Atomic Force Microscopy, Far-field and Near-Field Fluorescence Microscopy, three complementary techniques to visualize the spatial distribution of behenic acid

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Abstract

Thermographic materials have been developed in which silver behenate (AgBeh) acts as a silver source and in which behenic acid and silver clusters are formed during the thermal decomposition of silver behenate. Atomic force microscopy (AFM), laser scanning confocal microscopy (LSCM) and Near-field Scanning Optical Microscopy (NSOM) have been used to establish the spatial distribution of the behenic acid formed in the polymer binder, polyvinylbutyral.

Differences in hardness between the behenic acid formed and the polymer binder enable the visualization of the areas of behenic acid by atomic force microscopy. A method based on fluorescence microscopy has been developed to measure changes in pH in thin polymer layers. Sodium fluorescein acts as a pH sensitive fluorophore, due to the quenching of the fluorescence of fluorescein in acidic environments.

Silver behenate crystals embedded in thin layers of polyvinylbutyral were investigated. Heating in situ enables the same area to be studied both before and after heating. After complete characterization of the silver behenate/polyvinylbutyral model system, the influence of additional components on the 'sphere of influence' was investigated. The results obtained with both techniques led to the same conclusions.

Introduction

During the thermal development of (photo)thermographic systems containing silver behenate, behenic acid is one of

the main reaction products. The presence of behenic acid in a polyvinylbutyral matrix depresses its glass transition temperature¹. The phase diagram of behenic acid in polyvinylbutyral has been investigated with differential scanning calorimetry and it was found that only 7% by weight of behenic acid is soluble in the polymer matrix. If this value is exceeded, the behenic acid will exist as a separate phase in polyvinylbutyral.

In the neighborhood of a developed silver behenate crystal, it is therefore logical that phase separation could occur. Atomic force microscopy in combination with force modulation is a technique that might create the possibility of visualizing phase separation².

Alternatively, this phase separation could be visualized using laser scanning confocal fluorescence microscopy including a microenviromental pH sensor in a polymer matrix³. The sodium dianion of fluorescein was used as the pH sensitive compound. This form of fluorescein is characterized by a high fluorescence quantum yield⁴. However, the fluorescence is quenched in more acidic media. An analogous method was applied in the study of acid generation in chemically amplified photoresist⁵.

To enhance the spatial resolution obtained in laser scanning confocal fluorescence microscopy, near-field scanning optical microscopy was used⁶. NSOM is a high-resolution scanning probe technique, which provides simultaneous topographic and optical images of thin film materials.

Using the above-mentioned techniques, it may also be possible to study the influence of additional compounds on the behavior of silver behenate in polyvinylbutyral.

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Figure 1 : Thermal decomposition of silver behenate crystals in a polyvinylbutyral matrix: topographic image A before heating (25µm x 25µm, height from 0nm (black) to 100nm(white)); Topography line profile B, place is indicated by a line and arrow in A; Topographic image C after 60 seconds at 140°C (25µm x 25µm, height from 0nm (black) to 90nm (white)) and corresponding force modulation image D after 60 seconds at 140°C (25µm x 25µm, dark areas represent the softer regions, while bright gives the rigid regions). The force modulation image was taken simultaneously with the topographic image.

Experimental

Preparation of the polymer films

The polymer films were spincoated from a solution in methylethylketone on a glass support, and had a thickness of the order of a few 100nm. The films consisted of (1) polyvinylbutyral as polymer matrix and (2) 2 to 3% by weight of silver behenate. To study the influence of the addition of an extra component, tone modifier or reducing agent was added.

Films of behenic acid (7wt%) in a polyvinylbutyral matrix were prepared in a similar way.

For studies in LSCM and NSOM, a second layer, which contained the acid sensitive fluorophore, was applied by spincoating a polyvinylalcohol layer containing 0.25wt% sodium fluorescein (Aldrich).

Instrumentation

AFM images were obtained with a TopoMetrix TMX 2010 controller system with a Discoverer AFM-unit, using standard Siliconitride AFM-tips (topometrix model 1520), in contact mode. The force modulation experiments⁷ were performed with a modulation amplitude of 15Å. In situ heating was possible by the installation of a hot-stage on the scanner.

The laser scanning confocal microscopy setup consisted of a Bio-Rad MRC600 unit connected to a Nikon Diaphot 300 inverted microscope. The excitation light had a wavelength of 488nm and was generated by a TiSa (Tsunami, SP model 3690) pumped by an Ar^+ laser, and all fluorescence from the sample above 515nm was detected. A detailed description of the setup can be found elsewhere⁸.

The SNOM measurements were made on a modified Topometrix Aurora near-field microscope. The apparatus has been described in detail elsewhere⁸. The 488nm line from Ar^+ ion laser excited the samples and all emission was collected above 530nm. Optical images were obtained before and after heating the samples on a hot-stage.

Results and discussion

Atomic Force Microscopy

The surface of the polymer films was investigated by atomic force Microscopy (AFM) in contact mode. In Figure 1A a topographic image of silver behenate crystals embedded in a thin layer of polyvinylbutyral is presented. The silver behenate crystals can clearly be distinguished from the matrix. As shown in the line profile 1B the height differences vary between 5 and 70nm.

Figure 1C and 1D shows the AFM results of the sample that has been heated for 60 seconds at 140°C. The area, indicated by the ellipse, was analyzed, the two longer crystals acting as reference points. The topographic image 1C shows that the smaller crystals disappeared completely, while residuals of the larger crystals were still visible. In the corresponding force modulation image 1D, a softer zone around the crystal is detected which contains the behenic acid phase. The images in figure 2 show the evolution of the behenic acid phase around a single crystal. After successive heating cycles, the behenic acid dissolves in the matrix. Around the crystal, the droplets of behenic acid in the polymer gave the direction of diffusion away from the crystal. The dimensions of the softer phase became smaller. An important observation was that at the ends of the crystal, a depression in the matrix was detected in the topographic

images, suggesting that the behenic acid flowed from the crystal via the length direction and displaced the matrix to a certain extend.



Figure 2: Diffusion of behenic acid around a single silver behenate crystal in polyvinylbutyral matrix: Images taken after 60 seconds at 140°C topographic image A (10µm x10µm, height from 0nm to 90nm), and corresponding force modulation image B(10µm x 10µm); Images taken after 120 seconds at 140°C: topographic image C (10µm x10µm, height from 0nm to 90nm) and corresponding force modulation response D (10µm x 10µm). The force modulation images were taken simultaneously with the topographic images. The darker zones represent the behenic acid areas, which were softer than the matrix.

To prove that the behenic acid phase could be detected with the force modulation set up, a sample of polyvinylbutyral containing behenic acid was investigated. These results are presented in figure 3. The film matrix contained 7% by weight of behenic acid. At the start a rather rough surface, resulting from dewetting problems due to depositing this film on a glass support, can be seen in the topographic image 3A. After 15 seconds heating at 140°C, a more flat surface was seen in the topographic image 3C. In the force modulation image 3D, phase separation of the behenic acid in polyvinylbutyral was visualized. After an extended heating period, the behenic acid dissolved in the polymer matrix (figure 3E). This is in accordance with the phase diagram of behenic acid in a polyvinylbutyral matrix, which indicates that below 7wt%, behenic acid is molecularly soluble. At higher concentrations of behenic acid, solubilisation of the behenic acid in the matrix was not observed (these results are not presented in this paper).



Figure 3: Phase separation of behenic acid (7wt%) in polyvinylbutyral matrix: Images taken before heating: topographic image A (7 μ m x 7 μ m, height from 0 to 85nm) and corresponding force modulation response B (7 μ m x 7 μ m); Images after 15 seconds at 140°C: topographic image C (7 μ m x 7 μ m, height from 0 to 12nm) and corresponding force modulation response D (7 μ m x 7 μ m, bright corresponds to the rigid regions, dark to the softer phase); Images after 30 seconds at 140°C and 120 seconds at 80°C: topographic image E (7 μ m x 7 μ m, height from 0 to 12 nm) and corresponding force modulation response F (7 μ m x 7 μ m, bright areas correspond to the rigid regions, dark to the softer phase). The force modulation images were taken simultaneously with the topographic images.

In contrast with the results presented in figure 1, the behenic acid phase is more rigid than that of the polymer. This suggests that the behenic acid could be found in two different forms, one that was softer (amorphous) than the polymer and one that was harder (crystalline) than the polymer. This is consistent with DSC measurements in which, upon complete thermal decomposition of silver behenate in a polyvinylbutyral matrix, only a fraction of the behenic acid could be found in the crystalline phase.



Figure 4: Influence of tone modifier on the thermal decomposition of silver behenate in polyvinylbutyral matrix: Images before heating: topographic image A (25µm x 25µm, height from 0 to 35nm) and corresponding force modulation response B (25µm x 25µm); Images after 15 seconds heating at 140°C: topographic image C (25µm x 25µm, height from 0 to 35nm) and corresponding force modulation response D (25µm x 25µm, bright areas correspond to the more rigid regions, dark areas to the soft regions); Images of a single silver behenate crystal after 15 seconds heating at 140°C: topographic image E(10µm x 10µm,from 0 to 35nm) and corresponding force modulation response F(10µm x 10µm, bright areas correspond to behenic acid regions). The force modulation images were taken simultaneously with the topographic images.

Addition of a tone modifier affected the thermal decomposition of silver behenate. These results are presented in figure 4. An important difference compared with the system in the absence of tone modifier, was the presence of droplets of behenic acid after a heating period of only 15 seconds at 140°C.

Scanning confocal microscopy

In the laser scanning confocal fluorescence microscopy study, the polyvinylbutyral layer was overcoated by a polyvinylalcohol layer in which the pH-sensitive probe was Decreased intensity of fluorescence in the dissolved. behenic acid areas could be expected. Transmission and fluorescence of samples containing no additional components of tone modifier or reducing agent, all heated at 150°C for 5 seconds, were compared. In all three samples, behenic acid was observed as a region of decreased fluorescence. For the film containing no additional component, the shape of the original silver behenate crystal could still be recognized. Addition of tone modifier or addition of reducing agent led to an increased diffusion of behenic acid in the polymer matrix. The regions of fluorescence quenching extended beyond the original size of the crystal. This observation was in accordance with the above-mentioned AFM results.



Figure 5: Influence of additional components on the thermal decomposition of silver behenate in polyvinylbutyral matrix studied with LSCM: **A, B, C**: the optical transmission images ($80\mu m \times 60\mu m$) of a sample without any extra component, with tone modifier and with reducing agent respectively. The black lines represent the silver behenate crystals. **D, E, F** : The fluorescence images of the same area ($80\mu m \times 60\mu m$) after 5 seconds heating at 150°C respectively of a sample without any extra component, with tone modifier and with reducing agent, the areas with decreased fluorescence (darker) indicate the presence of behenic acid.

Near-Field Scanning Optical Microscopy

Near-field scanning optical microscopy confirmed that the quenching of fluorescein was due to the presence of behenic acid and not to a change in the topography of the overlying polyvinylalcohol layer. In figure 6A and 6C the topographic images clearly show no important difference in topography close to the silver behenate crystals after heating for 5 seconds at 150°C. While the fluorescence images 6B and 6D clearly indicate the original shape of the crystal, at the single crystal level, in the absence of tone modifier, in the presence of tone modifier the dark spot related to the fluorescence quenching was far greater than the dimensions of the silver behenate crystal. These results were in accordance with the above data obtained with AFM and LSCM.



Figure 6: NSOM images of silver behenate crystals in polyvinylbutyral matrix with and without tone modifier after 5 seconds heating on 150°C: topographic image **A** (20 μ m x 20 μ m, height from 0 to 100nm) and corresponding fluorescence image **B** (20 μ m x 20 μ m, intensity from 4800 to 8700 counts per second) of the sample without tone modifier; topographic image **C** (30 μ m x 30 μ m, height from 0 to 100nm) and corresponding fluorescence information **D** (30 μ m x 30 μ m, intensity from 640 to 13240 counts per second). The areas with decreased fluorescence indicate the presence of behenic acid.

Conclusions

Combination of optical surface analysis techniques showed that behenic acid was formed during thermal decomposition of silver behenate crystals and that addition of either tone modifier or reducing agent led to enhanced diffusion of behenic acid in the polymer matrix.

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Biography

Lieve Bastin received her Ph.D. in Chemistry from the Catholic University of Leuven (Belgium) in 1990. Since then, she has worked as a postdoctoral co-worker at the laboratory of Professor F. De Schryver of the same university. She has been involved in different research projects in collaboration with Agfa-Gevaert (Mortsel, Belgium). For the moment, the topic of her research concerns the study of the mechanism of (photo)thermographic materials.