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Polymer 42 (2001) 4329–4338

polymer

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Ultrathin sectioning of polymeric materials for low-voltage transmission electron microscopy☆

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Received 16 August 2000; received in revised form 12 October 2000; accepted 24 October 2000

Abstract

The relief in ultrathin sections is interpreted as one of the reasons for the image contrast observed in low-voltage transmission electron microscopy. In order to clarify this, processes taking place in the course of ultrathin sectioning were monitored using a polymeric material composed of hard matrix and soft particles (high-impact polystyrene). Volume changes leading to a surface relief were observed both on cut surfaces and in ultrathin sections. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Ultrathin sectioning; Low-voltage transmission electron microscopy; Polymer blends

1. Introduction

Low-voltage transmission electron microscopy (LV-TEM), which is a new visualisation technique [1,2], provides a new insight into the interpretation of TEM images of polymeric materials. Unlike the conventional voltage values of around 100 kV used in transmission electron microscopy, a voltage of the order of units of kV is exploited in LV-TEM. At those voltages, the instrument is able to provide an enhanced imaging contrast, nearly 20 times higher than for 100 kV [1,2], which is advantageous especially for low atomic number specimens to distinguish phases differing only slightly in their chemical constitution [3–5]. Then, very subtle differences in the structure of the material can be distinguished.

On the other hand, very thin specimens (films) must be prepared from the material to make it transparent for the low-energy electron beam. The required thickness of the ultrathin sections is at the lower limit (below 50 nm) in order to obtain good-quality images.

The results on the phase structure of polymer blends obtained with this technique [3] provide a surprisingly good resolution of the individual polymer constituents, when the chemical constitution is almost identical and the other physical properties of the constituents differ only very slightly (e.g. density). The good image contrast obtained suggests that there must be a physical reason for the effect.

The origin of the image contrast was ascribed to differences in density of the individual components [3–6]. The actual mechanism, however, remains unclear. More information is therefore needed to answer the question.

In the present study, the key to the solution was sought in the processes taking place in the course of the sample preparation (ultrathin sectioning), which control the final microstructure of the specimen. The details of the sample preparation (cutting ultrathin sections) are followed and the surface structure of the cut surfaces and of the ultrathin sections is inspected. High-impact polystyrene (HIPS) was selected to monitor the behaviour of various materials during ultrathin sectioning at room temperature or in cryogenic conditions.

2. Experimental

2.1. Materials

The HIPS was Krasten 562E, Kaučuk Kralupy, Czech Republic: the polystyrene (PS) is of molecular weight M_w 200 000–260 000, M_n 70 000–75 000; polybutadiene (PB) content is 7.5–8%.

At room temperature, the PS matrix is well below its glass transition temperature, T_g, while the other constituent, viz. PB is well above its T_g. Both polymers are below their T_g at the cryogenic conditions of the ultrathin sectioning.

Staining is a standard procedure both to achieve a good

☆ Presented in part at 12th European Congress on Electron Microscopy (EUREM), 9–14 July 2000, Brno, Czech Republic.

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PII: S0032-3861(00)00790-4

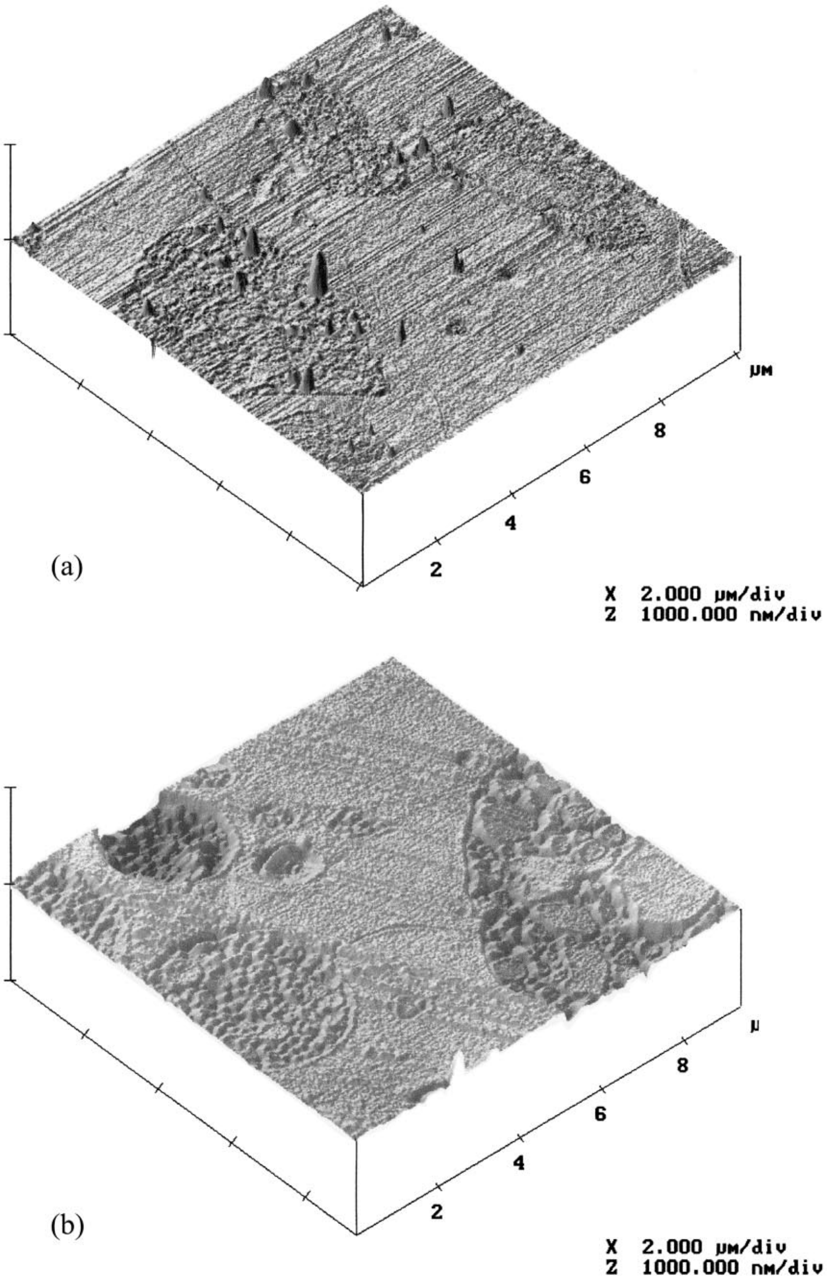

Fig. 1. Cut surface of HIPS with hardened PB inclusions: (a) OsO_4-stained room-temperature cut, (b) non-stained cryo-cut, and (c) OsO_4-stained cryo-cut. AFM.

image contrast and to harden the material to be sectioned. From the variety of techniques with various staining agents that have been used with polymers [7–11], OsO_4 staining was used in this study to harden PB prior to cutting the sections.

2.2. Equipment and techniques

Ultrathin sections (and the cut surfaces remaining after ultrathin sectioning) were prepared using an ultramicrotome Ultracut UCT with cooling system FC-S (Leica). A diamond knife with edge angle 45° when room-temperature sectioning and with edge angle 35° when low-temperature cutting was used. At low-temperature cutting, the sample temperature was −130°C, and the knife temperature, −70°C.

Cut surfaces and the surfaces of ultrathin sections placed on the freshly cleaved mica were observed by scanning electron microscopy (SEM) and atomic force microscopy (AFM).

LV-TEM can be performed using either a special instrument (newly developed TEM equipment, cf. Refs. [1,2]) or a scanning electron microscope (SEM) that is equipped with a detector of transmitted electrons (STEM operation mode).

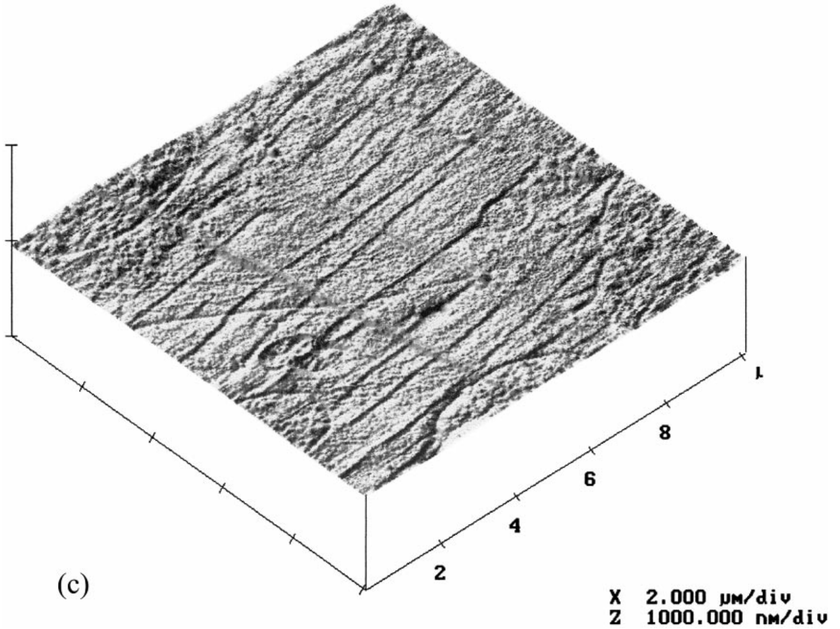

Fig. 1. (*continued*)

A SEM operates with lower voltages than a regular TEM and an advantage of using a SEM instrument to LV-TEM is that the operation voltage can be easily changed. We have used both types of instruments in this study; however, the presented results were obtained with STEM mode.

Scanning electron microscope JSM 6400 (JEOL) was used to obtain low-voltage transmission micrographs of all the ultrathin sections (STEM, transmission adapter, operating voltage 25 kV). The same instrument was used in the secondary electrons mode to obtain the regular scanning electron micrographs of the objects.

The AFM study was performed on a Multimode AFM Nanoscope IIIa, Digital Instruments, using tapping mode. Silicon microcantilevers OTESPA were used. Their resonance frequency was about 270 kHz, tip radius was about 5 nm. The results are presented here as stereoviews (3D images). Note that the baselines of the images of the ultrathin sections are *not related to their thickness*, i.e. the thickness of the ultrathin sections is a part of the vertical scale of the corresponding figures only.

3. Results and discussion

The material (HIPS) selected for the study of behaviour in the course of ultrathin sectioning is characterised by a hard matrix (PS) and dispersed particles composed of a soft PB matrix with hard PS inclusions at room temperature. Both the constituents are hard under cryogenic conditions (the cryo-sectioning conditions) and, moreover, the PB matrix of the particles can be hardened using OsO_4 staining. Both ultrathin sections and the remaining cut surfaces of the untreated and OsO_4-treated sample were observed after sectioning at room temperature and in cryogenic conditions. While SEM provides a qualitative evaluation of the surface topography, AFM comprises a fully quantitative measurement of the corresponding surface profile. Doubts concerning the hill/valley textures observed are thus excluded.

3.1. Cut surfaces

Relatively flat surfaces are produced when a hard, homogeneous material is cut. The PB component in HIPS can be hardened either by staining or by cooling down well below its glass transition temperature T_g. A comparison of cut surfaces of variously hardened HIPS is in Fig. 1. The cutting brings about relatively flat cut surfaces when soft PB particles are hardened by each of the above two ways (cooling, staining). It is necessary to mention here that all the results of the cutting are observed at room temperature. The smallest differences between the highest and the lowest sites on the cut surfaces (smallest relief of the surface) are in the chemically stained (OsO_4) sample, which was cut at room temperature (Fig. 1(a)), with the exceptions, however, of several protrusions, probably plastically deformed non-stained parts of the PB component. Cryo-cutting of a non-stained sample results in a very flat cut surface in the region of the PS matrix while a pronounced relief is observed in the regions of dispersed inclusions (Fig. 1(b)). Low-temperature cutting of an OsO_4-stained sample yields a relatively flat cut surface (Fig. 1(c)).

HIPS with non-hardened (non-stained) PB exhibits a very pronounced relief after sectioning at room temperature (Fig. 2). At this temperature, the PB phase is well above its glass transition temperature, where a negative influence on the section quality due to large plastic deformations is very

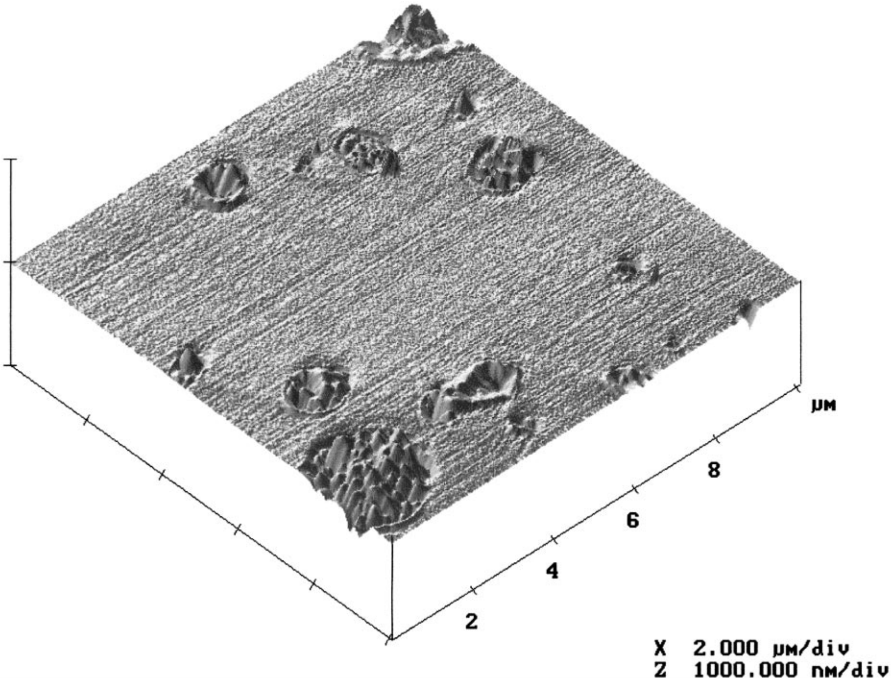

Fig. 2. Cut surface of HIPS with non-hardened PB inclusions: room-temperature cut. AFM.

likely (cf. Ref. [12]). It can be seen from Fig. 2 that the original structure of dispersed particles is visibly distorted. The cone-like textures inside the particles reflect the shape of the probe scanning the surface, viz. the convolution image of deformation textures (probably deformation fibres), which are thin and high.

SEM makes the phase structure visible, using either the materials contrast of stained samples (due to increased secondary electron emission from the osmium atoms in the stain, Fig. 3) or topographical contrast on non-stained samples (Fig. 4). Distortion of the cut surface can be seen; however, not so distinctly as to distinguish the substantial deformations that take place with room-temperature cutting of a sample with non-hardened PB phase (Fig. 4(b)).

3.2. Ultrathin sections

Phase structure can be observed from ultrathin sections on stained samples (using conventional-voltage TEM) and on non-stained samples with LV-TEM (cf. Ref. [3]). Both the techniques also give good results in the case of a HIPS blend (Fig. 5). Distortion of the sample (ultrathin section) is usually not very important when observing the distribution of individual phases in a blend. On the other hand, a closer watch for the localisation of the low-volume phases (e.g. a compatibiliser) requires information on the origin of possible artefacts. Therefore, surface reliefs of ultrathin sections (placed on freshly cleaved mica) were sought. The results (Fig. 6) correspond well with those obtained above on the cut surfaces.

A pronounced surface relief was found both on OsO_4-treated samples (Fig. 6(a),(b)) and after sectioning non-treated samples at low temperatures (Fig. 6(c),(d)). The extent of unevenness (roughness) is comparable in the two cases. On the other hand, when sectioning the non-treated sample at room temperature, the roughness of the sections at the sites of dispersed particles is much higher (Fig. 6(d)) as expected. In this case the textures are artefactual: almost the same slope of the “cones” reflect the shape of the scanning probe — the convolution image of thin and high textures, which arise as a result of large deformations due to cutting soft portions of the material. The relief is similar to that on the cut surfaces as discussed above.

A comparison of the light portions of Fig. 5(b), which correspond to the PB component (see Fig. 5(a)), with the relief of the ultrathin section of a non-stained sample (Fig. 6(c)) suggests an interpretation of the contrast origin with the LV-TEM. As valleys are in the spots of the PB phase (Fig. 6(c)) and the corresponding spots are light in LV-TEM, the lower thickness of the ultrathin section represents at least a substantial contribution to the image contrast.

3.3. Volume relaxation in the sectioning process

Cutting ultrathin sections is apparently the most precise procedure to produce flat cut surfaces with the minimal deformation possible. However, the sectioning comprises plastic deformation of the sections resulting in their shortening in the cutting direction (e.g. Refs. [7,13]). Time-dependent and material-dependent relaxation of the internal stresses involved were mentioned in a detailed discussion of the ultrathin sectioning technique [7,14].

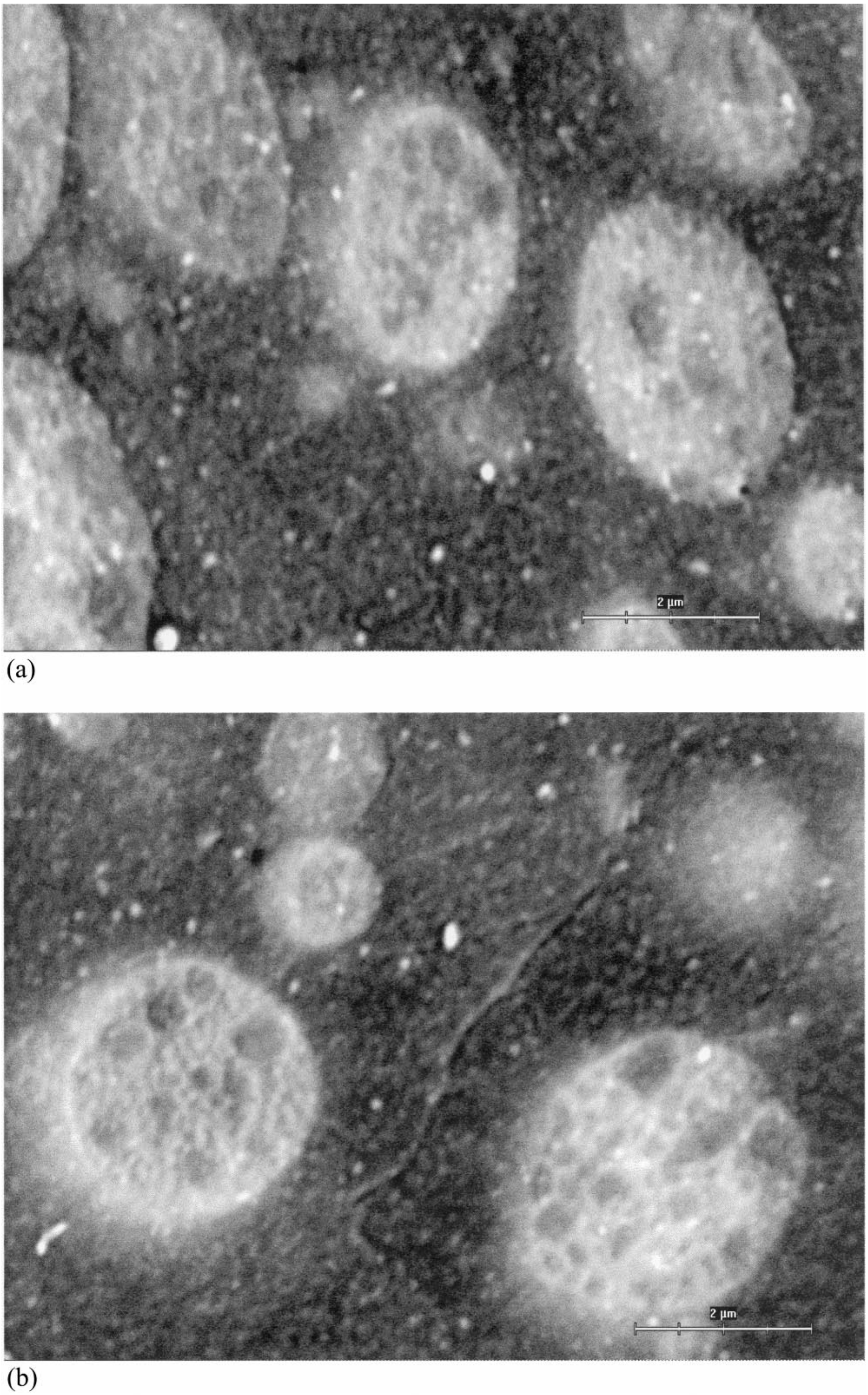

Fig. 3. Cut surface of HIPS with (a) OsO_4-hardened PB inclusions: cryo-cut; and (b) room-temperature cut. SEM of carbon-covered specimen (material contrast).

Several factors influencing the final topography of the ultrathin section (and of the cut surface) are involved:

- chemical treatment during staining;
- deformation of the material in contact with the knife;
- temperature history during specimen preparation.

All of them imply volume changes that have a subsequent

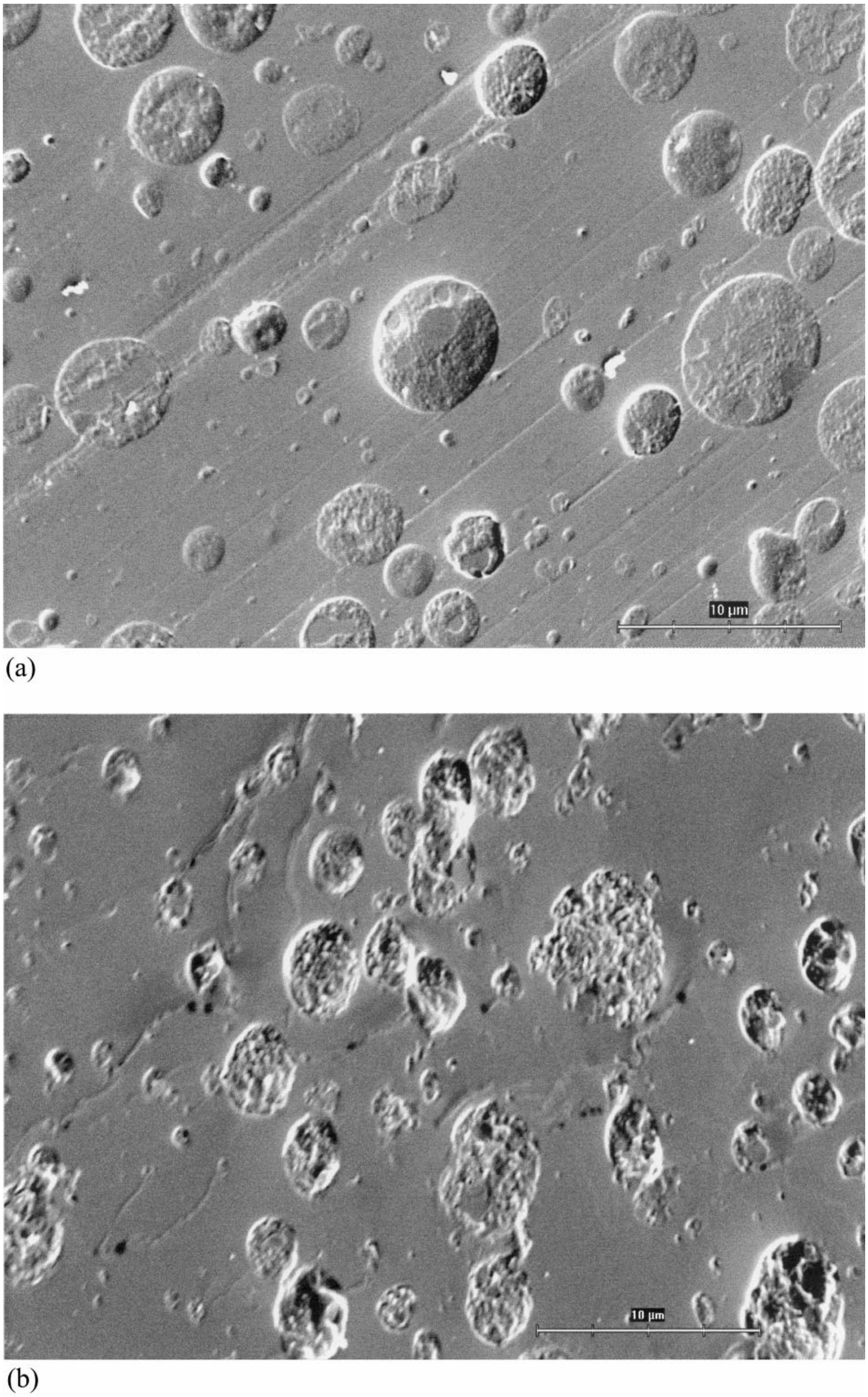

Fig. 4. Cut surface of HIPS with non-stained PB-containing inclusions: (a) cryo-hardened PB (cryo-cut), and (b) non-hardened PB phase (room-temperature cut). SEM of Pt-covered specimen (topographical contrast), tilt angle 30°.

influence on the shape, surface topography, and finally on the resulting image in the electron microscope.

Selective chemical reaction of the staining agent with the sample or its diffusion into a component change not only the properties of the particular component (density, electron density, glass transition temperature) but also its interaction with the adjacent material (internal stresses). Changes may then be expected in the subsequent heating and/or cooling.

Deformation of the material certainly reflects the local mechanical properties of the particular component at the

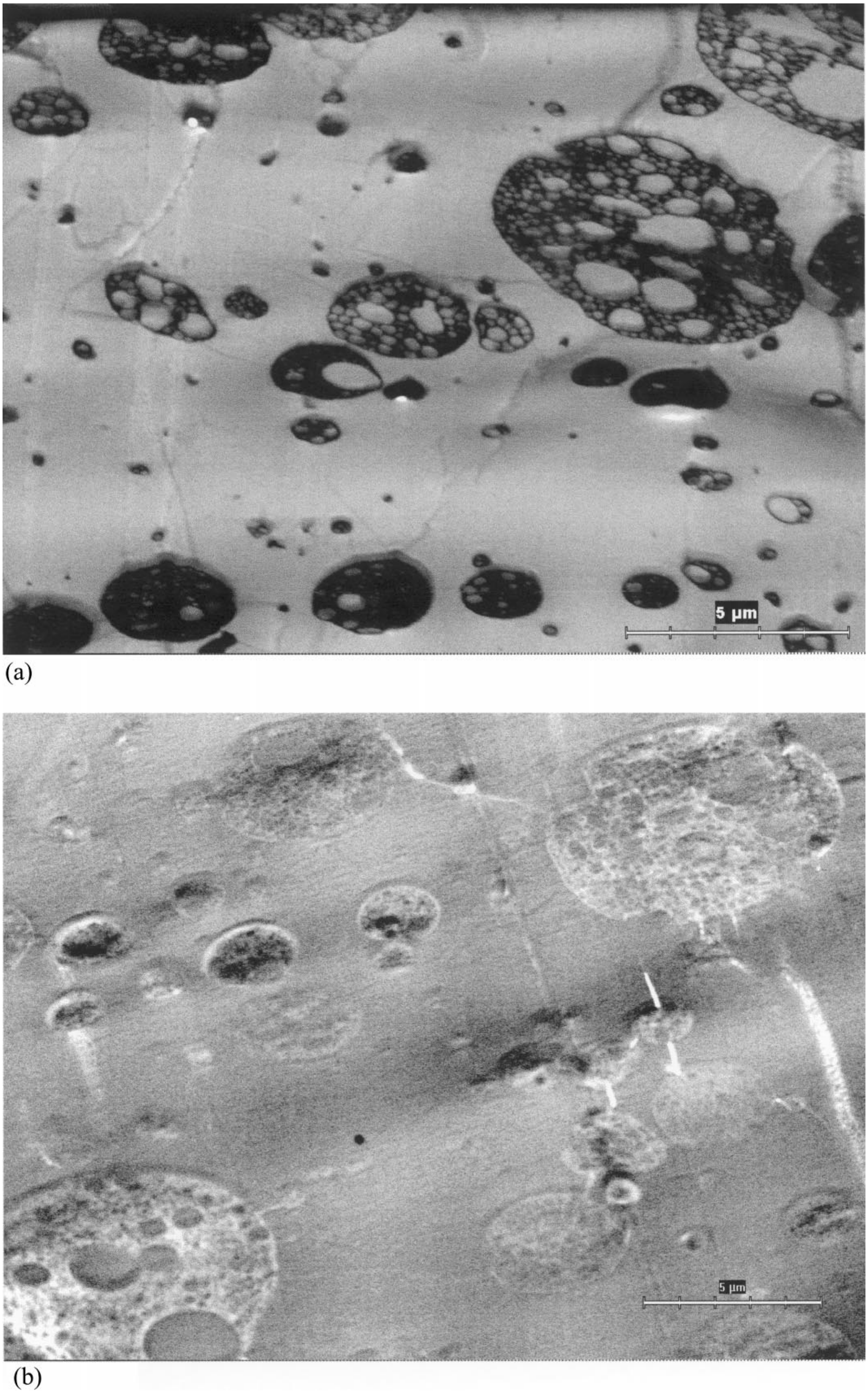

Fig. 5. Phase structure of HIPS blend as obtained from transmission electron microscopy of ultrathin sections: (a) TEM of OsO_4-stained samples, (b) LV-TEM of non-stained blend (25 kV).

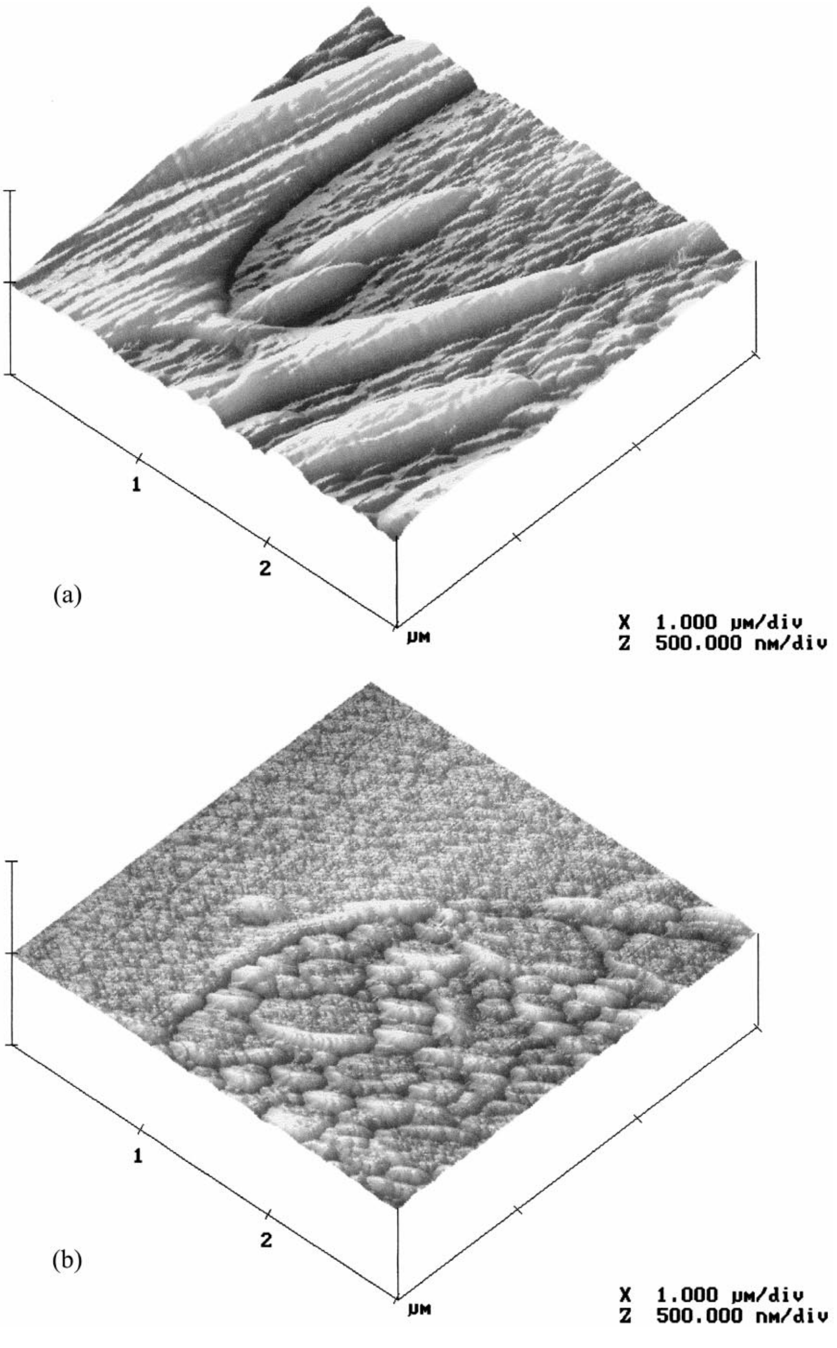

Fig. 6. Surface relief on ultrathin sections of HIPS: with OsO_4-hardened PB (a), (b), and non-stained ultrathin sections (c), (d): cryo-cut (a), (c), and room-temperature cut (b), (d). Ultrathin sections were placed on freshly cleaved mica. AFM.

cutting temperature (in addition to the knife quality, knife setting, etc.). Uneven mechanical compliance of the adjacent phases in the blend certainly results in local differences in thickness. Frozen-in stresses, which are also very likely, are released in subsequent heating and manifest themselves as volume changes. From this point of view, the danger of large plastic deformation during sectioning increases especially in plastics above the glass transition temperature (PB sectioned at room temperature in our case).

Sectioning at low temperatures involves cooling the sample to the cutting temperature and its subsequent heating to room temperature after cutting. Parts of the sample that are originally soft at room temperature become hard on cooling and this material transition from the rubber-like to glassy state implies a substantial change in the thermal expansion coefficient. Even if we assume that the original ultrathin cryo-section is flat and has a uniform thickness, the following heating to the room temperature (at which it is observed) will certainly introduce a detectable unevenness of the section. We are of the opinion that this mechanism

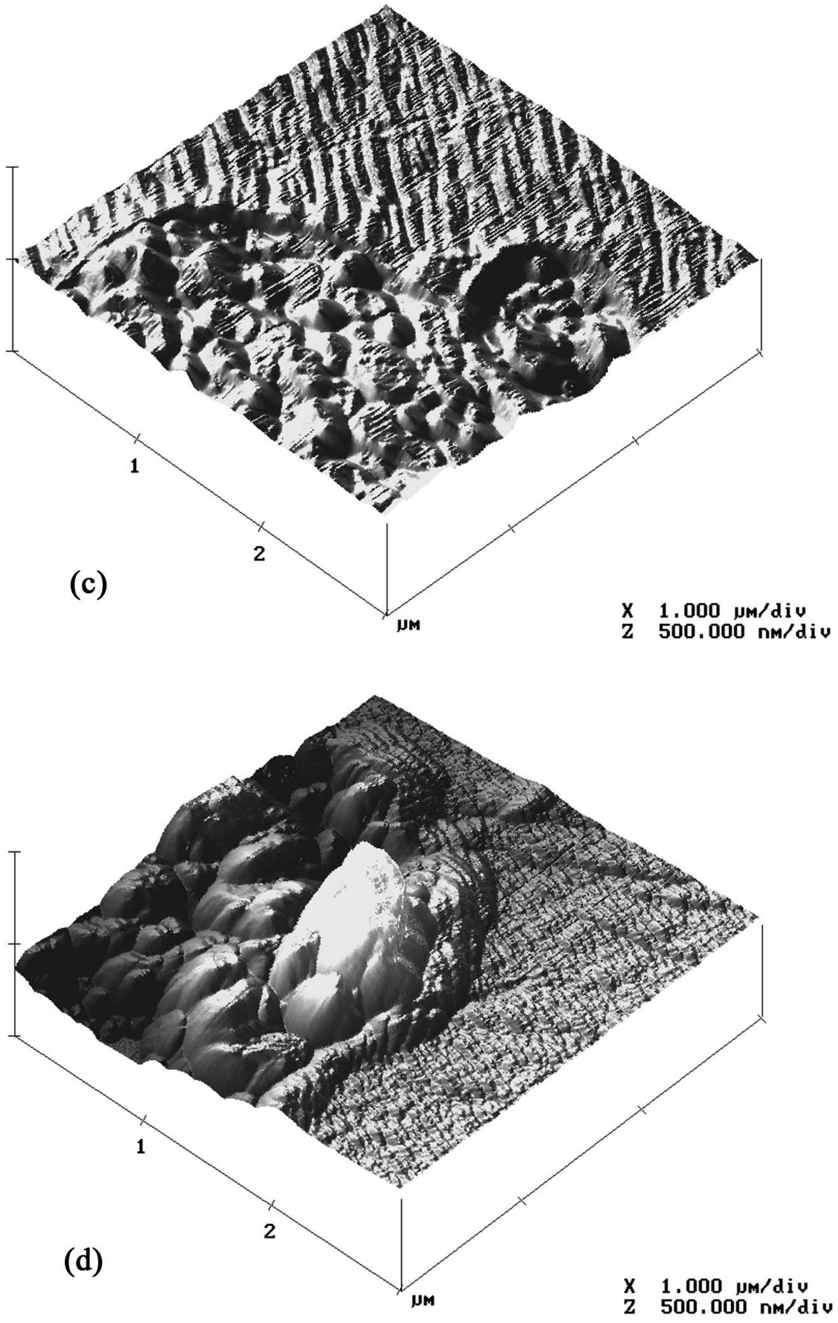

Fig. 6. (*continued*)

contributes to the contrast observed in low-voltage electron microscopy of polymers.

Volume changes involved in the relaxation of frozen-in stresses were observed after annealing of a blend (polycarbonate/poly(methyl methacrylate)) at a temperature between the glass transition temperatures of the two constituents [15]. The *volume relaxation technique* of sample preparation was proved to depict the true phase structure of polymer blends. The mechanism of volume changes is identical with that in cryogenic sectioning: after cutting in the frozen state (below the glass transition temperatures of all components), the sections are heated to room temperature, at which one component is well above its T_g, i.e. its volume increased and possible internal stresses relaxed, while the other component–matrix is well below its T_g.

The resulting differences in thickness at the sites of individual phases are certainly an effect that contributes to the image contrast of transmission electron micrographs. As the contrast is particularly enhanced in LV-TEM, the preparation effects can be advantageous for visualisation of such objects as cannot be stained for some reason.

3.4. Comparison of the sectioning techniques

The efficiency of hardening of PB with OsO_4 treatment and with freezing can be compared from both cut surfaces and ultrathin sections (Figs. 1, 2 and 6). Reduction of deformation effects (demonstrated in Figs. 2 and 6(d)) by any of the hardening techniques is evident and need not be discussed. Staining may not target the total volume of the component to be stained (Fig. 1(a)), and some deformed textures may then appear. Their origin remains unclear; they may arise as a result of cutting deformation of more plastic pieces (cutting artefact), reflecting probably the missing chemical reaction with the staining agent. Further examination of the effect is needed.

Incorporation of osmium into the chemical structure of the PB phase certainly increases its T_g above room temperature. Then the T_g of the stained PB is closer to that of PS, which explains the extent of volume changes in the compared samples. When cutting the hardened samples (low-temperature cutting), the extent of volume changes seems to be more pronounced with non-stained samples (cf. Fig. 1(b), (c)), though the differences observed from ultrathin sections are less convincing.

A comparison of stained samples sectioned at room temperature and low temperatures (Fig. 6(a), (b)) also reveals some differences. Cryo-sectioning certainly involves the volume relaxation effect, as can be seen from the different levels of the two phases, viz. matrix and dispersed particles. Sectioning of an OsO_4-treated sample at room temperature reflects the differences in mechanical behaviour as manifested during the cutting process. No volume changes can be expected as cutting is performed without any temperature change.

4. Conclusions

- A distinct surface relief can be observed on both ultrathin sections and remaining cut surfaces irrespective of cutting conditions, even when the best instruments and sample preparation technique are applied.
- The surface relief in ultrathin sections is a result of local differences in thickness at the sites of the sample components.
- The volume relaxation effect applies when cryo-sectioning is exploited.
- Cutting response depending on the mechanical properties of the sample components applies at room-temperature cutting. Cutting components at a temperature above their glass transition temperatures results in large plastic deformation at their sites.
- Differences in thickness at the sites of individual phases contribute to the enhancement of image contrast of transmission electron micrographs. The effect is particularly advantageous in LV-TEM.

Acknowledgements

The authors gratefully acknowledge financial support of the Grant Agency of the Czech Republic (grant No 106/00/1307). Support of the Academy of Sciences of the Czech Republic (programme No. S4050008) is also acknowledged. The authors want to express their thanks to Dr E. Coufalová for the low-voltage transmission electron microscopy.

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