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RADIATION FRAGMENTATION AND BIOLOGICAL ACTIVITY OF TABLETED DRUG IRRADIATED ON THE M-30 MICROTRON

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The results of the study of fragmentation of tableted aspirin without and after irradiation of the fast electrons of 18 MeV on the M-30 microtron are presented. The peculiarities of tablet fragmentation were studied by mass spectrometry from the aqueous phase; the data obtained show a significant effect of radiation treatment of tablets on the structure of their mass spectra. It was found that radiation destruction of the material of the active ingredient of tablets and its accompanying components, filler, coating, etc., affects the drug's biological activity. Using four test microorganisms from different phylogenetic groups, the selectivity of the antibacterial or stimulant effect of the tablets immediately after irradiation and in the time of evolution was shown.

INTRODUCTION

Fragmentation of medicines derived from biologically active molecules is an essential stage of their use for restoring or regulating human body functions [1–2]. There are several ways to fragment biomolecules, such as breaking chemical bonds to form fragments of fission and rearrangement, when the breaks in chemical bonds are accompanied by a change in the molecule's structure or by interaction with atoms or molecules in the environment to form new chemical clusters. The nature of fragmentation depends on the method of administration of drugs: enteric and parenteral, each of which provides specific effects on the human body [3].

Medicinal tablets are a widely used form of medicinal products administered orally. More than 90% of medicinal products sold on the market are manufactured in the form of tablets, as they have a number of advantages over other dosage forms: non-traumatic administration, the ability to use them independently, etc. The peculiarities of their use include the presence of filler based on typical food components, a binding agent, and a shell made of a biocompatible substance [4]. Such a composition is focused on oral use and has been carefully tested, but the peculiarities of fragmentation of tableted drugs and their biological activity require a particular study.

Radiation is a powerful tool in destroying biological molecules, forming arrays of fission fragments of different grades depending on the energy and type of irradiating nuclear particles [5, 6]. It can simulate all possible channels of their fragmentation and relaxation mechanisms after the radiation stops. This paper uses the mass spectrometric method to present the results of a study of the fragmentation of tableted aspirin irradiated on the M-30 microtron (18 MeV). The choice of aspirin, formula $C_9H_8O_4$, as the object of study stems from its availability and widespread use. Aspirin is widely used to treat numerous conditions such as pain and aches [7], fever [8], rheumatic and inflammatory diseases, rheumatoid arthritis, and in the prevention of heart attacks. It also has an antiplatelet effect, inhibiting thromboxane formation, which binds platelet molecules to form a stain on damaged blood vessel walls.

The subject of this study was the mass spectrum of tablet fission fragments, including aspirin itself, filler, binder, and coating material, which are also subject to chemical fragmentation in the body. The studies were conducted on aspirin tablets before and after radiation exposure.

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RESEARCH OBJECT AND EXPERIMENTAL CONDITIONS

In this study, the drug "Acetylsalicylic acid-Darnitsa No. 10" of the standard assortment was investigated; the weight of the tablets was 600 mg, the composition contained 500 mg of acetylsalicylic acid (Fig. 1,a), and minor amounts of excipients such as citric acid and potato starch. Salicylic acid reacts with excess acetic anhydride to make aspirin. A small amount of the acid is used as a catalyst to speed up the reaction (see Fig. 1,b). After crystallization, the product appears as a solid mass, and the crystals are collected by vacuum filtration. The tablet's weight is 0.6 g, i.e., the active ingredient of the tablet occupies more than 83% of the tablet's weight. According to [9], the tablet contains binders that ensure the formation of tablets and granules with the required mechanical strength.

The samples were irradiated using a microtron M-30 [11] with an accelerated electron energy of 18 MeV at an accelerated electron current of 0.37 μA. When irradiated, the samples were placed 50 cm from the M-30 microtron and packed in sealed plastic packaging. The uniformity of the irradiation field with an area of 100 cm^2 was not worse than 5%, and the irradiation conditions did not lead to the heating of the samples.

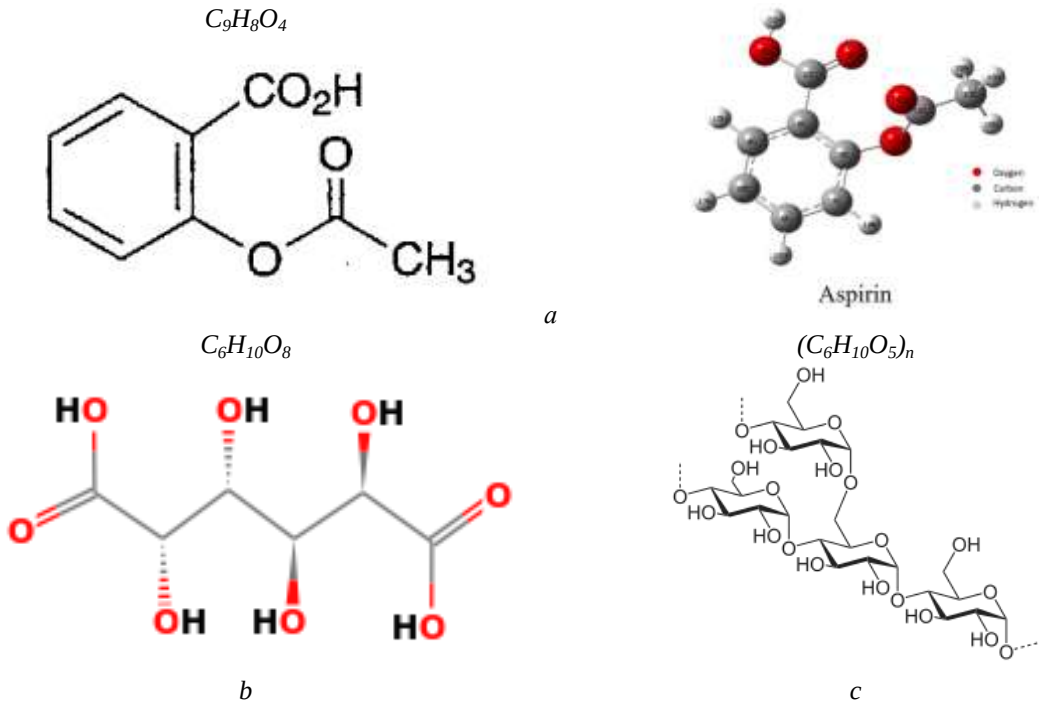

Fig. 1. Chemical-structural and molecular formula of aspirin (a) and its essential components in the tablet state: citric acid (b) and potato starch (c) [10]

The flux density over the irradiation area was $2.25 \cdot 10^{10}$ el.$\cdot cm^{-2} \cdot s^{-1}$ and was controlled by a Faraday cylinder. There are two series of irradiations of samples with fluences D1 – $4.04 \cdot 10^{13}$ eV/cm^2 and D2 – $2.02 \cdot 10^{12}$ and $4.04 \cdot 10^{13}$ eV/cm^2 were performed; the reference estimates of absorbed doses are 2.0 and 4.0 kGy.

The mass spectroscopic studies were carried out on an installation with a monopolar mass spectrometer with intersecting electron and molecular beams, see [5]. For this experiment, the molecular beams were formed from the aqueous phase of dissolved aspirin tablets by spraying into the active volume of the mass spectrometer. For the standard operating mode, the range of recorded masses of MX-7304A is 0…180 Da with a resolution of at least ΔM = 1 Da. The following modes of operation of the electron source are provided: measurement of spectral masses in the mode of fixed electron energy in the range of 10…70 eV; measurement of energy dependences of the yield of formed ion fragments with a smooth change in the energy of the scanning electron beam in the range of 5…70 eV. The ions formed due to the interaction of molecules with electrons were separated by mass and recorded by an automated system with digital indication of the mass number and intensity of particles.

Finally, the biological activity of all tested aspirin tablet solutions before and after irradiation was evaluated by the presence/absence of antibacterial or stimulating effects against selected test microorganisms. In total, we used four microorganisms in the experiment. In particular, the objects of the study were clinical isolates of opportunistic pathogens: gram-negative rod-shaped Escherichia coli (lac+), gram-positive cocci Staphylococcus aureus, gram-positive bacilli, spore probiotics Bacillus subtilis 090 and microscopic fungi Candida albicans.

OBTAINED RESULTS AND THEIR DISCUSSION

Figs. 2,a,b show the mass spectrum obtained from a solution of aspirin tablets in distilled water without and after irradiation. The main peaks of aspirin m/z=43, 63, 92, 120, 138, 180 are present here (see Fig. 2 c), but the intensity of the spectral lines differs significantly from the NIST data [12]. This may be because the mass spectrum of tableted rather than pure aspirin is measured, as well as the technology of measuring mass spectra from aqueous solution. The difference between the spectrum of Fig. 2,a from the reference aspirin (see Fig. 2,c) [12] can also be explained by the contribution of citric acid molecular ions (see Fig. 2,d) and potato starch, unfortunately, its mass spectrum is not available in NIST. Still, the complex structural formula allows for a possible influence on the mass spectrum of the tablet.

The general tendency of changes in the mass spectra of irradiated aspirin tablets from non-irradiated ones is the formation of intense mass spectral lines in the vicinity of m/z = 70…80, which, by the way, are present in the mass spectra of citric acid (see Fig. 2 d), as well as the preservation of all minor components of the spectrum in the range of m/z = 15…80.

The biological effects of the tested tablet samples were evaluated by the presence of antibacterial or stimulating effects against the test microorganisms selected by us.

Irradiated and non-irradiated aspirin tablets were dissolved in 20 ml of MRS broth. A bacterial suspension was prepared from the respective microorganisms' daily culture per the turbidity standard of 0.5 McFarland density units ($1.5 \cdot 10^8$ CFU/mL), which was set using a Den-1 densitometer. The purity of the culture, namely the morphological and tinctorial

properties of the test microorganisms, was determined by bacterioscopy (Gram's method) using immersion microscopy of a light microscope (Primo Star iLED, Carl Zeiss).

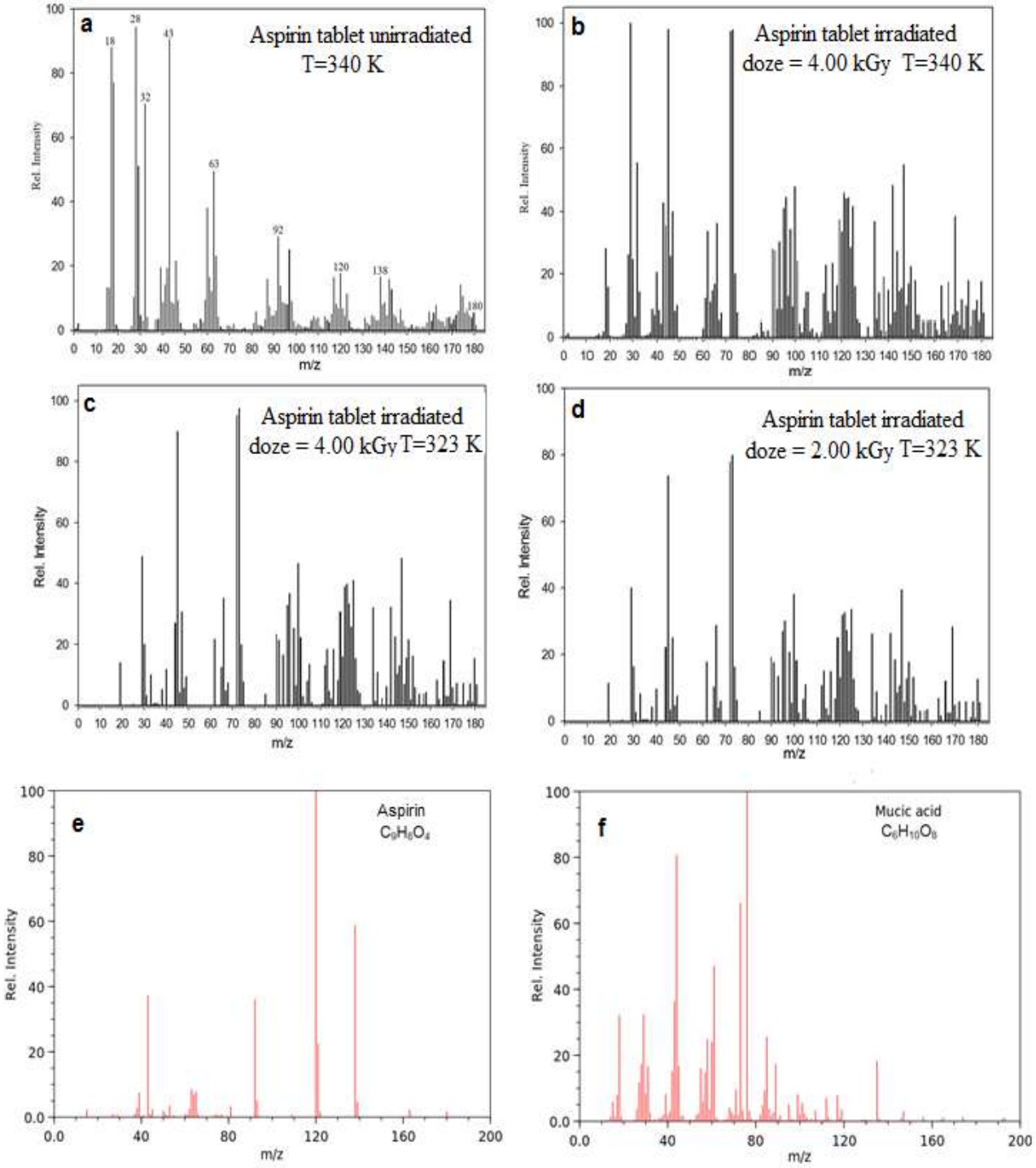

Fig. 2. Mass spectrum of tableted aspirin obtained from the aqueous phase: a – unirradiated, b and c irradiated with a dose of D1 at the different temperatures of heater, d – with dose D2 and e, f – literature data [12] on the mass spectrum of acetylsalicylic and citric acids, respectively

The number of microorganisms, determined by the indicator of colony-forming units in 1 ml (CFU/mL), was determined using the plate method for making tenfold serial dilutions [12]. The exposure time (co-cultivation) was 1 hour in a thermostat at 36.8…37 ºC. The suspensions were sown on appropriate nutrient media (Nutrien Agar, Mannitol Salt Agar, Sabouraud Dextrose Agar, Endo Agar, produced by Biolife, Italy). The studies were carried out in stage 1, immediately after irradiation, and in stage 2, 24 h later. The results of these studies are systematized in Table.

Characteristics of the biological effect of tableted aspirin without and with 18 MeV irradiation at M-30 on the population of selected bacteria, dose parameters D1 and D2 are given in the text

No.	The name of the solution	Initial concentration, CFU/mL	Concentration, CFU/mL			
			Bacillus subtilis 090		Escherichia coli (lac+)	
			(stage 1)	(stage 2)	(stage 1)	(stage 2)
1	Aspirin, control	$1.5 \cdot 10^8$	10^4	$>10^{10}$	10^4	$<10^4$
2	Aspirin, dose D1	$1.5 \cdot 10^8$	10^6	$>10^{10}$	$4 \cdot 10^6$	$<10^4$
3	Aspirin, dose D2	$1.5 \cdot 10^8$	10^4	$>10^{10}$	$<10^4$	$<10^4$
			Staphylococcus aureus		Candida albicans	
			(stage 1)	(stage 2)	(stage 1)	(stage 2)
1	Aspirin, control	$1.5 \cdot 10^8$	$<10^4$	$<10^4$	$<10^4$	$<10^4$
2	Aspirin, dose D1	$1.5 \cdot 10^8$	10^4	$<10^4$	$<10^4$	$<10^4$
3	Aspirin, dose D2	$1.5 \cdot 10^8$	$<10^4$	$<10^4$	$<10^4$	$<10^4$

It is advisable to analyze the results obtained separately for each type of microorganism, considering the peculiarities of their biological purpose. Thus, the effect of aqueous solutions of tableted aspirin on Staphylococcus aureus in irradiated and non-irradiated states has an antibacterial effect ($<10^4$ CFU/ml). The result of the spectrum of aspirin action on Candida albicans for irradiated and non-irradiated solution samples shows fungicidal properties, and this effect is maintained at both stages ($<10^4$ CFU/ml). The data obtained on the spectrum of aspirin action on Escherichia coli (lac+) showed that solution 2 inhibits bacterial growth at the 1st stage (from $1.5 \cdot 10^8$ to $4 \cdot 10^6$ CFU), the antibacterial effect persists and completely stops growth ($<10^4$ CFU/ml) at the second stage of observation.

Finally, the spectra of aspirin's effect on the probiotic Bacillus subtilis 090 showed that irradiated and non-irradiated solutions inhibit bacterial growth at the first stage of the study. However, in the second stage, the tested solutions, on the contrary, stimulate the growth of Bacillus subtilis 090. These conclusions require further in-depth research to clarify the interaction mechanisms and confirm the observed effects.

CONCLUSIONS

The results show that radiation modification of tableted pharmaceuticals can significantly affect the nature of their fragmentation and biological activity. The study of the nature of tablet fragmentation should consider the structuring of the active substance itself, in this case, aspirin, and related substances that play the role of fillers and protective coatings. The next stage of this task involves organizing the array of fragments of the tablet component separation and the possible formation of new chemical compounds with different chemical and therapeutic properties.

Additional study is needed to analyze aspirin's radiation fragmentation, separate its spectra from the overall picture of the tableted drug, and use the data obtained to explain new aspects of biological activity.

The established biological effects (see Table) indicate the presence of a selective effect of irradiated compounds on test microorganisms from different phylogenetic groups both immediately after radiation stimulation and in the time evolution. It is shown that the biological efficacy of irradiated aspirin tablets, whether antibacterial or stimulating, differs in direction and degree of manifestation for different types of test microorganisms, as well as in exposure time and method of solution preparation. The discovery of a dose dependence of the biological activity of certain microorganisms (Bacillus subtilis 090, Escherichia coli (lac+)) for the first time, while for others (Staphylococcus aureus, Candida albicans), such a dependence is absent, is a novel finding. A detailed understanding of the mechanisms of such effects is important for the practical application of the identified phenomena in medicine and pharmacology and requires additional research.

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РАДІАЦІЙНА ФРАГМЕНТАЦІЯ ТА БІОЛОГІЧНА АКТИВНІСТЬ ТАБЛЕТОВАНОГО ПРЕПАРАТУ, ОПРОМІНЕНОГО НА МІКРОТРОНІ М-30

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Представлено результати дослідження фрагментації таблетованого аспірину без та після опромінювання швидкими електронами 18 МеВ на мікротроні М-30. Особливості фрагментації таблеток досліджувалися мас-спектрометричним методом із водної фази; отримані дані засвідчують суттєвий вплив радіаційної обробки таблеток на структуру їх масових спектрів. Встановлено, що радіаційна деструкція матеріалу активної компоненти таблеток та її супутніх компонент: наповнювача, оболонки, інше, впливає на біологічну активність ліків. На прикладі чотирьох тест-мікроорганізмів із різних філогенетичних груп показана селективність антибактеріальної чи стимулюючої дії таблеток відразу після опромінення та у часовій еволюції.