ELSEVIER

Carbohydrate Polymers 37 (1998) 7–12

Carbohydrate Polymers

AFM in studies of thermoplastic starches during ageing

L. Kuutti[a], J. Peltonen[b], P. Myllärinen[c], O. Teleman[c], P. Forssell[c,*]

[a]*VTT, Chemical Technology, PO Box 1401, FIN-02044 Espoo, Finland*
[b]*Department of Physical Chemistry, Åbo Akademi University, Porthaninkatu 3-5, FIN-20500 Turku, Finland*
[c]*VTT, Biotechnology and Food Research, PO Box 1503, FIN-02044 Espoo, Finland*

Received 16 January 1998; revised 21 April 1998; accepted 5 May 1998

Abstract

Atomic force microscopy (AFM) and friction force microscopy (FFM) have been used to characterize the ageing of thermoplastic starch (TPS) films prepared by the extrusion technique, using water and glycerol as plasticizers. The fresh oat and barley starch films (1 week after extrusion) have flat and homogeneous surfaces. In the older starch films (2–5 weeks), the surface roughness has changed and the friction images reveal surface heterogeneity. The structural changes are supported by the tip-sample force–distance curve analysis. The changes in the structural properties during ageing i.e. starch–glycerol phase separation, crystallization of starch, or reorientation of polymers, are discussed.

Keywords: Starch; Atomic force microscopy; Friction force microscopy

1. Introduction

Starch can be processed to mouldable thermoplastic material at high temperatures under shear (Shogren et al., 1992; Sala and Tomka, 1993). During processing the semi-crystalline structure is lost and the molecules are partially depolymerized. If water is used as the plasticizer the product is brittle, but in the presence of another plasticizer, such as glycerol, materials with varying mechanical properties can be prepared. Even if stored at constant humidity and temperature, the mechanical properties of thermoplastic starch (TPS) films change with time (Forssell et al., 1998).

Starch occurs as semi-crystalline small particles, which are insoluble in water at room temperature. Starch is a homopolymer of glucose consisting of α (1,4)-linked, essentially linear and α (1,4)- and α (1,6)-linked amylopectin, with a highly branched structure. The chains of amylose easily form single or double helices (Zobel, 1992). The linear outer chains of the amylopectin form left-handed double helical structures, with strong hydrogen bonding of the hydroxyl groups. The crystalline regions are arranged as thin lamellar domains (Kassenbeck, 1975; Kassenbeck, 1978).

The atomic force microscope (AFM) (Binnig et al., 1986) creates a surface image by scanning a flexible cantilever mounted tip over the sample. AFM is now an established technique (see e.g. Hansma et al., 1988; Drake et al., 1989; Hansma et al., 1991; Butt et al., 1992; Peltonen et al., 1992; Bustamante and Keller, 1995; Zasadzinski, 1996) and will not be described in detail here. When the tip is scanned sideways, the friction force causes a torsion in the tip cantilever. A micrograph showing this torsion is termed friction force microscopy (FFM) (Mate et al., 1987; Meyer and Amer, 1990; Bhushan et al., 1995), and can be used, for example, to resolve different phases in phase-separated thin films (Overney et al., 1992).

AFM has been used directly in the structural investigation of biological specimens (Bustamante et al., 1994, Yang and Shao, 1995) and surfaces. Hanley and Gray applied the technique to cellulose microfibrils (Hanley et al., 1992; Hanley et al., 1994), and obtained information on the microfibril morphology at the 100 nm scale. More recently, we achieved a resolution of 4 Å in AFM micrographs of crystalline cellulose surfaces (Kuutti et al., 1995), and demonstrated that they corroborate the three-dimensional crystalline cellulose structures derived from electron diffraction experiments (Wada et al., 1993; Sugiyama et al., personal communication, 1992). AFM has also been applied to image real-time enzymatic degradation of starch granules (Thomson et al., 1994) and the surface structures of a starch–zein thermoplastic polymer (Gauldie et al., 1994).

* Corresponding author. Tel: 00 358 94565212; Fax: 00 358 94552103; e-mail: pirkke.forssell@vtt.fi

0144–8617/98/$ - see front matter
PII S0144-8617(98)00042-3

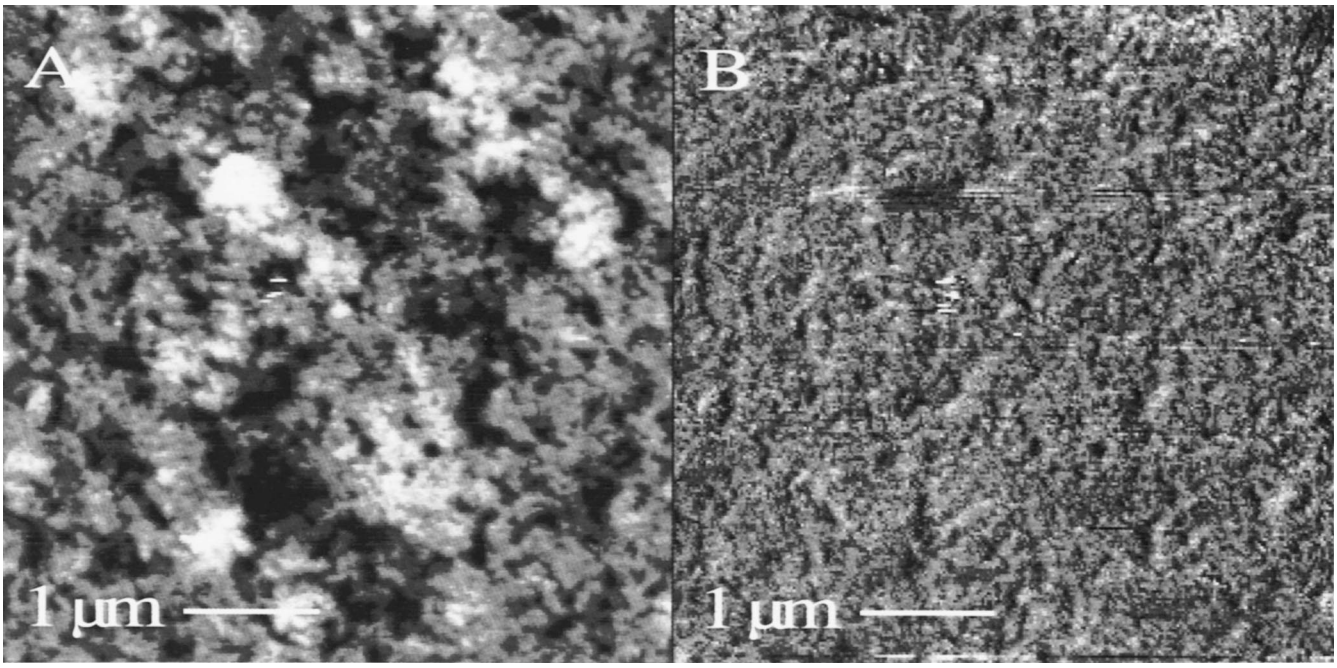

Fig. 1. (a) A top-view AFM image of the fresh barley film (1 week old), and (b) a simultaneously measured lateral force image. The image size is $5 \times 5\ \mu m^2$ and the black-to-white colour scale in (a) (*z*-scale) is 0–50 nm. The friction image (b) reveals a homogenous surface (relative scale).

Here, we use atomic and friction force microscopy to characterize the ageing phenomena at the surface of TPS films. Some results of a tip-sample force–distance curve analysis are also presented. The molecular origin of ageing is discussed.

2. Materials and methods

2.1. Materials

Barley and oat starch were obtained from Primalco Ltd (Rajamäki, Finland), and glycerol was obtained from Akzo Chemicals (Riedel-de Haën, Seelze, Germany), (85%). Thermoplastic barley and oat starch films were prepared by the plasticization of a starch/glycerol/water mixture in a twin screw extruder (Clextral BC-21, Firming, France), and processing the plasticized mixture to films in a single screw extruder (Brabender Plasti-Corder, Duisburg, Germany). The processing was repeated three times to ensure homogeneity. In both extruders the barrel temperature was 170°C and the die temperature 120°C. Water content of the feed was about 12% by weight and glycerol content 30% by weight (dry basis). The fresh films were near transparent. The ageing occurred under controlled conditions by storing for 1–5 weeks at constant humidity and temperature (RH 50%, 20°C).

2.2. Methods

A Nanoscope III AFM (Digital Instruments, Inc., Santa Barbara, USA) with 200 μm sharpened Si_3N_4 tips, with a spring constant of $k = 0.12$ N/m was used to image the samples. Small pieces of TPS films ($4 \times 4\ mm^2$) were cut and placed on sample stubs. All images (512×512 pixels) were measured in air in the constant force mode (constant deflection). The roughness values were measured for images of the same size, $5 \times 5\ \mu m^2$. The friction mode measures the lateral forces, i.e. the torsion of the cantilever spring in the direction of scanning. The friction images were measured during retrace scanning, so that the dark colour indicates high friction and the light colour low friction, only images with relative friction scale, are presented. The microscope was placed on a vibration damping table to eliminate external noise.

3. Results and discussion

3.1. Ageing of thermoplastic starch films

Fig. 1(a) shows a $5 \times 5\ \mu m^2$ AFM image of the fresh barley film (1 week old), and Fig. 1(b) the corresponding friction force micrograph. The mean roughness of the porous surface was typically 4 ± 1 nm. The friction image indicates a homogeneous surface, i.e. the friction varies little within the studied surface. The fresh oat film (images not shown) is similar, although somewhat rougher, 10 ± 2 nm, and very sticky.

The 2-week-old barley film is rougher (12 ± 2 nm) than the fresh, but remains homogeneous regarding friction. Fig. 2 shows $10 \times 10\ \mu m^2$ micrographs of the 2-week-old oat film. The height profile is similar to that of a 1-week-old sample. The surface roughness has, however, decreased clearly, being 6 ± 1 nm. The main change is observed in

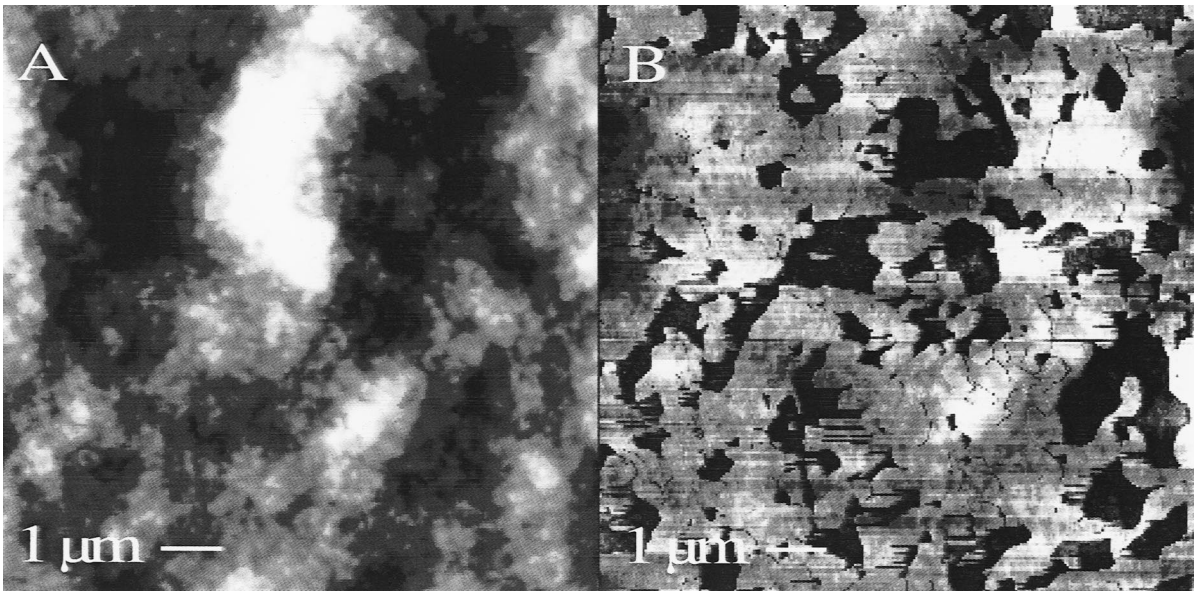

Fig. 2. (a) A $10 \times 10\ \mu m^2$ top-view AFM image of the 2-week-old oat film, and (b) the respective FFM image. The black-to-white colour scale of (a) (*z*-scale) is 0–150 nm. The friction image (b) reveals a partly heterogenous surface stucture, i.e. there are dark and light areas of high and low friction.

the friction image, revealing a partly heterogeneous surface structure [Fig. 2(b)] featuring high (dark) and low (light) friction areas. The areas of low friction dominate the surface. The differences in friction are not due to changes in topography, as seen by comparing Fig. 2(a) and (b). The stickiness of the oat film has decreased considerably.

In the 3–4-week-old oat samples, the same two-phase structure was observed on the surface. The relative amount of the low friction areas has increased even further. The topographic image shows small, round, island-like structures. The friction images captured for barley still indicate a homogeneous surface at an age of 3–4 weeks.

At 5 weeks, the initial higher friction phase has disappeared almost completely from the surface of the oat film, even if local differences are significant. The mean roughness has increased to 15 ± 5 nm. At this age the surface structure of barley also begins to change, as indicated by the friction data. The total surface roughness has increased, but flat areas on the surface are observed from both image types [Fig. 3]. These flat areas are approximately 10 nm thick and often hundreds of nanometers in lateral size (Fig. 4), and appear to have a multilayer lamellar structure. These lamellae are not always straight, parallel, or of uniform thickness, but are typically slippery. Thus, the ageing

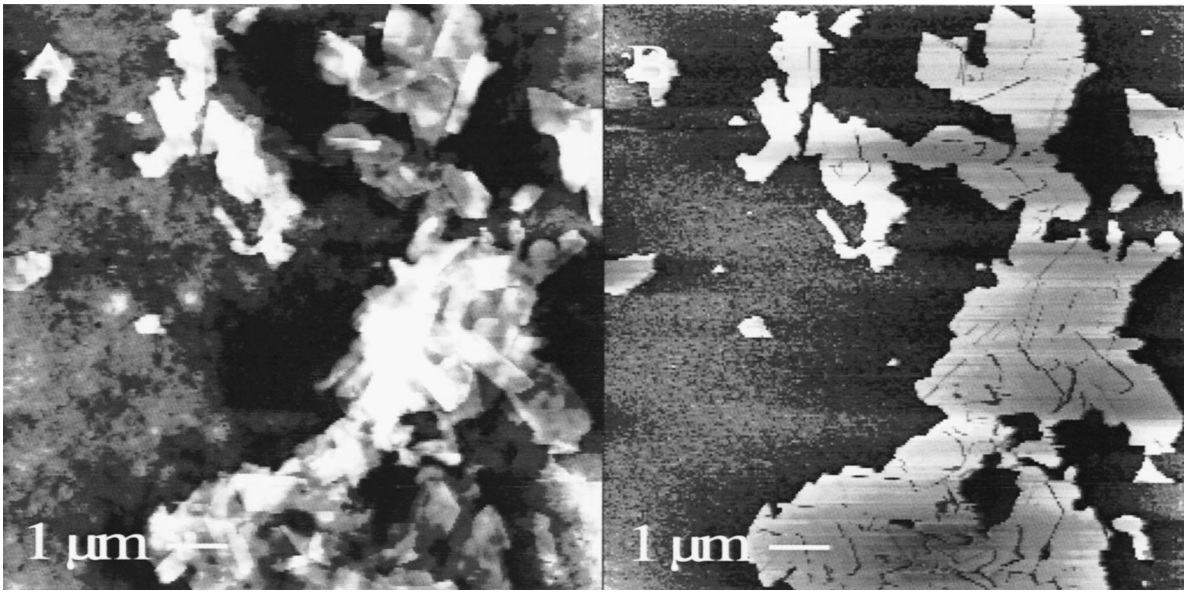

Fig. 3. The 5-week-old barley film, (a) a $10 \times 10\ \mu m^2$ top-view AFM image, and (b) the respective FFM image. The black-to-white colour scale of (a) (*z*-scale) is 0–100 nm.

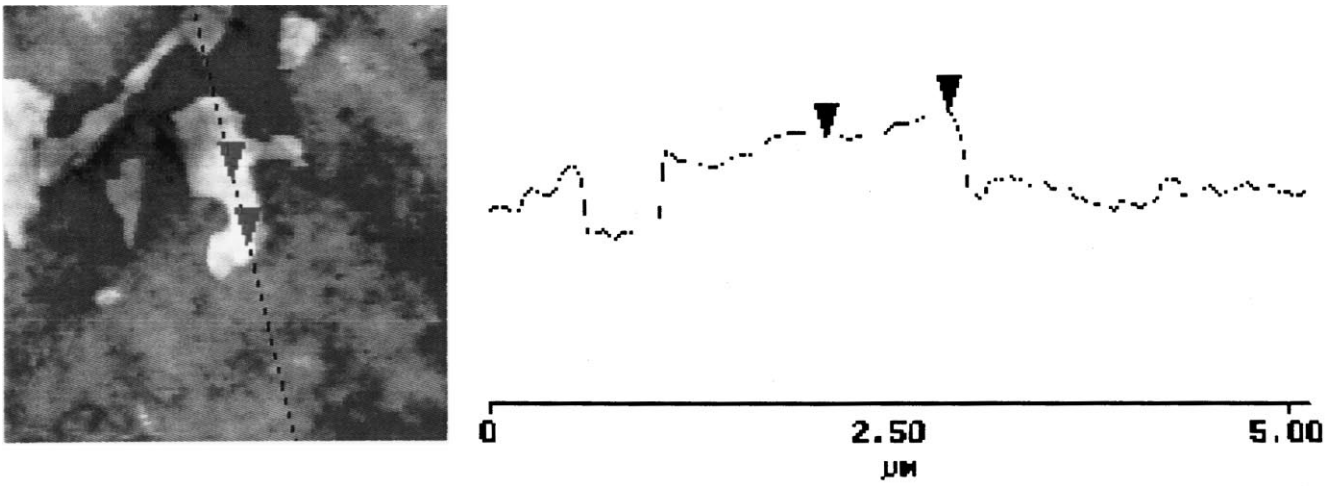

Fig. 4. The section analysis of one multilayer lamellar structure in the barley film. The vertical distance between the two arrows in the figure is 7.1 nm.

brings about a lower friction surface in both oat and barley based films.

An interesting detail ($4 \times 4\ \mu m^2$) of the suggested multilayer lamellar structure is shown in Fig. 5. The right-hand image was recorded immediately after the left-hand one. Part of the lamellar structure in Fig. 5(a) has been peeled off by the microscope tip, as seen in Fig. 5(b). The peeling occurs from the light areas but not from other topological features, which indicates that the flat, slippery areas are softer.

3.2. Force curve analysis

Measurement of the tip-sample, force–distance curves gave additional information about the ageing phenomenon, regarding the changes in the maximum attractive van der Waals force F_{attr} at the point of contact and the adhesion force F_{adh} at the pull-off point (Fig. 6). F_{attr} is directly proportional to the Hamaker constant A, according to the equation $F_{attr} = (AR)/(6d^2)$, where d is the tip-sample distance, normally in the range of 2–4 Å (Israelachvili, 1992), and R is the radius of the microscope tip, assumed to be 40 nm. In the studied system (tip–air–sample), only the sample properties change with time and thus affect the value of A. The adhesion force, F_{adh}, is proportional to the surface energy (Johnson et al., 1971) as $\gamma = F_{adh}/3\pi R$.

Both the Hamaker constant and the surface energy increased with age, although faster for the oat samples.

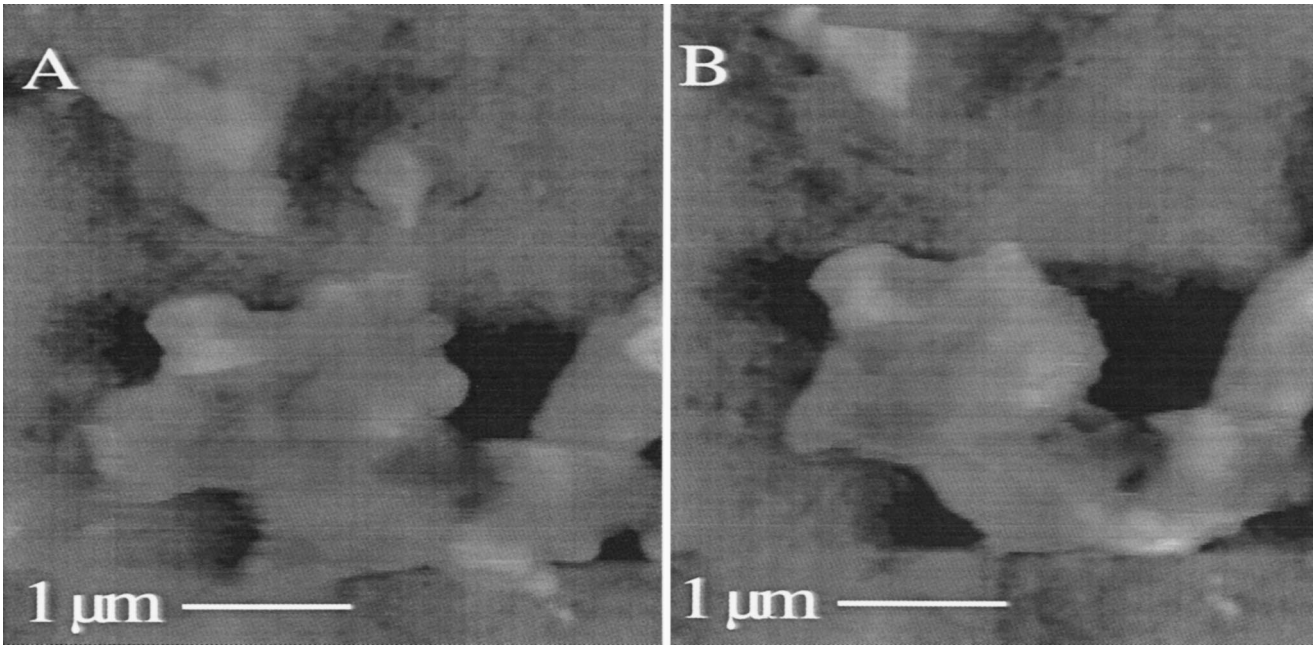

Fig. 5. An interesting detail of the suggested multilayer lamellar structure shown in Fig. 4. These $4 \times 4\ \mu m^2$ FFM images have been measured immediately after each other. Certain parts of the lamellar structure in (a) have been peeled off by the tip, as seen in (b).

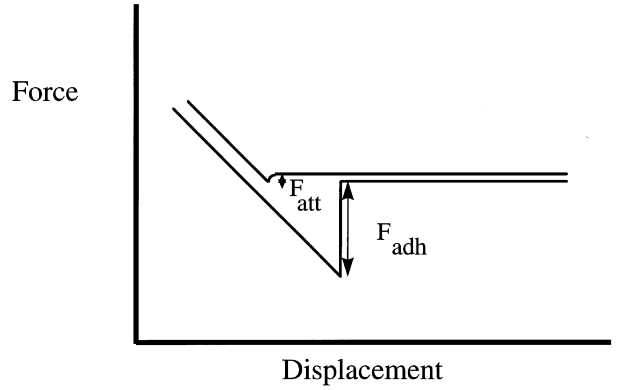

Fig. 6. The tip-sample force–distance curve. F_{attr} is the maximum attractive van der Waals force at the point of contact, and F_{adh} is the adhesion force at the pull-off point.

This is consistent with the observed FFM data which showed heterogeneous friction image already for the 2-week-old sample. The final surface energy of the oat sample is close to the literature value of glycerol, 64 mJ/m^2 (Israelachvili, 1992). However, these results should be considered as preliminary, due to the inaccuracy of the known tip radius and the geometry. The obtained value represents an average for the entire sample; statistics were not collected in a phase separated way. Further, the applied formula neglect any other interactions other than van der Waals.

3.3. Crystallinity changes

In a separate study we have determined the change in crystallinity in extruded starch films (Forssell et al., 1998). Oat and barley films were found to contain the same amount of B-type crystallinity. When fresh, the barley film contained a fair amount of Eh-type crystallinity, which slowly converted into Vh. The oat film contained only Vh-type crystallinity when fresh.

In connection with our results on surface changes, it appears that the Eh-type crystallinity may hinder glycerol diffusion, whereas the Vh-type does not, i.e. it allows the formation of pure glycerol patches on the film surface sooner for the oat film than for barley.

4. Conclusions

The aim was to study the surface of TPS films with FFM and to produce data, which could explain the obtained mechanical behaviour of TPS during ageing (Forssell et al., 1998). The frictional imaging enabled the observation of a contrast in a phase-separated substrate surface, even without topographical differences between the components.

Material-specific friction on the nanometer scale depends on the chemical and physical nature of the molecule. In particular, the stiffness of the molecule, the intra- and intermolecular forces, and the morphological state of the molecule are important. The starch–glycerol–water phase separation on the surface of oat and barley thermoplastic starch films differs from each other. On the surface of oat films, the reason seems to be the diffusion of glycerol. Glycerol, as a small and flexible molecule, seems to diffuse from the starch–glycerol–water matrix to the surface, making those low friction areas. The appearance of glycerol seems to be consistent with the increasing surface energy. In turn, the increasing surface energy coincides with increasing friction, which is in agreement with earlier reports (Green et al., 1995). Also, the decrease in the stickiness of the oat films point to glycerol diffusion.

In the case of barley starch films, the reason seems to be, at first, short-range reorientation of polymers, and finally, the crystallization of starch. The polymer chains form helical crystal structures, which seem to be rather soft material. Earlier results (Kassenbeck, 1975; Kassenbeck, 1978; Jenkins et al., 1993) concerning the thickness of the crystalline lamellae from different botanical sources support our result of approximately 10 nm. The crystalline lamellae in barley starch films are not so-called blockets (Gallant et al., 1997), which are spherical and range in diameter from 20–500 nm depending on starch type and location in the granule (Baldwin, 1995).

Acknowledgements

We thank Dr. A. P. Heiner for useful critical discussions and Prof. J. B. Rosenholm for providing the use of the microscope. Pirkko Forssell gratefully acknowledges financial support through the EC project AIR-2-CT94-1187.

References

Baldwin, P. (1995). Studies on the surface chemistry, minor component composition and structure of granular starches. PhD Thesis, The University of Nottingham, UK.

Bhushan, B., Israelachvili, J. N., & Landman, U. (1995). Nanotribology: friction, wear and lubrication at the atomic scale. *Nature, 374*, 607–616.

Binnig, G., Quate, C. F., & Gerber, C. (1986). Atomic force microscope. *Phys. Rev. Lett., 56*, 930–933.

Bustamante, C., Erie, D., & Keller, D. (1994). Biochemical and structural applications of scanning force microscopy. *Curr. Opin. Struct. Biol., 4*, 750–760.

Bustamante, C., & Keller, D. (1995). Scanning force microscopy in biology. *Physics Today, Dec*, 32–38.

Butt, H.-J., Guckenberger, R., & Rabe, J. P. (1992). Quantitative scanning tunneling microscopy and scanning force microscopy of organic materials. *Ultramicroscopy, 46*, 375–393.

Drake, B., Prater, C. B., Weisenhorn, A. L., Gould, S. A. C., Albrecht, T. R., Quate, C. F., Cannell, D. S., Hansma, H. G., & Hansma, P. K. (1989). Imaging crystals, polymers, and processes in water with the atomic force microscope. *Science, 243*, 1586–1589.

Forssell, P. M., Hulleman, S. H. D., Myllärinen, P., Moates, G. K. M., & Parker, R. (1998). Ageing of rubbery thermoplastic starches prepared from barley and oat starches. *Carbohydrate Polymers*, submitted.

Gallant, D. J., Bouchet, B., & Baldwin, P. M. (1997). Microscopy of starch: evidence of a new level of granule organisation. *Carbohydrate Polymers, 32*, 177–191.

Gauldie, R. W., Raina, G., Sharma, S. K., & Jane, J.-L. (1994). Atomic force microscopy images of starch polymer; crystalline and amorphous structures. In Cohen et al. (Eds.), *Atomic force microscopy/scanning tunneling microscopy* (pp. 85–90). New York: Plenum Press.

Green, J.-B. D., McDermott, M. T., Porter, M. D., & Siperko, L. M. (1995). Nanometer-scale mapping of chemically distinct domains at well-defined organic interfaces using frictional force microscopy. *J. Phys. Chem., 99*, 10960–10965.

Hanley, S. J., Giasson, J., Revol, J.-F., & Gray, D. G. (1992). Atomic force microscopy of cellulose microfibrils: comparison of transmission electron microscopy. *Polymer, 33*, 4639–4642.

Hanley, S. J., Gray, D. G. (1994). Atomic force microscope images of black spruce wood section and pulp fibres. *Holzforschung 48*, 29–34.

Hansma, P. K., Elings, V. B., Marti, O., & Bracker, C. E. (1988). Scanning tunneling microscopy and atomic force microscopy: application to biology and technology. *Science, 242*, 209–216.

Hansma, H. G., Weisenhorn, A. L., Edmundson, A. B., Gaub, H. E., & Hansma, P. K. (1991). Atomic force microscopy: seeing molecules of lipid and immunoglobulin. *Clin. Chem., 37*, 1497–1501.

Israelachvili, J. N. (1992). *Intermolecular and surface forces* (p.176). New York: Academic Press.

Johnson, K. L., Kendall, K., & Roberts, A. D. (1971). Surface energy and the contact of elastic solids. *Proc. R. Soc. Lond. Ser. A, 324*, 301–313.

Jenkins, P. J., Cameron, R. E., & Donald, A. M. (1993). A universal feature in the structure of starch granules from different botanical sources. *Starch/Stärke, 45*, 417–420.

Kassenbeck, P. (1975). Elektronenmikroskopischer Beitrag zur Kenntnis der Feinstruktur der Weizenstärke. *Starch/Stärke, 27*, 217–227.

Kassenbeck, P. (1978). Beitrag zur Kenntnis der Verteilung von Amylose and Amylopektin in Stärkenkörnern. *Starch/Stärke, 30*, 40–46.

Kuutti, L., Peltonen, J., Pere, J., & Teleman, O. (1995). Identification and surface structure of crystalline cellulose studied by atomic force microscopy. *J. Microscopy, 178*, 1–6.

Mate, C. M., McClelland, G. M., Erlandsson, R., & Chiang, S. (1987). Atomic-scale friction of a tungsten tip on a graphite surface. *Phys. Rev. Lett., 59*, 1942–1945.

Meyer, G., & Amer, N. M. (1990). Simultaneous measurement of lateral and normal forces with an optical-beam-deflection atomic force microscope. *Appl. Phys. Lett., 57*, 2089–2091.

Overney, R. M., Meyer, E., Frommer, J., Brodbeck, C., Ţthi, R., Howald, L., Güntherodt, H.-J., Fujihira, M., Takano, H., & Gotoh, Y. (1992). Friction measurements on phase separated thin films with a modified atomic force microscope. *Nature, 359*, 133–135.

Peltonen, J. P. K., Pingsheng, H., & Rosenholm, J. B. (1992). Order and defects of Langmuir–Blodgett films detected with the atomic force microscope. *J. Am. Chem. Soc., 114*, 7637–7642.

Shogren, R. L., Swanson, C. L., & Thomson, A. R. (1992). Extrudates of corn starch with urea and glycols: structure/mechanical property relations. *Starch/Stärke, 44*, 335–338.

Sala, R., & Tomka, I. (1993). Water uptake in partially frozen and plasticized starch. In J. M. V. Blanshard & P. J. Lillford (Eds.), *The glassy state in foods* (pp.483–489). Nottingham: Nottingham University Press.

Thomson, N. H., Miles, M. J., Ring, S. G., Shewry, P. R., & Tatham, A. S. (1994). Real-time imaging of enzymatic degradation of starch granules by atomic force microscopy. *J. Vac. Sci. Technol., B12*, 1565–1568.

Wada, M., Sugiyama, J., Okano, T. (1993). Native celluloses on the basis of two crystalline (Iα/Iβ) system. *J. Appl. Polym. Sci. 49*, 1491–1496.

Yang, J., & Shao, Z. (1995). Recent advances in biological atomic force microscopy. *Micron, 26*, 35–49.

Zasadzinski, J. A. (1996). Scanning force microscopy: new instrumentation and applications. *Curr. Opin. Colloid Interface Sci., 1*, 264–269.

Zobel, H. F. (1992). Starch granule structure. In *Developments in carbohydrate chemistry* (pp. 1–36). American Association of Cereal Chemists, St. Pauls, MN.