Self-Organization of Polymeric Lipids with Hydrophilic Spacers in Side Groups and Main Chain: Investigation in Monolayers and Multilayers

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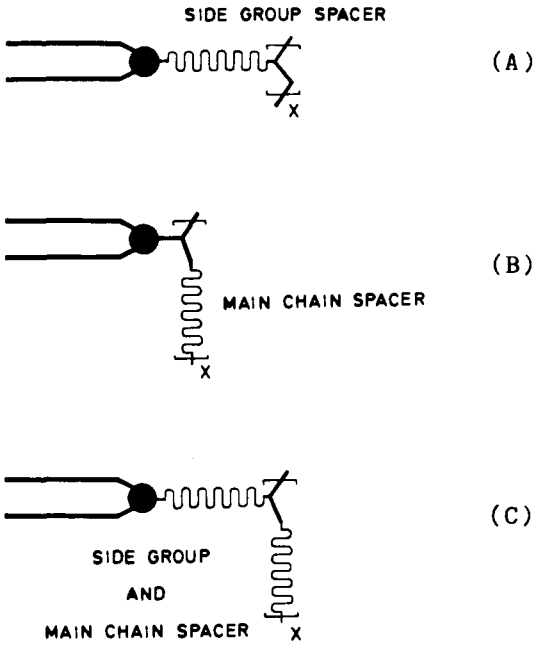
Abstract: Several polymerizable lipids were synthesized and polymerized to amphiphilic homopolymers and to copolymers with the help of hydrophilic comonomers. The self-organization of these polymeric lipids was investigated in monolayers and Langmuir–Blodgett multilayers. The self-organization of these polymers in model membranes is due to hydrophilic spacer groups in the amphiphilic side groups as well as to hydrophilic spacer groups in the polymer backbone. Thus, highly ordered monolayers and LB-multilayers are easily obtained.

Hydrophilic Spacer Groups in Polymeric Lipids. Currently, there is great interest in liposomes, monolayers, bilayer membranes (BLM), and Langmuir–Blodgett multilayers as biomembrane models and furthermore because of their manifold potential applicability.[1-6] In general, these types of aggregates show poor stability in comparison with biomembranes. This lack of stability can be overcome by the polymerization of reactive groups within the amphiphiles.[1b-3] In most cases, however, the resulting polymer chains interfere with the motion of the oriented side groups. Thus, a decrease or even the loss of the fluid phases of the membranes usually occurs.[2,3] More drastically, the reduced mobility, which is due to the polymer backbone, hinders the efficient self-organization of prepolymerized lipids.

To overcome this problem and to retain the fluidity, which is a fundamental property of biological membranes, the incorporation of hydrophilic spacer groups into polymerizable lipids has recently been realized.[7] Due to the decoupling of the motion of the polymer main chain and the bilayer via a side group spacer, these polymers directly form model membranes from prepolymerized lipids. Highly ordered monolayers from polymeric lipids were obtained and could be transferred onto solid supports to build up polymeric LB-multilayers.[7] The advantage of this concept is that side reactions and structural changes of the membranes induced by the polymerization reaction of oriented monomeric layers are avoided. Up to now, the spacer concept has mainly been used for rather complicated lipids with hydrophilic spacer groups between the polymer chain and the amphiphilic side groups. In this contribution it will be demonstrated that this spacer model can be expanded by using copolymers prepared from easily accessible unsaturated amphiphiles and hydrophilic comonomers forming a main chain spacer. A similar concept has successfully been realized in liquid crystalline side group polymers where spacers can be placed either in the side groups[8] or in the polymer backbone.[9] It has also been shown that the introduction of flexible comonomer units does not prevent the formation of LC-phases by the copolymers.[9]

The different possibilities for amphiphilic polymers containing spacer groups to decouple the motions of the polymer main chain from the membrane forming side groups are summarized in Scheme I. Polymeric amphiphiles with three types of spacers were obtained. The polymerization of lipids with hydrophilic spacer groups between the amphiphilic parts and the polymerizable units (spacer lipids)[7] leads to homopolymers (A) with side group spacers. The copolymerization of conventional monomeric lipids with hydrophilic comonomers results in copolymers (B) with main chain spacers only. The copolymerization of monomeric spacer lipids and hydrophilic comonomers leads to polymers (C) containing both main chain spacers as well as side group spacers (combined spacers).

Scheme I. Schematic Representation of Amphiphilic Polymers Containing Hydrophilic Spacergroups[a]



[a] (A) Side group spacer (hydrophilic segments); (B) main chain spacer (hydrophilic comonomers); (C) main chain spacer and side group spacer (spacercombination).

The self-organization of copolymers from monomeric lipids and hydrophilic comonomers is outlined in Scheme II. The spreading of amphiphilic copolymers (D) containing hydrophilic spacer groups on water surfaces should lead to monolayers (E). Their orientation is not disturbed by the polymer chain, whereas the isotherms are strongly affected by the length of the spacer group. In addition, the combination of order and mobility within the monolayers of these polymers might lead to LB-multilayers (F) with perfectly packed bilayers and a high-layer correlation. First

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(2) Bader, H.; Dorn, K.; Hupfer, B.; Ringsdorf, H. *Adv. Polym. Sci.* **1985**, *64*, 1.
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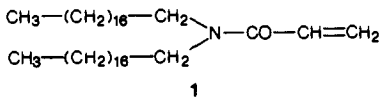
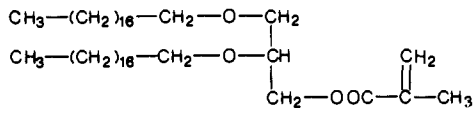
Chart I. Used Types of Monomeric Lipids (⊃●⌐) and Hydrophilic Comonomers (●⌐)

lipid monomers

nonionic, without spacer

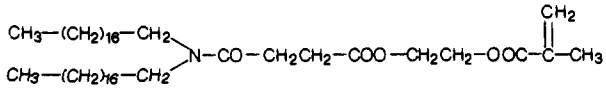


1

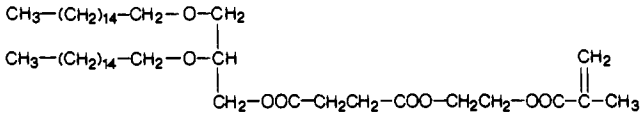


2

nonionic, hydrophilic spacer

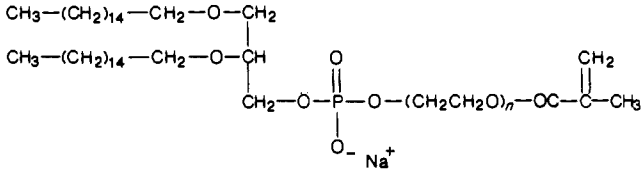


3

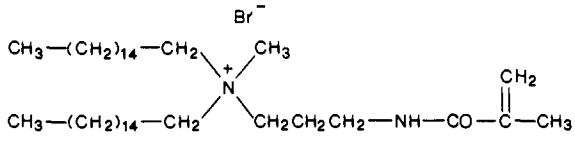


4

ionic, hydrophilic spacer

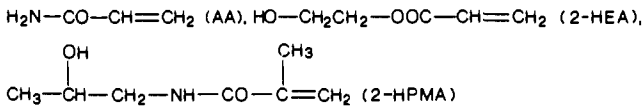


5, n = 1
6, n = 4



7

hydrophilic comonomers



attempts to use polymers for the self-organization in monolayers, multilayers, and liposomes have already been described in literature.[7,10-13]

This paper deals with the systematic investigation of the influence of side group spacers and main chain spacers in amphiphilic polymers on their self-organization in monolayers. Furthermore, the formation of LB-multilayers from polymeric monolayers was investigated.

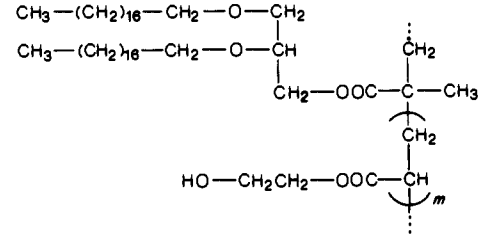
Material and Methods

The monomeric lipids and hydrophilic comonomers used for copolymerization are summarized in Chart I. All spreading experiments were performed with prepolymerized lipids. They were polymerized in isotropic solutions via radical initiation with AIBN and purified by reprecipitation and spread from organic solvents. Homopolymers were prepared from the polymerizable lipids **1**–**7**. Copolymers were prepared from **1** with acrylamide (AA), from **2**–**6** with 2-hydroxyethylacrylate (2-HEA), and from **7** with 2-hydroxypropylmethacrylamide[14] (2-HPMA). The molar ratios of polymerizable lipids and comonomers ranged from 1:1 to 1:10. In order to study the structure dependent spreading behavior and the multilayer formation, five representative

Chart II. Synthesized Amphiphilic Homopolymers and Copolymers[a]

polymers with hydrophilic main chain spacers

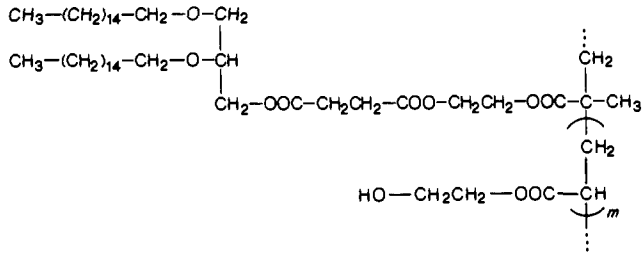
nonionic (based on lipid 2)



8, m = 0
8-1, m = 0.9
8-5, m = 5.0
8-10, m = 7.5

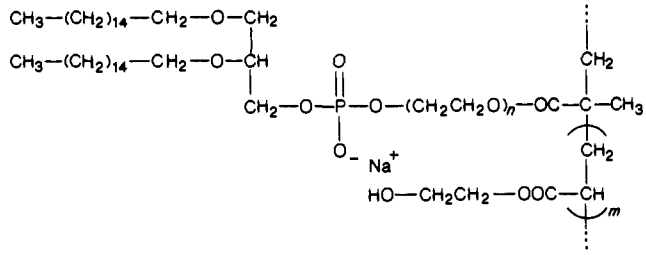
polymer with hydrophilic main chain and side group spacers

nonionic (based on lipid 4)



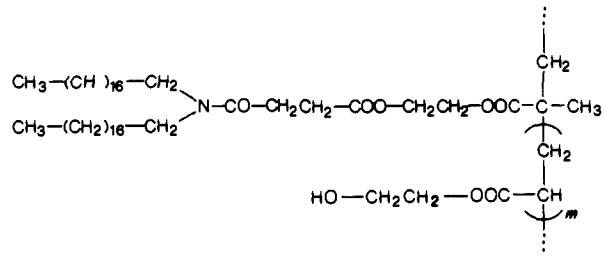
9, m = 0
9-1, m = 0.9
9-5, m = 4.5
9-10, m = 8.9

ionic (based on lipid 5 and 6)



10, m = 0; n = 1
10-5, m = 5.0; n = 1
10-10, m = 9.5; n = 1
11, m = 0; n = 4
11-2, m = 2.0; n = 4

nonionic (based on lipid 3)



12, m = 0
12-1, m = 1.2
12-5, m = 4.9
12-10, m = 8.5

[a] The strutures shown refer to statistical copolymers.

(10) Hodge, P.; Koshdel, E.; Tredgold, R. H.; Vickers, A. J.; Winter, C. S. *Brit. Polym. J.* **1985**, *17*, 368.
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(13) Kunitake, T.; Nagai, M.; Yanagi, H.; Takarabe, T.; Nakashima, N. *J. Macromol. Sci.-Chem.* **1984**, *A21*, 1237.
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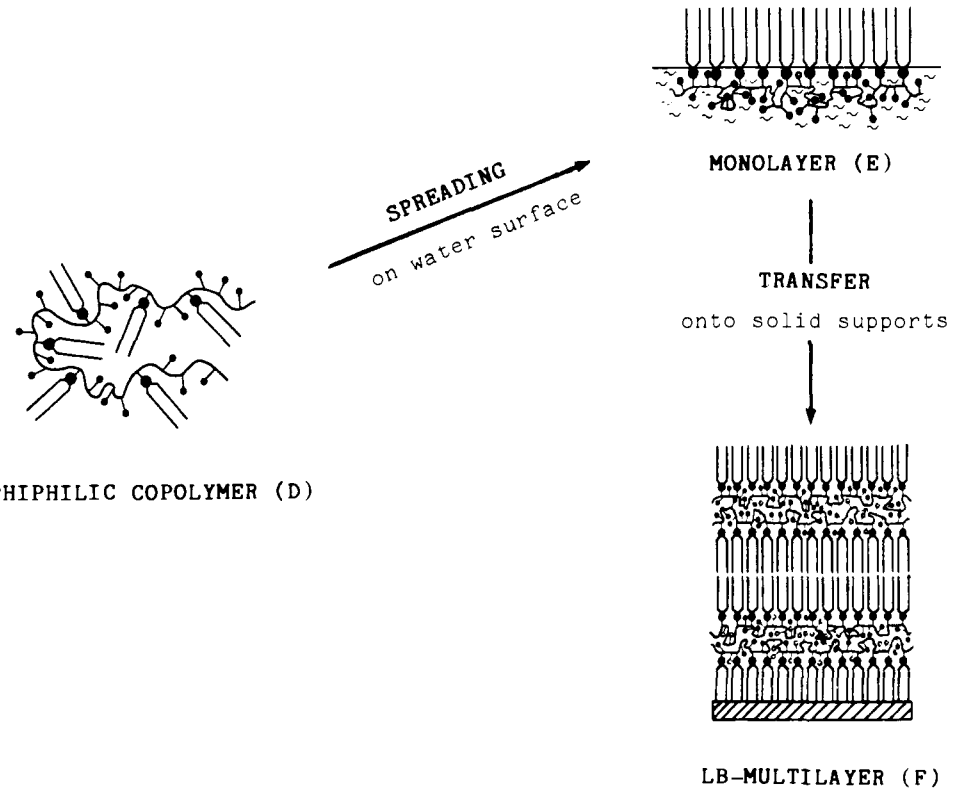
Scheme II. Self-Organization of Copolymers from Amphiphilic (⇒●—\) and Hydrophilic Monomers (●—\)



homopolymer and copolymer systems were selected. These polymers include nonionic as well as ionic lipids, as shown in Chart II.

Materials. Synthesis of the Monomeric Lipids 1–7. The monomeric lipids **1**,[13] **6**,[7] and **7**[16] were prepared according to published procedures.

1,2-Bis(octadecyloxy)-3-(methacryloyloxy)propane (2). A solution of methacryloylchloride (0.90 g, 8,6 mmol) in 5 mL of dichloromethane was added dropwise to an ice-cooled solution of 1,2-*O*-dihexadecylglycerol[17-19] (3.4 g, 5.7 mmol), triethylamine (0.58 g, 5.7 mmol), and 2,6-di-*tert*-butyl-*p*-cresol (ca. 5 mg, inhibitor) in 100 mL of dichloromethane and stirred overnight at room temperature. TLC (ethylacetate/hexane, 1:3) showed complete conversion into the methacrylate ester. The solution was washed with 1 N hydrochloric acid, followed by a dilute sodium hydrogen carbonate solution and water. After drying with sodium sulfate, the crude methacrylate ester was purified by means of liquid chromatography on a silica gel column by using hexane/diethyl ether: 20/1 as the eluent: yield 1.7 g (41 %); mp 35–36 °C; ^{1}H NMR ($CDCl_3$) δ (ppm) 0.87 (t, 6 H, $CH_3(CH_2)_{16}$), 1.2–1.6 (m, 64 H, $CH_3(CH_2)_{16}$, 1.9 (s, 3 H, $C(CH_3)=CH_2$), 3.3–3.6 (m, 7 H, CH_2O), 4.1–4.2 (m, 2 H, CH_2OOC), 5.6–6.1 (m, 2 H, $CH_2=C$); IR (KBr) ν (cm^{-1}) 2910, 2850, (CH_3, CH_2), 1720 (C=O), 1635 (C=C), 1465 (CH_3, CH_2), 1170 (C–O–C), 940 (C=C), 720 (CH_2). Anal. Calcd for $C_{43}H_{84}O_4$ (665.15): C, 77.65; H, 12.73. Found: C, 77.63; H, 11.94.

3-Methacryloyl-3-oxapropyl 3-(*N*,*N*-Dioctadecylcarbamoyl)-propionate (3). Dioctadecylamine (10.4 g, 20 mmol) (precipitated from chloroform/acetone and recrystallized in diethyl ether), succinic anhydride (4.0 g, 40 mmol), and pyridine (1.74 g, 40 mmol) were refluxed in dichloromethane for 2 days. The solution was washed with 2 N sulfuric acid, followed by a saturated sodium hydrogen carbonate solution and then water. After drying with sodium sulfate and the evaporation of the solvent, the crude reaction product was recrystallized from acetone: yield 12 g (98%); mp 56 °C.

A solution of dicyclohexylcarbodiimide (1.44 g, 7 mmol) in dichloromethane was added dropwise into an ice cooled solution of *N*,*N*-dioctadecylsuccinamide (4.0 g, 64 mmol), 2-(hydroxyethyl)methacrylate (1.7 g, 10 mmol), and 4-dimethylaminopyridine (30 mg) in dry dichloromethane. The mixture was allowed to react for 1 h in an ice bath and overnight at room temperature. The precipitated urea derivative was separated by filtration, and the organic solution was washed with water and dried with sodium sulfate. The crude methacrylate was purified by means of liquid chromatography on a silica gel column by using ethyl acetate/hexane (1/5) (by volume) as the eluent: yield 2.5 g (55%); mp 31–32 °C; ^{1}H NMR ($CDCl_3$) δ (ppm) 0.87 (t, 6 H, $CH_3(CH_2)_{16}$), 1.2–1.6 (m, 64 H, $CH_3(CH_2)_{16}$), 1.9 (s, 3 H, $C(CH_3)=CH_2$), 2.6–2.7 (m, 4 H, CH_2COO), 3.1–3.3 (m, 4 H, CH_2O, CH_2N), 4.3 (s, 4 H, CH_2OOC), 5.6–6.1 (m, 2 H, $CH_2=C$); IR (KBr) ν (cm^{-1}) 2910, 2860 (CH_3, CH_2), 1730, 1650 (C=O), 1650 (C=C), 1460 (CH_3, CH_2), 1150 (C–O–C), 940 (C=C), 720 (CH_2). Anal. Calcd for $C_{46}H_{87}NO_5$ (734.21): C, 75.25; H, 11.94; N, 1.91. Found: C, 73.88; H, 11.44; N, 1.83.

3-Methacryloyl-3-oxapropyl 2,3-Bis(hexadecyloxy)propyl Succinate (4). The synthesis of the 1,2-*O*-dihexadecylglycerol has been described previously.[17-19] The reaction of 1,2-*O*-dihexadecylglycerol with succinic anhydride was performed as described for **3** [yield 5 g (85%); mp 51 °C]. The carboxylic acid obtained was esterified with 2-(hydroxyethyl)methacrylate in the same manner as **3**: yield 2.5 g (80%); mp 33–34 °C; ^{1}H NMR ($CDCl_3$) δ (ppm) 0.87 (t, 6 H, $CH_3(CH_2)_{14}$), 1.2–1.6 (m, 56 H, $CH_3(CH_2)_{14}$, 1.9 (s, 3 H, $C(CH_3)=CH_2$), 2.6 (s, 4 H, CH_2COO), 3.3–3.6 (m, 7 H, CH_2O), 4.1–4.3 (m, 6 H, CH_2OOC), 5.6–6.1 (m, 2 H, $CH_2=C$); IR (KBr) ν (cm^{-1}) 2910, 2850 (CH_3, CH_2), 1730 (C=O), 1640 (C=C), 1460 (CH_3, CH_2), 1150 (C–O–C), 950 (C=C), 720 (CH_2). Anal. Calcd for $C_{45}H_{84}O_8$ (753.17): C, 71.76; H, 11.24. Found: C, 72.24; H, 11.00.

Sodium 2,3-Bis(hexadecyloxy)propyl 3-Methacryloyl-3-oxapropyl Phosphate (5). Freshly distilled phosphorus oxychloride (1.27 g, 8.3 mmol) in dry tetrahydrofuran (THF) (15 mL) was cooled to 0 °C in an ice bath, and triethylamine (0.84 g, 8.3 mmol) in THF (15 mL) was slowly added under stirring.[7,20] Then, the temperature was lowered to −5 °C with the help of an ice/sodium chloride bath and 1,2-*O*-dihexadecylglycerol (3.0 g, 5.5 mmol) in THF (30 mL) was added dropwise. The mixture was stirred for 1 h at −5 °C until thin-layer chromatography (eluents: chloroform/methanol/water, 100/15/1; ethylacetate/hexane, 1/5) showed complete conversion to the phosphoric acid dichloride. A solution of 2-(hydroxyethyl)methacrylate (1.4 g, 11 mmol) and triethylamine (1.6 g, 1.65 mmol) in THF (15 mL) was added to the reaction mixture at −5 °C. After having been stirred for 2 days at room temperature, no further change in thin-layer chromatography could be detected. Afterwards, the mixture was filtered to remove the precipitated triethylamine hydrochloride. The hydrolysis was performed by adding

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(18) Barry, P. J.; Craig, B. M. *Can. J. Chem.* **1955**, *33*, 716.
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(20) Eibl, H. J. *Angew. Chem.* **1984**, *96*, 267.

Table I. Molar Monomer Ratios (Comonomer Feeds) and Copolymer Composition

copolymers	comonomer feed	copolymer composition
	2:2-HEA	
8-1	1:1	1:0.9
8-5	1:5	1:5.0
8-10	1:10	1:7.5
	4:2-HEA	
9-1	4:1	1:0.9
9-5	1:5	1:4.5
9-10	1:10	1:8.9
	5:2-HEA	
10-5	1:5	1:5.0
10-10	1:10	1:9.5
	6:2-HEA	
11-2	1:2	1:2.0
	3:2-HEA	
12-1	1:1	1:1.2
12-5	1:5	1:4.9
12-10	1:10	1:8.5

20 mL of diluted acetic acid (10 vol%). After 30 min the pH of the mixture was adjusted to pH 9 by the addition of 0.1 M sodium hydroxide solution and then stirred in a closed vessel for 2 h. The methacrylate was obtained by evaporation of the solvent and the extraction of the residue with dichloromethane several times (the emulsion was destroyed by adding of sodium chloride). The crude product was purified by means of liquid chromatography on a silica gel column by using chloroform/methanol/water (100/20/1) as the eluent. Then the pure product was obtained by reprecipitation from chloroform/acetone: yield 0.5 g (12%); mp 46 °C; ^{1}H NMR ($CDCl_3$) δ (ppm) 0.87 (t, 6 H, $CH_3(CH_2)_{16}$), 1.1–1.6 (m, 56 H, $CH_3(CH_2)_{16}$), 1.9 (s, 3 H, $C(CH_3)=CH_2$), 3.3–3.6 (m, 7 H, CH_2O), 3.8–4.3 (m, 6 H, CH_2OP, CH_2OOC), 5.6–6.1 (m, 2 H, $CH_2=C$); IR (KBr) ν (cm^{-1}) 2910, 2845 (CH_3, CH_2), 1720 (C=O), 1630 (C=C), 1470 (CH_3, CH_2), 1120 (C–O–C), 1070 (P–O–C), 1240 (P=O), 970 (C=C), 720 (CH_2). Anal. Calcd for $C_{41}H_{80}O_8NaP$ (755.05): C, 65.22; H, 10.68; P, 4.10. Found: C, 64.72; H, 10.58; P, 4.16.

Methods. The infrared spectra (IR) were recorded with the help of a Perkin-Elmer 457 IR spectrometer. The IR bands were reported in wave numbers (ν) in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Aspect 3000 (400 MHz) spectrometer equipped with fourier transform data analysis. Chemical shifts were reported in ppm (δ) downfield in relation to tetramethylsilane. Small angle X-ray scattering experiments (SAXS) were performed by means of a powder defractometer, Siemens Type D 500, by using the Ni filtered Cu Kα line (λ = 0.1541 nm). The layer spacings were calculated by using the Bragg equation.

Liquid chromatography was performed on silica gel columns (Fa. Merck silica gel 60, 0.063–0.20 mm). Silica gel on AL-sheets (Fa. Merck TL-sheets, 60 F_{254}, thickness 0.2 mm) were used for thin-layer chromatography. Microanalysis was performed by Microanalysis Laboratories, University of Mainz.

Polymerization. The monomeric lipids were polymerized in a toluene/dioxane (1/1) mixture with 1 mol % 2,2'-azoisobutyronitrile (AIBN) as radical initiator. After the mixtures were flushed with nitrogen for 15 min, the polymerizations were carried out at 65 °C. Homopolymerizations were carried out at this temperature for 10 h whereas in the case of the copolymerizations only 5 h were needed (conversion about 50%). The polymeric lipids were obtained by precipitation in methanol or acetone and lyophilized in benzene. The polymers were characterized by TLC and ^{1}H NMR spectroscopy. The compositions of the statistical copolymers were determined by means of microanalysis. The values are given in Table I.

Preparation of Monolayers. The monolayers were characterized by using a computer-controlled film balance containing a Wilhelmy pressure pickup system.[21] The monomeric and polymeric lipids were spread as chloroform/methanol (9/1) solutions in concentrations of about 0.5 mg/mL. The surface pressure (π) was plotted vs. area/molecule for monomeric lipids and vs. area/repeat unit for polymeric amphiphiles. The water was purified by distillation and passed through a Milli-Q water purification system (Millipore Corp).

(21) Albrecht, O. *Thin Solid Films* **1983**, *99*, 227.

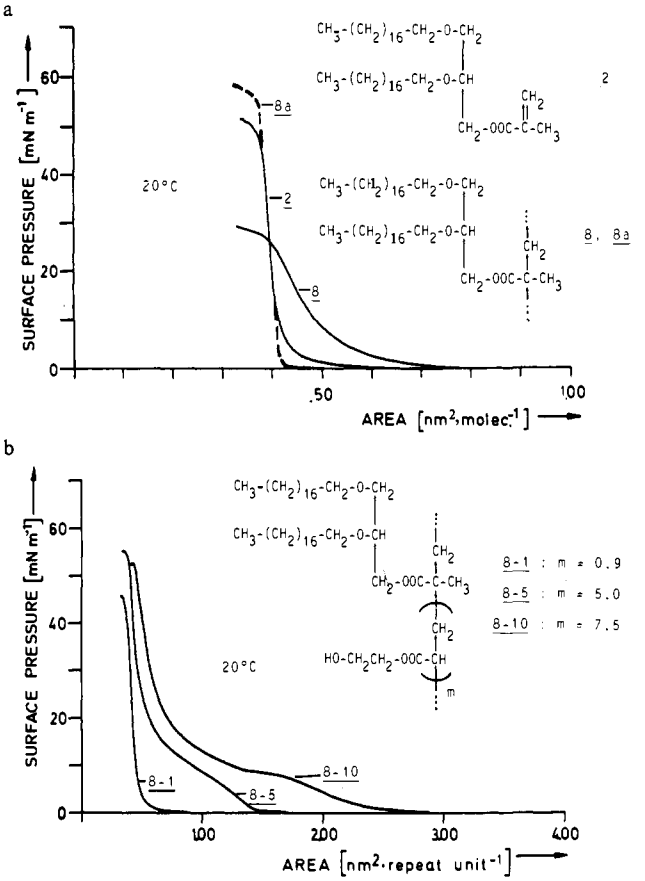


Figure 1. Isotherms of monomer **2**, homopolymer **8** (prepolymerized in solution before spreading) and homopolymer **8a** (polymerized in monolayer after spreading) on water at 20 °C. (b) Isotherms of the amphiphilic copolymers **8-1**, **8-5**, and **8-10** on water at 20 °C.

Preparation of Multilayers. Langmuir-Blodgett multilayers were prepared by means of a commercially available film balance (Lauda) on pure aqueous subphases at 20 °C. Multilayers could be deposited on various materials, such as silanized quartz, polyester, polypropylene, and polytetrafluoroethylene. The flexible polymer films were fixed by a Teflon sample holder.[22] The hydrophobic support materials were cleaned by ultrasonication in p.a. grade chloroform, washed twice with p.a. grade diethyl ether, and then rinsed with clean water several times. Polyester films (Hostaphan RE 3.0/Kalle, FRG) were used for the X-ray scattering experiments. The parameters for the transfer of monolayers are listed in Table III. Deposition takes place at each downward and upward dip (Y-type deposition).[4] Between subsequent dips, the samples were allowed to dry in the air for 5 min, to avoid retransferring of the last deposited monolayer to the water surface. The multilayers from the copolymer **11-2** were dried for 20 min between subsequent dips.

Results and Discussion

Monolayer Experiments. Spreading Behavior of Monomers. The amphiphiles **1–7** form stable monolayers at the air water interface. All amphiphiles exhibit solid analogue phases with the exception of the ammonium amphiphile **7**,[15] which shows a fluid analogue phase only. The collapse areas are approximately 0.4 nm^2/molecule, thus showing tight packing of the hydrophobic alkyl chains. The surface pressure-area diagrams (isotherms) of amphiphiles **2**, **4**, **5**, and **6** are shown representatively, in Figures 1–4, including the isotherms of the corresponding homopolymers and copolymers for direct comparison (see next paragraph).

The nonionic lipid **2** *without spacer* forms only solid analogue monolayers even up to 40 °C. With increasing temperature the collapse pressures decrease from 50 mN/m (20 °C) to 18 mN/m (40 °C).

In contrast to the lipids **1** and **2** without spacer groups, the lipids *with spacer groups* such as **4**, **5**, and **6** exhibit a coexistence of

(22) Albrecht, O.; Laschewsky, A.; Ringsdorf, H. *Macromolecules* **1984**, *17*, 937.

Table II. Characterization of Monolayers at 20 °C

	π_C[a]	A_C[b]	A_F[c]
(a) Monomeric Lipids			
2 (1a)[e]	48	0.39	
4 (2a)[e]	47	0.39	1.30
5 (3a)[e]	62	0.37	1.25
6 (4a)[e]	58	0.36	2.00
(b) Polymeric Lipids			
8 (1a)[e]	26	0.40	
8a (1a)[e]	55	0.38	
8-1 (1b)[e]	43	0.38	
8-5 (1b)[e]	53	0.39	1.50
8-10 (1b)[e]	52	0.41	2.75
9 (2b)[e]	28	0.38	
9-1 (2c)[e]	41	0.40	1.05[d]
9-5 (2d)[e]	58	0.41	1.80
9-10 (2e)[e]	60	0.42	3.60
10 (3b)[e]	31	0.40	
10-5 (3c)[e]	52	0.41	1.95
10-10 (3d)[e]	53	0.42	3.80
11 (4b)[e]	40	0.36	0.95
11-2 (4c)[e]	43	0.36	1.30

[a] π_C = Collapse pressure in mN/m. [b] A_C = Collapse area in nm²/molec. (nm²/repeat unit). [c] A_F = Area of the fluid phase in nm²/molec. (nm²/repeat unit). [d] Measured at 40 °C. [e] Number and letter denote particular figure.

solid and fluid analogue phases at 20 °C (Figures 2a–4a); the shapes of these isotherms show striking similarities, which are apparently due to a strong influence of the spacer groups. The spreading behavior in the fluid analogue phase is dominated by the spacer groups, whereas the behavior in the solid analogue phase is dominated by the hydrophobic interaction of the alkyl chains, as previously discussed.[7] An extended transition region between the fluid phase and the solid phase can be observed (Figures 2a–4a) for all spacer lipids investigated. The areas occupied per molecule in the fluid phase are exceptionally large, depending on the size of the hydrophilic spacer, which has to be considered as an integral part of the head group. The influence of the spacer length on the area occupied in the fluid phase is best illustrated by comparing the isotherms of the homologous phospholipids **5** and **6** (Figures 3a and 4a). In the case of **5** with a spacer of one ethyleneoxide unit, the area occupied in the fluid phase is 1.25 nm²/molecule compared to 2.00 nm²/molecule in the case of **6** which has a spacer of four ethyleneoxide units: the area occupied in the fluid phase increases with spacer length (see Table II).

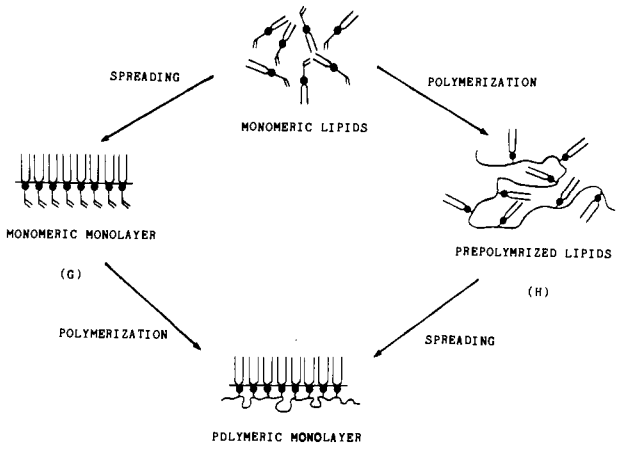
Spreading Behavior of Polymers. In general there are two strategies to obtain polymeric monolayers as pointed out in Scheme III.[7] Both strategies have already been realized in the past. Although the second pathway, i.e., the spreading of prepolymerized lipids (H), is much easier to perform experimentally, the resulting monolayers are less defined than polymeric monolayers obtained by method (G), i.e., the direct polymerization at the water surface. As discussed in the introduction, an efficient self-organization of the amphiphilic side groups is hindered by the polymer backbone.

These problems are demonstrated by comparing the polymeric monolayers prepared by the spreading of prepolymerized homopolymer **8** or by polymerization of an oriented monomer film **8a** (Figure 1a), as described in the following paragraph.

Spreading Behavior of Homopolymers without Spacers. The spreading behavior of polymeric lipid **2** is demonstrated in Figure 1a; polymeric monolayers were produced by using both strategies as shown in Scheme III. The "classical" way, i.e., the UV polymerization of monomeric monolayers[23] of lipid **2** leads to polymeric monolayers (homopolymer **8a**). Comparing the isotherm with the one of the monomer **2**, a similar spreading behavior of **8a** can be observed. The tight chain packing in the solid analogue phase is preserved after polymerization, whereas the collapse pressure is even slightly increased (Table II). However, if the prepolymerized lipid **8** is spread, much less defined monolayers are formed: there is no hint of a defined condensed phase,

(23) Gee, G. *Trans. Faraday Soc.* **1936**, *32*, 187.

Scheme III. Two Strategies To Obtain Polymeric Monolayers[a]



[a] (G) Self-organization of monomeric lipids with subsequent polymerization; (H) polymerization in solution and spreading of prepolymerized lipids.

because the slope of the curve is less steep and the collapse pressure is strongly reduced. This indicates a poor self-organization of the homopolymer without a spacer group (**8**, Figure 1a). The direct attachment of the amphiphilic side groups to the polymeric backbone, obviously reduces the capability of self-organization. The remarkable difference in the spreading behavior of the prepolymerized lipid **8** and in the monolayer polymerized lipid **8a** demonstrates the importance of the spacer concept: a separation of the amphiphilic side groups and the polymer backbone is necessary to allow an efficient self-organization of amphiphilic polymers. Such a separation can be provided by flexible spacer groups incorporated into the polymers as shown in the following paragraphs.

Spreading Behavior of Homopolymers with Side Group Spacers (Type A). The isotherms of the homopolymers **9**, **10**, and **11** are shown in Figures 2b–4b. All these homopolymers exhibit a solid analogue phase with collapse areas of ca. 0.4 nm²/repeat unit, indicating a tight packing of the alkyl chains, comparable to that obtained with the monomers. The isotherms are characterized by high collapse pressures (Table II) and steep slopes of the curves. Thus, the incorporation of side group spacers allows an efficient self-organization even of prepolymerized amphiphiles but not in the case of the prepolymerized lipid **8** (Figure 1a) without spacers. It is obvious, that the efficiency of the decoupling process is improved with increasing spacer length. This is demonstrated by comparing the homologous polymeric phospholipids **10** and **11** (Figures 3b and 4b). The homopolymer **10** with the short spacer group—one ethylene oxide unit—forms a solid phase only, up to 40 °C, whereas the coexistence of fluid and solid phases is observed for the homopolymer **11**[7] with the long spacer group—four ethylene oxide units. This means, that an extended spacer group favors the mobility of the hydrophobic chains in polymeric monolayers. The preservation of the phase transition which is due to the high mobility of the alkyl chains in the polymeric spacer lipids is an essential feature of this new class of amphiphiles: it makes them applicable as stable biomembrane models. The shift of the phase transition to higher temperatures and the decrease of the area occupied in the fluid phase for the homopolymer **11** compared to the monomer **6** (Figure 4a,b) point to a sufficient but reduced mobility of the amphiphilic side groups in the polymer. This partial decoupling effect agrees well with fluorescence bleaching studies of lateral diffusion in such polymeric monolayers of **11**, which is much slower than the lateral diffusion in monomeric monolayers of lipid **6**.[24]

The relatively high "viscosity" of the polymeric monolayers is also demonstrated by the unusual increase of the collapse pressure

(24) (a) Meller, P. Ph.D. Thesis, Technical University of München, FRG 1985. (b) Meller, P.; Peters, R.; Ringsdorf, H., submitted for publication.

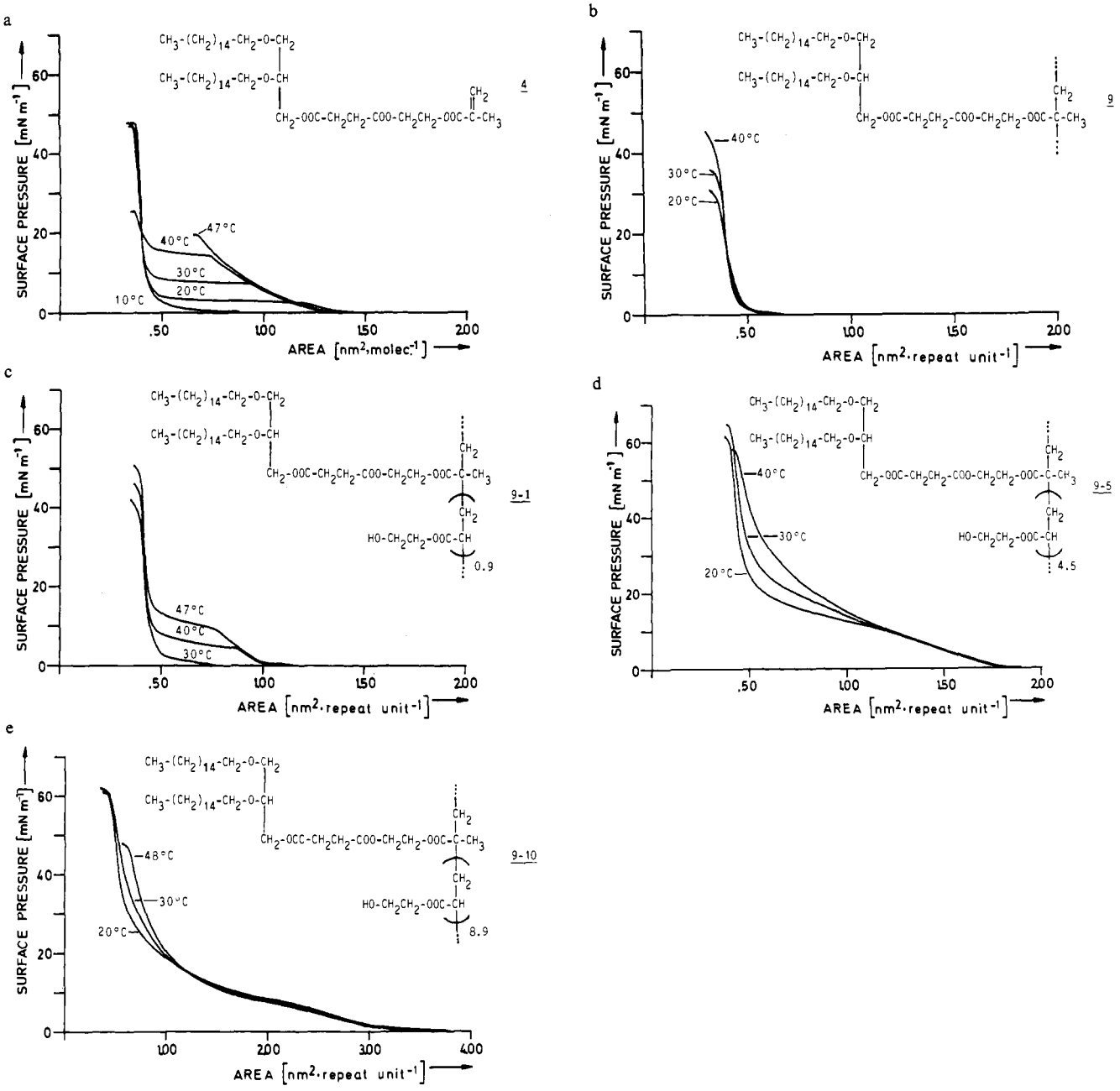


Figure 2. (a) Isotherms of the monomeric glycerolipid 4 on water at 10, 20, 30, 40, and 47 °C. (b) Isotherms of the amphiphilic homopolymer **9** on water at 20, 30, and 40 °C. (c) Isotherms of the amphiphilic copolymer **9–1** on water at 30, 40, and 47 °C. (d) Isotherms of the amphiphilic copolymer **9–5** on water at 20, 30, and 40 °C. (e) Isotherms of the amphiphilic copolymer **9–10** on water at 20, 30, and 48 °C.

with temperature (see Figures 2b, 2e, 3b, 4b, 4e). This is in contrast to the normal behavior of the monomers (see, e.g. **4** Figure 2a). The slow migration due to the viscosity of the polymers results in a premature collapse of the monolayers. With increasing temperature the migration is improved, and as a consequence the collapse pressures rise.

Spreading Behavior of Copolymers with Main Chain Spacers (Type B). In addition to the use of side group spacers, the efficient decoupling of polymer backbone and amphiphilic side groups is also achieved by the incorporation of flexible main chain spacers. For example this can be accomplished by copolymerization of the monomeric lipid **2** (without spacer) and 2-HEA as the hydrophilic comonomer. The effect of the main chain spacer can be seen in the isotherms of the resulting copolymers **8–1**, **8–5**, and **8–10** at 20 °C (Figure 1b). All copolymers show solid analogue phases with high collapse pressures and collapse areas of about 0.4 nm^2/repeat unit. The shapes of the curves in the solid phase correspond to the monomer curve of **2** (Figure 1a), with a steep slope hinting at an efficient decoupling process. A 1:1 ratio of lipid monomer to hydrophilic comonomer **8–1** already improves the spreading behavior compared to that of the homopolymer **8** without any spacer (Figure 1a). With increasing comonomer content, i.e., with increasing length of the main chain spacer, a coexistence of fluid and solid phases is observed at room temperature. Furthermore, the increase in mobility is accompanied by an increase of the area occupied in the fluid phase. This area is 1.50 nm^2/repeat unit for the 1:5 copolymer **8–5** and 2.75 nm^2/repeat unit for the 1:10 copolymer **8–10** (Figure 1b). This behavior corresponds to the spreading of homopolymers with side group spacers as already discussed in the case of the phospholipids **10** and **11** (Figures 3b and 4b). It should be noted that the main chain spacer does not interfere with the tight packing of the hydrophobic chains in the solid phase, which is exemplified by the collapse areas of ca. 0.4 nm^2/repeat unit. These results demonstrate that the main chain spacer is as efficient for decoupling purposes as the already reported side group spacer.[7] From a synthetic point of view, the much easier access to spacer groups and their variability are advantages of the main chain spacer. Furthermore the length of the spacer groups can also be varied very easily.

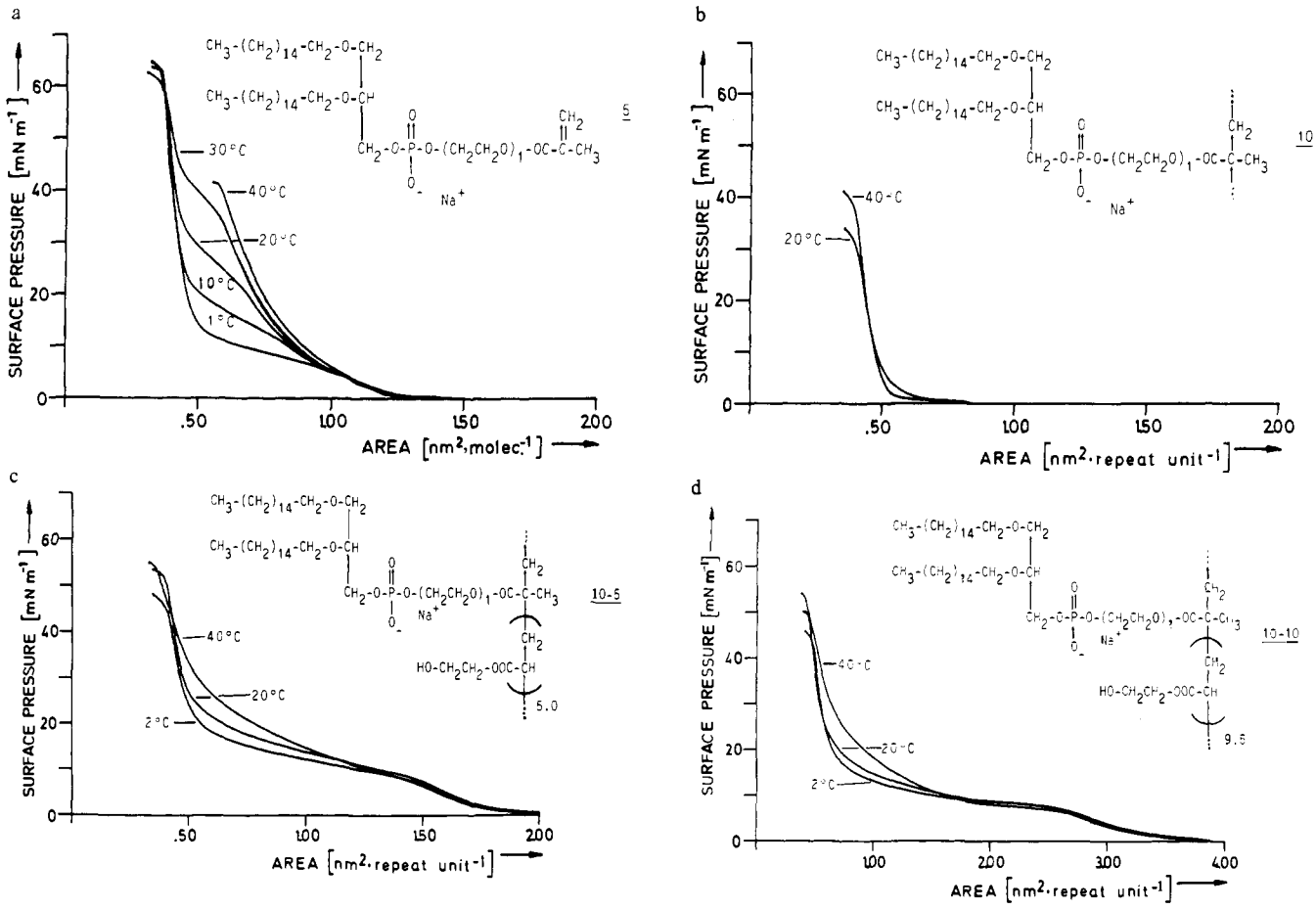


Figure 3. (a) Isotherms of the monomeric phospholipid **5** on water at 1, 10, 20, 30, and 40 °C. (b) Isotherms of the amphiphilic homopolymer **10** on water at 20 and 40 °C. (c) Isotherms of the amphiphilic copolymers **10–5** on water at 2, 20, and 40 °C. (d) Isotherms of the amphiphilic copolymers **10–10** on water at 2, 20, and 40 °C.

Spreading Behavior of Copolymers Combining Main Chain Spacers and Side Group Spacer (Type C). Copolymers with the combination of a main chain spacer and a side group spacer were produced by copolymerization of polymerizable spacer lipids such as **4–6** with hydrophilic comonomers such as 2-HEA.

The spreading behavior of the copolymers **9–1**, **9–5**, and **9–10** is illustrated in Figure 2c–e. In the case of the copolymers **9–1** to **9–10** solid analogue phases with tightly packed alkyl chains and high collapse pressures were observed as well as in the case of the monomer **4** and the homopolymer **9**. In contrast to the homopolymer **9**, which bears just a short side group spacer and shows only a solid analogue phase up to 40 °C, the additional main chain spacer leads to a coexisting fluid phase. Hence, the mobility of the hydrophobic chains is increased **8–1** to **8–10** the additionally incorporated spacer groups: the result corresponds to the increase of the spacer length of either type as shown in the case of the various copolymers **8–1** to **8–10** (Figure 1b). The formation of fluid phases of the copolymers **9–1** to **9–10** is favored by the increased content of hydrophilic comonomers (increased spacer length) which has already been demonstrated. At the same time, the area of the fluid phases is enlarged from 1.05 nm^2/repeat unit of the 1:1 copolymer **9–1** to 1.80 nm^2/repeat unit of the 1:5 copolymer **9–5** and to 3.60 nm^2/repeat unit of the 1:10 copolymer **9–10** (Figure 2c–e).

In agreement with the discussion above, a phase transition in the isotherms was observed for the copolymer **9–1** (Figure 2c), the same as for the monomer **4** (Figure 2b). The lengthening of the spacer group increases the mobility of the hydrophobic side chains in the polymer and, thus, reduces the phase transition temperature of the copolymer **9–1** compared to that of the homopolymer **9**. Nevertheless, the phase transition temperature of the monomer **4** is still lower and the area occupied in the fluid phase is larger than that of the copolymer **9–1** which only points to a partial decoupling. An analogous behavior has been discussed for the homologous polymeric phospholipids **10** and **11** with different length of the side group spacer. Interesting is the fact that the phase transitions seem to become independent of temperature with high comonomer contents, e.g., for the copolymers **9–5** and **9–10**. Within a temperature range of 1–40 °C the isotherms are basically the same. Slight differences are only noted in the solid analogue phase (Figure 2d,e).

These conclusions were confirmed by further investigations of other copolymer systems. The copolymers **10–5**, **10–10**, and **11–2** synthesized from the monomeric phospholipids **5** and **6** and 2-HEA show an analogous spreading behavior compared to the copolymers **9–1** to **9–10** (Figure 2c–e). The isotherms of the copolymers **10–5** and **10–10** (Figure 3c,d) with an extended main chain spacer are strikingly similar to the isotherms of **9–5** and **9–10** (compared to Figure 2d,e). The spreading behavior is obviously dominated by the extended main chain spacer. The phase transition again seems to be almost independent of temperature. However, in the case of the copolymer **11–2** (Figure 4c) with a short main chain spacer, the phase transition behavior is again temperature dependent and directly comparable to the phase transition of the monomer **6**. This indicates an increased mobility of the hydrophobic chains due to the additional main chain spacer: with side group spacers only, the homopolymer **11** (Figure 4b) shows a higher phase transition temperature. Corresponding to this, the area occupied by the copolymer **11–2** (Figure 4c) in the fluid phase is larger than that of the homopolymer **11** and almost reaches the area of the monomer **6** (see Table II). Due to the more efficient decoupling effect of the combination of a main chain spacer with a side group spacer, the spreading behavior of the copolymer **11–2** resembles the spreading behavior of the monomer **6**.

Multilayer Experiments. LB-multilayers show a great potential for various applications.[1,5,6,25] A sufficient stability of the

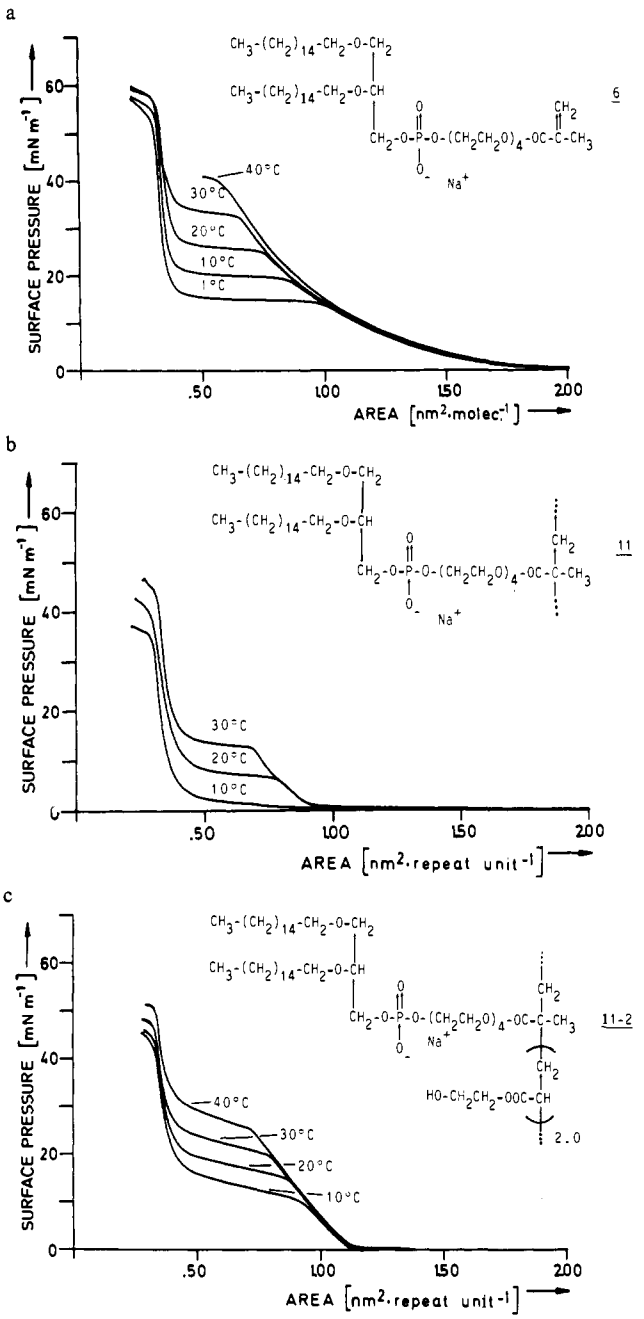


Figure 4. (a) Isotherms of the monomeric phospholipid **6** on water at 1, 10, 20, 30, and 40 °C. (b) Isotherms of the amphiphilic homopolymer **11** on water at 10, 20, and 30 °C. (c) Isotherms of the amphiphilic copolymer **11–2** on water at 10, 20, 30, and 40 °C.

multilayers is a basic requirement which can in general be achieved by covalent linkage of the amphiphiles, i.e., by polymerization.[26-28]

However, the classical route to polymeric multilayers, which is the transfer of monomeric layers and a subsequent polymerization in the multilayers,[26] gives rise to structural changes which can damage the layer structure and cause defects.[7,29-31]

First attempts to use prepolymerized amphiphiles to avoid these problems have already been published. Polymeric amphiphiles with side group spacers have been realized with the polymeric

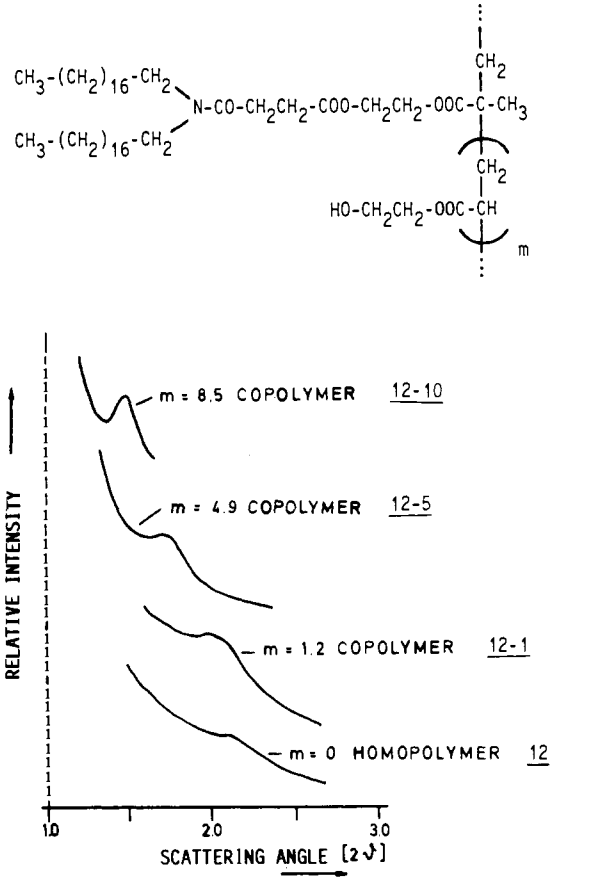


Figure 5. X-ray scattering diagrams prepared from polymeric amphiphiles (**12** to **12–10**) with various comonomer contents.

phospholipid **11**;[7] in addition the spreading behavior of alternating copolymers based on hydrophobized maleic anhydride derivatives were described.[10,32,33]

So the question arose whether prepolymerized lipids with combined spacers (see Scheme I) can be used to prepare LB-multilayers and to investigate the influence of the spacer length on the structure of the multilayers. The results of our first experiments will be described here. For this purpose, the polymers **12**, **12–1**, **12–5**, and **12–10** from the monomeric amide lipid **3** and the comonomer 2-HEA with comonomer ratios ranging from 1:1 to 1:10 (see Chart II) were chosen.

The corresponding monomer **3** used for the copolymer preparation did not form LB-multilayers at 20 °C because its monolayers exclusively form liquid analogue phases at this temperature; only irregular deposition of some lipid material was observed. SAXS experiments showed no layer reflections and, thus, no defined multilayer structure could be produced. In contrast to this, the polymers **12** to **12–10** could be deposited to form LB-multilayers as shown by means of small angle X-ray scattering (SAXS) experiments. The scattering diagrams are given in Figure 5. Layer reflections were observed for all polymers; with increasing comonomer contents there is a shift to smaller scattering angles. That leads to the conclusion that the layer spacings grow with increasing comonomer contents, as had been expected. The plot of the layer spacings vs. the copolymer composition as given in Figure 6 reveals a linear relationship between layer spacing (thickness of a sandwich bilayer) and comonomer content (spacer length). Furthermore, the SAXS diagrams in Figure 5 show that the scattering reflections become narrower and more intense with increasing comonomer content, i.e., with increasing spacer length. This points to the fact that the correlation of the bilayers is improved. The higher mobility in the system, which is caused

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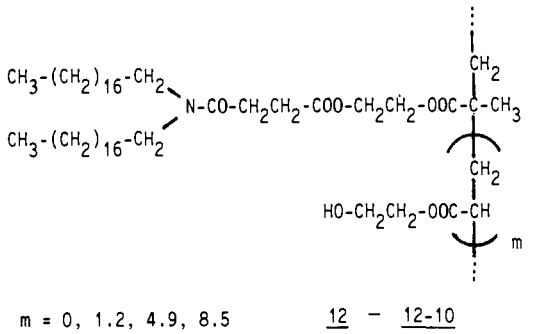

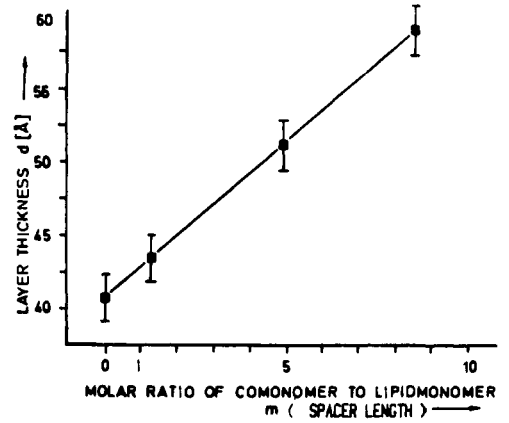

Figure 6. Layer spacings of LB-multilayers of the polymers **12** to **12–10** in dependence of the comonomer content *m* (spacer length).

Table III. Deposition Conditions and Characterization of LB-Multilayers at 20 °C

compd	surface pressure during deposition in mN/m	dipping speed downward	dipping speed upward in cm/min	thickness of sanwich bilayer in nm[a]
6	45	5	0.5	7.09 ± 0.03
11a[b]				6.95 ± 0.03
11b[c]	30	2.5	0.5	6.90 ± 0.20
11-2	30	2.5	0.5	6.80 ± 0.20
12	25	1.5	0.5	4.09 ± 0.15
12-1	30	2.5	0.5	4.29 ± 0.15
12-5	40	2.5	0.5	5.10 ± 0.15
12-10	40	2.5	0.5	5.90 ± 0.15

[a] Measured by SAXS, 15 dips = 30 layer deposited. [b] UV-polymerization in multilayers by 254-nm light, light intensity 0.5 mW cm^{-2}. [c] Polymerization in solution before spreading.

by the more efficient decoupling of the lipid bilayer from the polymer chain, due to the increased length of the main chain spacer, may be a possible explanation. Both the increase of the layer thickness (Figure 6) and the layer correlation (Figure 5) which are due to the length of the main chain spacer are illustrated in the model shown in Figure 7. However, there seems to be an optimum in the spacer length, beyond which the order of the polymeric multilayers declines. This was found by comparing polymeric multilayers of homopolymer **11** with those of copolymer **11–2**. The multilayers prepared from the homopolymer **11** having

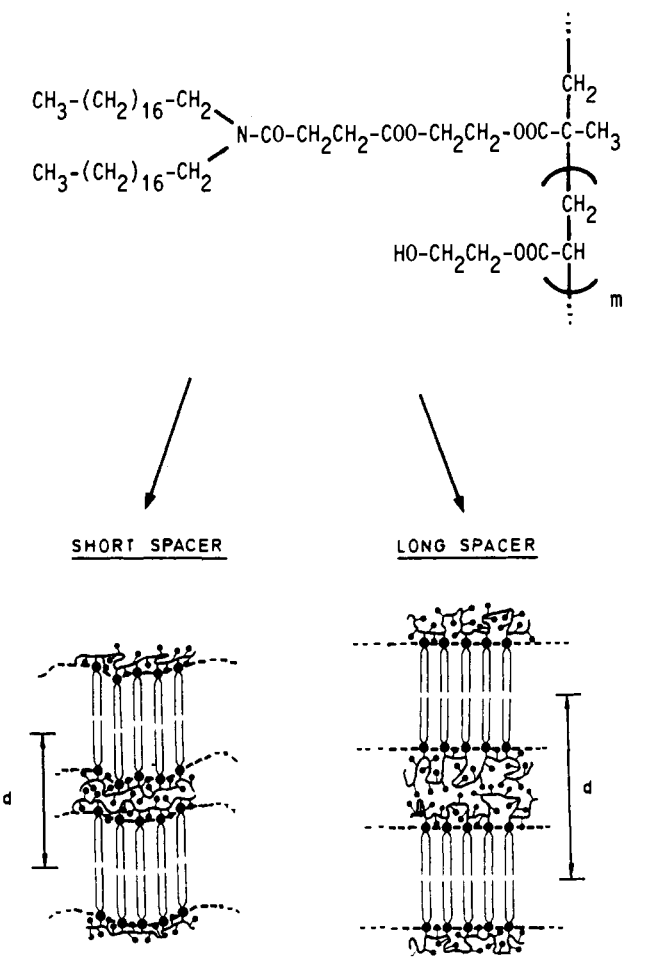

Figure 7. Schematic representation of the layer thickness (d) and layer correlation (---) in dependence of the length of the main chain spacer (short spacer: m = 1.2; long spacer: m = 8.5).

only a long side group spacer show a narrow, intense layer reflection indicating a rather high degree of order in the multilayers. Further, the obtained layer spacing agrees well with the spacing of the polymer formed by photopolymerization of the corresponding monomer **6** in multilayers[7] (Table III). In contrast, the SAXS plot of the multilayers of copolymer **11–2** which possesses the same side group spacer and in addition a short main chain spacer, shows a rather broad and less intense scattering reflection. Although, the calculated layer spacing of **11–2** agrees well with those observed in the case of homopolymer **11** (see Table III), the broadness of the SAXS reflection suggests that the multilayers of **11–2** are less ordered than the multilayers of **11**. Thus, the behavior of the multilayers based on homo- and copolymers derived from the uncharged amphiphilic monomer **3** stand in stark contrast to those derived from the charged monomeric phospholipid **6**.

Registry No. 2, 76282-10-5; **3**, 105473-57-2; **4**, 105473-51-6; **5**, 105501-11-9; **6**, 96326-73-7; **8**, 105473-49-2; **8-1**, 105473-50-5; **9**, 105473-52-7; **9-1**, 105473-53-8; **10**, 105501-12-0; **10**, 105501-12-0; **10-5**, 105501-13-1; **11**, 105473-55-0; **11-2**, 105473-56-1; **12**, 105473-58-3; **12-1**, 105473-59-4; $H_2C{=}C(CH_3)COCl$, 920-46-7; $H_3C(CH_2)_{15}OCH_2CH(O(CH_2)_{15}CH_3)CH_2OH$, 6076-35-3; $H_3C(CH_2)_{17}NH(CH_2)_{17}CH_3$, 112-99-2; $(H_3C(CH_2)_{17})_2NCO(CH_2)_2CONH_2$, 105473-60-7; $H_2C{=}C(CH_3)CO_2(CH_2)_2OH$, 868-77-9; $POCl_3$, 10025-87-3; succinic anhydride, 108-30-5.