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DRUG SYNTHESIS METHODS AND MANUFACTURING TECHNOLOGY

SYNTHESIS AND CHARACTERIZATION OF POLY(DL-LACTIC ACID) NANOPARTICLES LOADED WITH THE ANTITUBERCULOSIS DRUG ISONIAZID

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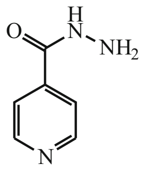
The possibility of creating a new dosage form of the antituberculosis drug isoniazid using nanoparticles of the polymer poly(DL-lactic acid) was studied. It was shown that nanoprecipitation was promising for preparing nanoparticles and loading them with drugs.

Keywords: poly(DL-lactic acid), drug delivery, isoniazid, nanoparticles.

New medical treatments for many diseases were made possible by utilizing nano-sized materials. In particular, polymer nanoparticles (NPs) and nanocapsules can be used to develop effective intracellular drug delivery systems [1]. The small size and large specific surface area in addition to adequate *in vivo* stability enable polymer NPs and nanocapsules to bind or adsorb a drug on their surface, deliver it to the target organ, and release the biologically active compound gradually, producing a prolonged effect.

Isoniazid (**I**) exhibits high bacteriostatic activity against various forms and strains of mycobacterium tuberculosis [2, 3]. However, it is quickly eliminated from the body, which makes it necessary to use high doses of the active ingredient. Furthermore, a large part of the administered drugs is biotransformed and does not exert antibacterial activity [3]. One technique for increasing the treatment effectiveness is to develop targeted drugs based on polymers. Herein, poly(DL-lactic acid) (PLA), a widely used biodegradable and biocompatible polymer, was selected as the polymer base.



I

EXPERIMENTAL PART

PLA was prepared by polymerization with opening of the cyclic lactide ring [4].

This method is based on production of a dilactide followed by polymerization. The first synthetic step includes production of the precondensate that is then depolymerized thermally to the dilactide. The dilactide polymerizes with opening of the ring to form a linear polylactide containing ~5% unreacted monomer.

Preparation of PLA NPs loaded with isoniazid. Compound **I** (5 mg) was dissolved beforehand in H_2O (0.2 mL), stirred, and treated with Me_2CO (0.5 mL). PLA (20 mg) in Me_2CO (1 mL) was mixed with the solution containing the drug. Then, this mixture was stirred gently and treated with H_2O (5 mL). The organic solvent was evaporated at reduced pressure for 40 min. The NP dispersion was diluted with H_2O

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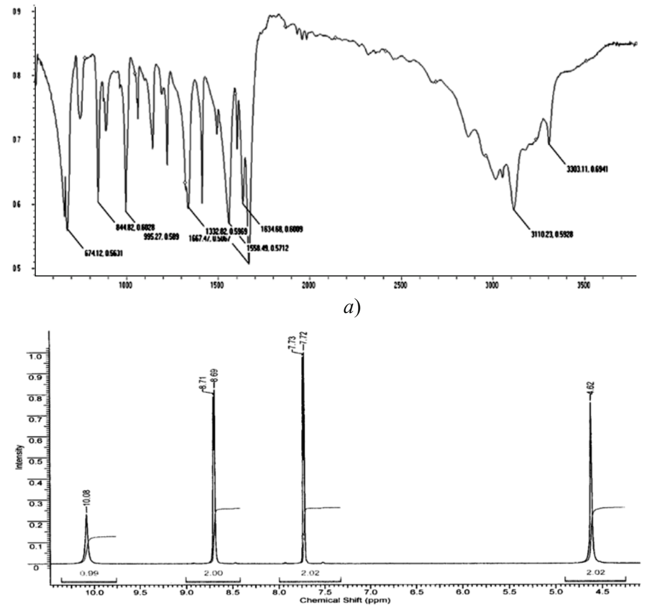
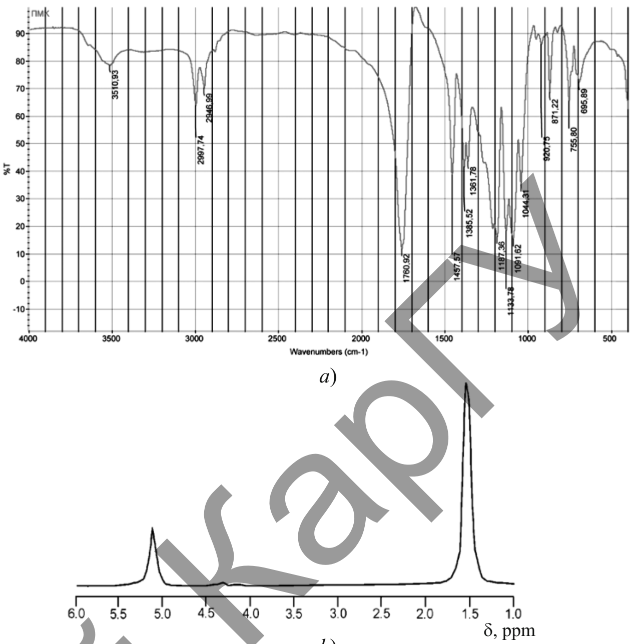


a)

b)

Fig. 1. IR (*a*) and PMR (*b*) spectra of isonicotinic acid hydrazide.



a)

b)

Fig. 2. IR (*a*) and PMR (*b*) spectra of poly(DL-lactic acid).

to 20 mL. Large aggregates, if present, were removed by passage through a 1-μm filter. The resulting polymer NPs were isolated by sedimentation using centrifugation at 14,000 rpm, rinsed with buffer solution, and dried to constant mass.

Drug substance for loading into the PLA matrix was supplied by TOO Romat (Pavlodar Pharmaceutical Plant, Pavlodar, Kazakhstan).

Isoniazid was a crystalline powder and was identified by IR and PMR spectroscopy (Fig. 1) and melting point (443 – 447 K).

The average NP diameter was measured by photon correlation spectroscopy (PCS) at 25°C at an angle of 173° in Zetasizer Nano S90 and ZS90 instruments (Malvern Instruments Ltd., Malvern, UK). Particle sizes were also determined by transmission electron microscopy on a CM12 transmission electron microscope (Philips) and FEI Tecnai 20 (type Sphera) TEM equipped with an LaB6 filament operating at 200 kV and 103 K.

The molecular mass of the synthesized polymer was determined on an Agilent 1200 instrument with a refraction detector [GPC/SEC (styrogel) column, 300 mm, 7.5 mm inner diameter, 1 mL/s eluent flow rate, calibration with polystyrene standards of known molecular masses].

The degree of incorporation of the NP dispersion was determined by centrifugation at 14,500 rpm (MiniSpin plus 14500 centrifuge, Eppendorf, Germany) for 30 min. Then, PLA NPs containing isoniazid were dissolved in the calculated amount of $CHCl_3$. Unincorporated drug was extracted (3×) with doubly distilled H_2O. The degree of drug incorporation into PLA NPs was determined spectrophotometrically (KFK-2 photocolorimeter) at $\lambda_{max} = 315$ nm after centrifuging the NPs at 14,500 rpm for 30 min at 25°C.

IR spectra were recorded from KBr pellets on an FSM 1201 FTIR spectrometer (Infraspek Ltd.).

PMR spectra were recorded with HMDS internal standard on a Bruker Avance AV 300 spectrometer at operating frequency 300 MHz and 25°C.

TABLE 1. Characteristics of Poly(DL-lactic acid) Particles

Solvent	Average particle diameter *d*, nm	NP polydispersion (PDI)	Fraction of nano-sized particles, %
THF	858.8 ± 5.4	0.342 ± 0.020	11.9 ± 0.6
Me_2CO	290.1 ± 4.3	0.316 ± 0.015	98.0 ± 0.2
Me_2CO—EtOH	635.8 ± 4.7	0.439 ± 0.019	92.7 ± 0.3

TABLE 2. Characteristics of Poly(DL-lactic acid) NPs Loaded with Antituberculosis Drug Isoniazid

Characteristics	Empty poly(DL-lactic acid) NPs	Poly(DL-lactic acid) NPs loaded with drug
Average particle diameter *d*, nm	290.1 ± 5	273.3 ± 9
NP polydispersion (PDI)	0.316 ± 0.013	0.384
ζ-potential, mV	– 35.0 ± 2.8	– 30.4 ± 1.9
Molecular mass, Da	1520.0 ± 2	1630.0 ± 9
Loading, %	–	50.0
Particle yield, %	76.6	78.4

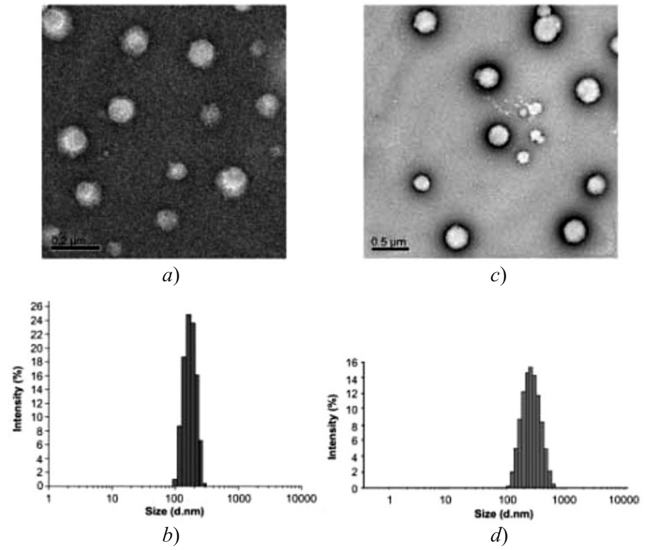


Fig. 3. Electron microscope image of empty PLA NPs (*a*) and those loaded with drug (*c*); particle-size distribution (*b*) of empty PLA NPs (*b*) and PLA loaded with isoniazid (*d*).

RESULTS AND DISCUSSION

Nanoprecipitation was first proposed in 1989 [5] and is a promising method for preparing NPs. We studied the possibility of preparing PLA NPs by nanoprecipitation followed by nanoencapsulation of the antituberculosis drug isoniazid. The nanoprecipitation system consisted of three components, i.e., polymer, solvent, and precipitant. The organic solvents were those that were water-miscible and easily removed by evaporation (EtOH, Me_2CO, dioxane, or mixtures). Therefore, series of experiments designed to select a solvent were performed first in order to select the optimal conditions for preparing the PLA NPs. THF, Me_2CO, and Me_2CO—EtOH (1:1, v/v) were selected for the tests. Table 1 lists the results.

Table 1 shows that the best results were obtained with Me_2CO as the solvent. The NPs had satisfactory physicochemical characteristics according to PCS. The system consisted mainly of ~290-nm particles (polydispersion 0.316) and contained an insignificant amount (2%) of microparticles. This corresponded to requirements for polymeric drug carriers [7] and enabled empty PLA NPs synthesized by nanoprecipitation to be used as delivery systems for biologically active compounds.

The product (polymer) was an odorless white powder. Figure 2a and 2b show its IR and PMR spectra, respectively.

Figure 2a shows that the IR spectrum contained characteristic absorption bands at 1758 – 1763 and 2994 – 2997 cm^{-1} for C=O and $–CH_2$ vibrations; 1184 – 1190, for C–O–C ester indicated that –COO– had formed. Also, absorption bands at 1452 – 1457 and 1382 – 1389 corresponded to $–CH(CH_3)$ vibrations. These bands indicated that PLA had formed. Typical –CH vibrations of the lactide ring appeared in the characteristic region 932 – 934 cm^{-1}. These peaks were not observed in all samples, which indicated that the lactide ring had opened.

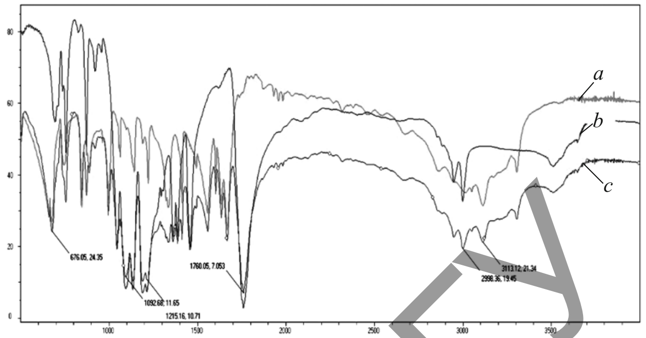


Fig. 4. IR spectra (KBr) of isoniazid (*a*), PLA (*b*), and PLA NP with drug (*c*).

The PMR spectrum of PLA (Fig. 2*b*) showed that the reaction was successful. Strong resonances for CH_3 and CH protons were observed at 1.52 and 5.13 ppm, respectively.

Next, we attempted to load the antituberculosis drug isoniazid into the PLA matrix. Series of experiments in which the drug:polymer ratio was varied from 1:10, 1:5, and 1:4 were conducted in order to prepare NPs with the optimal characteristics and high loading. The last ratio was considered optimal according to the results. Guided by the conditions developed for preparing PLA NPs by nanoprecipitation, NPs with loaded with drug were synthesized. Mixing two solutions (polymer solution in organic solvent and drug solution in H_2O) produced an effect similar to occlusion in which the drug particles were encapsulated by the forming precipitate (polymer) particles. In contrast with adsorption, drug was absorbed not only on the surface but also within the polymer NP mass. This suggested that a high degree of incorporation was achieved. PLA NPs were rinsed (3×) with H_2O to reduce the Me_2CO content to $<1.5 \cdot 10^{-4}$ mg (Me_2CO content determined on an Agilent 7890A GC). Thus, injection of PLA NPs produced by the aforementioned method would not affect the total Me_2CO blood content.

Measured particle sizes and their polydispersion showed that the fraction of nano-sized particles was 98.6%. The average particle size was 273.3 nm; polydispersion, 0.384.

Electron microscope images of empty PLA NPs and NPs loaded with the antituberculosis drug isoniazid were taken in order to study the obtained NPs in more detail (Fig. 3*a* and 3b). It can be seen that the particles had a narrow size distribution. The average diameter of empty NPs and those loaded with drug was 100 – 150 nm.

Figure 4 shows IR spectra from studies of the incorporation of **I** to the PLA NP matrix.

The IR spectrum of isoniazid (a) corresponded to that in the literature [6]. Characteristic bands in the spectrum at 3070 – 3020 cm^{-1} corresponded to pyridine ring vibrations; at 1653, to $–NH_2$ scissoring vibrations; at 1541 – 1547,

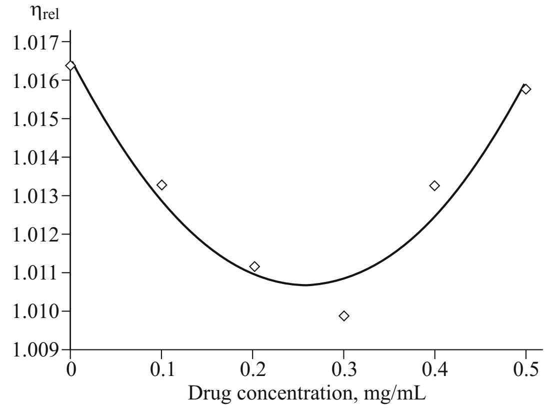


Fig. 5. Relative polymer viscosity as a function of amount of added drug.

–N–H vibrations; 1550 – 1620, characteristic C=O bands (Fig. 4*a*).

The IR spectrum of PLA (Fig. 4*b*) contained characteristic absorption bands at 1758 – 1763 and 2994 – 2997 cm^{-1} for C=O and $–CH_2$ stretching vibrations; 1184 – 1190, for C–O–C ester indicating that a –COO– bond had formed. Absorption bands at 1452 – 1457 and 1382 – 1389 corresponding to $–CH(CH_3)$ bonds were also present. These bands indicated that PLA had formed.

The spectrum of the polymer loaded with drug substance (Fig. 4*c*) showed that the principal characteristic peaks of the polymer and drug did not change and new absorption bands did not appear. This suggested that isoniazid was situated in the PLA matrix and was not bonded covalently to the polymer.

The degree of loading of a drug in the polymer is an important parameter when preparing polymer-loaded drugs. The degree of isoniazid incorporation into the polymer that was found by spectrophotometry (at 315 nm) was ~50%, which was a rather high value.

The high drug content in the polymer matrix could have resulted from the formation of a polymer–drug complex. Various types of critical effects occur during complexation of polymers to high- and low-molecular-mass compounds. Potentiometric, turbidimetric, and viscometric titration data can be used as quantitative criteria of the formation of a polymer–drug complex [7]. The last method is based on the fact that more compact structures are formed if the polymer is complexed by low-molecular-mass compounds. As a rule, conformational changes of the macromolecules cause the polymer solution viscosity to decrease during titration with the drug solution to a certain critical value. An analogous phenomenon was observed during titration of PLA by isoniazid solution in an aqueous–organic medium. The relative viscosities of solutions were determined from the ratio of the solution flow time to the solvent flow time in a glass Ubbelohde viscometer.

The results for the amount of drug consumed in the titration were used to determine that ~50% of the isoniazid was incorporated into the polymer (Fig. 5). This was indicated by the fact that the curve passed through a minimum on a plot of viscosity as a function of the amount of added drug.

Thus, physicochemical research methods indicated that isoniazid was effectively incorporated into the PLA matrix.

Table 2 presents data for the physicochemical characteristics of empty PLA NPs and those loaded with isoniazid.

The particle surface charge, characterized quantitatively by the ζ-potential and determining the dispersion stability, was equally as important a factor for preparing the colloidal dispersions. It is well known that the absolute value of the ζ-potential should be >30 mV in order to prepare stable colloidal systems because a system with a surface charge close to zero is unstable and tends to coagulate. Table 2 shows that both empty NPs (–35 mV) and those loaded with drug (–30.4 mV) had low ζ-potentials in addition to small particle sizes and a narrow range of polydispersion. This indicated that the NPs were highly stable over time.

Molecular masses of the polymers were determined by gel-permeation chromatography and were 1520 and 1630 Da for empty and drug-loaded NPs, which agreed with the literature [8, 9]. Furthermore, Table 2 shows that the degree of incorporation was rather high, indicating that nanoprecipitation was promising for preparing NPs loaded with drugs.

Thus, we prepared PLA NPs loaded with the antituberculosis drug isoniazid with particle size 273 ± 9 nm and polymer molecular mass 1630 Da. This opened new possibilities for administering this drug as NPs [9 – 12].

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