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ARTICLES

Possibilities of Cryogenic Autoradiography

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Abstract—Photographic properties of one of atomic photodetectors are studied at the temperature of liquid nitrogen. The characteristic curves obtained at room and cryogenic temperatures indicate that the detector retains its physical and photographic properties, and its possibilities can be expanded to studies of deeply frozen samples. The data obtained point to an increase in the sensitivity of the photographic material frozen to cryogenic temperatures at short exposures.

Keywords: autoradiography, nuclear photodetector, cryogenic temperature, Mossbauer sources, characteristic curve

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Autoradiography is a traditional instrumental method for the investigation of the local chemical heterogeneity of materials. It is used in quite different fields of science and engineering: microbiology [1], medicine [2], geology [3], materials science [4], and in solving astronomy and space science problems [5, 6]. The method is based on recording the ionization radiation of a radionuclide by photographic detectors. A visible picture of the distribution of radionuclides in the surface layer of a material (sample), an autoradiogram (ARG), is usually obtained after the photographic development of the detector. In addition to other conditions, an important condition for obtaining high-quality images with a good resolution is the immovability of the radionuclides in the studied material relative to the detector material within its exposure. This requirement restricts the choice of samples that can be investigated by autoradiography to materials in which the diffusion or motion (for example, due to Brown or convective motion) of the components or phases present is hindered or even impossible. Thus, autoradiography is usually limited by the study of only solid substances, whereas liquid solutions and dispersed systems with liquid dispersive media remain outside the possibilities of this technique. However, in many cases researchers can obtain and will suffice information on the distribution of components in a sample frozen to low temperatures. For example, the problem of the distribution of the ^{57}Co radionuclide in frozen solutions is important in their study by Mossbauer spectroscopy [7, 8].

The application autoradiography to the study of frozen samples was from time to time mentioned in the literature [9, 10]. The main objects of study by low-temperature autoradiography are biological tissues frozen to rather low temperatures: from –10, –20 [11], and –30 [12] to –76 [13] and –80°C [14]. However, these works were occasional and did not include the analysis of the possibility of changes in the properties of photographic materials used as detectors at low temperatures.

It is clear that, if a latent image is formed at a significantly reduced exposure temperature, this, undoubtedly, should be reflected in the qualitative properties of the detector. It is known that many events of the formation of latent images and also of accompanying and competing processes are thermally activated and their rates depend on temperature. On examples of traditional photographic materials it was known that a change in exposure temperature can result both in horizontal and possible vertical shifts of the isodensity curve in the reciprocity failure diagram [15]. As a result, temperature effects can be separately distinguished among a number of photographic effects; these can be expressed as a monotonous increase in the optical density of a photographic material with decreasing temperature at low illumination intensity [16] or with increasing temperature at high illumination intensity [17] and as a complicated nonmonotonous temperature dependence at moderate illumination intensities. This is associated with the complex temperature dependence of the photosensitivity of traditional photographic materials [18]. We could not find in the literature any description of the effect of exposure temperature on the photodetectors used in autoradiography. It is clear that, for a particular detector–ionization source pair, such temperature effects cannot be qualitatively and quantitatively predicted without special experiments.

The aims of this work were the study of the effect of low temperatures on the registration of audioradiographic images of the Mossbauer ^{57}Co radionuclide by one of widely used nuclear detectors, the development of a procedure of cryogenic autoradiography, and the design of corresponding devices and facilities.

EXPERIMENTAL

The test source of ionization radiation was a standard closed ^{57}Co/Rh MK57.BP5.01 source, used in Mossbauer spectrometry and manufactured by the Cyclotron Joint-Stock Company (Obninsk, Russia) in 2005 according to Technical Specifications TU 95 2407 [19]. The source represented a cylindrical capsule made of aluminum (outer diameter 10 mm) with a cylindrical niche 7.4 mm in diameter and 1 mm in depth (according to our data). Rhodium foil with the radionuclide incorporated into rhodium crystal lattice was arranged at the bottom of the niche under a layer of aluminum foil (30 μm according to the manufacturer). The diameter of the active part of the source was 5 mm according to the manufacturer. The radionuclide in the source was ^{57}Co with a half-life period of 271.79 days. The decay of the radionuclide proceeds with the emission of gamma quanta (used in Mossbauer spectroscopy) and also of X-rays and beta-rays [20, 21]. The calculated radioactivity of the source in the experiment varied from 58 to 33 μCi.

The detectors of ionizing radiation were single-coated nuclear films BioMax MR Film from KODAK, developed for registering beta emission of ^{14}C, ^{35}S, and ^{33}P radionuclides.

The detector was exposured at room temperature in a special device, in which the source of ionizing radiation and the photographic detector were brought in contact and fixed relative to one another with a spring mechanism. After the exposure, the contact of the detector with the source was broken and they were removed from each other.

In the case of detector exposure at cryotemperatures, the ionization source, separating lead screen, and photographic detector were preliminarily fixed relative to one another in the above device and cooled by immersing the whole ensemble in liquid nitrogen. After cooling the lead screen was removed and the source was pressed to the detector with a spring mechanism. After the exposure, the contact between the source and the detector was broken and the detector was removed from the source activity range. All the specified operations, including exposure, were performed in the medium of liquid nitrogen. After the detector was removed, no formation of significant amounts of condensate on it was observed in air.

After latent images were obtained, the detectors were developed in batches, each of 48 ARG. Only freshly prepared solutions were used for the chemical treatment of each batch. In the further work ARG were compared with one another only within each batch, i.e., only ARG obtained within one week, when the radioactivity of the source varied slightly (by less than 2%), and which were developed simultaneously.

The ARG images were digitized using an Epson scanner, model Epson Perfection V200 Photo with a resolution of 4800 × 9600 dpi and dynamic range to 3.2 D. The scanner was calibrated using a standard KODAK Q60 target. The transmission scanning of the target was done with a resolution of 4800 dpi and disabled image correctors. The image obtained was saved as a bmp file in the grayscale mode with 8 bit color depth. In the image obtained, we determined average brightness in the "Luminosity" channel in the areas 100 × 200 pic in each grey zone of the calibration target using the Adobe Photoshop Elements 6.0 software. The values obtained were used to construct a calibration dependence for the luminance of a corresponding zone on the image "brightness" recorded by the scanner, declared by the manufacturer of the calibration target. The curve obtained can be well approximated by a third-order polynomial, which was then used to process the experimental data (Fig. 1).

The images of nuclear films, exposed and developed according to the recommendations of the manufacturer, were scanned and saved as in the procedure described above. To determine of the optical density of the image in the ARG recorded in this work, we estimated the average value of brightness in the "Luminosity" channel in areas 300 × 300 pic in an ARG zone of interest. The value obtained was used to determine the luminance (L^*) of the area using the calibration polynomial specified above (Fig. 1), which in its turn was used to determine optical density by the equation $A = 3\log(116/(L^* + 16))$ [22] (this equation is valid for luminance higher than ≈8). The resulting optical density of an ARG area of interest was obtained by the subtraction of the background, an area of equal sizes with no image in the immediate proximity to the studied image, from the optical density obtained above.

RESULTS AND DISCUSSION

The visual appearance and physical properties of nuclear detector film samples subjected to freezing in liquid nitrogen within from several minutes to several days were not different from those of similar samples exposed at room temperature. In other words, after unfreezing films under the conditions preventing the formation of condensate on their surfaces, we observed no mechanical changes neither of the photographic layer nor of the base (cracking, flaking, curling, deformations, and so on).

All ARG obtained at room temperature and at the temperature of liquid nitrogen under irradiation with a Mossbauer source were well reproduced (Fig. 2). The images on ARG were circles with sufficiently clear boundaries corresponding to the inner boundary of the cylindrical glass of the source holder. The circle exhibited reproducible distortions, corresponding to the deformation of the source holder. Darkening inside

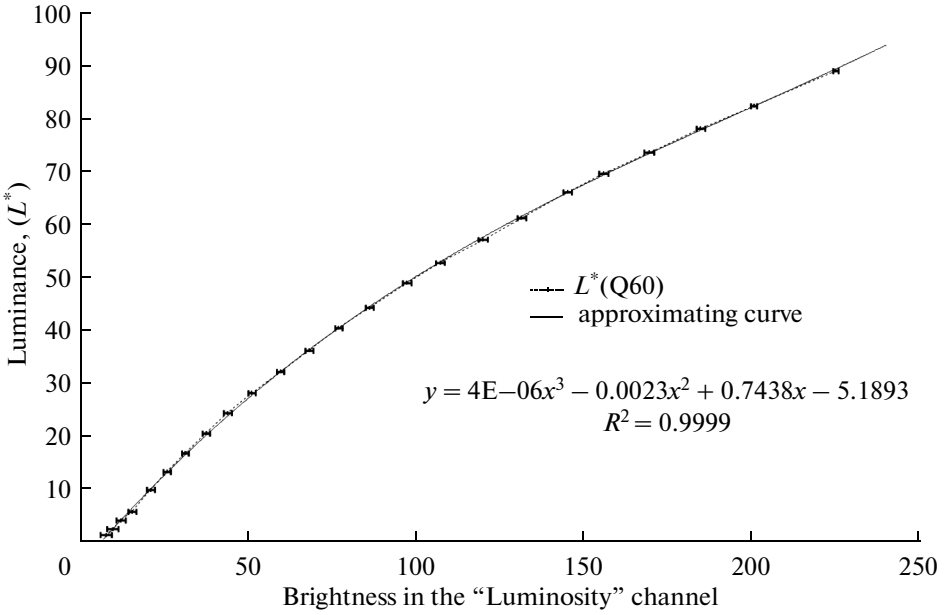

Fig. 1. Calibration curve of the scanner.

the circle, corresponding to the action of the radioactive part of the source, diffusely decayed to the borders. One could quite easily distinguish a rather uniformly exposed disk, slightly shifted from the center to one of the image boundaries, and evidently corresponding to the asymmetric position of the rhodium foil with the radioactive isotope in the Mossbauer source. The maximum of the optical density of this exposed area was also shifted to the boundary of both the central, most dense disk, and the circle containing this disk. We can suppose that the radionuclide atoms are also nonuniformly distributed within the foil. Thus, the most active area of the source used is wholly shifted to its border by approximately 1.5–2.0 mm. In quantitative measurements, the value of brightness was determined just in this area of the ARG, i.e., in the region of the maximum optical density. In many ARG of the source recorded at the temperature of liquid nitrogen (most often at short exposures), we observed chaotic areas of light exposure around the sharp border of the circle corresponding to the inner section of the source, such as points, horseshoes, and rings arranged in a circle (Fig. 2). The reason for the appearance of similar "satellites" of the main image can be either the mechanical action of the source holder contacting with the detector on the photoemulsion layer or the radioactive pollution of the source holder. The second version was proved in an additional autoradiography study of scrapes from the source surface.

In addition to demonstrated possibility of using cryogenic autoradiography for the qualitative characterization of the studied samples, it would be important to study the possibilities of the proposed procedure for quantitative measurements. The latter ones in autoradiography become possible because of the dependence of the optical density of the photodetector on the value of the accumulated dose (fluence) of the recorded radionuclide radiation [23]. The fluence is proportional to the concentration of radionuclide at the place of contact of the detector with the studied sample. The complex dependence of the recorded optical density on the accumulated source dose is usually described by a characteristic curve built on the basis of the experimental data [24]. In this work an ARG image was digitized for the above purpose using a flatbed scanner. The possibility of using commercial flatbed scanners for determining the optical density of films, also in autoradiography, was shown in a number of works [25–28]. When proposing the above procedure of quantitative densitometry, we relied on the technical characteristics of the equipment used, physical models incorporated into the equipment by the manufacturer, the possibilities of the software, and the properties of the studied samples.

The dependence of the optical density of the studied ARG on exposure found as described above (exposure logarithm, product of the radioactivity of the source by the time of exposure) is shown in Fig. 3. To construct it, we used ARG data obtained at the times of exposure from 2.5 min to 5 h and source radioactivity from 33 to 58 μCi.

The characteristic curves constructed as described above based on ARG data obtained both at room temperature and temperature of liquid nitrogen exhibit typical S-shapes. It can be seen in Fig. 3 that, at equal exposures, the optical density for ARG exposed at the temperature of liquid nitrogen is, as a whole, lesser than that for ARG exposed at room temperature. An exception is provided by the region of low exposures (initial section), in which the characteristic curves

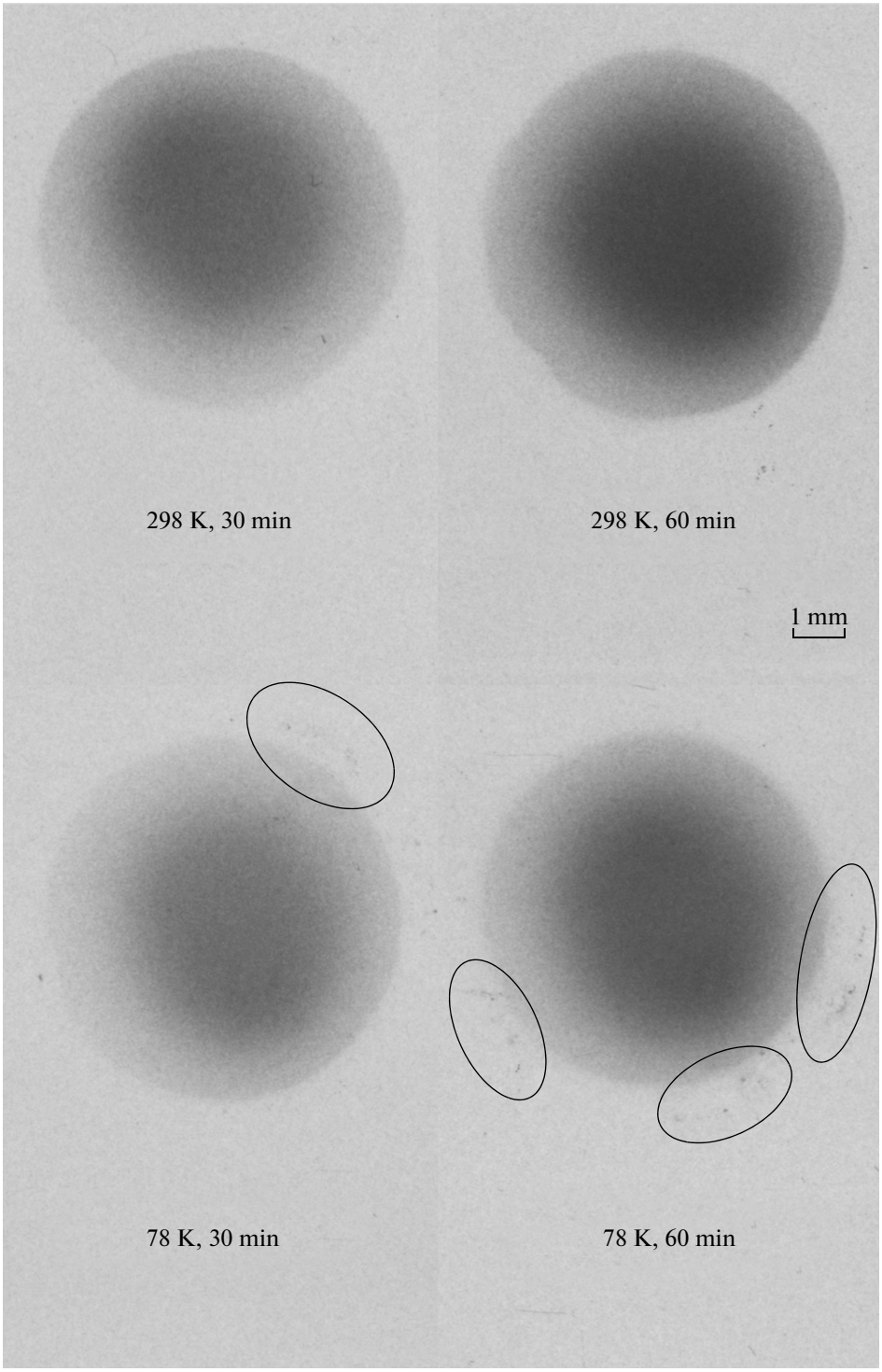

Fig. 2. Typical autoradiograms obtained under different exposure conditions (regions corresponding to the pollution of the source holder are noted).

intersect (Fig. 3). It can be seen that the initial section in curve *1* is much longer than the similar section in curve *2* (Fig. 3). The next section of direct proportionality for the curve corresponding to room temperature exhibits a steeper slope than that for the curve corresponding to cryogenic conditions. This means that the degree of contrast for the photographic material exposed at room temperature is higher. Both curves change from the regions of correct exposures to the top sections at approximately equal exposures. However, the longer region of high expo-

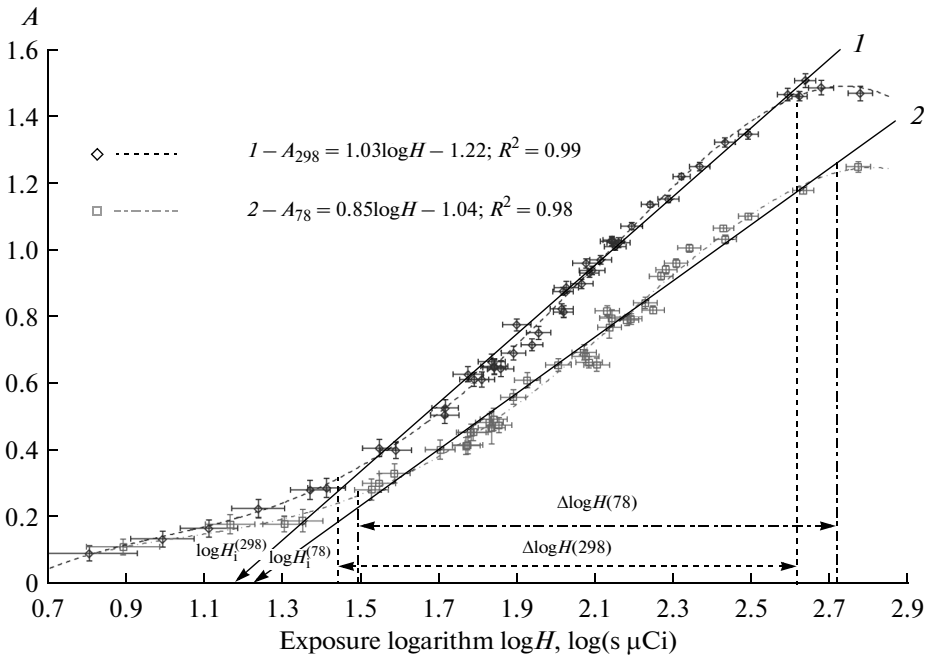

Fig. 3. Characteristic curves obtained under different conditions: *1*, at room temperature; *2*, at the temperature of liquid nitrogen (experimental data, approximating straight lines of the regions of normal exposures, regions of photographic latitude, and inertia points are shown).

sures in this case was observed just for low-temperature curve *2* (Fig. 3). The maximum optical density (~1.5) for the high-temperature curve agrees with the data obtained in [24].

Because of the above features of the characteristic curves, the lengths of regions of normal exposures of the photographic material for different temperatures (shown by vertical lines in Fig. 3) in the logarithmic expression differ by only 0.05 units. However, if we take into account the regions of low exposures, in which the gradients of the curves are higher than 0.2 (the minimum useful gradient at which a change in image tint can still be visually detected [29]), the useful photographic width for both curves expands to the whole studied region of low exposures. However, for the low-temperature curve, the useful photographic width for the same reason also expands to the studied region of high exposures (Fig. 3).

Let us note that the optical density of the background (photographic fog, A_0) virtually depends neither on duration nor on exposure temperature and that its average values were 0.09 ± 0.02 for room temperature and 0.09 ± 0.01 for cryotemperature, which is much lower than the value reported in [24].

As follows from the characteristic curves obtained in this work, the decrease of exposure temperature to the temperature of liquid nitrogen led to the formal reduction of the sensitivity of the photographic detector. Thus, if we determine the sensitivity of the detector by the value of the logarithm of exposure at the inertia point [29, 30], we can show that at freezing it makes 89% of the corresponding value at room temperature (Fig. 3). Note that the determination of the sensitivity of a photographic material by the value of the inertia point characterizes only the data obtained in the region of normal exposures. When determining the sensitivity of the studied photographic material by the procedure based on the value of exposure corresponding to an optical density equal to $A_0 + 0.10$ units [29, 30], we will find that sensitivity of the frozen photographic material of the detector, at least, did not change if not increased (Fig. 3).

The results described above show that the reduction of the temperature of the detector to 78 K in registering actinic radiation slightly changes the properties of the detector, and some of them are even improved. For example, the reduction of temperature reduces the contrast of the detector, but, at the same, time considerably extends the useful range of exposures, thus allowing the researcher to record areas with significantly differing radioactivities.

As a whole, the characteristic curve obtained at the cryotemperature possesses smaller ordinates compared to the curve obtained at room temperature. One of the reasons for this can be a decrease in the effective sensitivity of the detector with cooling, which is due to the delay of processes of the formation of latent images. However, we must take into account that the procedure of ARG recording assumes the presence a medium (about 1 mm thick) between the radiation source and the detector. At room temperature this medium is gaseous air and, at the temperature of liquid nitrogen, liquid nitrogen. A substantial growth of the density of medium between the radiation source and

the detector should also lead to the reduction of the detected radiation intensity. The role of this factor will be elucidated in a separate publication. Here let us only note that the specially conducted studies have shown that gamma radiation (the main radiation of the source used) is not actinic for the given detector. The detector obviously records X-radiation with energies lower than 7.2 keV.

Taking into account the presence of different media between the source and the detector at room temperature and cryotemperatures, we must note the intersection of characteristic curves in the regions of low exposures. This is indicative of a considerable increase in the effective sensitivity of the detector at cryotemperatures for low exposures [16, 18]. Though the possibility of quantitative measurements by the data autoradiography in this region is virtually zero, there appears a possibility of recording regions with very low radioactivity at a qualitative level. This can be illustrated by isolated spots, visually distinguished in cryogenic ARG and arranged in a circle around the main image (Fig. 2); these, probably, correspond to the radioactive pollution of the holders of the used radiation source.

Thus, it was found experimentally that the nuclear photographic detector BioMax MR Film from KODAK at the temperature of liquid nitrogen retains its physical properties (the base does not crack, does not undergo curling, the photolayer does not flake). It was shown that this detector can be used to record the radiation of ^{57}Co both at a room temperature and at the temperature of liquid nitrogen. The characteristic curves for the studied nuclear photographic detector obtained at room temperature and at the temperature of liquid nitrogen indicate that the detector as a whole retains its properties at cryotemperatures, and can thus be used to study deeply frozen samples. In addition, an increase in the sensitivity of the frozen detector at low exposures allows the registration of sample areas containing very small amounts of radionuclide atoms.

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