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PREPARATION OF NANOPARTICLES OF LOW-MOLECULAR CHITOSAN COMPLEXES WITH ISONICOTINIC ACID HYDRAZIDE

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Аннотация. Получены наночастицы комплексов низкомолекулярного хитозана с гидразидом изоникотиновой кислоты (изониазида), сшитого триполифосфатом натрия (TPP). Методом ИК-спектроскопии подтверждено состав и структура образованных комплексов. Установлено, что полимерные комплексы содержат до 50% изониазида, а средний размер синтезированных наночастиц изменяется в диапазоне от 120 до 200 нм. Методами ПЭМ и АСМ показано, что образующиеся наночастицы имеют сферическую форму с высокими значениями дзета-потенциала (от 25 мВ до 50 мВ).
Ключевые слова. Хитозан, наночастицы, гидразид изоникотиновой кислоты

Abstract. Isonicotinic acid hydrazide (isoniazid) loaded nanoparticles of chitosan (CS) cross-linked with tripolyphosphate (TPP) were prepared. The prepared nanoparticles were characterized by FT-IR spectroscopy to confirm the cross-linking reaction between CS and cross-linking agent. Up to about 50% of isoniazid were loaded into the nanoparticles and the average size of nanoparticles ranged from 120 to 200 nm. The nanoparticles formed were spherical in shape with high zeta potentials (from +25 mV to +50 mV).
Keywords. Chitosan, nanoparticles, isonicotinic acid hydrazide

Over the past decades has been actively working to create systems drug delivery, allowing to increase the circulation time of the drug in the blood, protect it from the enzyme systems of the body, including molecules that provide the target delivery, potentially leading to the creation of a new generation of drugs. As matrixs for establishing such systems use various materials , mainly organic polymers non-protein nature, since they are characterized by low immunogenicity one them promising materials to create drug delivery systems is chitosan - deacetylated derivative of chitin, a natural polysaccharide [1,2]. Chitosan is compatible with mammalian tissue, low toxicity, body hydrolases degraded to oligosaccharides and glucosamine. A significant advantage of chitosan compared to other is reactive polysaccharides (hydroxo- and amino groups that are allows to obtain derivatives with the required characteristics. In a polymer matrix on the basis of chitosan and its derivatives can be incorporated substances of different nature - proteins, peptides, nucleic acids, vitamins, antineoplastic agents, etc. [3,4]. Further more, depending upon the molecular weight and the degree of modification of the polymer by various substituents can be prepared nanoparticles with different size and charge [5]. Despite the great potential of chitosan as a material for biomedical - changes , to date, the global pharmaceutical market only appear first registered drugs for external use [6]. Chitosan active positions tsioniruetsya on the market as a dietary supplement with immunostimulatory properties. Use chitosan particle formations on the

basis there of is for intravenous administration step Studies [7,8]. Besides chitosan exhibits antimicrobial properties [9].

Isonicotinic acid hydrazide (isoniazid) is an effective antituberculosis (anti-TB) drug widely used in medical practice. However, it has several disadvantages, among which should be attributed high toxicity and low activity due to which the drug is administered in large doses. One of the greatest challenges in the treatment of tuberculosis is the necessity to maintain a constant high concentration of anti-tuberculosis drug not only in blood but also in macrophages. Usual treatment is aimed at destroying the pathogen (Mycobacterium tuberculosis) only in the bloodstream, so it is inefficient and requires significant chemotherapy using extremely high doses of drugs over a long period. It is known that the introduction of carbohydrate type polymers into the structure of drugs leads to a favorable modification of their physical properties, a significant reduction in adverse side effects on an organism, increases bioavailability and prolongs their positive effect [4]. The preparation of nanoparticles of polymeric chitosan complexes with drugs allows creating formulations that increase the ability of a drug to penetrate into cells. Accordingly, the preparation of nanoparticles of low-molecular chitosan complexes with isonicotinic acid hydrazide in the presence of sodium tripolyphosphate (TPP) was investigated.

MATERIALS AND METHODS

Chitosan (degree of deacetylation = 91%, M_w = 600,000) was purchased from Sigma-Aldrich Co., Hungary. The DD of the chitosan material employed has been determined from integral intensities of all protons by its 1H NMR spectrum. Low molecular weight chitosans (M_w = 10.000) were prepared by oxidative degradation with H_2O_2 performed at room temperature [10,11]. The viscosity average molecular weights were determined by measuring relative viscosity with an Ostwald viscometer. Molecular weights were calculated from the intrinsic viscosity based on the Mark-Houwink equation.

Preparation chitosan-izonikotinic acid hydrazide complex nanoparticles. In three-neck round-bottom flask, equipped with a mechanical stirrer, reflux and a thermometer is placed 0.064 g (0.00059 mol) izonikotinic acid hydrazide, add 100 ml of 2% aqueous solution of CH_3COOH and dissolve at stirring at a temperature of 70 °C. After the dissolution of 0.1 g (0.1 masses. %) pre-purified chitosan (average molecular mass of 10,000). Stirred with a speed of about 200 adverting min and adds dropwise aqueous solution of sodium tripolyphosphate in 2 hours. Particle Size Distribution. The average particle size and size distribution were determined by quasielastic laser light scattering with a Malvern Zetasizer (Malvern Instruments Limited, United Kingdom). Nanoparticle distilled water solutions of 3 mL (1 mg/mL) were put into polystyrene latex cells and measured at a detector angle of 90°, a wavelength of 633 nm, a refractive index of 1.33, a real refractive index of 1.59, and a temperature of 25 °C.

Electrophoretic Mobility Study. Electrophoretic mobility measurements (μ_e) were performed with a Zeta-sizer Nano ZS (Malvern Instruments, UK) and μ_e was measured against waiting time.

Transmission Electron Microscopy (TEM). Specimens were prepared by dropping the sample solution onto a copper grid. The grid was held horizontally for 20 s to allow the molecular aggregates to settle and then at 45° to allow excess fluid to drain for 10 s. The grid was returned to the horizontal position, and one drop of 2% phospho- tungstic acid was added to give a negative stain. The grid was then allowed to stand for 30 s to 1 min before the excess staining solution was removed by draining as above. The specimens were air- dried and examined using a Zeiss Libra 120 transmission electron microscope (Carl Zeiss, Germany).

(FT-IR) spectroscopy. The IR spectrum of samples was recorded on a Fourier Transform Infrared spectrometer Vertex 70 (Brucker, Germany).

To determine the activity of polymeric forms of TB Isoniazid and Streptomycin against strain H37Rv two fold dilution method was used for the liquid medium Sotona. MIC was recorded using a culture medium containing 10 % normal horse serum incubation at month 1.5. Acute toxicity drug analogues synthesized chitosan determined using out bred mice weighing 18-20 g. Polymers injected intraperitoneally in saline, the monitoring was conducted 10 days.

RESULTS AND DISCUSSION

The formation of nanoparticles of low-molecular chitosan homologues with isoniazid in the presence of TPP complex was investigated. It is known that the use of the TPP leads to the formation of stable chitosan nanoparticles. It was found that the particle size is significantly affected by the concentration of chitosan and TPP. When the concentration of the chitosan is less than 0.1 mg/ml, the dispersion with a wide range of particle sizes is obtained. The most consistent results are obtained when the concentration

of chitosan is 0.2 mg/ml; with its further growth the particle size increases, while maintaining a narrow size range. The investigation of the effect of the concentration of TPP (Fig. 1) showed that with its increase the particle size decreases.

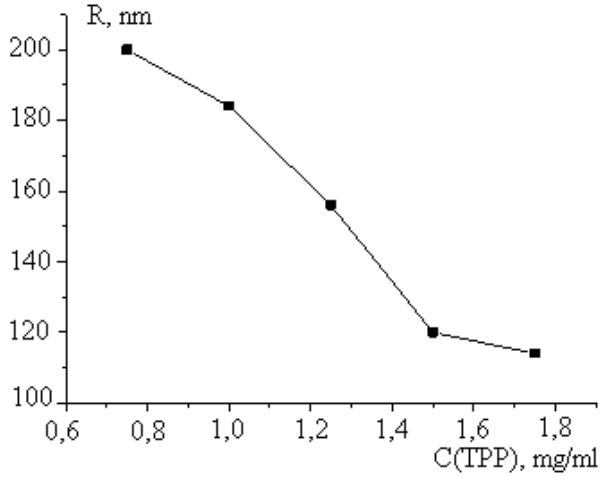

Fig.1. Dependence of particle size of chitosan (MW = 10 000) on the TPP concentration (pH = 4.0, C (chitosan) = 2 mg/ml).

The study of the effect of pH change on the process of producing dispersions of chitosan showed that the dependence is non-linear (Fig. 2, curve 2). At low pH the particle size decreases, reaches its minimum, then the growth of particles is observed. This growth is associated with the decrease in the stability of the particles, as indicated by reduce of the ξ-potential (Fig. 2, curve 1).

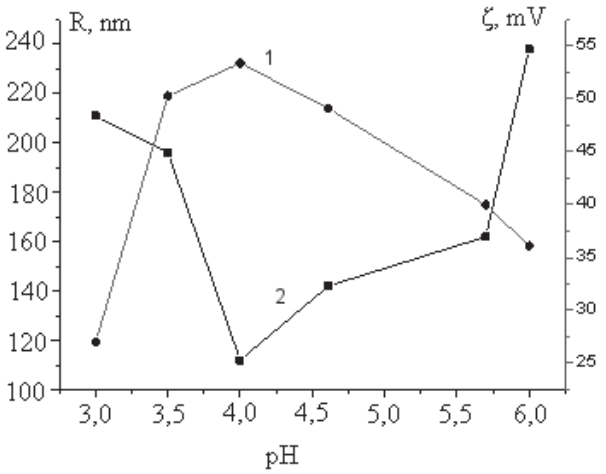

Fig.2. Dependence of the particle size of chitosan (MW = 10 000) (2) and the ξ-potential (1) on the pH of the medium (C (chitosan) = 2 mg / ml, C (TPP) = 1.75 mg / ml).

The studies on the effect of chitosan molecular weight in the range of 5-30 kDa showed that the size of its nanoparticles does not change significantly. Apparently, chitosan macromolecules are extremely prone to association, so macromolecular associates are involved in formation of nanoparticles.

The study of the effect of TPP concentration on the degree of binding and particle size of the complexes formed. As it was found, the degree of binding increases with the growth of TPP concentration, reaching a maximum value of 25 wt.% (Fig. 3), then it reaches a plateau. It should be noted that the average particle size decreases (Fig.4).

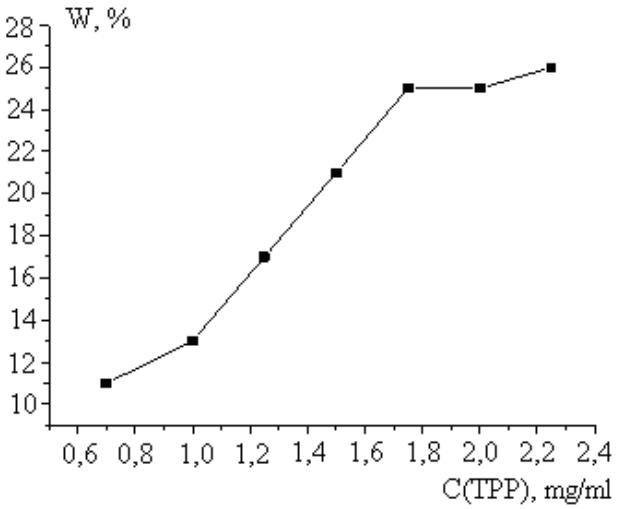

Fig. 3. Dependence of the mass fraction of isoniazid in isoniazid-chitosan complex ($\overline{M}_W$ = 10 kDa) on TPP concentration.

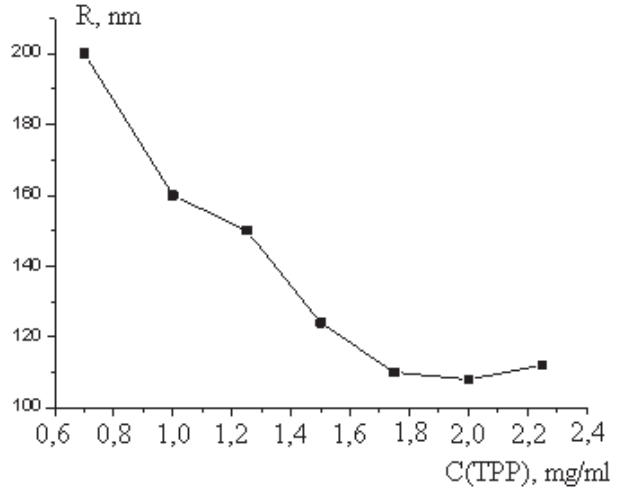

Fig. 4. Dependence of the particle size of chitosan-isoniazid complex on TPP concentration.

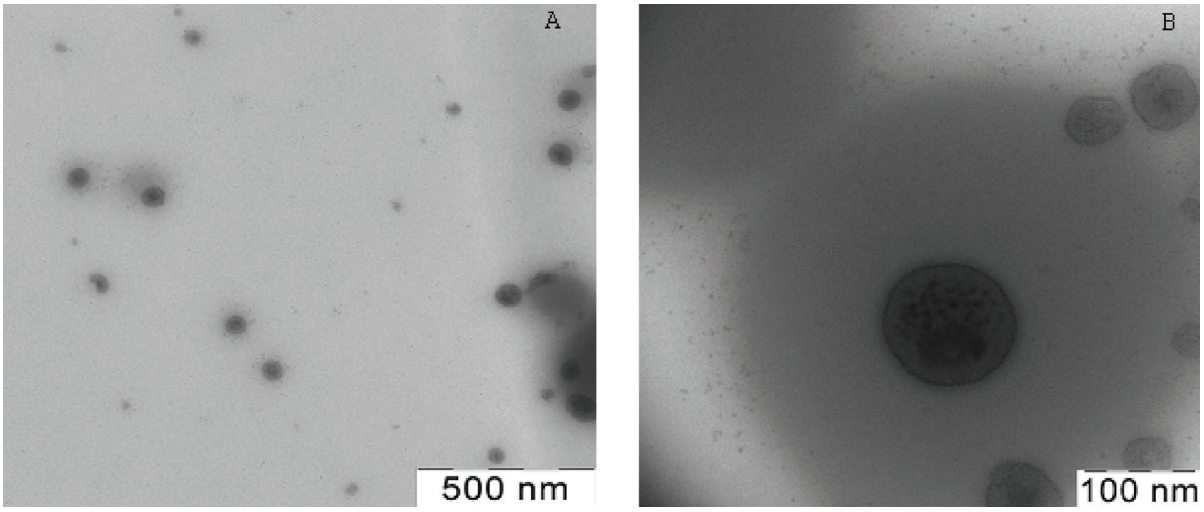

Fig.5. TEM of isoniazid-chitosan complex

Table 1.

Anti-tuberculosis activity and acute toxicity of polymeric forms izoniazid and streptomycin.

Compound	Contents related drugs, %	MIC, m	Number of mouse	LD50, g/kg
I	7.6	2.6	40	13.3
II	22	0.9	40	4.8
III	25	0.8	40	4.3
izoniazid	100	1.0	40	0.23

In order to determine the optimum conditions for interaction between chitosan and isoniazid, we studied the effect of their molar ratio on the degree of binding. The study was conducted in the range of 1.0-1.8 molar excess relative to chitosan. It was found that the introduction of initial molar ratio greater than 1.0 in the range studied does not cause any increase in the mass fraction of isoniazid in the resulting complex. The control over the content of isoniazid in the complex was exercised by FT-IR spectroscopy similar to the previous case.

To confirm the formation of chitosan-isoniazid complexes their study was conducted by the means of FT-IR spectroscopy. The FT-IR spectra of the complexes show a shift of the characteristic absorption bands in comparison with the initial chitosan and isoniazid spectra. In particular, the shift of the absorption band corresponding to the stretching vibrations of the hydrazide >C=O bond from 1654 cm^{-1} for initial isoniazid to 1663 cm^{-1} indicates the participation of this bond in the formation of complexes, probably with the primary or secondary amino group of chitosan. The same is indicated by the shift of the absorption band of 1616 cm^{-1} corresponding to bending vibrations in the primary and secondary amino groups in chitosan to 1631 cm^{-1} in the complex. According to the data obtained by IR spectroscopy, the "pyridine" nitrogen atom of isoniazid also participates in complexing, which is indicated by the shift of the absorption band corresponding to the stretching vibrations of the -C=N-aromatic ring of isoniazid from 1584 cm^{-1} to 1560 cm^{-1}. Apparently, the "pyridine" nitrogen atom of isoniazid interacts with P=O bonds of TPP molecules.

Transmission electron and atomic force microscopy the shape and size of the synthesized nanoparticles are investigated. It is shown (Fig.5,6) that they have a spherical shape. The obtained results correlate with the dynamic light scattering.

Tests have TB activity of the synthesized polymeric forms of chitosan-isoniazid complexes when used as a test strain of Mycobacterium tuberculosis culture N37Rv compared to the corresponding drug substance showed practically identical IPC considering the quantitative content of the substance in the composition polimeranalogov . Acute toxicity studies in animals indicate objects decline LD_{50} polymeric forms of drugs in comparison with the corresponding substances in 2.5-4.5 times (table 1).

CONCLUSION

Nanoparticles of chitosan of low molecular complexes with isonicotinic acid hydrazide are prepared. The dependence of the particle size

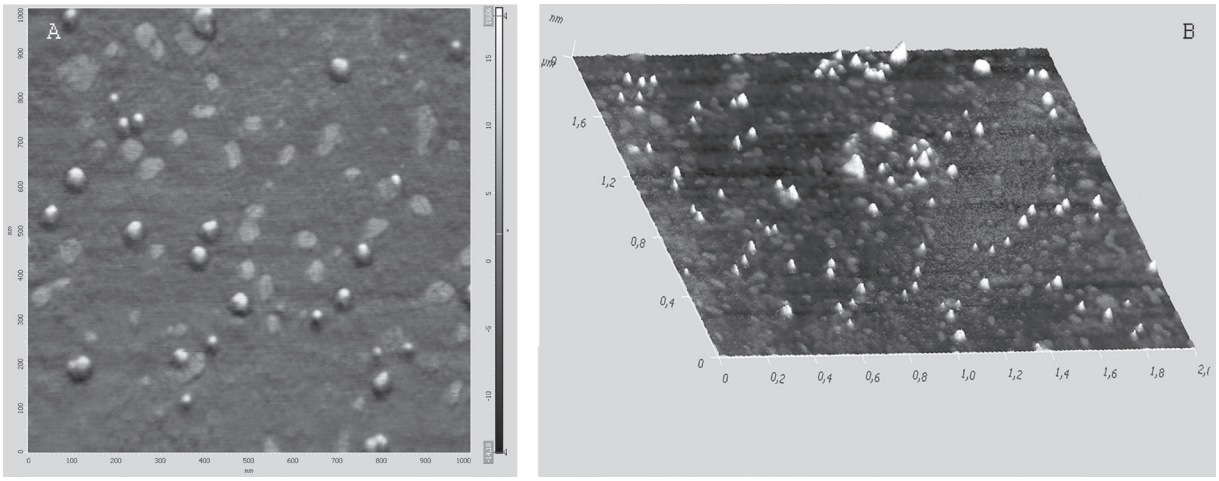

Fig.6. AFM of isoniazid-chitosan complex

and stability of the concentration of sodium tripolyphosphate, and the pH of the system has been found. Minimum amount of low molecular weight chitosan nanoparticles complexes with isonicotinic acid hydrazide corresponds to maximum value ξ potential. The resulting complexes exhibit tuberculocidal activity comparable to native isoniazid with acute toxicity is reduced 2.5-4.5 times.

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