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OF SURFACE PHENOMENA

Mass Spectrometric Imaging of Surfaces: Effect of the Way of Applying a Marker Substance on the Quality of Obtained Data

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Abstract—A study is performed of the SALDI mass-spectrometric imaging of surfaces of structural materials. The search for the best marker substance is described. It is shown that nickel and copper salts cannot be used to solve the problem. Good signal reproducibility over a surface is shown by silver chloride, bromide, and silver iodide, the mass spectra of which are a large set of cluster ion peaks. Silver halides have a high ionization cross section as well. The best reproducibility is found for nitrobenzoic acids, due to the stable aromatic cycle in the molecule and the procedure's softness of ionization. It is also shown that the most uniform distribution of a marker substance over a surface is obtained by immersing the sample in its solution. Single drops and sprays can also be applied with some modifications.

Keywords: imaging, mass spectrometry, surface distribution, surface diagram

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INTRODUCTION

Mass spectrometric surface imaging is an important area of mass spectrometric research, which allows us to obtain unique data on the surface composition and distribution of compounds in thin films. Imaging is used for both high- and low-molecular weight compounds. Recent advances in the field of mass spectrometric imaging were reviewed in [1]. The widespread use of MALDI imaging is due primarily to the universality of mass spectrometry with respect to analytes, and the possibility of MALDI ionization to obtain mass spectra from each point of a surface. The most common works have been on analyzing the content of different compounds in animal tissues for histology [2], searching for pathologies [3], and other clinical studies [3, 4].

MALDI mass spectrometry has all the advantages of mass spectrometric means, e.g., high sensitivity (at the level of femtograms of a substance [6, 7]) and a wide range of analytes, ranging from low molecular weight organics [8, 9] to the highest molecular weight proteins and industrial polymers [10–12]. At the same time, surface mass spectrometry has a number of characteristic disadvantages, most of which are due to problems of uniformly applying a sample to a surface. This complicates quantitative analysis and requires

special ways of preparing samples and the modification of equipment [13–15].

A poorly studied area of using MALDI is investigating the state of thick surfaces and searching for surface contamination. Only ways of studying thin films are described in the scientific literature on investigating surfaces [16, 17]. We proposed a way of studying surface morphology using marker substances in [18, 19], where it was shown they can be used as detectors of chemical surface homogeneity. A necessary condition for obtaining a correct display is leveling irregularities in the distribution of matter not related to surface morphology. Uneven crystallization of the substance can produce such effects. These are mainly associated with the wrong choice of solvent and the way it is applied. The marker substance itself also plays an important role. The aim of this work was a systematic study to identify the effect of these factors and develop ways of eliminating them.

EXPERIMENTAL

Mass spectrometric imaging was performed using a Bruker Daltonics Ultraflex laser desorption/ionization mass spectrometer equipped with a time-of-flight mass analyzer. Ionization was done using a pulsed nitrogen laser with an operating wavelength of 337 nm. The energy of one pulse varied from 20 to 110 μJ . The

Table 1. Mode of laser radiation used to study the distribution of different marker substances on surfaces

Marker substance	E , $\mu\text{J}/\text{pulse}$	F , Hz
Silver chloride	30–35	20
Silver bromide	30–35	20
Silver iodide	30–35	20
Nitrobenzoic acid	50–55	20
Dinitrobenzoic acid	45–50	20
Nickel chloride	70–80	20
Copper chloride	70–80	20

E is energy of laser radiation, F is pulse frequency.

number of pulses varied from 1 to 100. The pulse frequency was 20 to 150 Hz. The mode of imaging for a particular compound was chosen separately in each experiment.

Different marker substances were used to prepare samples: silver halides (dry salts, Reakhim, Russia), nitrobenzoic and dinitrobenzoic acids (99.9%, Sigma-Aldrich, Germany), nickel chloride (Reakhim, Russia), and copper chloride (Reakhim, Russia).

Water (deionized; Milli-Q class, at least 18 M Ω), dichloromethane (Panreac, Spain), acetonitrile (JTBaker, United States), ethyl alcohol (96% Ferein), and acetone (Reakhim, Russia) were used as solvents.

The chosen solvents were the ones most common and readily available to the laboratory.

RESULTS AND DISCUSSION

Selecting the Frequency and Energy of Laser Radiation

Conditions of observing the mass spectrum were selected for each marker compound at the start of our study. Relatively high sensitivity to the marker compound and changes in its concentration on a surface was required. The problem was thus reduced to determining the maximum of derivative $\Delta I/\Delta c$, where I is the peak intensity of the marker compound in the mass spectrum, and c is the concentration of the marker substance on the surface of the material in terms of unit area. Results from our study and the mathematical processing of our results are presented in Table 1.

Table 1 shows that ionization was most effective for silver halides, since their ionization requires the least energy. At the same time, the dynamic range was very small, since the signal goes off scale at energies above 60 μJ . Silver halides generate a wide range of peaks of cluster ions in the mass spectrum with different ionization cross sections (Fig. 1). However, any of them can be used to determine changes in concentration on the surface.

Nitrobenzoic acids show somewhat lower efficiency of ionization, which allows their use in a wider dynamic range of concentrations. The mass spectra of nitrobenzoic acids also consist of only a molecular ion peak and several low-intensity ion fragments.

Nickel and copper chlorides display the lowest efficiency of ionization and a large set of cluster adduct ions with water and alkali metals. Varying the concentrations of these salts on a surface showed that the

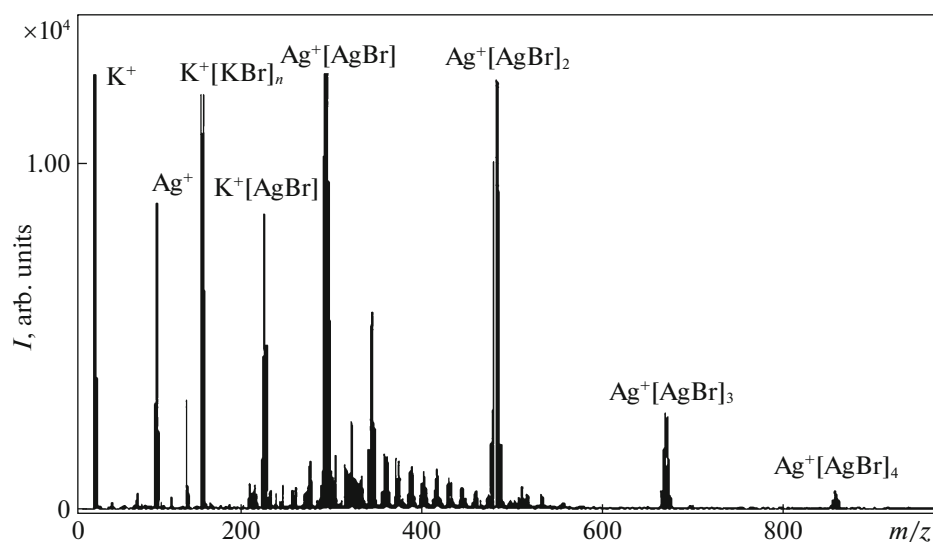


Fig. 1. SALDI mass spectrum of silver bromide, recorded from a surface of stainless steel in the mode of positive ion detection.

intensity of adduct ions depends little on the concentration of the marker substance, which extremely limits the scope of these compounds.

From Table 1 we can also see the frequency of laser pulses was chosen to be the same for all of the studied compounds, since the efficiency of ionization depends little on the frequency. It is maximum at 20 Hz, regardless of the given marker compound.

From the above, we may conclude that silver halides can be used at the lowest working concentrations, while the use of nitrobenzoic acids requires concentrations several orders of magnitude higher.

Selecting the Optimum Concentration of the Marker Substance

The choice of the optimal concentration of a marker substance is an important parameter in constructing surface diagrams. The concentration determines the intensity and quality of the peaks used for quantitative analysis. The requirements for such peaks are:

- it must have an intensity in the middle of the working scale of the mass spectrometer;
- it must adequately reflect the change in the concentration of the substance on the surface at a given point;
- peak width must be such that it does not overlap adjacent isotopic peaks; and
- the area around the peak must not have appreciable chemical noise.

The peaks characteristic of each marker substance were selected to create surface diagrams. These peaks are listed in Table 2.

It can be seen from Table 2 that using a silver halides as a marker substance is most promising, since these compounds form a wide range of cluster ions with a large molecular weight. The absence of appreciable chemical noise is in this case guaranteed. If it is

Table 2. Characteristic peaks for markers of the studied substances (modes of recording mass spectra are given in parentheses)

Marker substance	Main peak, Da	Auxiliary peak, Da
Silver chloride	394.67 (+) 322.74 (–)	538.55 (+) 464.61 (–)
Silver bromide	482.55 (+) 454.56 (–)	670.37 (+) 642.38 (–)
Silver iodide	576.52 (+) 596.52 (–)	812.33 (+) 830.33 (–)
Nitrobenzoic acid	166.01 (–)	–
Dinitrobenzoic acid	211.00 (–)	–
Nickel chloride	–	–
Copper chloride	–	–

impossible to use one main peak, we can always use another (the right column of Table 2). Silver halides also have high ionization cross sections and sensitivity to changes in surface concentration [20].

Nitrobenzoic acids are widely used to clean rocket fuel tanks of toxic propellant components and can be easily detected on their aluminum surfaces [21]. It was found that in addition to molecular ions, the mass spectra of nitrobenzoic acids contain fragments of ions $M-COOH$, MH_2O , along with cluster ions of molecular dimers (Fig. 2). Such ions are characteristic of organic acids and can in some cases be used as auxiliary ions for surface studies. In this work, the ratio of ion fragments changed along with the concentrations of nitrobenzoic acids. This shows that ion fragments cannot adequately reflect the change in the amount of matter on a surface.

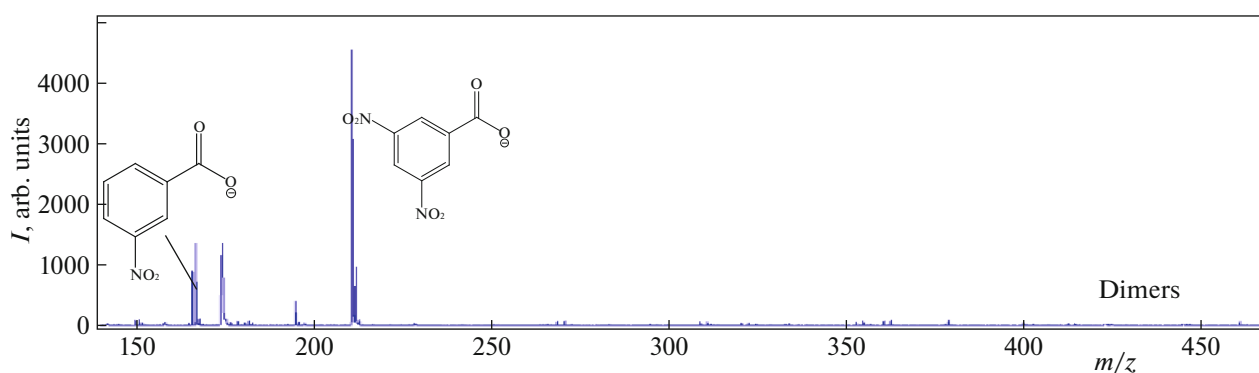


Fig. 2. Mass spectrum of a mixture of nitrobenzoic acids on a surface of aluminum alloy AMg-6, recorded in the mode of negative ion detection.

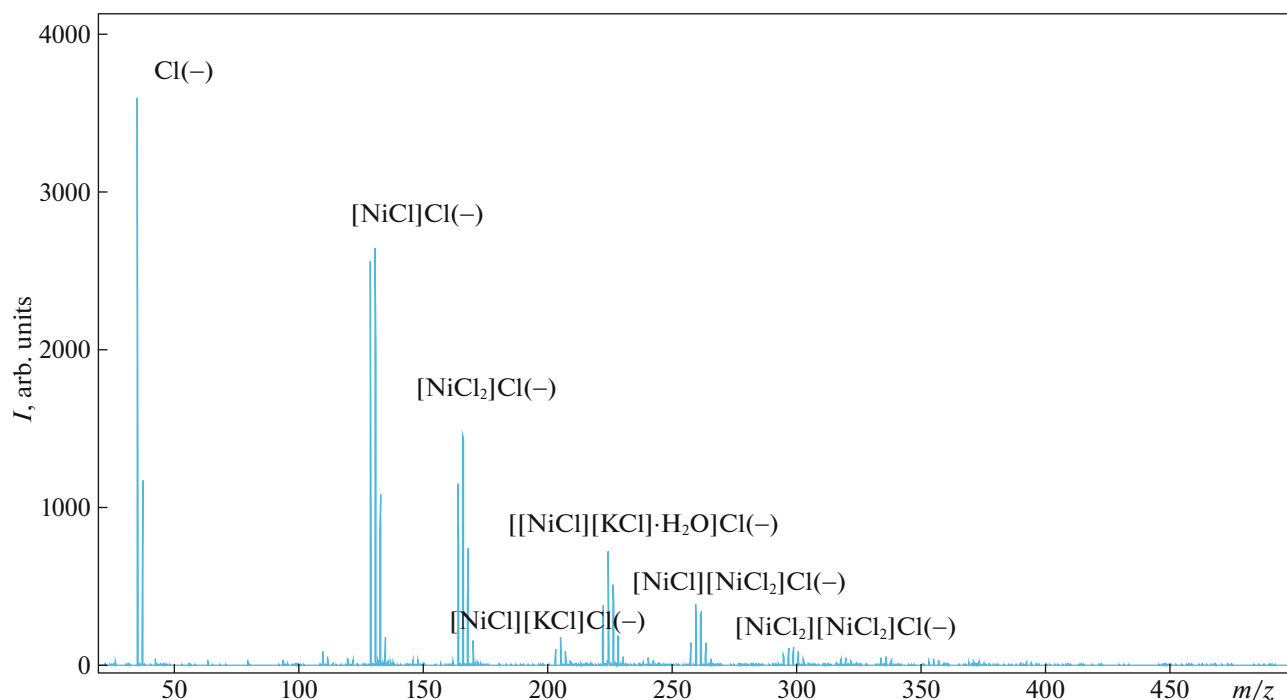


Fig. 3. Mass spectrum of nickel chloride on the surface of aluminum alloy AMg-6, recorded in the mode of negative ion detection.

Like many other inorganic salts, nickel chloride is prone to cluster formation under SALDI conditions of ionization.

Figure 3 shows that cluster ions were associated with one another and potassium chloride, which entered the region of ionization from the water that was used. Despite the purity of the water (Mili-Q grade), it contained several picograms per liter of potassium and sodium salts. These impurities actively associated with dissolved salt molecules. Experiments have shown the relative intensities of the peaks of such clusters both vary with concentration and depend on the properties of a surface at a particular point. No single peak reflects quantitative content on a surface because of the effect the amount of salt and the type of surface have on the degree of fragmentation. This salt can be used to search for damaged areas of a surface, but it is difficult to find a quantitative characteristic that reflects a change in fragmentation. Nickel chloride is a promising substance and can be used as a marker of the state of a surface. It cannot be used in the context of this work, however, since at this stage an accurate quantitative characteristic of the concentration of the substance on the surface is required.

Studies have shown that copper chloride makes a poor marker. It was found that copper chloride forms cluster ions as large as 100 Da (i.e., in the region of the strongest chemical noise). Cluster ions form associates with water molecules and reflect quantitative charac-

teristics poorly. Copper chloride ions also have a small ionization cross section, and therefore do not accurately reflect a small change in the amount of the substance on a surface.

Based on our results, silver halides, nitrobenzoic acid, and dinitrobenzoic acid were selected as marker substances.

Imaging of Metal Surfaces

The main disadvantage of using mass spectrometric imaging to study the uniformity of surface morphology is the uneven deposition of a marker substance, caused by its uneven crystallization from a solution on a surface. With such application, it becomes impossible to distinguish between differences in concentration associated with crystallization and surface morphology. The ways that are used must ensure uniform application. The question then arises of how to accomplish this. We believe it is best to use mass spectrometric imaging on an model inert surface. The sample plate used in a SALDI mass spectrometer can serve as such a surface, since it is prepared for the most uniform application of a sample and has uniform morphology over the surface area. When using different ways of applying a marker substance, imaging on such a surface will show the degree of uniformity of

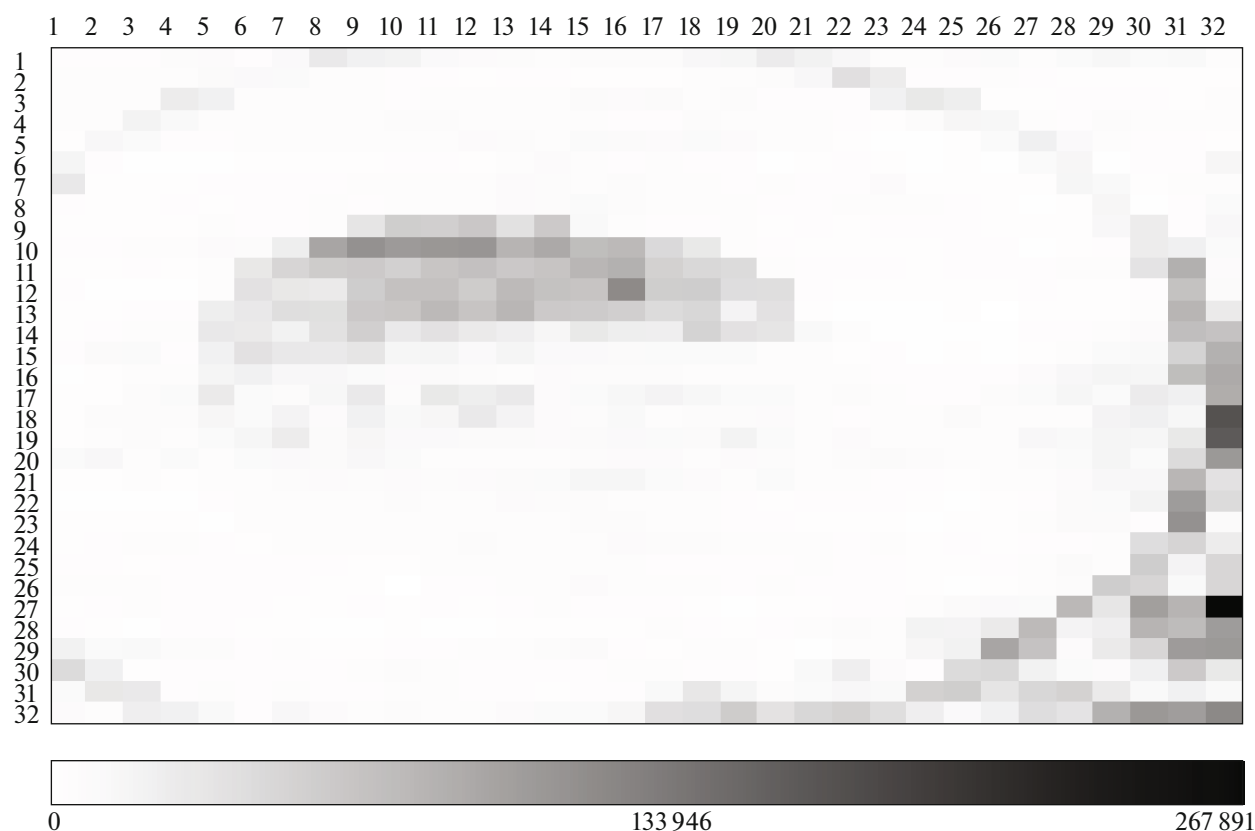


Fig. 4. Imaging of nitrobenzoic acid on a surface of inert stainless steel, applied in single drops. Molecular ion imaging was done at 166 Da with negative polarity.

application and eliminate all deviations of the signal associated with uneven surface morphology.

We tested three ways of applying marker substances: spraying a solution of the marker substance in the form of an aerosol onto the surface, applying one sub-microliter drop, and immersing the surface in a solution of the marker substance.

Table 3. Signal reproducibility in surface imaging using different means of application

Marker substance	Drops	Spraying	Immersion
	<i>S</i> , %		
Silver chloride	30–35	20–25	10–15
Silver bromide	25–30	25–30	10–15
Silver iodide	30–35	20–25	10–15
Nitrobenzoic acid	25–30	15–20	5–10
Dinitrobenzoic acid	25–30	15–20	5–10

S is standard deviation.

Of these three, the single-drop technique was excluded immediately because imaging data showed there was crystallization at the edges of a drop, which disturbed the distribution of uniformity considerably.

Figure 4 shows that crystallization proceeds along the edges of the drop and locally in its center. The spread of the intensity of the molecular peak from point to point is 30–35%.

Spraying the solution onto a surface produced much better results for all of the marker substances. Figure 5 shows the result from surface imaging by spraying a silver iodide slurry onto a surface. We can see the distribution was extremely uneven and the concentration fell from the center to the periphery.

Our data are summarized in Table 3. We can see the standard deviation of the signal over the surface is smallest when using sample immersion. Other ways can achieve more uniform application as well. When using a drop, we can increase its size and perform imaging inside it at a distance from the edges. This approach improves the standard deviation of the signal by 10–12%. However, the best reproducibility is achieved by spraying multiple times on one area of a surface. An important factor here is the amount of sample applied and the number of sprays. A more uni-

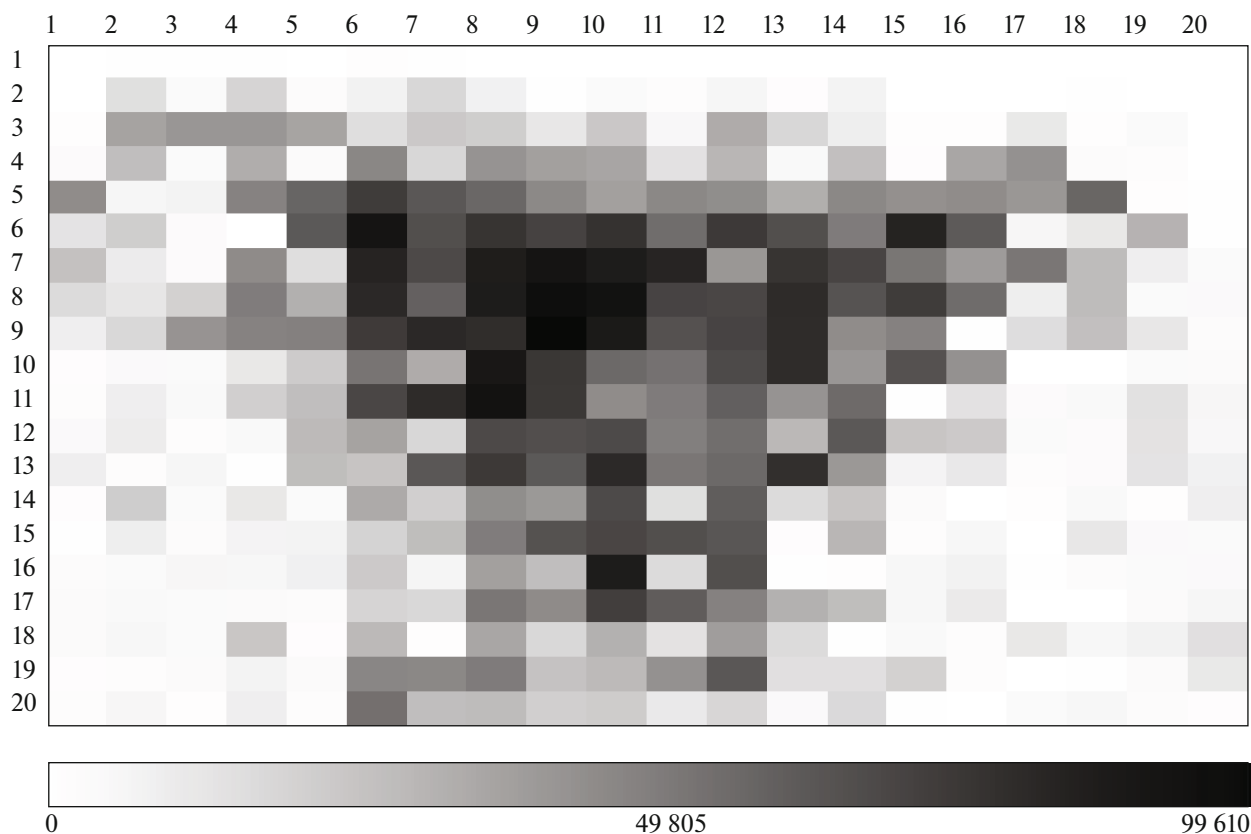


Fig. 5. Imaging of silver iodide on the surface of inert stainless steel, applied by spraying. Molecular ion imaging was done at 576.5 Da with positive polarity.

form distribution can be achieved by increasing the number of sprays. However, a surface is overloaded if we increase the amount of the sprayed marker substance, and the efficiency of ionization falls during mass spectrometric imaging.

It should be noted that when inorganic salts are used, the overall spread of peak intensity values is on average greater than for nitrobenzoic acids. This is due to the multistage fragmentation of silver halide cluster ions during ionization. It has been noted that ion fragmentation is spurred on by each ionization pulse and continues not only during the process, but in the mass analyzer during ion separation as well [22]. As with other aromatic compounds, the ionization of nitrobenzoic acids results in much less fragmentation under mild conditions. Fragmentation is therefore more reproducible. By correctly selecting the way of applying nitrobenzoic acids, we can obtain the best reproducibility for the imaging of structural materials.

CONCLUSIONS

We studied different ways of applying a marker substance to the surface of a metallic material for purposes of imaging and assessing the uniformity of

morphology. The procedure is already in demand for studying damage to the aluminum surfaces of fuel tanks of Russian launch vehicles after draining highly toxic propellant components. It was shown that the most uniform application without complicating sample preparation can be achieved by completely immersing a fragment of a material in a solution or suspension of a marker substance. Three silver halides, nitrobenzoic acid, and dinitrobenzoic acid were used as marker substances. The best results were obtained when applying nitrobenzoic acids to a surface by immersion in a solution.

Our results were used to develop ways of applying a marker substance to surfaces of structural materials for purposes of studying surface morphology in the mode of mass spectrometric imaging.

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