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Modification of Optical Spectra and Cytostasis of Doxorubicin and Conium Solutions with High-Energy Electron Irradiation

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The characteristics of optical absorption and cytostatic activity under high-energy electron irradiation of a solution of doxorubicin (of anthracycline class) in sodium chloride are studied. The energy of irradiating electrons was of 1 MeV, and the absorbed dose was within 2–90 kGy. As proven, when sodium chloride is irradiated, the absorbed dose affects the optical absorption spectra of samples and leads to an increase in the cytostatic effect of the drug. As shown, the optical spectra and cytostatic activity change with time in a co-ordinated manner. The dissolution of conium (of alkaloid class) in irradiated sodium chloride causes shifts of the maxima of the infrared absorption spectra of conium. A possible reason for the changes in solution properties, when using pre-irradiated sodium chloride, is the conformational rearrangement of the antitumour drug molecules. This effect can be caused by the interaction with bubbstons and their clusters, which are formed under the impact of irradiation.

У роботі досліджено характеристики оптичного вбирання та цитостатичної активности під час опромінення високоенергетичними електронами розчину доксорубіцину (класу антрациклінів) у Натрію хлориді. Енергія електронів опромінення дорівнювала 1 МеВ, а увібрана доза знаходилася у межах 2–90 кГр. Доведено, що за опромінення Натрію хлориду величина увібраної дози впливає на спектри оптичного вбирання зразків і приводить до посилення цитостатичної дії препарату. Показано, що зміни оптичних спектрів і цитостатичної активности відбуваються узгоджено в часі. Розчинення коніуму (хемічного класу алкалоїдів) в опроміненому Натрію хлориді зумовлює зсуви максимумів ІЧ-спектрів увібрання коніуму. Можливою причиною зміни властивостей розчину за використання попередньо опроміненого Натрію хлориду є конформаційна перебудова молекул протипухлинного препарату. Такий ефект може зумовлюватися взаємодією з бабстонами та їхніми кластерами, що виникають під впливом радіяційного опромінення.

Key words: NaCl, doxorubicin, conium, bubbstons, optical absorption, high-energy electrons, radiation dose, cytotoxicity.

Ключові слова: NaCl, доксорубіцин, коніум, бабстони, оптичне вбирання, високоенергетичні електрони, доза опромінення, цитотоксичність.

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1. INTRODUCTION

The problem of antimicrobial resistance in 2020 was proclaimed by the World Health Organization as one of the ten global public health threats facing humankind.

It should be noted that the use of anticancer chemotherapy drugs is associated with a number of significant problems. These problems include nonspecific action and high toxicity of anticancer drugs to organs and tissues not affected by the tumour. A serious obstacle to the therapeutic effect of drug therapy is the resistance of malignant tumours to cytostatics [1, 2].

One of the most promising new approaches for modifying water-soluble antitumour drugs has been developed at the Taras Shevchenko National University of Kyiv [3, 4]. It is based on the use of high-energy (of 1 or 2 MeV) electron irradiation of sodium chloride for injections before dissolving antitumour drugs in it.

Studies [5, 6] have shown that the cytostatic activity of doxorubicin and conium increases. The magnitude of the activity increase depends on the antitumour drug concentration, the radiation dose I absorbed by sodium chloride, and the time interval between the irradiation and determination of cytostatic activity. It is important that the above-mentioned drugs are related to different chemical compounds: doxorubicin belongs to the anthracycline class, while conium is an alkaloid.

The aim of the study is to investigate the mechanisms of high-energy electron irradiation effect on the optical spectra and cytostatic activity of doxorubicin and conium solution in sodium chloride pre-irradiated with electrons, and to assess the possibility of modifying existing antitumour drugs.

2. MATERIALS AND EXPERIMENTAL PROCEDURE

The absorption spectra of doxorubicin (Pharmacia Italia SpA, Italy) and conium (*Conium maculatum*, Weleda, Germany) solutions in sodium chloride were studied. Conium contains the alkaloids coniine ($C_8H_{17}N$), N-methylconiine ($C_9H_{19}N$), γ-coniceine ($C_8H_{15}N$), conhydrine ($C_8H_{17}NO$), and pseudoconhydrine ($C_8H_{17}NO$)). Sodium chloride for injections (0.9% NaCl, YURiA-PHARM, Ukraine) was used as a solvent for antitumour drugs.

The solvent was irradiated with electrons with energy of 1 MeV using a resonant linear electron accelerator Argus (pulse duration of 3.3 μs, pulse frequency of 400 Hz). The average current density of the electron beam was of 0.1 μA/cm^2, which corresponded to a flow density of $6.25 \cdot 10^{11}$ cm$^{-2} \cdot$s^{-1}. The absorbed dose I was determined, considering that one gray (Gy) corresponds to a fluence of $4.5 \cdot 10^9$ cm^{-2}. The dose I absorbed by the solvent ranged from 2 to 90 kGy.

The IR absorption spectra were recorded with a Bruker IFS-66 spectrometer; the absorption spectra in the visible and ultraviolet (UV) ranges were recorded with a Shimadzu UV-260 spectrophotometer with a measuring range of 190–900 nm.

The luminescence was recorded with a CaryEclipse spectrofluorometer (Varian) using a 1×1 cm^2 quartz cell.

The cytostatic activity of doxorubicin was determined using Lewis lung carcinoma (LLC) cell line.

All samples for recording IR spectra were formed as KBr tablets. The IR spectra were recorded using a Bruker IFS 66 Fourier-transform IR spectrometer (Germany) in transmission mode. The accuracy of the wave-number determination was 0.2 cm^{-1}, the transmittance was determined with accuracy of 0.1%. The OPUS 5.5 software package was used for the spectra recording and processing.

3. RESULTS AND DISCUSSION

The effect of preliminary electron irradiation of sodium chloride for injections on the optical and cytostatic properties of doxorubicin and conium dissolved in it was studied. The Fourier-transform infrared (FTIR) absorption spectra $A(I, k)$ in the UV range for doxorubicin after its dissolution in sodium chloride for injections, which was pre-irradiated with high-energy electrons, are shown in Figs. 1, 2.

The data shown in Fig. 1 indicate significant changes in the spectra, which was caused by solvent irradiation. In the range of va-

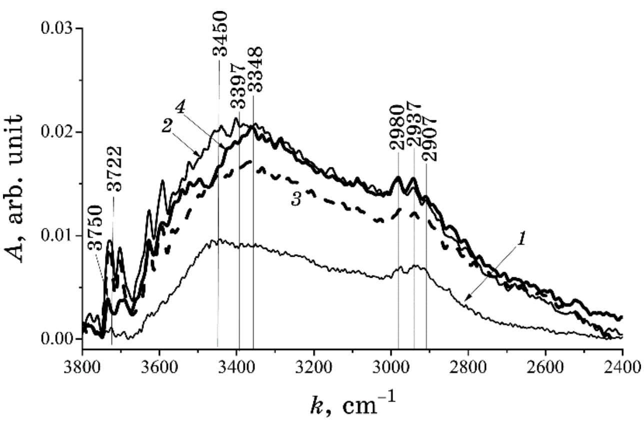

Fig. 1. Absorption spectra for doxorubicin in the range 3800–2400 cm^{-1}: curve *1* ($I = 0$ kGy), curve *2* ($I = 10$ kGy), curve *3* ($I = 40$ kGy), curve *4* ($I = 80$ kGy).

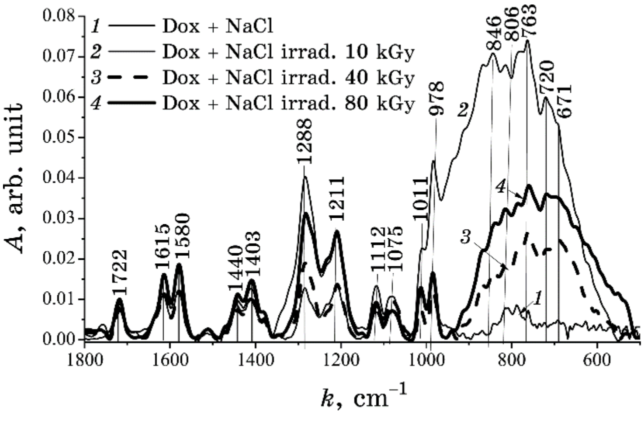

Fig. 2. Absorption spectra for doxorubicin in IR the range: curve *1* ($I = 0$ kGy), curve *2* ($I = 10$ kGy), curve *3* ($I = 40$ kGy), curve *4* ($I = 80$ kGy).

lence vibrations of OH–NH–CH molecular groups at 3800–2400 cm^{-1}, the intensity of this band increases for all samples with irradiated solvent without exception (curves *2–4*). Besides, a shift of the maximum of this band to higher frequencies is observed compared to the non-irradiated sample (*1*).

Analysis of the spectra in Figs. 1, 2 and the data listed in Table 1 indicates that the position of the absorption maxima corresponds to the values of the corresponding normal vibration of a certain molecular group. A comparison of the positions of the peaks, which correspond to vibrations of the same groups of atoms of unirradiated sodium chloride and solution samples irradiated with different doses, showed that some absorption maxima shifted, split and sometimes vanished. These effects can be associated with specific interactions between molecular groups, bubbstons and free radicals that appeared after irradiation.

The complete vanishing of a maxima compared to the control

TABLE 1. Maximums of IR absorption spectra of doxorubicin with saline irradiated by 1-MeV electrons with different doses.

Wave-number, cm^{-1}			
Dox + NaCl	Dox + NaCl (irrad. 10 kGy)	Dox + NaCl (irrad. 40 kGy)	Dox + NaCl (irrad. 80 kGy)
671.374	—	—	671.374
720.436	720.436	—	720.436
763.186	763.186	759.225	763.186
806.824	806.824	806.824	806.824
—	846.113	846.113	846.113
978.772	978.772	978.772	978.772
1011.502	1011.502	1011.502	1011.502
1075.962	1075.962	1075.962	1075.962
1288.453	—	—	1282.387
1403.152	1403.152	1403.152	1403.152
1572.995	1580.123	1575.697	1575.697
1615.075	1615.075	1615.075	1615.075
2925.531	2937.000	—	2925.531
—	2980.385	2980.385	2980.385
—	–	—	3348.112
—	3450.845	3450.845	3450.845
—	3600.645	—	3600.645
—	3625.685	3625.685	3625.685
3693.217	3700.760	3700.760	3700.760
3722.331	3722.331	3722.331	3722.331
—	3750.509	3750.509	3750.509

sample occurs due to changes in the conformational characteristics of molecules belonging to those molecular groups whose vibrations have corresponding frequencies (Table 1). Since vibrations are active in the IR spectrum only when they lead to a change in the dipole moment of a molecule, those molecular groups, whose maxima vanish after irradiation, do not affect the total dipole moment of the molecule because they oscillate near the centre of symmetry.

This conclusion correlates with the findings of the analysis of the spectra in the range 500–1000 cm^{-1}, in which the deformation vibrations of hydrogen bonds contribute. A significant increase in the absorption intensity is also observed, which is the largest in the spectrum *2*. At the same time, no significant shifts of bands are observed in the range corresponding to vibrations of C=O double bonds and deformation vibrations of CH.

The increase in the intensity of the bands along with high-frequency shifts can indicate an increase in the number of hydrogen bond groups in the case of valence vibrations. The distortion of the planar shape of the doxorubicin molecule due to the free rotation of its parts around single bonds can lead to a change in the positions of the maximums of the absorption bands with a frequency shift to the low-frequency range of the spectrum. These effects can be caused by nonspecific interactions between the molecular groups of doxorubicin and the radiolysis products of the solvent water. This conclusion is confirmed by the data presented in Fig. 3, which shows the effect of irradiation by value $\Delta A(I,k)/A(0,k)$, where $\Delta A(I, k) = A(I, k) - A(0, k)$ ($A(0, k) = A(I = 0$ kGy)) in the FTIR spectra of doxorubicin solution.

The curves *1*, *2*, and *3* in Fig. 3 are given by functions

$$\frac{\Delta A(I = 10, \mathrm{kGy})}{A(0,k)}, \ \frac{\Delta A(I = 40, \mathrm{kGy})}{A(0,k)}, \text{ and } \frac{\Delta A(I = 80, \mathrm{kGy})}{A(0,k)},$$

respectively. According to the spectra in Fig. 3, $A(I, k_{1,2})/A(0, k_{1,2}) > 7$ for all used values of I and wave-numbers $k_1 = 668.94$ cm^{-1} and $k_2 = 900.60$ cm^{-1}. This behaviour of $A(I, k)$ may indicate the invariance of the power centres responsible for the absorption of waves with wave-numbers k_1, k_2, and their coagulation under the influence of irradiation with high-energy electrons.

The dependence of the optical absorption spectra of doxorubicin solution in the UV and visible range on the absorbed dose was investigated (see Fig. 4).

The time interval between the irradiation of the samples and the measurements shown in Fig. 4 did not exceed ten days. During this time, no changes in each of the spectra were observed for all I under study. The absorption value of doxorubicin solution in the visible range (Fig. 4) does not monotonically depend on I that is consis-

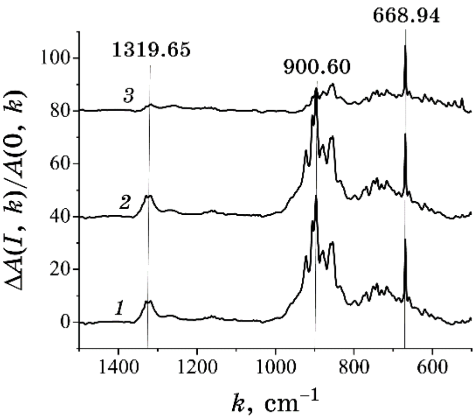

Fig. 3. Ratio of difference in absorbances of irradiated and non-irradiated doxorubicin solution to absorbance of non-irradiated doxorubicin.

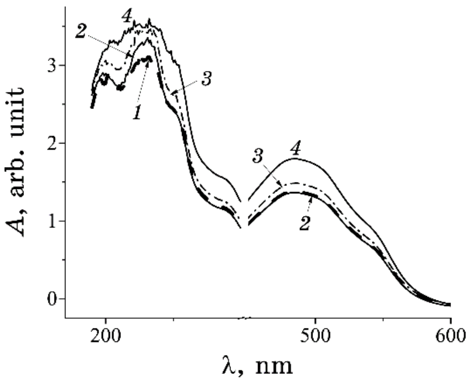

Fig. 4. Optical absorption spectra of doxorubicin solution in the UV and visible range: curve *1*—$I = 0$ kGy, curve *2*—$I = 10$ kGy, curve *3*—$I = 40$ kGy, curve *4*—$I = 80$ kGy.

tent with the data obtained for the infrared range (Figs. 1, 2). The optical spectra recorded within ten days after solvent irradiation were analyzed using the OriginLab software package. The experimental spectra were approximated by linear combinations of Lorentzian functions (Fig. 5).

The dose dependence of the position of the maxima of the absorption spectra in the UV and visible range, recorded immediately after solvent irradiation and preparation of doxorubicin solutions, is presented in Table 2.

The results of the analysis of the obtained spectra indicate shifting of the maxima (Table 1) and dramatic changes in their intensities, depending on the solvent irradiation dose.

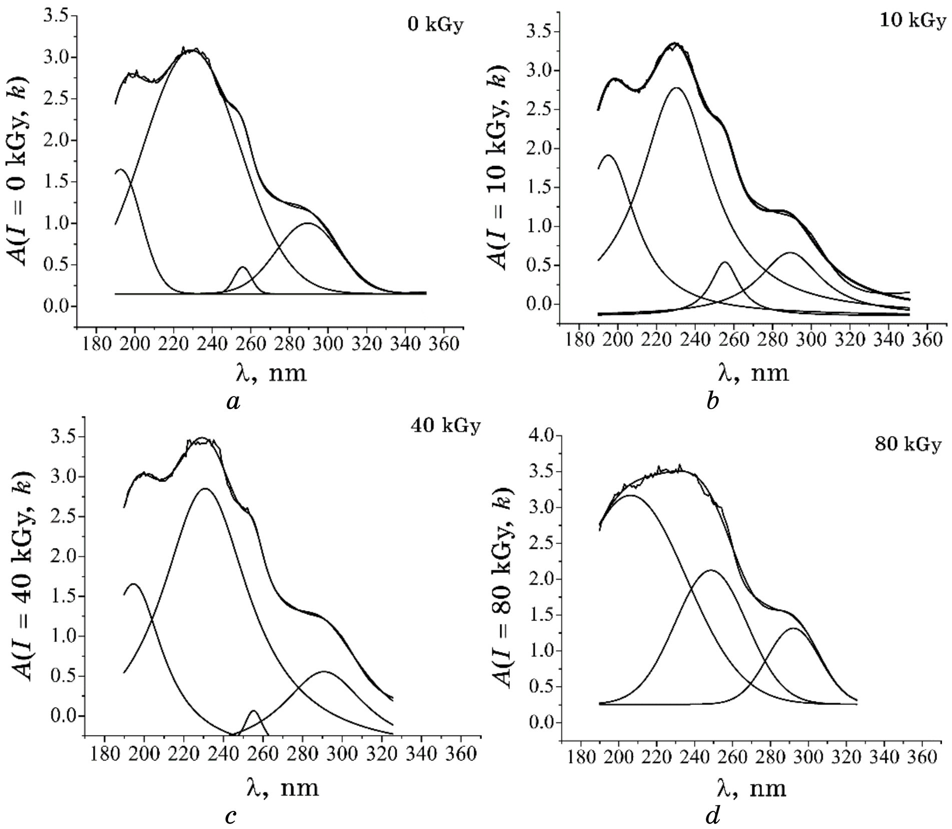

Fig. 5. Approximation of absorption spectra of doxorubicin solution in sodium chloride in the UV range by linear combinations of Lorentzian functions: (*a*) $I = 0$ kGy, (*b*) $I = 10$ kGy, (*c*) $I = 40$ kGy, (*d*) $I = 80$ kGy.

TABLE 2. Position of maxima of absorption spectra in the UV and visible range as a function of dose in optical absorption spectra.

λ/*I*, kGy	irrad. 0 kGy	irrad. 10 kGy	irrad. 40 kGy	irrad. 80 kGy
λ, nm	192.57	195.10	194.59	206.20
λ, nm	229.32	230.31	230.83	248.51
λ, nm	255.81	255.34	255.27	—
λ, nm	289.45	290.06	290.79	292,04
λ, nm	471.44	474.53	474.71	474.34
λ, nm	509.07	510.72	510.81	510.13

The effect of irradiating solvent on an alkaloid drug (conium) was also studied in order to compare it with an anthracycline drug

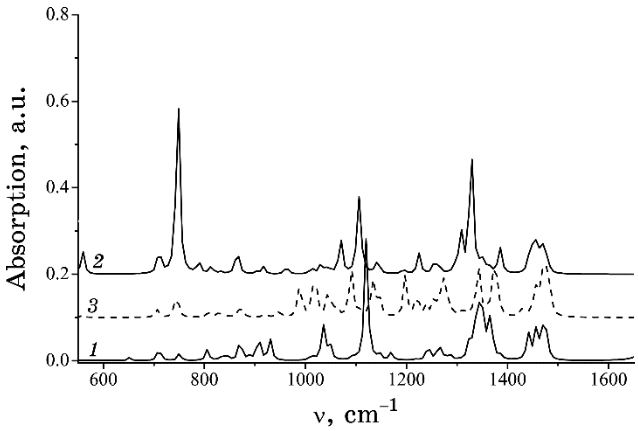

Fig. 6. Dependence of normalized absorption of conium on wave number for 0.9% NaCl solution non-irradiated (curve *1*) and irradiated with different doses of absorbed electron radiation: *2*—5 kGy, *3*—10 kGy.

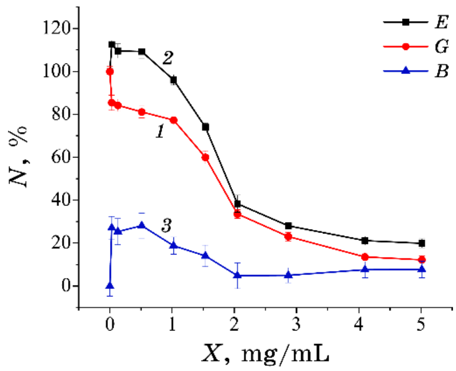

Fig. 7. Cytostatic effect of doxorubicin dissolved in irradiated and non-irradiated sodium chloride solvent.

(doxorubicin).

The addition of solvent (Fig. 6) irradiated with different doses of electron radiation affected the position of the maxima of the conium absorption bands: 1120 cm^{-1} line (curve *1*) shifted to 1106 cm^{-1} (curve *2*), then, to 1089 cm^{-1} (curve *3*). This shift of the absorption lines of conium in the vibrational range of the IR spectrum indicates a change of conformational state caused by the interaction with bubbstons (their clusters) present in the irradiated solvent.

The cytostasis of doxorubicin dissolved in sodium chloride irradiated with high-energy electrons in comparison to a non-irradiated solvent was studied. Figure 7 shows the numbers of live LLC cells in percentage (N) after 24-h incubation of doxorubicin solution for different concentrations (X).

In Figure 7, curve *1* shows the percentage of live LLC cells after incubation of doxorubicin dissolved in irradiated ($I = 40$ kGy) sodium chloride for different concentrations of doxorubicin; curve *2* shows the percentage of live cells for the non-irradiated solvent; curve *3* shows difference of curves *2* and *1*. The dependences in Fig. 7 show the effect of the irradiated solvent depending on the doxorubicin concentration.

A possible reason for the changes in the spectra of doxorubicin solution, when using a pre-irradiated solvent, is the conformational rearrangements in the antitumour drug molecules caused by the interaction with bubbstons and their clusters under the radiation exposure. The space distribution of the electric field of single bubbstons was studied in Refs. [6–8], but the formation of bubbston clusters has not been fully investigated. On the one hand, solving this problem is complicated due to the nonlinear nature of the equations describing the electric field of the double electric layer of a bubbston; on the other hand, it is due to the lack of information about the polarization, deformation, and forces of individual bubbstons and their clusters. When bubbstons coagulate, their spherical symmetry is broken; the fractal dimension of coagulants depends on the mechanisms of cluster growth [7].

Usually, the electric potential distribution $\varphi(k)$ in the vicinity of a single spherically symmetric bubbston is determined using the Poisson–Boltzmann equation [9] and information on the distribution of counter-ions (n_0) and density $\rho(r)$ around the inner positively charged surface of the air–liquid interface [10].

In the case of spherical symmetry of the nanoparticle and zero total charge of the nanoparticle and the adjacent layer of singly-charge counter-ions with a concentration of n_0, the corresponding equations are [10, 11] as follow:

$$Q_0 + 4\pi\int_{R_0}^{\infty} \rho(r) r^2 dr = 0\,, \quad \frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2 \frac{\partial\phi(r)}{\partial r}\right) = -\frac{\rho(r)}{\varepsilon\varepsilon_0} = \frac{2en_0}{\varepsilon\varepsilon_0}\sinh\left(\gamma\phi(r)\right), \quad (1)$$

where R_0 is the radius of the bubbston air bubble, ε_0 is the vacuum permittivity, ε is the dielectric constant, T is the ambient temperature, k_B is the Boltzmann constant, and $\gamma = e/(k_B T)$.

At low values of electrical energy,

$$\frac{e\phi(r)}{k_B T} << 1\,, \quad (2)$$

the Debye radius (R_D) is supposed to be $R_D = \sqrt{\dfrac{\varepsilon\varepsilon_0 k_B T}{2e^2 n_0}}$. Therefore, the R_D decreases with increasing n_0 caused by irradiation.

Using the Maple 2019 software, Eqs. (1) were solved numerically for $n_0 = 10^{21}$, $3{\cdot}10^{21}$, 10^{22} m^{-3}. The parameter R_D was estimated by the co-ordinate of the point r_0, at which the derivative $\phi(r)/r = r_0$ differs from the tangent of the angle at the point R_0. This analysis showed that the space dimensions of bubbstons decrease, when sodium chloride is irradiated with electrons, causing changes in the distribution of ponderomotive forces near a bubbston.

4. SUMMARY

The mechanisms of antitumour drug modification of doxorubicin and conium solutions in sodium chloride for injections, irradiated with high-energy electrons, are investigated. Modifications of existing antitumour drugs without adding foreign nanoparticles are developed. It should be noted that doxorubicin belongs to the chemical class of anthracyclines, and conium belongs to the class of alkaloids.

It is shown that the absorption spectra of doxorubicin solution in the infrared range are characterized by a non-monotonic dependence of the ratio of intensities of high- and low-frequency maxima on the absorbed dose. This behaviour is caused by the coagulation (clustering) of bubbstons, which can occur at high bubbston concentrations (at high doses of absorbed radiation).

The radiation dose absorbed by sodium chloride affects the spectral dependence of the absorption of doxorubicin dissolved in it (in the visible range). In the range 175–250 nm, the absorbance decreases, however, in the range 250–600 nm, it increases as compared to the absorbance of doxorubicin solution prepared with non-irradiated solvent. The value of the absorption difference modulus in the visible range of the spectrum increases monotonically with increasing absorbed radiation dose, which does not coincide with the behaviour in the infrared range. This is associated with the dependence of molecular absorption processes on the frequency of electromagnetic radiation.

It is shown that the addition of an irradiated solvent affects the positions of the maximums of the conium absorption bands. The absorption line at 1120 cm^{-1} shifts to 1106 cm^{-1}, and then, to 1089 cm^{-1}. This shift of conium absorption lines in the vibrational range of the IR spectrum can indicate changes in its conformational state caused by interaction with bubbstons (or their clusters) present in the irradiated solvent. The effect of irradiation ($I = 2$ kGy, 5 kGy) on the IR spectra of the conium solution was observed over the course of two weeks.

The study of the doxorubicin pharmacological activity performed using the Lewis lung carcinoma cell line proved that pre-irradiation of sodium chloride with high-energy electrons before dissolving doxorubicin increased the cytotoxic/cytostatic effect of the drug. This effect was most pronounced at relatively low concentrations.

The optical effects of doxorubicin dissolved in pre-irradiated sodium chloride decreases significantly after two–four months; the higher the radiation dose, the slower the relaxation processes proceed. The forces of interaction of bubbstons during their clustering depend on their mutual orientation and are non-central.

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