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To the application of the emission Mössbauer and positron annihilation spectroscopies for detection of carcinogens

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Abstract Being the main cause of cancer, almost all chemical carcinogens are strong electrophiles, that is, they have a high affinity for the electron. We have shown that positron annihilation lifetime spectroscopy (PALS) is able to detect chemical carcinogens by their inhibition of positronium (Ps) formation in liquid media. Electrophilic carcinogens intercept thermalized track electrons, which are precursors of Ps, and as a result, when they are present Ps atom does not practically form. Available biophysical data seemingly indicate that frozen solutions model better an intracellular medium than the liquid ones. So it is reasonable to use emission Mössbauer spectroscopy (EMS) to detect chemical carcinogens, measuring the yield of $^{57}Fe^{2+}$ ions formed in reactions of Auger electrons and other secondary electrons they produced with $^{57}Fe^{3+}$. These reactions are similar to the Ps formation process in the terminal part the positron track: $e^+ + e^- \Rightarrow$ Ps. So EMS and PALS are complementary methods for detection of carcinogenic compounds.

Keywords Positrons · Annihilation · Positronium · Screening of carcinogens · Emission Mössbauer spectroscopy · Track electrons

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1 Introduction

Chemical carcinogens are considered as a main cause of oncological diseases. They penetration into human body with food, water, cosmetics and even drugs ultimately leads to the appearance of malignant tumors. Chemical carcinogens are mostly appearing as by-products of new technologies. Their number increases each year by several thousands. In this situation, their systematic monitoring in products and wastes becomes very important. However, traditional biological methods of their analysis are expensive and long lasting. So rather fast physico-chemical methods may be useful in this case.

The idea to use the emission Mössbauer spectroscopy (EMS) for detection of carcinogenic-hazardous substances was born during our positron annihilation investigations. Both methods are based on the following two findings made by American researchers at the end of the last century.

The first observation was made by John and Elizabeth Miller, who found that molecules of almost all carcinogenic substances are strong electrophiles [1]. Getting inside cells, they are able to oxidize nucleic acids (take away their electrons), causing mutations. It is believed that mutation is the first stage of cancer development. Miller's discovery cardinally changed the situation with detection of carcinogens. Electrophilicity is a chemical property, so several chemists promptly became involved in study of this problem with a help of their own specific tools.

The second finding, important for nowadays discussion, was established by American radiation chemists John Bakale [2]. He decided that electrophilicity of molecules may be revealed as their ability, being dissolved in an inert solvent, more or less efficiently capture free excess electrons, which are created by ionizing radiation passing through the solvent. It turned out that a carcinogenic molecule captures a free electron immediately, just at a first encounter with it. This reaction proceeds without activation, i.e. it is controlled only by the diffusion of reagents. Values of its rate constant may serve as a criterion of the carcinogenicity of the testing substance. Based on this fact, Bakale suggested a method for determining the carcinogenicity of chemical compounds. Prediction ability of his method turned out to be good, about 80%. However, for its implementation, a cumbersome, complex and expensive installation (the pulse radiolysis spectrometer) based on an electron accelerator is required. Number of such installations in the world is not so large (several tens), so this method is not able to provide the required rate of analysis. Nowadays in Russia there are no such installations at all.

2 Applicability of the positron annihilation spectroscopy for detection of carcinogens

Along with two fundamental observations discussed above concerning properties of carcinogens, there is additional argument in favor of application of positron annihilation lifetime spectroscopy (PALS) and EMS to this problem. It is similarity of the early chemical reactions in tracks of fast electrons, positrons, muons and Auger electrons emitted by ^{56}Fe, the daughter product of radioactive nuclei ^{57}Co [3–6] (see Figs. 2 and 5). Their essence becomes clear, if we trace a fate of a fast positron, for example, in liquid water.

General scheme of PALS spectrometer is shown in Fig. 1. In PALS we measure lifetime of each positron implanted into studied sample (liquid), i.e. the time interval between the "starting" nuclear γ-quantum accompanying e+ birth in a $\beta+$ decay of ^{22}Na nucleus and one of the "stopping" (511 keV) annihilation γ- quantum, which produces in two-gamma annihilation process: $e^+ + e^- \rightarrow 2\gamma$.

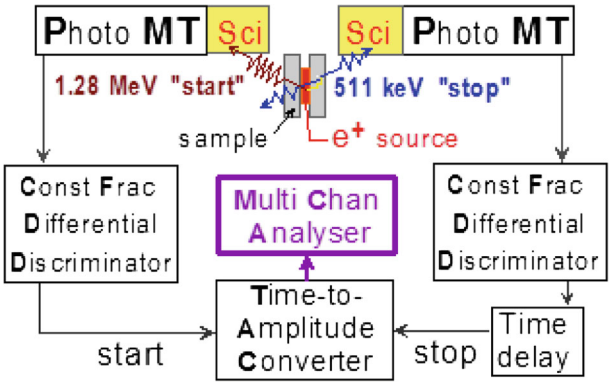

Fig. 1 Scheme of the positron annihilation lifetime spectroscopy (here "Sci" are BaF_2 scintillators, PMT stands for a photomultiplier tube)

Entering the liquid, a fast positron looses its energy in ionization collisions with molecules and at the end of its track (i.e. in its blob, which is a nanoscale spherical region of about 100 Å in diameter) it creates 30-40 closely separated ion-electron pairs (each of which consists of an electron and a radical-cation H_2O^+). According to the terminology adopted in radiation chemistry, this part of the positron track is called the positron blob.

Recombining with thermalized, but yet not solvated electrons, e^-, radical-cations regenerate water molecules (electronically excited) or form new radiolytic products. One of them is molecular hydrogen [3]:

$$\begin{aligned} &(H_2O)^+ + e^- => H_2O*, \\ &(H_2O)^+ + e^- + H_2O => H_2 + 2OH, \text{ (reaction rate constant is } k_{ei}) \end{aligned} \quad (1)$$

Obviously, these reactions take place not only at the end, but throughout the entire track of the fast positron. Same reactions occur in the track of any other ionizing particle, including Auger electrons emitted by ^{56}Fe, the daughter product of ^{56}Fe, the daughter.

In the terminal e^+ blob the positron competes with radical-cations H_2O^+ for the presolvated electrons. If it is succeeded to capture one of the track electrons, one of two types of positronium atoms, ortho-Ps or para-Ps, is formed, depending on the total spin of the Ps atom (spin=1 for ortho- and spin=0 for para-state). Formation probability of the long-lived ortho-positronium state is 3 times as large then that of the short-lived para-positronium [7]:

$$e^+ + e^- => \text{o-Ps or p-Ps, (reaction rate constant is} k_{ep}). \quad (2)$$

Experimentally Ps formation probability can be obtained by means of exponential deconvolution of raw PALS spectra with a help of the standard fitting programs [http://prac.us.edu.pl/~kansy/index.php?id=lt10].

Quite analogously, at the end of a muon track, as a result of combination of a thermalized μ^+ with one of thermalized electrons, the muonium atom is formed [9]:

$$\mu^+ + e^- => \text{Mu, (reaction rate constant is} k_{em}). \quad (3)$$

When scavenger S of presolvated (track) electrons is added into solution in concentration c_S, the reaction:

$$e^- + S => S^-, \text{ (reaction rate is} k_{eS}) \quad (4)$$

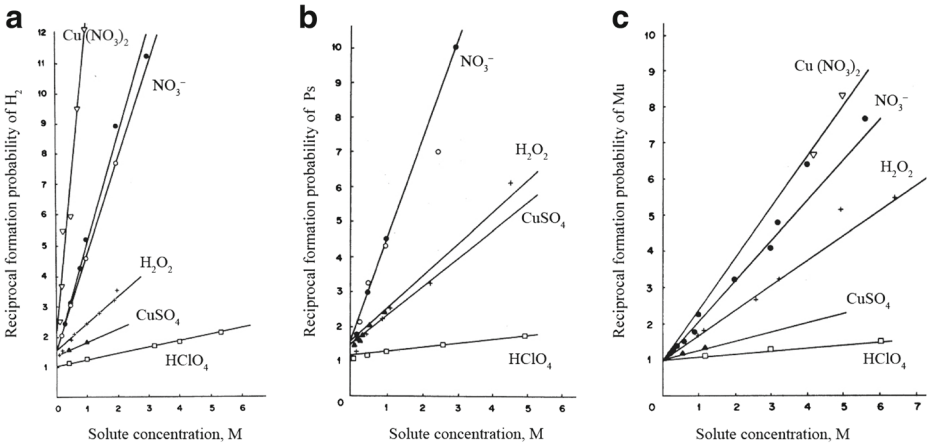

Fig. 2 Dependencies of reciprocal yields of H_2, Ps and Mu in aqueous solutions of different electron scavengers vs. their concentrations [4]

competes for the track electrons with the reactions (1)–(3) and must inhibit (suppress) formation probabilities (yields) of radiolytic hydrogen, Ps and Mu atoms. As a result of this competition, the reciprocal yields of these species should linearly increase with increasing c_S, in accordance with the following asymptotic equations [8]:

$$\frac{G^0_{\mathrm{H2}}}{G_{\mathrm{H2}}} = 1 + q^{\mathrm{H2}}_S c_S \quad , \quad q^{\mathrm{H2}}_S \approx \frac{k_{eS} V_0}{k_{ie} n_0}. \tag{5}$$

Here $G^0_{\mathrm{H2}} = G_{\mathrm{H2}}(c_S = 0)$is the hydrogen yield, extrapolated to zero concentration of electron acceptor and q^{H2}_S is the inhibition coefficient of H_2 formation by S, n_0/V_0 is the typical concentration of ion-electron pairs in a track of a high energy electron (in spurs). Same relationships are valid for Ps and Mu [4, 9]:

$$\frac{P^0_{\mathrm{Ps}}}{P_{\mathrm{Ps}}(c_S)} = 1 + q^{\mathrm{Ps}}_S c_S, \quad q^{\mathrm{Ps}}_S \approx \frac{k_{eS} V_0}{k_{ep} n_0} \quad , \tag{6}$$

$$\frac{P^0_{\mathrm{Mu}}}{P_{\mathrm{Mu}}(c_S)} = 1 + q^{\mathrm{Mu}}_S c_S, \quad q^{\mathrm{Mu}}_S \approx \frac{k_{eS} V_0}{k_{em} n_0} \quad . \tag{7}$$

Here P^0_{Ps} and $P_{\mathrm{Ps}}(c_S)$ are the yields of Ps in the pure solvent and when S is added, respectively; q^{Ps}_S is the inhibition coefficient of Ps formation. V_0 is the typical volume of the terminal e^+ blob and n_0 is the initial number of ion-electron pairs in it. Meaning of quantities entered equation (7) are similar.

Figure 2 illustrates how (1)–(3) work for different electron scavengers in aqueous solutions. It is seen that the inhibition effect of the yields of these species are almost the same (same q_S values).

It is clear from this approach that if a substance S is an efficient electron scavenger, it almost completely suppress formation of the Ps atom, if its concentration is enough large. But as we mentioned above, efficient electron scavengers are very probably carcinogens. Therefore, a negligible yield of Ps in a solution containing a compound S, indicate that this substance should be a carcinogen. This is the idea of the positron annihilation method of detecting potential carcinogens.

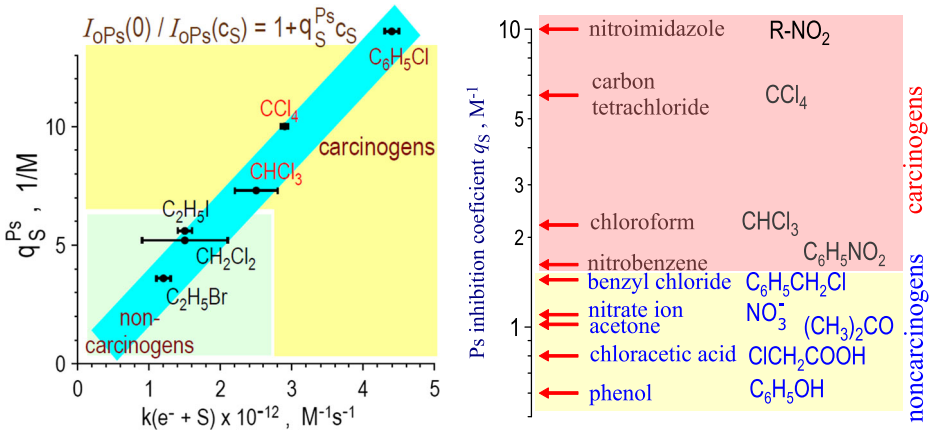

Fig. 3 Left: Inhibition coefficients of Ps formation in cyclohexane by different electron scavengers vs. the reaction rate constant k(S+e$^-$). Right: Inhibition coefficients of Ps formation by different track electron scavengers dissolved in ethanol

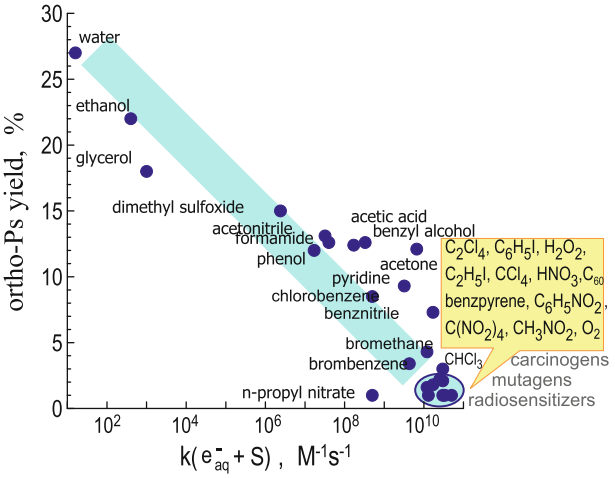

Fig. 4 Ortho-Ps yields (in %) in various chemical compounds vs. their reaction rate constants with hydrated electrons. The chemicals were not dissolved in a solvent, but were taken "as is" (as a monophase substance)

3 Experimental results

Tests of several dozens of well-known chemical compounds (carcinogens and non-carcinogens) confirmed the above statement and demonstrated efficiency of the positron method [10]. Figure 3 (Left) demonstrates a rather good correlation between inhibition coefficients q_S of Ps formation in cyclohexane by different scavengers S of quasifree track electrons against the reaction rate constants k(S+e$^-$). The strongest Ps inhibitors (q_S >6 M^{-1}), being the most effective electron scavengers, k_{eS} >3•10^{12} $M^{-1}s^{-1}$, are really carcinogens according to biological data. Since polar media could also be considered as appropriate models of intracellular milieu surrounding DNA molecules, measurements have

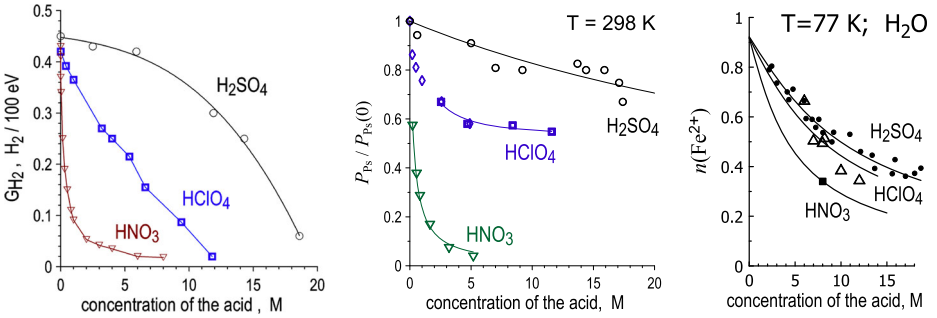

Fig. 5 Correlation between yields of radiolytic hydrogen, positronium and $^{57}Fe^{2+}$ ions in aqueous solutions of acids [5, 6]

been carried out also in ethanol solutions, Fig. 3 (Right). The electrophilic molecules with large Ps inhibition coefficients, $q_S > 2\ M^{-1}$, are carcinogens too.

Probably more visual results are obtained when we consider variation of the Ps yield in substances not dissolved in something, but simply taken in their pure form (Fig. 4). It is seen that Ps is not formed practically in most substances that are identified as carcinogens in animal tests. In these substances Ps formation does not exceed 2-3 %.

In connection with this, we note another such interesting result. It turns out that in the cancer-affected skin of mices [11], the yield of Ps is abnormally low. This suggests that in the cells of such tissues there is an accumulation of a significant concentration of carcinogens. Thus, the positron method not only facilitates, accelerates and reduces costs of identifying carcinogens, but also opens up new opportunities in studies of carcinogenic action.

Emission Mössbauer spectroscopy studies chemical transformations in the vicinity of radioactive nuclei ^{57}Co, which transform into ^{57}Fe as a result of e^--capture [12]. After that the excited daughter atom emits several Auger electrons with a total energy about 6 keV and transforms into a multicharged ion $^{57}Fe^{n+}$ [5, 6]. Around this ion (in 1 nm vicinity) one appears 200-300 ion-electron pairs (we call it as the Auger blob). Because of very high electron affinity of the multicharged iron cation, $^{57}Fe^{n+}$, during several femtoceconds it transforms into chemically stable state of Fe^{3+}, picking up electrons away from neighboring water molecules:

$$^{57}Fe^{n+} + H_2O \rightarrow {}^{57}Fe^{(n-1)+} + H_2O^+, \qquad {}^{57}Fe^{(n-1)+} + H_2O \rightarrow {}^{57}Fe^{(n-2)+} + H_2O^+.$$
$$^{57}Fe^{4+} + H_2O \rightarrow {}^{57}Fe^{3+} + H_2O^+, \qquad H_2O^+ + H_2O \rightarrow H_3O^+ + OH. \quad (8)$$

Further reduction of the Mössbauer ion $^{57}Fe^{3+}$ to $^{57}Fe^{2+}$ state

$$^{57}Fe^{3+} + e^- \rightarrow {}^{57}Fe^{2+} \quad (9)$$

is similar to the Ps, H_2 and Mu formation. Reaction (9) may be inhibited by the electron scavenging reaction (4). In concentrated solution of electron scavenger S the $^{57}Fe^{2+}$ yield tends to zero [5, 6].

Above reactions indicate that intratrack quasifree electrons are common precursors of H_2, Ps, Mu and $^{57}Fe^{2+}$. This becomes apparent in correlations of the yields of these species shown in Fig. 5.

Existence of such a correlation indicates that emission Mossbauer spectroscopy can be used for detection of chemical carcinogens.

It is also worth mentioning that recent biological data indicate that for simulation of the intracellular milieu might be better to use non liquid, but frozen glassy solutions. Their structure seems to be closer to the structure of intracellular water. If it is so, detection of carcinogenic properties of chemical compounds by means of the emission Mossbauer spectroscopy can be more adequate. Anyway combination of the positron annihilation and emission Mössbauer spectroscopies seems very promising for detection of carcinogens.

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