

RESEARCH ARTICLE

Production of Polylactide Nanoparticles Loaded With Isoniazid and Vitamin C: A Promising Candidate for the Treatment of Resistant Forms of Tuberculosis

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Received: 10 May 2024 | **Revised:** 5 November 2024 | **Accepted:** 5 November 2024

Funding: This work was supported by Ministry of Education and Science of the Republic of Kazakhstan AP14871344.

Keywords: antituberculosis drug | multidrug-resistant tuberculosis | nanoparticles | polylactic acid

ABSTRACT

Nowadays, the method of directed transport of drugs is becoming increasingly important in medicine and pharmacology. It allows for increasing the concentration of delivered substances in a certain place and blocking or severely limiting their accumulation in healthy organs and tissues. One of the promising drug carriers used in the development of controlled drug delivery systems is polylactic acid (PLA). We were interested in using biodegradable polymeric nanoparticles (NPs) loaded with isoniazid (INH) and vitamin C (VC) which have sustained action, targeted delivery, high efficacy, and low toxicity. It will help to expand the use of these simple drugs for the rapid treatment of multidrug-resistant forms of tuberculosis. Thus, in this work, PLA-INH-VC NPs were prepared by double emulsion method where several factors affecting particle size were investigated. TGA/DSC and IR spectroscopy studies identified the presence of molecularly dispersed drugs (INH and VC) in polylactide nanoparticles. VC was found to reduce the degradation of the polymer and thus prolonged the release of the drug from the polymer matrix. PLA-INH-VC nanoparticles were analyzed for mycobacterial activity and the nanoparticles were found to inhibit the growth of INH-resistant mycobacteria.

1 | Introduction

The modern pharmaceutical industry has extensively used polymers to improve the efficiency and targeting of drug delivery. The use of polymeric nanoparticles for drug delivery represents an advanced approach in the field of medical technology which opens up new possibilities in the treatment of various diseases, one of which is tuberculosis (TB). TB remains one of the most serious infectious diseases caused by the bacillus *Mycobacterium tuberculosis* (MTB). This pathogen often attacks the lungs although it can also affect other organs such as the kidneys, bone tissue, and brain. TB is a global public health threat, requiring comprehensive and effective treatments.

Since 1968, isoniazid (INH) and rifampicin (RIF) have been widely used for the treatment of TB. They kill more than 99% of TB bacilli within 2 months of starting treatment. Unfortunately, INH resistance soon set in and the urgent need for multitherapy was recognized [1]. At that time, drug-resistant TB was overcome by the development and introduction of combination therapy with these drugs. This approach provided a good chance for treatment as it rapidly killed both dividing and long-lived MTB bacteria. Despite the high efficacy of this combination therapy, serious complications including hepatotoxicity and persistent cells have been reported [2]. Other side effects such as unexplained fever, cytopenia, and neurological have also been reported and have resulted in adverse effects on treatment adherence and clinical outcomes [3].

Vilche'ze et al. [4] observed that MTB cultures were sterilized faster with a combination of vitamin C (VC) and INH than with VC alone. Since TB treatment is based on the use of multiple drugs rather than monotherapy, they tested a sub-inhibitory concentration of VC added to the combination of INH and RIF. While the combination of INH and RIF sterilized the MTB culture for 3–4 weeks, the addition of VC to this treatment reduced the sterilization time by 7 days. This suggests that VC can be used as an optional treatment.

Vitamin C (ascorbic acid) is a strong antioxidant that is formed during cellular metabolism and destroys toxic free radicals and other reactive oxygen species. Combining INH with an antioxidant substance can reduce INH-induced liver damage. Ascorbic acid readily reacts with superoxide radicals, hydrogen peroxide, and singlet oxygen and neutralizes them [5, 6]. VC alone or in combination with other antibacterial agents showed promising antibacterial activity against various pathogens such as *Helicobacter pylori*, *Staphylococcus aureus*, and multidrug-resistant *Pseudomonas aeruginosa* [2]. This can be explained by two mechanisms: for one thing when VC is transported into bacterial cells, it is metabolized in the presence of oxygen and hence bacteria are subjected to oxidative stress; for another, acetic and lactate acids are formed from VC [1].

Therefore, we were interested in using biodegradable polymeric nanoparticles loaded with INH and VC with sustained action, targeted delivery, high efficacy, and low toxicity, which will help to expand the use of these simple drugs for rapid treatment of TB.

2 | Experimental

2.1 | Materials

Polylactic acid poly (D, L-lactide, Mn 15,000), INH ($\geq 99\%$), polyvinyl alcohol (PVA) (hydrolyzed, Mw 9000–10,000) (all by Sigma Aldrich), and VC (California Gold Nutrition) were used in this work without further purification. All other materials and reagents used in this study were of analytical grade of purity.

2.2 | Production of Polylactide Nanoparticles Loaded With INH and VC

Polylactide nanoparticles loaded with INH and VC (polylactic acid (PLA)-INH-VC NPs) were prepared by double emulsion method. The polymer was dissolved in 5 mL of dichloromethane (DCM), and INH (2.5 mg/mL) and VC (2.5 mg/mL) were dissolved in water. The polymer and drug solution (drug to polymer ratio varied from 1:1 to 1:3) was homogenized (5–10 min). The produced solution was added to 0.5%–1% aqueous solution of PVA (the ratio of organic to aqueous phase varied from 1:1 to 1:5 by volume) and then the mixture was homogenized. The solvent was evaporated at room temperature for 12 h with magnetic stirring. PLA nanoparticles loaded with INH and VC were isolated after 20 min of centrifugation at 14,000 rpm (Eppendorf Microcentrifuge 5420, Eppendorf, Hamburg, Germany). After centrifugation, the supernatant

was collected and the amount of unbound drug was determined. The precipitate was washed with distilled water and centrifuged again at 14,000 rpm for 20 min. The washing process was repeated three times.

2.3 | Determination of Particle Size, Polydispersity, and Zeta (ζ) Potential

Particle size and polydispersity of nanoparticles were determined using photon correlation spectroscopy on a Malvern Nano S90 Zetasizer (Malvern Instruments Ltd. Malvern, UK). For all measurements, each sample was diluted to the appropriate concentration with distilled water. Each dimensional analysis lasted 120 s and was performed at 25°C with 90° angle determination. Zeta potential was measured with a ζ -potential analyzer (Zetasizer Nano ZS90, Malvern Instruments, Worcestershire, UK) using electrophoretic laser Doppler anemometry. The surface morphology of PLA nanoparticles was analyzed by scanning electron microscopy (MIRA 3LM TESCAN, Brno, Czech Republic, EU).

2.4 | Drug Loading and Yield of Nanoparticles

The amount of drug in solution was determined by high-performance liquid chromatography (HPLC) (Shimadzu LC-20 Prominence). The detector instrument was adjusted to a wavelength of 262 nm. The mobile phase was water—acetonitrile—formic acid (94:5:1) with a flow rate of 1.5 mL/min. An Agilent 300 Extend (Agilent Technologies, Tokyo, Japan) C-18 column (5 μ m sorbent grain size, 100 Å, 4.6 $\times$ 150 mm) was used. The operating temperature of the column was maintained at 40°C. The instrument was set up for an injection volume of 10 μ L (loop injection).

The amount of INH and VC encapsulated in the polymer nanoparticles was determined by measuring the amount of unencapsulated drug in the aqueous solution recovered after ultracentrifugation and washing of the particles. The drug loading degree and nanoparticle yield were calculated as follows:

$$\text{Drug loading (\%)} = \frac{\text{Mass of the total drug} - \text{Mass of free drug}}{\text{Mass of total nanoparticles}} \times 100\%$$

$$\text{Nanoparticles Yield (\%)} = \frac{\text{Mass of total nanoparticles}}{\text{Mass of the total drug} + \text{Mass of total PLA}} \times 100\%$$

2.5 | Study of the Produced Nanoparticles by Infrared Spectroscopy, Thermogravimetric Analysis and Differential Scanning Colorimetry

Infrared (IR) spectroscopy (FSM 1202, Infracpek LLC Russia) was used to identify the samples. FT-IR spectra were produced by the KBr method. A 3 mg of sample was mixed with 100 mg KBr and a pellet was prepared. The IR range investigated was 4000–400 cm^{-1} .

Thermogravimetric and differential thermal analysis were carried out on a LabSYS evo TGA/DTA/DSC analyzer (Setaram,

France) in the temperature range of 30°C–550°C in an aluminum oxide crucible at a heating rate of 10°C/min in an inert nitrogen environment and a flow rate of 30 mL/min by sample decomposition.

2.6 | In Vitro Study of Drug Release From Polymer Nanoparticles

Kinetics of INH release from polymer matrix was determined by dialysis method, in phosphate-buffered saline (pH 7.4) at 37°C. For this purpose, nanoparticles loaded with the drug were dispersed in phosphate buffer and treated with ultrasound for 10 min. The produced dispersion was transferred into a dialysis membrane (MWCO 1000 Da). The membrane, sealed with clamps, was placed in a dialysis beaker with buffer solution, covered with a lid, and stirred on a magnetic stirrer at 200 rpm. Dialysate samples were taken periodically. To study the extent of release from the polymer nanoparticles, the amount of drug released was determined by HPLC and calculated according to the formula.

$$\text{Drug release (\%)} = \frac{\text{Mass of released drug}}{\text{Mass of the total drug in nanoparticles}} \times 100\%$$

2.7 | Determination of Mycobacterial Activity of the Produced PLA-INH-VC Nanoparticles

In vitro studies were performed in Airstream AC2-4E8 biosafety cabinets (Esco Micro Pte. Ltd. Singapore), which provided sterile conditions through negative pressure and air filtration through HEPA filters. Determination of mycobacterial activity was carried out on a dense Lowenstein–Jensen medium. INH-resistant MBT strain isolate was used in the experiment. The MBT culture was removed from the jamb, ground in a test tube, and suspended with 0.9% physiological solution. The MBT suspension was taken by pipette and transferred to another tube. The MBT suspension was seeded into the liquid medium at the rate of 0.2 mL per 2.0 mL of nutrient medium.

Poly lactide NPs were synthesized with INH and VC and the VC concentrations were 25, 50, and 100 mg/mL. The concentration of INH was constant at 25 mg/mL. Blank PLA NPs without drugs were used as control. All tubes of each experimental row were seeded with 0.2 mL of bacterial suspension. The tubes were sealed with silicone stoppers and incubated in the thermostat at 37°C for 21 days.

The results of suppressive activity were evaluated by counting the number of colony-forming units (CFU) of MTB on a nutrient

medium and comparing the control with the experimental cultures. For samples showing positive results, smears were made and stained according to the Ziehl–Neelsen method (Dzodanu et al., 2019). The smear was allowed to air dry and examined microscopically using a Leica DMLS Binocular Microscope.

2.8 | Design of Taguchi Experiment (DoE)

Taguchi Design of Experiments (DoE) method was applied in the study to evaluate the impact of various parameters on the optimization and reduction of the number of experiments. This method is suitable for studying multiple factors using Design Expert software (version 13, Stat-Ease, Minneapolis, USA). We evaluated four factors, including Drug/PLA ratio, PVA concentration, organic to aqueous phase ratio, and homogenization time using the L9 orthogonal matrix (see Table 1).

2.9 | Statistical Processing of the Produced Data

All studies were carried out at least three times. The results are presented as mean values with standard deviations. To compare the independent groups, one-way analysis of variance (ANOVA) was performed using Minitab19 software.

The effects of the proposed experiments on responses were analyzed using Design Expert software to obtain the main effects of different factors independently, and then ANOVA was performed to determine statistically significant factors. The optimal conditions were determined by selecting the function.

3 | Results and Discussion

One of the promising drug carriers used in the development of controlled drug delivery systems is PLA [7–10]. PLA has attracted attention due to its biodegradable and biocompatible properties, as well as low toxicity; in addition, the polymer is used to create a wide range of delivery systems containing several drugs [11–13]. PLA is widely used for the preparation of sustained-release drugs, and PLA nanoparticles have been successfully used as a carrier for anti-TB drugs [6, 7].

The aim of this study is to optimize the production of nanoparticles based on polylactic acid loaded with INH and VC. Some factors affecting the processes of the preparation of nanoparticles were analyzed: ratio of drug to polymer, ratio of organic to aqueous phase, surfactant concentration, and homogenization time. The physicochemical properties of the produced NPs

TABLE 1 | Taguchi L9 experimental scheme for the production of PLA-INH-VC NPs.

	Name	Units	Level 1	Level 2	Level 3
A	Drug/PLA ratio		1/1	1/2	1/3
B	PVA concentration	%	0.5	0.75	1
C	Organic to aqueous phase ratio		1/1	1/2.5	1/5
D	Homogenization time	min	5	7	10

were studied and the in vitro release of bioactive substances was determined.

We used the Taguchi orthogonal array (OA) to determine the optimal conditions and identify the key parameters affecting the size of PLA nanoparticles. PLA nanoparticles were synthesized using the double emulsion method. We performed nine experiments to determine the best synthesis conditions for the nanoparticles. Table 2 shows the structure of the orthogonal matrix and the results of particle size and polydispersity measurements. The particle size of each nanoparticle was measured using photon correlation spectroscopy, and the drug loading and nanoparticle yield were calculated.

The particle size and polydispersity data of PLA-INH-VC NPs were analyzed using Design Expert software. ANOVA table was used to determine the key process parameter affecting the properties of PLA-INH-VC NPs. The ANOVA tables for the Taguchi method are presented in Table 3. The analysis using ANOVA revealed that homogenization time did not significantly affect the average particle size and also the concentration of PVA did not affect the polydispersity. Hence, these factors were not considered in the ANOVA analysis.

For particle size, *p* values less than 0.05 indicate that the model conditions are significant. Drug/PLA ratio, PVA concentration, and organic to aqueous phase ratio are significant model conditions, while homogenization time can be considered as not significant. Although the *p* value for the drug/PLA ratio is 0.0791, it was still retained in the model because when it is retained, the model becomes significant. A model *f* value of 21.62 means that the model is significant. The probability of getting such a large *f* value due to noise is only 4.49%.

In the ANOVA table for polydispersity (Table 3), the *p* value for the model is less than 0.05, indicating that the model is significant. The model's *f* value of 7.71 indicates that the model is statistically significant. This indicates that there is only a 3.65% probability that such a large *f* value can be produced as a result of random noise. This builds confidence in the model's ability to explain the variability in the data and emphasizes its importance for further analysis and interpretation. In this case, drug/PLA ratio and organic to aqueous phase ratio are significant conditions to support the model structure. Figure 1 shows the different effects of independent variables on the average size and polydispersity of nanoparticles.

Particle size increases with increasing PLA content in the system. The drug/PLA ratio also affects the mean PDI value. When the polymer mass increased from 5, 10 to 15 mg, the polydispersity values decreased. The ζ potentials of NPs ranged between -10 and -23 mV for all the drug-to-polymer ratios were evaluated. This negative charge was due to the presence of terminal carboxyl groups ($-\text{COOH}$) on the nanoparticle surface [14]. As the polymer mass increases, the yield of NPs also increases from 11% to 56%, but the degree of drug loading decreases for both INH and VC (Table 2).

At a PVA concentration of 1%, the minimum mean particle size of 301 ± 5 nm was recorded (Table 2). The polydispersity index increased with increasing PVA concentration. The value

TABLE 2 | Particle size and polydispersity index of the produced PLA-INH-VC NPs.

Run	A: Drug/ PLA ratio	B: PVA concentration (%)	C: Organic to aqueous phase ratio	D: Homogenization time (min)	Size (nm)	PDI	ζ potential (mV)	Drug loading (%)		
								INH	VC	NPs yield (%)
1	1/3	0.5	1/5	7	540 ± 1	0.282 ± 0.18	-15 ± 0.3	39 ± 7	41 ± 9	34
2	1/3	0.75	1/1	10	644 ± 2	0.357 ± 0.05	-21 ± 1.4	21 ± 4	22 ± 2	56
3	1/2	1	1/1	7	427 ± 6	0.460 ± 0.07	-13 ± 0.4	14 ± 5	18 ± 4	41
4	1/1	0.75	1/2.5	7	380 ± 6	0.426 ± 0.04	-18 ± 0.3	39 ± 4	48 ± 4	34
5	1/3	1	1/2.5	5	447 ± 8	0.346 ± 0.04	-14 ± 5	35 ± 7	13 ± 6	52
6	1/2	0.75	1/5	5	313 ± 7	0.263 ± 0.03	-17 ± 2	42 ± 5	50 ± 3	32
7	1/1	0.5	1/1	5	629 ± 2	0.840 ± 0.008	-22 ± 6	21 ± 7	66 ± 5	29
8	1/2	0.5	1/2.5	10	638 ± 9	0.272 ± 0.005	-10 ± 0.1	22 ± 7	35 ± 4	41
9	1/1	1	1/5	10	301 ± 5	0.334 ± 0.10	-23 ± 0.8	18 ± 2	15 ± 4	11

TABLE 3 | ANOVA table for the average particle size and polydispersity index of PLA-INH-VC NPs.

Size						
Source	Sum of squares	Degree of freedom	Mean square	<i>f</i>	<i>p</i>	
Model	3.669E-06	6	6.115E-07	21.62	0.0449	Significant
A-Drug/PLA ratio	6.586E-07	2	3.293E-07	11.64	0.0791	
B-PVA concentration	1.581E-06	2	7.905E-07	27.94	0.0345	
C-Organic to aqueous phase ratio	1.430E-06	2	7.148E-07	25.27	0.0381	
Residual	5.657E-08	2	2.829E-08			
Cor total	3.726E-06	8				
PDI						
Model	4.92	4	1.23	7.71	0.0365	Significant
A-Drug/PLA ratio	1.91	2	0.9573	6.00	0.0625	
C-Organic to aqueous phase ratio	3.00	2	1.50	9.42	0.0307	
Residual	0.6380	4	0.1595			
Cor total	5.56	8				

of ζ potential varied from -13 ± 0.3 to -23 ± 0.8 mV. Reducing the particle size with increasing PVA concentration can improve emulsion stabilization by preventing droplet coalescence. When the concentration of PVA increases, the polymer molecules surround the droplets more effectively, creating a barrier to their merging and aggregation. This prevents the formation of larger particles in the process of nanoparticle synthesis and promotes the formation of a more stable emulsion with smaller particles, which is confirmed by the ζ potential results. The authors of [15–17] also confirmed this mechanism of the influence of PVA concentration on emulsion stabilization and particle size.

As we can see, the phase ratio (organic/aqueous phase) in the double emulsion method plays an important role. The average particle size and polydispersity decreased as the ratio of the organic phase to aqueous phase increased. Thus, at a 1:5 phase ratio of organic phase to aqueous phase, a minimum mean particle size of 301 ± 5 nm and a maximum percentage of drug absorption of about 80% was observed (Table 2). This is because increasing the aqueous phase can improve emulsion stability and prevent droplet coalescence, which also encourages the formation of smaller particles. This effect is supported by experimental data and literature sources [18].

With increasing homogenization time, the average particle size increases and the polydispersity decreases. With increasing homogenization time, more stirring of the components occurs, which promotes a more uniform distribution of particles and decreases the differences in particle size. This can lead to an increase in the average particle size as smaller particles can become larger due to fusion or aggregation during prolonged homogenization [19]. At the same time, the reduction of polydispersity is because more intensive mixing helps to reduce the

variation of particle sizes and makes them more homogeneous in size [15].

Applying Design Expert software and a desirability function, we performed computer optimization to identify the influence of independent parameters on the results. Based on the ANOVA results obtained using Design Expert software, we proposed optimal solutions for the production of PLA-INH-VC nanoparticles. We used a desirability function to determine the levels of factors that minimize particle size and polydispersity index. A solution with a desirability factor of 88.6% was selected as the most preferred solution, which provides satisfactory values of PDI and particle size. Then we synthesized nanoparticles by following the optimal conditions: drug/polymer ratio 1/2 (by weight), PVA concentration 1%, organic to aqueous phase ratio 1:5 (by volume) at a homogenization time of 5 min.

The experimental result of average particle size and drug loading showed acceptable agreement with the model (Table 4). There is a big difference between the predicted and experimental polydispersity index and the NPs yield. However, the experimental polydispersity index is lower than the model, which proves that the nanoparticles are quite monodisperse. Also, NPs yield is higher than predicted, which gives us more produced nanoparticles. We believe that a high value may arise from the transformation of the results required for the prediction. This study shows that the Taguchi method is one of the suitable methods for the optimization of experimental conditions to achieve the minimum nanoparticle size of PLA-INH-VC NPs.

The shape and surface morphology of the produced nanoparticles were evaluated by scanning electron microscopy (SEM).

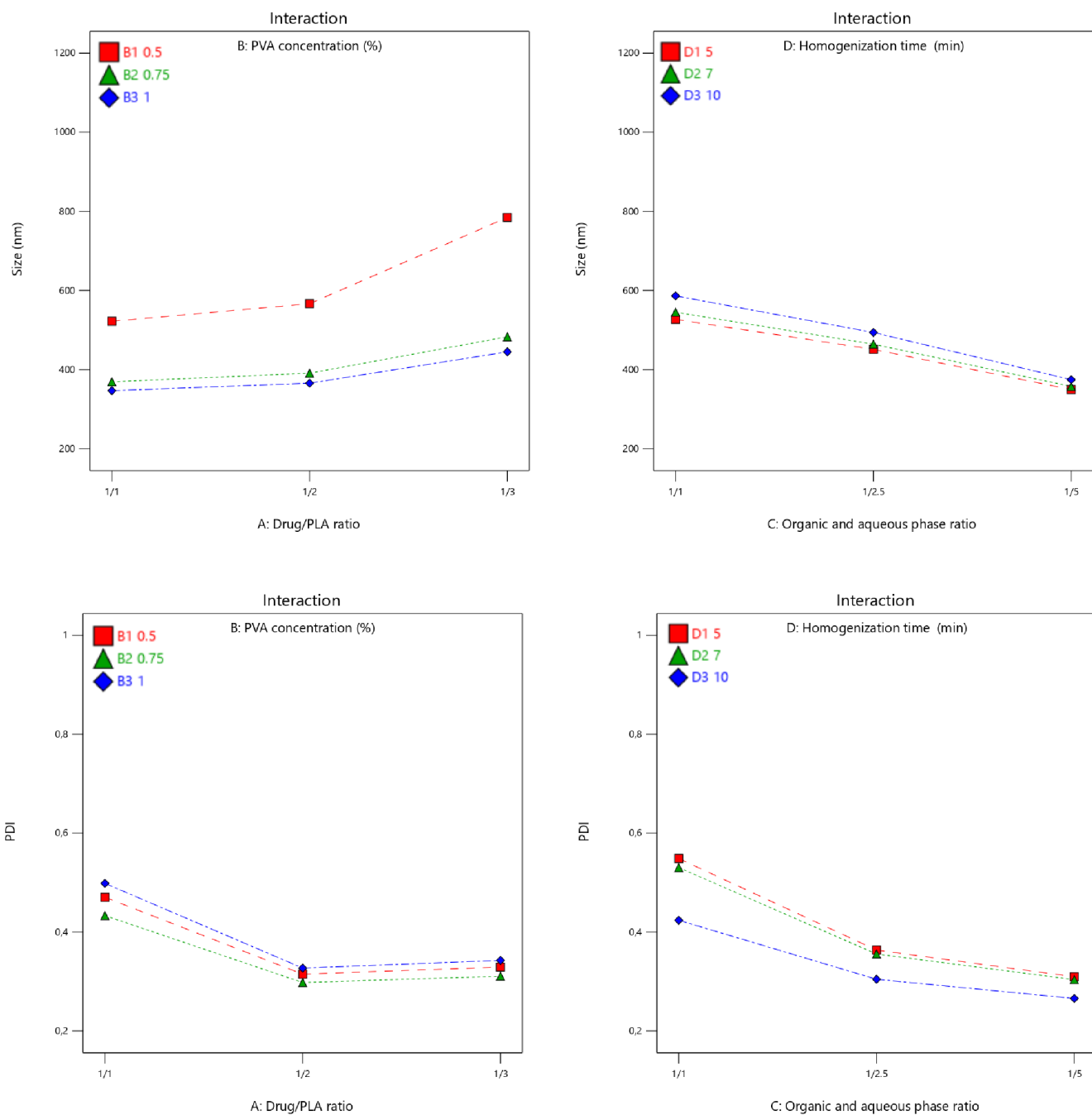


FIGURE 1 | Effect of parameters on average particle size and polydispersity of PLA-INH-VC NPs.

TABLE 4 | Predicted and experimental results for PLA-INH-VC NPs.

	Size (nm)	PDI	Drug loading (%)		NPs yield (%)
			INH	VC	
Predicted	298	0.287	40	41	37
Experimental	296 ± 10	0.216 ± 0.004	38 ± 4	39 ± 6	42
Error %	0.67	25	5	5	13

The study showed that most of the nanoparticles possessed spherical shape. The surface of the particles had a characteristic smoothness (Figure 2).

Thermal gravimetric analysis (TGA) usually provides information on mass loss as a function of temperature. The TGA and differential scanning calorimetry (DSC) thermograms of

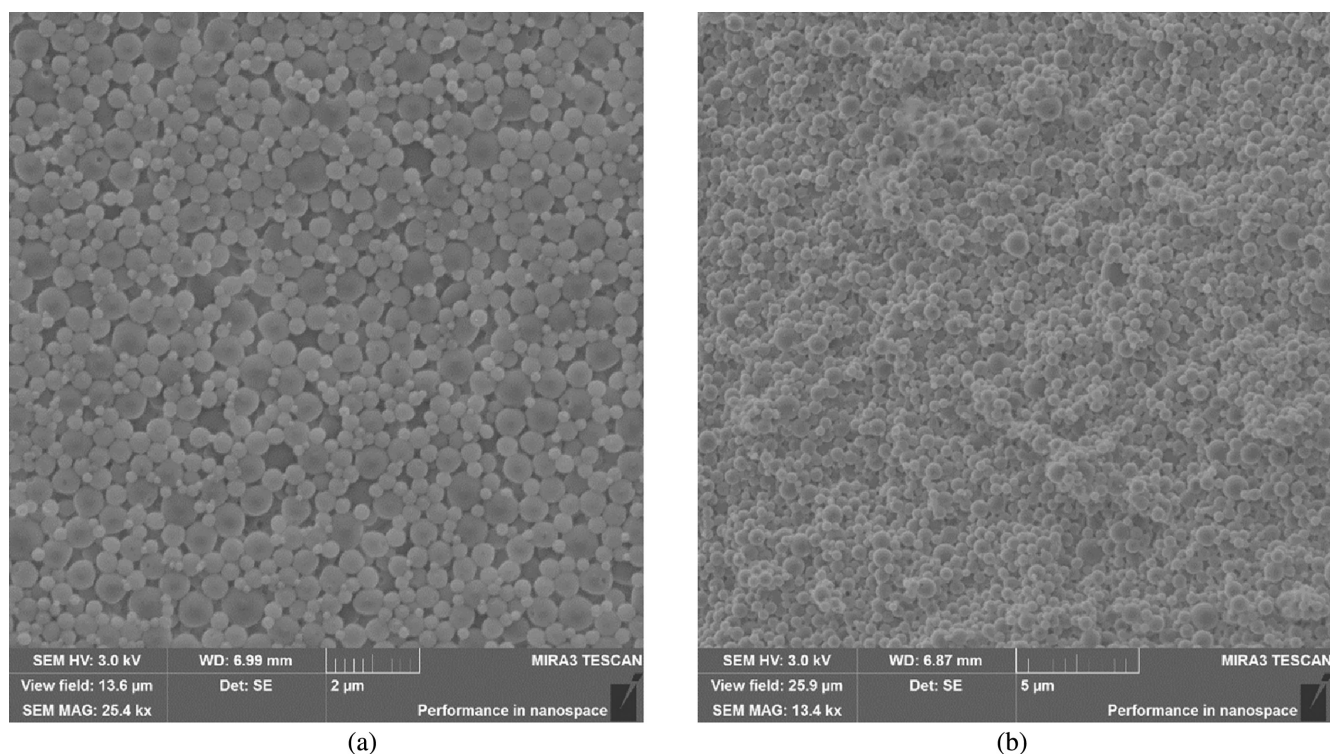


FIGURE 2 | Scanning electron micrograph of PLA-INH-VC nanoparticles prepared under optimized experimental conditions (a) scale = 2 μm and (b) scale = 5 μm .

pure INH, VC, PLA, and PLA-INH-VC NPs were presented in Figure 3.

INH shows a characteristic endothermic peak at 179.5°C with enthalpy change (Figure 3a), indicating phase transition (melting point 170°C–174°C) of this drug without loss of mass. In the temperature range from 260°C to 360°C, a mass loss of up to 68% is observed, which probably corresponds to the decomposition temperature of the drug [20]. Figure 3b shows the TGA/DSC of VC. In a nitrogen atmosphere, VC began to decompose at approximately 195°C, and the maximum rate of decomposition was observed at approximately 250°C. At 600°C, approximately 30% of the original sample remained. Analysis of the DSC curve shows that VC changes its composition at about 198°C, which is characterized by a strong endothermic peak. For pure PLA (Figure 3c), an endothermic peak at 350°C was observed, which was accompanied by mass loss. The maximum decomposition temperature of pure PLA was at 373°C and the mass loss was about 90%. During thermal degradation, PLA decomposes to lactic acid and further to carbon dioxide and water [21]. When the PLA-INH-VC complex is heated, it starts to decompose at 307°C (Figure 3d). The curve is analogous to the thermogram of the polymer, which indicates the absence of chemical interaction between the polymer and drugs.

FT-IR spectra of pure drug and vitamin, pure polymer, and nanoparticles were compared and shown in Figure 4. For pure INH, it shows broad and intense characteristic peaks at 3306 cm^{-1} , which are related to N–H stretching. The C–H stretching vibrations of the heteroaromatic structure are evident in the range of 3100 cm^{-1} for asymmetric stretching, while the

peaks at 1412 cm^{-1} are related to the symmetric ring vibrations of the C–C bonds. The C–N stretching absorption of aliphatic amines is weakly pronounced and is noted at 1060 cm^{-1} [22]. The spectrum includes bands at 671 and 1556 cm^{-1} corresponding to C=C=O and H–N–N bends, respectively [23]. The spectra of pure VC revealed characteristic peaks for the stretching and bending frequencies of functional groups as the identity of its molecular structure and purity. It showed intense peaks at 3526 cm^{-1} for alcoholic O–H stretching, 3031 and 2916 cm^{-1} for C–H stretching of alkenes and alkanes, 1755 cm^{-1} for C=O stretching, 1670 cm^{-1} for C=C stretching, and 1320 cm^{-1} for C–O stretching [24]. The spectra of pure PLA polymer were identified with specific peaks for the presence of relevant functional groups such as 3440 cm^{-1} for O–H stretching, 2989 cm^{-1} for symmetric and asymmetric C–H valence vibrations from CH_3 , 1750 and 1180 cm^{-1} which are related to C=O stretching and C–O–C stretching [25, 26].

Significant peaks of drug and VC were overlapped with PLA due to the presence of common functional groups in both drug and polymer. The chemical nature of the drug remained undisturbed and stable which is confirmed by other characterization studies such as HPLC analysis in the process of determining the extent of drug encapsulation and the drug release from the nanoparticles. Thus, it was confirmed that there were no significant interactions between the drug and the polymer.

Drug and VC release from the polymeric matrix were studied in phosphate-buffered saline at pH 7.4 by dialysis at 37°C. The in vitro release process is presented in Figure 5. The results demonstrated a biphasic release process, where a high burst of

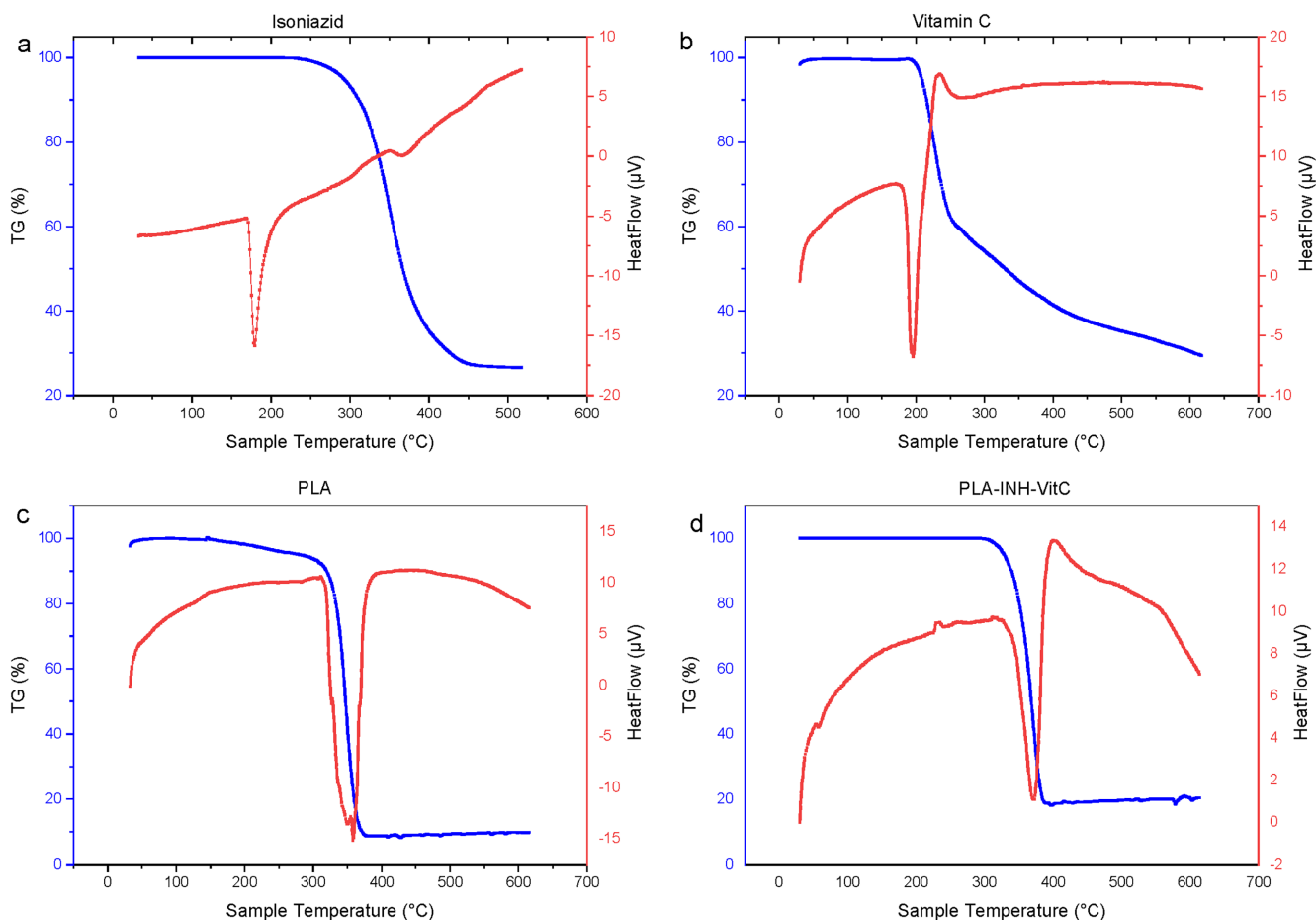


FIGURE 3 | TGA and DSC analysis results for isoniazid, vitamin C, polylactide, and polylactide nanoparticles loaded with isoniazid and vitamin C.

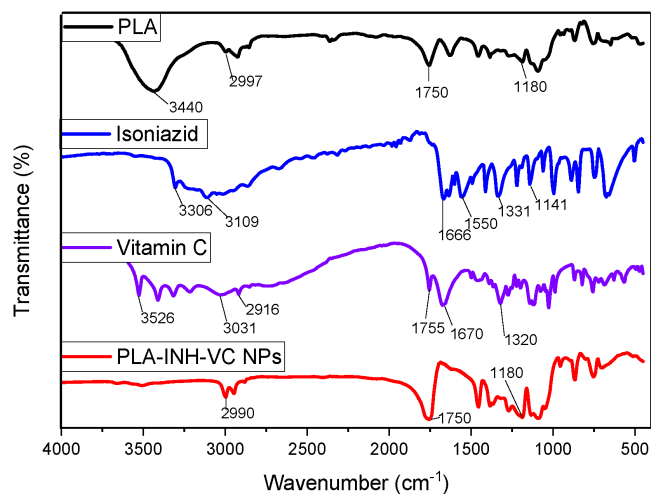


FIGURE 4 | FT-IR spectra of poly(lactic acid) (PLA), isoniazid, vitamin C, and PLA-INH-VC nanoparticles.

release in the first hour (more than 50% for INH and more than 30% for VC) is observed due to the presence of the drug on the surface of the nanoparticles. The burst release is followed by a slower release of the remaining drug and vitamin encapsulated in the PLA matrix. Not all of the loaded INH and VC are released after 24 h, only 70% and 50%, respectively.

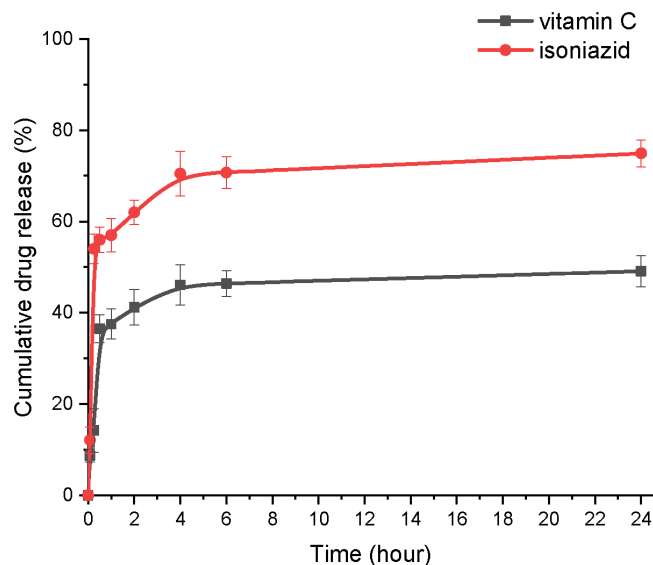


FIGURE 5 | In vitro release rate of isoniazid and vitamin C from polylactide nanoparticles.

Adsorbed molecules on the particle surface are rapidly desorbed upon contact with the dissolution medium. Diffusion of drug, erosion and swelling of the polymer matrix, and degradation of polymer are the main mechanisms of drug release. Since the

degradation of PLA is slow, the release of INH from nanoparticles will mainly depend on the diffusion of the drug during the experimental studies.

To confirm the mycobacterial activity of the produced nanoparticles, studies were carried out. To determine the mycobacterial activity, polylactide NPs were synthesized with INH and VC with VC concentrations of 25, 50, and 100 mg/mL. The concentration of INH was constant at 25 mg/mL. Blank PLA NPs without drugs were used as control. In the in vitro experiment, studies were performed on cultures of drug-resistant INH-resistant MBT strains. The study of antitubercular activity was carried out on a dense Lowenstein–Jensen medium. The results are presented in Figure 6.

While PLA-INH NPs inhibited the growth of MTB cultures for 1 week, the addition of VC increased the time of culture growth

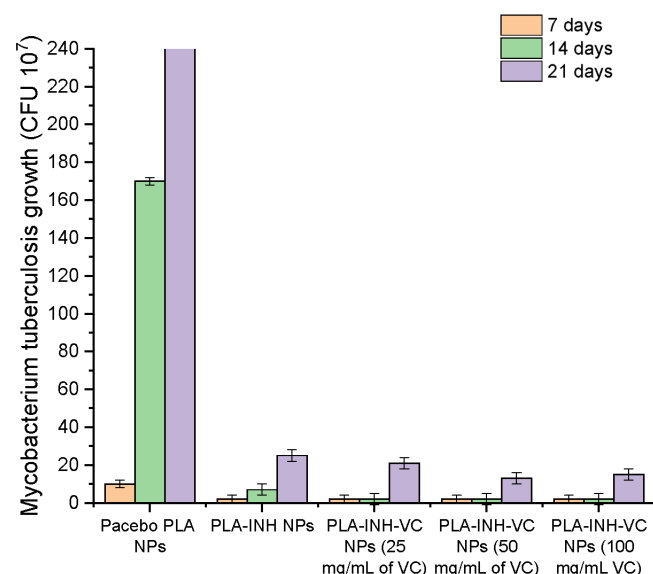


FIGURE 6 | Results of mycobacterial activity of PLA-INH-VC NPs.

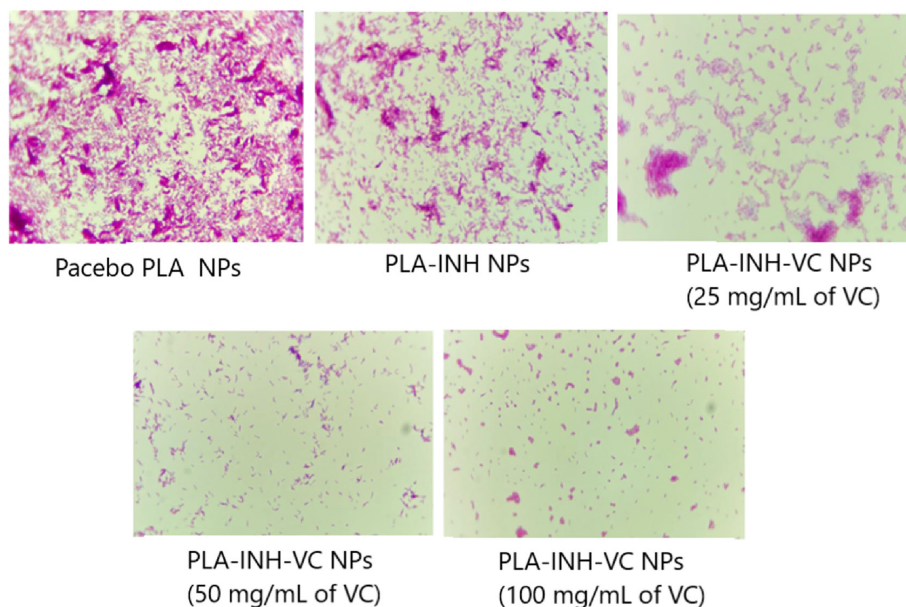


FIGURE 7 | Ziehl–Neelsen staining of MTB smears of specimens with positive results.

suppression to 2 weeks. This suggests that VC can be used as an adjunctive therapy. The concentration of VC from 25 to 100 mg/mL had no effect on mycobacterial growth suppression.

As a result of the study of the potential for antitubercular activity, it was found that in an in vitro experiment, nanoparticles demonstrated antitubercular activity against an INH-resistant strain with moderate growth suppression. Nanoparticles altered the morphology of mycobacterium. Surface medium growth was visualized on 7–21 days as small or large lump-like matt or shiny isolated colonies, or as a wrinkled milky or white with a yellowish tinge (Figure 7). In addition, the use of VC at a low dose may be a protective strategy to reduce INH-induced liver damage in humans [5]. At high concentrations of VC, its antioxidant effect was not increased compared to the low doses.

Based on the obtained results of the study of mycobacterial activity, there is hope for the further application of polymeric NPs with anti TB drugs for the treatment of TB. Thus, the use of NPs with prolonged action will help to solve such problems of non-compliance with the dosage and frequency of drug administration, reducing side effects and overcoming multidrug resistance of mycobacteria to INH.

4 | Conclusion

Thus, in this work, PLA-INH-VC NPs were produced by the double emulsion method, where several factors affecting particle size were investigated: polymer-to-drug ratio, organic-to-aqueous phase ratio, surfactant concentration, and homogenization time. Stable nanoparticles were produced at a drug/polymer ratio of 1:2 by weight, PVA concentration of 1%, organic to aqueous phase ratio of 1/5 by volume, homogenization time of 5 min with minimum average particle size of 296 ± 10 nm, and narrow particle distribution of 0.216 ± 0.004 . The NPs yield was 42%, and the INH and VC loading were 38% and 39%, respectively. TGA/DSC

and IR studies revealed that molecularly dispersed drugs (INH and VC) were present in the polylactide nanoparticles. Based on the results of the study of the mycobacterial activity of the produced nanoparticles, it was determined that PLA-INH-VC nanoparticles inhibited the growth of INH-resistant mycobacteria. These results indicate the potential of nanoparticles for the efficient delivery of drug components, which opens up prospects for their medical applications.

Acknowledgments

This research was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan under Grant No. AP14871344, titled "Development of colloidal biopolymer-based drug delivery systems for tuberculosis chemotherapy."

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Supporting Information

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