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SURFACE-IONIZATION MASS SPECTROMETRY OF ACETYLCHOLINE HALIDES

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The surface ionization of acetylcholine iodide has been investigated mass-spectrometrically. It has been shown that surface-ionization mass spectrometry with the successive use of catalytically active and catalytically passive ionizers (ion emitters) permits a highly sensitive and reliable identification of micro amounts of acetylcholine molecules, including those present in complex mixtures.

The determination of micro amounts of acetylcholine, especially its identification in mixtures with other organic substances, is an urgent problem. The use of chemical and biological methods is either insufficiently sensitive and nonselective or is extremely laborious [1]. On the use of mass spectrometry with the classical methods of ionization by electrons and photons, and also of secondary-ion mass spectrometry [2] and fast-atom bombardment mass spectrometry [2], only ions of fragments of the molecule are observed, and intact acetylcholine cations are not recorded.

The surface ionization (SfI) of nitrogen-containing organic and bioorganic compounds as a simple and effective method of obtaining polyatomic ions is finding use not only for determining a number of properties of polyatomic particles and unique characteristics of their interaction with a solid surface but also for the highly sensitive and selective analysis of organic substances even when they are present in complex mixtures [3-5]. The general laws of the SfI of amines and their derivatives, including compounds of biogenic origin, that have been found permit the prediction of the SfI mass spectra and the densities of such ions [6].

Thus the SfI mass spectra of alkylamines and their derivatives consist of the quasimolecular ions $(M - H)^+$, $(M + H)^+$, and $(M - R)^+$, where H is a hydrogen atom eliminated from or added to the initial molecule, M, and R is a radical present in the β- position to the nitrogen atom. They all have unsaturated bonds and can be represented in the form of ions with a tetravalent positively charged nitrogen atom having sp^2- and sp^3-hybridized orbitals [2, 6].

The current densities of the $(M - H)^+$ and $(M - R)^+$ ions in the SfI of tertiary amines on metal oxide emitters amount to $5 \cdot 10^{-2}$ A/Pa·cm². The possibility of the SfI mass-spectrometric analysis of salts of quaternary alkylamines has been shown in [7]. It was found that when a catalytically passive iridium surface coated with a monolayer of carbon having a graphitic structure was used as the emitter the SfI mass spectrum consisted of lines of intact cations of the quaternary amines R_4N^+.

In the present paper we demonstrate the possibility of SfI mass spectrometry in the analysis of micro amounts of acetylcholine, even in complex mixtures.

The experimental procedure for surface-ionization mass spectrometry has been described in [7]. Micro amounts of acetylcholine chloride or iodide were enclosed in a quartz vaporizer. When the latter was heated, molecules passed to the surface of the heated emitter and were recorded mass-spectrometrically. As emitters we used high-melting metals (W, Mo, Ir) and their oxides (W_xO_y, Mo_xO_y), and also a film emitter — an iridium wire coated with a monolayer of carbon having a graphitic structure [8, 9].

On ionization on metal and oxide surfaces the mass spectrum consisted of ions of the products of the dissociation of the initial acetylcholine molecules. Below, we give a typical mass spectrum from ionization on a tungsten oxide emitter. Apart

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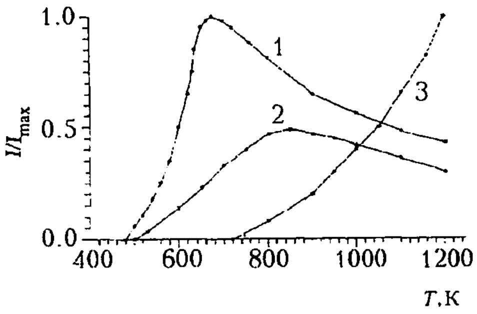

Fig. 1. Temperature dependences of the ion currents in the surface ionization of acetylcholine on oxidized tungsten: *1*) the $(M_1 - R_1)^+$ ions with *m/z* 58, $I \sim 10^{-7}$ A/cm^2; 2) the $(M_1 - R_2)^+$ ions with *m/z* 72, $I \sim 10^{-7}$ A/cm^2; *3*) the $(M_1 - H)^+$ ions with *m/z* 130, $I \sim 10^{-7}$ A/cm^2.

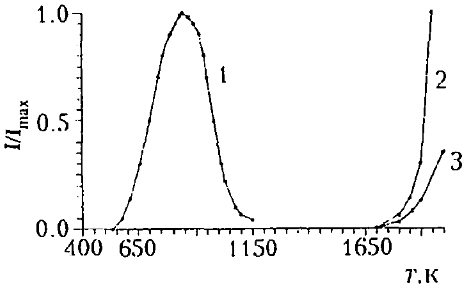

Fig. 2. Temperature dependences of the ion currents in the surface ionization of acetylcholine on iridium coated with a monolayer of graphite: *1*) $(CH_3)_3N^+C_2H_4OOCCH_3$ ions with *m/z* 146, $I \sim 10^{-10}$ A/cm^2; *2*) $(M_1 - R_1)^+$ ions with *m/z* 58, $I \sim 10^{-9}$ A/cm^2; *3*) $(M_1 - R_2)^+$ ions with *m/z* 72, $I \sim 10^{-9}$ A/cm^2.

from the lines shown, the mass spectrum included lines of ions with *m/z* 84, 86, 94, 100, and 162 having intensities of 1-3% changing with time and apparently due to impurities in the sample. No lines of intact cations were observed. Only at high vaporizer temperatures ($T > 500$ K) and large currents of molecules on the surface of metallic emitters, when, as a result of the decomposition of the arriving molecules, carbon islands were formed here, did we observe weak lines of ions with *m/z* 146, corresponding to intact acetylcholine cations.

m/z	%	Presumed structure of the ion	Additional information
130	0.7	$(CH_3)_2N^+{=}CH{-}CH_2{-}O{-}CO{-}CH_3$	Emitter temperature 1000 K
72	60	$(CH_3)_2N^+{=}CH{-}CH_3$	Vaporizer temperature 420 K
58	100	$(CH_3)_2N^+{=}CH_2$	$J_{58}=10^{-7}$ A/cm^2

Acetylcholine and its salts are thermally unstable compounds. Consequently, not only molecules of acetylcholine salts but also products of the thermal decomposition of these molecules in the quartz vaporizer may reach the emitter surface. In actual fact, as experiments on the coverage of the beam from the vaporizer by a screen have shown, the main contribution to the mass spectrum is made by ions formed on the arrival at the emitter surface of volatile substances not condensing at room temperature produced by the thermal decomposition of acetylcholine in the quartz vaporizer. The coverage of the beam changed the intensity of the lines of ions with *m/z* 58, 72, and 130 only slightly, by not more than 10-15%, and completely "covered" only the line of ions with *m/z* 146.

It is known that the main channel for the thermal breakdown of molecules of tertiary alkylamine salts is the reaction $R_4N^+Cl \rightarrow NR_3 + RCl$.

If this channel is the main one in the decomposition of acetylcholine salts, the result should be the formation of molecules of dimethylaminoethyl acetate $(CH_3)_2N-CH_2-CH_2-O-CO-CH_3$.

According to the rules for the formation of ions in the SfI of amines and their derivatives [2, 6], the SfI mass spectra of such molecules corresponds to that given above.

The ions $(M - R_1)^+$ $(R_1 = -CH_2-O-CO-CH_3)$, having *m/z* 58, are formed with a high efficiency, the ions $(M - R_2)^+$ $(R_2 = -O-CO-CH_3)$, having *m/z* 72, with a smaller but comparable efficiency, and, finally, the ions $(M - H)^+$, having *m/z* 130, with an efficiency 10^2 times smaller because of the influence of the electronegative carboxylate group on the ionization potential. As experiments with coverage of the beam by a screen have shown, some of the molecules of acetylcholine salts may reach the emitter surface without decomposing. On the surface of metal and metal oxide emitters they dissociate with the formation of the same dimethylaminoethyl acetate molecules [2, 7]. The SfI of the latter leads to the desorption of the ions shown above. The dependence of the ion currents on the emitter temperature (Fig. 1) is characteristic for the SfI of such molecules [6]. The ratio of the lines in the mass spectrum scarcely changed when the vaporizer temperature was brought to 430 K.

All this permits the conclusion that it is possible to judge the presence of acetylcholine in the vaporizer from these lines. The high efficiency of the ionization of the decomposition products ensures a high sensitivity of the analysis. An experimental evaluation of the sensitive threshold showed that, when an oxidized tungsten emitter with an area of 1 cm^2 is used, $\sim 10^{-12}$ g of an acetylcholine halide is sufficient, for recording the ion currents $I_{58} = 10^{-11}$ and $I_{130} = 10^{-13}$ A, stable for 30 sec.

However, the use of this method for analyzing acetylcholine in a mixture may be difficult if, for example, the mixture contains amines similar to the products of the thermal decomposition of acetylcholine in the vaporizer or if it contains substances the thermal decomposition of which will lead to the same products as the thermal decomposition of acetylcholine.

A selective ionization of acetylcholine with the formation of only its intact cation would be ideal for its reliable mass-spectrometric identification, particularly when it is present in mixtures. To achieve this aim we have investigated the SfI of acetylcholine salts on an iridium surface coated with a monolayer of carbon having a graphitic structure. Such a valence-saturated surface is catalytically passive for dissociation reactions of inorganic and organic molecules [8, 9]. For example, the degree of dissociation of alkali metal halides on such a surface is only $\sim 10^{-4}$, which corresponds to the degree of defectiveness of this surface [8].

Unlike "ordinary" molecules, the weakly bound molecules of quaternary alkylamine halides may experience dissociation on such a surface, with the splitting out of the halogen atom and the desorption of the intact cation [7]. In actual fact, as experiments have shown, when the molecular current was directed from a quartz vaporizer to an Ir–C surface the composition of the desorbed ions was represented only by the lines of ions of the intact acetylcholine cation with *m/z* 146. At the same time, together with the molecules of the acetylcholine salts, the products of their thermal decomposition in the vaporizer also arrived at the Ir–C surface. However, they were not ionized, since these molecules do not dissociate on the "smooth" valence-saturated Ir–C surface, i.e., they cannot form the ionizable particles (M – H) and (M – R). This was confirmed by experiments with an additional feed to the emitter of various organic substances, from alkylamines to hydrocarbons. In all cases the mass spectrum consisted only of lines of intact acetylcholine cations. In this case coverage of the beam of molecules from the vaporizer by a screen led to the complete disappearance of ions in the mass spectrum.

The dependence of the current of intact acetylcholine cations on the temperature of the emitter is shown in Fig. 2. The ion current density at the maximum amounted to $I \sim 10^{-10}$ A/cm^2. The fall in the ion current with a rise in the temperature can be explained only by a dissociation of the acetylcholine molecules, since the thermal emission properties of an Ir–C emitter are constant up to T 1700–1800 K [8, 9]. For example, the molecules of acetylcholine salts may dissociate. The products of this reaction — stable molecules — vaporize from an Ir–C surface without dissociating. At an emitter temperature $T \geq$ 1700-1800 K a breakdown of a monolayer carbon film on Ir begins, the carbon vaporizes from the surface and "bare" sections of the Ir appear. On these sections the product of the decomposition of acetylcholine in the quartz vaporizer and the products of the dissociation of acetylcholine salts on the emitter surface may undergo dissociation reactions with the formation of the ionizable particles $(M_1 - R_1)$ and $(M_1 - R_2)$, and the lines of ions with *m/z* 58 and 72 appear in the SfI mass spectrum (see Figs. 1 and 2).

Thus, surface-ionization mass spectrometry permits the detection and qualitative analysis of micro amounts of acetylcholine in various mixtures with high sensitivity and selectivity. Quantitative analysis always requires calibration.

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