 Schematic drawing in vertical perspective of drop spreading on the target liquid surface [2]. The impacting drop coming from the left hand side, forms the *grey area* which moves further and forms the anchor pattern – the *area inside the solid black line*. The outlines of the drop patterns are extracted from experimental visualizations, the induced velocity field was drawn tentatively with arrows to give a qualitative impression

Figure 3 was taken from Leneweit et al. [2] and represents a sketch of the drop spreading pattern in vertical perspective. The impacting drop induces a velocity field in the target liquid, which expands the front part of the spread drop liquid to an anchor-like pattern and compress its rear part.

When lipid monolayers are deposited on the drop and on the target liquid the drop impact patterns depend on the monolayers rheological properties.

The Proof of Bilayer/Multilayer Formation Under the Drop Liquid

The main goal of this study is to prove the synthesis of bilayer structures with lipids from the two monolayers: drop and target monolayers. The place where the lipids from the drop monolayer could come in contact with the lipids from the target monolayer is under the thin liquid sheet of the drop impact pattern. If the insoluble lipids are captured under the drop bulk liquid they are in an aqueous medium and they form spontaneously bilayer and/or multilayer phases, like vesicles and liposomes, as shown in the literature [7–12]. Due to the fact that the new bilayer structures are formed with lipids from both drop and target monolayer, they are more or less asymmetric.

An experimental verification is necessary to prove whether lipids from the drop and/or target monolayer are under the drop liquid after impact. The impacting drop could push and displace the lipids from the two monolayers and the two bulk liquids could come in contact without separation lipid monolayers in between them. In this case there should be no lipids in the contact zone of the two aqueous media which means that no bilayer formation would take place.

To prove if there are lipids from the drop and from the target monolayer captured under the drop liquid after drop impact we made the drop bulk liquid to sink into the target liquid. This was done by using for the drop bulk liquid a water/glycerol mixture with 1.2% more glycerol than for the target bulk liquid, slightly increasing in this way the drop liquid density. After the impacting drop comes to rest in the anchor pattern, the drop liquid starts to sink into the target bulk liquid, due to the density difference, as shown schematically in Fig. 4.

To see the behaviour of the drop bulk liquid during and after impact, we labelled it fluorescently with calcein, as presented in Fig. 5.

The pictures in vertical perspective show the formation of the anchor form pattern, like described in Fig. 3 by Leneweit et al. [2]. After impact, the drop bulk liquid spreads as a thin liquid sheet on the target liquid surface in an anchor-form pattern and when the horizontal motion come to rest, it starts sinking into the target liquid due to the density difference, as seen in the lower line of Fig. 5 and presented schematically in Fig. 4. In the last three pictures from the vertical perspective the anchor form pattern cannot be clearly seen anymore because of the fluorescence of the sinking liquid.

Once we know the behaviour of the drop bulk liquid, which sink almost completely as shown in Fig. 5, we check if there are lipids captured under it. For this we labelled fluorescently the target or the drop monolayer. The sinking drop liquid submerges into the target liquid the fluorescently labelled lipids captured under it.

Figure 6A shows the lateral perspective pictures when the target liquid was fluorescently labelled. As can be observed there are lipids from the target monolayer withdrawn into the subphase. This means that the target monolayer was not displaced by the impacting drop, but was actually covered by the drop liquid. Due to the density difference the drop liquid is sinking into the target liquid withdrawing the target lipids captured under it. These lipids have to have the conformation of bilayer or multilayer structures as discussed above.

When the fluorophore substance is in the drop monolayer (Fig. 6B) it can be observed that the drop phospholipids are also submerged into the target bulk liquid once the drop liquid starts to sink. The quantity of the phospholipids from the drop, which are caught between the drop

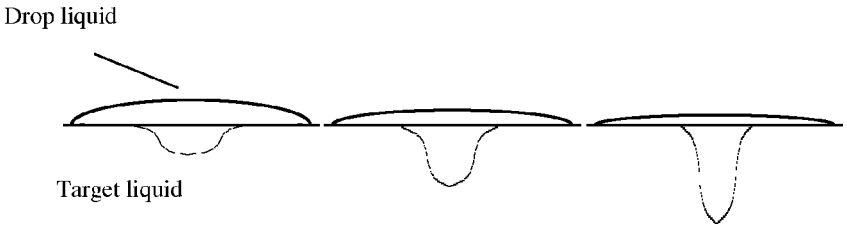

Fig. 4 The drop liquid sinks into the target liquid once the impacting drop comes to rest due to the density difference and interfacial instability

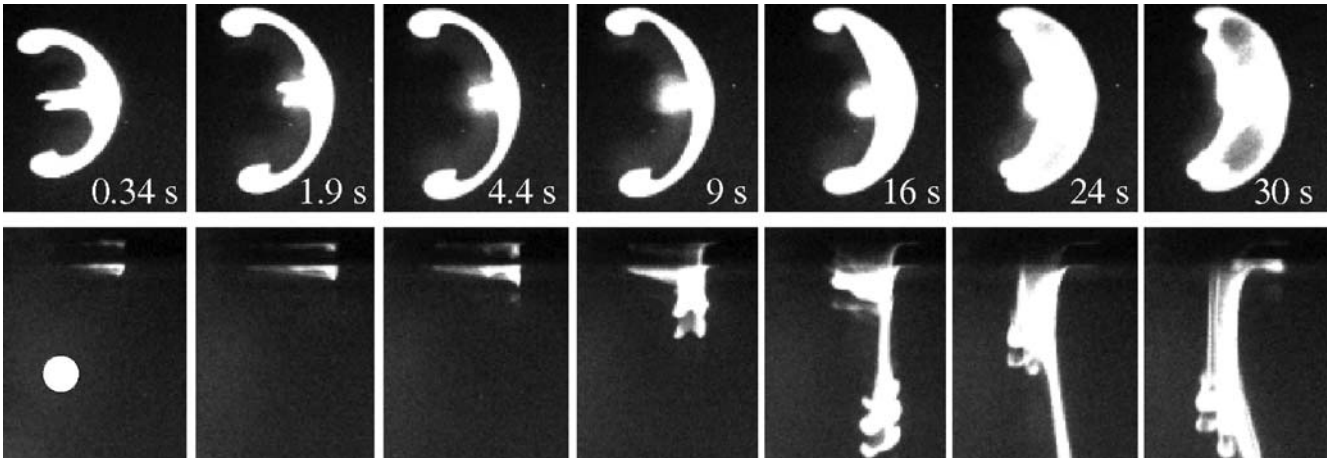

Fig. 5 Drop impact in vertical (*upper line*) and lateral (*lower line*) perspective with the drop bulk liquid fluorescently labelled with calcein. Drop and target monolayers: DOPC/cholesterol/DPPE = 64/33/3 (molar). The *white circle* from the first picture represent the drop before impact, $D = 1.8$ mm

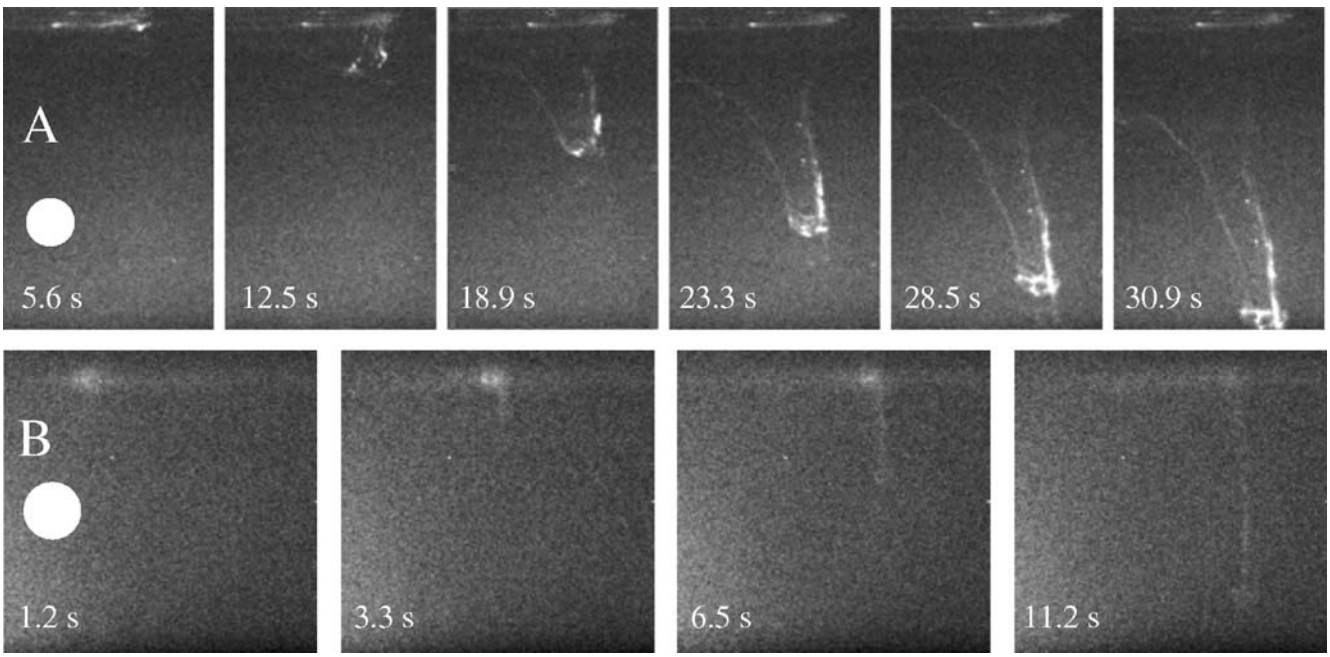

Fig. 6 Drop impact in lateral perspective: **A** Target monolayer: DOPC with 3 mol % NBD-PE; drop monolayer: DOPC with 3 mol % DPPE; **B** Target monolayer: DOPC/cholesterol/DPPE = 64/33/3 (molar); drop monolayer: DOPC/cholesterol/DPPE = 64/33/3 (molar). The sinking flow of the drop bulk liquid involves the phospholipids from the target and drop liquid monolayers, which were captured under the drop liquid

and the target liquid, seems to be much diminished compared to the phospholipids from the target liquid. This fact will be discussed in Sect. 4.

In this way we are able show that phospholipids from both drop and target monolayers are captured under the drop liquid in all experiments. As discussed above, lipids in a water medium means bilayer structures, like uni- or multi-lamellar liposomes.

To conclude we can say that bilayer structures can be formed by oblique drop impact on monolayers. The details of their generation will be shown in the following sections.

Drop Impact on Monolayers with Different Binary Mixtures of Either Unsaturated (DOPC) or Saturated (DPPC) Phospholipids with Cholesterol

The drop impact patterns, and the bilayer structures formed by drop impact, are highly influenced by the rheological properties of the drop and target monolayer. The rheological properties of monolayers can be modified by using different lipids, saturated or unsaturated, and their mixtures with different amounts of cholesterol [3]. The advantage of using cholesterol is that its presence stabilises the bilayer structures [13] and in high amounts inhibits the multilayer formation [14].

Drop Impact on Unsaturated Monolayers.

DOPC Monolayers Without Cholesterol. Figure 7 shows the vertical and lateral perspectives of a drop impact with pure DOPC monolayers. As seen as well in Fig. 6A, lipids from the target monolayer, captured under the drop liquid pattern, are submerged by the drop bulk liquid into the subphase.

To check if for DOPC monolayers, lipids from the drop monolayer are also captured under the drop liquid, we did experiments where the drop monolayer was fluorescently labelled, see Fig. 8. From these experiments we observe that fluorescently labelled lipids are also submerged into the subphase.

The anchor-formed pattern of the spreading drop liquid appears dark for the unlabelled drop monolayer in Fig. 7 and bright in Fig. 8 when the drop monolayer was fluorescently labelled. The anchor pattern is formed by the drop liquid, as shown in Figs. 3 and 5. The fact that the anchor form appears dark in Fig. 7 and bright in Fig. 8 means that

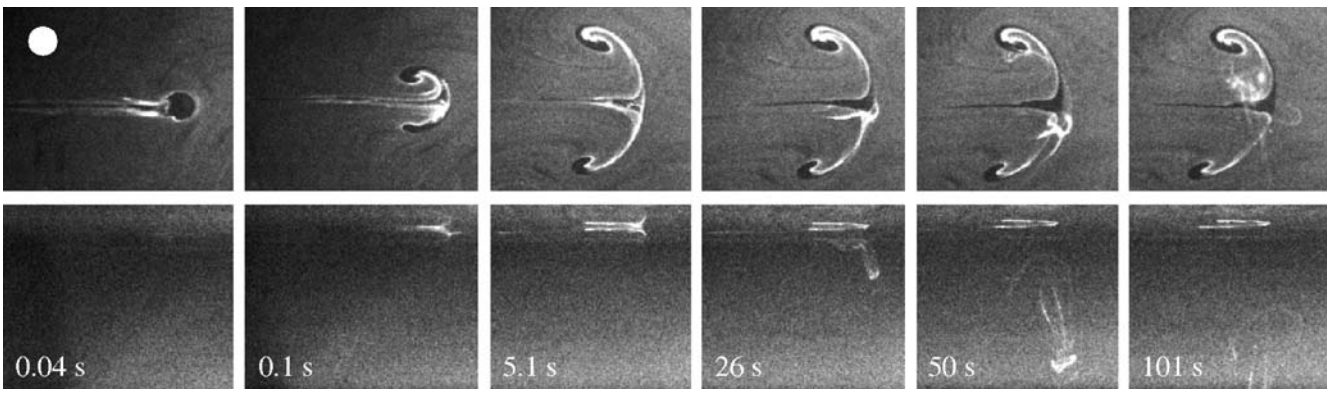

Fig. 7 Drop impact in vertical (*upper line*) and lateral (*lower line*) perspective, target monolayer: DOPC with 3 mol % NBD-PE; drop monolayer: DOPC with 3 mol % DPPE

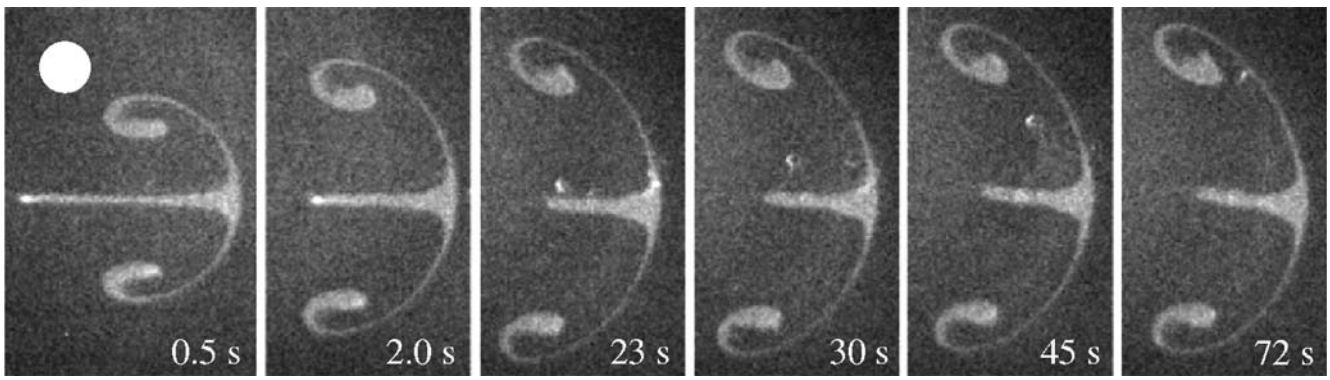

Fig. 8 Drop impact in the vertical perspective, target monolayer: DOPC with 3 mol % DPPE; drop monolayer: DOPC with 3 mol % NBD-PE

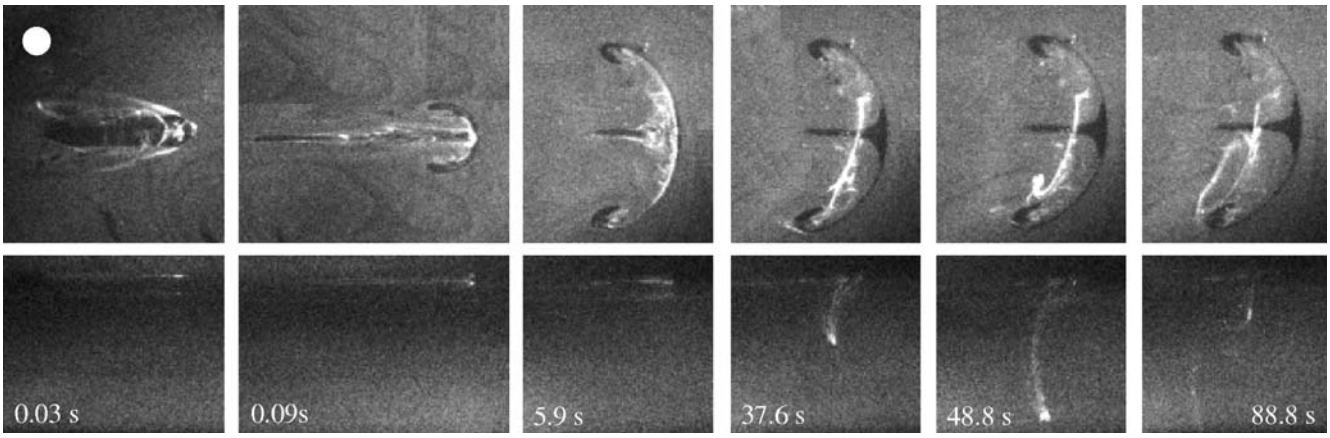

Fig. 9 Drop impact in vertical (*upper line*) and lateral (*lower line*) perspective, target monolayer: DOPC/cholesterol/NBD-PE = 37/60/3 (molar); drop monolayer: DOPC/cholesterol/DPPE = 37/60/3 (molar)

the drop liquid pattern is covered with lipids from the drop monolayer at its air-exposed surface. This monolayer from the top side of the anchor pattern is stable in time and the lipids forming it are not submerged into the subphase by the sinking of the drop bulk liquid.

For pure DOPC monolayers, if the target monolayer is fluorescently labelled (Fig. 7), extra-bright areas are formed in the first stage of drop impact, along the tail, and come to rest in the surrounding of the drop fluid at the backside of the anchor pattern. These areas are brighter than the fluorescently labelled target monolayer and remain on the target liquid surface. This is not the case when the drop monolayer is fluorescently labelled (Fig. 8), meaning that these areas contain lipids only from the target monolayer.

To conclude we can say that for drop impacts on DOPC monolayers, lipids from the drop and target monolayers are captured under the drop liquid pattern and extra-bright areas are formed at the back-side of the drop impact pattern with lipids from the target monolayer.

DOPC Monolayers with 60 mol % Cholesterol. Drop impact pictures on DOPC monolayers with 60 mol % cholesterol are shown in Fig. 9 where the target monolayer is fluorescently labelled.

During drop impact bright lipid structures are formed in the centre part of the anchor pattern and in the front part of the tail region. These bright structures are under the drop bulk liquid because once the drop bulk liquid sinks they are withdrawn into the subphase. No extra-bright areas are formed on the target monolayer like the case of pure DOPC (Fig. 7). The unlabelled drop monolayer remains on the target surface as a dark anchor pattern.

Drop Impact with Saturated Monolayers.

DPPC Monolayers Without Cholesterol. Drop impact pictures with pure DPPC monolayers are shown in Fig. 10, where the drop monolayer is fluorescently labelled.

We observe that the tail of the drop impact pattern is very long and does not form an anchor pattern. The first picture taken at 0.03 s after impact looks similar with the last one which was taken at more than 16 s after impact. From these pictures and from experiments where the target monolayer was fluorescently marked (results not shown), it can be seen that the target monolayer is stable and does not move at all. The impacting drop lands and stops quickly without inducing any movements on the target liquid.

From pictures taken in lateral perspective (results not shown), it can be observed that in the front part of the drop impact pattern, where the main mass of the drop liquid come to rest, the stable DPPC monolayer is broken and the drop liquid submerges into the target bulk liquid due to the density difference. This involves as well the drop and target monolayers lipids captured under the front part of the drop liquid.

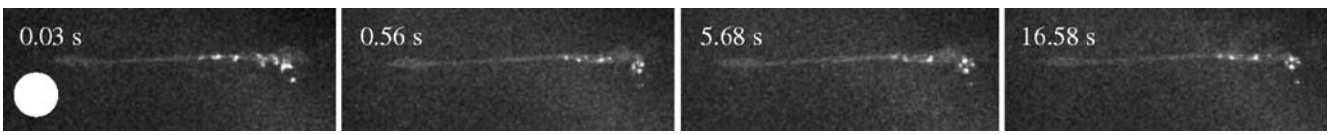

Fig. 10 Drop impact in vertical perspective, target monolayer: DPPC/DPPE = 97/3 (molar); drop monolayer: DPPC/NBD-PE = 97/3 (molar)

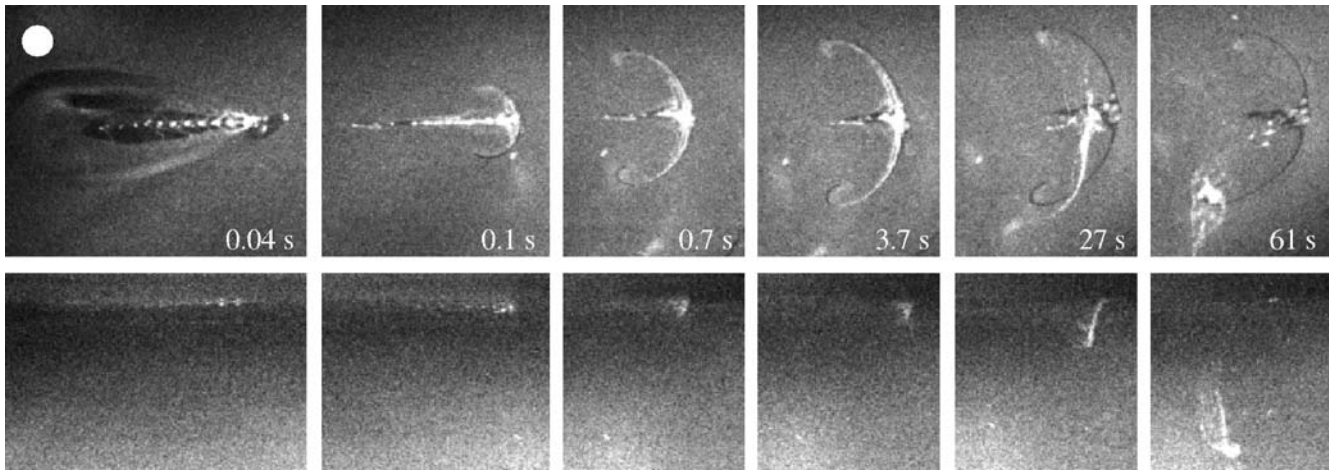

Fig. 11 Drop impact in vertical (*upper line*) and lateral (*lower line*) perspective, where the target liquid monolayer was formed by DPPC/cholesterol/NBD-PE (37/60/3 molar) and the drop monolayer by DPPC/cholesterol/DPPE (37/60/3 molar)

Extra-bright areas – appearing like spots, or fragments, are formed from both target (results not shown) and drop monolayers and remain at the target liquid surface.

DPPC Monolayers with 60 mol % Cholesterol. Drop impact pictures on DPPC monolayers with 60 mol % cholesterol are shown in Fig. 11 with the target monolayer being fluorescently labelled.

As can be seen there is a big difference between the drop impact on pure DPPC (Fig. 10) and on DPPC with 60 mol % cholesterol (Fig. 11). In Fig. 11 the drop impact pattern has an anchor pattern, like drop impacts on DOPC and DOPC-cholesterol monolayers.

From the vertical and lateral perspective, for the both cases: drop or target fluorescently labelled monolayers, it can be seen that the drop and target lipids are captured under the drop liquid and submerged into the subphase.

As can be observed in Fig. 11, during the drop impact some extra-bright areas, appearing like spots, are formed with lipids from the fluorescently labelled target monolayer. This happens as well when the drop monolayer is fluorescently labelled, results not shown. These extra-bright spots are not submerged by the drop bulk liquid into the subphase. They suddenly disappear, their lipids reintegrate back into the existing monolayer, and some of them remain at the liquid surface for more than 100 s. No extra-bright areas similar with the ones from the back-side of the anchor pattern of the pure DOPC monolayers are formed in this case.

Discussion

Monolayer Rheology

Before discussing our results regarding bilayer formation by oblique drop impact on different monolayers we have to take into account the differences between saturated and unsaturated monolayers and the effect of cholesterol on monolayer rheology.

DOPC monolayers, due to the unsaturation, i.e. kinks of the alkyl chains, are in the liquid expanded phase, which is a fluid phase at all film pressures Π [3, 13, 15]. At 21 °C and $\Pi > 25\,\mathrm{mN\,m^{-1}}$ DPPC monolayers are in the solid analogous phase [3, 13, 16], which is highly incompressible and condensed [13, 16]. Shah and Schulman [13] show that the effect of cholesterol on either saturated or unsaturated phospholipids is strikingly different. Cholesterol increases the surface elasticity, the dilational and the shear viscosity of unsaturated phospholipid monolayers [3, 13, 14, 17]. In saturated monolayers cholesterol disturbs the order between phospholipid molecules fluidifying the solid monolayer [13, 14, 18] and lowering its shear viscosity [18]. Pure cholesterol monolayers are liquid [13] and have very low surface shear viscosities which are hardly detectable [18].

In a previous study [3] we found that the surface elasticity and the surface dilational viscosity are higher for DPPC/cholesterol than for DOPC/cholesterol monolayers

Table 1 The surface elasticity and the surface dilational viscosity of DPPC-cholesterol and DOPC-cholesterol monolayers as determined in [3]

	Chol. (%)	ε (mN/m)	η (mN s/m)
DOPC-Chol.	0	120	40
	60	250	140
DPPC-Chol.	0	80	95
	33	180	200
	60	670	530

and both are increasing with the cholesterol content (see Table 1).

As a function of film pressure and cholesterol content, the DPPC-cholesterol monolayers are either in a solid, liquid/solid coexistence or in a liquid state, whereas DOPC-cholesterol monolayers are always in a liquid state [3, 15].

Bilayer Formation

As seen in the drop impact pictures (Figs. 6–11) lipids from the target and drop monolayers are captured under the drop bulk liquid and submerged into the target liquid. In Fig. 12 A we schematically present a possible mechanism of the drop and target monolayer dynamics, and bilayer formation under the drop bulk liquid. If the target monolayer is stiff enough, it is not displaced by the impacting drop, which rolls and spreads on it. In this way, asymmetric planar bilayers with lipids from the drop and target monolayers can be formed under the thin sheet of the drop liquid. Due to the sinking of the drop liquid the planar bilayer structures are bended and can form three dimensional bilayer structures as vesicles and liposomes. This is sustained by the observations of Ridsdale et al. [8], who showed that large bilayer structures, like folds, convert into more stable vesicular structures. For monolayers containing 60 mol % cholesterol it is expected that the vesicular structures are unilamellar because the high amount of cholesterol inhibits multilayer formation as was shown by Malcharek et al. [14].

We will discuss now the extra-bright areas formed during the drop impact and coming to rest at the back-side of the anchor pattern for pure DOPC monolayers. As presented in the results section, these extra-bright areas are formed only with lipids from the target monolayer and not from the drop monolayer. A possible mechanism of formation of these extra-bright lipid structures is presented in Fig. 12B and C. Pure DOPC monolayers are fluid and have low shear viscosity, low surface dilational viscosity and low surface dilational elasticity [3]. It is assumed that during the drop impact the target monolayer is compressed in the front part of the drop impact pattern. As the drop moves, this front compression forms the surrounding surface of the drop pattern. Compressed lipid monolayers are stable up to a maximum film pressure above which they collapse and form 3D bilayer structures [10–12]. The sketch C of Fig. 12 shows as an enlargement the collapsed target monolayer. Here the compressed target monolayer is shown as it folds and forms bilayers or multilayers. Gopal and Lee [10] show that monolayers in the liquid expanded phase, as is the case for DOPC monolayers, are not able to sustain large-scale folding, and collapse on a smaller length scale by forming vesicle-like structures. These structures are symmetric, being formed only with lipids from the target monolayer.

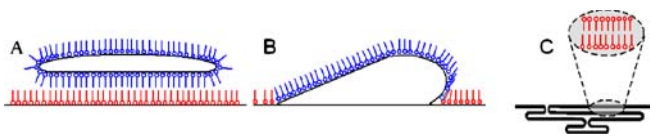

Fig. 12 Two mechanisms to describe monolayer dynamics during drop impact. **A** The target monolayer is stable and the drop liquid spreads on it. Asymmetric bilayers are formed under the drop liquid. **B** The target monolayer is displaced, compressed and expanded by the impacting drop. No bilayers are formed under the drop liquid. **C** As an enlargement of sketch (**B**) the compressed target monolayer is shown as it folds and forms symmetric bilayers or multilayers

The fact that the extra-bright areas are 3D and not 2D structures anymore is sustained by the observation that there is no way to obtain such a extra-bright monolayer by increasing the monolayer film pressure (results not shown). A higher film pressures and higher fluorophore content does not give a brighter monolayer because of the quenching effect between the fluorophore molecules when they are restricted to form a monolayer.

As shown in Figs. 6–8 and discussed above, for drop impact on DOPC monolayers the first proposed mechanism (Fig. 12A) is as well correct. This means that for DOPC monolayers a combination of the two proposed mechanisms (Fig. 12A and B) seems to occur.

DOPC-cholesterol monolayers are liquid, but stiffer than pure DOPC monolayers, as discussed above, having higher dilational viscosity and dilational elasticity [3]. For drop impacts on DOPC monolayers containing 60 mol % cholesterol (Fig. 9), no extra-bright areas are formed on the target monolayer like in the case of pure DOPC monolayers (Fig. 7). In this case the impacting drop neither displaces, compresses or folds the target monolayer. The presence of cholesterol makes the target monolayer more stable than the pure DOPC monolayer.

We will discuss now the drop impact on saturated DPPC-cholesterol mixtures.

As is well known, at 21 °C and 30 mN/m film pressure a pure DPPC monolayer is solid and stiff, having high shear viscosity [18], but a relatively low surface dilational viscosity and elasticity [3]. The drop impact pattern on pure DPPC monolayers has a long tail and no anchor form (Fig. 10). The target monolayer is so stiff that the impacting drop cannot induce any surface movement in it as schematically drawn in Fig. 3.

During the drop impact some extra-bright small areas – appearing like discrete spots, or fragments, are formed from both target and drop monolayers and remain at the target liquid surface or sink into the subphase with the drop bulk liquid. The fact that these extra-bright spots are formed when the drop monolayer is fluorescently labelled, means that the drop monolayer fractures as well during the drop impact. This is not the case for pure DOPC, where only the target monolayer collapses and form continuous bright areas.

Using the imaging software ImageJ and its "Multi Cell Outliner" plugins we determined the areas of the drop impact patterns. These areas depend on the monolayers composition, being larger than the drop area before impact with approximately 140–160% for a pure DPPC monolayer and with approximately 220–250% for the others monolayers studied. There is a difference between the collapse of a liquid expanded monolayer like DOPC and that of a solid monolayer like DPPC. As discussed above, DOPC monolayers are not able to sustain large-scale folding, and collapse on a smaller length scale by forming vesicle-like structures [10], whereas the solid DPPC monolayers are apparently too brittle to bend, and collapse by fracture, as Lipp et al. [9] have shown. This means that in the case of DPPC the both drop and target monolayers fracture during drop impact and form asymmetric bilayer or multilayer structures which appear as extra-bright spots.

DPPC monolayers with 60 mol % cholesterol are in a fluid/fluid phase. Microscopically, they show domains of condensed complexes surrounded by a cholesterol rich phase [3, 19], but this μm-sized structures are at least two orders of magnitude smaller than the bright spots which we show in Fig. 11. As already shown in the literature [13, 18], cholesterol greatly reduces the shear viscosity of DPPC monolayers. The fluidifying effect of cholesterol in a saturated monolayer can be seen very well in our results by comparing the drop impact patterns of pure DPPC monolayer with the ones of DPPC with 60 mol % cholesterol. The last one present an anchor forming pattern similar to the drop impact pattern on the liquid DOPC and DOPC-cholesterol monolayers.

For monolayers of DPPC with 60 mol % cholesterol (Fig. 11) during the drop impact some big 3D structures, appearing like extra-bright spots, are formed with lipids from the drop and target monolayers. Earliest at 2.1 s after impact and at approximately one second after the drop liquid comes to rest in the anchor form, and after the drop bulk liquid starts to sink into the target liquid, the 3D structures start to disappear. We assume that their lipids reintegrate back in the existing drop or target monolayer because they transform into a larger bright round-area with approximately the same light intensity as the existing fluorescently labelled monolayer. Usually all extra-bright spots disappear in 12 to 33 s, whereas in some isolate cases the 3D structures are stable for more than 100 s.

We will discuss now the difference between the extra-bright 3D collapsed structures in the case of saturated DPPC monolayers with 60 mol % cholesterol and the ones of the pure DOPC monolayers. The former are formed from both drop and target monolayers and appears like extra-bright spots, unstable in time, the later ones are formed only from the target monolayer and appear like extra-bright large areas and are stable in time. We assume that this is due to the difference in structure of the two monolayers. The saturated monolayer with 60 mol % cholesterol contains two different phases: one of condensed complexes surrounded by a second phase of liquid cholesterol. Gopal et al. [10] and Leep et al. [9] show that the nucleation of the collapse takes place at the boundaries between the condensed domains and the fluid phase. This is in concordance with observations of Malcharek et al. [14] which found that the collapse of monolayers with high amount of cholesterol produces only isolated collapsed structures.

The unsaturated DOPC monolayer is in a liquid expanded homogeneous phase. Gopal and Lee [10] show that the liquid monolayers are not able to sustain large-scale folding and collapse on a smaller length scale by forming vesicle-like structures. These structures are stable in time and do not reintegrate into the existing monolayer like in the case of saturated DPPC with 60 mol % cholesterol.

Conclusions

Oblique drop impact was studied with phospholipid monolayers on both drop and target liquid surfaces. These experiments visualize the rheological properties of monolayers giving rise to complex pattern formation. During drop impact the monolayers composition and mechanical properties influence their dynamics and bilayer/multilayer formation. Asymmetric bilayer structures are formed by spontaneous aggregation of phospholipids captured under the thin sheet of the drop liquid. Symmetric bilayer structures are formed by collapse of the drop and target monolayers. Solid monolayers fracture during drop impact and bilayer/multilayer fragments are formed. For some monolayer compositions the 3D bilayer/multilayer structures reintegrate into the existing monolayer, as bilayer formation is reversible.

Acknowledgement Financial support by the DFG (LE 1119/3-1,2) is gratefully acknowledged.

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