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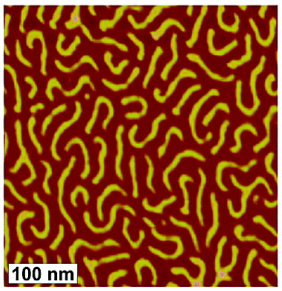

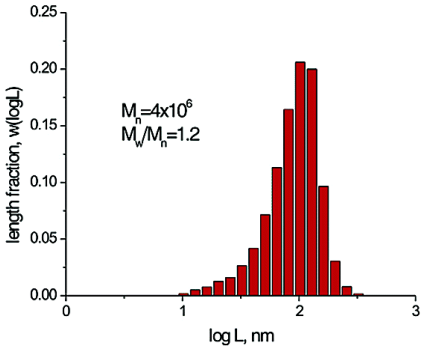

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Measuring Molecular Weight by Atomic Force Microscopy

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Abstract: Absolute-molecular-weight distribution of cylindrical brush molecules were determined using a combination of the Langmuir Blodget (LB) technique and Atomic Force Microscopy (AFM). The LB technique gives mass density of a monolayer, i.e., mass per unit area, whereas visualization of individual molecules by AFM enables accurate measurements of the molecular density, i.e., number of molecules per unit area. From the ratio of the mass density to the molecular density, one can determine the absolute value for the number average molecular weight. Assuming that the structure of brush molecules is uniform along the backbone, the length distribution should be virtually identical to the molecular weight distribution. Although we used only brush molecules for demonstration purpose, this approach can be applied for a large variety of molecular and colloidal species that can be visualized by a microscopic technique.

Introduction

Accurate characterization of molecular weight distribution is very important since many physical properties of polymers depend on the chain dimensions. The characterization is straightforward for low molecular weight polymers with a simple chemical structure, e.g., linear chain homopolymers without ionic and associating groups. However, experimentalists face severe difficulties when studying large molecules possessing a complex architecture, heterogeneous chemical composition, charged moieties, and/or surface active groups. Here, we propose to use a combination of two well-known techniques, i.e., Atomic Force Microscopy (AFM) and Langmuir−Blodget (LB) technique, to determine the number average molecular weight and the molecular weight distribution. This approach does not require any prior information about the chemical composition and the architecture of macromolecules. The only necessary condition for the practical application of this method is visualization of individual molecules.[1]

Methods

The method includes several steps. First, one should prepare a stock solution of a known concentration c. Second, a certain amount (volume V) of the solution is spread over the water surface in a Langmuir trough to form a monolayer of adsorbed molecules. In the third step, the monolayer is compressed laterally to a certain area S_{LB} at which a dense monolayer forms. Step four is the transfer of the monolayer onto a solid substrate for AFM studies. One should also measure the transfer ratio T—the ratio of the change in area of the water supported monolayer during the transfer onto a solid substrate to the area of the substrate. Finally, step five, the transferred monolayers are scanned by AFM for visualization of individual molecules.

From the concentration, volume, and transfer area S_{LB} one can calculate the mass per unit area as

$$m_{LB} = c \cdot V / S_{LB} \tag{1}$$

One should note that the film transfer could be performed at any area S_{LB}, provided that the monolayer is dense and the molecules can be clearly resolved. Visualization of individual molecules by AFM enables their counting within the micrograph area S_{AFM} to determine the number of molecules per unit area n_{AFM}

$$n_{AFM} = N / S_{AFM} \tag{2}$$

The error associated with the molecular density decreases as $1/\sqrt{N}$. In this work, we counted approximately 3000 molecules for each sample to obtain a relative standard deviation of 4%.

From the mass and molecular densities, one can calculate the number average molecular weight M_n using the following equation

$$M_n = \frac{m_{LB}}{n_{AFM}} \frac{T}{m_{am}} \tag{3}$$

where T is the transfer ratio and m_{am} is the atomic mass unit $m_{am} = 1.6605 \times 10^{-24}$ g. The transfer ratio corrects for the difference between the mass density of the water supported monolayer and the mass density of the transferred film.

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In addition, AFM images give length distribution of the visualized molecules. The length fraction of molecules with length L can be calculated as

$$w_L = L/L_n n_L \quad (4)$$

where L_n is the number average length for an ensemble of counted molecules and n_L is the number fraction of molecules with length L. Assuming that the molecular weight is proportional to the contour length ($M \approx L$), the length distribution should be identical to the molecular weight distribution from GPC (weight fraction w_M versus molecular weight M).

Experimental Section

Materials. A series of PBA brushes (samples A, B, C, and D) with different lengths of the side chains was prepared by grafting of *n*-butyl acrylate from a poly(2-(2-bromopropionyloxy)ethyl methacrylate) (pBPEM) macroinitiator as described elsewhere.[2] Atom transfer radical polymerization (ATRP)[3] allowed preparation of brushes with a well-defined degree of polymerization of the main chain and uniform distribution of the side chains along the backbone.[4] The macroinitiator, which was the same for all four brushes, was characterized to give the number average molecular weight $M_n = 1.5 \times 10^5$ and the polydispersity index $M_w/M_n = 1.4$. This polydispersity is relatively high. Typically, for ATRP, polydispersities may be as low as $M_w/M_n < 1.1$ for short chains (DP < 100). However polydispersity may increase with the chain length due to inevitable transfer/termination reactions. The M_n gives the number average degree of polymerization of the backbone $N_n = 567 \pm 35$. From the N_n value and the total number average molecular weight of the brush molecules, one could determine the degree of polymerization of PBA side chains to be 9, 27, 35, and 51 for samples A, B, C, and D, respectively.

Characterization. Average molecular weights and molecular weight distribution were measured by gel permeation chromatography (GPC) equipped with Waters microstyragel columns (pore size 10^5, 10^4, 10^3 Å) and three detection systems: a differential refractometer (Waters Model 410), multi-angle laser light-scattering (MALLS) detector (Wyatt, DAWN EOS), and a differential viscometer (WGE Dr. Bures, η-1001). The 90° detector was calibrated using toluene. All other detectors were normalized to the 90° signal. Static light scattering (SLS) measurements were done using a Brookhaven Goniometer equipped with a Coherent argon laser using the 514 nm line, an operating power of 20−100 mW, and an angle range of 15−155°. Solutions were made with a concentration range from 10^{-4} to 10^{-2} g/mL in THF that had been filtered using 0.2 μm NALGENE PTFE filters.

Sample Preparation. Langmuir-Blodget experiments were carried out using a KSV 5000 Instrument filled with double-distilled water (Mili-Q). The film was transferred onto a mica substrate using a transfer speed of 0.5 mm/min. During transfer, the pressure was maintained constant. The transfer ratio was determined separately by using a larger substrate at the same transfer speed. From the ratio of the change in the trough area $\Delta S_T = 2241$ mm^2 and the covered substrate area $\Delta S_S = 2250$ mm^2, one could determine a transfer ratio of 0.996. A value close to unity indicates that the transfer did not cause significant changes in the mass density of the water supported monolayer.

Measurements. AFM images were collected using a Multimode IIIa Atomic Force Microscope (Veeco Metrology Group) in tapping mode. To ensure accurate counting of visualized molecules, several images were collected from the same sample but in different areas, using different scan sizes and scan directions. As already mentioned, for every sample about 3000 molecules were counted. The counting was performed using a custom software program for analysis of digital images. The program is designed to identify the molecular contour, and to determine the contour length, the end-to-end distance, and the curvature distribution.

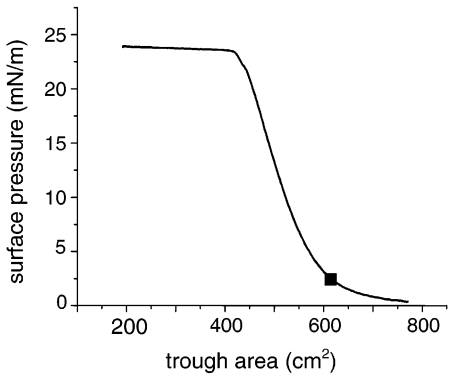

Figure 1. Surface pressure−area diagram for Polymer D. The dot on the curve indicates the transfer area and pressure.

Table 1. Characterization of Molecular Layers by the Langmuir-Blodget and AFM Techniques

polymer[a]	n_n[b]	m_{LB},[c] 10^{-16} g/μm^2	n_{AFM},[d] μm^{-2}	M_n[e] $\times 10^6$
A	9	7.8 ± 0.4	589 ± 54	0.8 ± 0.11
B	20	6.5 ± 0.4	255 ± 10	1.5 ± 0.15
C	31	7.6 ± 0.4	183 ± 7	2.5 ± 0.22
D	51	8.2 ± 0.4	124 ± 5	4.0 ± 0.35

[a] Cylindrical brush molecules with different degrees of polymerization n_n of the PBA side chains. The number average degree of polymerization of the main chain $N_n = 567 \pm 35$ was the same for polymers A, B, C, and D. [b] The number average degree of polymerization of the side chains was determined as $n_n = (M_n - m_n)/N_n M_0$, where M_n—number average molecular weight of the PBA brush measured by MALLS-GPC, $m_n = 0.15 \times 10^6$ number average molecular weight of the main chain determined by MALLS-GPC of the macroinitiator, and $M_0 = 128$ g/mol—molecular weight of BA monomeric unit. [c] Mass per unit area from eq 1. [d] Number of molecules per unit area from eq 2 [e] Number average molecular weight from eq 3.

Results

Figure 1 shows a surface pressure versus film area isotherm for polymer D with the longest side chains. The isotherms for polymers A, B, and C were somewhat different[2], however the difference was not essential and furthermore not relevant for the molecular mass determination. Similar to other fluids, compression of the PBA brushes was fully reversible pointing to equilibrium conditions of the experiment. Typically, the monolayer was transferred to a mica substrate at surface pressures from 1 to 3 mN/m. Table 1 presents masses per unit area (m_{LB}) at the transfer pressure. The low surface pressure was used to prevent the formation of globules and agglomeration of molecules[2].

Figure 2 shows an AFM image of Sample B on mica. The image demonstrates the uniform coverage of the substrate, which enables accurate counting of molecules. However, the image also reveals two issues, which may affect the quantitative analysis: (i) crossing of molecules and (ii) partial visualization of molecules at the image borders. Because the image analysis program automatically captures all kinds of individual species, it considers both the crosses and the molecular fragments as molecules, i.e., two crossed molecules are counted as one and partially imaged molecules are counted as whole molecules.

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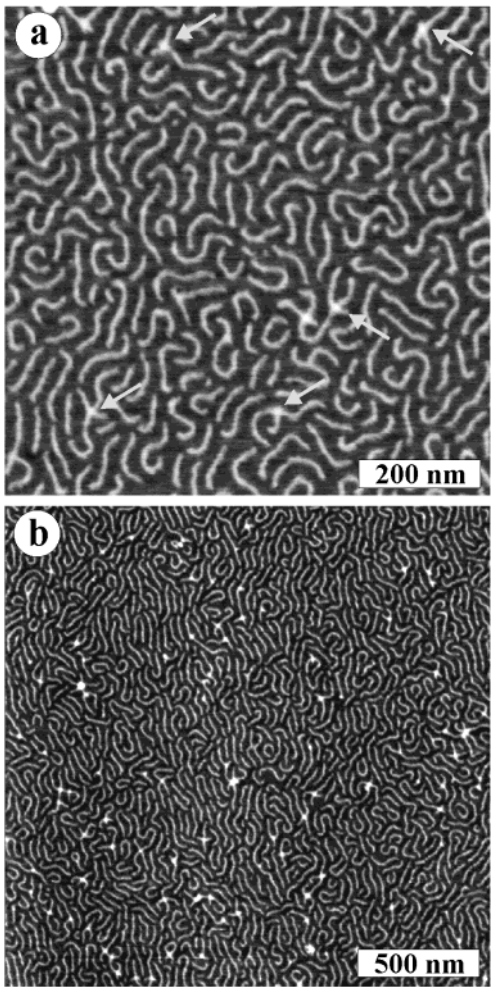

Figure 2. Individual molecules of polymer B were clearly resolved by tapping mode AFM. The higher resolution image (a) demonstrates details of the molecular conformation including crossing molecules indicated by arrows. The larger scale image (b) demonstrates the uniform coverage of the substrate.

Table 2. Molecular Weights of PBA Cylindrical Brushes Determined by SLS, MALLS-GPC and the AFM-LB Methods

	SLS	MALLS-GPC		AFM		
polymer	M_n,[a] 10^6	M_n,[b] 10^6	M_w/M_n[c]	M_n,[d] 10^6	L_n,[e] nm	L_w/L_n[f]
A	1.1	0.8	1.39	0.8 ± 0.11	110 ± 8	1.24
B	1.4	1.6	1.54	1.5 ± 0.15	108 ± 6	1.33
C	2.5	2.4	1.39	2.5 ± 0.22	115 ± 5	1.24
D	3.9	4.7	1.46	4.0 ± 0.35	113 ± 5	1.20

[a] The number average molecular weight was calculated from the weight average molecular weight determined by SLS using the polydispersity index M_w/M_n from GPC.[c] [b] Number average molecular weight of brush molecules determined by MALLS-GPC. [c] Polydispersity index of the molecular weight measured by MALLS-GPC. [d] Number average molecular weight determined by the AFM-LB approach (eq 3). [e] Number average length measured for an ensemble of 300 molecules with a statistical deviation of 5 nm. [f] Polydispersity index of the molecular length obtained from AFM images.

Therefore, crossed molecules will overestimate the M_n, whereas the partially imaged border molecules increase the number of molecules per unit area, i.e., underestimate the M_n.

The problem of crossings was resolved by increasing the number of counted species by the number of crosses. This approach can be applied to relatively short molecules that do not cross themselves to form complex topologies such as cycles, knots, and networks. As to the border molecules, the total

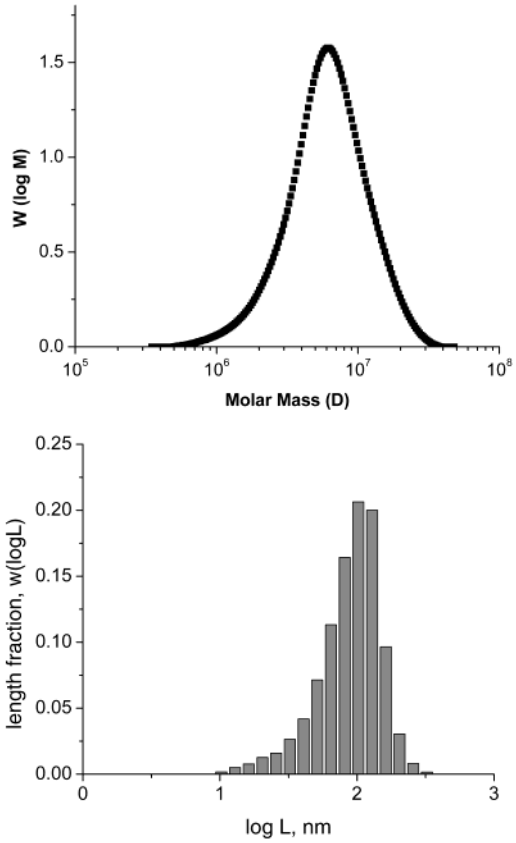

Figure 3. (top) MALLS-GPC diagram presents molecular weight distribution of Sample D. (bottom) The molecular length distribution (eq 4) was measured by AFM for an ensemble of 3060 molecules.

number of molecules was recalculated as $n_{AFM} = n - n_p + n_b$, where n is the number of individual molecular species visualized by AFM, n_p is the number of partially imaged molecules, and n_b is the number of equivalent border molecules of complete length. The number of the equivalent molecules was determined as $n_b = \sum_i L_{b,i}/L_n$, where $L_{b,i}$ is the length of the partially imaged molecules and L_n is the number average length of the complete molecules. The L_n value was determined separately as $L_n = \sum_i L_i/N$, where L_i is the length of complete molecules.

After the above corrections, the molecular density of the LB films was calculated. For example, sample D gave $n_{AFM} = 124 \pm 5$ molecules/μm^2. Using eq 3 one could calculate the number average molecular weight $M_n = (4.0 \pm 0.4) \times 10^6$. Table 1 also depicts molecular weights of samples A, B, and C including errors of the corresponding measurements. The relatively large error associated with Sample A is due to less clear visualization of the smaller molecules. The error in M_n can be reduced by counting more molecules. The molecular weights obtained from the AFM-LB method were compared to SLS and MALLS-GPC data obtained for the same polymers. Table 2 demonstrates remarkably good agreement between the methods. The agreement is indeed remarkable because the AFM-LB and SLS/GPC measurements were carried out independently and are based on different principles.

In addition to the number average molecular weight, the AFM-LB method allows characterization of the molecular weight distribution. The latter can be derived from the molecular length distribution assuming that the molecular weight is directly

proportional to the length, i.e., $M \sim L$. This assumption is often reasonable, especially in this work where the ATRP synthesis yields brushes with a uniform structure along the backbone. This property was confirmed by GPC analysis of the side chains detached from the backbone.[5] A general procedure for statistical analysis of the contour length is well established for different types of linear molecules.[6−11] Figure 3 shows molecular weight and molecular length distributions determined for Sample D by MALLS-GPC and AFM, respectively. In both diagrams, the Y-axes correspond to weight fraction. One can see that the distributions obtained by the different methods are very similar. Note that in both cases the distributions cover three decades of the molecular sizes. It would also be instructive to notice that the GPC distribution of cylindrical brushes is virtually identical to the distribution of the macroinitiator ($M_n = 1.5 \times 10^5$, $M_w/M_n = 1.4$) used for preparation of the brush molecules. This observation is consistent with the above assumption of the uniform composition of the brushes along the backbone. Table 2 presents the polydispersity indexes obtained by GPC and AFM. The GPC values are somewhat larger than those from AFM. The difference can be attributed either to the intrinsic broadening of elution curves in GPC or to undercounting of small fractions of very small and very large molecules in AFM images. The undercounting issue becomes relevant for samples with broader distributions. Their analysis would require scanning of larger areas with more molecules to improve statistical representation of minority fractions. This and other discrepancies between the molecular weights in Table 2 are currently under investigation.

Conclusions

The combination of AFM and LB techniques allowed accurate determination of the number average molecular weight and molecular weight distribution. The method relies on visualization of individual molecules which enables their counting. The AFM-LB data demonstrated remarkably good agreement with results obtained by the MALLS-GPC technique. Although the application of the method was demonstrated for brush molecules, it can be applied for other kinds of "visualizable" species.

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