Chitosan Macromolecules on a Substrate: Deposition from Solutions in sc CO$_2$ and Reorganisation in Vapours

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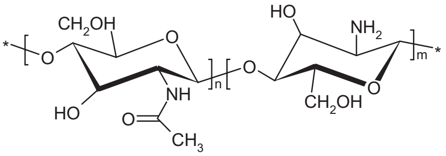
Summary: Specially pretreated chitosan macromolecules possess limited solubility in supercritical carbon dioxide. When deposited on mica substrate from such solutions they adopt somewhat extended conformation. The adsorbed macromolecules become mobile on the interface when exposed to water vapour as revealed by environmental scanning force microscopy. During the exposure in the presence of coadsorbed water layer the chitosan strands demonstrate slight tendency to adopt more compact but still two-dimensional conformation on the substrate.

Keywords: atomic force microscopy (AFM); chitosan; conformational analysis; single macromolecules; surfaces

Introduction

Chitosan is a copolymer of D-glucosamine and N-acetyl-D-glucosamine (see the scheme below) and can be obtained in industrial quantities from rather abundant natural polysaccharide chitin.



Chitosan is considered to be a very important material for possible biomedical usages because it demonstrates a unique complex of promising properties: it is non-toxic, biodegradable, bio- and haemo-compatible, anticoagulating, antimicrobial, apyrogenic, hypoallergenic, etc.[1,2] A chitosan molecule contains free amino groups that allows it to bind protons in aqueous media at low pH and thus to get an extra positive charge. These polycationic properties are rather unique for a natural polymer and may be very useful from the viewpoint of a variety of applications.[1,2] On the one hand, such polycations may be used to coat and hide possible bio- or haemo- incompatible residual sites at the surface of artificially stabilised xenotissue to be used inside a body as a bioprosthetic material.[3,4] On the other hand, such polycations may be applied together with polyanionic chitosan derivatives to prepare layer-by-layer stabilised coatings at the surface of biomedical articles.[3]

Recently we focused on preparation of chitosan coatings from water-free solutions in supercritical carbon dioxide (sc CO$_2$).[5] This fluid is not a liquid and may be continuously converted in a closed vessel from a dense sc state (a solvent for some polymers) to a gaseous state (a non-solvent for polymers) without formation of a liquid phase. Such a scheme eliminates coexistence of drying droplets or films of a liquid at the surface with the polymer structures

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being deposited. As a result, a morphology reorganisation of the material on a substrate due to inevitable influence of surface tension gradients or capillary forces is suppressed (such reorganisation is a typical drawback when a deposition scheme from usual liquid solutions is applied). Following this approach, we achieved before high uniformity and mechanical stability of sc-deposited nanosized ultrathin films of different fluoropolymers, including commercial copolymer Teflon AF 2400[6] and commercial low molecular weight PTFE.[7] Special benefits of sc CO_2 are most advantageous when it is applied to modify porous substrates. Indeed, in distinct from liquid solvents sc CO_2 ensures both: i) absolute permeating ability (into a porous matrix), and ii) complete removal from the matrix after decompression. Thus, superhydrophobic surfaces were produced by a treatment of porous structures having highly developed (rough) surfaces in solutions of hydrophobic polymers in sc CO_2.[8] Further, regularly packed fluorinated dendrimers were sc-deposited as small ordered patches of 2D hexagonal arrays with the ca. 6 nm lattice parameter.[9] Also high degree of regularity was detected for sc-deposited self-assembled toroidal nanoobjects formed by semifluorinated (perfluoroalkyl)alkanes.[10] Therefore, it seems to be very promising to perform systematic studies of individual macromolecules, supramolecular structures and ultrathin films as deposited directly from such a peculiar non-liquid solvent.

Possible additional advantages of the technique of direct deposition from solutions in sc CO_2 may be related also to the expected improved mechanical stability of the chitosan coatings thus obtained. Indeed, we have shown before that single chitosan macromolecules are deposited from solutions in sc CO_2 on a substrate in a rather extended conformation, which ensures a high amount of entanglements of polymer chains and thus good mechanical properties of the coatings finally formed.[5] Besides, there are no acidic residues in the coatings prepared from sc CO_2 in distinct from the films typically cast from aqueous (acidic) solutions of chitosan. Such acidic residues may deteriorate mechanical stability and adhesion of the coatings in the presence of water.

Unfortunately, there is a serious obstacle for the proposed approach due to the fact that the all so far tested chitosan materials and their derivatives either are completely insoluble in sc CO_2, or show only very poor solubility, not suitable for real applications. It is not clear yet, whether the solubility may be improved further either by chemical modification of the macromolecular structure, or by admixture of some non-toxic and hypoallergenic cosolvents (e.g., water) to the sc CO_2 media.

Nevertheless, the achieved poor solubility is already enough for deposition of single isolated chitosan macromolecules onto different substrates. On model ultraflat substrates (such as, for example, mica) they may be directly observed using scanning force microscopy (SFM). This technique has been extensively applied in order to study directly the conformational properties of single individual chains of different polysaccharides, including chitosan, as adsorbed, mainly, from aqueous solutions.[11–15]

In the present paper we address the question about the stability of macromolecular conformation for single chitosan chains as deposited from sc CO_2 and then exposed to water vapour. Indeed, it is known that conformation of adsorbed macromolecules may become unstable in different vapours.[16–20] But in real biomedical applications the chitosan coatings are to be subjected to aqueous surroundings. Thus, even provided that one may indeed manage to deposit very uniform coatings from solutions sc CO_2, the next important question concerns stability of the films in contact with water. This is especially important if the films are ultrathin (with a nanometer scale thickness). Model experiments would be useful to predict the stability of such films and thus to draw a conclusion about benefits of using the coatings prepared from sc CO_2 in real applications. The most gentle and controllable way to introduce model contact with water is to expose the hydrophilic substrate with the adsorbed polymer structures to saturated water vapour. If adsorbed single macromolecules will demonstrate considerable conformational changes in the presence of coadsorbed water on a substrate, one may expect strong tendency towards morphology reorganisation for the more dense chitosan coatings as well. In that case the mechanical stability and adhesion of the coatings will be presumably poor. The real-time following of the possible vapour-induced transformations using environmental SFM seems to be a rather powerful tool to study macromolecular response onto such an external stimulus at the really single-macromolecule level. Therefore, the results of such systematic observations may be useful not only to verify general uniformity but also to predict the stability and mechanical properties of the chitosan coatings as prepared by direct deposition from solutions in sc CO_2.

Experimental Part

We used chitin and chitosan from ZAO Sonat (Russia). The chitosan samples had different degrees of N-acetylation (DA): 2% and 15%. The samples with 26%, 35%, and 50% DA were prepared by means of N-reacetylation of the chitosan with 15% DA as received. The N-reacetylation was performed in methanol solutions of acetic anhydride in two steps: first at pH 5.0 up to 26% or 35% DA, and then at pH 8.0 (in the presence of NaOH) up to 50% DA as described in Ref. [21]. The homogeneous N-reacetylation results in the products with uniform statistic distribution of acetyl groups and even the sample with 50% DA is quite soluble in acidic aqueous solutions. The molecular weights (M_v) of the obtained samples were in range of 100–150 kg/mol. These values were determined by viscosity measurements (25 °C, aqueous solution, 2% acetic acid, 0.2 M sodium acetate) using Mark-Kuhn-Houwink equation with previously described coefficients according to Ref. [22].

Two different types of pre-treatments among several tested procedures indeed improved polymer solubility in sc CO_2. The first type of the pre-treatment was a reprecipitation of the chitosan material from dichloracetic acid aqueous solutions into an alkaline medium (this reprecipitation procedure was performed for chitosan samples with 2%, 15%, and 50% DA, here labelled as chitosan-0.98, chitosan-0.85, and chitosan-0.50, respectively). The second type of the pre-treatment included a preparation of chitosan-Ca complex by means of N-deacetylation of chitin impregnated with $CaCO_3$, that resulted in a sample with 15% DA incorporating some amount of calcium hydroxide (here labelled as chitosan-0.85-Ca). The deacetylation was performed with NaOH as described in Ref. [23]. The amount of inorganic residual calcium hydroxide in the sample was 17% by weight, as determined by elemental analysis. The molecular weight (M_v) was about 150 kg/mol.

In the experiments we used CO_2 of high purity (>99.997%, Linde Gas Rus, Russia). The experimental setup and the experimental procedure were the same as described before.[6-10] The setup consists of a pressure generator equipped with pressure sensors and a thermostatically controlled stainless steal reactor (inner volume 10 ml), which are united together by a set of capillaries. The setup can sustain the pressures up to 80 MPa (as limited by the reactor). Mica (muscovite, Plano GmbH, Germany) was used as a substrate for the deposition of polymer structures from solutions in sc CO_2.

The purity of the reactor was of extreme importance for the success of the experiments on deposition of ultrasmall quantities of the chitosan material. Before each experiment with a new chitosan sample the reactor was wiped with a new clean cotton tissue and then washed successively with acidic water solutions (freshly prepared using Milli-Q water), then with liquid solvents of an analytical grade (for liquid chromatography, Aldrich) and, finally, with sc CO_2. The purity of the system was checked by means of a control exposure of freshly cleaved mica substrate in the reactor with sc CO_2 but without any polymer at the same conditions (temperature, pressure, time of exposure), as in real experiments in the presence of polymer. After the control exposure the substrate was checked with SFM. The purity of the system was considered to be acceptable only if there were no adsorbed structures visualized by SFM. Otherwise the cleaning procedure was repeated. The applied procedure is apparently not very optimal as far as we indeed occasionally have to repeat it several times.

Gravimetric tests on solubility were performed with Ohaus AP250D balance (guaranteed reproducibility 0.02 mg) for chitosan samples with weights in range of 1–10 mg. We measured and analysed the mass loss of the samples as a result of the exposure to sc CO_2 in the reactor.

The deposition of macromolecules was performed as illustrated by Figure 1. In the reactor we put a freshly cleaved piece of mica with a surface area of about 1.5 cm^2 and small amount of chitosan (0.2–0.5 mg). The reactor was closed, tightened, and thoroughly purged with CO_2. Then it was filled with sc CO_2 and exposed for 2–4 hours at constant temperature (70 °C) and pressure (70 MPa). The exposure was finished by a rather fast decompression: the complete removal of CO_2 from the reactor took about 10 sec. Then the substrate was retrieved from the disassembled reactor and scanned with SFM. When successive series of several exposure cycles were repeated, at every cycle a new freshly cleaved mica substrate was put into the reactor (for the following SFM analysis after the exposure), but the chitosan was not added: the amount still remaining in the reactor was supposed to be redissolved and redeposited.

The static SFM observations were carried out in a tapping mode in air at room conditions (relative humidity ca. 40–60%) using a MultiMode microscope with a Nanoscope-IIIa controller (Digital Instruments, USA). When imaging dynamics of vapour-induced transformations we applied constant water vapour flow throw a hollow tip directly to the small surface area just being scanned. The air flow was generated by an air pump and was saturated with vapours in a bubbling system. SFM images were collected with an information density of 512 × 512 points at 1 Hz scanning frequency. We used silicon NCH-W cantilevers (NanoWorld AG, Switzerland) with a resonance frequency of about 320 kHz. Editing of the SFM images and image analysis including height measurements were performed by means of NanoScope software (Digital Instruments, USA). In order to estimate reliably the heights of the deposited macromolecules above a substrate level we performed many repetitive measurements in different places of the sample and then applied averaging procedure. This

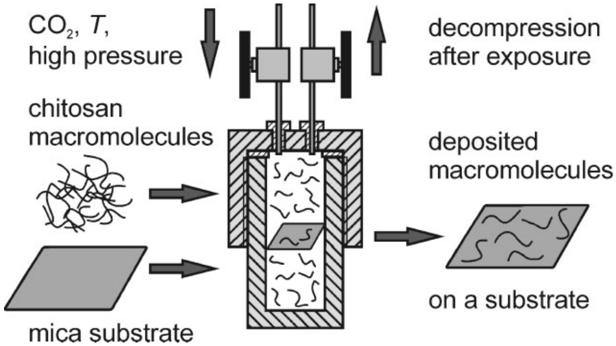

Figure 1.
Scheme of direct deposition of chitosan macromolecules onto mica substrate from solutions in sc CO_2.

scheme resulted in a confidence range of the measured heights.

Results and Discussion

At the first stage, we tested solubility of several different chitosan samples with different DAs and different molecular weights, including some chitosan derivatives. As it was already discussed in Ref. [5], mostly, they were completely insoluble in sc CO_2. In order to check solubility we applied two approaches: i) SFM testing of a precipitate on a substrate after exposing it to sc CO_2 in the presence of polymer, ii) gravimetrical analysis of the difference between the amount of chitosan put in the reactor initially and the amount found in the reactor after the exposure. The approach with the SFM analysis of the precipitate is a very sensitive method to check solubility as far as it may allow one to detect even single polymer molecules on a substrate after dissolution and following precipitation. Yet, in many cases we did not find any structures, which might be related to the precipitated polymer material. Thus in such cases we drew a conclusion about complete insolubility of the material tested. Such a conclusion was always in general agreement with the gravimetric data, in spite of the fact that reproducibility of gravimetric analysis was rather poor from one experiment to another (apparently, due to occasional sample losses not related to real dissolution and reprecipitation).

Chitosan-0.85-Ca (15% DA) complexed with calcium hydroxide is the one of the samples, which has demonstrated some solubility (yet also very limited one). For that material the reason for the enhanced solubility may be related to some affinity between calcium ions and CO_2 molecules. On the other hand, it may also be related to a reduced amount of hydrogen bonds in such a rather bulky macromolecular complex. In our previous paper[5] we reported high degree of supramolecular aggregation for that material as deposited on mica from solutions in sc CO_2. Later on we have found out that the chitosan-0.85-Ca macromolecules may be deposited also as more isolated strands if the exposure/decompression cycles are repeated a few times with the same chitosan material remaining in the reaction chamber after every decompression step (the piece of mica substrate is to be changed for a new one at the every cycle). Typical images of chitosan-0.85-Ca macromolecules deposited from sc CO_2 are presented in Figure 2.

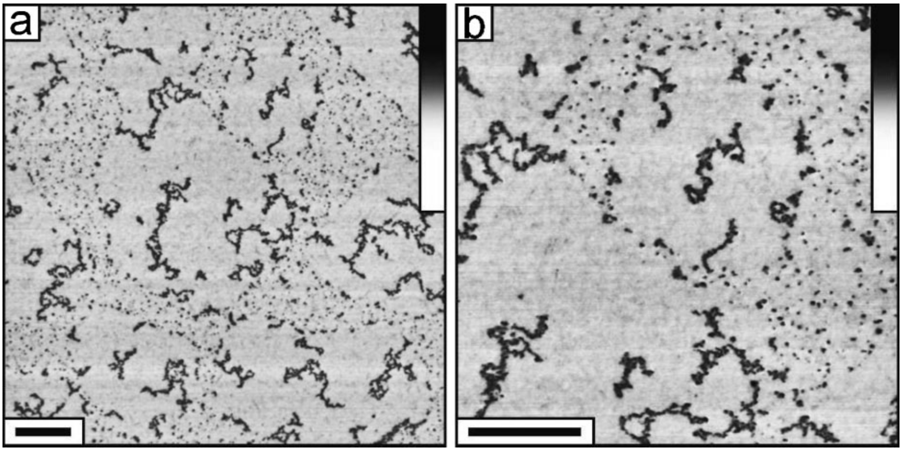

Figure 2.
Micrographs of Ca-complexed macromolecules of chitosan-0.85-Ca sample on mica substrate as deposited directly from solution in sc CO_2 (exposure conditions: 70 MPa, 70 °C, 4 h) as a results of the *second* exposure/decompression cycle. SFM images of different sizes: a) $4 \times 4\,\mu m^2$, b) $2 \times 2\,\mu m^2$. Bar size 500 nm, height scale 10 nm.

One can see here well resolved individual polysaccharide chains as deposited from the solution in sc CO_2. They adopt somewhat extended conformation of an adsorbed two-dimensional coil. Thus, solubility of this material is enough to be deposited on a substrate as single individual macromolecules from the sc CO_2-solution for the following SFM observation, but that implies still very small concentrations. Indeed, from the viewpoint of possible applications the detected solubility is yet discouragingly poor both for that macromolecular complex and for the all other so far tested materials as well (among those demonstrating any detectable solubility at all).

The heights of the visualised polymer chains (Figure 2) above the substrate level are typically in range of 1.5–1.9 nm coinciding well with the previously reported values for chitosan-0.85 sample.[5] Taking into account these values it is clear, that molecular conformation is not highly extended. Instead, the macromolecules keep some elements of a secondary structure and demonstrate pronounced local folding and bending. In the images of Figure 2 one can notice some helical formations and hairpins. Such features of the macromolecular conformation contribute to the increased height as compared to the one expected for a hypothetic fully stretched chain, that is below 1.1 nm.[24–30] It is interesting to relate this peculiarity of the observed macromolecular morphology to the behaviour of the polysaccharide chains in aqueous solutions. Chitosan macromolecules in aqueous solutions are typically described as semi-rigid (or semi-flexible) worm-like chains.[31–53] At low pH the mutual electrostatic repulsion of different macromolecular segments is supposed to contribute significantly to the increased stiffness. But with increase of the ionic strength and thus screening of the electrostatic interaction, the macromolecular flexibility is increasing and finally the intrinsic stiffness of the polymer chains may be revealed. Still, even this intrinsic stiffness is supposed to be rather large because of some ordered, presumably, helical macromolecular conformation.[1,51,52] It should be pointed out that the conformation of the macromolecules dissolved in nonpolar sc CO_2 is not affected by electrostatics. Thus the observed hairpins and helical structures as well as some other clearly visible (Figure 2) indicators of strong intra- (and inter-) macromolecular association for polymer chains deposited from solution in sc CO_2 may be the very reasons for the rather large intrinsic stiffness of chitosan macromolecules as typically observed in experiments.[31–53]

Of course, complexation with Ca may also affect a macromolecular conformation. Thus we tested solubility in sc CO_2 for pure (non-complexed) chitosan samples with different DAs. Among the tested materials, we did not detect any solubility of the samples prepared by precipitation from acetic acid into alkaline media. On the contrary, some of the exactly the same materials became soluble in sc CO_2 after precipitation from dichloracetic acid into alkaline media. In distinct from acetic acid, the dichloracetic acid is known to be an effective acceptor of hydrogen bonds thus such a pre-treatment produces a chitosan material with reduced hydrogen bonding.[54,55] It appears that reduction of hydrogen bonding promotes solubility.

Nevertheless, for chitosan-0.98 (2% DA) the solubility was still rather small even after such a pre-treatment and we were not able to resolve reliably any deposited macromolecules on a substrate surface after the exposure in sc CO_2 in the presence of this material. For chitosan-0.85 (15% DA) the solubility was the highest one as detected both gravimetrically and by means of SFM analysis of the precipitate. Some typical images of the chitosan-0.85 macromolecules as deposited from sc CO_2 are presented in our previous paper.[5] The macromolecules adopt rather extended conformation thus it is possible to measure their contour lengths and then to perform a quantitative conformational analysis, see Ref. [5]. The measured heights of the macromolecular SFM-profiles above the substrate levels are mostly in the range of 1.3–2.0 nm similarly to that for chitosan-0.85-Ca sample.

Thus, the increased heights here are apparently again explained by some local compaction of the macromolecules: folding, bending, formation of hairpins and helixes.

For chitosan-0.50 (50% DA) the solubility was intermediate one, i.e. it was higher as compared to the chitosan-0.98 sample, but smaller as compared to the chitosan-0.85. Typical images of some sc-deposited chitosan-0.50 macromolecules are presented in Figure 3 (see also Figure 4). Usually, the contours of the adsorbed from sc CO_2 chitosan-0.50 macromolecules are noticeably shorter as compared to chitosan-0.85-Ca and chitosan-0.85 samples and the heights of the macromolecular SFM-profiles above the substrate level are larger: they are in range of 1.9–2.9 nm. Apparently, the chitosan-0.50 macromolecules during adsorption from sc CO_2 adopt more compact conformation on a substrate because of different chemical composition (larger amount of acetamido groups).

Sc CO_2 is an unusual solvent possessing rather peculiar properties. One of them is high intensity of density fluctuations, which may affect strongly the conformation of a dissolved polymer chain.[56–61] The influence of such fluctuations on the macromolecular conformation is especially profound in the proximity of a critical point.[56–61] Our system is far away from the CO_2 critical point (7.4 MPa, 31 °C) during the exposure stage (70 MPa, 70 °C): both pressure and temperature are much higher than the critical values. Yet during the subsequent decompression stage the system may approach the critical point more closely as far as at this stage the pressure decrease is always associated with some decrease of temperature. Indeed, when density of sc fluid is being reduced (i.e. the mean intermolecular distance is increasing), the potential energy of CO_2 molecules interaction is increasing too and that increase is to be compensated by corresponding decrease of their kinetic energy (thus, reduction of temperature). Therefore, the conformation of adsorbed macromolecules as prepared by deposition from sc CO_2 may be affected by strong density fluctuations of the solvent and thus may be rather atypical as compared to the same macromolecules deposited from usual liquid media. Indeed, the general appearance of the chitosan macromolecules in the SFM images as presented here and in Ref. [5] is dissimilar as compared to typical appearance of the polysaccharide chains deposited from aqueous solutions.[11–15] Therefore, the deposited from sc CO_2 macromolecules may adopt rather peculiar

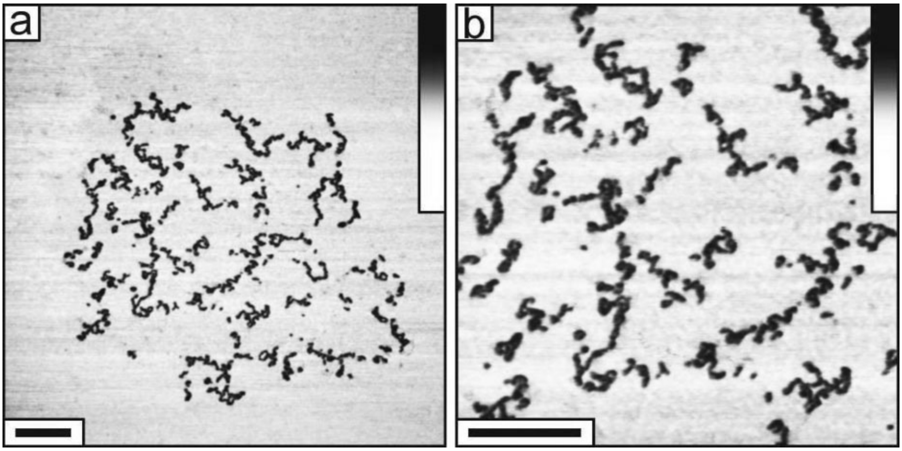

Figure 3.
Micrographs of chitosan-0.50 macromolecules on mica substrate, deposited from sc CO_2 (70 MPa, 70 °C) as a results of the *third* exposure/decompression cycle. SFM images of different sizes: a) 2 × 2 μm^2, b) 1 × 1 μm^2. Bar size 250 nm, height scale 10 nm.

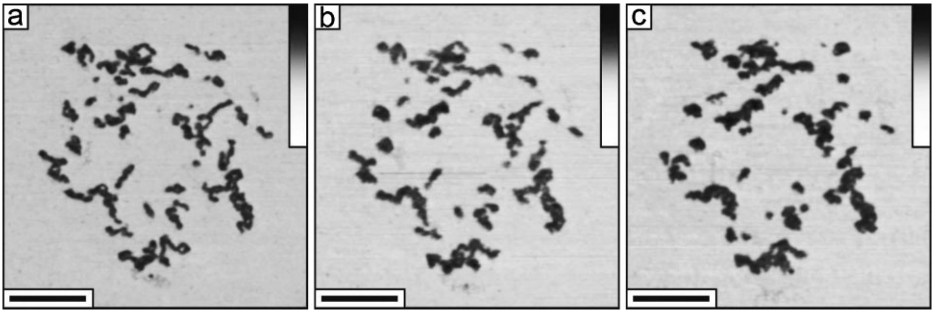

Figure 4.
Micrographs of chitosan-0.50 macromolecules on mica substrate: influence of exposure to water vapour. a: macromolecules as deposited from sc CO_2 (70 MPa, 70 °C); b–c: the same macromolecules during exposure to water vapour being performed for 40 min (b) and in dry conditions after 2 h of exposure to water vapour (c). Scan size: $1 \times 1\,\mu m^2$, bar size 250 nm, height scale 10 nm.

conformation determined by such a specific solvent influence. After solvent removal this conformation become non-equilibrium one, trapped by adhesion to the substrate. Indeed, the macromolecular mobility on mica is strongly suppressed after solvent removal and reorganisation is kinetically frozen.[16–20]

Yet, it is known that surface-trapped conformations may be transferred into more relaxed ones if the adsorbed macromolecules are exposed to water vapour.[18,20,62–65] This effect is well established for amphiphilic polymer chains adsorbed on substrates with high surface energy (e.g. mica, silicon, etc.) when there is an ultrathin water layer coadsorbed from vapour phase.[16–20,62–65] The coadsorption of water molecules unfreezes macromolecular mobility and the amphiphilic polymer chains having surface active properties expand in order to maximize occupied surface area at the interface (thus reducing interfacial tension). This effect is similar to the long known extension of some amphiphilic polymer molecules at water surface (though they are insoluble in water in a bulk).[66–70]

Taking into account the results of those observations[16–20,62–70] we expected that chitosan-0.50 strands would unfold on a substrate during exposure to water vapour. Indeed, the chitosan-0.50 macromolecules have amphiphilic nature comprising both hydrophilic and hydrophobic moieties in the macromolecular structure. The factual dynamics observed using environmental SFM during exposure to water vapour is depicted in Figure 4.

In the images of Figure 4 one can see that the macromolecules indeed acquire some mobility and demonstrate some reorganisation as a result of the exposure to water vapour. But they do not expand: on the contrary, they are getting more compact. Still, the height of the macromolecules above the substrate level is not changing, that means they are not being displaced from the interface and are keeping the same occupied surface area. Thus, such behaviour is not in contradiction with the model proposed in Refs. [16–20] The observed compaction is of a two-dimensional type and may be explained by hydrophobic attraction of acetamido groups resulting in intramolecular association.

Conclusion

We performed SFM studies of individual chitosan macromolecules as deposited directly from such a peculiar non-liquid solvent as sc CO_2. Using the procedure of direct deposition from polymer solutions in sc CO_2 one can eliminate the disturbing influence of surface tension gradients or capillary forces on the morphology of the structures being deposited. We have systematically tested several chitosan materials with different DAs and pre-treated in different manners for the possibility to be dissolved and reprecipitated on a substrate surface during an exposure in a reactor with sc CO_2. Only a few among many tested chitosan samples have demonstrated any detectable solubility (yet very limited one) at the laboratory achievable conditions (regarding temperature and pressure of the sc medium). The employed deposition strategy has allowed us to visualise single individual chitosan macromolecules adsorbed on a substrate from sc CO_2 in a somewhat extended conformation.

At the same time, atypical nature of a highly fluctuating sc fluid raises the question about possible specific influence of the solvent on the conformation of the deposited macromolecules. If the acquired conformation is strongly non-equilibrium one, the adsorbed macromolecules may show pronounced tendency towards relaxation, provided the macromolecular mobility on a substrate is somehow unfrozen. In that case one may expect that for increased amount of deposited macromolecules (i.e. for thin films of the polysaccharide) there will be also strong tendency towards morphological reorganisation. Consequently, the sc-deposited coatings will apparently demonstrate poor stability, deterioration of mechanical properties and delamination. In order to model and check this mechanism it is possible to enhance macromolecular mobility on a substrate by means of an exposure to water vapour. Possible vapour-induced reorganisations can then be followed in real time by environmental SFM. For chitosan macromolecules deposited from sc CO_2 and exposed to water vapour we have found only slight tendency towards transformation into more compact but still two-dimensional structures. More experiments are yet required to reveal general features of vapour-induced conformational transitions of different chitosan macromolecules (with different chemical composition, DAs, molecular weights) directly adsorbed on a substrate from solutions in sc CO_2.

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[1] M. Rinaudo, *Prog. Polym. Sci.* **2006**, *31*, 603.
[2] C. K. S. Pillai, W. Paul, Ch. P. Sharma, *Prog. Polym. Sci.* **2009**, *34*, 641.
[3] A. I. Gamzazade, N. P. Bakuleva, E. M. Belavtseva, M. O. Gallyamov, *Bull. Russ. Acad. Sci: Phys.* **2009**, *73*, 468.
[4] G. M. Nogueira, A. C. D. Rodas, R. F. Weska, C. G. Aimoli, O. Z. Higa, M. Maizato, A. A. Leiner, R. N. M. Pitombo, B. Polakiewicz, M. M. Beppu, *Mater. Sci. Eng. C* **2010**, *30*, 575.
[5] M. O. Gallyamov, I. S. Chaschin, A. I. Gamzazade, A. R. Khokhlov, *Macromol. Chem. Phys.* **2008**, *209*, 2204.
[6] M. O. Gallyamov, R. A. Vinokur, L. N. Nikitin, E. E. Said-Galiyev, A. R. Khokhlov, I. V. Yaminsky, K. Schaumburg, *Langmuir* **2002**, *18*, 6928.
[7] M. O. Gallyamov, V. M. Buznik, A. K. Tsvetnikov, R. A. Vinokur, L. N. Nikitin, E. E. Said-Galiev, A. R. Khokhlov, K. Schaumburg, *Khimicheskaya Fizika* **2004**, *23*, 76.
[8] M. O. Gallyamov, L. N. Nikitin, A. Yu. Nikolaev, A. N. Obraztsov, V. M. Bouznik, A. R. Khokhlov, *Colloid J.* **2007**, *69*, 411.
[9] N. A. Shumilkina, V. D. Myakushev, E. A. Tatarinova, M. I. Buzin, N. V. Voronina, T. V. Laptinskaya, M. O. Gallyamov, A. R. Khokhlov, A. M. Muzafarov, *Polym. Sci. A* **2006**, *48*, 1240.
[10] M. O. Gallyamov, A. Mourran, B. Tartsch, R. A. Vinokur, L. N. Nikitin, A. R. Khokhlov, K. Schaumburg, M. Möller, *Phys. Chem. Chem. Phys.* **2006**, *8*, 2642.
[11] M. K. Cowman, M. Li, A. Dyal, E. A. Balazs, *Carbohydr. Polym.* **2000**, *41*, 229.
[12] M. Milas, M. Rinaudo, I. Roure, S. Al-Assaf, G. O. Phillips, P. A. Williams, *Biopolymers* **2001**, *59*, 191.
[13] M. K. Cowman Ch. Spagnoli, D. Kudasheva, M. Li, A. Dyal, S. Kanai, E. A. Balazsy, *Biophys. J.* **2005**, *88*, 590.
[14] Ch. Spagnoli. A. Korniakov, A. Ulman, E. A. Balazs, Y. L. Lyubchenko, M. K. Cowman, *Carbohydr. Res.* **2005**, *340*, 929.
[15] T. Ueno, Sh. Yokota. T. Kitaoka, H. Wariishi, *Carbohydr. Res.* **2007**, *342*, 954.
[16] M. O. Gallyamov, B. Tartsch, A. R. Khokhlov, S. S. Sheiko, H. G. Börner, K. Matyjaszewski, M. Möller, *Chem. - Eur. J.* **2004**, *10*, 4599.

[17] M. O. Gallyamov, B. Tartsch, A. R. Khokhlov, S. S. Sheiko, H. G. Börner, K. Matyjaszewski, M. Möller, *J. Microsc.* **2004**, *215*, 245.

[18] M. O. Gallyamov, B. Tartsch, A. R. Khokhlov, S. S. Sheiko, H. G. Börner, K. Matyjaszewski, M. Möller, *Macromol. Rapid Commun.* **2004**, *25*, 1703.

[19] M. O. Gallyamov, A. R. Khokhlov, M. Möller, *Macromol. Rapid Commun.* **2005**, *26*, 456.

[20] M. O. Gallyamov, B. Tartsch, P. Mela, I. I. Potemkin, S. S. Sheiko, H. Börner, K. Matyjaszewski, A. R. Khokhlov, M. Möller, *J. Polym. Sci. B: Polym. Phys.* **2007**, *45*, 2368.

[21] A. I. Gamzazade, ''Chitin/chitosan derivatives of controlled structure as prospective new biomaterials'', DrSci thesis, INEOS RAS, Moscow **2005**.

[22] A. Gamzazade, M. Chlenov, A. Kriger, E. Frontchek, in: ''*Chitin and chitosan*'', H. Struszczyk, H. Pospieszny, A. Gamzazade, Eds., Polish Chitin Society, Lodz 1999, p 51.

[23] S. V. Nemtsev, A. I. Gamzazade, S. V. Rogozhin, V. M. Bykova, V. P. Bykov, *Appl. Biochem. Microbiol.* **2002**, *38*, 521.

[24] G. L. Clark, A. F. Smith, *J. Phys. Chem.* **1936**, *40*, 836.

[25] K. Mazeau, W. T. Winter, H. Chanzy, *Macromolecules* **1994**, *27*, 7606.

[26] K. Okuyama, K. Noguchi, M. Kanenari, T. Egawa, K. Osawa, K. Ogawa, *Carbohydr. Polym.* **2000**, *41*, 237.

[27] K. Okuyama, K. Osawa, Y. Hanafusa, K. Noguchi, K. Ogawa, *J. Carbohydr. Chem.* **2000**, *19*, 789.

[28] A. Lertworasirikul, S.-i. Tsue, K. Noguchi, K. Okuyama, K. Ogawa, *Carbohydr. Res.* **2003**, *338*, 1229,

[29] A. Lertworasirikul, S. Yokoyama, K. Noguchi, K. Ogawa, K. Okuyama, *Carbohydr. Res.* **2004**, *339*, 825.

[30] A. Lertworasirikul, K. Noguchi, K. Ogawa, K. Okuyama, *Carbohydr. Res.* **2004**, *339*, 835.

[31] A. I. Gamzazade, V. M. Slimak, A. M. Skljar, E. V. Stykova, S.-S. A. Pavlova, S. V. Rogozin, *Acta Polym.* **1985**, *36*, 420.

[32] M. W. Anthonsen, K. M. Vårum, O. Smidsrød, *Carbohydr. Polym.* **1993**, *22*, 193.

[33] M. Rinaudo, M. Milas, P. L. Dung, *Int. J. Biol. Macromol.* **1993**, *15*, 281.

[34] N. Errington, S. E. Harding, K. M. Vårum, L. Ilium, *Int. J. Biol. Macromol.* **1993**, *15*, 113.

[35] R. G. Beri, J. Walker, E. T. Reese, J. E. Rollings, *Carbohydr Res.* **1993**, *238*, 11.

[36] M. L. Tsaih, R. H. Chen, *Int. J. Biol. Macromol.* **1997**, *20*, 233.

[37] G. Berth, H. Dautzenberg, M. G. Peter, *Carbohydr. Polym.* **1998**, *36*, 205.

[38] R. H. Chen, M. L. Tsaih, *Int. J. Biol. Macromol.* **1998**, *23*, 135.

[39] R. Signini, J. Desbrières, S. P. C. Filho, *Carbohydr. Polym.* **2000**, *43*, 351.

[40] E. Buhler, M. Rinaudo, *Macromolecules* **2000**, *33*, 2098.

[41] E. Buhler, O. Guetta, M. Rinaudo, *Int. J. Polym. Anal. Charact.* **2000**, *6*, 155.

[42] K. Mazeau, S. Pérez, M. Rinaudo, *J. Carbohydr. Chem.* **2000**, *19*, 1269.

[43] J. Brugnerotto, J. Desbrières, G. Roberts, M. Rinaudo, *Polymer* **2001**, *42*, 9921.

[44] J.-H. Pa, T. L. Yu, *Macromol. Chem. Phys.* **2001**, *202*, 985.

[45] G. Berth, H. Cölfen, H. Dautzenberg, *Progr. Colloid Polym. Sci.* **2002**, *119*, 50.

[46] G. Berth, H. Dautzenberg, *Carbohydr. Polym.* **2002**, *47*, 39.

[47] S. Trzciński, K. M. Vårum, D. U. Staszewska, O. Smidsrød, M. Bohdanecký, *Carbohydr. Polym.* **2002**, *48*, 171.

[48] J. Desbrieres, *Biomacromolecules* **2002**, *3*, 342.

[49] V. I. Pedroni, P. C. Schulz, M. E. Gschaider, N. Andreucetti, *Colloid. Polym. Sci.* **2003**, *282*, 100.

[50] C. Schatz, C. Viton, T. Delair, C. Pichot, A. Domard, *Biomacromolecules* **2003**, *4*, 641.

[51] M. Rinaudo, *Macromol. Symp.* **2006**, *245-246*, 549.

[52] M. Rinaudo, *Macromol. Biosci.* **2006**, *6*, 590.

[53] G. A. Morris, J. Castile, A. Smith, G. G. Adams, S. E. Harding, *Carbohydr. Polym.* **2009**, *76*, 616.

[54] A. I. Gamzazade, A. M. Sklyar, S. A. Pavlova, S. V. Rogozhin, *Vysokomolek. Soed. A* **1981**, *23*, 594.

[55] E. F. Titova, E. M. Belavtseva, A. I. Gamzazade, A. M. Sklyar, S. A. Pavlova, S. V. Rogozhin, *Acta Polym.* **1986**, *37*, 121.

[56] V. V. Vasilevskaya, P. G. Khalatur, A. R. Khokhlov, *J. Chem. Phys.* **1998**, *109*, 5108.

[57] V. V. Vasilevskaya, P. G. Khalatur, A. R. Khokhlov, *J. Chem. Phys.* **1998**, *109*, 5119.

[58] A. Dua, B. J. Cherayil, *J. Chem. Phys.* **1999**, *111*, 3274.

[59] T. Sumi, H. Sekino, *Chem. Phys. Lett.* **2005**, *407*, 322.

[60] T. Sumi, H. Sekino, *J. Chem. Phys.* **2005**, *122*, 194910.

[61] T. Sumi, N. Imazaki, H. Sekino, *Phys. Rev. E* **2009**, *79*, 030801.

[62] J. Kumaki, Y. Nishikawa, T. Hashimoto, *J. Am. Chem. Soc.* **1996**, *118*, 3321.

[63] J. Kumaki, T. Hashimoto, *J. Am. Chem. Soc.* **2003**, *125*, 4907.

[64] J. Kumaki, T. Kawauchi, E. Yashima, *Macromolecules* **2006**, *39*, 1209–1215.

[65] J. Kumaki, T. Kawauchi, E. Yashima, *Macromol. Rapid Commun.* **2008**, *29*, 406.

[66] R. Vilanove, F. Rondelez, *Phys. Rev. Lett.* **1980**, *45*, 1502.

[67] A. Takahashi, A. Yoshida, M. Kawaguchi, *Macromolecules* **1982**, *15*, 1196.

[68] A. Takahashi, A. Yoshida, M. Kawaguchi, *Macromolecules* **1983**, *16*, 956.

[69] R. Vilanove, D. Poupinet, F. Rondelez, *Macromolecules* **1988**, *21*, 2880.

[70] F. Monroy, H. M. Hilles, F. Ortega, R. G. Rubio, *Phys. Rev. Lett.* **2003**, *91*, 268302.