УДК 543.429.23: 615.038

Yu. G. Chernetskaya, head of laboratory (RUE "Belmedpreparaty");
S. A. Lamotkin, PhD (Chemistry), assistant professor (BSTU);
T. V.Trukhachyova, deputy director general (RUE "Belmedpreparaty")

APPLICATION OF NMR SPECTROSCOPY IN PREDICTING HYDROGEL MATRIX STABILITY BY "STRESS" TESTING METHOD

The results of the study of hydrogel matrix stability by means of nuclear magnetic resonance spectroscopy are described in the article. Ionizing radiation effect on auxiliary substances and active ingredients of hydrogel polymeric matrixes have been studied. It has been proved that under the influence of high-velocity electrons at absorbed doses 25–35 kGy the formation of a three-dimensional polymeric structure of the matrix occurs. It has been ascertained that active ingredients of hydrogels miramistin and gentamycin are stable under absorbed radiation doses in the range 5–35 kGy.

Introduction. Hydrogel matrixes are radiation crosslinked biologically compatible polymers of medical purpose (polyvinylpyrrolin (PVP), polyethelene oxide (PEO) and agar) which are used for local wound therapy and burns. Radiation treatment provides the formation of a three-dimensional polymeric structure and sterility of drug products. To impart antimicrobial properties, the composition of wound-healing drugs (in the form of hydrogel plates) is added with antibiotic gentamycin and antiseptic miramistin [1, 2]. Hydrogel matrixes are characterized by elasticity, mechanical strength, air impermeability and sorption properties with regard to the wound effluent.They prevent reinfection, normalize metabolic and stimulate reparative processes in the wound. Hydrogel plates are used to treat green traumatic, granulating (infected and noninfected) wounds, bed sores, trophic atonic granulating ulcers, sacred fire, thermal 2–3a and 3b–4 degree burns in regeneration phase [3, 4].

The aim of the research is to investigate hydrogel matrix stability and its drug components under the action of ionizing radiation.

Main part. The structure of the intact compounds in the composition of hydrogel polymeric matrixes and their decomposition products have been analyzed using NMR spectroscopy.

NMR spectra were recorded by an AVANCE-500 spectrometer with the operational frequency 500 MHz for the nuclei ^{1}H and 125 MHz for the nuclei ^{13}C. Solutions of the compounds in D_2O added with CD_3COCD_3 as an internal standard were analyzed. Chemical signal shifts of the compound protons were determined by acetone admixture signal (CHD2COCD3, $\delta = 2.05$ ppm in the spectrum ^{1}H) and by methyl group CD_3 of the standard $\delta = 30.2$ ppm. The spectra records were done with regard to the proton relaxation of all the compounds. ^{1}H and ^{13}C NMR spectra of polyvinylpyrrolidon, polyethelene oxide, agar, miramistin and gentamycin as well as those of miramistin and gentamycin hydrogel matrixes have been recorded and analyzed. Active ingredients and polymer mixtures were exposed to ionizing radiation at the electron acceletator -10-10 of the SSI "JIOandNR – Sosny" of the National Academy of Sciences of the Republic of Belarus, the absorbed dose range being 5–35 kGy.

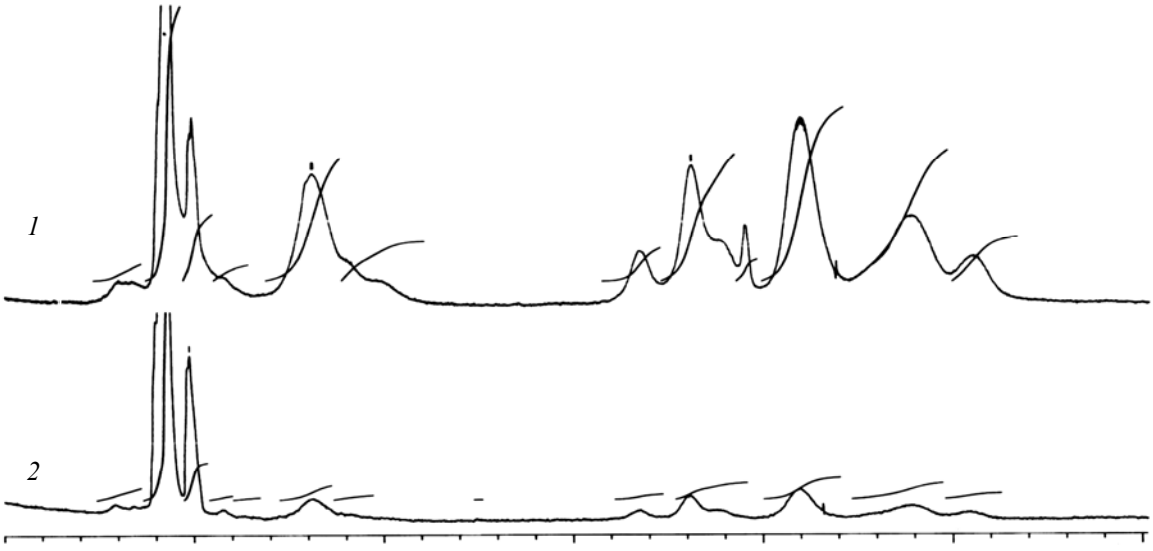

Fig. 1. H NMR hydrogel spectra:
1 – before radiation; *2* – after radiation by 25 kGy dose

The action of the ionizing radiation on polymers occurs indirectly through water radiolysis products ($H_2O \rightarrow e^-_{aq}$, H·, OH, H_2, O, H_2O_2, H^+_{aq}, OH^-_{aq}), i. e. radiolytic transformations of dissolved substances (ingredients) in water solutions are accomplished due to the indirect effect mechanism of ionizing radiation [4]. Hydroxyl radicals (OH) eliminate hydrogen atoms from the structural units of PVP and PEO with the formation of macroradicals. Macroradicals which undergo recombination reactions, form new intromolecular and intermolecular covalent bonds (crosslinking). This results in the formation of hydrogel matrixes which are actually a three-dimensional polymeric network. Agar doesn't crosslink with PVP and PEO, but acts as a filler. The obtained hydrogels don't dissolve in water, don't melt when heated, they become elastic and mechanically strong. It is possible to identify PEO signals in the region 3.56–3.58 ppm of the ^{1}H NMR spectrum. The main component – PVP – has the largest number of signals in the spectrum. It is proved by comparing the initial PVP spectrum with its calculation model and with the hydrogel matrix spectrum. The most characteristic signals for the PVP hydrogel spectrum are signals in the region 1.45–2.32 and 3.18–3.67 ppm.

While examining ^{1}H NMR hydrogel spectra radiated with doses 25 and 35 kGy (Fig. 1), it is obvious that PVP signals disappear almost completely (the residual amount being 3 and 1.5% respectively). This is due to the formation of the three-dimensional polymeric structure of the hydrogel matrix. To identify the structure and to study stability, drug spectra were recorded.

Antiseptic miramistin is a quaternary ammonium compound salt – miristamydopropyldimethylbenzylammonium chloride monohydrate.

The obtained data of the chemical shifts correspond to the given formula (Fig. 2) ^{1}H δ, ppm: 1–2.9; 2–1.95; 3–3.11; 2`–1.95; 3`–1.29; 4`÷13`–1.05÷1.25; 14`–0.82; 2``, 6``–7.38; 3``, 5``–7.27; 4``–7.22; 2``–4.35 and 8``, 9``–2.89.

The spectra obtained by the experiment correspond to the calculated ones.

After radiation treatment the analysis of miramistin showed that in the ^{1}H NMR spectrum of its aqueous solution there appeared additional low intensive signals with different multiplicity

These signals belong to the decomposition products formed under radiation. Comparing the integral intensity of the initial compound and the resultant products it can be stated that the total destruction level is 0.3–0.5%.

Gentamycin is water soluble antibiotic of the aminoglycoside group. The main components of the gentamycin complex are C group gentamycins – C_1, C_{1a}, C_2, C_{2a}, C_{2b}.

In the NMR gentamycin sulphate spectrum there exists a considerable amount of superposition signals of different intensity. Hence, it's rather problematic to distribute individual signals between different structures.

Besides, further researches showed that radiation treatment of the gentamycin sulphate practically doesn't affect isomer skeleton (very weak signals appear in the region 3.61–3.67 ppm).

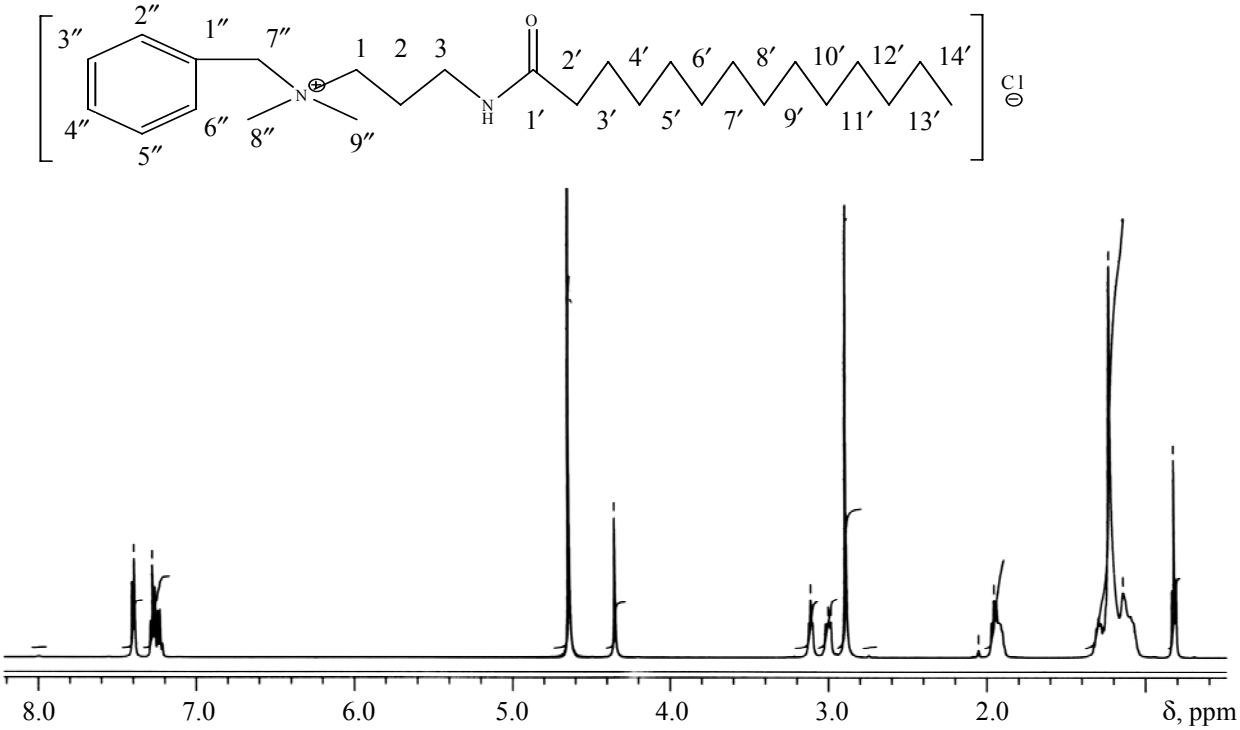

Fig. 2. ^{1}H NMR miramistin spectra after radiation

It should be noted that in the spectra of the radiated substance along with the water signals in combination with the hydroxyl and amine groups of gentamycin and sulphuric acid which exchange protons (δ, 4.64 ppm) there appear broad signals of an acid character (δ, 7.00 и 8.00 ppm). The reason of it is salt destruction and appearance of a free sulphuric acid which doesn't exchange protons with water.

Comparison of the integral intensities in the initial and the resultant spectra (the spectra after radiation) proves that the total degree of destruction is about 1%.

Ionizing radiation effect on miramistin and gentamycin in the composition of hydrogel polymeric matrixes has been investigated. It was stated that due to the action of high-velocity electrons on hydrogels at doses 5–35 kGy miramistin and gentamycin retain their structure. The degree of their destruction is not higher than that of the ingredients radiated in the dry state (less than 1%).

Summary. NMR spectroscopy research entirely ascertained the structure of hydrogel matrix components and active ingredients. The influence of ionizing radiation on the stability of hydrogels, miramistin and gentamycin at absorption doses in the range 5–35 kGy has been studied. Comparison of hydrogel spectra before and after radiation made it possible to estimate the degree of polymer crosslinking (nearly 98%) at doses 25–35 kGy. It has been determined that different dose radiation of miramistin and gentamycin results in their slight destruction (not less than 1%).

^{1}H and ^{13}C NMR spectra of hydrogel matrixes containing miramistin and gentamycin have been recorded. Classification of resonance signals has been performed. The retention of the structure of the researched pharmaceutical ingredients in the hydrogel polymeric matrix composition after the action of ionizing radiation at doses 5–35 kGy has been proved.

References

1. Противомикробное и ранозаживляющее средство на основе гидрогелевой полимерной матрицы: пат. 11060 Респ. Беларусь, МПК (2006) A 61L 15/16 / П. Т. Петров [и др.]; заявитель и патентообладатель РУП «Белмедпрепараты»; заявл. 21.10.05; опубл. 30.06.07 // Афіцыйны бюл. / Нац. цэнтр інтэлектуал. уласнасці. – 2008. – № 4 (63). – С. 62.

2. Бенько, А. Н. Новые технологии лечения травматических дефектов мягких тканей конечностей с использованием лекарственных форм на основе гидрогеля: автореф. дис. канд. мед. наук: 14.00.22 / А. Н. Бенько; Респ. науч.-практ. центр травматологии и ортопедии. – Минск, 2008. – 27 с.

3. Карман, А. Д. Обоснование комплексного метода лечения рожистого воспаления: автореф. дис. … канд. мед. наук: 14.00.27 / А. Д. Карман; Бел. гос. мед. ун-т. – Минск, 2008. – 20 с.

4. Пикаев, А. К. Современная радиационная химия. Радиолиз газов и жидкостей / А. К. Пикаев. – М.: Наука, 1986. – 440 с.

Received 16.03.2012