Atomic Force Microscopy on Self-Assembled Polymer Structures

Emmanouil Glynos

A thesis submitted to the University of Edinburgh for the Degree of Doctor of Philosophy.

March 2007
To my parents and my brother
Declaration

I declare that this thesis has been composed by myself and is all my own work except where otherwise stated.

Emmanouil Glynos
March 2007
Acknowledgements

This work could have not been possible without the help, guidance and support of many people.

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Summary

The atomic force microscopy (AFM) is perhaps the most versatile member of a family of microscopes known as scanning probe microscopes (SPM). The AFM has provided an unprecedented spatial and force resolution of the order of Angstroms and subnanonewtons, respectively. In this work the AFM was used to study self-assembled polymeric structures at surfaces. The thesis is divided in two main parts; Part A: polymers at surfaces and Part B: Polymer microbubbles.

In Part A we started our studies by investigating the morphology of physisorbed linear and star polybutadiene (PB) on a freshly cleaved mica surface from dilute solution after solvent evaporation. AFM has been widely used to study polymer films. Although many studies have been reported on thin film polymer blends, block copolymer micelles adsorbed on surfaces, end-grafted polymers adsorbed or chemisorbed on surfaces and several other complex systems the rather simple case of homopolymers, linear or star, physisorbed on a solid substrate has been largely ignored.

For the case of linear PB, we found that the dependence of the molecular weight on the observed polymer structures is crucial for samples with relatively high surface density where the interactions among the adsorbed polymers become significant. For a relatively high surface density we observed a tendency of the adsorbed polymers to aggregate and form an isotropic structural pattern. We explained these structural phenomena with increasing surface density in terms of the molecular interactions of the adsorbed polymers when in good solvent conditions and after the abrupt solvent evaporation.

For the case of star PB we present a study of the structure and growth of star shaped polymer monolayers on mica. We found that the monolayer morphology depends strongly on the functionality (number of arms) of the star polymer. We observed a strong dependence of the evolution of the average island height and surface coverage in different surface densities on the functionality of the star polymers. We explained these structural phenomena in terms of the molecular and conformational properties of the different star polymers. We found that the structural regimes are highly affected by the expected change of the star polymer behaviour from polymer-like to colloidal-like when the star functionality increases.
We have also used the AFM in order to observe time-dependent changes of polymeric structures on surfaces. We studied poly(isoprene-\(b\)-ethylene oxide) block copolymer micelles on mica under ambient conditions. We found that the time dependent behavior of the polymeric islands arises from the surface “aging” of freshly cleaved muscovite mica. Mica transforms from highly hydrophilic when freshly cleaved to less hydrophilic with the exposure time in ambient conditions.

In part B the AFM is proposed for the first time as a tool to image the surface of polymer microbubbles at the nanometre range in liquid and to perform reproducible measurements on the nano/micro mechanical properties. The introduction of microbubbles as contrast agents in ultrasonic imaging has expanded to areas such as drug/gene delivery. The progress of microbubble research has been slower than originally envisaged, partly due to its evolution into a multidisciplinary scientific area. However, the most significant obstacle in the development of microbubble technology remains the understanding of microbubble behaviour, which has been compromised by the lack of experimental data. The techniques used today however, remain largely empirical, as they draw from signal processing rather than thorough knowledge of microbubble behaviour. Such knowledge would lead to optimal use of contrast microbubbles and improve visualisation of microvasculartiy. Furthermore microbubble modelling still has limited predictive value, and requires untested assumptions, the most important being about the microbubble shell properties.

Motivated from this lack of information for the microbubbles we applied the AFM to assess structural aspects of the microbubble shell and probe their mechanical properties. As microbubbles are large objects compared to the overall size of usual AFM tips a convolution between the AFM tip and the microbubble was typical of the acquired topographies. However, a small part of the top of the bubble was imaged with nanometer resolution and roughness measurements are reported. Using contact mode AFM force-distance curves were captured and the range of stiffness (or effective spring constant) of biSphere® microbubbles was systematically measured. Applying a simple continuum model, based on elasticity theory, we found that the elastic modulus of the microbubble shell increased significantly as the microbubble dimensions decreased. Furthermore, we investigated non-linear mechanical effects at higher deformations and showed the conditions under which the microbubbles were permanently damaged.
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Chapter 1

Introduction
Introduction

Self-assembly is defined as the fundamental principle which generates structural organization on all scales from molecules to galaxies. It is defined as a process in which pre-existing parts or disordered components of a system form well-defined structures or patterns. Examples of self-assembling systems include weather patterns, solar systems, histogenesis and self-assembled monolayers.\(^1\)

The most well-studied subfield of self-assembly is the molecular self-assembly, which expresses the ability of molecules to form supramolecular structures. The self-assembling molecules are often polymers with the ability to assemble from the random coil conformation into a well-defined supramolecular polymer structure.\(^2\) A very simple and well known example of a supramolecular polymer structure is the micelle formation of amphiphilic diblock copolymers in aqua solutions.\(^3,4\)

Self-assembly is an important technique as a manufacturing method in nanotechnology. In self-assembly the final/desired structure is “encoded” in the shape and properties of the molecules that are used, while in the traditional techniques, such as lithography, the desired final structure must be carved out from a larger block of matter. Self-assembly is thus referred to as a “bottom-up” manufacturing technique, as compared to lithography being a “top-down” technique. In general, molecular self-assembly can be applied instead of the expensive and slow lithography to produce similar materials in larger quantities.

In this thesis we report the use of the atomic force microscope (AFM) for the study of self-assembled polymer structures. This work has been divided in two main parts:

Part A: Polymers at surfaces (Chapters 3, 4 and 5)

Polymers on surfaces play an important role for many technological applications such as microelectronics, colloidal stability,\(^5,6\) surface nanopatterning,\(^7\) adhesion,\(^8\) and friction modification.\(^9,10\) They can form a dense polymer layer which is a soft interface and can have a wide range of exciting research interest. Polymer monolayers are formed by anchoring or grafting chains on an appropriate substrate. The grafting mechanism can be chemical (chemisorption), i.e. covalent bond, hydrogen bond, or purely physical (physisorption), i.e. van der Waals interactions, and can occur either at the backbone or at the end(s) of the chain. The systems used were linear and star polybutadiene polymers and poly(isoprene-\(b\)-ethylene oxide) block copolymers.
Part B: Polymer microbubbles (Chapters 6 and 7).

Microbubbles are stable, haemodynamically inert\textsuperscript{11,12}, micrometer-sized, biocompatible spheres consisting of a self-assembled ultra-thin shell (10s of nm) encapsulating an inert gas. They are smaller than the smallest blood vessel of a human body, and so are used as ultrasound contrast agents to allow improved visualisations of the vascular bed\textsuperscript{13, 14}, and to differentiate vascular patterns on tumours non-invasively. Furthermore, they have shown potential as carriers of drugs/genes for targeted drug/gene delivery. In this part we used the AFM to nanointerrogate these microbubbles.

The work in this thesis is organized as follows:

In Chapter 2 we review some basic concepts of atomic force microscopy and its basic operation.

In Chapter 3 we applied the AFM to investigate the morphology and self-assembly of linear polybutadiene (PB) physisorbed from dilute solutions onto freshly cleaved mica surfaces after abrupt solvent evaporation. Several different molecular weights ($M_w$) of linear PB were used in order to see the effect of the $M_w$ on the observed structures.

In Chapter 4 we moved to the case of star-shaped PB molecules physisorbed on freshly cleaved mica after abrupt solvent evaporation. Several molecules with different functionalities (number of arms) were used to study the effect of the functionality of the star polymer on the observed structure on the polymer modified mica.

In Chapter 5 we applied the AFM to study the time-dependent changes in the structure of poly(isoprene-$b$-ethylene oxide) block copolymer micelles on mica under ambient conditions. The effect of surface "aging" of freshly cleaved muscovite mica from highly hydrophilic, when freshly cleaved, to less hydrophilic with time, on the morphology of the adsorbed ampliphilic diblock copolymers micelles is discussed.
In **Chapter 6** we are introducing for the first time the AFM as a tool to image the surface of ultrasonic contrast agent polymer microbubbles at the nanometre range. Our data revealed roughness information of the microbubble shell.

In **Chapter 7** we used the AFM to investigate the mechanical properties of the contrast agent polymer microbubbles. A wide range of different spring constant cantilevers was used resulting in a systematic study of the microbubble shell properties. A simple model for the deformability of the spherical shell was applied to estimate the Young’s modulus \((E)\) of the microbubble shell.
References

Chapter 2

Introduction to Atomic Force Microscopy (AFM)
2.1 General Aspects

The atomic force microscopy (AFM) is perhaps the most versatile member of a family of microscopes known as scanning probe microscopes (SPM). Since its introduction from Binning and Roher\(^1\) the AFM has provided an unprecedented spatial and force resolution of the order of Ångstroms and sub-nanonewton. The importance of their invention was recognized through the Nobel Prize in Physics in 1986. The significance and the development of the AFM since its introduction is apparent by the size of literature, both in published review papers\(^2-9\) and books\(^10-16\), owing to its applicability to a wide range of research fields.

The AFM generates images by "feeling" rather than "looking at" the surface. A good analogy is a blind person who, in order to understand objects, feels them with his fingers and then builds up a mental image of what he touched. The main features of the AFM are illustrated in Figure 2.1. The most important part of an AFM is the tip, which is mounted on a miniature cantilever. It is the part of the AFM which touches the surface giving rise to the image through its force interaction with the sample surface. Typical tips have a μm scale pyramidal shape with a nm size end radius (for standards tip the typical values are 2-3 μm tall pyramid and 30-50 nm radius apex). Although in most of the cases the sharpness of the tip determines the resolving power of the instrument quite often the so-called atomic resolution on crystals is obtained more easily and better with standard tips.

The sharp tip at the end of the cantilever is brought into contact with the sample through a piezoelectric tube and especially from the the z-piezo extension. The deflection of the cantilever is monitored through a detection mechanism. In modern instruments a laser beam is focused on the cantilever and reflected from its back side onto a position sensitive four segment photodetector. The photodetector is sensitive enough to detect atomic scale movement of the tip. When the tip is in contact with the surface the electronics of the instrument make the tip to scan the area of interest through x- and y- extension of the piezo in a raster fashion. The whole system works in a feedback or control loop so that the tip can precisely scan the sample in a controlled manner at all times. The AFM images are generated by recording the interactions forces of the tip with the surface as the tip scans the sample.
The main advantage of AFM over the other routine imaging techniques, such as optical microscopy and scanning electron microscopy (SEM), is that the AFM is not a far-field technique and does not offer only spatial resolution, but can map the sample in 3D. Furthermore, the resolution of AFM is much higher than the traditional optical microscopy which is a far-field technique and therefore limited from the diffraction limit of light which is roughly half its wavelength, i.e. 200 nm. Although the SEM can provide a higher resolution than the optical microscope and in some cases resolution comparable with the AFM by using high energy electrons, the disadvantage of SEM over AFM is that the SEM has to be used mainly under vacuum and at low temperature, limiting the range of applications over the AFM. The AFM can explore samples under a variety of environmental conditions. For example imaging biomolecules under aqueous conditions was not achievable until the introduction of the AFM and especially its fluid cell\textsuperscript{17,18}.

### 2.2 Contact Mode AFM

The most obvious demonstration of the AFM operation is when the AFM is running the so-called contact mode. During this mode the tip is brought into contact with the sample, driven in the $z$-direction by the piezo, causing the cantilever to be deflected. After the initial contact of the tip with the surface, the deflection level of the cantilever can be pre-defined by the user and this is known as the operating set-
point of the instrument. From the deflection of the cantilever, \( \Delta \), the force felt by the tip can be determined from Hooke's law:

\[
F = k_c \Delta
\]

(2.1)

where \( k_c \) is the spring constant of the cantilever. This situation corresponds, at the molecular scale, to repulsive Coulomb forces between ion cores. These forces vary rapidly with the position of the outermost atom, which is a true near-field interaction.

During scanning the feedback or control loops try to maintain the set-point deflection of the cantilever constant. When the tip encounters an object the cantilever begins to bend and the feedback loop adjusts the z-channel of the piezo to move the tip (or the sample for some AFMs) and return the cantilever deflection to each set-point. Plotting the z correction signal from the feedback loops against the x and y move of the piezo the AFM image is generated.

### 2.3 Tapping Mode AFM

The major problem in operating the AFM in contact mode is that this mode can induce mechanical deformation of the imaged surface due to the tip-sample forces, especially due to the presence of lateral/friction forces. The need to avoid surface damage, which is even more pronounced in soft materials, motivated the development of the tapping mode operation of AFM. During this mode the cantilever vibrates at a frequency close to its resonance and is moving towards the sample to a distance smaller than its oscillation amplitude. Consequently, the tip “touches” the sample on each cycle. In this case, the feedback loop tries to keep the vibrating cantilever at constant amplitude during scanning. The user can define the cantilever amplitude during scanning and in this case it is called amplitude set-point (\( A_{sp} \)). Variation of the tip-sample force can be achieved in this mode by changing the driving amplitude, \( A_0 \), and the \( A_{sp} \). When the tip passes over an object the cantilever has less space to oscillate and the amplitude decreases. In analogy when the cantilever passes over a depression the cantilever due to the more available space oscillates with higher amplitude. In both cases the feedback loop adjusts the z-position of the piezo in order to maintain the cantilever amplitude at the pre-defined set-point. The effective forces experienced by the sample during this mode are smaller than the contact mode\(^{19} \). For example, the comparison of contact and tapping mode images of
3 nm wide nanofibrils, in a stretched PE tape, showed that the nanofibrils are less damaged during tapping mode\textsuperscript{20}.

\textbf{2.4 Phase Imaging in Tapping Mode AFM}

Phase imaging is a powerful extension of tapping mode AFM that can provide useful information by recording the phase delay of the measured signal and can be performed at the same time as the topographic imaging in tapping mode. Phase imaging takes advantage of the fact that the tip-sample interactions do not only depend on the sample topography but also on different sample characteristics such as hardness, elasticity, adhesion, friction, viscoelasticity and other properties of the sample. Hence, phase imaging can go beyond simple topographical imaging to detect variations in the composition. Information is obtained from the phase shift between excitation and oscillation of the cantilever due to different materials on the sample. Contrast on a phase image has been interpreted as variations in stiffness or adhesive properties\textsuperscript{21}. It is generally accepted\textsuperscript{22,23,24} that in phase AFM image the brighter areas usually correspond to hard materials and dark to soft materials (elastic and adhesive), where the repulsive force dominates in the tip-sample interaction. However exceptions have been reported where the bright areas correspond to soft materials and the dark to harder materials\textsuperscript{22,25}. In addition contrast reversion in phase images have been reported to take place\textsuperscript{26-29} because of the dependence of phase shift to the set point amplitude ratio $r_{sp}$ ($r_{sp}=A_{sp}/A_0$). To find which region, the dark or the bright, is rigid force-versus-distance curves, also known as force curves, can be performed in the different regions\textsuperscript{30}. Concluding, without additional experiments only qualitatively conclusions can be obtained from the phase images, such as that different colour regions correspond to different materials. Also, researchers seem to agree on the fact that apart from the extra information, phase images provide a better contrast than topographic images\textsuperscript{21}.

\textbf{2.5 Force Spectroscopy}

The AFM, as already has been mentioned, has been proven to be a powerful tool for imaging surfaces, measuring surface topography on a scale from Ångstroms to microns. However the instrument can go beyond topographical information and can
be used to record the interactions between the tip and the sample. These measurements are usually known as “force measurements” and forces in the nano-Newton scale can been routinely recorded. In a force measurement the tip attached to the cantilever spring is moved towards and away from the sample in the normal direction. The vertical position of the tip and deflection of the cantilever are recorded and converted to force-vs-distance curves, briefly called “force curves” or “f-d curves”.

Figure 2.2 shows a typical force-distance curve. A complete curve consists of the approach (red line) and the retrace (blue line) part. The cantilever approaches the surface from a distance from the surface that can be considered as relatively large so no interactions between the tip and the surface occur and the deflection of the cantilever does not change (region A in Figure 2.2). As the separation between the tip and the sample decreases and due to the fact that long and short range interaction forces become effective, the cantilever starts to bend. When the gradient of the interaction force exceeds the stiffness of the cantilever, a “jump” into contact may occur (region B in Figure 2.2). After the contact of the tip with the surface the continuation of the piezo movement results in the cantilever to bend away from the surface, changing its deflection and giving rise to the straight line of the curve. Depending on the relative stiffness of the cantilever with respect to the sample during this portion of the curve the tip can indent or compress the surface. After the maximum approach, the cantilever then is withdrawn (region D) and a hysteresis may appear due to the variety of attractive tip-sample interactions (position E), such as adhesive forces. As the withdrawal continues the cantilever detaches from the sample and returns to its resting position (region F).
The direct results of an AFM force measurement is a measure of the photodetector current, $I_p$, versus height position of the piezo, $Z_p$. To obtain a force-vs-distance curve the $I_p$ and $Z_p$ have to be converted into force and distance\textsuperscript{31, 32}. For these conversions two parameters have to be determined; the photodetector sensitivity, $s$, and the contact point, i.e. the zero distance or else zero separation. Both of these parameters are inferred from the force curve itself. To avoid false interpretations of the data the above two parameters have to be derived from a force curve on a hard surface where no penetration or compression occur after contact. In this case, the slope of the linear part in the contact area on a hard surface, region c in Figure 2.2, determines the photodetector sensitivity (i.e. $s$ is slope of the linear part), and the point of contact is the beginning of this linear part, i.e. point B in Figure 2.2. The cantilever deflection, $\Delta$, then is calculated as:

$$\Delta = \frac{I_p}{s} \quad (2.2)$$

and the applied force of the cantilever on the sample can be estimated from Hooke’s law according to Equation 2.1.

The piezo height position can be converted into separation, $D$, by subtracting the cantilever deflection, $\delta_c$, on a hard surface due to the applied force from the movement of the piezo after the contact point:

$$D = Z_p - \delta_c \quad (2.4)$$
Figure 2.3 demonstrates how the correction, described above, modifies the force-vs-piezo position curve (or force-vs-distance curve) to the “true” force-vs-separation. In Figure 2.3a two characteristic force-vs-piezo position curves are shown. The red one corresponds to a curve on a hard surface and the blue curve to a compliant surface. The (0,0) on the axis corresponds to the contact point of the tip with the surface. By subtracting the deflection of the cantilever on a hard surface after the contact, the force-vs-separation curves can be obtained in Figure 2.3b. In this case, it is clear that after the above correction, and after the initial contact of the tip with the hard surface, no penetration or compression occurs and the curve is vertical after the contact. On the other hand the blue line, which corresponds to the compliant surface, appears with a finite slope indicating that a real compression or indentation occurred.

Figure 2.3: a) Two typical force-vs-piezo position curves on an infinitely stiff surface (red line) and on a compliant material (blue line) and b) the corresponding converted force-vs-separation curves obtained by subtracting the deflection of the cantilever during contact on a hard surface.

2.6 Artefacts of AFM and Convolution Effect

AFM images are always a convolution of the geometry of the tip and the shape of the object or sequence of objects being imaged. A typical AFM tip when viewed at the µm scale appears to be pyramidal (Figure 2.4, left) while at the nm level the tip is spherically caped (Figure 2.4, right). Hence the way that the AFM tip convolutes the
image depends on the scale of the object-features being imaged. Detailed ways to deconvolute the AFM images obtained in this thesis and estimate the real dimensions of the objects imaged will be presented for each case separately in the following chapters.

Figure 2.4: Scanning electron microscopy (SEM) images of a chip: (left) the cantilevers and (right) the tip apex.


2.7 References

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Part A

Polymers at Surfaces
Chapter 3

Adsorption and Clustering after Solvent Evaporation of Linear Polymers on Surfaces
3.1 Introduction

We investigated the morphology of physisorbed linear polybutadiene (PB) polymers on freshly cleaved mica after solvent evaporation by atomic force microscopy (AFM). We found that the (sub-)monolayer morphology depends strongly on the molecular weight, $M_w$, of the linear polymer and is influenced significantly by the conformations/positions of the adsorbed polymer chains when in good solvent conditions. The dependence of the observed polymer structures on $M_w$ is significant for samples with relatively high surface density where the interactions among the adsorbed polymers become significant. For relatively high grafting densities, the adsorbed polymers tended to aggregate and an isotropic structural pattern was observed. We explain these structural phenomena in terms of the molecular interactions of the adsorbed polymers when in good solvent conditions and after the abrupt solvent evaporation.

We have to note that the scope of this study was not to study the adsorption kinetics, but to investigate structural aspects of the organisation of PB on mica when the solvent conditions change from good to bad. Other techniques have been proven to be more powerful for adsorption kinetics studies: ellipsometry\textsuperscript{1-3}, attenuated total reflection (ATR)\textsuperscript{4,5}, attenuated total reflectance Fourier transform infrared (ATR-FTIR)\textsuperscript{6}, two dimensional Fourier transform infrared (2D-FTIR)\textsuperscript{7}, quartz crystal microbalance technique (QCM)\textsuperscript{8-10}, surface plasma spectroscopy (SPS)\textsuperscript{11}, second harmonic generation (SHG)\textsuperscript{12}. Additionally it has been recently reported that AFM is not suitable for in-situ studies of adsorption due to the fact that the tip is near the surface and can affect the adsorption process; e.g. polymers can adsorb on the tip and be transferred onto the surface with the tip-surface contact\textsuperscript{13}.

In this chapter, we report on two aspects: 1) the structure of the polymer (sub-)monolayer in air after it has been adsorbed onto mica from very dilute solutions for different surface densities; 2) The effect of the surface density and the molecular weight on the formation of aggregates.

3.2 Background

In this section we will review some general aspects regarding the adsorption of linear polymers on surfaces that will help in the interpretation and understanding of
our results. This section is divided in four parts: in the first one we discuss the case of a single chain adsorbed on a surface; in the second one we concentrate on the effect of the $M_w$ on the conformation of a single adsorbed chain; in the third part, we move to the problem of many chains adsorbed on a surface; and at the end in the fourth part we do simple theoretical calculations to demonstrate the importance of the polydispersity index of polymers on the size variation of the chains.

### 3.2.1 Adsorption of single chain

A chain in a dilute solution near a weakly adsorbing surface would like to increase the number of monomers in contact with the surface in order to gain adsorption energy. Nevertheless, this will lead to loss of entropy due to confinement. Therefore, the adsorption behaviour of an individual chain is the result of a competition between the energy gain from binding monomers to the substrate and the entropic repulsion due to lose of conformational energy. An adsorbed chain gains energy usually much less than $kT$ from each monomer in contact with the surface but the whole chain, due to the many monomer contacts, can gain energy much higher than the lost conformational energy (which is of the order of few $kT$). Hence, the chain has a tendency of maximizing the number of contacts with the substrate$^{14}$.

An homopolymer chain physisorbed on a substrate can consist of: *trains*, where all the segments are in contact with the substrate; *loops*, where these parts of the chain have no contact with the surface and connect two trains; and *tails* which are the non-adsorbed ends of the chain, Figure 3.1$^{15}$.

![Figure 3.1: Illustration of an adsorbed single chain in good solvent conditions. Tail, loop and train are indicated.](image-url)
3.2.2 Effect of molecular weight on the conformation of a single adsorbed chain

A blob is defined as a length scale of the chain in which the cumulative interaction energy is of the order of $kT$ (thermal energy). Using scaling arguments the blob size of an ideal chain adsorbed from a dilute solution can be derived. As we have already mentioned the chain would like to increase the contact points with the surface to gain adsorption energy but this competes with the loss of conformational energy. If we assume a chain adsorbed on a surface, the thickness of the adsorbed chain determines the blob size. The energy gain of the chain for an adsorbed monomer with the surface is $-\delta kT$ (for the case of weak adsorption is $0 < \delta << 1$). The adsorption blob size is determined as\(^{14}\):

$$b \approx \frac{v}{(1-v)} \left( \frac{3}{5} \right)$$

In good solvent conditions $v = 3/5$. Therefore, for the case of a single isolated chain the blob size is independent of the number of monomers and consequently is also independent of the molecular weight of the polymer chain.

3.2.3 Adsorption of many chains from a dilute solution

When many chains are present in the solution, the adsorption process and eventual structure for each polymer chain is influenced by the interactions (repulsion in a good solvent) with the surrounding already adsorbed chains. It has been shown that the length of the tails and loops depends both on the number of polymers which have been adsorbed on the surface and on the length (related directly to $M_w$) of the chain\(^{15}\). Scheutjens-Fleer theory predicts that for very low surface coverage (small number of chains on the surface) the chains have the tendency to "flatten" on the surface in order to lower the energy of the system, i.e. the chains appear with small loops and tails. By increasing the surface coverage the chains appear with longer loops and tails. For the case of high adsorption the tails are longer than the loops, which have been verified by a very long decay of the segment density profiles beyond the loop layer\(^{16}\). Semenov and Joanny\(^{17}\) have also shown by using scaling arguments that the outer structure of adsorbed polymer layers is dominated primarily by the tails of the adsorbed polymer chains rather than the loops. The average number of segments in the tail (and hence the tail length), increases linearly with the chain $M_w$.  

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Figure 3.2: Illustration of adsorbed chains in good solvent conditions. From top to bottom, the surface polymer density increases.

The adsorbed chains structure at different densities is illustrated schematically in Figure 3.2. Note the increased thickness as the density increases.

3.2.4 Effect of $M_w$ distribution of nominally monodisperse polymers on the collapsed chain volume

The polydispersity effect on the adsorption has been already reviewed\(^\text{15}\). For the case of dilute solutions the main conclusion is that at equilibrium (long incubation time and at the highest adsorbed amount) high molecular weight polymers adsorb preferentially over lower molecular weight ones. This is driven by entropic reasons. The entropy of mixing in the solution decreases strongly with increasing chain length and this drives the adsorption of longer molecules on the surface.

Generally little attention has been paid to the effect of the distribution of molecular weights for polymer samples which are considered monodisperse. Using simple arguments we will prove that even for monodisperse polymers (i.e. $M_w/M_n \approx 1$) the distribution of the molecular weight cannot be fully ignored. For given $M_w$ and $M_n$ the standard deviation of a distribution of molecular weight can be determined\(^\text{18}\):

\[
\sigma = M_n \left( \frac{M_w}{M_n} - 1 \right)^{1/2}
\]  

(3.2)
For a normal distribution the 68.3% of the $M_w$ values are within one standard deviation of the mean. Using the data of Table 3.1 the standard deviation for each molecular weight can be determined. For PB with $M_w = 38.6$ kg/mol we find $\sigma = 6.7$ kg/mol, for $M_w = 78.8$ kg/mol, $\sigma = 17.6$ kg/mol, and for $M_w = 962$ kg/mol, $\sigma = 304$ kg/mol.

For $M_w = 78.8$ kg/mol, the smallest chain within one standard deviation can have a molecular weight of 61.2 kg/mol which corresponds to a molecule of mass $101.66 \times 10^{-21}$ g and by using the bulk density of PB we find a molecular volume of 123.07 nm$^3$. The biggest molecular weight within one standard deviation is 96.6 kg/mol or molecular mass of $160.46 \times 10^{-21}$ g which corresponds to a volume of 194.26 nm$^3$. The volume of the chain for molecular weight 78800 g/mol is 158.47 nm$^3$ (Table 3.1). So using only the range of one standard deviation we can see that a chain can vary from 123.07 nm$^3$ up to 194.26 nm$^3$, which is a difference of about 58%. Within two standard deviations (97.7% of all particles) the volume difference between the smaller to the bigger chain is about 246%. This leads us to the conclusion that even for relatively monodisperse polymers the distribution of molecular weights can not be fully neglected when individual chains/globules are considered.

In Figure 3.3 the distribution of the volume is plotted. This was obtained by assuming a normal distribution for the volume with an average volume $\mu = 158.47$ nm$^3$ and standard deviation, $\sigma = 33.60$ nm$^3$, i.e. half of the difference of the minimum value ($123.07$ nm$^3$) from the maximum value ($194.26$ nm$^3$).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure33.png}
\caption{Volume distribution for PB with molecular weight $M_w = 78.8$ kg/mol and $M_w/M_r=1.05$. The hatched area corresponds to: (left) one standard deviation from the mean and (right) two standard deviations from the mean.}
\end{figure}
3.3 Experimental Section

3.3.1 Materials

We used Polybutadiene of different molecular weights, Table 3.1, dissolved in an appropriate volume of toluene. Toluene was used as received (Fisher Scientific). The concentrations of the solutions \( c \) were kept below the critical overlap concentration \( c^* \). Mica (Agar Scientific, Essex, UK) was cleaved before used.

<table>
<thead>
<tr>
<th>Linear PB molecular characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>( M_w ) (kg/mol)</td>
</tr>
<tr>
<td>38.6</td>
</tr>
<tr>
<td>78.8</td>
</tr>
<tr>
<td>962</td>
</tr>
</tbody>
</table>

\(^a\)Radius of gyration in dilute good solvent conditions from light scattering measurements\(^1\)
\(^b\)Calculated volume of a collapsed molecule (based on the molecular weight and bulk density of PB\(^4\))

3.3.2 Sample preparation

All the samples were prepared by exposing freshly cleaved mica to a toluene solution (good solvent for PB) of a desired concentration of the linear PB with \( c < c^* \). A range of incubation times was used. The mica surface was removed from the solution and placed in 100 ml toluene bath for 24 h and then rinsed exhaustively with 100 ml toluene. The samples were dried gently under a stream of nitrogen. Subsequently, the samples were imaged in air by using atomic force microscopy (AFM) in tapping mode.

3.3.3 Atomic Force Microscopy (AFM)

All experiments were carried out using a PicoSPM (Molecular Imaging, USA) operating in tapping mode (intermittent contact mode). In this mode the cantilevers are made to oscillate vertically at their natural resonance frequency, and move in a raster fashion within a specified region of interest. Commercially available \( \text{Si}_3\text{N}_4 \)

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Linear Polymers on Surfaces

(MikroMasch, Tallinn, Estonia), rectangular cantilevers, with a spring constant of 1.75 N/m and resonance frequency of 130-160 kHz were used. We deduced the tip radii by SEM images and values of 50 nm were measured. Each sample was imaged at several different areas and similar structures were obtained demonstrating the reproducibility of the sample preparation protocol. All the images are shown without any image processing except horizontal levelling using the free software WSxM (http://www.nanotec.es). Particle sizes in the AFM images were obtained using the grain analysis module of the commercially available SPIP software (http://www.imagemet.com). The grain analysis module was applied in at least four different areas of the sample. Care has been taken on the detected particles to be in flat areas of the mica and not near steps and edges. In order to ensure accurate measurement of the dimensions in the interrogated 3D space a calibration of the piezo that controls the movement of the cantilever was performed by imaging a calibration grid with known dimensions.

3.3.4 Convolution effect

It is well known that the AFM tip can make an object lying on a surface look wider due to the convolution of the geometry of the tip and the shape of the object being imaged. For example this effect has been reported for DNA. Simple numerical procedures for subtracting the tip influence from objects with simple shapes such as paired helical filaments (PHF) (spherical shape) air-dried on mica, or for spherical-cap globules of thiol-terminated polystyrene (PS-SH) on gold surface, have been reported. The calculations used the measured height and width of the objects. In our case the polymeric islands were considered as spherical caps and we calculated their volumes and heights using grain analysis module of the commercial software Scanning Probe Image Processor (SPIP, Image Metrology). The influence of the tip shape was subtracted using simple geometric arguments, and the true volume \( V_t \) was estimated in terms of the apparent height \( h \), apparent volume \( V_a \) and the radius of the tip \( R_t \):

\[
V_t = \frac{1}{3} \pi h^2 \left( \frac{2V_a}{\pi h^3} + \frac{h^2}{3} \right) - \frac{h}{2} - R_t \]

(3.3)
All the tips used for the experiments were imaged with scanning electron microscopy (SEM) and the measured tip radii were in the range of about 50 nm as it is shown in the SEM image in Figure 3.4 for one of the tips used in this study.

Figure 3.4: SEM images of a chip. In the first (left) the cantilevers are shown and in the second one (right) the tip apex.

Assuming the shape of spherical caps for the interrogated particles, simple numerical calculations were performed using equation 3 to check the effect of the tip on how the spherical caps would appear on an AFM image, i.e. the convoluted volume was calculated. In our calculations the real volume, \( V_r \), of the spherical cap was kept constant while the height took values from 0.3 nm (flat conformation) to 10 nm (vertical conformation) and the convoluted volume, \( V_a \), was calculated trying to see when the effect of the tip is more important with respect to spherical caps of higher or lower aspect ratios. The radius of the tip was considered to be 50 nm. For our calculations we used three different spherical caps of (real) volumes similar to the theoretical volumes of the molecules used in this study, Table 3.1.

Figure 3.5: The ratio of the real and the convoluted (measured) volume of a globule against its height.
From Figure 3.5, it is obvious that the effect of the tip is more important when the globule is higher, and hence when it takes a vertical conformation, i.e. for high aspect ratio. The shorter the particle (and hence wider) the less is affected from the convolution due to the tip. Nevertheless, it has to be noted that this type of simple deconvolution is very approximate and as has already been reported even the deconvoluted volumes can be 100% or even 200% bigger than the real volume of the object imaged\textsuperscript{22}. Thus precise quantitative measurements of the chain volumes are difficult to obtain.

Nevertheless, these calculations can give the order of magnitude of the real volumes and will help to check if the observed structures represent single collapsed molecules or not by comparing the calculated volumes with the observed convoluted volumes.

3.4 Results

The linear PB monolayers were formed by immersing the mica substrate into the toluene solution with a procedure explained in the experimental section and the characterization was done by employing tapping mode AFM in air. Several samples using different adsorption/incubation times were prepared for each different polymer. Several $3 \times 3 \ \mu m^2$ images of each sample were collected.

Figure 3.6a, b and c show some typical AFM height images of linear PB with $M_w = 38.6 \ \text{kg/mol}$ adsorbed on mica for three different adsorbed amounts (seen at the top of each image) with the corresponding phase images Figure 3.6d, e and f (below each height image). In all images, we observed the formation of polymeric islands. The contrast in the corresponding phase images for all the cases indicates difference in materials ("hard" mica against "soft" PB). Analysing at least four images for each adsorbed amount we obtained the corresponding height histograms of the polymeric islands which are shown in Figure 3.7.
<table>
<thead>
<tr>
<th>$\Gamma = 2.61 \times 10^{-3} \text{mg/m}^2$</th>
<th>$\Gamma = 8.84 \times 10^{-3} \text{mg/m}^2$</th>
<th>$\Gamma = 1.85 \times 10^{-2} \text{mg/m}^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image (a)" /></td>
<td><img src="image2.png" alt="Image (b)" /></td>
<td><img src="image3.png" alt="Image (c)" /></td>
</tr>
<tr>
<td><img src="image4.png" alt="Image (d)" /></td>
<td><img src="image5.png" alt="Image (e)" /></td>
<td><img src="image6.png" alt="Image (f)" /></td>
</tr>
</tbody>
</table>

Figure 3.6: Tapping mode AFM images, 3x3 µm², image of a freshly cleaved mica and immediately exposed in linear PB solution with $M_w = 38.6$ kg/mol. Top: topography, bottom: phase. a) and d) correspond to $\Gamma = 2.61 \times 10^{-3} \text{mg/m}^2$; b) and e) correspond to $\Gamma = 8.84 \times 10^{-3} \text{mg/m}^2$ and c) and f) correspond to $\Gamma = 1.85 \times 10^{-2} \text{mg/m}^2$.

Figure 3.6a corresponds to low incubation time and hence low adsorbed amount ($\Gamma = 2.61 \times 10^{-3} \text{mg/m}^2$), the polymeric islands appear flat (short) and circular. The chains took the globular conformation since the imaging took place in air (i.e. bad solvent conditions result in collapsed chains). The height distribution of the islands (Figure 3.7a) is nearly symmetric with a small tail at larger heights and a clear peak at about 1.0 nm. As the adsorbed amount increased, Figure 3.6b ($\Gamma = 8.84 \times 10^{-3} \text{mg/m}^2$), the number of the polymeric islands increased and some larger ones started to form. The height distribution retains a nearly symmetric part with a peak at approx. 1.3 nm but some higher islands (3-4.5 nm) started to appear, corresponding to the large islands clearly seen in the corresponding AFM image, Figure 3.7b. For even higher adsorbed amounts, Figure 3.6c ($\Gamma = 1.85 \times 10^{-2} \text{mg/m}^2$), the number of islands increased further and some bigger particles started to appear. The height distribution of the islands (Figure 3.7c) has again a nearly symmetric part with a peak at approx. 1.7 nm and a “tail” of islands with heights of 3.8 - 5.1 nm.
Figure 3.7: Total distribution of islands height for $M_w$ of 38.6 k, from a) to c) correspond to $\Gamma = 2.61 \times 10^{-3}$ mg/m$^2$, $\Gamma = 8.84 \times 10^{-3}$ mg/m$^2$ and $\Gamma = 1.85 \times 10^{-2}$ mg/m$^2$, respectively.

In Figure 3.8a, b and c we present some typical AFM images of the linear PB with $M_w = 78.8$ kg/mol adsorbed on mica for three different incubation times resulting in different adsorbed amounts. For short incubation time, and hence small adsorbed amount $\Gamma = 3.76 \times 10^{-3}$ mg/m$^2$ the islands appeared again, as in the case of linear PB with $M_w = 38.6$ kg/mol, flat on the surface (short) and circular. By increasing the adsorbed amount, Figure 3.8b and $\Gamma = 7.19 \times 10^{-2}$ mg/m$^2$, the number of the islands increased and some bigger islands started to appear. For even higher adsorbed amount (Figure 3.8c and $\Gamma = 3.71 \times 10^{-1}$ mg/m$^2$) even larger islands formed. In all cases, the shape of the polymeric islands remained circular.
Analyzing at least four AFM images for each adsorbed amount, for the case of the linear PB with $M_w = 78.8$ kg/mol, we measured the corresponding island heights and volumes. The real volume, or deconvoluted volume, was estimated by applying equation 3.1, as it has been described in the experimental section. For each adsorbed amount, the corresponding height histograms are presented in Figure 3.9 while the island height against the deconvoluted volume plots are presented in Figure 3.10, and the 2D fast Fourier transform (FFT) of the AFM images can be seen in Figure 3.11. In the height against deconvoluted volume plots each point was obtained by averaging the height of particles corresponding to similar deconvoluted volumes.
For $\Gamma = 3.76 \times 10^3 \text{ mg/m}^2$ the height histogram has a peak at around 1.2 nm with a small secondary peak at 3.8 nm, Figure 3.9a. The polymeric islands with height up to 3.5 nm in the distribution correspond to single collapsed polymer chains as the average deconvoluted volume, 274 nm$^3$, of these islands is similar to the theoretical one, Table 3.1. The islands of height around 3.8 nm (corresponding to the secondary peak) occupy volumes of about 400 nm$^3$ and thus can be considered as aggregates of around 2 polymeric chains. By increasing the adsorbed amount, $\Gamma = 7.19 \times 10^2 \text{ mg/m}^2$, the height distribution of the polymeric islands obtained a bimodal character, with a first peak at 1.8 nm and a second at 5 nm.

Figure 3.10 shows that the first part of the bimodal distribution corresponds to single collapsed polymer chains while islands with height higher than 3.5 nm, i.e. the second part of the bimodal distribution, correspond to aggregates of several polymeric chains. For higher adsorbed amount, $\Gamma = 3.71 \times 10^1 \text{ mg/m}^2$, the height distribution of the polymeric islands maintained a clear bimodal character with a first peak at 2.2 nm.
and a second peak at 7 nm. Again from Figure 3.10, it becomes clear that the first part of the distribution corresponds to single collapsed chains while the second part corresponds to aggregates of several polymeric chains.

![Figure 3.10](image)

**Figure 3.10:** Height against deconvoluted volume of the polymeric islands for the case of PB with $M_w = 78.8$ kg/mol and for different adsorbed amounts

In Figure 3.11, the radial average distributions of the 2D FFT were considered for all the different patterns obtained by different adsorbed amounts (and hence different incubation times) for the case of linear PB with $M_w = 78.8$ kg/mol. In general, if the shape of an FFT is centrosymmetric, this implies an isotropic distribution of the pattern. The radial average distribution of a centrosymmetric shape FFT shows a maximum at a specified wavevector $q$ ($q = 2\pi/\lambda$) which corresponds to a characteristic length scale which is the preferred/characteristic distance of the undulation/particles.

For the case of $M_w = 78.8$ kg/mol and $\Gamma = 3.76 \times 10^{-3}$ mg/m$^2$, the 2D FFT is not centrosymmetric and hence without a clear maximum in the radial average intensity implying an anisotropic distribution of the particles (collapsed polymers), Figure 3.11a. For higher adsorbed amount, $\Gamma = 7.19 \times 10^{-2}$ mg/m$^2$, the 2D FFT shows a centrosymmetric shape and a clear maximum can be seen in the radial average intensity at a specified wavevector $q$ which corresponds to a characteristic distance of 221 nm, Figure 3.11b. Similar behaviour is observed for the case of $\Gamma = 3.71 \times 10^{-1}$ mg/m$^2$, with a characteristic distance of 372 nm, Figure 3.11c.
Figure 3.11: Relative average intensity as a function of the wave number \((q = 2\pi/h)\). These plots were obtained by the radial average of the 2D FFT image (inset); for a) \(\Gamma = 3.76 \times 10^{-3} \, \text{mg/m}^2\), b) \(\Gamma = 7.19 \times 10^{-2} \, \text{mg/m}^2\) and c) \(\Gamma = 3.71 \times 10^{-1} \, \text{mg/m}^2\). The characteristic distance from a) to c) is: 928 nm, 221 nm and 372 nm, respectively.

In Figure 3.12 we present some typical AFM images of linear PB with \(M_w = 962 \, \text{kg/mol}\) adsorbed on mica for three different adsorbed amounts. For a low adsorbed amount, \(\Gamma = 1.32 \times 10^{-2} \, \text{mg/m}^2\), the polymeric islands appeared flat on the surface (short) and circular. For higher adsorbed amounts, \(\Gamma = 3.88 \times 10^{-2} \, \text{mg/m}^2\), the number of the islands increased. By increasing the adsorbed amount even more, \(\Gamma = 6.03 \times 10^{-1} \, \text{mg/m}^2\), big circular polymeric islands appeared. In all cases, the shape of the polymeric islands remained circular.
Analyzing several AFM images for each absorbed amount, for the case of linear PB with $M_w = 962$ kg/mol, the corresponding islands heights and volumes were measured. Figure 3.13 shows the corresponding height histograms for each adsorbed amount. The island height against the deconvoluted volume plots for each adsorbed amount are presented in Figure 3.14 while the 2D-FFT graphs of the AFM images can been seen in Figure 3.15.
For a low adsorbed amount, $\Gamma = 1.32 \times 10^{-2}$ mg/m$^2$, the height distribution of the polymeric islands appear nearly symmetric (with a small tail at larger heights) with a peak at approx. 1.8 nm, Figure 3.13a. The polymeric islands in this case correspond to single collapsed chains as the average deconvoluted volume, about 3000 nm$^3$, of these islands is similar to the theoretical one, Table 3.1. For a higher adsorbed amount, $\Gamma = 3.88 \times 10^{-2}$ mg/m$^2$, the height distribution of the polymeric islands remains nearly symmetric but the peak shifted to around 5.2 nm, Figure 3.13b. The polymeric islands correspond again to single collapsed polymer chains as their average deconvoluted volume is around 3000 nm$^3$. For even higher adsorbed amount, $\Gamma = 6.03 \times 10^{-1}$ mg/m$^2$, the height distribution of the polymeric islands retained its nearly symmetric shape. This time the distribution peak increased further to about 12 nm, Figure 3.13c. In this case the polymeric islands correspond to aggregates of several polymeric chains as the deconvoluted volume of the polymeric islands is much larger than the theoretical volume of the single collapsed chain.
Figure 3.14: Deconvoluted volume against height of the polymeric islands for the case of PB with $M_w = 962$ kg/mol and for different adsorbed amounts.

Figure 3.15 shows the radial average intensity of the 2D-FFT graph for each adsorbed amount for the case of linear PB with $M_w = 962$ kg/mol. For $\Gamma = 1.32 \times 10^{-2}$ mg/m$^2$, the 2D-FFT is fairly centrosymmetric with a maximum just emerging at a low wavevector in the radial average intensity, Figure 3.15a. For higher adsorbed amount, $\Gamma = 3.88 \times 10^{-2}$ mg/m$^2$, the 2D-FFT shows again a fairly centrosymmetric shape and a maximum can be seen in the radial average intensity at a specified wavevector $q$ which corresponded to a characteristic distance of 300 nm, Figure 3.11b. A clear centrosymmetric shape with a clear maximum in the corresponding radial average intensity can be observed for the case of $\Gamma = 6.03 \times 10^{-1}$ mg/m$^2$; the characteristic distance is at 428 nm, Figure 3.11c.
Figure 3.15: Relative average intensity as a function of the wave number \( q = 2\pi/\lambda \) for a) \( l = \exp(1.32 \times 10^2 \text{ mg/m}^2) \) b) \( l = \exp(3.88 \times 10^2 \text{ mg/m}^2) \) and c) \( l = \exp(6.03 \times 10^4 \text{ mg/m}^2) \). The characteristic distance from a) to c) is: 600 nm, 300 nm and 428 nm, respectively.

3.5 Discussion

3.5.1 Medium/long chain order by increasing adsorbed amounts

As we have seen in Figure 3.11 and Figure 3.15, for the cases of linear PB with \( M_w = 78.8 \text{ kg/mol} \) and \( 962 \text{ kg/mol} \) respectively, for high adsorbed amounts the polymers acquired isotropic distributions revealing some medium/long-range ordering behaviour. It is highly unlikely that this effect is connected to solvent instabilities due to its rapid evaporation (last step before imaging in dry conditions). It has been shown in other studies\(^{23-25}\) that the rapid solvent evaporation in various polymers-on-surfaces systems produces “frozen” conformations corresponding to the molecular structure just before the rapid evaporation. Furthermore, we have also observed similar isotropic distributions for star-shaped PB molecules adsorbed on mica using the same sample preparation protocol (results presented in the chapter 4). These results indicates that in our system it is more likely that during adsorption and more essentially during the immersion of the sample in a pure toluene bath for 24 hours, the
polymers can reconfigure their position/conformation and expand on the surface obtaining equilibrated structures. In others words, our results suggest that the adsorbed polymers show to some extent a mobility on the surface. Although at the whole polymer chain scale the molecules were expected to be relatively strongly adsorbed on the surface through the many monomer-surface contacts, at the monomer scale the adsorption was relatively weak and dynamic monomer adsorption-desorption effects can easily occur driven by thermal fluctuations and affected by external forces (repulsive in the case of good solvent conditions and attractive in the case of bad solvent conditions). Thus, we believe that our morphologies observed in the air (bad solvent) are highly affected by the possible equilibrated adsorbed polymer structure in good solvent conditions and are related to conformations just before the abrupt evaporation of the solvent. During good solvent conditions, the polymer chains repel each other and at increased surface densities they acquire a two-dimensional liquid-like order within the plane of mica. When the solvent conditions change, they collapse either individually or within clusters depending on the extent of overlaps and entanglements. The distribution of the collapsed structures (islands) reflects the liquid-like order when in good solvent conditions.

3.5.2 Growth of Linear PB monolayers on Mica

We now discuss the growth of linear PB (sub-)monolayers on mica with respect to the shape, height and volume evolution of the observed polymeric islands. We will divide the discussion in two parts: (i) single collapsed chains; in this section, we will discuss how the chain molecular weight and the adsorbed amount affects the conformation and size of the observed single-chains when the solvent conditions change from good to bad. (ii) aggregation; in this section we will discuss the tendency of linear polymers to form aggregates in relatively high surface densities and the factors affecting the shape and sizes of the aggregates when the solvent conditions change from good to bad.
i) Single collapsed chains

At first we concentrate on the lowest adsorbed amount observed in our experiments for each case; \( I = 2.61 \times 10^3 \) mg/m\(^2\), \( J = 3.76 \times 10^3 \) mg/m\(^2\), \( K = 1.32 \times 10^2 \) mg/m\(^2\) for linear PB with \( M_w \) of 38.6, 78.8, 962 kg/mol, respectively.

The vast majority of polymer islands correspond to single collapsed linear PB chains for all the cases. The existence of a minority number of small aggregates cannot be excluded. This is certain in the case of \( M_w = 78.8 \) kg/mol, where for the lowest observed amount the distribution certainly included some aggregates (as noted in the result section). However, in this section we will focus on structural properties of the single collapsed chains. For all the cases, the polymeric islands appeared circular in the AFM images since the samples were imaged in air (bad solvent for PB) and hence, the adsorbed polymer chains took globular conformations to minimize their surface energies and avoid many contacts with the unfavourable air.

The structure of adsorbed polymers on surfaces in good solvent conditions depends strongly on the surface coverage due to the repulsive excluded volume interactions with the surrounding adsorbed chains (as has been explained in the background section). In the case of the linear PB with \( M_w \) of 38.6, 78.8 and 962 kg/mol and for the lowest adsorbed amount presented here the number of the adsorbed chains is similar, \( 16 \pm 3 \) chains/\( \mu \)m\(^2\), \( 15 \pm 2 \) chains/\( \mu \)m\(^2\) and \( 5 \pm 1 \) chains/\( \mu \)m\(^2\), respectively. If we assume that the molecules were homogenously and isotropically distributed on the surface when in good solvent conditions and before the abrupt solvent evaporation the average distance between the centres of the molecules is between 250-300 nm, for linear PB with \( M_w \) 38.6 kg/mol and 78.8 kg/mol and about 450 nm for the molecule with \( M_w = 962 \) kg/mol. In all cases the chain-chain distance is at least one order of magnitude higher than their radius of gyration when in good solvent conditions. Hence, we can assume that interactions among them are weak and the adsorbed chains can be considered as isolated.

As it has been mentioned in the background section, the blob size and consequently the effective height of a single isolated chain in good solvent conditions should be fairly independent of the molecular weight. Thus, in good solvent conditions all the different molecular weight linear PB samples, for the case that they can be considered as single adsorbed chains, are expected to have taken conformations with the same/similar heights. After the abrupt solvent evaporation the molecules appeared collapsed, forming pancakes (Figure 3.6a, Figure 3.8a and Figure
3.12a) with their height distributions showing a peak at 1 nm, 1.2 nm and 1.8 nm for the cases of linear PB with $M_w$ of 38.6 kg/mol, 78.8 kg/mol and 962 k/mol, respectively.

We now define the volume fraction of the non-adsorbed monomers of an adsorbed chain, $\Phi_{na}$, as the ratio of the monomers not in contact with the surface over the total number of monomers of the adsorbed chain. For the case of single/isolated molecules adsorbed on mica in good solvent conditions, $\Phi_{na}$ is expected to be independent of the $M_w$. Furthermore it is to be expected that $\Phi_{na}$ increases with the surface density as the increase of the surface density results in a chain structure with higher loops and tails and thus higher number of non-adsorbed monomers.

![Figure 3.16: Height of the collapsed single chains against their $M_w$ for the case of low adsorbed amounts; the red line depicts the corresponding linear fit.](image)

Figure 3.16 shows the plot of the single collapsed PB chains height, for the lowest observed adsorbed amount, against their molecular weight with the corresponding linear fit. The slope has a value of $8 \times 10^{-4}$ nm·mol/kg indicating that the effect of the molecular weight on the height of the isolated collapsed PB chains on mica is minimal. We note that a more than 10-fold increase of $M_w$ results in a less than double increase in height.

For the two smallest molecular weights and for higher adsorbed amounts larger islands of aggregated chains co-existed with single collapsed chains. This is better illustrated by the case of 78.8 kg/mol where a clearly bimodal height distribution of the polymeric islands was observed. In contrast, for the polymer PB with $M_w = 962$ kg/mol and for $\Gamma = 3.88 \times 10^{-2}$ mg/m^2 only single collapsed chains
were observed while for $\Gamma = 6.03 \times 10^{-1}$ mg/m² the vast majority of the polymeric islands were aggregates of several linear PB molecules.

Table 3.2 shows the average height of single collapsed polymer chains for every polymer used and for every adsorbed amount observed in this study. For each one of the different $M_w$ polymers used the height of the single collapsed chains increases with the increase of the adsorbed amount. Additionally the chains with higher $M_w$ collapsed in higher single chain globules.

<table>
<thead>
<tr>
<th>$\Gamma$ (mg/m²)</th>
<th>Height (nm)</th>
<th>$\Gamma$ (mg/m²)</th>
<th>Height (nm)</th>
<th>$\Gamma$ (mg/m²)</th>
<th>Height (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2.61 \times 10^3$</td>
<td>$1.04 \pm 0.06$</td>
<td>$3.76 \times 10^3$</td>
<td>$1.15 \pm 0.15$</td>
<td>$1.32 \times 10^2$</td>
<td>$1.84 \pm 0.16$</td>
</tr>
<tr>
<td>$8.84 \times 10^3$</td>
<td>$1.28 \pm 0.06$</td>
<td>$7.19 \times 10^2$</td>
<td>$1.84 \pm 0.15$</td>
<td>$3.88 \times 10^2$</td>
<td>$5.15 \pm 0.16$</td>
</tr>
<tr>
<td>$1.85 \times 10^2$</td>
<td>$1.55 \pm 0.06$</td>
<td>$3.71 \times 10^1$</td>
<td>$2.16 \pm 0.15$</td>
<td>$6.03 \times 10^1$</td>
<td>--------</td>
</tr>
</tbody>
</table>

As it has been discussed in the background section in good solvent and by increasing the surface density, the adsorbed chains will appear with longer loops and tails through the increase of the repulsive excluding volume interactions with the surrounding adsorbed chains resulting in a higher $\Phi_{na}$. Furthermore, the length of the loops and tails increases linearly with the $M_w$ resulting again in higher $\Phi_{na}$.

Our results show a strong relation of the collapsed single chain height with the $\Phi_{na}$. When the $\Phi_{na}$ of an adsorbed chain increases, for example due to increasing surface density in good solvent conditions, the height of the corresponding collapsed chain increases. This is to be contrasted with isolated adsorbed chains which were expected to have $\Phi_{na}$ independent of $M_w$ and collapsed in globules of similar height. Consequently, the size of collapsed single chains depend on the conformation of the chain when in good solvent conditions, and hence on the $\Phi_{na}$. After the abrupt solvent evaporation the chains obtain a "frozen" conformation reflecting the conformational properties when in good solvent conditions.
When the solvent conditions changed from good to bad, the chains would collapse trying to minimize their contact with the air. The chain would try to maintain the contacts with the surface to preserve the favourable adsorption energy. The non-adsorbed monomers of the chain would collapse on top of the adsorbed monomer "layer" trying to minimize the contact with the air forming a polymer globule/nanodroplet. Therefore the height of a single collapsed globule should be larger for higher grafting density as the $\Phi_{na}$ is higher and hence higher is the number of the monomers collapsed on top of the adsorbed monomer "layer" (Figure 3.17 right). Table 3.2 shows that for higher molecular weights a single chain collapsed and formed a higher globule.

**Figure 3.17**: Left: Conformation of polymer chains of the same molecular weight adsorbed on a surface in good solvent conditions. From top to bottom, the surface density increases leading to repulsive interactions between the chains. In this way, they occupy less space laterally and take more vertical conformations with less number of monomers adsorbed. Right: Corresponding conformation of collapsed chains when in bad solvent conditions. At high surface densities the chains form higher globules.

It is important to note that for higher adsorbed amounts, the height of single collapsed chains vary strongly with the deconvoluted chain volume (Figure 3.10 and Figure 3.14). As it was discussed in the background section even for relatively monodisperse polymers the distribution of $M_w$ can not be fully neglected when individual chains/globules are considered. The monomer number variation of chains within the same sample can lead to height variations of the globules as bigger chains will have higher $\Phi_{na}$ and hence will collapse in higher globules. In contrast, for isolated adsorbed chains, $\Phi_{na}$ is expected to be independent of the chain size.
Consequently, the height dependence on the polymer size polydispersity is much less pronounced.

**ii) Aggregations**

In all cases (different $M_w$), the number of the islands increased (higher surface density) with the incubation time. The tendency of chains to fuse together and form homogeneous aggregates after the abrupt solvent evaporation is pronounced in the case of the higher observed adsorbed amounts (Figure 3.6c, Figure 3.8c and Figure 3.12c). The formation of aggregates results in a discontinuity in the plots of island heights against their deconvoluted volumes, as aggregates have volumes of several times bigger than the volume of single collapsed chains (Figure 3.10 and Figure 3.14).

For low adsorbed amounts the polymer chains are expected to lie relatively flat on the surface and no overlap/entanglements between polymer chains takes place. By increasing the adsorbed amount (and hence the number of the adsorbed chains) the conformation of the chains change and they appear with higher loops and tails. The conformational change of the adsorbed polymers on the surface, in good solvent conditions, by increasing the adsorbed amount, can be looked in two different ways; as a 2D problem corresponding to the adsorbed monomer and the surface area occupied by them and as a 3D problem for the non-adsorbed part of the chains.

De Gennes proposed that a polymer chain in 2D will segregate from other chains due to the local high concentration of the single chain and this segregation behaviour is independent of the molecular weight of the chain\textsuperscript{26}. Therefore in 2D we expect to have no significant number of entanglements between different chains. Furthermore, this 2D segregation behaviour was verified experimentally by Sato \textit{et al.}\textsuperscript{27} by studying polymers at the air/water interface. Furthermore Ito \textit{et al.}\textsuperscript{28} by studying the morphology and the shear viscosity of the polymer-modified, water surface, also found that the polymer chains were segregated in 2D.

Moving now to the 3D consideration, we can take into account the non-adsorbed parts of the adsorbed chains (loops and tails). These parts are expected to appear with higher mobility than the adsorbed trains as they are not confined/adsorbed on a plane. When the adsorbed amount increases the chains are forced to be close together. For linear PB with $M_w = 78.8$ kg/mol and $M_w = 962$ kg/mol and for the highest adsorbed amount if we assume isotropic distribution of the molecules on the surface when in good solvent conditions, the average distance between the chain
centres is in the order of 20 nm and 80 nm, respectively. In 3D this closed-pack conformation of the adsorbed chains would result in the free part of the chains (loops and chains) to be close together leading to a local high concentration of the non-adsorbed monomers near the surface.

A simple way to understand the behaviour of the non-adsorbed parts of the adsorbed chains near the surface is to employ the well known and widely studied case of a high concentration polymer solution, semi-dilute regime \((c > c^*)\). In general, in high concentration polymer solutions, entanglement between chains can occur if the molecular weight of the chains is above the entanglement molecular weight \((M_e)\). Nevertheless for the case of entanglement between adsorbed chains the case is complicated as entanglements are expected to appear only within the non-adsorbed parts of the chains. The effective \(M_w\) of these sub-chains is not easy to be determined. Hence, even though the \(M_w\) of the whole adsorbed chain can be higher than the corresponding \(M_e\), the effective \(M_w\) of the loops and tails will be smaller and perhaps smaller than \(M_e\).

For PB, \(M_e \approx 2\ kg/mol\) and it is much smaller than the \(M_w\) of all the different polymer chains used in this study. Considering that the effective \(M_w\) of the sub-chains is a small fraction of the total chain \(M_w\), one can qualitatively assume that the higher the \(M_w\) of the adsorbed chain is the more probable the effective \(M_w\) of the sub-chains to be higher than the corresponding \(M_e\). Hence, bigger adsorbed chains could make entanglements in a more efficient way between their non-adsorbed loops and tails. In this way, we would expect higher molecular weights to have an increased tendency for aggregates. This is what we observed is our results: for the highest adsorbed amount observed, the ratio of the aggregates over the total number of islands increased with the \(M_w\); for \(M_w = 38.6 \ kg/mol\) few aggregates were observed, for \(M_w = 78.8 \ kg/mol\) the number of aggregates increased and a bimodal distribution is obtained, while for \(M_w = 962 \ kg/mol\) almost exclusively big aggregates were observed.

On this point it is also important to note that there is an additional effect which leads longer chains to form aggregates in a more efficient way. We expect that longer chains are easier to appear with longer loops and tails, for higher grafting densities, because \(M_w\) should affect \(\Phi_{na}\) in a non-uniform way. A shorter chain with each monomer-surface contact loses a larger percentage of its conformational entropy than a longer one. Consequently, in order to remain adsorbed would have to balance the
entropic loss, by keeping a larger proportion of its monomers in contact with the surface and hence a lower $\Phi_{na}$. Assuming a maximum necessary $\Phi_{na}^{\text{max}}$ for a chain to stay adsorbed, this is expected to be smaller for shorter chains (smaller $M_w$).

In all cases, the height of the aggregates was higher than the single collapsed chains, within the same molecular weight, and slowly increased as the number of the chains in the aggregate increased. Higher molecular weights produce larger/higher aggregates: For highest observed adsorbed amounts, the height distribution of aggregates of linear PB with $M_w = 38.6$ kg/mol shows a peak at about 4.5 - 5 nm, while for $M_w = 78.8$ kg/mol the aggregate peak is about 7 nm and for $M_w = 962$ kg/mol the aggregate peak is about 12 nm. In all cases, the aggregates are characterised by a relatively narrow distribution of heights while they can vary widely in volume (Figure 3.10 and Figure 3.14). This translates to a relatively low variation of height and large variation of lateral sizes. A schematic illustration of the behaviour is shown in Figure 3.18. The formation of the aggregates was driven from the fact that the chains try to decrease their surface energy fusing together, avoiding the many contacts with the unfavourable air (bad solvent for PB) and also retaining their contact with the substrate to maintain their adsorption energy. The lateral size of the aggregates is determined by the polymer length but also critically by the number of segregated chains in 2D. The height of the aggregates is mainly determined by the total number of the non-adsorbed mobile monomers which are more numerous when many and longer chains are involved. Nevertheless, the mobility of the non-adsorbed monomers is severely restricted by the adsorbed part of the chains resulting in low variation of heights. The determination of height by the number of non-adsorbed monomers also explains the observation that samples with higher $M_w$ formed higher aggregates after the abrupt solvent evaporation. As we have seen above, higher molecular weight chains are characterised by higher $\Phi_{na}$ in good solvent conditions.
3.6 Conclusions

Linear polymer adsorbed (sub-)monolayers on flat surfaces were studied by atomic force microscopy. (i) At sufficiently low surface densities, where the molecules can be considered as isolated, the polymers take flat conformations. The height of the collapsed chains appeared almost independent of the molecular weight. We have argued that this behaviour stems from the conformations of the isolated adsorbed chains when in good solvent conditions and before abrupt solvent evaporation. (ii) At higher surface densities, aggregates of several polymer chains formed for all molecular weights. Our results show that higher molecular weight molecules can self-assemble in aggregates in an easier manner. We explained this in terms of the higher length of loops and tails of the higher molecular weight chains. (iii) In most cases, at sufficiently high surface densities, an isotropic distribution of adsorbed polymers was observed indicating near to equilibrium positions and conformations just before the sudden change of solvent conditions.

Figure 3.18: Different conformations of multichain adsorption in good (left) and bad solvent conditions (right). From top to bottom, the drawings correspond to an aggregation of few chains to many chains. The aggregate is much wider when many chains are involved, bottom, but without significant difference in height.
3.7 References

Chapter 4

Adsorption and Clustering after Solvent Evaporation of Regular Star Polymers on Surfaces
4.1 Introduction

In this chapter, we investigate the structural regions of sub-monolayers and monolayers of adsorbed PB star polymers of three different functionalities \( f = 18, 32 \) and 59) on mica surfaces. The adsorption was attained by immersing the freshly cleaved mica surface into star solutions in toluene (good solvent for PB) at different concentrations well below the critical overlap concentration, \( c^* \). An important aspect of our work is the pre-treatment of the polymer modified surfaces: extensive rinsing with toluene to remove any non-adsorbed molecules followed by an exposure in a pure toluene bath for several hours to attain as far as possible equilibrated conformations and finally a rapid solvent evaporation under flow of nitrogen. Imaging was performed using a tapping mode atomic force microscopy (AFM) in air. We observed a strong dependence of the evolution of the average island height and surface coverage with increasing surface density on the functionality (number of arms) of the star polymers. The observed dependence of the monolayer growth and structure on the star functionality and surface density have also allowed us to argue on the probable conformation and behaviour of the adsorbed star polymers in good solvent conditions \textit{just before} the evaporation of the solvent. We explain these structural phenomena in terms of the molecular and conformational properties of the different star polymers. Our study provides real space observations of the star polymer surface structures near the overlap surface density for the first time. The structural regions are highly affected by the change of the star polymer behaviour from polymer-like to colloidal-like when the arm functionality increases. Furthermore, although this study concentrates on the star polymer behaviour on surfaces, indirectly some of the insights might reflect the structure of star polymers in bulk polymer solutions. Our study provides real space observations of the star polymer surface structures near the overlap surface density for the first time.
4.2 Background

Recent advances in polymer chemistry led to the synthesis of a wide range of new materials, such as star-shaped macromolecules with large number of homopolymer arms covalently joined to a dendritic core. Star polymers can be considered molecules with intermediate character between colloids and polymers; thus, they represent a soft matter system bridging two very important members with long history and important industrial applications such as polymers and colloids. The intermediate behaviour of star polymers renders them ideal for the design of novel systems and materials with fine-tuned intermediate physical properties. This fact is the reason why star polymers attracted considerable interest during the last few years and several studies have already been reviewed. These reviews focused on experiments, theory and simulations of star polymer systems mainly away from surfaces. From the experimental point of view, the fact that star polymers which are nearly monodisperse in both number of arms and the degree of polymerization can be synthesized by anionic polymerization by chemists, has played an important role in the development of the field since it made possible the exploration of well defined model systems.

The conformation of an isolated star was meticulously explained by Daoud and Cotton, using scaling theory to introduce the blob model of a star polymer. In this model the star polymer was considered as a succession of concentric shells of blobs, the size of which, $\xi(r)$, depends on the distance of the blob from the centre of the star leading to a non-uniform monomer density distribution, $c(r)$, which has been verified experimentally and by computer simulations. The non-uniform monomer density distribution results in a non-uniform osmotic pressure of the star which decreases from its maximum at the centre (core) to the outer part (corona) of the star polymer. Star polymers can be considered as molecular entities with two characteristic length scales: a small one of polymeric nature corresponding to interaction blobs (self avoiding segment) and a large one of colloidal nature which corresponds to its overall size.

A lot of studies have focused on the star polymer dynamics and results have already been reviewed by Vlassopoulos et al. The generic behaviour of the star is described in terms of the competition between its polymeric and colloidal character which strongly depends on the functionality, $f$ (number of arms), of the star. More specifically, they showed experimentally that the interpenetration between stars
increases with decreasing $f$. This stems from the fact that the osmotic pressure within
the star increases with the star polymer functionality preventing interpenetrations
between different star polymers. Studies on the concentration dependence of the
viscosity of star polybutadiene (PB) solutions (similar systems with the one studied in
this work) showed molecular tuneable softness from entangled polymers ($f <\approx 32$) to
soft colloidal spheres ($f >\approx 32$)$^{3,7}$. The softness of the star with respect to $f$ has also
been studied comprehensively by Likos et al$^8$ using fluid-state theory and Monte
Carlo simulations. By calculating the star-star interactions for arbitrary distance, $r$,
between their cores they showed that the softness of a star polymer decreases by
increasing $f$. Hence, low functionality leads to low osmotic pressure of the star
polymer which in turn makes the star polymer softer.

An interesting line of investigation is the structural regions and possible
transitions of star solutions as one move from the dilute region of isolated stars to
progressively dense structures as the concentration increases. Several structural
regions have been predicted by theory$^9$ and investigated experimentally$^{10}$ depending
on the star functionality. Watzlawek et al.$^{11}$ employed simulations in combination
with small angle neutron scattering (SANS) data and found that the core-core
structure factor of dense star polymer solutions exhibit unusual behaviour above the
concentration at which the star molecules start overlapping (overlap concentration).
This is explained in terms of two competitive length scales: the average interparticle
distance and the star corona diameter. However, concentration dependent structural
studies have not been performed for star homopolymers adsorbed on surfaces.

AFM studies of star polymers adsorbed on surfaces have been so far restricted
to the structure of isolated stars. Kiriy et al.$^{12}$ studied the structure of a heteroarm star
copolymer, polystyrene/poly(2-vinyl-pyridine) (PS-P2VP) with seven arms adsorbed
on mica and Si wafers by tapping mode atomic force microscopy (AFM) in air. They
used dilute solutions of various good and selective solvents and rapid evaporation.
They found that samples prepared from a good solvent resulted in globular
conformations while those from selective solvents yielded extended surface
conformation of the one type of segments. In some cases, they used metal clusters
permitting the enhanced AFM visualisation of the few P2VP arm-segments. An
important conclusion of this work was that the history and treatment of samples have
profound consequences in the polymer conformations on the surface. However, in
several cases, mainly due to the rapid evaporation of the solvent, one can claim that
the surface polymer structure resembles the structure of the polymer in the bulk solution.

4.3 Experimental Section

4.3.1 Materials

A series of regular 1,4-polybutadiene multiarm stars were synthesized by Roovers and co-workers using chlorosilane chemistry, yielding central dendritic cores of spherical shape and different generations on which the desired number of polymeric arms were grafted\(^4\). The characteristics of the star shaped molecules used in this study are shown in Table 4.1.

<table>
<thead>
<tr>
<th>Star Polybutadiene</th>
<th>Code</th>
<th>(f)</th>
<th>(M_w) (g/mol)</th>
<th>(R_g) (nm)(^a)</th>
<th>(R_h) (nm)(^b)</th>
<th>(R_b) (nm)(^c)</th>
<th>(c^*) (g/g)</th>
<th>(V_{molecule}) (nm(^3))</th>
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</thead>
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<tr>
<td>1518</td>
<td>18</td>
<td>311k</td>
<td>12.4</td>
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<td>5.3</td>
<td>8.11 x 10(^{-2})</td>
<td>625</td>
<td></td>
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<tr>
<td>3216</td>
<td>32</td>
<td>558k</td>
<td>13.4</td>
<td>17.2</td>
<td>6.4</td>
<td>1.19 x 10(^{-1})</td>
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<tr>
<td>6480</td>
<td>59</td>
<td>4200k</td>
<td>34.1</td>
<td>47.7</td>
<td>12.6</td>
<td>5.11 x 10(^{-2})</td>
<td>8450</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Radius of gyration in dilute good solvent conditions from light scattering measurements\(^7\),\(^14\).

\(^b\) Hydrodynamic radius in dilute good solvent solutions\(^7\).

\(^c\) Calculated radius in bad solvent conditions (compact sphere) using the bulk PB density value\(^15\), \(\rho = 0.826 \text{ g/cm}^3\).

\(^d\) Calculated volume of a collapsed molecule (based on the molecular weight and bulk density of PB).

4.3.2 Preparation of Star PB Monolayers on Mica

All the samples were prepared by exposing freshly cleaved mica to a toluene solution (good solvent for Polybutadiene, PB) of the desired concentration of the star PB. Three star polymers were used with their molecular characteristics shown in Table 4.1. The concentration of the solutions for all the molecules was 100 times less than the critical overlap concentration, i.e. \(c/c^* = 0.01\). The \(c^*\) was determined from the radius of gyration, \(R_g\), of this star measured in dilute good solvent solution by light scattering\(^7\). A variety of incubation times were used, from approx. 3 min up to 5 days. After incubation the polymer-modified mica was rinsed extensively with toluene and placed in a toluene bath for several hours (more than three) to remove any non-adsorbed chains and allow the adsorbed polymers to attain closer to equilibrium
conformations. Subsequently, the sample was rapidly dried under flow of nitrogen and imaged in air by atomic force microscopy (AFM) in tapping mode.

4.3.3 Atomic Force Microscopy (AFM)

All the AFM experiments have been performed in air (bad solvent for the star PB) using a Veeco AFM, multimode/nanoscope IIIa (Veeco, Santa Barbara, CA, USA). The samples were imaged in tapping mode (tip in intermediate contact with the surface). The RTESPA Veeco cantilevers with a nominal spring constant and resonance frequency of 40 N/m and 300 kHz respectively were used to image the samples. The cantilevers were oscillated vertically 5% below their natural resonant frequency, and moved in a raster fashion within a specified region of interest. Each sample was imaged at several different areas.

4.3.4 Island Volumes, Heights and Island Surface Coverage Calculations

The free software WSxM (Nanotech Electronica S. L. www.nanotech.es) was used for simple levelling and display. The island heights, volumes and the surface coverage (percentage of substrate surface covered by polymers) were determined by either using the grain analysis or the roughness analysis module (height distributions of pixels) of the commercial software Scanning Probe Image Processor (SPIP, Image Metrology). The total adsorbed amount, $\Gamma$ (mg/m$^2$), was calculated using the observed volumes and the bulk PB density. The volume values calculated directly from AFM image analysis (and consequently the deduced adsorbed amounts also) suffer from the convolution effect which is explained below.

4.3.5 Convolution Effect

AFM images are always a convolution of the geometry of the tip and the shape of the object or sequence of objects being imaged. For the case of an isolated object, the convolution affects only the lateral dimensions of the object being imaged as the corresponding height is accurately measured (Figure 4.1a). In general, if the tip is much smaller than the object of observation the AFM topography will contain minimal artefacts revealing almost the real shape of the object. It has been reported in
AFM studies on single molecules that the convolution effect can generally overestimate the real volume of the object\textsuperscript{16} by an order of magnitude larger than it is in reality.

For the case of a sequence of objects being close together, i.e. for a high particle surface density, the convolution due to the geometry of the tip results in images where the height of the objects is underestimated (Figure 4.1b). This effect has already been reported for the case of a dense monolayer of thiol-terminated polystyrene adsorbed on a gold surface\textsuperscript{17}. Deep valleys between the objects are inaccessible to the tip; only the top of the objects can be imaged making the objects to appear short and hence smaller in the image.

\[ V_a = \frac{\pi h}{2} \left( \frac{3V_r}{\pi h^2} + h + R_t \right) \left( \frac{\pi h^3}{6} \right) \]

\textbf{Figure 4.1:} Schematic drawing showing (a) how a spherical cap appears (green) due to the convolution of the geometry of the AFM tip and the shape of the object being imaged (yellow spherical cap) and (b) how spherical caps sitting next to each other appear shorter, black line.

A calculation in order to check the effect of the tip geometry on the measured volume of an object was performed. We assumed that the objects are spherical caps as this has been proven to be a satisfactory assumption for the case of single molecules on surfaces in bad solvent conditions\textsuperscript{16,17}. The tip usually has the shape of a pyramid (of a size approximately 3-5 μm) ending in a nano-scale sharp apex with an effective radius of curvature (about 40 to 50 nm for the tips we used in this study as measured by SEM). Using simple geometrical arguments the apparent volume, \( V_a \), of the apparent spherical cap (green) in Figure 4.1a can be connected with the real volume, \( V_r \), of the real spherical cap (yellow), the real measured height, \( h \), and the tip radius, \( R_t \).
For our calculations we used three different spherical caps of volumes similar to the theoretical volumes of the polymers (in collapsed state, bad solvent conditions) studied (Table 4.1). In our calculations, the theoretically estimated volume (or real volume), $V_r$, of the spherical cap was kept constant while the maximum height took values from nearly 0.4 nm (flat conformation) to 16 nm (narrow, vertical conformation) and the convoluted volume (or apparent volume), $V_a$, was calculated. The tip was considered to be a perfect sphere.

![Graph](image)

Figure 4.2: The ratio of the real and the convoluted volume (apparent volume) of a globule against the height for three spherical caps of different volume: a) 625 nm$^3$ (black squares) b) 1120 nm$^3$ (red circles) and c) 8450 nm$^3$ (green triangles) for $R_i = 40$ nm.

These calculations were used to check if the observed structures/globules represent single collapsed molecules or not by comparing the experimentally observed and measured apparent volumes with the calculated convoluted volumes using Equation 1. The accurate estimation of the convolution effect for arbitrary shapes of particles and different surface densities is complex. All the volumes and total adsorbed amounts reported here (unless stated otherwise) are apparent ones taken directly from AFM images.

### 4.4 Results

Several samples using different adsorption/incubation times were prepared for each of the different polymers. Several series of $6 \times 6$ μm$^2$ images for every sample
were collected. Analysing these images, the average island height, surface coverage and the total adsorbed amount were determined.

In Figure 4.3a, the adsorbed amount against the immersion time for the samples of star polymers with 18-arms, 32-arms and 59-arms are plotted. It is clear that all the curves follow similar behaviour; the adsorbed amount increased with time and a plateau was finally reached. This indicates that there is a specific, to each polymer, maximum adsorbed amount that can be attained at high enough immersion times. It is clear that for the case of 18-arms the adsorption converged faster to the plateau than for the case of 32-arms, while the adsorption for the 59-arms converged with even slower kinetics.

![Figure 4.3: Plot of the adsorbed amount against the immersion time for the samples of star PB with 18-arms (black squares), 32-arms (red circles) and 59-arms (green triangles).](image)

It should be noted that any quantitative comparison between the maximum adsorbed amount values for the different samples cannot be easily made due to the difficulty to take fully into account the convolution effect. For example the adsorbed amount of the 18-arm stars is overestimated as the individual islands were well separated and appeared larger (Figure 4.4). On the other hand, the maximum adsorbed amount for the case of 32-arms and 59-arms could be underestimated since the structures were very close together (Figure 4.7 and Figure 4.9) and due to the corresponding convolution effect the islands could appear shorter than they really were resulting in an artificially lower maximum adsorbed amount. Nevertheless and in spite of these effects, the monotonic shape of the adsorption isotherms ensures that
the apparent adsorbed amount is a good indicative measure of the increasing adsorption with the immersion time for each polymer separately and that we have used sufficiently long immersion times to arrive to a plateau in the adsorbed amount for all the polymers used.

Figure 4.4 shows some typical AFM images of 18-arm star PB adsorbed on mica for six selected incubation times resulting in adsorbed amounts seen at the top of each image. Analysing at least three images for each immersion time we measured the corresponding average island height and surface coverage. These values are plotted against the increasing adsorbed amount and shown in Figure 4.5. In Figure 4.4a, which corresponds to a low incubation time (hence, low adsorbed amount) the polymeric islands appear flat (short) and many of them are non-circular and somewhat elongated on the surface. As the adsorbed amount increased but still for relatively low values, (region I in Figure 4.5), the height of the islands increased while the surface coverage remained relatively constant. The islands started to appear circular (Figure 4.4b) at the end of region I. For adsorbed amounts higher than approx. $2.8 \times 10^{-1}$
mg/m\(^2\) the average height of the islands remained constant while the island coverage increased linearly with the adsorption (region II in Figure 4.5). The shape of the islands remained mainly circular but some asymmetric ones started to appear (Figure 4.4c). When the adsorbed amount reached a value of approx. \(7.4 \times 10^{-1}\) mg/m\(^2\) the average height of the islands started again to increase while the surface coverage remained almost constant (region III in Figure 4.5). The islands appeared again to take non-circular/elongated shapes and also became wider (Figure 4.4e and f).

![Figure 4.5](image)

**Figure 4.5:** This plot shows the variation of the 18-arm star-PB island average height (black solid points, left scale) and the surface coverage (open blue points, right scale) versus the adsorbed amount. Island average heights and surface coverage data were obtained by averaging the measurements over at least three different areas for each incubation time.

The volumes of the islands for adsorbed amounts up to around 0.284 mg/m\(^2\) are similar with the convoluted volumes calculated using Equation 1. For example, the measured, from the AFM images, average island volume for adsorbed amount \(2.84 \times 10^{-1}\) mg/m\(^2\) was around 6500 nm\(^3\) while the calculated convoluted volume using Equation 1 (for a real volume equal to the volume of a single collapsed 18-arm star PB, Table 4.1, and height 4.5 nm (the average height of the polymeric islands for this adsorbed amount)) is 4500 nm\(^3\), (see also Figure 4.2). Hence the islands can be considered to be mainly single collapsed star polymers for adsorbed amounts within the region I in Figure 4.5. For higher incubation times some bigger islands started to form. For these higher adsorbed amounts we can roughly estimate the number of star molecules in each island by dividing the apparent volume of each island with the apparent volume of the single collapsed star polymer, as it appeared in the low
coverage AFM images. In this way, the corresponding probability of finding a set number of molecules in any island can be determined. For example, Figure 4.6 shows this probability plot for samples with adsorbed amounts at the end of region I and within region II.

For an adsorbed amount equal to $4.01 \times 10^{-1}$ mg/m$^2$, it is clear that the island constitutes mainly of single collapsed stars and in some cases of few aggregates of two stars. For a higher adsorbed amount ($6.02 \times 10^{-1}$ mg/m$^2$) the number of single collapsed chains decreased while aggregates of three and four stars are more often. For an even higher adsorbed amount (e.g. $7.41 \times 10^{-1}$ mg/m$^2$, not shown) the number of single polymer chains decreased dramatically while the number of multi-aggregated chains increased even more, with many of them including 5 or 6 molecules. For even higher adsorbed amounts, i.e. within the region III (Figure 4.5), most polymeric islands can be considered as aggregates of several stars.
Figure 4.7: 32-arm star PB molecules adsorbed on freshly cleaved mica from a toluene solution; images in air, $6 \times 6 \, \mu m^2$ scans using tapping mode AFM. The mica was left in the solutions from 5 min up to approx. 2 days. The adsorbed amount of the star PB polymers on mica is given above each image. From image a) to image f) the adsorbed amount increased from around 0.173 mg/m$^2$ up to 1.81 mg/m$^2$.

Figure 4.7 shows some typical AFM images of the 32-arm stars adsorbed on mica for six selected incubation times. The average island height and surface coverage where determined by analysing at least three images for each immersion time we measured. These are plotted versus the adsorbed amount in Figure 4.8. For the case of the 32-arm star PB and low adsorbed amount the polymeric islands appeared flat on the surface (short) and some of them are asymmetric and elongated (Figure 4.7a). By increasing the adsorbed amount up to around 0.33 mg/m$^2$ the average height increased linearly while the island coverage appeared approximately constant (region I in Figure 4.8). The shape of the islands started to appear circular at the border between region I and II. When the adsorbed amount is higher than 0.33 mg/m$^2$ the surface coverage started increasing while the average height of the islands stayed relatively constant, with a small decrease for the highest adsorbed amount measured. The latter could be attributed to the convolution effect due to the formation of a denser structure. For the two highest adsorptions measured (region III), a semi-continuous network was observed (Figure 4.7e and f).
Figure 4.8: This plot shows the variation of the 32-arm star-PB island average height (black solid points, left scale) and the surface coverage (open blue points, right scale) versus the adsorbed amount. Island average heights and coverage data were obtained by averaging the measurements over at least three different areas for each incubation time.

As in the case of the 18-arm star PB sample, for the 32-arm star PB and for low adsorbed amounts, the volumes of the islands were similar to the convoluted volumes calculated using Equation 1. For example, for the adsorbed amount $3.26 \times 10^{-1}$ mg/µm$^2$ the measured average volume of the polymeric islands was around 11000 nm$^3$ while the theoretical convoluted volume of this molecule is around 8400 nm$^3$. Hence, the polymeric islands for adsorbed amounts within the region I in Figure 4.8 can also be considered as mainly single collapsed 32-arm star PB molecules. For adsorbed amounts higher than $3.26 \times 10^{-1}$ mg/µm$^2$ (region II of Figure 4.8), aggregates of 32-arm star molecules started to form resulting gradually to the semi-continuous network of Figure 4.7f (within region III).
In Figure 4.9, we present some typical AFM images of 59-arm star PB adsorbed on mica for six selected incubation times. Figure 4.10 shows the average island height and surface coverage plotted against the adsorbed amount for the case of the 59-arm PB. The island surface coverage increased while the average height of the islands remained nearly constant, apart from the highest adsorbed amount where the average height decreased (Figure 4.10). The shape of the islands remained symmetric and circular even for low adsorbed amounts. We did not observe any formation of aggregates for any adsorbed amounts. The volumes of the islands for most adsorbed amounts (except for the highest one) were similar to the convoluted volumes calculated from Equation 1. For example, for the adsorbed amount 0.37 mg/μm² the measured/apparent average volume of the polymeric islands was around 34000 nm³ while the theoretical convoluted volume of this molecule is around 26500 nm³.
Figure 4.10: This plot shows the variation of the 59-star PB island average height (black solid points, left scale) and the surface coverage (open blue points, right scale) versus the adsorbed amount. Island average heights and coverage data were obtained by averaging the measurements over at least three different areas for each immersion time.

Figure 4.11 shows a high contrast image of the maximum adsorbed amount (Figure 4.9f). It is clear that the islands are close together, even touching each other in many cases. As it has been mentioned in the convolution section, when particles are close together they can appear shorter in an AFM image than they really are. Also the height distribution of the islands (Figure 4.11b) shows a bimodal distribution, with the first peak being around 6.5 nm and the second just above 9 nm. This can be explained by the fact that some of the islands were so close together that appeared shorter, due to the convolution effect, contributing to the first peak of the bimodal distribution while some others did not have any close enough neighbours and thus their height was not affected giving rise to the second peak of the bimodal distribution with height of 9nm. Therefore, the decrease of the average height from around 9 nm to less than 8 nm observed for high adsorbed amounts for the case of the 59-arm PB (Figure 4.10) can be an artefact and a direct consequence of the convolution effect.
In Figure 4.11, we present the radial averaged intensity of a 2D fast Fourier transform (FFT) of two typical images for each star polymer: one for low and one for the highest observed adsorbed amount. The shape of the FFT is centrosymmetric implying an isotropic distribution of the islands/structures. The radial averaged intensity shows a maximum at a specified wavevector which corresponds to a characteristic length scale for each case indicating the average distance from the 1st neighbours. Similar isotropic distributions hold for almost all of our observed structures when more than approx. 5 min adsorption times were used (i.e. when a sufficiently large number of molecules were adsorbed). It is worthwhile noting that the smallest characteristic length was observed for the intermediate functionality star. Furthermore, moving from a low to the highest adsorbed amount, the characteristic length decreased for the 18-arm star polymer while increased for the 32- and 59-arm star polymers.
Figure 4.12: Relative average intensity as a function of the wave number \(q=2\pi/\lambda\) for star PB of (a) 18-arms and \(\Gamma = 2.84 \times 10^1 \text{ mg/m}^2\), (b) 18-arms and \(\Gamma = 1.64 \text{ mg/m}^2\), (c) 32-arms and \(\Gamma = 1.73 \times 10^1 \text{ mg/m}^2\), (d) 32-arms and \(\Gamma = 1.81 \text{ mg/m}^2\), (e) 59-arms and \(\Gamma = 3.70 \times 10^1 \text{ mg/m}^2\) and (f) 59-arms and \(\Gamma = 1.94 \text{ mg/m}^2\). These plots were obtained by the radial average of the 2D FFT image (small inset, left) resulting from the corresponding AFM image (large inset, right) and show a maximum at a specific wave vector \(q\) which corresponds to a characteristic length scale of 171 nm, 389 nm, 300 nm, 204 nm, 311 nm and 255 nm for images (a) to (f), respectively.
4.5 Discussion

4.5.1 Adsorption of Star PB on Mica

In Figure 4.3a, the curves of the adsorbed amount against the immersion time of 18-arm, 32-arm and 59-arm star PB indicate that the higher molecular weight (larger molecular size) polymers exhibited slower adsorption kinetics as one would expect owing to their slower diffusion coefficient in the solution. In any case, all curves converge to a specific adsorbed amount value which was different for each polymer. This is an indication that there is a specific maximum attainable adsorption for each star polymer. As it has already been discussed, the calculation of the exact maximum adsorbed amount from AFM images and hence the exact maximum number of molecules adsorbed on the surface is not easy due to the convolution effect. Nevertheless, in the case of the 59-arm star PB and for high adsorption (Figure 4.9f) the number of star molecules adsorbed on the surface can be estimated from the corresponding AFM image since each of these globules corresponds to a single collapsed 59-arm star molecule, as it was discussed in the experimental section. The number of islands for this case of the highest measured adsorbed amount correspond to 92 ± 10 particles/μm².

Assuming a surface fully covered with hard spheres of radius \( R \), equal to the star corona radius \( R_c = (11/5)^{1/2} R_g = 1.48 \) nm of the 59-arm star PB (Table 4.1, good solvent conditions), we can estimate a maximum adsorbed number of particles. For the calculation we assumed the hexagonal lattice, the densest packing of circles in a plane which has a packing density of \( n = (1/6) \pi \sqrt{3} = 0.907 \), Random Closed Packing (RCP), that gives a packing density of \( n = 0.82 \) and Random Loose Packing (RLP) of \( n = 0.772 \). For the hexagonal lattice we would expect 113 spheres/μm², for RCP there will be 102 spheres/μm² while for RLP there will be 96 spheres/μm². These calculations show that the maximum number of molecules adsorbed on the surface within our experimental protocol compare well with the estimated values for a random packing. This means that at least for the largest \( f \) star polymer used in our study, the combination of its high osmotic pressure and the repulsive excluded volume interactions in good solvent conditions do not allow the penetration and adsorption of other molecules within the pervaded volume of the already adsorbed ones; i.e. the overlap surface density in good solvent conditions is
the maximum density that can be attained on the surface using our experimental protocol.

4.5.2 Growth of Star PB monolayers on Mica

We now discuss the growth of the star PB monolayers on mica with respect to the shape of the observed islands and the evolution of the average island heights and surface coverage. We will divide the discussion for the monolayer growth on mica in three parts: (i) Region I: low adsorbed amount region, (ii) Region II: intermediate adsorbed amount region and (iii) Region III: high adsorbed amount region. In Figure 4.13 we plot separately the adsorbed amount, average island height and surface coverage for all polymers noting the extent and boundaries of each region.

![Graphs showing adsorbed amount, average height, and surface coverage for different star PB monolayers on mica.](image)

**Figure 4.13:** (top left) Adsorbed amount versus immersion time; (top right) island average height versus adsorbed amount and (bottom) surface coverage versus adsorbed amount for 18- (black squares), 32- (red circles) and 59-arm star PB (green triangles). The regions I, II and III are depicted in each plot.
Star Polymers on Surfaces

i) Region I: low adsorbed amounts

At first, we concentrate on the lowest adsorbed amount observed in our experiments for each case; \( \Gamma = 9.72 \times 10^{-2} \text{ mg/m}^2 \), \( \Gamma = 1.73 \times 10^{-1} \text{ mg/m}^2 \) and \( \Gamma = 5.43 \times 10^{-2} \text{ mg/m}^2 \) for 18-, 32-, 59-arm star PB, respectively.

The polymer islands correspond to single collapsed star PB molecules for all cases, as it has been discussed above by comparing the measured island volume with the convoluted volume for each case. In the AFM images of the 18-arm stars the single molecules appeared mostly asymmetric on the surface (Figure 4.4a). For the 32-arm stars few collapsed molecules appeared asymmetric and their asymmetry is less prominent (Figure 4.7a) while for the 59-arms all globules retain a circular shape (Figure 4.9a).

In general, the structure of polymers adsorbed on surfaces in good solvent conditions depends strongly on the surface polymer density due to the repulsive excluded volume interactions with the surrounding adsorbed chains\(^{21}\). In the case of 18-arm and 32-arm star polymers and for the lowest adsorption presented here the number of the adsorbed chains are similar, 16±3 chains/\( \mu \text{m}^2 \) and 14±2 chains/\( \mu \text{m}^2 \), respectively. If we assume that the molecules were homogenously and isotropically distributed on the surface when in good solvent conditions (before the abrupt solvent evaporation) the average distance between the centres of the molecules is between 250-300 nm, an order of magnitude higher than their radius of gyration. Hence, we can assume that the interactions among them are weak. The average distance of the 59-arm star PB for the case of the lowest adsorption is even higher (> 1\( \mu \text{m} \)) since even fewer particles are adsorbed on the surface.

Asphericity in the shape of single star polymers in 3D good solvent conditions has been reported in molecular dynamics (MD) simulations; where an evolution of the star polymer conformation from aspherical to spherical object with increasing functionality is observed\(^{2-3}\). In our experiments we have observed the same trend albeit for frozen surface conformations in the dry state. We suggest that the degree in the asymmetry observed in these conditions could be due to the different degree of asphericity/asymmetry when the adsorbed polymers were in good solvent conditions. For the 59-arm star polymer, the molecule is expected to be relatively spherical in good solvent conditions, due to the high functionality, leading to a symmetric, circular collapsed conformation. For the lower-functionality star polymers, their asymmetry in
the good-solvent conditions was captured in the dry state when the molecule abruptly collapsed due to the rapid evaporation of the solvent. This interpretation also indicates that the shape and size (parallel and normal to the surface) of the collapsed star polymer depend strongly on the conformation of the adsorbed star polymer in the initial good solvent conditions just before drying.

The 18-arm stars at the lowest adsorbed amount appeared as single chains with a height of approx. 1.5 nm (Figure 4.5 or Figure 4.13). It has to be noted that for isolated collapsed linear PB chains in air we found an average height of approx. 1.5 nm and almost independent of the molecular weight. For the 32-arm stars the average height of the single molecules appeared to be higher, approximately 2.3 nm (Figure 4.8 or Figure 4.13), while for the 59-arm star polymer the measured height appeared much higher, approximately 9 nm, than that of their low functionality counterparts (Figure 4.10 or Figure 4.13). These observations indicate that in the case of star polymers and for low surface densities (where the adsorbed molecules can be considered nearly isolated) the average height of a single collapsed star polymer depends strongly on its functionality alone. This observation can be explained by the intramolecular crowding effect of the arm-chains for stars with increased functionality. In general, a single physisorbed linear homopolymer gains an energy of $\delta kT$ with each surface contact. Although $\delta$ in most cases is much smaller than unity, it is energetically favourable for the molecule to form many contacts with the surface and attain a total enthalpy much larger than $kT$ (larger than its entropic contribution to free energy) resulting in a flat conformation. Similarly, in the case of a star physisorbed polymer and for low surface densities and hence negligible star-star interactions, the polymers would lie relatively flat on the surface trying to balance the loss of entropy due to confinement with the enthalpy gain by both (i) adsorbing many arms and (ii) increasing the number of monomer contacts of each adsorbed arm with the surface. However, there is a further complication due to the star architecture as any arm conformation is affected by the excluded volume interactions with the other arms of the same star which are joined together in its centre. This intramolecular crowding effect is expected to play a particularly important role for star polymers with a high functionality.

We now define the volume fraction of the non-adsorbed monomers of the star polymer, $\Phi_{na}$ (i.e. $\Phi_{na}$ is the ratio of the monomers not in contact with the surface over the total number of monomers of the star polymer). Owing to the crowding effect, in
good solvent conditions the increase of the star polymer functionality results in an increased number of non adsorbed chain-arms and consequently to an increase in $\Phi_{na}$ (Figure 4.14 left). Additionally, the non-adsorbed arms would be more stretched for higher functionality due to the increased local density and the intramolecular repulsive excluded volume interactions, in analogy with the well known polymer “brush” where the increase of the surface grafting density results to a thicker swollen brush with the chains stretched away from the surface (balanced only by their entropic elasticity)\textsuperscript{22}. Therefore, the height of the adsorbed star polymer should be larger for higher functionalities in good solvent conditions (Figure 4.14 left).

When the solvent conditions change from good to bad, the chain-arms will collapse trying to minimize their contact with the air. Each arm will collapse in a way that depends on whether it is adsorbed or not. If the arm is adsorbed it will try to keep many contact points with the surface to retain the favourable adsorption energy. If the arm in not adsorbed it will collapse on top of the adsorbed chain-arm “layer” trying to minimize the contact with the air forming a polymer globule/nanodroplet. Consequently, the height of the globule is expected to be larger for higher $\Phi_{na}$ and hence for stars with higher functionality (Figure 4.14 right).

By increasing the adsorbed amount the average height of the islands increased for the cases of 18-arm and 32-arm star PB (similar results have also been found for
the case of linear PB on mica), while for the 59-arm the average height increased only slightly. The islands appearing in the region of low adsorbed amounts (region I) correspond to single collapsed star polymers for all the cases. In region I, the increase of the adsorbed amount will result in the decrease of the average distance between the adsorbed molecules, leading to higher local densities and due to the repulsive excluded volume interactions between the molecules in good solvent conditions to a confinement effect. The available space for each adsorbed star polymers is decreased leading to a re-conformation of the adsorbed chains to higher loop and tail distributions and possibly to (at least partial) desorption of some chain-arms; these changes lead to an increase of $\Phi_{na}$. As discussed above higher $\Phi_{na}$ will lead to higher collapsed globules. This interpretation explains the height increase of the 18-arm star and 32-arm PB in this region. Moreover, this confinement and re-conformation effect also explains why the collapsed globules appeared more symmetric/circular and narrower (Figure 4.4b and Figure 4.7c) than in the case of the lowest adsorbed amount. The island surface coverage remained relatively constant while the adsorbed amount increased, as the increase of the number of molecules adsorbed on the surface was balanced from the fact that the corresponding collapsed molecules were narrower and occupied less space laterally.

It has already been mentioned in the introduction that the star softness decreases by increasing the functionality, thus for the high functionality stars adsorbed on a surface the stiffness is expected to be high and hence the intramolecular structure would not be significantly affected from the intermolecular (star-star) excluded volume repulsions in good solvent conditions. Consequently, the proposed re-conformation for the case of the 59-arm star polymer is expected to be less important and hence the molecule would collapse into a globule with dimensions (both in height and laterally) largely independent of the adsorbed amount changes. In agreement with these arguments, our results indeed show that the height of the individual 59-arm star globules increased only slightly with the adsorbed amount while the island surface coverage increased as more stars were adsorbed (Figure 4.10 and Figure 4.13).

If we compare the space available for each individual molecule within the region of low adsorbed amounts, we find that the values are larger than the bulk $2R_e = 2 \left( \frac{11}{5} \right)^{1/2} R_g = 2.96 R_g$. In all star polymer cases for the highest adsorbed amount within this region, the ratio of the bulk $R_e$ to the effective average half distance
between the molecules (calculated from AFM images) takes values close to 0.3 while the above discussion has indicated the start of star-star interactions for the highest surface densities within region I. Thus, we can conclude that the effective size of a star polymer adsorbed on a surface is much larger than its $R_c$ in a 3D bulk solution. This is not surprising since an adsorbed molecule is expected to take laterally an extended conformation owing to (i) the enthalpically favourable contacts with the surface leading to a relatively extended flat shape and (ii) the increased intramolecular crowding effect (compared to a 3D bulk solution) combined with the repulsive excluded volume interactions since many chain-arms have to be accommodated within a 2D space.

**ii) Region II: intermediate adsorbed amounts**

Increasing further the adsorbed amount for the case of 18-arm star PB and 32-arm star PB resulted in the average island height to remain constant. This constant value for the 18-arm star PB was observed at approximately 4.5 nm while for the 32-arm star PB was approximately 6.3 nm (Figure 4.13).

The average island height of 4.5 nm for the case of the 18-arm star PB was reached at $\Gamma = 0.284$ mg/m$^2$ (Figure 4.4b, end of region I). From island volume analysis, we deduced that these islands correspond mainly to single collapsed molecules. By increasing the adsorbed amount, some aggregates of star polymers started to form. Because of the low functionality, the osmotic pressure of these star polymers is low and increasing the number of stars resulted in significant interpenetration between stars and thus in the formation of aggregates upon the sudden evaporation of the solvent, i.e. they collapsed together when the monomer-monomer interactions changed from repulsive (good solvent conditions) to attractive (bad solvent conditions). This interpenetration is expected to mainly occur between the non-adsorbed parts of arm chains in 3D. The frequency of the observed aggregates and the number of star molecules per aggregate varies according to Figure 4.6. We found that increasing the adsorbed amount, more aggregates with higher number of molecules within the aggregates were formed. The average island height did not change significantly indicating that their interpenetration in this region was relatively small. This behaviour led to the increase of the surface coverage with the increase of the adsorbed amount in region II.
For the case of the 32-arm star PB similar effects were observed and similar interpretation arguments can be made. However, in this case the interpenetration between different stars is expected to be less as the osmotic pressure within the 32-arm star is higher than in the case of the 18-arm one. Few aggregates started to form for $\Gamma = 0.566$ mg/m$^2$ (Figure 4.7d) and their number increased for higher adsorbed amounts. The average height of the collapsed polymeric islands remained approximately constant (Figure 4.8 or Figure 4.13). In turn this led to an increase of the island coverage of the surface with the increase of the adsorbed amount.

For star PB with 59-arms, the average height of the islands did not change (Figure 4.10 or Figure 4.13) and no aggregation was observed. The 59-arm star polymers can be considered relatively hard and are not affected from the confinement/crowding effect due to the increase of the adsorbed amount. Moreover, owing to the relatively high osmotic pressure inside the star, interpenetrations are more difficult to occur and no aggregates were observed. Hence, the surface coverage increased linearly with the adsorbed amount.

**iii) Region III: high adsorbed amounts**

For the 18-arm star polymers the island average height increased with the adsorbed amount in this region (Figure 4.5 or Figure 4.13). These islands correspond mainly to aggregates of several star polymers and the average mass of individual aggregates increased with the adsorbed amount indicating that the number of the associated molecules in the aggregates increased. Because of the high adsorbed amount the average distance between the centres of the stars in good solvent conditions was smaller than in the case of intermediate adsorbed amount and the interpenetrations between star polymers were larger leading to partial fusion upon the change of the solvent conditions. Hence, the height of the collapsed aggregates became larger as progressively higher interpenetrations occurred with the increase of the adsorbed amount. Similarly, the aggregation behaviour of linear PB on mica showed that the height of the aggregates was significantly larger than the single collapsed linear molecules. It is clear that although the adsorbed amount increased and hence more particles had adsorbed on the surface the surface coverage remained relatively constant (Figure 4.5 or Figure 4.13). AFM images (d), (e) and (f) in Figure 4.4 show the presence of fewer islands but they are laterally larger and higher balancing the total surface coverage.
For the case of the highest adsorbed amount in our experiments with 18-arm star polymers the structure looks as a discontinuous pattern of asymmetric islands of aggregates (Figure 4.4f). Experiments on linear PB on mica (presented in the previous chapter) and for high adsorbed amount showed the formation of big aggregates. However, the aggregate shapes were circular/symmetric as the aggregated linear chains could penetrate each other fusing together (loosing their individuality) via their non-adsorbed sub-chains of loops and tails. Although the low functionality results in a polymer like behaviour allowing interpenetrations between different stars, still the specificity of the star architecture does not allow very large penetrations and entanglements like in the case of their linear counterparts. Thus, they retain to some extent their individuality preventing the formation of symmetric/circular aggregates under bad solvent conditions.

In the case of the 32-arm star PB, some aggregates were formed (Figure 4.7e) and at the highest adsorbed amount the island growth led to a dense semi-continuous network of elongated narrow ripples (Figure 4.7f). The width of the ripples is comparable to the width of single collapsed 32-arm PB (in the intermediate adsorbed amount region). The average height of the aggregates remained relatively constant (Figure 4.8 or Figure 4.13) and similar to the average island height at intermediate adsorbed amounts. The aggregates height at the very highest adsorbed amount were slightly smaller but as discussed in the results section this height is underestimated due to convolution effects. Contrary to the case of the 18-arm star PB, for the 32-arm star PB the highest adsorbed amount does not result in the formation of individual aggregate islands but to a dense semi-continuous network of supramolecular stripes of star polymers with a much higher total surface coverage (almost double). The higher functionality stars have higher osmotic pressure and thus lower ability for interpenetrations and consequently more colloid like behaviour. Thus, they take laterally less extended conformations and they connect laterally only to few neighbours forming these linear semi-continuous aggregation patterns in analogy to two dimensional stripe-like structures predicted in simulations.\textsuperscript{23,24} It has to be noted that these simulations predict stripe-like structures stemming from purely repulsive interactions between particles "consisting of an exclusion zone plus a fine shoulder"\textsuperscript{23} which can be a simplified model of the repulsion associated with soft particles such as star polymers with sufficient number of arms. This raises the possibility that these structures were formed on the surface under good solvent
conditions before the evaporation of the solvent. However, there is also the possibility that these patterns arise upon the evaporation of the solvent due to the resulting attractive interactions in dry state. Measurements in good solvent conditions are required to clarify this issue which is beyond our current study and they will be pursued in the future.

The colloid-like behaviour is even more pronounced in the case of the 59-arm star PB where even for the highest observed adsorbed amount no aggregates were observed. In this case, the very high functionality leads to a very high osmotic pressure within the star preventing the interpenetrations between the polymers in good solvent conditions resulting in their individual collapse in bad solvent conditions, i.e. the star polymer has a stronger colloidal like behaviour. The average height again slightly decreased compared to the heights associated with the intermediate adsorbed amounts but this is also explained in terms of the convolution effect.

It is interesting to note that for the highest grafting densities, the smallest characteristic length (Figure 4.12) was observed for the 32-arm star. This is due to the fact that its functionality is not low enough to promote easy interpenetration and consequently large isolated aggregates (as in the case of 18-arm star) and not high enough to inhibit interpenetrations and consequently to result in isolated collapsed molecules (as in the case of 18-arm star). The preference for large isolated aggregates in the case of the low functionality polymer results in a relatively large characteristic length (at the highest adsorbed amount) compared to the characteristic length at low adsorbed amount.

4.5.3 Medium/long range order

As we have seen in Figure 4.12 the polymers have acquired isotropic distributions on the surface revealing some liquid-like ordering behaviour. As in the case of linear PB, it is highly unlikely that this effect is related to solvent instabilities due to its rapid evaporation and consequently our structures correspond to "frozen" conformations reflecting the molecular structure just before the rapid evaporation as has been reported in other studies. Hence, during adsorption and during the immersion of the sample in the pure toluene bath for several hours, the polymers have acquired close to equilibrated arrangements. In other words, our results suggest that the adsorbed polymers are capable of reconfiguring their position and conformation.
when in good solvent for sufficiently long times, expand on the surface and “feel” to some extent their neighbours at sufficiently high surface coverage. This might be due to the fact that although at the whole polymer scale, the molecule is expected to be relatively strongly adsorbed through the many monomer contacts with the surface, at the monomer scale the adsorption is relatively weak (to be expected for the non-specific interactions between mica and PB) and dynamic monomer adsorption-desorption effects can easily occur when in good solvent leading to a slow but finite mobility of the whole molecule on the surface. In support of our arguments, we note that the ordered structures we obtained are reminiscent of some ordered heteroarm star polymer morphologies originating from Langmuir-Blodgett monolayers (within which the mobility at the liquid-air interface is certainly enhanced)\textsuperscript{28} while they are very different to the relatively disordered (at the long and medium range) structures of polymers which have not been subjected to a good solvent bath treatment\textsuperscript{25}. This means that our adsorbed polymers when in good solvent behaved like a two-dimensional system of repulsive soft spheres; indeed, some of our structures (e.g. Figure 4.7f) are similar to theoretical predictions of “stripe phases from isotropic repulsive interactions”\textsuperscript{23, 24}. Thus, we conclude that our morphologies observed in dry state (bad solvent) are highly affected by the possibly equilibrated adsorbed polymer structure in good solvent conditions just before the evaporation of the solvent. It is also worthwhile noting that our results show that the formation of dimples (Figure 4.7d, Figure 4.9f) and/or ripples (Figure 4.7e, f) morphology at the highest surface coverage is not an exclusive property of mixed block-copolymer (linear\textsuperscript{27} or star\textsuperscript{29}) brushes but can also be observed in homopolymer systems such as ours at sufficiently high surface densities.

\textbf{4.6 Conclusions}

The star polymer adsorbed structures on flat surfaces was studied by atomic force microscopy. (i) At sufficiently low surface densities, we observed isolated molecules with flat conformation for low functionality stars while for stars with high number of arms the overall shape is more compatible with a colloidal like particle. We have argued that this behaviour stems from an intramolecular crowding effect related to multiple arms. Increasing the surface density, the results indicate a molecular re-conformation for low functionality stars due to the intermolecular crowding effect and their low internal osmotic pressure. The high functionality stars
appeared unperturbed. (ii) The increased interactions at intermediate surface densities for lower functionality stars resulted in small interpenetrations leading to aggregation. (iii) At even higher surface densities, increased and/or multiple interpenetrations of the low functionality stars led to the formation of elongated discontinuous islands and in some cases to the formation of semi-continuous networks of connected molecules. The high functionality star polymer continued to behave as a soft colloid and no aggregation behaviour was observed.

In most cases, at sufficiently high surface densities, an isotropic distribution of adsorbed polymer was observed indicating near to equilibrium positions and conformations just before the sudden change of solvent conditions. However, despite the new insights from our study in the dry state, it is important to do systematic AFM measurements directly in good solvent conditions. Furthermore, it has to be noted that our experimental protocol does not allow the development of surface densities much higher than the overlap density ($\sigma >> \sigma^*$) in which for high functionalities, more regular structures are expected to form.
4.7 References


Chapter 5

Conformational Time-Dependent Changes of Poly(isoprene-b-ethylene oxide) Block Copolymer (PI-PEO) Micelles on Mica in Ambient Conditions
5.1 Introduction

Smart responsive polymer coatings are surfaces designed to respond to external stimuli. The goal is to produce polymer modified surfaces able to change their properties (wetting, adhesion, roughness etc) through an external stimuli. Recent advances in this field include the synthesis of functional polymers with specific compositions and architectures, blending polymer materials with small amounts of additives and surface modifications by various physicochemical treatments. Most of these techniques have complicated surface preparation protocols and their fabrication is not always a trivial procedure.

In this chapter the atomic force microscopy (AFM) was used in tapping mode to study the time-dependent changes in the structure of poly(isoprene-b-ethylene oxide) block copolymer micelles on mica under ambient conditions. A conformational evolution of the polymer modified surface was observed with time. The time dependent behavior of the polymeric islands arises from the transformation of freshly cleaved muscovite mica from highly hydrophilic to less hydrophilic with the exposure time in ambient conditions.

5.2 Background

Mica has been proven to be a very "popular" substrate for studies of polymers on surfaces by atomic force microscopy (AFM). There are several reasons for the popularity of mica as it is cheap and easy to prepare by cleaving resulting in a clean atomically flat substrate free of contaminations. The latter is of great importance in AFM studies of polymer modified surfaces as low roughness will eliminate complexities originating from substrate surface roughness and the polymer-related features can be easily detected. Although polymer modified mica samples are widely imaged with AFM in ambient conditions not much attention has been given to the effect of the specific environment conditions on the sample morphology. A factor that makes mica not always a straight-forward substrate for the observation of adsorbed polymer structures on its surface is that mica in ambient conditions has a thin layer of water due to its high hydrophilicity when freshly cleaved. Moreover, it is known that after long exposure to air, mica becomes gradually less hydrophilic and
this occurs possibly by the accumulation of contaminant materials of organic origin always present in ambient conditions. This was verified by contact angle measurements of water on a freshly cleaved mica and on mica that was left after cleavage in ambient conditions\textsuperscript{5}. For the case of the freshly cleaved mica the contact angle was not possible to be measured (complete wetting) while the hydrated mica after several days showed a contact angle of $22.7 \pm 0.7^\circ$. Thus, extra care has to be paid for studies of polymers deposited/adsorbed on mica surfaces especially in the case of water soluble polymers.

Several studies using different techniques have focused on the adsorption of water on mica. Beaglehole et al.\textsuperscript{7} used ellipsometry to study the water adsorption on mica at $18^\circ$C, within a controlled relative humidity (RH) environment. They found that the average thickness of the water layer on mica increased with RH and varied from 0.2 nm for RH about 50% up to 2 nm for RH larger than 50%.

Hu et al.\textsuperscript{8, 9} studied the structure of water on mica by developing a new technique that they called scanning polarization force microscopy (SPFM) which exploits the electrical polarization of the substrate by a charged AFM tip. They found that for RH < 20% there was a uniform layer of water which they called phase I. It is important to note that a water layer was found even for RH < 5% and indicates that water on clean mica can be adsorbed even for really low RH values. For RH > 20% a second, bulk layer formed which grew as the humidity increased; this second layer was called phase II and was found immediately after the mica sample was cleaved pointing out that the adsorption of water happened rapidly. Mica exposed to ambient air for several hours did not reveal any surface structure related to phase II, even for really high humidity of 99%, confirming that mica becomes less hydrophilic with long exposure to ambient conditions.

Cheng et al.\textsuperscript{10} using high-resolution X-ray reflectivity to probe the water structure on muscovite mica under ambient conditions. Their results showed the adsorption of a first layer of water at height around 0.15 nm followed by a secondary layer of water with height of 0.25 nm. The second layer of water was immobilized through hydrogen bonds with the first and they proposed that this layer had a liquid structure.

Cantrell et al.\textsuperscript{11} used Fourier transform infrared (FTIR) spectroscopy and they found the first adsorbed layer of water on mica (phase I) to be more structured and rigid than bulk water due to a strong bonding with mica. The second layer (phase II)
was much less bound and can be considered to have the properties of bulk water. Their measurements show that the phase II layer thickness was around 1.25 nm.

Spagnoli et al.\textsuperscript{5} used tapping mode AFM to image mica in ambient conditions. They observed a discontinuous layer of interconnected water islands of several layers. Furthermore, by observing the behaviour of a deposited anionic polysaccharide on mica they confirmed the existence of an ordered bound first layer. The existence of the bound and rigid phase I has been also verified by molecular dynamics simulations\textsuperscript{12}.

Xu et al.\textsuperscript{13} used an AFM tip as a tool to deliver to or possibly remove water from mica in different RH environments. They brought an AFM tip in contact with mica for different contact times and subsequently imaged the mica by SPFM. For RH < 20\%, where only the presence of phase I was expected, they observed dielectric holes to appear in the water layer of phase I at the area of contact, which disappeared relatively fast with time (within some minutes). For RH in the region of 20-45\% the tip can form small water islands around the contact area even if very short contact times were used. The contact time controls the dimensions of the affected area. For RH > 45\% similar effects were observed as in the case of RH in the region of 20-45\% but this time the observed structures were mobile. Nevertheless, these phenomena of water transfer on mica due to the tip contact seems to have negligible importance for the case of imaging mica by tapping mode AFM as it has already been reported\textsuperscript{5}. Furthermore, even if the fast intermediate contacts of the tip with the surface during tapping mode disturb the water film on mica, i.e. create holes, the equilibrium reformations of the water film is rapid\textsuperscript{8,9}.

The importance of the water layer on mica when using AFM to image molecules in ambient conditions has been reported by Thomson\textsuperscript{4}. He reported that under common laboratory conditions of about 40\% RH, he could not get reproducible imaging of antibodies due to their motion on the mica owing to the adsorbed thin layer of water. Furthermore, Crampton et al. recently reported the mobility of monofunctional aminosilane, aminopropyltrimethoxysilane (APDMMS)\textsuperscript{14} and of Aminopropyl-triethoxysilane (APTES)\textsuperscript{15} on mica under ambient RH by AFM and they found a time dependent behaviour of the APDMMS layers on mica.

Kumaki et al.\textsuperscript{2} studied the conformation of isolated poly(styrene)-\textit{b}-poly(methylmethacrylate) diblock copolymers onto mica by AFM. They spread a very dilute benzene solution of the copolymer on a water surface and subsequently
deposited it onto mica. They reported changes of the diblock copolymer conformation on mica for different RH and explained this in terms of the different thickness of the water layer on mica due to the changes in the RH.

Amphiphilic block copolymers are copolymers consisting of a hydrophilic and hydrophobic block. In water (selective solvent for amphiphilic block copolymers as it is a good solvent for one of the blocks, but a bad one for the other) the copolymer tends to self-assemble above a given concentration (critical micelle concentration or cmc) forming well-defined micellar structures (multimolecular spherical micelles). The core of the micelle is formed by the insoluble blocks whereas the corona by the soluble blocks. Hence, it provides a very good model system for the study of water adsorption on mica and its surface properties as the behaviour changes from strong hydrophilic to less hydrophilic with the exposure time in ambient conditions.

The copolymer micelle conformations on surfaces has attracted considerably attention and many AFM studies have been published for both dried films\textsuperscript{16-18} and in solution with \textit{in situ} imaging\textsuperscript{19-21}. In the case of dried films the block copolymer micelles appeared collapsed and formed spherical caps. For adsorbed micelles in solution the corona, due to the good solvent conditions, would appear swollen and the micelle would maintain the spherical conformation it had in the bulk solution.

In this paper we present the shape evolution of amphiphilic block copolymers, single molecules and micelles, under ambient conditions on mica surface. The mica was left in ambient conditions and its evolution in behaviour from strong hydrophilic to less hydrophilic affected the structural conformation of the adsorbed amphiphilic molecules. We observed a difference in the behaviour between the shape evolution of micelles and small aggregates or single molecules. This system provides a proof of concept for the development of tunable roughness responsive surfaces.

\section*{5.3 Experimental Section}

\subsection*{5.3.1 Synthesis and characterization of the PI-PEO block copolymer}

The poly(isoprene-b-ethylene oxide) block copolymer (PI-PEO) was synthesized by anionic polymerization high vacuum techniques. Monomers and
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Solvent were purified to the standards of anionic polymerization using well established purification techniques\(^{22-24}\). Polymerizations were conducted in benzene using s-BuLi as the initiator. Isoprene was polymerized first at room temperature. The resulting polyisoprene (PI), under these experimental conditions, has a high percentage of 1,4 microstructure (~92% as determined by \(^1\)H-NMR spectroscopy). After completion of the polymerization of the first monomer, the predetermined amount of purified ethylene oxide was added in the polymerization mixture. The colour of the solution changed immediately from pale yellow to colorless due to the crossover reaction from living polyisoprenyllithium (PILi) to polyisoprene-ethylene oxide lithium (PI-EOLi). Under these conditions only one ethylene oxide unit is added to the PILi chain end, since anionic polymerization of ethylene oxide by Li as the counterion is not possible\(^{22}\). A predetermined amount of phosphazine base, as a solution in hexane, was subsequently added (phosphazine:Li = 0.9:1 molar ratio)\(^{24-26}\). The temperature of the solution was subsequently raised to 40°C and the reaction mixture was left for two days for completion of ethylene oxide polymerization. Active chain ends were deactivated with degassed methanol in the presence of a small amount of HCl. The polymers were precipitated in acetone, stabilized with 2,6-di-tert-butyl-p-cresol, at −20°C and dried in vacuum.

Molecular weights and molecular weight distributions of the precursor block copolymers were determined by size exclusion chromatography (SEC) using a Waters system, composed of a Waters 1515 isocratic pump, a set of three \(\mu\)-Styrargel mixed bed columns, with a porosity range of \(10^2\) to \(10^6\) Å, a Waters 2414 refractive index detector (at 40°C) and controlled through Breeze software. Tetrahydrofuran was the mobile phase used at a flow rate of 1.0 mL/min at 30°C. The set-up was calibrated with polystyrene standards having weight average molecular weights in the range 2,500 to 900,000 g/mol. No peaks corresponding to the PI block were observed in any case leading to the conclusion that the block copolymer is free from PI homopolymer impurities. Average composition of the copolymer and microstructure of the PI blocks were determined by \(^1\)H-NMR spectroscopy using a Bruker AC 300 spectrometer in CDCl\(_3\) at 30°C. The weight average molecular weight of the copolymer was found to be \(M_w=20,700\) with a molecular weight polydispersity, \(M_w/M_n=1.03\). The diblock copolymer contains 29wt% PI.
Dynamic light scattering measurements on aqueous solutions of the block copolymer were performed on an ALV/CGS-3 Compact Goniometer System (ALV GmbH, Germany), using a JDS Uniphase 22mW He-Ne laser, operating at 632.8 nm, and an avalanche photodiode detector, interfaced with an ALV-5000/EPP multi-tau digital correlator with 288 channels and an ALV/LSE-5003 light scattering electronics unit for stepper motor drive and limit switch control. Autocorrelation functions, from DLS measurements, were collected five times for each solution and they were analyzed by the cumulants method and the CONTIN routine. Typically correlation functions were collected for 20s. Fits to the correlation functions were made using the software provided by the manufacturer. A "probability one to reject" of 0.5 was used routinely in the CONTIN analysis software. Measurements were made at a scattering angle of 90° and at a temperature of 25°C on solutions of the amphiphilic block copolymer. Apparent hydrodynamic radii, \( R_{h,\text{app}} \), at different polymer concentrations were calculated by aid of the Stokes-Einstein equation,\( R_{h,\text{app}} = \frac{kT}{6\pi \eta_o D_{\text{app}}} \), where \( k \) is the Boltzmann constant, \( T \) the absolute temperature, \( \eta_o \) the solvent viscosity and \( D_{\text{app}} \) the diffusion coefficient calculated from the analysis of the correlation function at the particular polymer concentration. The copolymer was found to form micelles with a constant \( R_h = 41 \pm 1.0 \) nm, having a narrow size distribution (\( \mu_2/\Gamma^2 < 0.1 \)), where \( \mu_2 \) is the second cumulant and \( \Gamma \) the decay rate of the correlation function) in the concentration range studied (2 x 10^{-4} to 4 x 10^{-3} g/mL).

For fluorescence measurements pyrene (Aldrich, recrystallized from ethyl acetate) was used as a hydrophobic probe. A pyrene solution in acetone was prepared first at a concentration of 1mM. Fixed volumes of this solution were introduced with a micropipette in glass vials, the acetone was allowed to evaporate, and an appropriate volume of the aqueous block copolymer solutions of different concentration were introduced, in order to give a final pyrene concentration lower than 3 x 10^{-7} M. These solutions were allowed to equilibrate overnight and measured the next day.

Steady-state fluorescence spectra of pyrene probe in aqueous solutions were recorded with a double-grating excitation and a single-grating emission spectrofluorometer (Fluorolog-3, model FL3-21, Jobin Yvon-Spex) at room temperature (ca 25°C) using air-equilibrated solutions. Excitation wavelength was \( \lambda = 335 \) nm and emission spectra were recorded in the region 350-500 nm, with an increment of 1 nm, using an integration time of 0.5 s. The \( I_1/I_3 \) ratio was determined
as the average of three measurements (where $I_1$, $I_3$ are the intensities of the first and the third peaks of the pyrene fluorescence spectra at 372 nm and 383 nm respectively). The critical micellar concentration (cmc) for the PI-PEO micelles is found at a concentration of $1.44 \times 10^{-5}$ g/mL.

5.3.2 Preparation of the Micellar Solutions

The solutions were prepared by dissolving a known amount of the sample in the appropriate volume of ultra-pure deionized water with resistivity of 18.2 MΩ-cm. As an extra precaution the solutions were heated at 60 °C overnight before use to ensure complete dilution of the sample. The concentrations of the solutions were kept well above the cmc, around $2 \times 10^{-3}$ g/ml. Before use the solution was filtered through 0.45μm hydrophilic Teflon Millipore filters.

5.3.3 Samples preparation for AFM measurements

A droplet of a deionized water solution of PI-PEO block copolymer was deposited on freshly cleaved mica (Agar Scientific, Essex, UK) for 5 minutes and the sample was rinsed very gently to remove some of the solution from mica. The sample was gently dried under a stream of nitrogen. Subsequently, it was placed in a clean glass Petri-dish and imaged with AFM in ambient conditions.

5.3.4 Atomic Force Microscopy (AFM)

All the AFM experiments have been performed in air using a Veeco AFM, Multimode/Nanoscope IIIa (Veeco, Santa Barbara, CA, USA) equipped with an E-scanner™ (x-y scan range: around 14 μm). The samples were imaged in tapping mode (tip in intermediate contact with the surface). The RTESPA Veeco cantilevers with a nominal spring constant and resonance frequency of 40 N/m and 300 kHz respectively were used to image the samples. In general “light tapping” was used by keeping the set-point quite high (but without losing contact) in order to minimize the interaction force between the tip and the substrate. The cantilevers were oscillated vertically 5% below their natural resonance frequency, and moved in a raster fashion within a specified region of interest. Images were processed and the island heights were measured using the commercial software Scanning Probe Image Processor (SPIP,
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Image Metrology). In order to avoid any influence of the sample from the laser (e.g. temperature increase) or any capillary necking events between the tip and the sample, the laser was switched off and the tip was retracted few micrometers during the time between consecutive scannings.

5.4 Results and Discussion

In this section we will discuss the time evolution of PI-PEO micellar structures adsorbed on mica. The preparation of the sample was extremely crucial as the micelles should be considered to be deposited rather than adsorbed on mica. This is because the hydrophilic PEO at the corona of the micelles does not interact strongly with the hydrophilic mica resulting in polymer structures that can be washed easily by an aggressive rinse. The mica sample was placed in a clean glass Petri-dish in ambient conditions. During the experiments the RH was higher than 55% and the temperature was approximately 21 °C. In the series of experiments presented here, the first measurement, was obtained 133 min after the sample was prepared and the last after 3240 min.

In Figure 5.1, AFM images (3.66 x 3.66 μm²) of mica from 133 min up to 2740 min after the sample was made are shown. AFM images were taken also for 2980 and 3240 min but no significant changes were observed for both the lateral and vertical dimensions of the polymeric island indicating that the time evolution of the structures had reached a plateau. As it was described in the experimental section between scans the tip was retracted a short distance away from the mica substrate and upon the re-approach, short displacements of the area of interest were observed. The observed displacements on the images originate from thermal drifts and hysteretic behaviour of the piezocrystal scanner. Hence, few islands were present in all captured images. Four of these structures in this series are indicated within the white boxes in Figure 5.1. For simplicity from now on we will refer to these islands as; structure A for the island in the top left box, structure B for the island in the top right box, structure C for the island in the bottom left box and structure D for the island in the bottom right box.
The images in Figure 5.1 show the shape evolution of the polymeric islands with time. For short time, i.e. 133 min, the islands appear in irregular, non spherical shape in the lateral directions (Figure 5.1a). This contradicts other micelle structure observations, both in good and in bad solvent conditions, according to which micelles appeared circular in lateral directions\(^ {16-21} \). In our case the laterally circular structure is a gradual time evolution product, Figure 5.1h).

The RH during the experiment was higher than 55% hence phase II of water is expected to be present on mica. As it has already been discussed in the introduction part the phase II of the adsorbed water on mica appears as a bulk layer of water and conforms as a discontinuous layer of interconnected water islands with high mobility\(^ {5, \textit{8,9}} \). Studies by ellipsometry showed that this non-continuous layer of water (phase II) can be 2 nm in height for RH > 50% (similar RH conditions as in our experiments).

The micelles have a PI core and a PEO corona. The diblock copolymer molecule used contains 71wt% PEO resulting in a water soluble corona as the dominant part of the micelle. Consequently, the PI blocks of the molecules forming the core are relatively short with molecular weight of 6 kg/mol. Krishnamoorti \textit{et al.}\(^ {27} \) measured the entanglement molecular weight \( (M_e) \) for PI to be 6.37 kg/mol. Thus, in our case the molecular weight of the PI block is smaller than the \( M_e \) and hence we do not expect significant number of entanglements between the PI chains in the core. Moreover, in room temperature the PI is expected to be liquid-like since its \( T_g \) is -67
°C (or even lower as the $T_g$ of thin polymer films decreases from its value in bulk$^{28}$). Furthermore, studies showed that small PS-PVP-PEO micelles and hence with small PS cores are soft and deformed after deposition on mica$^{29}$ while larger PS-PMA micelles with larger PS cores deposited on mica appeared stiff and they did not deform$^{30}$. Hence, it is reasonable to expect for our much more flexible PI-PEO micelles to easily take the shape of the asymmetric water islands of phase II.

In Figure 5.2 (left) horizontal profiles of structures A, B, C and D for the case of 133 min after the sample was prepared show that all the structures were flat and with similar heights of approx. 5 nm even though, as it is clear from the AFM images of these structures, they do not have the same lateral dimensions. The lateral asymmetry of the islands in combination with that fact that they were flat, with similar heights along the surface, indicate that the micelles were confined within the adsorbed water on mica and the water-air interface. The measured height of the phase II adsorbed water of mica was found$^7$ to be 2 nm while here the flat conformation of the polymeric islands is approx. 5 nm in height. This increased height is compatible with a relatively swollen layer of hydrophilic PEO blocks and a collapsed PI layer on top at the water-air interface. We can assume that the micelles through the hydrophilic PEO blocks took the shape of the phase II irregular water islands but they also acted as a "sponge" and absorbed the bulk mobile water of phase II. Owing to the local good solvent conditions the PEO corona of the micelle will be relatively swollen.

By zooming in the islands that appeared in all the AFM images in this series more details were captured concerning the conformational evolution of the islands (Figure 5.3). It is clear that the structures shrank taking more circular shape with time. With time the amount of the adsorbed water on mica decreases and the absorbed
micelles will start to shrink as the attractive interactions of the hydrophobic PI core will dominate. For the case of structure A, left column in Figure 5.3, it is clear that a "secondary" sub-structure, indicated with an arrow, evolved with time in a different fashion and almost disappeared with time. We will discuss in detail and give possible explanation of this phenomenon later on.
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Figure 5.3: AFM topography images of structures A, B, C and D at various times after the sample was prepared. The columns from left to right correspond to structures A, B, C and D respectively.
In Figure 5.4a, b c, d and e AFM images—snapshots of the time evolution of structure C are shown for 133, 598, 745, 1555 and 2740 min respectively after the sample was prepared. Vertical profiles indicated with the white line in each AFM image are plotted in Figure 5.4f. With time the polymeric island shrank laterally and expanded vertically.

![AFM images of structure C](image)

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**Figure 5.4**: AFM height images of structure C for a) 133 min, b) 598 min, c) 745 min, d) 1555 min and e) 2740 min after the sample was prepared with the corresponding profiles f) (indicated with the white line in each AFM image).

The profiles appear clearly higher ultimately by a factor of almost four and the shape changes from flat to progressively narrower. It is well known that AFM images are always a convolution of the geometry of the tip and the shape of the object being imaged. As it has been reported in AFM studies on single molecules/particles the
convolution effect can generally overestimate the lateral dimensions of the object while the height is much more realistic\textsuperscript{31}. Using simple calculations the effect of the tip geometry on the measured volume of an object was checked. Assuming spherical caps of constant volume and changing the maximum height it has been found that the convolution effect is higher for the higher spherical caps (chapters 3 and 4). This is reflected on the profiles in Figure 5.4 where it is obvious that while the height changes dramatically (400\%) the width changes weakly (maximum of approx. 30\%). Higher objects “suffer” more form the convolution effect and the width will be much more overestimated.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5_5.png}
\caption{Height of the island against the time for; (top left) structure A, (top right) structure B, (bottom left) structure C and (bottom right) structure D.}
\end{figure}

In Figure 5.5 the height against the time is plotted for each structure and it can be clearly seen that the height evolution shows similar behavior. The height converges to a specific plateau value after a long time, 2740 min, indicating that the observed evolution was terminated. In Figure 5.6 the ratio of the height over the maximum/plateau height of each structure, obtained after 2740 minutes, is plotted.
with the corresponding logarithmic fit for each case. It is clear that for all cases this evolution has a logarithmic behavior with a similar coefficient.

![Graph](image)

**Figure 5.6:** Normalised height versus time for structures A (red circles), B (blue squares), C (green triangles) and D (pink diamonds) with the corresponding logarithmic fit (red, blue, green and pink respectively).

In Figure 5.7a to h show high contrast AFM images of the “secondary” sub-structure of structure A, indicated by the arrow in Figure 5.3. The height evolution of this sub-structure (Figure 5.7i), shows that the height increased up to 598 min and then decreased. At the beginning, i.e. 133 min after the sample was prepared the island appeared with similar height as the other structures, i.e. around 5 nm. For the case of 2740 min after the sample was prepared the island appeared short with a maximum height of approx. 1 nm. The AFM image profile for this case (Figure 5.7j) shows that this island appeared with a bimodal conformation as there are areas with height of approx. 1 nm, corresponding to the red regions of the high contrast AFM image, and a wider area of the island with height around 0.4 nm, corresponding to the green region. After a long exposure of mica to air the phase II water on mica is expected to be absent as the mica with time becomes less hydrophilic. Nevertheless, water layer of phase I still exists on the mica with a height of approx. 0.2 nm. This height is slightly less than the measured height of the green layer in the area of the sub-structure after 2740 min since the sample was prepared.
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In a selective solvent diblock copolymer solution and above the cmc, it is expected that there is a co-existence of micelles with single molecules. Hence during the deposition of the solution on the freshly cleaved mica both micelles and single diblock PI-PEO would be deposited-adsorbed on mica. Owing to the fact that the PEO blocks within the large micellar structures can act as a sponge for water (there should be a coupling between the water and the PEO blocks) we would expect the single diblocks to be concentrated around the micelles. For a short time after the sample was prepared and due to the presence of the thick water layer of phase II both micelles and single diblock molecules would concentrate in the regions where the phase II water exists since it is a good solvent for the PEO block of the molecule. Nevertheless as the phase II water gradually disappears single diblock molecules will behave differently.
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since the large hydrophilic block will take laterally extended flat conformations and will dominate over the smaller PI block. At the same time the hydrophobic PI blocks come together in order to minimize the area of contact with the unfavourable water, forming a "small" assembly of a few diblock copolymer molecules. The low height (0.4 nm) areas (green) of the sub-structure can be associated with the laterally extended PEO blocks while the 1 nm height areas (red) can be associated with the PI block aggregates (Figure 5.7h).

Kumaki et al.² found a similar behaviour for the poly(styrene-\textit{b}-methyl methacrylate) block copolymers (PS-PMMA) on mica. They deposited PS-PMMA molecules on mica by the Langmuir-Blodgett (LB) method using a low surface pressure during deposition to achieve isolated molecules of PS-PMMA. The spreading conditions were similar with the conditions used in previous published studies where using this method PS single-chain particles were obtained³², ³³. By imaging the samples with AFM in their laboratory ambient conditions, RH of 50-60%, they found aggregations of a few PS-PMMA molecules. The aggregates appeared with a relatively high core, consisting of assembled PS blocks, with a short and more extended monolayer of the PMMA blocks around them. They argued that the decrease of the water layer thickness made the PMMA layer expand laterally and the whole structure to appear shorter. They measured that at the maximum lateral extension the thickness of the PMMA monolayer was 0.2-0.5 nm. It has to be noted that although PMMA is not a water soluble molecule it can interact with water due to attractive interactions between the \textit{–COOCH}_3 groups with water. Roiter et al.³⁴ studied the conformation of adsorbed flexible polyelectrolyte chains. They found that for pH corresponding to good solvent conditions for the polymer the molecules appeared as extended coils with height of around 0.4 nm. Their AFM images also revealed points of chain self-superposition which were considered as chain loops with height of around 0.8 nm. These observations are in good agreement with our measured height of 0.4 nm for the PEO region observed in our case. Due to the small height of the phase I water layer no chain loops were expected in our case. Furthermore, the PEO monomer has \textit{~0.44 nm} length and less than 0.2 nm height³⁵.

This secondary sub-structure is certainly not unique. Similar observations were made for areas around other islands. A very interesting result is that the areas rich in single PI-PEO diblock copolymers are around the regions where the big islands/micelles were at the beginning, i.e. 133 min after the sample was prepared.
This strengthens our assumption that the polymer structures, micelles and single chains, are concentrated and take the shape of the asymmetric mobile water islands of phase II, probably slightly modified from the phenomenon described above as the "sponge" action of the hydrophilic corona of the micelles. With time the phase II water layer started to disappear making on the one hand the micelles to shrink and on the other hand the small and more flexible single molecules to gradually confine themselves within the short water layer of phase I.

5.5 Conclusions

The time-dependent evolution of the shape and height of the PI-PEO block copolymer modified mica in ambient conditions was observed by atomic force microscopy. The PI-PEO micelles adsorbed/deposited on mica showed a logarithmic growth of their height with time. The AFM images revealed that during this growth the lateral shape of these initially flat micelles changed gradually from asymmetric/irregular to circular. The observed phenomena were driven by the change of mica from strongly hydrophilic to less hydrophilic with the exposure to air in ambient conditions. During this change the initial mobile water layer of phase II disappeared resulting in weaker interactions of the hydrophilic PEO corona of the micelles with the surface making more effective the attractive interactions of the hydrophobic PI core. Hence, the micelles shrunk obtaining, the expected behaviour for bad solvent conditions, spherical-cap like shapes. The behaviour of the co-existing single diblock copolymer molecules was different as they laterally expand and become shorter with time. These molecules do not belong to any supramolecular structure, are small, mobile and "free" to take advantage of the existence of the very thin (~0.2 nm) water layer of phase I.

We consider this study to be early evidence of roughness tunable responsive surfaces with the changes of the RH. In any case, our system cannot be considered reversible as the mica changes permanently its properties with time (it becomes less hydrophilic). Nevertheless inspired from our observations we believe that a surface with tunable hydrophilicity in a controlled and predicted manner (i.e. surfaces that alter their hydrophilicity by changing the applied voltage) in combination with amphiphilic polymers (single molecules or assembled in supramolecular structures) can lead to the fabrications of smart and "intelligent" surfaces.
5.6 References

Conformational Time-Dependent of PI-PEO on Mica

Part B

Polymer Microbubbles
Chapter 6

Structural Investigation of Polymer Microbubbles
6.1 Introduction

Microbubbles are stable, haemodynamically inert\(^1\),\(^2\) and sub-capillary sized gas, encapsulated in thin shell and are smaller than the smaller blood vessel of a human body, that are used as ultrasound contrast agents (UCAs) to allow improved visualisation of vascular bed\(^3\),\(^4\) and differentiate vascular patterns of tumours non-invasively. Animal and human studies in the myocardium\(^5\)-\(^8\), kidney\(^9\) show that is possible to quantify perfusion non-invasively by means of UCAs-enhanced imaging. The differentiation of vascular patterns for tumours\(^10\)-\(^16\), as well as transplant pathology\(^17\) are also on going areas of investigation.

The potential of site-targeted microbubbles expands the field beyond perfusion imaging and allows attachment of specific markers of disease\(^18\). Post-ischemic inflammatory responses in the kidney\(^19\) or in the myocardium\(^20\) can be detected. The techniques promise to detect inflamed plaque in its early stage. Thrombus targeting\(^18\), \(^21\), and imaging therapeutic angiogenesis\(^22\) are possible. The expansion of microbubble technology to include localised drug/gene delivery is potentially very valuable. Myocardial transfection using microbubbles has recently been reported\(^23\)-\(^25\). Microbubble research illustrates that a powerful gene delivery tool with simultaneous pathology monitoring is feasible.

It is important to appreciate that the UCAs are different from all other medical imaging contrast media in that they have a very complex interaction with the ultrasound. Microbubbles oscillate, coalesce, explode or fragment in the presence of ultrasound field and as a result emit the ultrasound pulses or variable spectral and energy characteristics. By altering a parameter of the ultrasound beam (frequency, pulse length, amplitude, phase, pulsing rate) and/or a parameter of microbubble constitution (shell elasticity, gas solubility etc) it is possible to control the microbubble response and as a result improve the sensitivity of imaging them\(^26\). New modalities that detect microbubble non-linear signals\(^9\),\(^27\), make use of microbubble destruction\(^28\)-\(^31\), and reject background tissue signals\(^32\)-\(^34\) have been developed and included in the ultrasonic equipment, but are based on a very basic understanding of the microbubble properties.

Nevertheless the most important obstacle in the development of the microbubble technology remains the lack of understanding of microbubble behaviour,
which has been compromised by the lack of experimental data. A thorough scientific knowledge of these properties would lead to optimal use of contrast microbubbles and improve visualisation of microvascularity. Up to date the mathematical modelling of microbubble behaviour still limited predictive value, due primarily to a lack of reliable methods for establishing the mechanical properties of the microbubble shell\textsuperscript{35-40}. Models either assumed a fixed behaviour for the shell properties\textsuperscript{41} or used a best fit to experimental data\textsuperscript{42}. Microbubble science is evolving today into a complex multidisciplinary research area, but there are still important cross-disciplinary barriers yet to be bridged.

Since its introduction\textsuperscript{43}, the atomic force microscopy (AFM) has provided an unprecedented spatial and force resolution of the order of Angstroms and subnanonewtons, respectively\textsuperscript{44-46}. It is, therefore, ideally suited to study the morphology and the mechanical properties of materials at the nanometer scale. AFM can simultaneously record structural\textsuperscript{47-49}, viscoelastic\textsuperscript{50, 51}, and frictional properties\textsuperscript{52}. Detailed mapping of the forces of the gas-liquid interface of bubbles is possible\textsuperscript{53}. The introduction of the AFM into microbubble science is proposed here as a means of nanoscale imaging.

### 6.2 Experimental Section

#### 6.2.1 Materials

The biSphere\textsuperscript{®} (Point Biomedical Corp, San Carlos, CA, USA) microbubbles were used in this study. The microbubble size (diameter) ranges from 2 to 6 µm (mean diameter about 4 µm) and its structure is presented in Figure 6.1. The inner core is nitrogen gas, there is a thin shell of a stiff structural biodegradable polymer to stabilize the bubble (red layer) and there is an albumin gel layer to make the biocompatible microbubble harmless for the human body. According to the manufacturers the bulk (macroscopic) elastic modulus of the material constituting the structural shell is in the range of 1.4 to 2.8 GPa.
According to the manufacturers the thickness of the outer layer (hydrogel) is in the range of 10 nm and should not change with the microbubble size. The thickness of the structural polymer shell can be 30 nm for a microbubble with 4 \( \mu m \) diameter. The shell thickness is approximately linear to the microbubble size, i.e. the ratio of the shell thickness to the microbubble diameter is similar across the population of the microbubbles. Figure 6.2 shows the relation of the polymer shell thickness with the size of the microbubble according to the data provided from the manufacturers. The linear fit shows that the increase of the microbubble shell with the size of the microbubble is 7.5 nm/\( \mu m \)

Although the absolute values of the shell thickness are very approximate (e.g. variations could be in the range of 50\%) the linear behaviour between the shell thickness and the microbubble radius is reliable\textsuperscript{54}.
6.2.2 Sample Preparation

As AFM experiments require the interrogated objects to be motionless, Petri dishes were coated with a poly-L-lysine solution (1:10 with deionised water). Poly-L-lysine was purchased from Sigma-Aldrich (Sigma-Aldrich Co. St. Louis, USA). As a microbubble is air-filled it behaves as buoyant in a water suspension and will float at the water-air interface. Hence, the adsorption of the microbubbles took place by positioning the coated dishes on the top of microbubble suspension in order to bring them into contact with the floating microbubbles, Figure 6.1.

![Schematic illustration of the adsorption of biSpheres® on a Petri-dish pre-coated with Poly-L-lysine](image)

Figure 6.3: Schematic illustration of the adsorption of biSpheres® on a Petri-dish pre-coated with Poly-L-lysine

6.2.3 Atomic Force Microscopy (AFM)

For the present study we used the Bioscope® AFM with a Nanoscope IV controller (Veeco, Santa Barbara, CA, USA), which was mounted on a Zeiss Axiovert-200 inverted optical microscopy (Carl Zeiss Ltd., Herts, UK). Tapping mode AFM imaging was employed to investigate the biSphere® microbubble topography. We used NP-20 Veeco V-shaped cantilevers with nominal spring constant 0.06 N/m. This tip has a pyramidal shape with a tip half-angle 35° and radius of curvature of 20 nm. In this mode, the cantilever is made to oscillate vertically at 5% below their natural resonant frequency and to move in a raster fashion within a specified region of interest. Optical microscopy was used in order to bring the tip at the vicinity of the microbubble and image the microbubble. The piezo scanner used had a lateral range of 115 µm. The resonant frequency of the cantilever in liquid was between 5 to 8 kHz; their tuning was performed at 200 nm above the surface of the microbubbles as the
resonant frequency shifts slightly in the vicinity of solid surfaces. All measurements were taken in filtered and deionised water (18.2 MΩ-cm) and at room temperature. To ensure accurate distance measurement in the interrogated 3D space, a calibration of the piezo that controls the movement of the cantilever was performed by imaging a standard calibration grid of known dimensions.

As the microbubble sizes range from 2 to 6 μm initial individual microbubble topographies were acquired for square areas of 10 to 20 μm in a raster fashion with scanning rates of 0.1 to 0.5 kHz. The cantilever oscillation amplitude fraction that achieves contact with the sample during imaging, known as the amplitude set point, was set at approximately 95% of the full amplitude of the cantilever oscillation. The experiment turned out to be very delicate because the z-range of the piezo is in the range of the bubble size. Image processing of the raw data of the captured topographies were performed the using the commercial software Scanning Probe Image Processor (SPIP, Image Metrology). The images were flattened with a standard plane fit to correct for the cantilever tilt and the piezo bow. Microbubbles sizing and roughness measurements were carried out using the same software.

6.3 Results and Discussion

Several microbubbles were imaged and in Figure 6.4 a typical one is presented. The area of imaging is 7.4 x 7.4 μm². Figure 6.4b is a 3D display of Figure 6.4a. The scanning rate was 0.4 Hz, and the sampling was 256 lines by 256 points per line. Figure 6.4b allows easier observation of the three dimensional shape of this topography. It can be seen that the structure is not spherical but a convolution of a sphere and a pyramid, as the AFM images are a convolution of the tip shape, which in this case is pyramidal, and the microbubble. Similar images have been captured using bacteria and small spheres⁵⁵. As the schematic drawing in Figure 6.4c demonstrates, the sides of the pyramid structure in Figure 6.4a are created by the contact of the sidewall of the tip on the microbubble surface, whereas the top of the structure is created by the contact of the tip apex with the top of the microbubble. If the tip shape is sharper, which can practically be achieved with a carbon nanotube⁵⁶, then the topography will depict the entire top half of the microbubble sphere. As the pyramidal angle is larger than zero the topography depicts a section of the sphere that is a section
of the top hemisphere of the microbubble. In our case, the solid angle of the sphere section is approximately $180^\circ - 2 \times 35^\circ = 110^\circ$ ($35^\circ$ being the tip half-angle). In addition, in the case of Figure 6.4 the cantilever is tilted at an angle of $5-10^\circ$ with respect to the horizontal so this angle is not centred over the microbubble. Using a well-characterised topography, it is possible to reconstruct the tip geometry with 1 nm accuracy\textsuperscript{57}, and subsequently to deconvolve its geometry from the captured topography of interest\textsuperscript{58}. This can alter the appearance of the topography to make it closer to a spherical cup, but not necessarily provide a significant improvement in the topography information at the top of the microbubble.

Apart from the tip geometry the topography may also be affected by the difference in compliance between the tip and the sample\textsuperscript{44}, especially when the loads applied from the cantilever tip are significant for the stiffness of the sample which may lead to sample deformation. Provided that this is not the case tapping mode is ideal for imaging soft and compliant samples since the forces are minimal.

Figure 6.4d shows a zoom into the $3.5 \times 3.5 \mu m^2$ dashed square area shown in Figure 6.4b, while Figure 6.4e shows a zoom into the $2.8 \times 2.8 \mu m^2$ dashed square area shown in Figure 6.4d. All zooms demonstrated that the topography was acquired highly reproducibly, and the features of the microbubble were accurately reproduced in all scales. In Figure 6.4e details of the microbubble top surface as small as 30 nm in lateral size and 2 nm in height are revealed. The lateral resolution on the top of the microbubble is mainly determined by the radius of curvature of the tip apex which is nominally 20 nm. Apart from using a sharper tip, in order to improve the spatial resolution in the present case, a lower range AFM piezo can be used to improve spatial resolution. We note that the AFM is, in principle capable of atomic resolution but only on very flat surfaces\textsuperscript{59, 60}. This is not the case for our interrogated microbubbles. The advantage of AFM over SEM is that the microbubbles need to be subjected to a low temperature or low-pressure environment. The microbubbles were interrogated in a liquid environment, which could potentially simulate physiological environment.
Figure 6.4: a) Topography of a biSphere® microbubble using tapping mode AFM (7.4 μm x 7.4 μm). This image can also be displayed in 3D (b) which shows a pyramid-like structure. The topography is a convolution of the tip of the AFM cantilever with the microbubble. The top of this pyramid is the microbubble topography. The sides of this pyramid are due to the contact of the sides of the tip with the microbubble, as shown in (c). A zoom into the dashed area of (b) was performed and the resulting topography is (d). A further zoom into the dashed area of (d) gave (e). The horizontal line in (a) corresponds to the topography profile shown in (f). The zero-point in the z-range is arbitrary.

Figure 6.4f is the height profile along the line shown in Figure 6.4a. The microbubble shape appears asymmetrical in this profile owing to the cantilever tilt mentioned above. The profile of Figure 6.4f provided the highest point of the microbubble which was at 2.77 ± 0.02 μm. However, as the microbubble was rough
Structural Investigation of Polymer Microbubbles

this does not represent the average height or diameter of the microbubble. A sphere was fitted to the microbubble profiles and the highest point of this sphere was found at $2.65 \pm 0.06 \, \mu\text{m}$. However, the assumption of sphericity may seem reasonable for the top part of the microbubble, as it appears also from the topographic traces, but it is not known whether it holds for the whole microbubble as the adhesion with the Petri dish might affect this sphericity at the bottom of the microbubble. The measured height is therefore an approximate estimate of the true microbubble diameter. The diameter can also be assessed by measuring the lateral dimensions of the convolved topography (Figure 6.4b), and then calculate the contribution of the tip geometry in order to deduce the diameter of the microbubble. This schematic is shown in Figure 6.5.

![Figure 6.5](image.png)

Figure 6.5: Schematic drawing showing how a spherical object (red) appears due to the convolution of the geometry of the AFM tip and the shape of the object being imaged (yellow).

By using simple geometrical arguments and considering a solid spherical object on a flat substrate we calculated the real width ($d$) in terms of convoluted width ($w$), height ($h$) and angle $a$, equation 6.1:

\[
d = w - h((1 + \sin a) \tan a - (1 - \cos a))
\] (6.1)

From the corresponding profile of the microbubble topography in Figure 6.4f we obtained the measured convolute width ($w$) and height ($h$) to be $w = 6 \, \mu\text{m}$ and $h = 2.8 \, \mu\text{m}$, respectively. Using equation 6.1 we calculated the real width ($d$) to be $d = 3.9 \, \mu\text{m}$, i.e. the width is similar but not equal to the measured height, leading to an aspect ratio of 0.71. The fact that the width appears bigger than the height can be due to a variety of reasons; the tip angle can be bigger than what the manufacturers claim, also
the tip is slightly tilted and the above model assume that the cantilever is parallel to the horizontal is not very accurate and as it was mentioned above the adhesion of the microbubble with the Petri dish might affect this sphericity. In any case, This indicates that a simple deconvolution of the size of the microbubble is not straightforward.

Information on the microbubble structure and roughness was obtained using the SPIP image analysis software once the topographies were highly reproducible. The root-mean-square roughness $S_r$ was 85 nm the roughness average $S_a$ was 64 nm and the peak-to-peak roughness, which represents the height difference between the highest and the lowest points on the microbubble topography, was 617 nm. These measurements are the first, to our knowledge, to demonstrate that microbubble shells have can be defected. It has been shown that these microbubbles have displayed more than one distributions of scatter behaviour when subjected to an ultrasound field$^{61}$. Today, we know that some biSphere® microbubbles leak their gas outside their shell to produce an efficient ultrasound scatter, in what has been termed “sonic cracking”$^{62}$. This has been speculated to be due to shell defects, but also to other ultrasound wave related effects. The observations in this chapter confirm the former and may provide in future investigations detailed statistics of the state of the shell topography. Indeed, such observations should have an impact in the design and engineering of microbubbles, and assess the reproducibility of the manufacturing process. In conclusion, AFM offers a valuable bridge between the biochemistry that is responsible for the manufacturing of microbubbles, and ultrasound physics and engineering that are set to exploit their properties as efficient scatterers of sound for usage in diagnostic imaging applications.

6.4 Conclusions

The applications of microbubbles range from ultrasound contrast imaging to biologic targeting and drug/delivery. AFM was used in tapping mode imaging to reveal topographical detail of the biSphere® microbubbles and it was introduced here as means to interrogate microbubbles that are used as contrast agents in ultrasonic imaging. Because microbubbles are comparable to the size of usual AFM tips, a convolution between the AFM tip and the microbubble was typical of the acquired topographies making the microbubble appear with pyramidal asymmetrical shape and
with overestimated width. Part of the half microbubble was imaged with nanometre resolution and detailed roughness measurements were reported. The advantage of AFM over scanning electron microscopy, a technique that also can provide topographical images with equal high resolution, is that it can give 3D information of the topography and also can be performed in liquid environment (that potentially could be a simulated physiological environment) and room temperatures in contrast with the SEM that requires low pressures and temperatures.
6.5 References


Structural Investigation of Polymer Microbubbles


Chapter 7

Mechanical Behaviour of Polymer Microbubbles
7.1 Introduction

Mechanical properties are among the most important and certainly the least understood physical properties of supramolecular polymer structures. Similarly in microbubble science the quantitative understanding of these parameters remains a significant challenge for microbubble modelling and experiments. The existing experimental techniques used in this field, such as optical and acoustical ones, can not quantify the microbubble mechanical properties. Furthermore, mathematical modelling of microbubble behaviour still has limited predictive value, due primarily to a lack of reliable methods for establishing the mechanical properties of the microbubble shell. Hence, measurements on individual microbubble properties are essential for the advancement of microbubble science and have the potential of guiding/aiding both modelling and applications. In particular the measurement of the mechanical properties of the microbubbles can provide insights of the shell structure and behaviour.

Motivated by the fact that the AFM has been proven to be not just a tool to image the topography of solid objects at high resolution but it can be used to give information for local material properties such as elasticity, hardness, Hamaker constant, adhesion and surface charge densities, we applied the AFM to assess the mechanical properties of individual microbubbles. The AFM has become an essential tool for researchers in various fields such as physics, materials science, surface science and biology. The contribution of the AFM in all these fields has already been reviewed\(^1\). We have already shown in the previous chapter that AFM is capable of probing structural properties of the microbubble shell at the nanometer scale. We now present a systematic study of the mechanical properties of the microbubbles using AFM and especially force-vs-distance curves.

7.2 Materials and Methods

For the present study we used the MFP-1D (Asylum Research, Santa Barbara, CA, USA). The AFM was mounted on a Nikon TE2000U (Nikon UK Limited, Surrey, UK) inverted optical microscope in order to allow both the placement of the
microbubble below the cantilever before the force measurements and the determination of the measured microbubble size (Figure 7.1).

![Figure 7.1: a) Optical microscopy image of the cantilever placed in the vicinity of a microbubble and b) the cantilever placed on top of the microbubble. The red scale bar at the right bottom of the images corresponds to 15 μm.](image)

To apply compression of microbubbles and interrogate their mechanical properties tipless cantilevers NSC-12 and CSC-12 (MikroMasch, Tallinn, Estonia) with aluminium back coating and typical spring constant ranging from 0.08 to 2.3 N/m were used. The spring constant of the cantilevers were determined automatically using a routine incorporated within our instrument. It is based on a method proposed by Hutter and Bechhofer and involves the measurement of the intensity of the thermal noise.

The raw data obtained from the MFP-1D AFM represents the deflection $\Delta$ of the cantilever versus the position of the piezo during loading (approach or trace) and unloading (retraction or retrace). The force $f$ was determined from the cantilever deflection by multiplying $\Delta$ with the spring constant ($k_c$) of the respective cantilever. The cantilever sensitivity during the measurements was determined from a reference curve on a hard surface taken prior to measurements on microbubbles. The deformation was calculated as the difference between the position of the piezo and the deflection of the cantilever measured on a hard surface. The zero of the separation was taken at the point of the first measurable force. To ensure that no changes occurred in the sensitivity during the measurement on microbubbles, a reference curve on a hard surface was also recorded after a microbubble measurement.

The Gaussian profile of the diode light beam’s cross section can introduce non-linearity effects which can be severe at high deflections. For this reason a tipless cantilever ($k_c = 1.26$ N/m) was used to acquire a force-vs-distance curve on a clean
hard surface (optical microscopy glass slide) in order to check the extent of the effect on the recorded data.

Figure 7.2: Force-vs-separation curve on a hard surface using the MFP-1D AFM.

Figure 7.2 shows a force-vs-separation (both trace and retrace) curve obtained by processing the raw data recorded from the AFM with the method described in chapter 2. The point (0,0) corresponds to the contact point of the cantilever with the surface, and the positive separation corresponds to compression or indentation. It is clear that after a certain amount of the applied force (~ 200 nN) the curve departs from the zero separation. This is in contradiction with the expected behaviour of a force-vs-separation curve on a hard surface, where after contact no compression-indentation was expected with further increase of the applied force. Looking at the corresponding force-vs-position curve of the cantilever on a hard surface, it is clear that the curve is non-linear (contrary to the expected Hooke's law) and there is a small bend. This non-linearity is revealed by the fact that a second order polynomial fits better the curve than a linear fit.
By subtracting the cantilever deflection from the force-vs-distance data using the polynomial equation acquired from the 2nd order polynomial fit, the force-vs-separation curve on a hard surface did not diverge from the vertical axis for any applied force showing that no compression or penetration occurs on a hard surface with the applied load (Figure 7.4). The results presented in this chapter were processed in a similar way using a subtraction of the corresponding non-linear curve. The determination of the non-linear equation was derived by fitting a 2nd order polynomial equation in a force-vs-position curve on a hard surface acquired before measurements on microbubbles were performed. It is also important to point out that Figure 7.4 shows that even for microcompression curves on hard surfaces there is a small hysteresis between loading (trace) and unloading (retrace) curves.
Experiments on silica microspheres (MO-SCI Specialty Products, L.L.C, Rolla, MO 65401) and on glass slides were performed in water in order to check the possible effects of our experimental geometry (tipless cantilever on spherical objects). In this case hard spheres were used (silica spheres) in order to avoid deformation during contact and have a direct comparison with AFM data on flat hard surfaces. Relatively stiff cantilevers \((k = 1.26 \text{ N/m})\) were used to reach high applied forces on both colloidal particles and flat surfaces. The curves had the same behaviour for both spheres and flat surface. No movement of the spherical particles were observed during compression.

Using the above experimental set up force-vs-distance curves were acquired on biSpheres\textsuperscript{®} microbubbles in water with 1 Hz scan rate and about 8 μm ramp size. A schematic drawing of the experiment is presented in Figure 7.5. The tipless cantilever moved towards an individual microbubble in normal direction. After the initial contact between the cantilever and the microbubble, the continuation of the cantilever movement towards the bound microbubble results in micro-compression testing which is recorded and subsequently analyzed. Images of microbubbles were captured from a digital Hamamatsu Orca-ER C4742-80 camera (Hamamatsu Photonics, Hamamatsu city, Japan) attached to the microscope. The images were transferred and processed in a PC using the provided with the microscope image analysis software IPLab v3.7 (BD Bioscience Bioimaging, Rockville, USA). Using a x60 objective it was possible to size the microbubbles with a half micron accuracy.

Figure 7.5: a) and b) schematic of microbubble deformation due to the compression by a tipless AFM cantilever.
A minimum of 6 force-vs-distance curves was acquired per microbubble in order to assess the variability and reproducibility of the experiments. As it will be shown in the results section the vast majority of the force-vs-separation curves on microbubbles had a linear part. This linear part was associated with an effective stiffness of the microbubble. The number of the minimum required measurements on its microbubble was determined by plotting the average slope of the linear part of $N$ number of force-vs-separation curves on a microbubble against $N$. A typical example is given in Figure 7.6. It is clear that after the 6th measurement the average slope converges to a specific value. Hence, for most cases, 7 curves were captured on each microbubble.

![Figure 7.6](image.png)

**Figure 7.6**: Average slope of the linear part of $N$ force-separation curves on a microbubble against $N$.

### 7.3 Theoretical Background

Several studies have focused on the mechanical properties of spherical shells. In particular, the mechanical properties of polyelectrolyte multilayer capsules (PMCs) have been the subject of several studies using different methods; micropipette technique, osmotic pressure difference, and AFM. It is worth pointing out that even though the same poly(styrene sulfonate) / poly(allylamine) (PSS/PAH) PMCs have been studied from different groups in recent years, significant differences were reported in the elastic properties measured.

The first quantitative estimates for the Young’s modulus of the shell material of the PSS/PAH PMCs were reported by Gao and co-workers using osmotic pressure differences between the inside and outside of the shell. In their study they found that the values for the Young’s modulus were about 1.5 GPa, concluding that the
multilayer material was in a glassy state. Furthermore, they managed to monitor the onset of the buckling instability as a function of the shell radius and thickness.

The same system was studied further from two groups using AFM force distance curves employing tipless cantilevers on which large spherical glass beads (diameter of tens of micrometers) were glued. In this way the contact can be considered as an extended plate-like contact. Vinogradova and co-workers found much lower elastic constants for this system using the AFM (~100 MPa) and concluded that the wall material is in an elastomeric state. Nevertheless, some assumptions made in this work, such as volume conservation during deformation, elasticity at large deformations were not in line with previous findings of others, which can explain the underestimation as compared to results from Gao and co-workers.

Another AFM study on the same system was performed by Fery and co-workers. They limited their analysis on the order of the shell thickness to avoid problems occurring at larger deformations and additionally no assumptions concerning the permeability were necessary. In their first paper on the mechanical properties of these microcapsules in order to analyze their AFM data they applied continuum elastic theory on the small deformation regime where only shell bending was expected. Based on the classical thin shell theory one would expect a linear force deformation for deformation up to few times the wall thickness. The applicability of thin shell theory is limited to shells with a ratio of the shell thickness over the radius smaller than 0.05. They used the following relation of the applied force and the corresponding capsule deformation: \[ f_{\text{bending}} = A \frac{Eh^2}{R_0} \Delta \] (7.1)

Where: \[ A = \frac{1}{(1 + \nu)\sqrt{6(1 - \nu)}} \] , for pure shear deformation (7.2) and \[ A = \frac{1}{(1 - \nu)\sqrt{6(1 + \nu)}} \] , for pure bending deformation, (7.3)

\( \nu \) is the Poisson ratio, \( E \) the Young's modulus of the material, \( h \) is the thickness of the shell and \( R_0 \) the radius of the PMCs. They assumed only shear deformation and a value of the Poisson ratio to be between 1/3 and 1/2. With this assumption the main
Mechanical Behaviour of Polymer Microbubbles

A contribution to the deformation of the capsules was from the stretching and $A$ will be in between $3/4 \sqrt{2}$ and $2/3$. Fitting their data with Equation 7.1 they found the Young's modulus of the capsules to be about 1.5 GPa and consequently consistent with the results obtained by Gao and co-workers\textsuperscript{5,6}.

Nevertheless Fery and co-workers recently reported a correction of the above model\textsuperscript{14,15}. They performed finite element modelling (FEM) and they showed that an exact analytical solution of the force deformation dependency can be given by the Reissner approximation\textsuperscript{19,20}, which shows the same scaling with Equation 7.1, but with a different prefactor:

$$f_{\text{bending}} = \frac{4}{\sqrt{3(1-\nu^2)}} \frac{Eh^2}{R_0} \Delta$$  \hspace{1cm} (7.4)

It is worth mentioning that in Reissner approximation a point-like force is assumed. Nevertheless, their FEM results matched well with the prediction of the Reissner result in the small deformation region. Their FEM results showed that the finite size of the contact area does not lead to strong deviations from the analytical theory based on point like contact. Furthermore, they showed that this model is independent of the particular system as long as the capsule wall material reacts in a linear elastic manner. Consequently, Equation 7.4 can serve as a guide for the measurements of other capsule-like systems studied by AFM. After the above prefactor correction, the Young's modulus was determined with a higher accuracy than previously to be about 300 MPa, assuming a Poisson ratio of $1/3$. This value of the Young's modulus now agrees better with Vinogradova and co-workers results. However, there is still a disagreement on whether the capsule shells should be characterized as elastomeric (Vinogradova group) or glassy (Gao group and Fery group) material. Fery and co-workers carried out speed-dependent measurements to answer this question. Their results showed no speed dependency which clearly rules out a viscoelastic material.

Equation 7.4 shows that for small deformations, the microcapsule behaves like a spring with an effective spring constant ($k_{\text{eff}}$):

$$k_{\text{eff}} = \frac{4}{\sqrt{3(1-\nu^2)}} \frac{Eh^2}{R_0}$$  \hspace{1cm} (7.5)
The linear part of a force-vs-separation curve can be associated with the \( k_{\text{eff}} \) of the object. Using equation 7.5 the corresponding Young's modulus of the material can be calculated.

Fery and coworkers AFM data showed also that for large deformations the linear dependency of the force on the deformation was lost and in some cases instabilities and/or buckling events were observed. Their analytical analysis focused only on the small deformation regime as the buckled capsule shape was very difficult to evaluate and moreover the behaviour in this regime is described by the non-linear von Karman equations\(^{21,22}\) making the analysis of these particular events complicated. Nevertheless, the post-buckling case has been described by Pogorelov\(^ {23}\). In the case of buckling a dimple is created by a geometrical inversion of the spherical domain around the force. Under the main assumption that all the deformation energy is concentrated in the rim around the dimple, proportionality between the force and the square root of the deformation is obtained, or more explicitly\(^ {23}\):

\[
 f_{\text{buckling}} = \frac{1.68}{1 - \nu^2} \frac{h^{3/2}}{R_0} \sqrt{\Delta} \quad (7.6)
\]

The intersection point between the Equations 7.4 and 7.6 should indicate a rough indication of the onset of buckling. Numerical and experimental results showed that this intersection occurs at a value well bellow the wall thickness, \( h \), and the results showed buckling for the deformations of a few shell thicknesses\(^ {24}\).

By defining the relative deformation, \( \varepsilon \), as

\[
 \varepsilon = \frac{\Delta}{2R_0} \quad (7.7)
\]

and assuming \( \nu = 1/3 \), Equation (7.4) can be rewritten as:

\[
 f_{\text{bending}} = 4.9Eh^2\varepsilon \quad (7.8)
\]

7.4 Results

BiSpheres\(^ {\circledR} \) microbubbles were positioned on a pre-coated with poly-L-lysine Petri dish using a procedure described in the experimental section of the previous chapter. Micro-compression tests (in water) were performed on individual microbubbles using the AFM. A range of tipless cantilevers with spring constant \( k_c \)
varying from 0.08 N/m to 2.3 N/m were used in order to perform a systematic study and assess the mechanical properties of the microbubbles. All the AFM data were processed using the method described in the experimental section of this chapter.

![Graph showing force vs. separation for a 4.4 μm microbubble](image)

**Figure 7.7**: Typical force-vs-separation curve (approach (black squares) and retraction (red circles)) for a 4.4 μm diameter biosphere microbubble.

Figure 7.7 shows a typical force-vs-separation curve on a microbubble using a cantilever with $k_c = 0.08$ N/m. Upon approach and before the contact of the cantilever to the microbubble the deflection was zero as there were no forces that would impose a deflection. When the cantilever contacted the microbubble at 0 nm separation its force was recorded (Figure 7.7) as positive. At some point the direction of the cantilever movement changed and the cantilever was retracted. The force reduced until eventually the cantilever detached from the microbubble. The approach (or trace) and retraction (or retrace) have some hysteresis which is comparable with the hysteresis observed on a hard surface.

Figure 7.8 (left) shows three typical force separation curves on three different microbubbles for a cantilever with $k_c = 0.08$ N/m. The curves have a soft repulsive force part at the beginning which quickly becomes linear. In any case this behaviour was fully reversible and subsequently reproducible. The loading and unloading curves for all the tests had very similar shape with a small hysteresis, comparable to the hysteresis observed on hard surfaces. The effective microbubble stiffness ($k_{eff}$) associated with the linear part of the micro-compression curves was determined by measuring the slope of this linear part. The $k_{eff}$ of each microbubble was determined by averaging slopes of at least 6 force-vs-separation curves on the same microbubble. The $k_{eff}$ for several microbubbles is plotted in Figure 7.8 (right). The plot shows a
significant dispersion in the measured $k_{\text{eff}}$ even for microbubbles with very similar size. For example a microbubble with size of about 4 μm can appear with $k_{\text{eff}}$ from about 0.75 N/m up to 1.8 N/m. There is no clear trend and there seems to be an independence of the $k_{\text{eff}}$ on the microbubble size. In many occasions, the curves on microbubbles suffer from a wavy pattern which is attributed to a low amplitude (~nm), high frequency (~1-10 kHz) oscillation of the microbubble shell. There may be many sources of this oscillation: hydrodynamic coupling effects with the movement of the piezo, instabilities of the microscope base (This is an inevitable compromise when an AFM is combined with an optical microscope and parasitic noise is always present at the very small scale).

Figure 7.8: (left) Three typical force-separation curves taken on three microbubbles; a microbubble with $k_{\text{eff}} = 1.53$ N/m and $d = 5.43$ μm (black squares), a microbubble with $k_{\text{eff}} = 0.87$ N/m and $d = 4.44$ μm (red circles) and a microbubble with $k_{\text{eff}} = 0.53$ N/m and $d = 3.26$ μm for (blue triangles) for a cantilever with $k_c = 0.08$ N/m. (right) Plot of $k_{\text{eff}}$ against the size of the microbubbles.

Figure 7.9 (left) shows three typical force-vs-separation curves using a cantilever with $k_c = 0.12$ N/m. The curves have a soft repulsive force part that quickly becomes linear. No significant hysteresis was observed in the loading/unloading curves. Our results show a slightly increase of the microbubble effective stiffness with the size, Figure 7.9 (right). Nevertheless, there is a significant dispersion of the measured $k_{\text{eff}}$ for microbubbles with very similar size.
Figure 7.9: (left) Three typical force-separation curves taken on three microbubbles; a microbubble with \( k_{\text{eff}} = 1.08 \text{ N/m} \) and \( d = 2.76 \mu\text{m} \) (black squares), a microbubble with \( k_{\text{eff}} = 1.81 \text{ N/m} \) and \( d = 4.78 \mu\text{m} \) (red circles) and a microbubble with \( k_{\text{eff}} = 2.24 \text{ N/m} \) and \( d = 5.25 \mu\text{m} \) (blue triangles) for a cantilever with \( k_c = 0.12 \text{ N/m} \). (right) Plot of \( k_{\text{eff}} \) versus the size of the microbubbles.

Figure 7.10 (left) shows three typical force-vs-separation curves for the case of micro-compression tests on microbubbles with a tipless cantilever of \( k_c = 0.45 \text{ N/m} \). The curves have a soft repulsive force part at the beginning followed by a linear-part (the beginning of a non-linear part observed at higher deformation in some curves will be presented later on). The microbubble \( k_{\text{eff}} \) monotonically decreases with the microbubble size, Figure 7.10 (right). The \( k_{\text{eff}} \) data again are dispersed even for microbubbles with very similar size.

For a cantilever with spring constant \( k_c = 0.61 \text{ N/m} \) two different types of behaviour in the force-vs-separation curves on microbubbles were observed. The first type of behaviour can be seen in Figure 7.11 (left). The curve appeared with a soft repulsive force part at the beginning which quickly became linear. Figure 7.11 (right)
Mechanical Behaviour of Polymer Microbubbles

depicts the second characteristic behaviour observed by using a cantilever with $k_c = 0.61 \text{ N/m}$. At the beginning, the curve has a soft repulsive force part followed by a linear part, which gradually becomes non linear with a progressively lower slope which develops into an "inflection point" (zero or almost zero slope and change of curvature sign) or in some cases even negative slope indicating an instability (for simplicity, we will refer to this area as instability area). After a certain separation the slope obtains again positive values.

**Figure 7.11:** Force-vs-separation curves, trace (black squares) and retrace (red circles), for (left) a microbubble with $k = 4.11 \text{ N/m}$ and $R_0 = 2.02 \mu\text{m}$ without an instability area and (right) a microbubble with $k_{\text{eff}} = 3.02 \text{ N/m}$ and $R_0 = 2.45 \mu\text{m}$ with an instability area. TO SXHMA PANW FAINETAI DIPLO!!!!!

For curves with an instability area the number of acquired curves was higher (10 and more) in order to check if there is a permanent damage on the microbubble that could alter the measured microbubble properties. In spite of this instability, no permanent damage was observed on microbubbles that were microcompressed with a tipless cantilever with $k_c = 0.61 \text{ N/m}$. In general, all the force-vs-separation curves (with or without the instability area) appeared to be highly reproducible (Figure 7.12 right and left) with the number of acquired curves.
Figure 7.12: Sequence of force-vs-separation curves on microbubbles (left) without an instability area and (right) with an instability area.

In any case, the microcompression curves, with or without the instability area, had a linear part that was associated with the microbubble $k_{\text{eff}}$. Figure 7.13 shows that the microbubble $k_{\text{eff}}$ decreased with the size of the microbubble. The data again demonstrate a significant dispersion on the measured $k_{\text{eff}}$. Open, blue triangles in the plot correspond to microbubbles with an instability area. It is clear that the instability area appeared for the relatively soft ($k_{\text{eff}} < 4 \, \text{N/m}$) and large microbubbles ($d > 4 \, \mu\text{m}$) of the population.

Figure 7.13: Plot of $k_{\text{eff}}$ versus the size of the microbubbles for tipless cantilever with $k=0.61 \, \text{N/m}$. Blue solid triangles correspond to microbubbles without an instability area while open blue triangles correspond to microbubbles with an instability area.

Two types of behaviour in the force-vs-separation curves were also observed with spring constant of 1.14 N/m. Figure 7.14 shows force-vs-separation curves without an instability area. At the beginning all the curves have a soft repulsive force part, for relatively small applied forces (around 40 nN) and small separations (around 12 nm), followed by a linear part which develops into a non linear part with
progressively lower slope. This behaviour was highly reversible after many acquired curves on the same microbubble and no significant hysteresis between the trace and retrace curves was observed.

**Figure 7.14:** Three typical force-vs-separation curves taken on three microbubbles; a microbubble with $k_{\text{eff}} = 11.87$ N/m and $d = 2.61 \mu$m (black squares), a microbubble with $k_{\text{eff}} = 8.60$ N/m and $d = 3.51 \mu$m (red circles) and a microbubble with $k_{\text{eff}} = 7.25$ N/m and $d = 4.14 \mu$m (blue triangles).

Figure 7.15 shows some characteristic curves for microcompression tests with an instability area. At the beginning the curves have a small repulsive force part followed by a linear part which develops into the instability area. After a certain separation the slope obtains again positive values.

**Figure 7.15:** Some typical force-vs-separation curves with an instability area taken on microbubbles; for a microbubble with $k_{\text{eff}} = 9.05$ N/m and $d = 3.23 \mu$m (dark yellow diamonds); for a microbubble with $k_{\text{eff}} = 6.88$ N/m and $d = 3.12 \mu$m (black squares); for a microbubble with $k_{\text{eff}} = 5.22$ N/m and $d = 3.98 \mu$m (red circles); for a microbubble with $k_{\text{eff}} = 4.48$ N/m and $d = 4.95 \mu$m (blue triangles) and for a microbubble with $k_{\text{eff}} = 3.49$ N/m and $d = 5.46 \mu$m (green triangles).

They were some cases where the instability area appeared in a highly reproducible fashion after many consecutive acquired curves, Figure 7.16 (left), while
in some other cases, the characteristics of the instability area changed with the number of the acquired curves, Figure 7.16 (right). From this last plot, it is clear that the instability area appeared progressively at smaller forces with consecutive acquired curves.

![Figure 7.16](image_url)

**Figure 7.16:** (left) Sequence of reproducible measurements on a microbubble and (right) sequence of poorly reproducible curves on a microbubble.

Figure 7.17 shows the trace and retrace of a force-vs-separation curve on a microbubble where the instability area appeared not fully reproducibly with the number of the acquired curves (consecutive traces). It is clear that the retrace curve takes a path very different to the trace and crucially drops to zero at the separation associated with the instability in the trace indicating a permanent damage on the microbubble.

![Figure 7.17](image_url)

**Figure 7.17:** Force-vs-separation curve, trace (blue squares) and retrace (red circles), for a microbubble with an instability area that appeared non-reproducibly with consecutive acquired curves.

Similarly to previous cantilevers, the slope of the linear part of the force-vs-separation curves was associated with an effective, microbubble stiffness. For
microbubbles where the microcompression curves were not fully reproducible we confined ourselves in measuring the slope of the linear part at the first acquired curve alone. Figure 7.18 shows that the $k_{\text{eff}}$ monotonically decreased with the microbubble size. Open green triangles correspond to microbubbles with an instability area while solid green triangles correspond to microbubbles without an instability area. The plot shows that no instability area was observed for microbubbles with an effective stiffness higher than 9.2 N/m. There is a region of microbubble stiffness ($6.5 \text{ N/m} < k_{\text{eff}} < 9.2 \text{ N/m}$) where the microcompression curves can appear with or without an instability while all the microbubbles with $k_{\text{eff}} < 6.5 \text{ N/m}$ appeared with an instability area.

![Figure 7.18: Plot of $k_{\text{eff}}$ versus the size of microbubbles for tipless cantilever with $k=1.14 \text{ N/m}$. Solid green triangles corresponds to microbubbles without an instability while open green triangles correspond to microbubbles with an instability area.](image)

Using a tipless cantilever with $k_c = 2.3 \text{ N/m}$ all the microcompression curves appeared with an instability area. Figure 7.19 shows some typical force-vs-separation curves on microbubbles.
Figure 7.19: Four typical force-vs-separation curves taken on microbubbles with a tipless cantilever with \( k_c = 2.3 \text{ N/m} \); a microbubble with \( k_{\text{eff}} = 10.61 \text{ N/m} \) and \( d = 3.02 \mu \text{m} \) (black squares), a microbubble with \( k_{\text{eff}} = 7.38 \text{ N/m} \) and \( d = 3.65 \mu \text{m} \) (red circles), a microbubble with \( k_{\text{eff}} = 6.41 \text{ N/m} \) and \( d = 4.43 \mu \text{m} \) (blue triangles) and a microbubble with \( k_{\text{eff}} = 4.07 \text{ N/m} \) and \( d = 4.85 \mu \text{m} \) (green triangles).

Figure 7.20 shows a force-vs-separation curve with two instability areas. This was observed for a relatively soft \( (k_{\text{eff}} = 3.8 \text{ N/m}) \) and big \( (d = 5.22 \mu \text{m}) \) microbubble.

Figure 7.20: Force-vs-separation curve with two instability areas for a microbubble with \( k_{\text{eff}} = 3.8081 \text{ N/m} \) and \( d = 5.22 \mu \text{m} \)

All the measurements on individual microbubbles with a tipless cantilever of \( k_c = 2.3 \text{ N/m} \) were not fully reproducible. There were cases where the necessary force for the instability area was decreasing with consecutive acquired curves (Figure 7.21a) and cases where after a number of acquired curves, the shape of the curve changed dramatically and no instability area was observed (Figure 7.21b and c).
In any case (curves with a single instability area or two instability areas) the microcompression curves using a cantilever with $k_c = 2.3 \text{ N/m}$ had a linear part which again was associated with the effective stiffness of the microbubble. For every microbubble the $k_{eff}$ was estimated using the first acquired curve. Figure 7.22 shows that the $k_{eff}$ decreased with the microbubble size.
Figure 7.22: Plot of $k_{\text{eff}}$ versus the size of microbubbles for a tipless cantilever with $k_c = 2.3$ N/m.

7.5 Discussion

Since we are the first, to our knowledge, to study the mechanical properties of the biShpere® microbubble we performed a systematic study to check the response of the microbubble for small applied forces and we subsequently moved to higher applied forces. To check the response of the microbubble for small applied forces with high accuracy and high force resolution compliant cantilevers were used. Employing cantilevers with low spring constant, i.e. $k_c = 0.08$ N/m and $k_c = 0.12$ N/m we managed to probe the response of the microbubble for forces up to 40 nN and 70 nN, respectively. The magnitude of the applied force on the microbubble was gradually increased by using stiffer cantilevers ($k_c = 0.45$ N/m, $k_c = 0.61$ N/m, $k_c = 1.14$ N/m and $k_c = 2.3$ N/m). It has to be stressed that cantilevers with different spring constants are not only capable of applying a different range of forces but also have different sensitivity and can probe mechanically different parts of the sample. Although the interpretation of the results is not trivial, if it is done carefully it can reveal a wealth of information on the mechanical response of the interrogated sample.

It has to be noted that most of the acquired force-vs-separation curves on microbubbles exhibit a wavyness (both trace and retrace). The oscillation amplitude varies across the microcompression curve. Its frequency increases with the $k_{\text{eff}}$ of the microbubble (Figure 7.23) indicating an oscillation of the microbubble shell. Various reasons can generate this oscillation as for example the hydrodynamics of the water-
immersed cantilever-microbubble system at the specific scan rate (1 Hz) or possibly microscope base instabilities.

The discussion section has two parts; i) small deformations: we discuss the behaviour of the microbubbles for small deformations where the relation of the applied force with the deformation (separation) of the microbubble was linear and ii) higher deformations: we focus on the area of the force-vs-separation curve where the behaviour of the curve departs from the linear behaviour and in some cases instabilities were observed.

![Figure 7.23: Frequency of microbubble oscillation versus the effective microbubble stiffness.](image)

i) Small deformations

For all the different cantilevers used, a linear part of the force-vs-separation was observed and its slope was associated with an effective stiffness, $k_{\text{eff}}$, of the microbubble. Figure 7.24 shows the plot of the $k_{\text{eff}}$ against the size of the microbubble for all the different cantilevers used in this study. For the most compliant cantilever with $k_c = 0.08$ N/m the measured effective stiffness of the microbubbles was about 1.25 N/m and appeared independent of its size. For the cantilever with $k_c = 0.12$ N/m, we found a gentle increase of $k_{\text{eff}}$ with the microbubble size. For $k_c = 0.45$ N/m the microbubbles appeared stiffer and their effective stiffness decreased with the size. For $k_c \geq 0.61$ N/m the microbubbles appeared with higher stiffness, especially the small ones, and the effective stiffness again decreased with the size. The $k_{\text{eff}}$ values and its dependence on size remained more-or-less similar for the stiffer cantilevers.
Figure 7.24: Effective stiffness against the size of microbubbles for the different spring constant cantilevers used in this study.

Figure 7.8 (left) shows three typical force-vs-separation on microbubbles. There is a soft repulsion at the beginning of the curve, with a range of about 10-20 nm. The microbubbles used in this study, as it was discussed in the previous chapter, have a biocompatible gel layer which according to the manufacturer is expected to have a thickness in the region of 10 nm. Hence, this soft repulsion can be associated with the compression of this soft gel layer and the possible extraction of the water from its structure. The force-vs-separations in Figure 7.8 show that for applied forces up to 40 nN the corresponding deformation is in the region of 20 to 80 nm. The effective stiffness of the microbubble appeared independent of the microbubble size with an average value of about 1.25 N/m. It is clear that by using compliant cantilevers we mainly probed the soft gel layer and possibly the outer part of the stiff shell where the shell roughness and statistical variations (root-mean-square roughness $S_p$ was 85 nm and the roughness average $S_a$ was 64 nm) can dominate the measurements. The applied force is not high enough to bend the shell and since we probed the characteristics of the outer shell alone there is no significant variation with the size and curvature of the microbubble.

Using a slightly stiffer cantilever of $k_c = 0.12$ N/m, the applied force on the microbubble was up to about 70 nN. The microbubbles appeared with slightly higher effective stiffness of about 1.75 N/m than in the case of the cantilever with $k_c = 0.08$ N/m. We still mainly probed the outer part of the shell. However, there is a weak dependence of the $k_{\text{eff}}$ on the microbubble size (Figure 7.9, right) as some “stiffer” microbubbles appeared with $k_{\text{eff}}$ around 2.25 N/m at sizes of about 5 μm. According to
the theoretical model the larger bubbles are more compliant and for this reason using this cantilever we started to gently bend their shell resulting in a higher $k_{eff}$. The curves show again a soft repulsion for about 10 nm separation that still can be connected with the outer gel layer of the microbubbles.

For the case of a tipless cantilever with $k_c = 0.45$ N/m the microbubbles appeared stiffer. The $k_{eff}$ was found to depend on the microbubble size, but this time decreased with microbubble size from 4.5 N/m for microbubbles with size 5 µm down to 2 N/m for microbubbles with a size of 3 µm (Figure 7.10, right). The soft repulsion at the beginning of the curves (Figure 7.10, left) appeared for a separation of about 20 nm. Since the cantilever is stiffer, it is expected to be less sensitive to small restoring forces from the gel and the outer part of the shell. Consequently, the linear part of the force-vs-separation curve is now mainly associated with the effective stiffness of the microbubble due to the shell deformation.

For the cantilever with $k_c = 0.61$, the measured effective stiffness of the microbubble overall slightly increased, especially for small microbubbles. We found that for this cantilever there is a strong dependence of $k_{eff}$ on microbubble size: $k_{eff}$ decreased with the size of the microbubble. Using even stiffer cantilevers, i.e. $k_c = 1.14$ and 2.3 N/m, the behaviour of the measured $k_{eff}$ did not change. This indicates that these cantilevers were strong enough and the applied forces were big enough to overcome the resistance of the gel and of the outer shell anomalies and deform the shell.

What is very important in Figure 7.24 is the dispersion of the measured microbubble stiffness amongst the population of the microbubbles. There were several microbubbles of similar size with significant different stiffness (for the same cantilever), which is a strong evidence of structural dispersions in the microbubble shells.

Equation 7.4 in the background section shows that for small deformations, when the bending of the shell dominates, the relation of the applied force with the separation is linear. As we mentioned in the previous chapter the manufacturers claimed that the shell thickness of the microbubble is around 30 nm for a 4 µm microbubble and changes approximately linearly with the size at 7.5 nm/µm per diameter, which introduces a correlation of $h$ with $R_0$ of:

$$h = 1.5 \times 10^{-2} R_0$$  

(7.9)

By substituting equation 7.9 in equation 7.5 and for $\nu=1/3$ we obtain:
\[ k_{\text{eff}} = 5.51 \times 10^{-4} E R_0 \quad (7.10) \]

Equation 7.10 shows that for \( E \) independent of the microbubble size, the \( k_{\text{eff}} \) increases with the microbubble radius. This is in contradiction with our experimental results where the \( k_{\text{eff}} \) decreased with the microbubble size. Nevertheless, using equation 7.10 the corresponding \( E \) of each microbubble can be determined, Figure 7.25 (left). This plot shows that \( E \) decreases with the size from 20 GPa for a microbubble with \( R_0 \) of 1.25 \( \mu \text{m} \) (shell thickness 18 nm, Figure 7.25 right) to 1.5 GPa for a microbubble with \( R_0 \) of 2.75 \( \mu \text{m} \) (shell thickness of 40 nm Figure 7.25 right).

![Figure 7.25: Young's modulus of microbubbles versus (left) microbubble radius and (right) microbubble shell thickness with the corresponding fits using equation 7.11 and 7.12 respectively.](image)

We now assume that \( E \) varies with the microbubble radius according to:

\[ E = c_1 R_0^{c_2} + c_3 \quad (7.11) \]

and with shell thickness according to

\[ E = c_4 h^{c_5} + c_6 \quad (7.12) \]

We fitted the experimental data with equations 7.11 and 7.12 (red lines in Figure 7.25) using \( c_1, c_2, c_3, c_4, c_5 \) and \( c_6 \) as fitting parameters; we obtained that:

\[ c_1 = 33.4 \pm 2.2 \text{ GPa/ } \mu \text{m}^{3.14} \quad (7.13) \]
\[ c_2 = -3.14 \pm 0.30 \quad (7.14) \]
\[ c_3 = 0.58 \pm 0.59 \text{ Gpa} \quad (7.15) \]
\[ c_4 = 1.4 \times 10^5 \pm 1.2 \times 10^5 \text{ GPa/ } \text{nm}^{3.07} \quad (7.16) \]
\[ c_5 = -3.07 \pm 0.29 \quad (7.17) \]
\[ c_6 = 0.47 \pm 0.61 \text{ GPa} \quad (7.18) \]
By substituting these values in equations 7.11 and 7.12 we obtain that:

\[ E = 33.4R_0^{-0.314} + 0.58 \]  

(7.19)

with \( R_0 \) in \( \mu \text{m} \), \( E \) in GPa, and

\[ E = 1.4 \times 10^5 h^{-3.07} + 0.49 \]  

(7.20)

with \( h \) in \( \text{nm} \), \( E \) in GPa.

Figure 7.26 left shows the variation of \( E \) with the microbubble radius according to Equation 7.19. Figure 7.26 right shows the variation of \( E \) with the microbubble shell thickness according to Equation 7.20. The plots show the \( E \) can vary from 35 GPa for a microbubble with \( R_0 = 1 \mu \text{m} \) (or with a shell thickness \( h = 15 \text{ nm} \)) to 1.6 GPa for a microbubbles with \( R_0 = 3 \mu \text{m} \) (or with a shell thickness \( h = 45 \text{ nm} \)). Hence, bigger microbubbles have a Young’s modulus within the range of the corresponding values provided by the manufacturers (based on the Young’s modulus of the bulk material: 1.4 to 2.8 GPa) while small microbubbles appeared with much higher \( E \).

Our experimental data showed that \( E \) depends strongly on the microbubble size and consequently on the shell thickness. This indicates a size effect on the measured mechanical properties of the microbubble shell. The observed behaviour of \( E \) is very similar to what have been previously observed in other nanosystems. Cuenot \textit{et al.}\textsuperscript{25} found that the elastic modulus of a polymeric nanotube strongly increased when its thickness decreased. They showed that the elastic modulus increased from around 2 GPa for a tube thickness of about 20 nm to 5 GPa for a tube thickness of about 16 nm and finally to 60 GPa for a tube thickness of 6.5 nm. They showed that the elastic modulus increased non-linearly and dramatically when the thickness
obtained very small values. Furthermore, Cuenot et al.\textsuperscript{26}, in another study, measured the elastic modulus of polypyrrole nanotubes using resonant contact atomic force microscopy (RCAFM). The measurement of the system resonance frequency shift to higher values with respect to the resonance frequency of the free cantilever enabled the determination of the nanotube effective stiffness and consequently its elastic modulus. Again their results showed that the elastic modulus increased rapidly when the nanotube thickness significantly decreased. Additionally, a similar size effect was observed by the same group using again RCAFM for silver and lead nanowires\textsuperscript{27}.

It is important to point out that the above studies showed that the measured $E$ was converging to (and gradually obtained) the value of the bulk material for samples with thickness $> 100$ nm. For very small thickness (about 20 to 15 nm) $E$ dramatically increased and obtained values much higher (one order of magnitude higher) than the value of the bulk material. In our measurements the thickness of the measured material (microbubble shell) varied from 15 nm to 45 nm. For a microbubble with shell thickness ($h$) of about 45 nm the measured $E$ was 1.6 GPa, i.e. the same $E$ of the bulk material. When $h$ becomes much smaller the size effect is more pronounced and any further reduction results in the dramatically increase of $E$ (taking a value of 35 GPa for $h = 15$ nm).

\textit{ii) Higher deformations}

We defined as higher deformation the region where the linear dependence of the force on the deformation was lost. This behaviour was observed mainly for tipless cantilevers with $k_c \geq 1.14$ N/m and in very few cases with $k_c = 0.61$ N/m. As it was discussed in the background section, the compression of a spherical shell surpasses the linear regime, during which the shell is bent, and moves to a non-linear regime for higher deformations where the shell stretches and buckling events have been predicted (see theoretical background).

For a tipless cantilever with $k_c = 0.61$ N/m non-linear behaviour in the force-vs-separation curves was mainly observed for microbubbles with small $k_{\text{eff}}$. Figure 7.27 shows a typical force-vs-relative separation for a soft microbubble with $R_0 = 2.36$ \(\mu\)m and $k_{\text{eff}} = 2.2$ N/m. The linear part holds up to about $\varepsilon \approx 0.012$ after which a non-linear behaviour starts to appear.
The instability appeared preferentially for microbubbles with small $k_{\text{eff}}$ (open blue triangles in Figure 7.13). Figure 7.28) shows a force-vs-relative deformation for a microbubble (with an instability area) with $k_{\text{eff}} = 3.02$ N/m and $R_0 = 2.45$ μm. This type of curves were highly reproducible in consecutive tests on the same microbubble and trace and retrace curves had similar shapes with relatively small hysteresis indicating that for the applied force reached with a cantilever of $k_c = 0.61$ N/m, (about 250 nN) no permanent damage occurred.

Using a tipless cantilever with $k_c = 1.14$ N/m the number of curves having an instability area increased. Similarly for this cantilever, the linear part in the force-vs-separation curves was concluded for relative deformations of about 0.01. The instability areas appeared also for stiffer microbubbles than in the case of the tipless cantilever with $k_c = 0.61$ N/m. Contrary to the case of a tipless cantilever with $k_c =$
0.61 N/m not all the force-vs-separation curves on the same microbubble were highly reproducible. There were cases where the instability area appeared for progressively lower applied force after several consecutive curves (Figure 7.16). Furthermore, the retrace curves in these cases appeared much different than the trace indicating a permanent damage on the microbubble. Figure 7.29 shows the $k_{\text{eff}}$ against the microbubble size for microbubbles with an instability area (using a tipless cantilever with $k_c = 1.14$ N). This plot shows that stiffer microbubbles can get permanently damaged more easily.

![Graph](image)

**Figure 7.29**: Plot of $k_{\text{eff}}$ against the size for microbubbles with an instability area. Data taken with a tipless cantilever of $k_c = 1.14$ N. Black open circles correspond to microbubbles where the microcompression curves appeared reproducibly while solid black circles correspond to microbubble where the microcompression curves appeared with poor reproducibility.

Using a tipless cantilever of 2.3 N/m, higher applied forces of around 900 nN were achieved. Instability areas appeared for all the microbubbles. Relatively soft microbubbles appeared with two instability areas during the compression. In any case the force-vs-separation curve on a microbubble was changing shape during consecutive tests and in some cases after a number of acquired curves no instability area was observed (Figure 7.21). All the curves with an instability area appeared with much different retrace with respect to the trace indicating permanent damage.

Figure 7.30 shows the force at which the instability appeared against the effective stiffness for microbubbles with instability areas for all cantilevers $k_c \geq 0.61$ N/m. The plot shows that stiffer microbubbles exhibit instabilities at higher applied forces. The data demonstrate a large dispersion of the applied force at the instability area with the $k_{\text{eff}}$. We have already seen Figure 7.13 that using a tipless cantilever with $k_c = 0.61$ N/m that an instability area can appear only in relatively soft microbubbles.
where the required amount of the applied force is small. By increasing the $k_c$ of the cantilever instability phenomena can be observed to stiffer microbubbles as higher forces can be applied with stiffer cantilevers.

![Graph showing force at the instability area against the effective stiffness of the microbubble.](image)

**Figure 7.30:** Force at the instability area against the effective stiffness of the microbubble.

Instability areas in the force-vs-separation curves have been observed on capsule deformability experiments. Their experimental set-up involved an optical microscope used in reflection interference contrast microscopy (RICM) mode. This experimental set up allowed the observation of the capsule shell shape across the contact area with the substrate during compression. In the single force-vs-separation curve reported in this publication, two instability areas were observed. The first instability area appeared as a small plateau of the force-separation curve. According to their RICM data during this instability area the contact area of the capsule with the substrate was increased. The second instability area appeared for a higher applied force and the RICM data showed that during the second instability area buckling events of the capsule shell took place. Hence, according to this study an instability area can be connected to both the increase of the contact area of the capsule with the substrate and to buckling events for even higher deformations. Moreover they mentioned that their AFM and RICM data appeared reproducible even for cases of high deformation and buckling events were recorded.

Considering our data it is clear that force-vs-separation curves with instability appeared reproducibly only for soft microbubbles and for applied forces up to 400 nN. For higher forces, all consecutive curves on the same bubble showed poor reproducibility and soft microbubbles appeared with two instability areas. Based on the study described above we could associate the fully reproducible instability with
the abrupt and fully reversible increase of the contact area of the microbubble with the
substrate. Of course, it can also be connected with the fact that extended deformation
of a large part of the shell/microbubble started to become important at higher
deformations and larger parts of the shell structure started to play a role on the
mechanical behaviour. In any case, the trace and the retrace curves have similar shape
indicating that non-permanent damage took place on the microbubble. When the
instability did not appear fully reproducibly, the trace and the retrace had different
behaviour indicating a permanent damage on the microbubble. In this case, we can
associate this instability with an irreversible buckling event. The microbubbles used in
our study are expected to be relatively stiff (high Young's modulus) and possibly
relatively brittle. Within a buckling event, some sort of permanent damage on the
microbubble structure can take place. A permanent damage will result in a "memory"
effect and can lead the next buckling event in the following curve to appear for
smaller applied forces.

Postema et al. reported gas release of the biSphere® in the present of ultrasound indicating microbubble cracking. Hence, during buckling it is possible that the shell can crack resulting in a partial gas release. The fact that in some cases after a number of consecutive force-vs-separation curves no instability area was observed can be connected with a (partial) "destruction" of the microbubble.

To demonstrate that the observed instability area on the force separation curves can be associated with a possible buckling phenomenon we performed a test showed in Figure 7.31. On a microbubble where no buckling event was observed when the cantilever was placed properly on top of the microbubble (Figure 7.31, left) we moved the cantilever at a position shown in Figure 7.31, right. In this position the microbubble is at the very edge of the cantilever. The microbubble is compressed from only one side and we expect a concentration of stress at the edge. We expect this geometry to lead easily to a buckling type of instabilities. Indeed, the force-vs-separation curve showed an instability area. We repeated this test several times and for different microbubbles and similar behaviour was observed. This supports our hypothesis that the instability area can be connected to buckling events on the microbubble.
7.6 Conclusions

The mechanical properties of the biSpheres® microbubbles were investigated using AFM tipless cantilevers. Force-distance curves were employed to microcompress the microbubbles. We performed a systematic study using several cantilevers with spring constants varying from 0.08 to 2.3 N/m and acquired more than two thousand force-distance curves on more than 200 microbubbles with sizes from 2 to 6 micrometers. All the force-vs-separation curves showed a linear part which was associated with an effective stiffness of the microbubble. We found that using relatively compliant cantilevers \( k_c \leq 0.12 \) N/m our measurements were affected by the outer gel layer and the outer part of the stiff shell as the applied forces are not big enough to bend the shell. Using cantilevers with \( k_c \geq 0.61 \) N/m we found the \( k_{\text{eff}} \) of the microbubbles to decrease with the size showing that smaller microbubbles are stiffer. Applying a simply model for the deformability of the spherical shell of the microbubble, the Young’s modulus, \( E \), was estimated. The measured \( E \) was found to decrease dramatically with the microbubble radius and consequently with the thickness of the shell indicating a strong size effect. For higher applied forces instability areas were observed in the microcompression curves. In some cases, these events can be associated with a buckling transition and shell cracking that can lead to partial release of the encapsulated gas.

The usefulness of the current data lies on the fact that we have measured directly the mechanical properties of a population of individual microbubbles. Performing a systematic study and using a large population, we can demonstrate the
dispersion of their properties and variations in their structure. In this way we can aid the manufacturing process. Furthermore, microbubble models have assumed uniform shell characteristics in the absence of such data. The present experimental set-up and study offers a tool that may be used to bridge manufacturing, mathematical modelling and acoustic or optical experiments with microbubbles. Certainly some other techniques should be applied simultaneously with the AFM experiments that will provide more information for the shell structure during compression.
7.7 References

Chapter 8
Conclusions and Future Work
Conclusions and Future Work

In Part A, we investigated self-assembled polymer structures on surfaces. At first, we concentrated on the simple but largely ignored case of physisorbed homopolymers on surfaces. We used several molecular weights of two architectures: linear and star-shaped polymers. In all cases, we observed a variety of reproducible structures which in many cases develop into regular isotropic nanopatterns consisting from (i) spherically-shaped polymer nanodroplets (ii) asymmetrically-shaped polymer nanodroplets, (iii) semi-continuous networks of connected molecules and (iv) packed single collapsed polymer molecules. We have shown that there is an intimate relationship between the molecular characteristics of the polymers with the resultant pattern. In other words, the observed structures are “encoded” in the molecular weight and architecture of the molecules. This study has shown our experimental protocol involving a relatively weak physisorption from dilute solutions and long incubation times in good solvent conditions can provide a general “bottom-up”, self-assembly-based methodology for nanopattern formation. These nanopatterns can be used as templates for creating functional nanostructures such as nanowires for nanoelectronics, quantum dots etc. Nevertheless, our studies have shown some particular molecular mechanisms of polymer interactions on surfaces which should be investigated further by other techniques and model polymer systems (e.g. single chain fluorescence imaging using DNA in situ with AFM) which have the potential to provide complementary information.

Unique opportunities arise if one uses functional/responsive polymers such as appropriate block-copolymers. In this case, the polymer modified surfaces will have a functional/responsive character to certain external stimuli. We embarked in this direction by using a well-studied self-assembled system based on amphiphilic diblock copolymer micelles. We found that resultant structures can evolve from very flat irregular nano-islands to spherically-shaped nanodroplets due to the changing interactions with the surface. We consider this study to be early evidence of roughness tunable responsive surfaces. Although our particular system cannot be considered reversible, the principle proved with our study can lead to the fabrication of smart and “intelligent” surfaces.

In part B, we have shown that AFM can be used successfully interrogating biomedically relevant microobjects such as ultrasound contrast agent microbubbles. With AFM one can probe individual microbubbles and acquire their specific individual properties in contrast to other techniques which measure averages. In this
way, one can evaluate the dispersion of their properties and variations in their structure. Microbubble models have assumed uniform shell characteristics in the absence of such data. We revealed the unexpected rough structure of the microbubble outer shell and the surprising size effect when the thickness of their shell becomes as miniscule as a few nanometres. This has important implications not only for microbubbles (and their specific applications and manufacturing) but also for the mechanical properties and functionality of a variety of miniaturised systems and nanoparticles within the broad nanotechnology field. Although AFM can be used to bridge the manufacturing, mathematical modelling and acoustic or optical experiments with microbubbles, some other techniques (e.g. high resolution confocal fluorescence microscopy, reflection interference contrast microscopy) should be applied \textit{in situ} with the AFM experiments in order to provide more direct information of the shell structure during compression.