How to Cite: Galiyeva, A.R., Tazhbayev, Ye.M., Zhumagaliyeva, T.S., & Daribay, A.T. (2022) Encapsulation of Isoniazid in Polylactide-Co-Glycolide Nanoparticles by Nanoprecipitation. *Bulletin of the University of Karaganda – Chemistry, 107(3), 208-217.* https://doi.org/10.31489/2022Ch3/3-22-17

Article Received: 31 May 2022 | Revised: 28 June 2022 | Accepted: 19 July 2022 | Published online: 28 July 2022

UDC 541.64 https://doi.org/10.31489/2022Ch3/3-22-17

A.R. Galiyeva*, Ye.M. Tazhbayev, T.S. Zhumagaliyeva, A.T. Daribay

Karagandy University of the name of academician E.A. Buketov, Kazakhstan
*(*Corresponding author's e-mail: aldana_karaganda@mail.ru)*

Encapsulation of Isoniazid in Polylactide-Co-Glycolide Nanoparticles by Nanoprecipitation

The use of polymeric materials as drug carriers has several advantages, such as prolongation of drug action and reduction of drug side effects. In this study, we consider the methods for the preparation of polylactide-co-glycolide (PLGA) polymeric nanoparticles with the anti-tuberculosis drug (ATD), isoniazid, by nanoprecipitation. Polymeric nanocarriers were obtained by varying individual parameters such as the nature of solvent and non-solvent, drug/polymer ratio, and stabilizer concentration. It was determined that the average particle size depends on the type of non-solvent. When alcohols were used, the average size increased in the sequence: ethanol < isopropanol < isobutanol. The type of solvent is an important factor in the formation of nanoparticles and their final characteristics. With an increase in the drug/polymer ratio, the average size of nanoparticles also increased. The size of obtained nanoparticles varied from 93 to 869 nm. Thermogravimetric and differential scanning calorimetry analyses were carried out to confirm the incorporation of the drug into the polymer matrix. In addition, polymer degradation and the degree of release of isoniazid from the polymeric matrix at different pH were studied. It was identified that the nanoprecipitation method can be used not only for hydrophobic but also for hydrophilic drugs.

Keywords: nanoparticles, polylactide-co-glycolide, PLGA, isoniazid, nanoprecipitation, hydrophilic drugs, anti-tuberculosis drugs.

Buketov University

Introduction

There are currently many drugs available for the treatment of tuberculosis. Due to the need for intensive use, they have significant toxic effects on the body and lead to the development of resistance to many drugs [1–3]. When using new dosage forms in the form of polymer nanoparticles (NPs) for the delivery of anti-TB drugs (ATD), the following results can be achieved: providing prolonged drug release while maintaining effective drug concentration in blood for a given period of time; reducing the side effects of the drug, thereby increasing its therapeutic effect [4–7].

However, the choice of polymer materials and methods should be based on many factors such as particle size, solubility and stability in water, permeability and charge of drug molecules, degree of biodegradation, biocompatibility, and toxicity [8, 9]. Encapsulation of hydrophilic compounds is not easy and remains a problem for highly loaded drug delivery systems, mainly due to drugs leaking into the external aqueous phase during nanoparticle production [10]. Since water-soluble drugs can be immobilized into nanoparticles in various ways, the nanoprecipitation method was chosen.

Nanoprecipitation was patented by Fessi et al. in 1989 [11]. It has mainly been used to encapsulate hydrophobic drug molecules since its development. Several polymers have been developed for this purpose, in particular biodegradable polyesters such as polylactide (PLA), polylactide-co-glycolide copolymer (PLGA), and poly-e-caprolactone (PCL) [3, 12–15]. As nanoprecipitation is a direct method, it has many advantages: easy to perform, no energy or time consumption.

At the same time, the nanoprecipitation method has a number of disadvantages. This method is mainly suitable for compounds of a hydrophobic nature, soluble in ethanol or acetone, but exhibiting limited solubility in water. Consequently, reduced or even zero leakage of the drug into the external medium results in an unsatisfactory degree of binding [11, 16, 17]. Some studies concerning the immobilization of hydrophilic drugs have also given promising results. Yoo et al. [18] proved that the correct choice of solvent and non-solvent can lead to the formation of nanoparticles, which means that the nanoprecipitation method can be carried out with other solvents and can be extended to hydrophilic preparations.

This study aims to optimize the nanoprecipitation method for obtaining PLGA-based nanoparticles immobilized with isoniazid, which exhibit a controlled particle diameter up to 300 nm, a narrow size distribu-

 © 2022 The Authors. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

tion, with high encapsulation efficiency and loading capacity. For this purpose, we choose different alcohols as non-solvents, different solvents, and varied parameters, such as stabilizer concentration and drug/polymer ratio.

Experimental

Materials

Isoniazid (INH) (≥ 99 %), polyvinyl alcohol (PVA) (hydrolyzed, MW 9000–10000), polylactide-co-glycolide copolymer (PLGA 50:50, MW 24 000 to 38 000), ethyl acetate (≥ 99 %) (all from Sigma Aldrich, Steinheim, Germany) were used without further purification. Ethanol was purchased from DosFarm (Almaty, Kazakhstan). The organic solvents (Dichloromethane (DCM), Dimethyl Sulfoxide (DMSO), and Chloroform) were obtained from Component Reagent (Russia). Sodium hydrophosphate and potassium dihydrophosphate were used to prepare the phosphate-buffered saline solution.

Preparation of polylactide-co-glycolide nanoparticles immobilized with isoniazid by nanoprecipitation

To obtain nanoparticles by nanoprecipitation, the polymer was dissolved in a suitable organic solvent and isoniazid in alcohol (drug/polymer ratio from 1:1 to 1:5). The resulting polymer solution is then mixed with the isoniazid. The obtained solution is then added to the dispersed phase (0.1–1 % PVA) by stirring on a magnetic stirrer. The resulting suspension was stirred at room temperature for 6 h on a magnetic stirrer to remove the solvent completely. INH-loaded PLGA nanoparticles were extracted by centrifugation (MiniSpi, Eppendorf, Hamburg, Germany) (14,000 rpm, 20 min). The obtained nanoparticle suspension was washed with distilled water using three centrifugation steps at 14,000 rpm for 15 min each to remove dissolved solids and organic solvent from the mixture. The obtained nanoparticles were dried to a constant weight.

Particle size, polydispersity, and morphology of the obtained nanoparticles

Particle size and polydispersity index (PDI) were determined by dynamic light scattering (DLS) using the Malvern Zetasizer Nano S90 (Malvern Instruments Ltd., Malvern, UK). Each batch of nanoparticles was appropriately diluted with distilled water immediately after production. The shape and surface morphology of the PLGA-INH NPs were examined by scanning electron microscopy (MIRA 3LM TESCAN, Brno, Czech Republic, EU).

Encapsulation efficiency, loading capacity, and PLGA-INH NPs' yield

The amount of isoniazid encapsulated in the polymer nanoparticles was determined by measuring the amount of unencapsulated isoniazid in the aqueous solution recovered after ultracentrifugation and particle washing. The amount of drug in solution was determined by high-performance liquid chromatography (HPLC) (Shimadzu LC-20 Prominence). The encapsulation efficiency, loading capacity, and yield of nanoparticles were calculated as follows:

$$\text{Encapsulation Efficiency}\,(\text{EE}\%) = \frac{\text{Mass of the total drug} - \text{Mass of free drug}}{\text{Mass of total drug}} \times 100\,\%.$$

$$\text{Loading Capacity}\,(\%) = \frac{\text{Mass of the total drug} - \text{Mass of free drug}}{\text{Mass of total nanoparticles}} \times 100\,\%.$$

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

In vitro study of drug release from polymer NPs

Isoniazid release kinetics from the polymeric matrix was determined by dialysis, in phosphate-saline buffer (pH 7.4) at 37°C. The obtained nanoparticles were resuspended in phosphate-buffered saline using ultrasound and placed in a dialysis membrane. The membrane, sealed with clamps, was placed in a beaker with 250 ml of buffer solution, closed with a lid and stirred on a magnetic stirrer at 200 rpm. Dialysates were sampled periodically (3 mL at a time). To study the degree of release from the polymer nanoparticles, the amount of drug released was recorded by HPLC and calculated by the formula:

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

The amount of degraded polymer was determined on a UV spectrophotometer (HP LC-20 Prominence, Shimadzu, Japan) at wavelength λ_{max} = 240 nm for the polymer.

Buketov University

Thermogravimetric analysis and differential scanning calorimetry

Thermogravimetric and differential thermal analyses were performed on a LabSYS evo TGA/DTA/DSC analyzer (Setaram, France) in the 30–550 °C temperature range in an aluminum oxide crucible at a heating rate of 10 °C/min in nitrogen inert medium and flow rate was 30 mL/min by decomposition of a nanoparticle sample.

Statistical analysis

The data are expressed as mean ± standard deviation and analysis was carried out statistically by Minitab 19 Statistical Software. The analysis of data was taken place via one-way analysis of variance (ANOVA).

Results and Discussion

Burkeev et al. [19] synthesized polylactide nanoparticles immobilized with isoniazid by the nanoprecipitation method. In this method, they used water as a non-solvent and acetone as a solvent and obtained NPs with a drug loading of 50 %. Since we used a more hydrophilic polymer, PLGA, and using distilled water as a non-solvent, NPs were not obtained. Therefore, to select a suitable non-solvent, the polymer was dissolved in organic solvent (DMSO) and the drug was dissolved in various non-solvents such as ethanol, isobutanol, isopropanol. The obtained drug solution was added to the polymer solution while stirring on a magnetic stirrer. The suspension was then added to the PVA solution, resulting in the formation of nanoparticles. Table 1 demonstrates the results of the average particle size and PDI of the obtained NPs.

Table 1

The effect of non-solvent on nanoparticle formation and characteristics

№	Type of non-solvent	Average particle size, nm	PDI	NPs' yield, %
1	Ethanol	182±6	0.104±0.031	68±9
2	Isopropanol	415±8	0.701±0.072	28±6
3	Isobutanol	870±9	1±0	32±4

As can be seen from Table 1, the average particle size depends on the nature of the dispersing solvent. Ethanol results in smaller nanoparticles, while the use of isobutanol or isopropanol gives even larger nanoparticles. The size values measured by DLS for ethanol are obtained with high reproducibility (less than 2% deviation between triplicate instances) and with a PDI that shows a homogeneous suspension of nanoparticles. When the drug/polymer ratio and PVA concentration remains constant, the particle size gradually increases in the series of homologous alcohols used as non-solvent.

The interest in using alcohols as insoluble substances lies in their relatively low dielectric constant (ε value) [17]. If the dielectric constant is lower, the insoluble substance will dissolve hydrophilic compounds and prevent drug leakage. Therefore, ethanol is the most suitable in this regard, as its dielectric constant is 24.6, which is far from the value of water (80.1) [17].

In the next step of the study, four different organic solvents were chosen to select a suitable solvent for the polymer: DMSO, DCM, acetone, chloroform, and the drug were dissolved in ethanol. Table 2 presents the obtained results.

Table 2

The effect of solvent type on average size and yield of nanoparticles

№	Solvent type	Average particle size, nm	PDI	NPs' yield, %
1	DMSO	182 ±6	0.104±0.031	68±9
2	Acetone	289±7	0.229±0.052	34±8
3	DCM	385±9	0.613±0.300	29±4
4	Chloroform	378±7	0.598±0.200	32±4

As can be seen from Table 2, nanoparticles with the smallest size and polydispersity are formed in DMSO. Other solvents (acetone, chloroform, DCM) are able to dissolve the PLGA copolymer, but they form large nanoparticles, while the solubility of polymers in DMSO is higher, and this solvent mixes well with the external phase, in our case with distilled water. This leads to the fastest diffusion of the two phases and more

Buketov University

nanoparticles. Accordingly, the lower the phase tension or the lower the interaction parameter of the organic solvent and the external phase, the higher the yield of nanoparticles [20, 21]. Accordingly, a lower phase tension or a weaker interaction parameter of the organic solvent and the external phase corresponds to a higher yield of nanoparticles. Consequently, the particle size obtained with the DMSO solvent is smaller and the NPs' yield is higher than with other solvents.

Drug and polymer ratios were varying parameters. Thus, the dependence of the polymer-drug ratio on particle size was investigated; for best results, nanoparticles should be in the range of 50–500 nm for alveolar macrophage phagocytosis [22]. Other characteristics, such as the encapsulation efficiency and loading capacity, were also investigated during the study. PLGA nanoparticles immobilized with isoniazid were obtained in five different ratios. The average size of the obtained NPs did not exceed 400 nm. Table 3 shows the physicochemical characteristics of the nanoparticles obtained by the DLS method.

Table 3

Average size and polydispersity of NPs at different INH/PLGA ratios

INH/PLGA ratio	Average particle size, nm	PDI	Encapsulation efficiency, %	Loading capacity, %	NPs' yield, %
1:1	221±8	0.124±0.023	42±6	39±6	41±9
1:2	275±4	0.335±0.044	48±8	42±9	44±9
1:3	288±5	0.372±0.040	52±6	46±6	57±9
1:4	322±7	0.444±0.041	60±5	58±8	63±5
1:5	317±9	0.448±0.012	56±8	53±9	59±7

With an increase in the drug/polymer ratio from 1:1 to 1:5, the particle sizes changed from 221±8 to 322±7 nm, and PDI from 0.124±0.023 to 0.448±0.012. An increase in the PLGA concentration led to an increase in particle size. This can be explained by the fact that an increase in the polymer concentration leads to an increase in the viscosity of the organic phase, which in turn increases the forces that counteract particle breakdown, leading to the formation of larger NPs [23, 24].

Besides the average size and polydispersity of the NPs, the nanoparticle production efficiency, such as the encapsulation efficiency and loading capacity, as well as the NPs' yield, are also important factors. The values of encapsulation efficiency for PLGA-INH NPs ranged from 42±6 % to 60±5 % and loading capacity from 39±6 % to 58±8 %. It can be seen from these results that increasing the PLGA concentration increases the encapsulation efficiency and drug loading into the polymer matrix. This can be explained by the fact that increasing the polymer concentration is likely to increase the viscosity of the organic phase, thus increasing the diffusion resistance between the organic and aqueous phases, thereby capturing more drugs in the NPs [25]. Based on the obtained data, it can be concluded that the optimal INH/PLGA ratio is 1:4, although it gives the largest size, but also the highest encapsulation efficiency and NPs' yield (60±5 and 63±5 %, respectively).

The study of the effect of various stabilizer concentrations on the formation of nanoparticles was a continuation of our work. PVA was used as a stabilizer. Figure 1 illustrates the results.

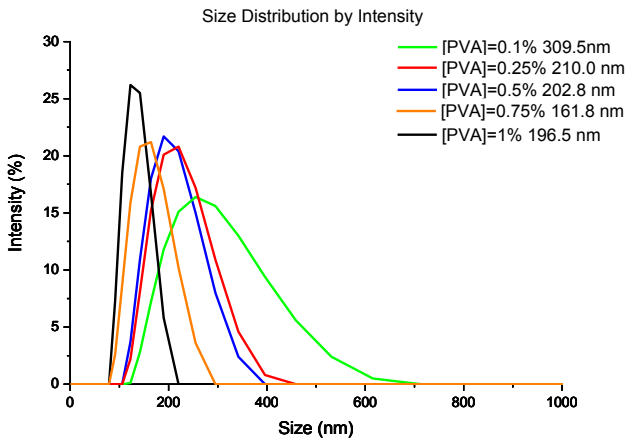
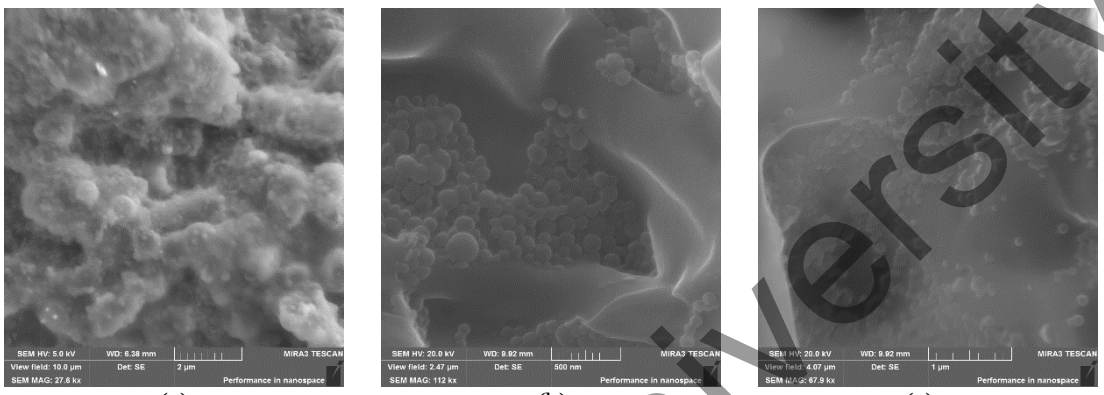


Figure 1. Distribution curves of NPs produced at different PVA concentrations

Buketov University

Figure 1 shows that with an increase in PVA concentration from 0.1 to 0.75 %, the average particle size of the NPs decreases (from 309.5±8 nm to 161.8±5 nm) due to stabilization of the medium. The small particle size of the obtained NPs is because of the high concentration of surfactant, which prevents coalescence of the globules, protects and stabilizes the droplets formed during emulsification, and leads to the formation of smaller emulsion droplets [26].

The morphological characteristics of the dried nanoparticles were determined by SEM, showing a regular spherical shape and smooth surface. Figure 2 shows a microphotograph of PLGA nanoparticles obtained by nanoprecipitation under the following conditions: non-solvent — ethanol, organic solvent — DMSO, INH/PLGA ratio 1:4, at different concentrations of PVA. The image shows both aggregates and individual parts of nanoparticles. The formation of aggregates can be explained by incomplete washing of the surfactant and the drying process.



(a) (b) (c)

a — 0.5 %; *b* — 0.75 %; *c* — 1 %

Figure 2. SEM image of PLGA – INH NPs at different PVA concentrations

Thermogravimetric analysis of the individual components of the system and the obtained NPs was performed to confirm the incorporation of isoniazid into the polylactide-co-glycolide NPs complex. Figure 3 illustrates TGA and DSC curves of isoniazid, empty PLGA nanoparticles, and PLGA nanoparticles immobilized with isoniazid.

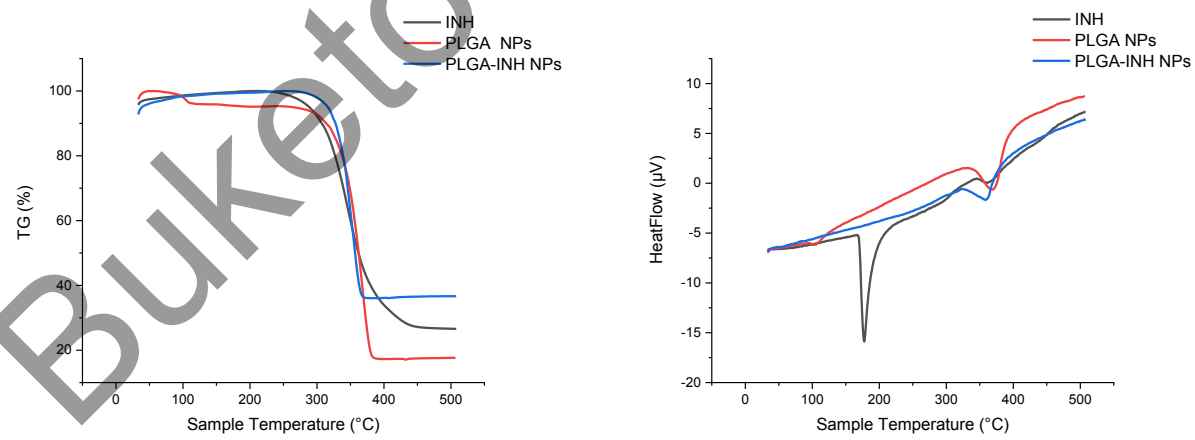


Figure 3. TGA and DSC of isoniazid, PLGA nanoparticles and PLGA particles immobilised with isoniazid

Thermal analysis (Figure 3) of pure INH showed an endothermic peak at 172 °C corresponding to its melting point and decomposition, which indicates that the drug could be in a crystalline form. Empty PLGA nanoparticles show an endothermic peak at 367 °C, which probably corresponds to their melting stage. The weight loss of the polymer NPs exceeds 76 % in the temperature range of 300–385 °C. For PLGA-INH NPs, the DSC curve shows an endothermic peak at 360 °C. On the TGA curve, the nanoparticles decompose at 300–370 °C and the weight loss exceeds 66 %. From the curves obtained, it can be concluded that the thermal degradation of polylactide-co-glycolide with the drug is slower than the degradation of free

Buketov University

polylactide-co-glycolide nanoparticles. In addition, a shift of the melting peak indicates an interaction of the drug with the polymer.

In vitro release of isoniazid from polylactide-co-glycolide NPs was studied by dialysis at different pH values, simulating the *in vitro* conditions of the gastrointestinal tract of people. Figure 4 shows a cumulative drug release from nanoparticles prepared by nanoprecipitation under the following conditions: non-solvent — ethanol, organic solvent — DMSO, INH/PLGA ratio 1:4, PVA concentration 0.75%. *In vitro* drug release from NPs typically consists of two phases: a first "burst release" phase followed by a second phase of prolonged release [27]. The first phase is caused by the release of the drug substance, which is adsorbed on the surface of the NPs or dispersed near the surface. The second phase is due to the release of the drug substance residing in the core [28–31]. As shown in Figure 4, "burst release" of the drug is observed at pH 6.86 after 2 hours; at pH 7.4 after 5 hours; and at pH 1.2 was 27 % within an hour. In the simulated stomach fluid (pH 1.2) there is a total (mean) release of the INH dose incorporated in the NPs after 2 h, which corresponds to the estimated transit time of the pharmaceutical form in the stomach environment [32]. After 6 hours, the release rate of isoniazid at pH 7.4 was 55 %, and at pH 6.86, a high release rate of 80 % was detected. This may be because the solubility of isoniazid increases with pH decreasing. We have previously studied the release of a drug from a dialysis membrane in a conventional dosage form (the conventional isoniazid tablet), which provides a single and short-term release of the drug [29]. At pH = 1.2, the drug is released normally and the next doses of the drug are required to achieve a therapeutic effect [33, 34].

From the experimental data, it can be concluded that the pH of the medium has a great influence on drug release. The solubility of the drug increased as the pH of the medium decreased [35, 36]. This partly explains the differences in drug release with changes in pH. However, this could not be the dominant factor in drug release. The release of small drug molecules from a biodegradable matrix may be due to polymer degradation [37].

Further, it was interesting to investigate the polymer degradation process. The polymer biodegradation was determined by dialysis at different pH values at 37°C [38]. Figure 5 shows data on degradation kinetics of PLGA NPs studied by UV-spectrophotometry.

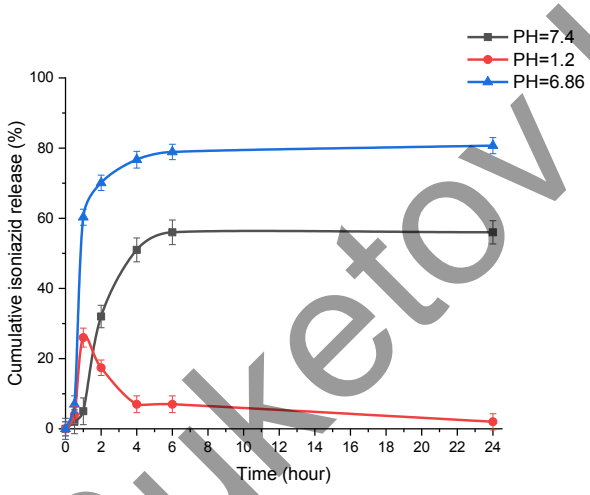


Figure 4. Degree of release of isoniazid from the polymeric matrix at different pH values

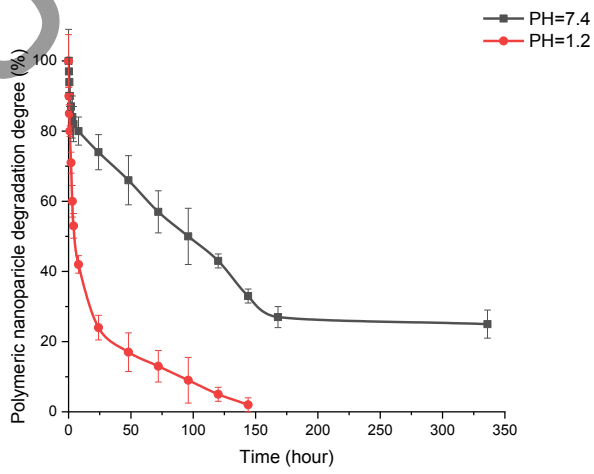


Figure 5. Degradation of PLGA NPs at different pH values

A decrease in the optical density of polymer solutions indicates a decrease in molecular weight as a direct result of degradation [36]. The degradation process over a period of 2 weeks shows that the polymer degrades faster in an acidic than in a neural medium. At pH 1.2, the polymer is completely degraded in six days, but in the beginning it degrades rapidly (60 %) and the remaining 40% is stable and slow to degrade. At pH 7.4, the polymer slowly, evenly degrades to 70 % within two weeks.

Conclusions

This study successfully developed INH-loaded PLGA NPs using the nanoprecipitation method. A number of studies have been carried out to select optimal conditions (non-solvents, solvents, drug-to-polymer ratio, and concentration of surfactant) for the immobilization of the anti-TB drug isoniazid in PLGA NPs. Optimized PLGA nanoparticles were prepared by nanoprecipitation under the following conditions:

Buketov University

non-solvent – ethanol, organic solvent — DMSO, INH/PLGA ratio 1:4, PVA concentration 0.75 % with satisfactory physicochemical characteristics and a loading capacity of up to 50 %. An *in vitro* release analysis confirmed that isoniazid has a controlled release in a neutral medium. It was determined that the degradation of polymeric nanoparticles is faster in an acidic medium and correspondingly the kinetics of isoniazid release from the polymeric matrix is higher than in a neutral medium. This study provides future opportunities for the development of polylactide-co-glycolide-based inhalation dry powders for aerosol delivery of anti-TB drugs to the lungs. In addition, TGA and DSC analyses were performed to confirm the incorporation of isoniazid into the polylactide-co-glycolide matrix. The study results indicate that optimized polymeric nanoparticle formulation could be potentially used for hydrophilic drug delivery systems for tuberculosis treatment.

Acknowledgments

The work was fulfilled within the framework of program-targeted funding of the Ministry of Education and Science of the Republic of Kazakhstan No. AP14871344 “Development of colloidal drug delivery systems based on biopolymers for tuberculosis chemotherapy”.

Buketov University

References

1 Fatima, R., Ashraf, M., Ejaz, S., Rasheed, M. A., Altaf, I., Afzal, M., & Anwar, K. (2013). In vitro toxic action potential of anti tuberculosis drugs and their combinations. *Environmental Toxicology and Pharmacology, 36*(2), 501–513. https://doi.org/10.1016/j.etap.2013.05.008

2 Arbex, M.A., Varella, M.D., Siqueira, H.R., & Mello, F.A. (2010). Antituberculosis drugs: drug interactions, adverse effects, and use in special situations. Part 2: second line drugs. *Jornal brasileiro de pneumologia : publicacao oficial da Sociedade Brasileira de Pneumologia e Tisilogia, 36*, 5, 641-56. https://doi.org/10.1590/S1806-37132010000500016

3 Tazhbayev, Ye.M., Burkeyev, M.Zh., Zhaparova, L.Zh., Zhumagalieva, T.S., & Arystanova, Zh.T. (2018). Nanoparticles on the basis of polylactic acid and polylactic-co-glycolic acids loaded with drugs. *Bulletin of the University of Karaganda – Chemistry, 90*(2), 31–39. https://doi.org/10.31489/2018ch2/31-39

4 Hussain, A., Singh, S., Das, S. S., Anjireddy, K., Karpagam, S., & Shakeel, F. (2019). Nanomedicines as Drug Delivery Carriers of Anti-Tubercular Drugs: From Pathogenesis to Infection Control. *Current Drug Delivery, 16*(5), 400–429. https://doi.org/10.2174/1567201816666190201144815

5 Nasiruddin, M., Neyaz, M. K., & Das, S. (2017). Nanotechnology-Based Approach in Tuberculosis Treatment. *Tuberculosis research and treatment, 2017*, 4920209. https://doi.org/10.1155/2017/4920209

6 Baranyai, Z., Soria-Carrera, H., Alleva, M., Millán-Placer, A. C., Lucía, A., Martín-Rapún, R.,la Fuente, J. M. (2020). Nanotechnology-Based Targeted Drug Delivery: An Emerging Tool to Overcome Tuberculosis. *Advanced Therapeutics, 4*(1), 2000113. https://doi.org/10.1002/adtp.202000113

7 Wang, W., Guo, H., Lin, S., Xiao, X., Liu, Y., Wang, Y., & Zhou, D. (2022). *Biosafety materials for tuberculosis treatment. Biosafety and Health.* https://doi.org/10.1016/j.bsheal.2022.03.013

8 Mitchell, M. J., Billingsley, M. M., Haley, R. M., Wechsler, M. E., Peppas, N. A., & Langer, R. (2020). Engineering precision nanoparticles for drug delivery. *Nature Reviews Drug Discovery, 20*(2), 101–124. https://doi.org/10.1038/s41573-020-0090-8

9 Elmowafy, E. M., Tiboni, M., & Soliman, M. E. (2019). Biocompatibility, biodegradation and biomedical applications of poly(lactic acid)/poly(lactic-co-glycolic acid) micro and nanoparticles. *Journal of Pharmaceutical Investigation, 49*(4), 347–380. https://doi.org/10.1007/s40005-019-00439-x

10 Becker Peres, L., Becker Peres, L., de Araújo, P. H. H., & Sayer, C. (2016). Solid lipid nanoparticles for encapsulation of hydrophilic drugs by an organic solvent free double emulsion technique. *Colloids and Surfaces B: Biointerfaces, 140*, 317–323. https://doi.org/10.1016/j.colsurfb.2015.12.033

11 Fessi, H., Puisieux, F., Devissaguet, J. P., Ammoury, N., & Benita, S. (1989). Nanocapsule formation by interfacial polymer deposition following solvent displacement. *International Journal of Pharmaceutics, 55*(1), R1–R4. https://doi.org/10.1016/0378-5173(89)90281-0

12 Martínez Rivas, C. J., Tarhini, M., Badri, W., Miladi, K., Greige-Gerges, H., Nazari, Q. A., Elaissari, A. (2017). Nanoprecipitation process: From encapsulation to drug delivery. *International Journal of Pharmaceutics, 532*(1), 66–81. https://doi.org/10.1016/j.ijpharm.2017.08.064

13 Govender, T., Stolnik, S., Garnett, M. C., Illum, L., & Davis, S. S. (1999). PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug. *Journal of controlled release: official journal of the Controlled Release Society, 57*(2), 171–185. https://doi.org/10.1016/s0168-3659(98)00116-3

14 Da Costa, D., Exbrayat-Héritier, C., Rambaud, B., Megy, S., Terreux, R., Verrier, B., & Primard, C. (2021). Surface charge modulation of rifampicin-loaded PLA nanoparticles to improve antibiotic delivery in Staphylococcus aureus biofilms. *Journal of Nanobiotechnology, 19*(1). https://doi.org/10.1186/s12951-020-00760-w

15 Rani, S., Gothwal, A., Pandey, P. K., Chauhan, D. S., Pachouri, P. K., Gupta, U. D., & Gupta, U. (2018). HPMA-PLGA Based Nanoparticles for Effective In Vitro Delivery of Rifampicin. *Pharmaceutical Research, 36*(1). https://doi.org/10.1007/s11095-018-2543-x

16 Barichello, J. M., Morishita, M., Takayama, K., & Nagai, T. (1999). Encapsulation of Hydrophilic and Lipophilic Drugs in PLGA Nanoparticles by the Nanoprecipitation Method. *Drug Development and Industrial Pharmacy*, *25*(4), 471–476. https://doi.org/10.1081/ddc-100102197

17 Bilati, U., Allémann, E., & Doelker, E. (2005). Development of a nanoprecipitation method intended for the entrapment of hydrophilic drugs into nanoparticles. *European Journal of Pharmaceutical Sciences*, *24*(1), 67–75. https://doi.org/10.1016/j.ejps.2004.09.011

18 Yoo, H. S., Choi, H. K., & Park, T. G. (2001). Protein-fatty acid complex for enhanced loading and stability within biodegradable nanoparticles. *Journal of pharmaceutical sciences*, *90*(2), 194–201. https://doi.org/10.1002/1520-6017(200102)90:2<194::aid-jps10>3.0.co;2-q

19 Burkeev, M. Z., Tazhbaev, E. M., Zhaparova, L. Z., Zhappar, N. K., & Zhumagalieva, T. S. (2016). Synthesis and Characterization of Poly(DL-Lactic Acid) Nanoparticles Loaded with the Antituberculosis Drug Isoniazid. *Pharmaceutical Chemistry Journal*, *50*(9), 608–611. https://doi.org/10.1007/s11094-016-1500-4

20 Song, K. C., Lee, H. S., Choung, I. Y., Cho, K. I., Ahn, Y., & Choi, E. J. (2006). The effect of type of organic phase solvents on the particle size of poly(d,l-lactide-co-glycolide) nanoparticles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *276*(1-3), 162–167. https://doi.org/10.1016/j.colsurfa.2005.10.064

21 Galindo-Rodriguez, S., Allémann, E., Fessi, H., & Doelker, E. (2004). Physicochemical Parameters Associated with Nanoparticle Formation in the Salting-Out, Emulsification-Diffusion, and Nanoprecipitation Methods. *Pharmaceutical Research*, *21*(8), 1428–1439. https://doi.org/10.1023/b:pham.0000036917.75634.be

22 Costa, A., Pinheiro, M., Magalhães, J., Ribeiro, R., Seabra, V., Reis, S., & Sarmento, B. (2016). The formulation of nanomedicines for treating tuberculosis. *Advanced Drug Delivery Reviews*, *102*, 102–115. https://doi.org/10.1016/j.addr.2016.04.012

23 Tefas, L. R., Tomuță, I., Achim, M., & Vlase, L. (2015). Development and optimization of quercetin-loaded PLGA nanoparticles by experimental design. *Medicine and Pharmacy Reports*, *88*(2), 214–223. https://doi.org/10.15386/cjmed-418

24 Elsewedy, H. S., Dhubiab, B. E. A., Mahdy, M. A., & Elnahas, H. M. (2020). Development, optimization, and evaluation of PEGylated brucine-loaded PLGA nanoparticles. *Drug Delivery*, *27*(1), 1134–1146. https://doi.org/10.1080/10717544.2020.1797237

25 Chishti, N., Jagwani, S., Dhamecha, D., Jalalpure, S., & Dehghan, M. H. (2019). Preparation, Optimization, and In Vivo Evaluation of Nanoparticle-Based Formulation for Pulmonary Delivery of Anticancer Drug. *Medicina*, *55*(6), 294. https://doi.org/10.3390/medicina55060294

26 Khan, R., Inam, M., Khan, S., Jiménez, A., Park, D., & Yeom, I. (2019). The Influence of Ionic and Nonionic Surfactants on the Colloidal Stability and Removal of CuO Nanoparticles from Water by Chemical Coagulation. *International Journal of Environmental Research and Public Health*, *16*(7), 1260. https://doi.org/10.3390/ijerph16071260

27 Chorny, M., Fishbein, I., Danenberg, H. D., & Golomb, G. (2002). Lipophilic drug loaded nanospheres prepared by nanoprecipitation: effect of formulation variables on size, drug recovery and release kinetics. *Journal of Controlled Release*, *83*(3), 389–400. https://doi.org/10.1016/s0168-3659(02)00211-0

28 Wang, Y., & Tan, Y. (2016). Enhanced drug loading capacity of 10-hydroxycamptothecin-loaded nanoparticles prepared by two-step nanoprecipitation method. *Journal of Drug Delivery Science and Technology*, *36*, 183–191. https://doi.org/10.1016/j.jddst.2016.09.012

29 Tazhbayev, Ye.M., Galiyeva, A.R., Zhumagaliyeva, T.S., Burkeyev, M.Zh., Kazhmuratova, A.T., Zhakupbekova, E.Zh., Zhaparova, L.Zh., & Bakibayev, A.A. (2021). Synthesis and characterization of isoniazid immobilized polylactide-co-glycolide nanoparticles. *Bulletin of the University of Karaganda – Chemistry*, *101*(1), 61–70. https://doi.org/10.31489/2021Ch1/61-70

30 Galiyeva, A.R., Tazhbayev, Ye.M., Zhumagaliyeva, T.S., Sadyrbekov, D.T., Kaikenov, D.A., Karimova, B.N., & Shokenova, S.S. (2022). Polylactide-co-glycolide nanoparticles immobilized with isoniazid: Optimization using the experimental Taguchi method. *Bulletin of the University of Karaganda – Chemistry*, *105*(1), 69–77. https://doi.org/10.31489/2022Ch1/69-77

31 Burkeev, M.Zh., Kreuter, J., Tazhbayev, Ye.M., Zhaparova, L.Zh., Zhumagalieva, T.S., Arystanova, Zh.T., & Mukhanova, D.A.. (2017). Preparation, characterization and investigation of in vitro release of anti-tuberculosis drug p-amino salicylic acid based on human serum albumin. *Bulletin of the University of Karaganda – Chemistry*, *87*(3), 38–44. https://doi.org/10.31489/2017ch3/38-44

32 De Almeida, J. M. F., Júnior, E. D., Verríssimo, L. M., & Fernandes, N. S. (2019). pH-Dependent release system of isoniazid carried on nanoparticles of silica obtained from expanded perlite. *Applied Surface Science*, *489*, 297–312. https://doi.org/10.1016/j.apsusc.2019.05.317

33 Pavani, J., Deepika, B., Nagaraju, K., Regupathi, T., Rao, K.N.V., & Rajeswar Dutt, K. (2017). Formulation development and in vitro evaluation of sustained release matrix tablets of tramadol hydrochloride. *Innovat International Journal Of Medical & Pharmaceutical Sciences*, *2*(6), 28–38.

34 Angajala, Gangadhara, & Subashini, R. (2014). Transdermal Nanocarriers: New Challenges and Prospectives in the Treatment of Diabetes mellitus. *Journal of Chemistry and Applied Biochemistry*, *1*(2): 107, 1–10.

35 Becker, C., Dressman, J. B., Amidon, G. L., Junginger, H. E., Kopp, S., Midha, K. K., Shah, V.P., Stavchansky, S., Barends, D. M. (2007). Biowaiver Monographs for Immediate Release Solid Oral Dosage Forms: Isoniazid**A project of the International Pharmaceutical Federation FIP, Groupe BCS, www.fip.org/bcs. *Journal of Pharmaceutical Sciences*, *96*(3), 522–531. https://doi.org/10.1002/jps.20765

36 Nkanga, C. I., Walker, R. B., & Krause, R. W. (2018). pH-Dependent release of isoniazid from isonicotinic acid (4-hydroxybenzylidene)-hydrazide loaded liposomes. *Journal of Drug Delivery Science and Technology*, *45*, 264–271. https://doi.org/10.1016/j.jddst.2018.03.016

Buketov University

37 Li, J., Jiang, G., & Ding, F. (2008). The effect of pH on the polymer degradation and drug release from PLGA-mPEG microparticles. *Journal of Applied Polymer Science*, *109*(1), 475–482. https://doi.org/10.1002/app.28122

38 Burkeev, M. Z., Zhaparova, L. Z., Tazhbaev, E. M., Zhumagalieva, T. S., Ali, S. I., & van Herk, A. M. (2013). In Vitro Studies of Capreomycin Sulfate Release from Polyethylcyanoacrylate Nanoparticles. *Pharmaceutical Chemistry Journal*, *47*(3), 154–156. https://doi.org/10.1007/s11094-013-0916-3

А.Р. Галиева, Е.М. Тажбаев, Т.С. Жұмағалиева, А.Т. Дәрібай

Полилактид-со-гликолид нанобөлшектеріне нанопретрация әдісімен изониазидті инкапсуляциялау

Дәрілік заттарды тасымалдаушы ретінде полимерлі материалдарды қолданудың бірқатар артықшылықтары бар, мысалы, препараттың әсерін ұзарту, дәрілік заттардың жанама әсерлерін азайту және т.б. Мақалада «Изониазид» туберкулезгеқарсы препараттың (ТҚП) полилактид-ко-гликолид (ПЛГА) полимерлі нанобөлшектерін нанотұндыру әдісі арқылы алу қарастырылған. Еріткіш емес пен еріткіш табиғатының, дәрі пен полимердің қатынасы және тұрақтандырғыштың концентрациясы сияқты жеке параметрлерді өзгерту арқылы полимерлік нанотасымалдаушылар алынды. Бөлшектердің орташа мөлшері еріткіш емес түріне тығыз байланысты екені анықталды. Спирттерді пайдаланған кезде орташа өлшем мына реттілікпен өсті: этанол < изопропанол < изобутанол. Нанобөлшектердің пайда болуының және олардың соңғы сипаттамаларының маңызды факторы еріткіштің түрі болып табылады. Дәрілік зат/полимер қатынасы ұлғайған сайын нанобөлшектердің орташа мөлшері де өсті. Алынған нанобөлшектердің өлшемі 93–869 нм аралығында болды. Препараттың полимерлі матрицаға қосылғанын дәлелдеу үшін термогравиметриялық және дифференциалды сканерлеуші калориметриялық талдаулар жүргізілді. Сонымен қатар, әртүрлі pH мәндерінде полимердің ыдырауы және полимер матрицасынан изониазидтің босап шығу дәрежесі зерттелді. Алынған нәтижелер нанотұндыру әдісін тек гидрофобты препараттарға ғана емес, сонымен қатар гидрофильді препараттарға да қолдануға болатынын көрсетті.

Кілт сөздер: нанобөлшектер, полилактид-со-гликолид, PLGA, изониазид, нанотұндыру, гидрофильді препараттар, туберкулезгеқарсы препараттар.

А.Р. Галиева, Е.М. Тажбаев, Т.С. Жумагалиева, А.Т. Дарибай

Инкапсуляция изониазида в наночастицы полилактида-со-гликолида методом наноосаждения

Применение полимерных материалов в качестве носителей лекарственных препаратов имеют ряд преимуществ, такие как пролонгирование действия препарата, уменьшение побочных эффектов лекарства и т.д. В данной статье рассмотрены способы получения полимерных наночастиц полилактида-со-гликолида (ПЛГА) с противотуберкулезным препаратом (ПТП) «Изониазид» методом наноосаждения. Варьируя отдельные параметры, такие как природа растворителя и нерастворителя, соотношение лекарства и полимера, концентрация стабилизатора, были получены полимерные нановносители. Было определено, что средний размер частиц тесно зависит от типа нерастворителя. При использовании спиртов средний размер увеличивался в последовательности: этанол < изопропанол < изобутанол. Немаловажным фактором для образования наночастиц и их конечных характеристик является тип растворителя. При увеличении соотношения лекарство/полимер средний размер наночастиц также увеличивался. Размер полученных наночастиц варьировался в пределах 93–869 нм. Для подтверждения включения лекарственного препарата в полимерную матрицу были проведены анализы термогравиметрии и дифференциальной сканирующей калориметрии. Кроме того, были исследованы деградация полимера и степень высвобождения изониазида из полимерной матрицы при различных pH среды. Полученные результаты показывают, что метод наноосаждения может быть применен не только для гидрофобных лекарств, но и для гидрофильных препаратов.

Ключевые слова: наночастицы, полилактид-со-гликолид, ПЛГА, изониазид, гидрофильные препараты, противотуберкулезные препараты, наноосаждение.

Information about authors*

Galiyeva, Aldana Rymzhanovna — 3rd year PhD student, Department of Organic Chemistry and Polymers, Karagandy University of the name of academician E.A. Buketov, Universitetskaya street, 28,

Buketov University

100024, Karaganda, Kazakhstan; e-mail: aldana_karaganda@mail.ru; https://orcid.org/0000-0002-8551-6297;

Tazhbayev, Yerkeblan Muratovich — Full Professor, Doctor of Chemical Sciences, Karagandy University of the name of academician E.A. Buketov, Universitetskaya street, 28, 100024, Karaganda, Kazakhstan; e-mail: tazhbayev@mail.ru; https://orcid.org/0000-0003-4828-2521;

Zhumagaliyeva, Tolkyn Sergazyevna — Candidate of Chemical Sciences, Associate Professor, Karagandy University of the name of academician E.A. Buketov, Universitetskaya street, 28, 100024, Karaganda, Kazakhstan; e-mail: zhumagalieva79@mail.ru; https://orcid.org/0000-0003-1765-752X;

Daribay, Arailym Turashkyzy — Department of Organic Chemistry and Polymers, Karagandy University of the name of academician E.A. Buketov, Universitetskaya street, 28, 100024, Karaganda, Kazakhstan; e-mail: arailymdaribay@gmail.com

*The author's name is presented in the order: *Last Name, First and Middle Names*

Buketov University