Influence of sample preparation methods on transmission electron micrographs of sol-gel materials

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The main goal of this research was to examine the influence of a sample preparation method on microscopic images obtained in transmission electron microscopy (TEM). The microscopic images of porous silica-based sol-gel materials were further processed with computer-aided methods, so thus to obtain information about distribution of pores. As a stain dopant osmium tetraoxide was used. Sol-gels were prepared from TEOS (tetraethyl orthosilicate) as a precursor and water as a solvent. The material was placed on microscopic grids, pure and with cellulose, or it was embedded in resin and then cut on a microtome. It has been demonstrated that the preparation procedure influences the pores distribution in a sol-gel silica network examined by TEM.

1. Introduction

The sol-gel process for the production of inorganic or hybrid organic-inorganic glass -like materials conducted at ambient temperatures, relies on the hydrolysis of the silica -based precursors (*e.g.*, TEOS $Si(OC_2H_5)_4$) mixed with alcohol or water and on the subsequent polycondensation to a glass-like form. The technology allows for the incorporation of organic or inorganic additives into the silica network at room temperature. Based on the sol-gel process a large number of sophisticated materials have been prepared and studied [1], [2].

Sol-gel glasses are classified as porous materials. A porous material is simply some kind of solid material with holes, also called pores. Generally, porous materials have the porosity of 0.2–0.95 [3]. The porosity means the fraction of pore volume to the

total volume [4]. Porosity of silica-based sol-gel materials depends on the R factor, defined as the ratio of the solvent moles number to the number of precursor moles.

Many porous materials have been used for various applications [5], [6]. They are classified according to many different criteria such as a pore size, pore shape, materials and production methods. Classification by the pore size and by pore distribution is useful while considering the applications of porous materials.

The pore size of porous material ranges widely from an atomic size to a millimeter order. Different pore sizes are required for different applications of porous materials. Most porous materials do not have uniform pores. Pore size distribution is also an important property. Narrow pore size distribution, *i.e.*, a uniform pore size, is for instance required in filters and bioreactor beds. Methods of evaluating exact pore sizes are important in manufacturing and applying porous materials. There are many methods of evaluating pore sizes in porous materials. Mercury porosimetry and gas adsorption methods are commonly used to measure pore sizes and pore distribution. Also the microscopic images of porous materials, optical [7] as well as electronograms, can be analyzed in order to receive information about their structure and properties.

Homogeneity of pure silica sol-gel films has not been completely explored yet. The results obtained from material examinations should lead to better understanding of the correlation between the material preparation process, microstructure and optical properties. To test the sol-gel properties, the TEM is often used [8]. TEM can reveal the finest details of an internal structure and magnifications of 350,000 times can be routinely obtained for many materials.

2. Materials preparation

The sol-gel silica materials for these studies were prepared by acid-based hydrolysis from the precursor -- TEOS (98% from Aldrich) mixed at room temperature with double distilled water with the molar ratio $R = 15$. As a catalyst hydrochloride acid (HCl 36% from Polish Chemicals) was used. The substrates were mixed for 4 hours by means of a magnetic stirrer (speed 400 rot./min). The final pH of the obtained hydrolyzate was 2.

Materials for the TEM must be specially prepared as far as thickness is concerned, which allows electrons to transmit through the sample. There are numerous ways of preparing material for the TEM examination. Technical methodology has reached a point where the reproducibility of specimen preparation is possible. A basic aim is to obtain morphological information and to get it by reproducible methods which can be repeated anywhere in the world, so the results worked out in one laboratory could be comparable with those obtained in another. Reproducibility strengthens the belief that micrographs are faithful reflections of the native state of the specimen. The risk of misinterpretation from technique-induced artifacts is lower as preparation techniques become more refined. It often helps to study a given sample with a variety of preparation techniques, as each can provide independent and self-consistent information.

The specimen and supporting film require the mechanical support of a metal grid which is necessarily electron opaque. The number of grid bars and percent open area can vary quite widely with different types of grids. The supports used for these studies (mesh 400 holes) are commonly used grids. Most TEM grids are made of copper because it is non-ferromagnetic and thus minimally distorts the magnetic field of the objective lens. In order to be able to perform the TEM examination and get a good contrast special stains are used. The popular stains are metal based compounds, like *e.g.*, osmium tetraoxide.

The sol-gel samples for the TEM investigation were prepared by means of three different methods. In the first method, to the pure hydrolyzate the 4% ethanol solution of the osmium tetraoxide (OsO_4 98% from Aldrich) was added. Then, the microscopic copper meshes were covered with sol-gel by dipping them in liquid hydrolyzate, so the material was deposited directly on the microscopic copper meshes and filled the grid holes. After 1 hour the solvent evaporated and the material was solid.

In the second method, the pure hydrolyzate was doped with the osmium tetraoxide, as well. Next, the microscopic meshes covered with cellulose membranes were coated with doped hydrolizate by the dip-coating method.

The third method was more complicated. Few drops of the ammonia solution (25%) were added to the pure hydrolyzate, what resulted in very quick gelation. Glycid ether 100 (from SERVA Electrophoresis GmbH), formerly known as EPON 812, is one of the most widely used embedding media. Sections of this resin show greater contrast in the electron microscope than that one shown by comparable Araldite sections. However, the inherent granularity of glycid ether 100 may limit high magnification and high resolution. Glycid ether cured with DDSA (from SERVA Electrophoresis GmbH, dodecenylsuccinic anhydride used as a hardener for epoxy resins) alone will result in rather soft blocks, whereas MNA (from SERVA Electrophoresis GmbH, methylnorbornene-2,3-dicarboxylic anhydride, methylnadicanhydride used as a hardener component for epoxy resins, for example EPON-embedding) yields very hard blocks. The applied method uses 2 different solutions – A and B. By using varying proportions of these two mixtures, blocks of the desired hardness can be produced. Mixture A contained 62 ml of glycid ether 100 and 100 ml of DDSA. Mixture B contained 100 ml of glycid ether 100 and 89 ml of MNA.

The embedding material used for the experiments described here contained 3 parts of mixture A and 7 parts of mixture B and 0.15 ml DMP30 (from SERVA Electrophoresis GmbH, 2,4,6-tris(dimethylaminomethyl)phenol used as an accelerator for epoxy polymerization). The polymerization process lasted for 6 days at 60 °C. After that, the prepared samples were cut by means of the ultra-microtome (section thickness 40 nm).

3. TEM examination

The optical microscopic methods can provide some information about the structure of the silica sol-gels [9], [10]. Transmission electron microscopy is an important tool in

performing the microstructural analysis [11], [12]. The TEM method allows to determine the distribution and the size of the dopant particles [13], which can be the compared with the distributions of pores and a silica network in sol-gels.

In the TEM technique an electron beam passing through a thin sample forms an image of the material internal structure. The image, formed by the interaction of the electron beam with the structure, magnified and focused, is observed on a fluorescent screen.

In recent years, digital image processing has evolved to the point where it is now possible to more fully exploit the high resolution potential of TEM [14]. In this study, all the electronograms were obtained using the transmission electron microscope JEM 100B.

4. Image processing

The micrographs obtained during the TEM investigation were digitalized using an analog-digital converter. They were further processed by means of Image Processing Toolbox under Matlab 6.0, The Math Works Inc.

The digitized images are in grayscale, where 0 represents black and 255 represents white color. For the subsequent analysis the image samples of size 747×747 pixels were taken. For each picture a histogram as well as three-dimensional luminance were calculated. The three-dimensional luminance function produces light intensity distribution in the region of interest. Pictures 1a–c presented in Fig. 1 are the TEM images of sol-gels prepared by the first method (placed on a pure copper grid). Pictures
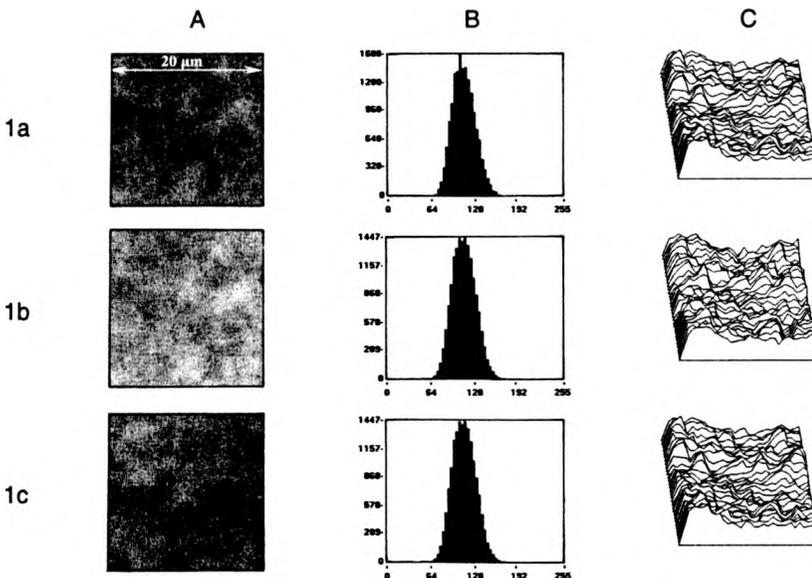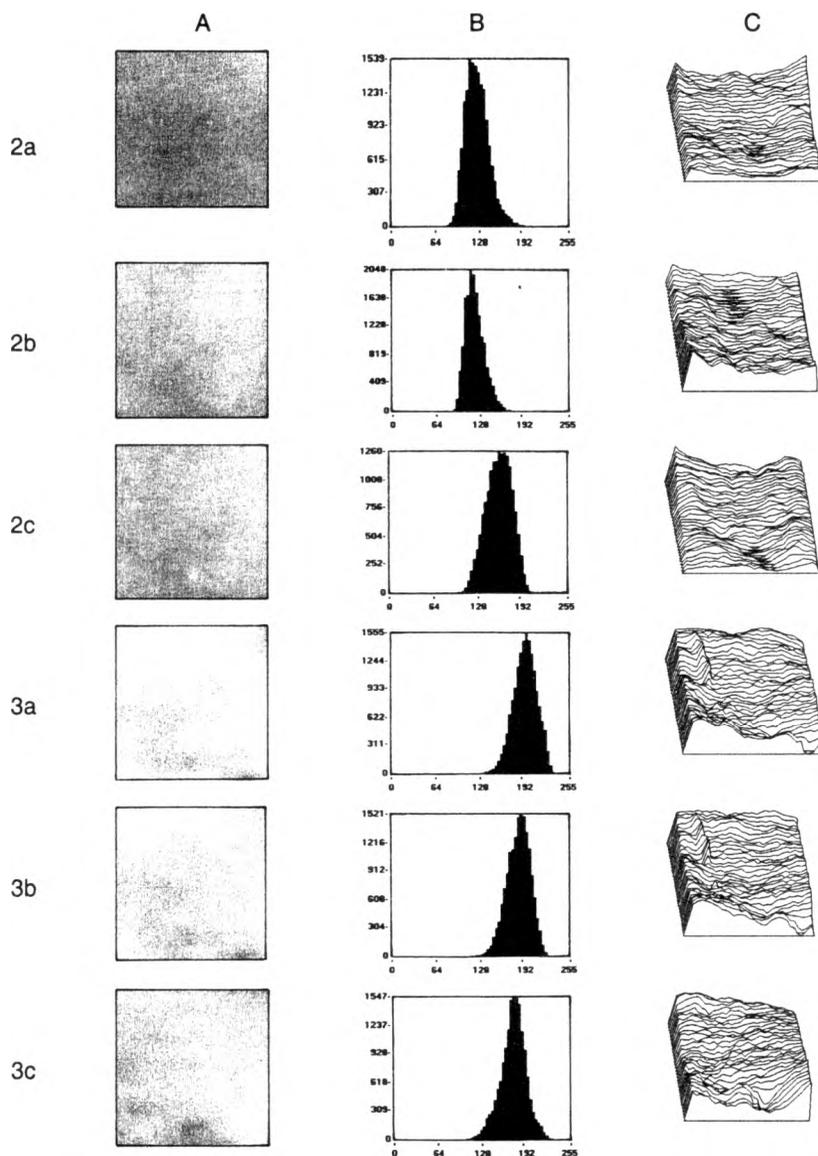

Fig. 1. To be continued.

Fig. 1. Orginal pictures, their histograms and three-dimensional luminance (for explanation, see the text).

2a–c present the images of samples prepared by the second method (copper grid covered with cellulose) and pictures 3a–c are the images of samples prepared by the third method (embedding in resin). In the column A the original pictures are placed, in the column B one can see the histograms of original digital images and in the column C the three-dimensional luminance of A is presented.

From the histograms in Fig. 1 one can see that the grayscale distribution is similar to the Gaussian distribution, but side shifted for some samples. This might be caused

by differences in exposure time when processing the micrographs. To correct this imprecision, the algorithm that shifts the histogram was written. First, the lock-up -table (LUT) matrix was created and the maximum of the histogram was calculated. Adding or subtracting the value for the desired spot (value 128) assigned the new values in LUT matrix. This procedure moved all of the histograms to the same place, what results in darkening or lightening (it depends on the initial point) of the image. All the histograms were shifted in this way, so thus the 128 gray level on x-axis was assigned as the point for which the biggest number of pixels exists.
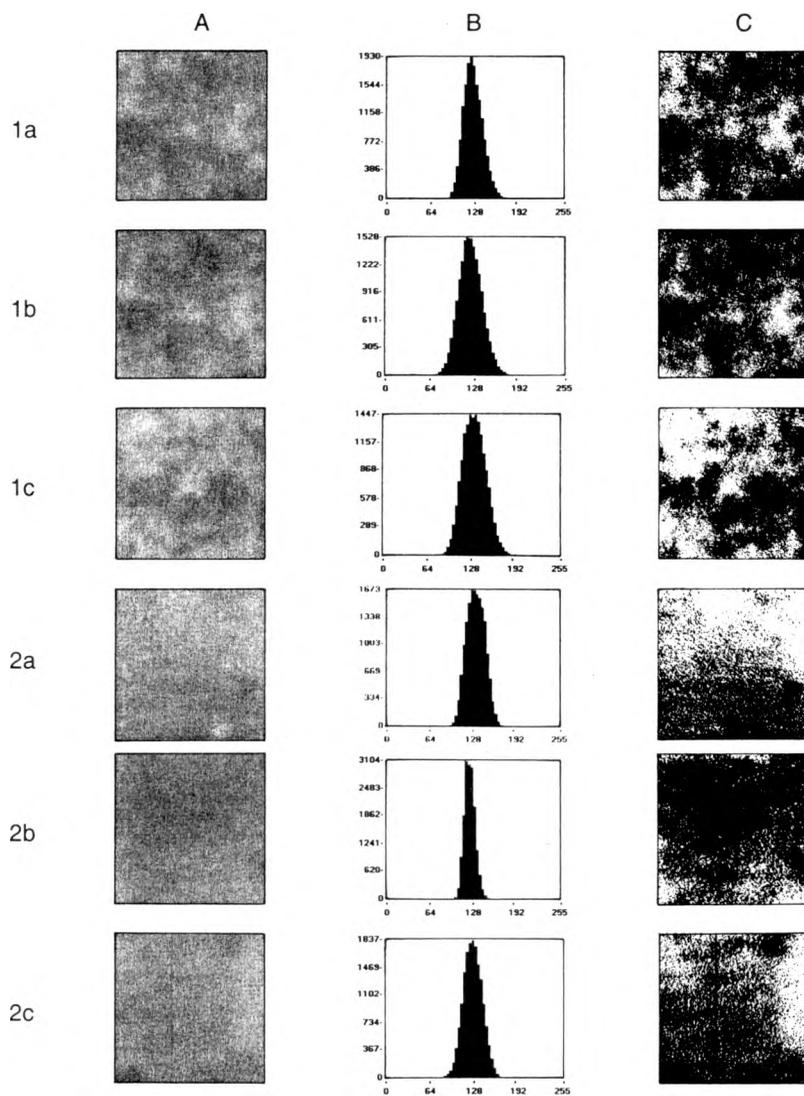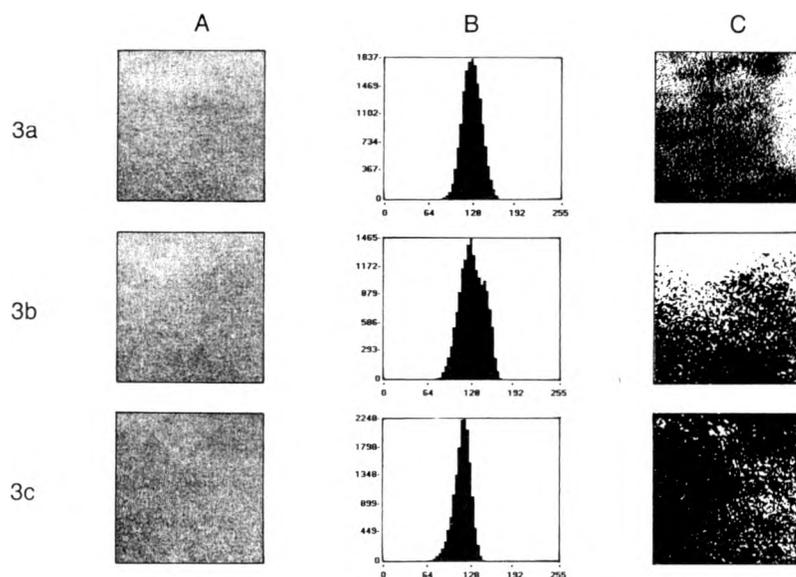

Fig. 2. To be continued.

Fig. 2. Processed picture with their histograms and images after binary filtering (for explanation, see the text).

In order to perform the analysis, the binary filter with the threshold level of 128 was used. Pixels with the gray level value below 128 were replaced by the level 0 and these above 128 were represented by white color (255). In Figure 2 the column A contains the images after histogram shifting, in the column B the histograms of the pictures from column A are shown. Column C in Fig. 2 contains the images after binary filtering.

5. Results

When observing the pictures in Fig. 2 (column C) one can see that the distribution of black and white pixels is different in each group of pictures. In the first group of images (1a, b and c – sol-gel material placed on a pure copper grid) black and white pixels are irregularly spread all over the picture. This is also visible when observing irregularities in the 3-D luminance (column C, Fig. 1). One can see that the black pixels are grouped in aggregations. In the second set of pictures (2a, b and c – sol-gel on a copper grid with cellulose membrane) the black pixels distribution is more regular than in the first group. In the last set of images (3a–c), the distribution is also different. Here, one can see that black pixels are collected in groups of clusters.

6. Conclusions

When testing the transparent material (in wavelengths corresponding to these used in electron microscopy), the material should be specially prepared and doped by contrast

compound. Very seldom one takes into consideration the fact that a sample preparation method can influence the material structure itself. As it was mentioned, the stain dopants fill the empty places in the silica-network, thus the black pixels distribution corresponds to the distribution of pores in material. It has been found that when performing the TEM analysis of porous materials such as sol-gels one has to be very careful with interpretation. Our studies have demonstrated that the pores distribution is strictly dependent on the method of sample preparation.

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