# STUDIES OF ADSORBED PHENOTHIAZINE ANTIDOTES BY TEMPERATURE-PROGRAMMED DESORPTION WITH MASS-SPECTROMETRIC ANALYSIS

## T.V. Kulik[1], N.P. Galagan[1], V.A. Pokrovskiy[1], and V.V. Khrapak[2]

[1] Institute of Surface Chemistry, National Academy of Sciences
Gen. Naumov Str. 17, 03680 Kyiv-164, UKRAINE
[2] Institute of Farmacology and Toxicology, E. Potiet Str. 14, 03057 Kyiv, UKRAINE

## Abstract

By spectrophotometric and kinetic methods of analysis a study has been made of adsorption of three antidotes of the phenothiazine series on the surface of fumed silica from water solutions. Physical and chemical parameters of adsorption have been calculated. Thermal decomposition of bulk antidotes has been investigated by the temperature-programmed desorption technique with the mass-spectrometric registration of volatile constituents (TPD MS) and the results achieved were compared with data for adsorbed samples. For all the samples at the first stage the maximum of HCl evolution is observed at 180°C. For methylene blue and toluidinee dark blue the first stage is accompanied by evolution of $CH_3Cl$ ($m/z$ 50). Then the decomposition proceeds through the rupture of thiazine rings. At this stage, at the temperature of maximum 240°C and higher in mass spectra there appear lines at $m/z$ 93, 66, 51 corresponding to molecular decomposition of aniline. Besides, sulfur-containing fragments originating from rupture of thiazine rings (lines at $m/z$ 32, 34, 107, 121, 135) are observed among the lines attributed to products of thermal decomposition of methylene blue and toluidinee dark blue at high temperatures. Comparison of TPD MS experimental data about thermal decomposition of the antidotes in the condensed and adsorbed states has shown that adsorption on highly dispersed silica surface changed dramatically the mechanism of thermal transformations and increased thermal stability of antidote molecules in comparison to that in the condensed state.

## Introduction

By the TPD MS technique a study has been made of the following three dyes of phenothiazine series: *Gen. Naumov Str. 17* chloride (Lauth's violet) (I), methylene blue (methylthionine chloride) (II), toluidine dark blue O (Textra) (III):



I   $R_1=R_2=R_3=R_4=R_5=H$;

II   $R_1=R_2=R_4=R_5=CH_3$, $R_4=H$;

III   $R_1=R_2=R_3=CH_3$, $R_4=R_5=H$; ×$ZnCl_2$.
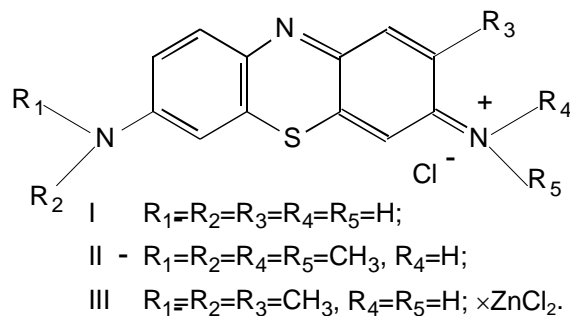
They are widely used in medicine as antidotes to provide treatment for toxic methemoglobinemia and cyanide poisoning, [1-6]. They belong to a biochemical (metabolic) class of antidotes because their use changes mechanisms of cell detoxication and enhances activity of enzymatic systems. Adsorption immobilization of these antidotes is a way to new medicinal compositions, which will differ from existing antidotes by a prolonged action, higher sorption capacity, and longer term of storage. These characteristics will allow preventive medicine practitioners to employ such compositions as multifunctional noninjection sorption antidotes in extreme situations.

## Experimental

The temperature-programmed mass spectrometry was used for analysis of volatile products of thermal decomposition of antidotes in the condensed state and in the state set up during their adsorption on the surface of fumed silica [7]. Weighed samples (of about 1 mg each) of antidotes adsorbed on the surface of aerosil A-300 were placed in a quartz-molybdenum tube, evacuated at $10^{-1}$ Pa, and then attached to the inlet system of a MX 7304 monopole analyzer (Sumy, Ukraine). The reactor-to-mass spectrometer interface included a high-vacuum valve (with an orifice 5 mm in diameter and an inlet tube 20 cm in length), its temperature was kept at 150°C. The reaction space was open in the ion source direction.

Adsorption of the dyes was effected from a water phase in steady-state conditions at a temperature 21–23°C. The preliminary thermal treatment of an adsorbent at 400°C for 2 h and proved to be sufficient to eliminate organic substances that could be adsorbed on its surface. The adsorbate concentration interval was 1–1000 $\mu$mol L$^{-1}$. An aqueous solution of a dye 10 mL in volume was mixed with 0.1 g of highly dispersed silica ($S$=300 m$^2$ g$^{-1}$), and the mixture obtained was let to stand for 2 h. The suspensions prepared were centrifuged, washed with water, and dried at room temperature. Adsorption values were determined by the spectrophotometry method (at a wave length of $\lambda$=540 nm for thionine and toluidine dark blue and of $\lambda$=670 nm for methylene blue) in terms of differences of concentrations in a solution in question before and after adsorption. The adsorption isotherms recorded were linearized on the Langmuir coordinates, with coefficients of determination $R^2$ being equal to 0.979–0.998. The samples used for the mass-spectrometric studies possessed surface concentrations of dyes of about 20 $\mu$mol g$^{-1}$. Desorption from aqueous solutions was effected under steady-state conditions.

## Results and Discussion

By the temperature-programmed desorption and mass-spectrometric analysis of volatile products (TPD MA) a study was made of adsorption and thermal stability of a number of phenothiazine dyes in the condensed state and in the state set up as a result of adsorption on the surface of highly dispersed silica (HDS).

The thermal decomposition of the dyes under investigation was distinguished for having two stages of release of hydrogen chloride (Fig. 1). The low-temperature stage starts at a temperature above 100°C and reaches its maximum yield at about 160°C. Besides, in the case of methylene blue and toluidine dark blue one can also observe a release of CH$_3$Cl (50 $m/z$), with the line intensity being equal to about 5% of that for the 38 $m/z$ line. The high-temperature stage of the release of HCl for toluidine dark blue and thionine exhibits a peak at about 400°C.

In the situation with free thionine in the temperature region between the peak temperatures for the release of hydrogen chloride there is a maximum (at 290°C) of aniline release in the molecular form (93, 66, 51 $m/z$) (Figs. 1, 2).
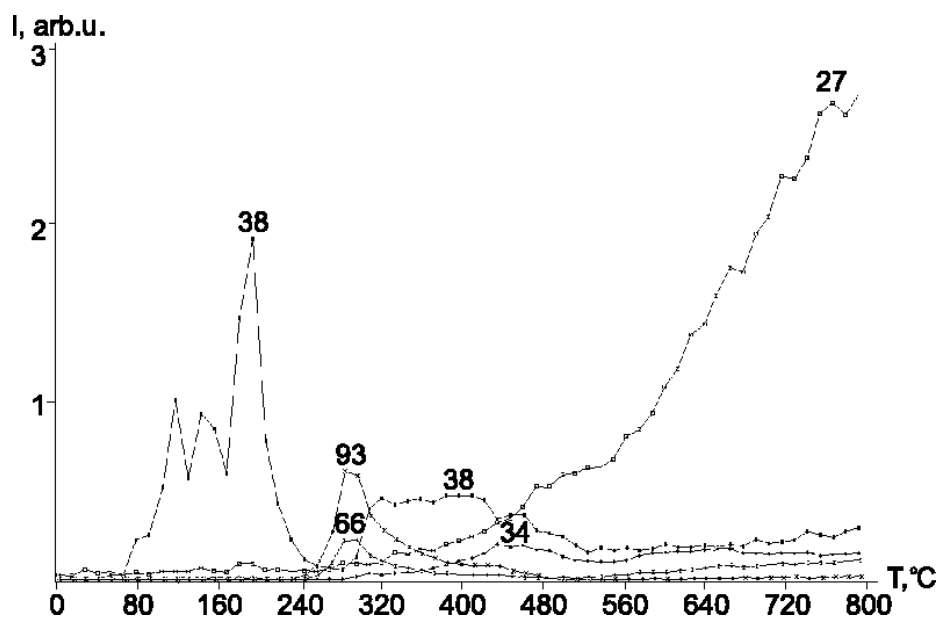
**Fig. 1.** Thermograms of fragments m/z: 93, 66, 38, 34, 27; decomposition of thionine chloride in the condensed state.
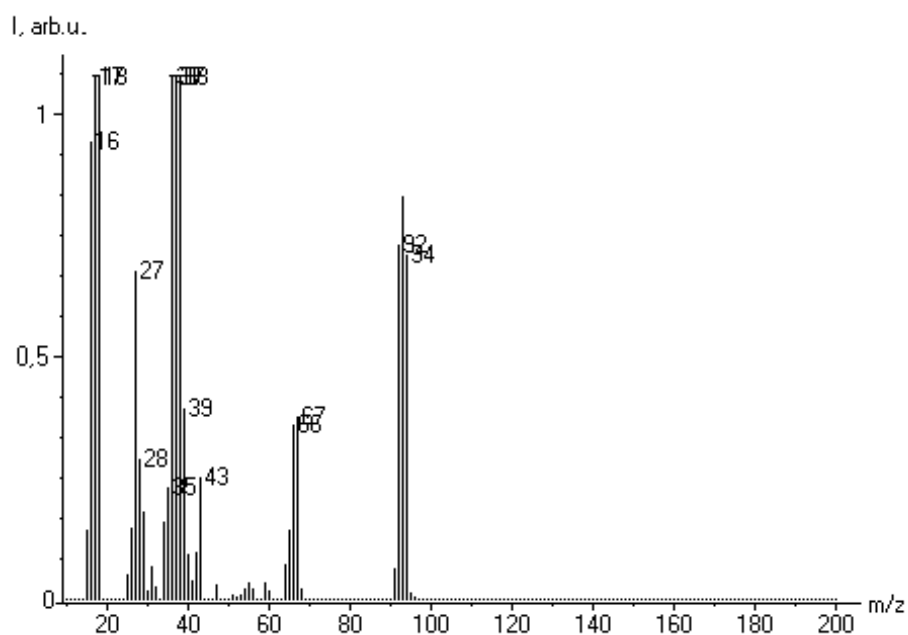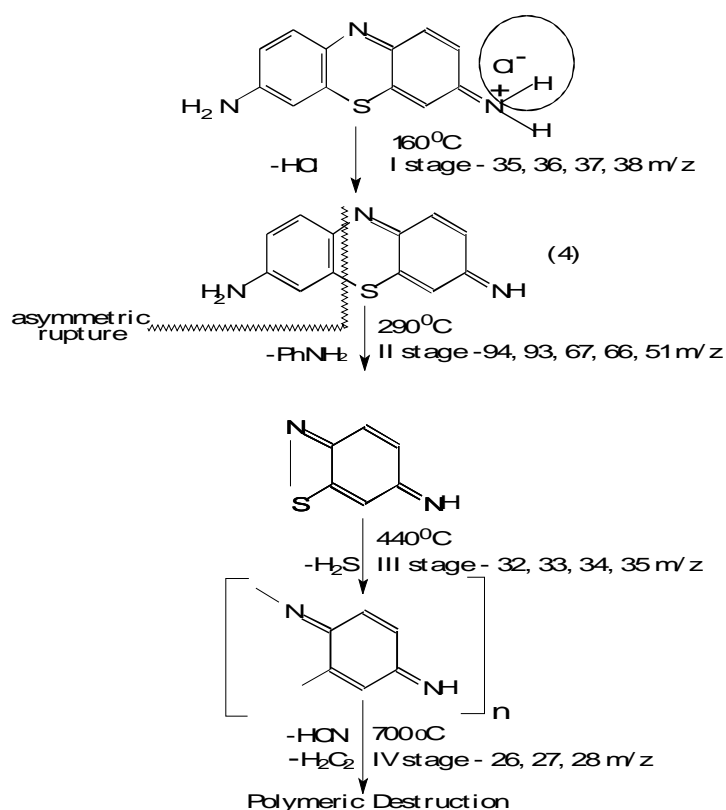


**Fig. 2.** Electron impact mass-spectrum of thionine chloride in the condensed state at 302°C.

This maximum is not related to the thermal desorption curve characteristic of hydrogen sulfide (35, 34, 33, 32 *m/z*). The onset of the hydrogen sulfide release coincides with the maximum thermal release of aniline, and the maximum yield of the hydrogen sulfide release is attained at about 440°C. This observation is attributed to the fact that aniline is formed as a result of the phenothiazine ring rupture which proceeds in such a way that sulfur-containing fragments continue to be retained in the solid phase and undergo polymerization with a release of hydrogen sulfide and formation of a nitrogen-containing polymer (analogous to polyaniline). With increasing temperature, one can observe destruction

of this polymer, which is corroborated by the fact that beginning from 290°C (aniline release maximum and sulfur release onset) there takes place a monotonous enhancement of the 14 $m/z$ signal intensity as well as considerable increase in the 27 $m/z$ signal intensity (Fig. 1). It is known that thermal decomposition of polymers based on aromatic amines leads to formation of hydrogen cyanide HCN (27 $m/z$). The thermolysis process can be represented by the following scheme 1:
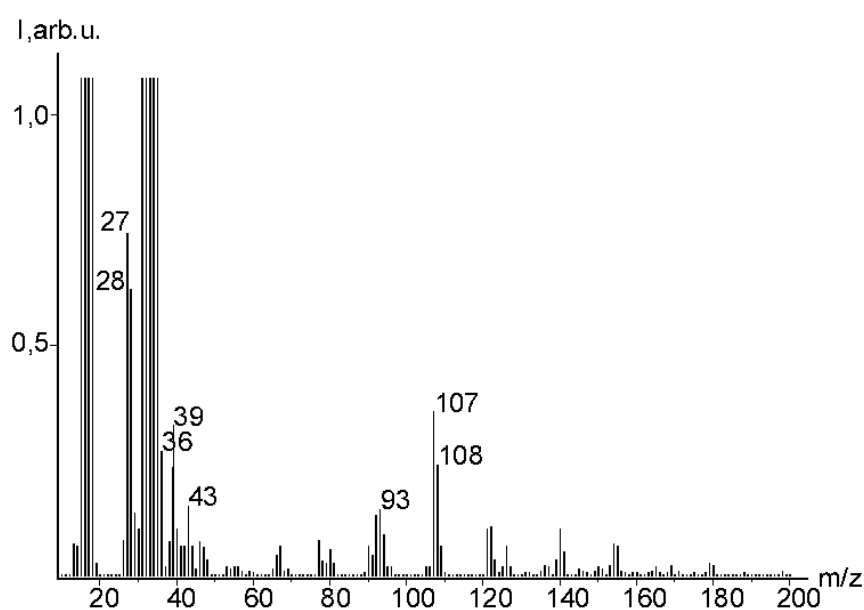


**Scheme 1**



**Fig. 3.** Electron impact mass-spectrum of thermolysis of condensed methylene blue at 444°C.
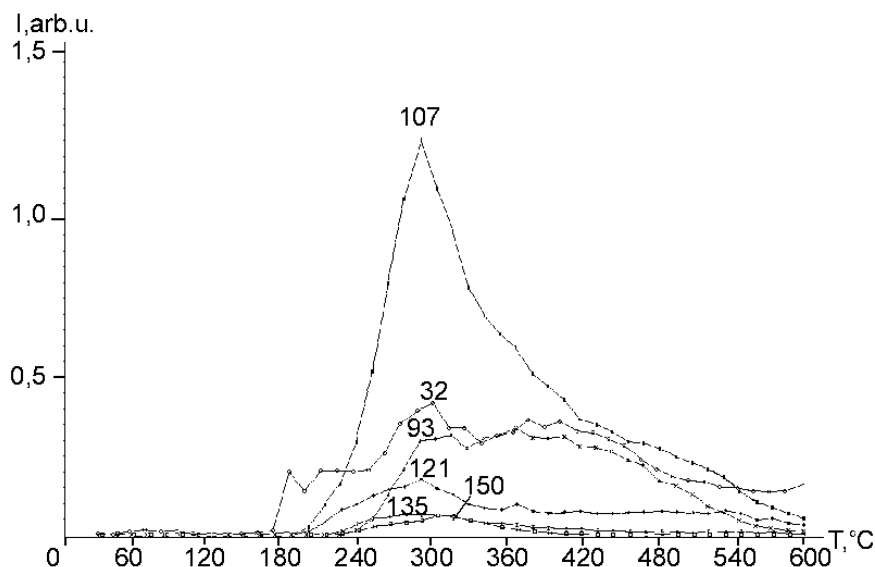
I,arb.u.



**Fig. 4.** Thermograms of fragments m/z: 150, 135, 121, 107, 93, 32; decomposition of toluidine dark blue in the condensed state.

Elucidation of mechanism of adsorption of antidotes on the HDS surface can furnish an explanation for adsorption mechanisms of bonding of antidote molecules with erythrocyte membranes. Therefore, a research has been made into adsorption of antidotes from aqueous solutions on the HDS surface. Adsorption methods and spectrophotometric technique of analysis allow us to determine physicochemical parameters of adsorption of molecules of these substances on the HDS surface and to obtain their adsorption isotherms (Fig. 5). These isotherms can be linearized on the Langmuir coordinates with high correlation coefficients. Thus, it becomes possible to determine such main physicochemical parameters of adsorption of dyes as limiting adsorption value, adsorption equilibrium constant, variation of the Gibbs free energy during adsorption, surface concentration of an adsorbate, surface area occupied by an adsorbate molecule, surface coverage. A study has also been made of the ability of these biomolecules to desorb from the HDS in a water solution. The physicochemical parameters attained are presented in Table. The limiting adsorption values are underestimated in view of the absence of saturation.

**Table 1.** Physicochemical parameters of adsorption of phenothiazine dyes on the surface of highly dispersed silica.

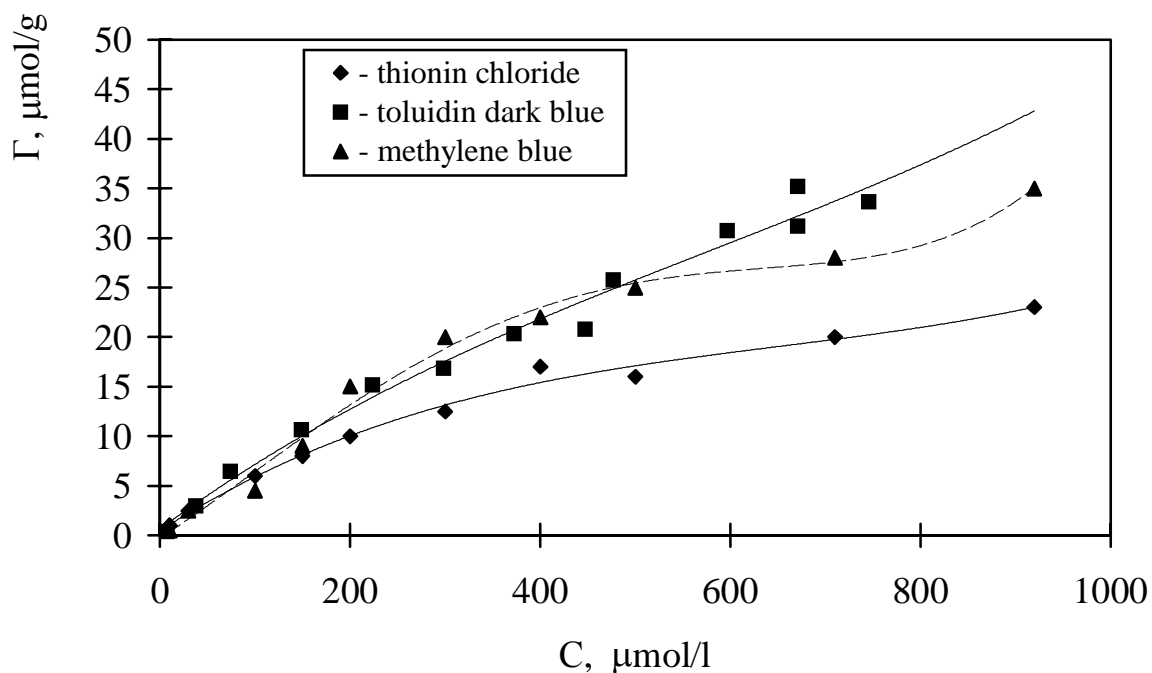| Antidotes | $\Gamma_\infty$ $\mu mol\,g^{-1}$ | $K \times 10^2$ $L\,mol^{-1}$ | $-\Delta G$ $kJ\,mol^{-1}$ | $\omega$ $nm^2$ | $\alpha$ $\mu mol\,m^{-2}$ | $\Theta$ % | $R^2$ | Desorption % |
|---|---|---|---|---|---|---|---|---|
| Thionine chloride | 29.7 | 25.41 | 19.44 | 16.81 | 0.099 | 3 | 0.979 | 18 |
| Toluidine dark blue | 112.4 | 5.34 | 15.58 | 4.43 | 0.375 | 11 | 0.997 | 3 |
| Methylene blue | 206.3 | 3.93 | 14.82 | 2.42 | 0.688 | 21 | 0.998 | 1 |

**Fig. 5.** Isotherms of thionine chloride, toluidine dark blue and methylene blue adsorption on fumed silica surface.

The phenothiazine dyes in the state set up in the course of their adsorption on the HDS surface have been studied by the TPD MS technique. The adsorbed samples are distinguished for the fact that during their thermolysis there is not a stage of release of hydrogen chloride in a high-temperature region (Figs. 6–8).
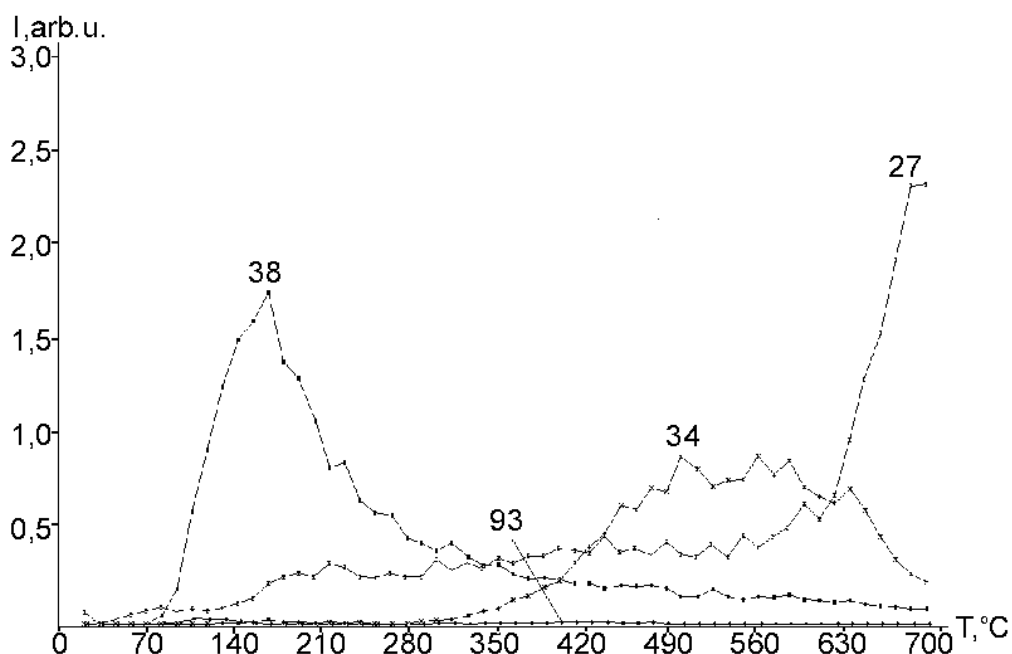


**Fig. 6.** Thermograms of fragments m/z: 93, 38, 34, 27; decomposition of toluidine dark blue in adsorbed on fumed silica surface.
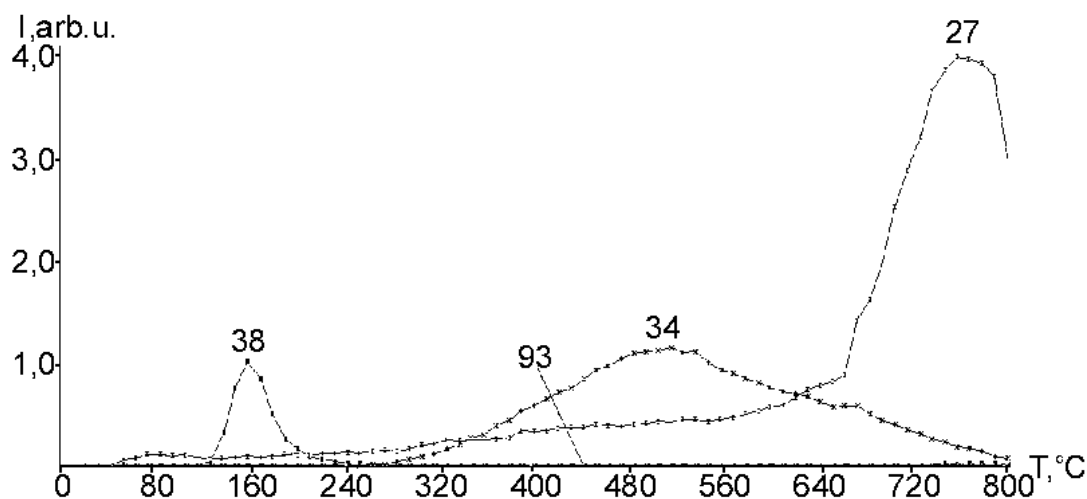
**Fig. 7.** Thermograms of fragments m/z: 93, 38, 34, 27; decomposition of methylene blue in adsorbed on fumed silica surface.
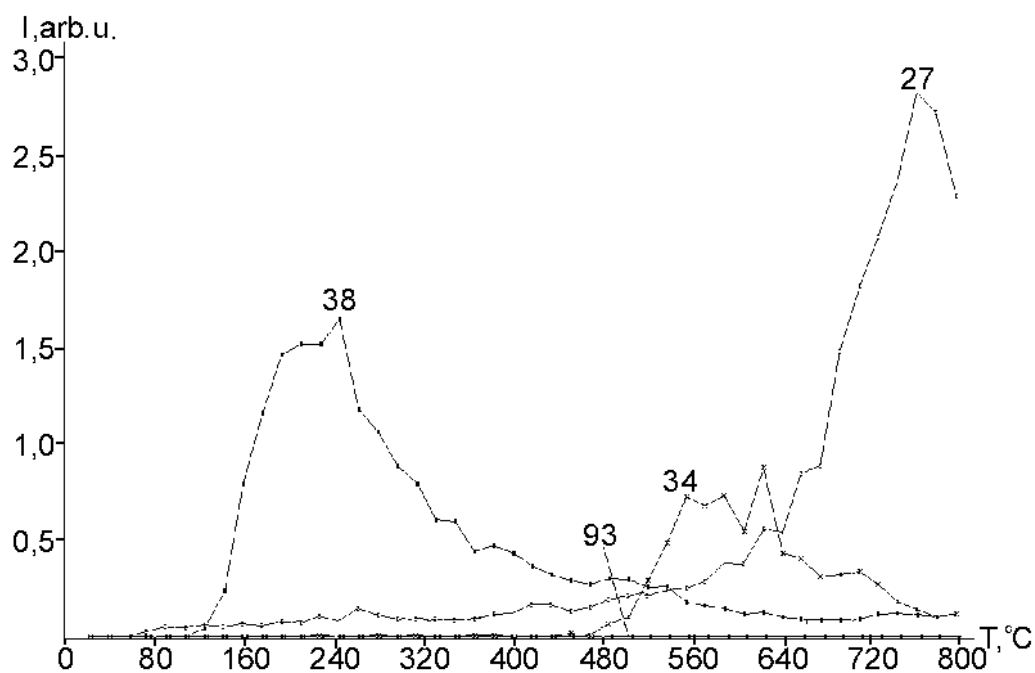


**Fig. 8.** Thermograms of fragments m/z: 93, 38, 34, 27; decomposition of thionine chloride in adsorbed on fumed silica surface.

In the case of adsorbed thionine there is not a stage of formation of aniline, what is also undoubtedly related to the dye adsorption on the surface (Fig. 9). It is possible that active sites of the silica surface stabilize phenothiazine rings, and their decomposition accompanied by the hydrogen sulfide release begins at a temperature above 500°C and leads to formation of a nitrogen-containing polymer which becomes chemically bonded with the silica surface so that its decomposition with the release of 27 *m/z* fragments (HCN) starts at a temperature over 560°C, i.e. its decomposition onset is shifted towards the high-temperature region by 100°C in comparison with the condensed state.
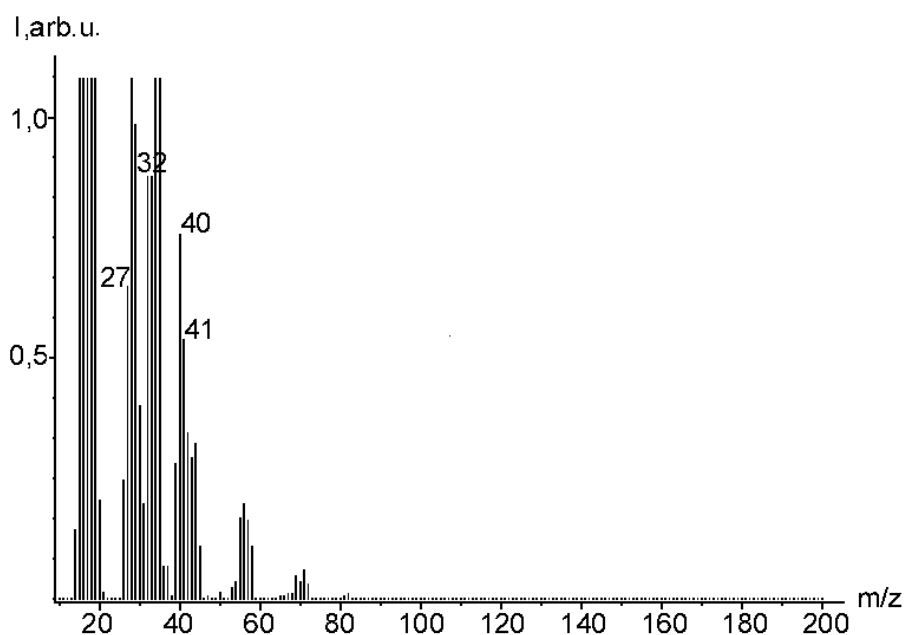
**Fig. 9.** Electron impact mass-spectrum of thermolysis of methylene blue adsorbed
on fumed silica surface at 473 °C.

Further, it should be mentioned that the thermal decomposition of methylene blue and toluidine dark blue in the adsorbed state is analogous with the decomposition of thionine in the adsorbed state (see Figs. 6–8). The both processes do not have a stage with the release of sulfur- and nitrogen-containing fragments, which is corroborated by the mass spectrum of volatile products of the adsorbed methylene blue thermolysis (Fig. 9). The difference in their chemical structure as compared to that of thionine (in particular, the presence of methyl groups in molecules of methylene blue and toluidine dark blue) exerts a weak effect on the mechanism of thermolysis of these dyes in the adsorbed state. There are differences only in the temperature intervals, positions of maxima on the temperature scale, and relative ionic current intensities while the main reaction products and sequence of stages do not vary. This fact indicates that the mechanism of adsorption for these three compounds is the same and, moreover, the bonding proceeds through species common for molecules of these compounds. The common and invariable species for all the three dyes under investigation is a thiazine fragment whereas their condensed benzene rings have different substituents (amine groups, dimethylamine groups, methyl groups) at different positions. Therefore, if the bonding to the silica surface had proceeded only through interactions with amine or dimethylamine groups, it would undoubtedly had manifested itself in the thermolysis mechanism and, as a result, in some differences between the thermolysis products for the three studied dyes.

From the data presented in Table it is evident that the difference between the chemical structures of the three compounds under study affects the nature of their adsorption from water solution on the HDS surface. In the investigated interval of concentrations the adsorption value increases with increasing number of methyl groups in molecules of the dyes, which seems to be related to an additional contribution of Van der Waals interactions to the bonding with the surface, as well as with increasing basicity of amine groups. At the same time, the fact that the largest change in the Gibbs free energy during adsorption is observed for thionine indicates that among the studied series of compounds it is thionine molecules that form the most strong bonds with the silica surface, which is corroborated by the most considerable increase (by 200°C in comparison with the condensed state) in the thermal stability of thiazine rings of thionine. In compliance with the Giles classification the adsorption isotherms of the dyes

under investigation are L-type isotherms (Fig. 5) and, consequently, during adsorption the principal plane of such a molecule should be in parallel to the silica surface. At this orientation of a molecule its bonding with the surface may involve both its amine groups and central thiazine ring. In the condensed state the positive charge of the corresponding cation will be delocalized on 16 atoms of the heteroaromatic system, with the largest part of the charge being delocalized on the sulfur atom of the thiazine ring (which is due to a larger atomic radius and lower electronegativity of sulfur in comparison with nitrogen). Besides, in the condensed state the delocalization of this charge may involve atoms and groups of atoms of the silica surface. It leads to stabilization of phenothiazine cations on the $SiO_2$ surface, and so the destruction of the phenothiazine heterosystem proceeds at substantially higher temperatures in comparison to the condensed state and begins with the release of hydrogen sulfide (i.e. of the fragment which makes the greatest contribution to the bonding with the surface), and it is this phenomenon that was more than once observed by us during thermolysis of a number of adsorbed biomolecules.

Bonding of a dye cation with the silica surface leads to a higher degree of delocalization of the charge and symmetry of its distribution so that phenothiazine system in the adsorbed state acquires a higher degree of symmetry in comparison with the condensed state. This manifests itself in the identical 'symmetric' mechanism of the thermal decomposition of the three dyes, which proceeds through rupture of chemical bonds symmetric about the center of a thiazine ring and involves abstraction of sulfur (Scheme 2). The compound formed as a result of this process undergoes polymerization at nitrogen atoms. The destruction of this polymer is evidenced for by the mass spectrum lines at 28, 27, 26 $m/z$ ($H_2CN^+$, $HCN^+$, $HC{\equiv}CH^+$). In this case the structural differences among these dyes are smoothed out. In the mass spectra of volatile products of thermolysis of adsorbed samples for the whole studied interval of temperatures (up to 800 °C) there are no lines which would correspond to nitrogen- and sulfur-containing fragments and which would be observed in the situation with an asymmetrical rupture of chemical bonds. It is this asymmetric mechanism that is characteristic of the condensed dye thermolysis (Fig. 8).
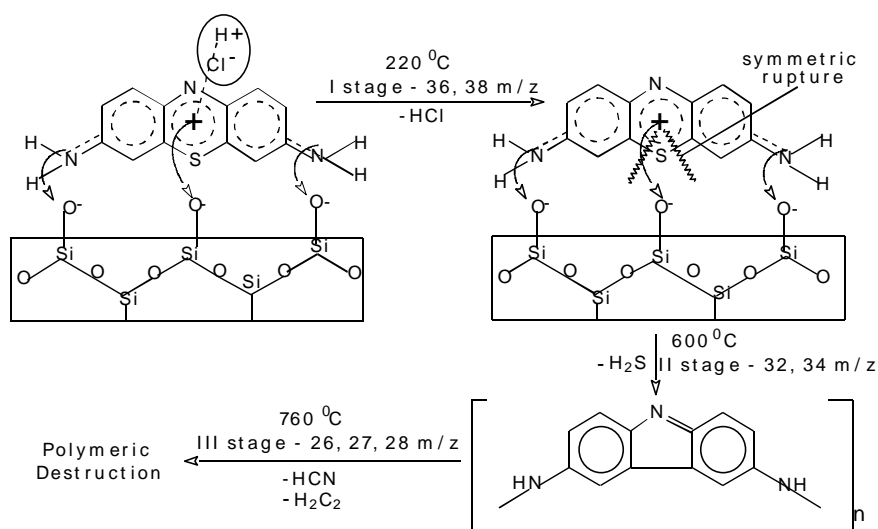


**Scheme 2**

The above-described observations are related to the fact that during the first stage of the thermolysis of the samples in the condensed state HCl is released as a result of abstraction of protons from dye molecules so that this process leads, in all probability, to formation of imine (stage I), i.e. uncharged asymmetric molecules. As a result, the subsequent thermolysis involves an asymmetric rupture of a phenothiazine molecule and release of sulfur- and nitrogen-containing fragments. In the situation with adsorbed forms of phenothiazines the release of HCl during stage I

proceeds at the expense of abstraction of protons of silanol groups on the silica surface. The phenothiazine cation remains unchanged and is stabilized due to a more symmetric delocalization of the charge. The methyl groups that are contained in molecules of methylene blue and toluidine dark blue create conformational hindrances to this stabilization, which seems to be related to the greater adsorption equilibrium constant and higher thermal stability of thiazine rings for thionine. That is, one can say that the difference in the chemical structure manifests itself in that the studied dyes in the condensed state require different mechanisms of their thermolysis while in the case of the adsorbed state the difference does not manifest itself. But this statement is inconsistent with a long-established fact that the difference in the structure does not affect the high antidotal action of these three compounds, i.e. does not affect their bonding with receptors *in vivo*. Thus, it is thiazine rings that perform a determining role in the process of bonding and detoxication. This inference is corroborated by the absence of any antidotal activity of compounds of the phenoxazine series (where sulfur atoms are replaced with oxygen atoms). Another support for the inference is that in the case of methemoglobinemia the function of antidotes is performed by such sulfur-containing compounds as aminosulfides (e.g. cystamine). Besides, it has been shown that methylene blue exerts a profound antidotal effect in the case of acute intoxications with hydrogen sulfide, which is likely to be related to its ability to block the toxic action through bonding with receptors of sulfhydryl groups that contain metal cations.

To sum up, it is possible to suggest the following mechanism of the biological effect. At the first stage on the erythrocyte membrane surface there proceeds bonding of a dye cations with receptors of sulfhydryl groups (these dyes are known to find use as metallochrome indicators for ions of such metals as Mg, Ca, Cd, Co(II), Ni, Zn (methylene blue) and Fe(II), Al, Pb, Hg(II) (thionine) [8]). At the second stage the metal that is present in a receptor reduces a dye cation to a leuco-derivative which is uncharged and, therefore, is capable of penetrating into an erythrocyte through its membrane. During the third stage the leuco-derivative reduces methemoglobin to hemoglobin.

## Conclusions

Because of the asymmetric charge distribution in a phenothiazine cation (the charge is predominantly localized on one of amine groups) it is possible to observe an asymmetric rupture of molecules with release of sulfur- and nitrogen-containing fragments in the case of condensed samples. In the case of adsorbed dyes due to the symmetric charge distribution among two amine groups and sulfur atom of a thiazine ring one can observe a symmetric rupture of a molecule, with the thiazine ring center being on the rupture plane. The cation adsorption observed takes place through bonding with deprotonated silanol groups of the silica surface so that bonding of an erythrocyte with the surface involves, in all probability, negatively charged oxygen and nitrogen atoms on the membrane surface. Adsorption of phenothiazine cations on the highly dispersed silica surface and, possibly, on the receptor surface favours stabilization of the cations owing to the charge delocalization, which facilitates the charge transfer and reduction of the cations to leuco-derivatives, with the reducing agent function being performed by a metal present in the receptor.

## References

1. Pronczuk de Jarbino J. Evaluation of antidotes: international activities // IPCS INTOX Workshop on Poisons Control in Eastern European Countries // Erfurt., 11-15 November, 1996. – Germany. - P.30-35.
2. Kouides P.A., Abboud C.N., and Fairbanks V.F. Flutamide-induced cyanosis refractory to methylene blue therapy // Br. J. Haematol. – 1996. – V.94 (1) – P.73-75.
3. Sadeghi-Hashjin G., Folkerts G., Henricks P.A.J., Van de Loo P.G.F., Dik I.E.M., and Nijkamp F.P. Relaxation of guinea-pig trachea by sodium nitroprusside: cyclic GMP and nitric oxide not involved // Br. J. Pharmacol. – 1996. – V.118 (3). – P.466-470.

4. Brown G., Frankl D., and Phang T. Continuous infusion of methylene blue for septic shock // Postgrad. Med. J. – 1996. – V.72 (852). – P.612-614.

5. Oksengendler G.I. Poisons and Counterpoisons // Nauka. – Leningrad. – 1982 – 192 p. (in Russian).

6. Khrapak V.V. and Kulik T.V. Toxic military substances as a cause of chemical catastrophes // Suchasni Problemy Toksikologii. - 2000. - V.2 - P. 3-19.

7. Kulik T.V., Galagan N.P., and Pokrovskiy V.A. TPD MS studies on adsorption immobilization of phenothiazine antidotes // 49th ASMS Conference on Mass Spectrometry and Allied Topics. - Chicago. - Illinois. - May 27-31. – 2001. - Book Abstr. (CD ROM).

8. Bishop E. (Ed.), Indicators, Pergamon Press, Oxford. New York. Toronto. Sydney. Braunschweig, 1972.