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FULL PAPER

# Vancomycin prolonged release *via* PLGA system loaded with drug-containing chitosan nanoparticles as a novel *in situ* forming drug delivery system

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Bone infection (Osteomyelitis) is an inflammation of the bone that usually results in infection. Nowadays, *in situ* forming systems are investigated for the osteomyelitis treatment. These systems are in the form of viscous liquid, but they become solid or semi-solid and the drug is released slowly after injection into the infection site. The aim of present study was the long-term release of vancomycin using a PLGA system loaded with drug-containing chitosan nanoparticles. The *in situ* formulations formed in this study were composed of three main components: polymer, water-miscible solvent, and active pharmaceutical ingredient. PLGA 504H polymer and PEG 250DME solvent with a polymer to solvent ratio of 3:1 was used to prepare the *in situ* forming system. Chitosan nanoparticles were designed using gelation ionic method by designing the experiment of chitosan nanoparticles with encapsulation efficiency of 51% and drug loading of 25%. Then, by adding different ratios of released drug to loaded drug through nanoparticles in the system, their release profile was examined. The results revealed that adding chitosan nanoparticles reduced burst release by 44% and increased the release time. In this system, the drug can be added to the polymer solution in different proportions of the free form and the drug-containing nanoparticle. Furthermore, in this system, it is possible to use the combination of different drugs in free form or loaded in nanoparticles to improve the treatment process in the system. The use of biodegradable polymers eliminates the need for surgery in the use of this medicinal system. Moreover, this system is biocompatible and non-toxic due to the non-use of organic solvent in the preparation of the system and the use of PEG 250 DME solvent.

**KEYWORDS**

Chitosan; vancomycin; poly lactic-co-glycolic acid (PLGA); *in* situ forming; bone infection; drug delivery system.

## Introduction

Osteomyelitis is an inflammation of bone that usually leads to infection. Due to the high resistance of healthy bone to infection, this disease is less common. However, factors such as diabetes, AIDS, the presence of infection in the body, the use of intravenous drugs, and

surgery increase the risk of developing this disease. In spite of extensive medical advances, more than 10% of orthopedic surgeries result in this disease [1,2]. Osteomyelitis can occur at any age due to its pathogenicity. Acute osteomyelitis can be treated with antibiotics within 4 to 6 weeks [3], but in chronic osteomyelitis, drug consumption, surgery, and removal of dead tissue will be needed. Common therapies used to treat this disease include oral and injectable methods. The oral method has many side effects due to the high dose of the drug and the injectable method is not patient-friendly due tothe long hospital stay and its high cost [4,5]. Nowadays, *in situ* forming systems are investigated for the osteomyelitis treatment [6,7]. These systems are in the form of viscous liquid, but they become solid or semi-solid and the drug is released slowly after injection into the infection site [8]. The main problem with these systems is their high burst release, which occurs in the first few hours of implant injection and, in addition to causing toxicity, reduces the release period due to the release of large amounts of drug [9-11]. An ideal drug system should release the right dose of the drug evenly and at a constant rate so that the final release rate remains in the drug treatment window. *In situ* forming systems are one of the types of drug release control systems. These systems are often made of biodegradable polymers that can be injected into the body with a syringe and are solidified to form a drug store once injected at the site. *In situ* forming systems are divided into two types of *in situ* forming microparticles and *in situ* forming implants. According to the solidification mechanism in the *in vivo* environment, *in situ* forming implants are divided into four groups: thermoplastic pastes, *in situ* cross-linked polymers, thermally induced gelling systems, and *in situ* polymer precipitation [12,13]. This precipitation can be produced by the release of a solvent, a change in temperature or a change in pH [14].

These systems include water-insoluble polymers that dissolve with the drug in an organic, water miscible, and biocompatible solvent. After injecting this system into the aqueous medium, the solvent penetrates into the medium and water penetrates into the cross-linked polymer. Due to its insolubility in water, the polymer precipitates and forms a solid implant system that can control the release rate of the drug [15,16]. One of the problems with this system is the possibility of burst release of the drug, especially in the first few hours after injection into the body. Since this injectable implant system is administered as a liquid, it is quite reasonable to assume that there is a lag between injection and formation of precipitate [8]. During the lag time, the burst release of the drug may increase its concentration in blood plasma compared to the conventional implant systems. The initial burst release of the drug causes tissue inflammation and in some cases general toxicity. Due to the existence of such unwanted phenomena, these systems are used only for specific therapies [11, 17-20].

Vancomycin is a tricyclic glycopeptide antibiotic and the first choice for prophylaxis and treatment of infections caused by gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) like *osteomyelitis* [21].

Based on the findings of our previous studies, N-methyl-2- pyrrolidone (NMP) and acetone are two solvents that could improve the release profile of vancomycin from *in situ* forming systems based on poly(D,L-lactide-co-glycolic acid). Chitosan nanoparticles are used as drug carriers due to their biocompatibility [22,23].

The present study aims to investigate the Vancomycin prolonged release via PLGA system loaded with drug-containing chitosan nanoparticles as a novel in situ forming drug delivery system. These nanoparticles can be loaded in these drug-containing, drug-free systems, PLGA 504H polymer, and PEG 250DME solvent with a polymer to solvent

ratio of 3:1 was used to prepare the *in situ* forming system.

## Materials and methods

### *Preparation of drug-containing chitosan nanoparticles*

Chitosan nanoparticles containing vancomycin were designed and constructed by gelation ionic method by Calvo [24] for long-term release of the drug from system and reduce the burst release that causes the release of a large amount of drug in the early hours of implantation and side effects, and also prevents controlled release to examine the reduction in the initial release of drug to system.

To load vancomycin in chitosan nanoparticles, according to the studies, an experiment was designed by Box-Behnken method with three variables of chitosan solution concentration, chitosan to TPP ratio and chitosan to drug ratio at three levels. Variables such as initial solution volume, stirrer speed, TPP solution concentration, and stirring time were kept constant.

To prepare the drug-containing nanoparticles, chitosan solution with a certain concentration in 10 mL of water was distilled twice. For this purpose, 1% volume/volume solution of acetic acid was prepared and chitosan was added. The solution was placed on a magnetic stirrer for one night so that the chitosan to be completely dissolved. Then, the solution was passed through a 0.45 µm filter to obtain a completely transparent solution. After that, the desired amount of drug was added to the solution. Next, TPP solution with a concentration of 1 mg/mL was added to the drug-containing chitosan solution to form an opaque solution due to the formation of nanoparticles. Then, nanoparticle solution was stirred on a magnetic stirrer at 600 rpm for 30 minutes for better stirring. Thereafter, the solution was poured into a microtube and centrifuged at 14000 rpm for 30 minutes at 10 °C. The precipitated nanoparticles were washed twice with distilled water and centrifuged again. The residual supernatant was collected from the microtube floor for analyzing the level of loading and the precipitated nanoparticles were separated by pipetting. Then, the nanoparticle suspension was distilled in double-distilled water for 5 minutes at 60 watts and placed in a -80 °C freezer to be dried by freeze-drying. The nanoparticles were dried by freeze-drying for 24 hours at -40 °C for further analysis.

### *Determining the amount of drug loaded in nanoparticles*

To determine the amount of loaded drug, the amount of drug left in the residual supernatant after centrifugation was measured by spectrophotometer and high-performance liquid chromatography (HPLC). Using the standard curve and measuring the absorption of the sample, the concentration of the drug in the sample can be obtained and the total amount of drug loaded in the nanoparticles can be calculated using the total volume of the supernatant.

Likewise, to confirm this value, the nanoparticles were re-dissolved in acetic acid after drying with a freeze dryer; the absorption rate of the sample was measured by a high-performance liquid chromatography (HPLC) at 280 nm.

The drug loading (DL) and its encapsulation efficiency (EE) in nanoparticles can be obtained using the obtained data and with the help of the following equations:

EE (%) = [Total weight of drug used/weight of drug loaded in nanoparticles] × 100.

DL (%) = [Weight of drug loaded in nanoparticles/total weight of nanoparticles] × 100.

### *Preparation of optimal nanoparticles*

The optimal values of chitosan concentration factors, the chitosan to TPP ratio, and the chitosan to vancomycin ratio were obtained to maximize the encapsulation efficiency and

rate of drug loading, respectively, were obtained at 1.04, 4.70, and 0.95. To measure the loading value, high-performance liquid chroma tographic (HPLC) analysis was performed for the residual supernatant from the nanoparticle solution as well as nanoparticles dissolved in acetic acid. Based on this analysis, the loading value was 25% and the encapsulated drug was 51%. In this research, all nanoparticles were prepared based on these values. To measure the size of nanoparticles, dynamic light scattering device was used. The drug-free particle size was 130 nm and the drug-containing particle size was 174 nm. Poly-dispersity index of drug-free nanoparticles was 0.217 and that of drug-containing nanoparticles was 0.305, indicating a narrow dispersity of particles size.

*In situ forming system*

To prepare this system, two separate syringes were used, one of which contained a drug active ingredient (Figure 1A) and the other contained polymer solution (Figure 1B). At time of its use, the two syringes are connected to each other (Figure 1C) and after mixing the components inside the two syringes, the system is prepared for injecting (Figure 1D). To prepare the formulations in this section, according to library studies and performing simple tests such as the possibility of removing the final formulations from the syringe, a constant concentration of 25% was selected for the polymer and 75% for the solvent (w/w), which was 330 mg in total. The drug ratio was 10:1 and 33 mg in all formulations. The formulations were based on PEG250 DME solvent and PLGA 504H polymer.

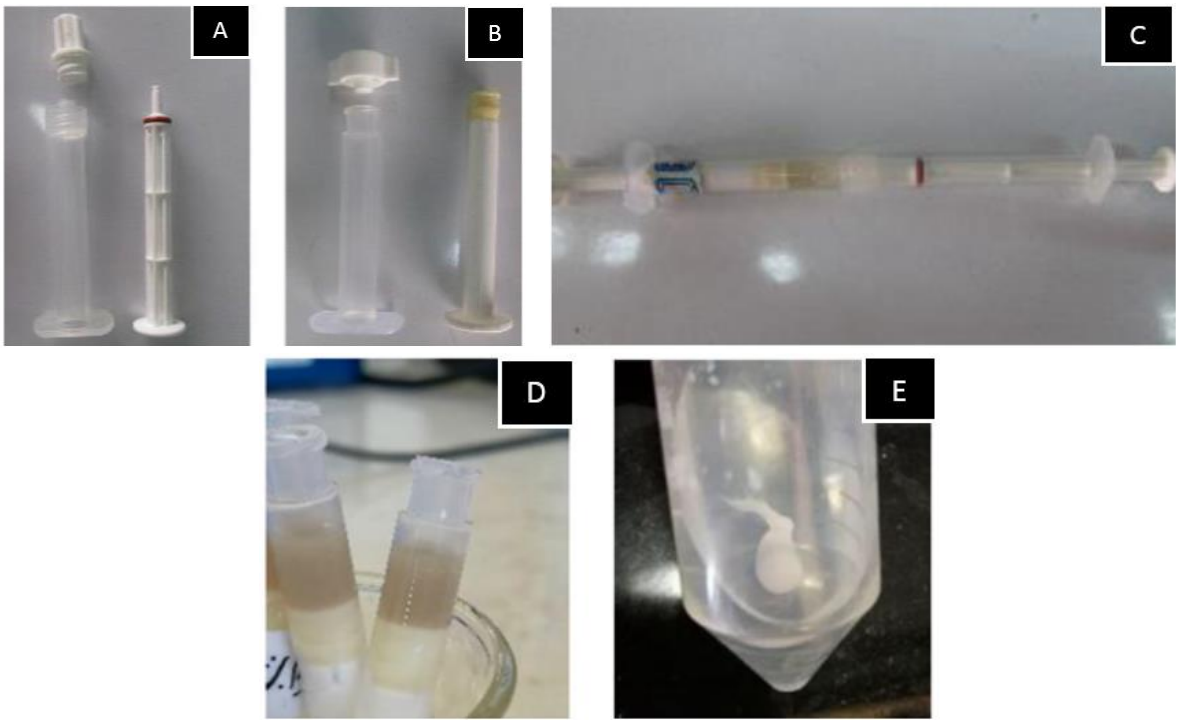


**FIGURE 1** Drug syringe (A), polymeric syringe (B), connection of two syringes (C), polymer solution (D), injection into aqueous medium, and implant formation (E).

To prepare the system, certain amounts of polymer and solvent were carefully weighed and poured into a polymer syringe to prepare a uniform solution. The, this mixture was kept in an oven at 37 °C for two hours to ensure complete dissolution of the polymer in the solvent and the release of air bubbles. Certain amounts of active ingredient and drug-containing nanoparticles were also added to the drug syringe.

At the time of injection, the two syringes were connected to each other and after complete mixing, they were immediately injected into the containers containing the release medium. This solution turned into semi-solid form after injection and exposure to the aqueous medium (Figure 1E).

Table 1 and 2 presents the prepared formulations as code: Vancomycin hydrochloride (H), Nanoparticles containing the drug (N).

**TABLE 1** Method of naming the formulations (mg)

| Formulation code | Amount of released drug (mg) | Amount of loaded drug on nanoparticles (mg) |
|---|---|---|
| **H33** | **33** | **0** |
| **H4N1** | **26** | **7** |
| **H1N1** | **17** | **15** |
| **H2N3** | **13** | **20** |

**TABLE 2** Method of naming the formulations (%)

| Formulation code | Amount of released drug (%) | Amount of loaded drug on nanoparticles (%) |
|---|---|---|
| **H33** | **100** | **0** |
| **H4N1** | **80** | **20** |
| **H1N1** | **51** | **49** |
| **H2N3** | **40** | **60** |

*Method of assessing the amount of drug release from the in situ forming system*

The amount of drug release was analyzed at certain hours determined by the conditions of the sink with a shorter time interval in the first days, and then a few days once (depending on the formulation type and the amount of release. The amount of drug release was calculated once every 1 to 4 days using spectrophotometer, depending on the drug stability.

*The effect of nanoparticles on morphology and drug release from the in situ forming PLGA system*

First, in different ratios of released drug to loaded drug in nanoparticles, scanning electron images and drug release from systems were investigated. In all systems, the total amount of drug was 33 mg. H33 formulation had the highest released drug without nanoparticles and H2N3 formulation contained the highest amount of loaded drug through nanoparticles among the formulations (60%) and the lowest amount of released drug (40%). Since the implant becomes very dense and non-injectable and it is not possible to disperse all nanoparticles in the solvent due to an increase in nanoparticles, the maximum amount of loaded drug through nanoparticles in systems is 60%.

Figure 2 shows the design and development of novel *in situ* forming drug delivery system based on long-term release of drug (Antibiotics) for osteomyelitis using a PLGA loaded with drug-containing chitosan nanoparticles.

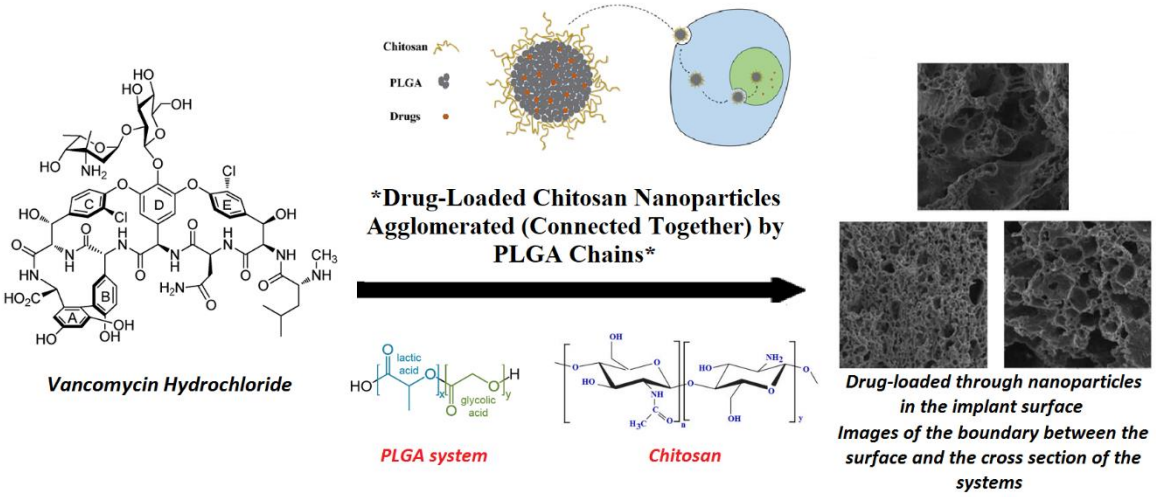


**FIGURE 2** The general schematic of osteomyelitis *in situ* forming drug delivery system

## Results

To examine the morphology of the systems, their surface and cross-section were imaged by scanning electron microscopy. The related results are depicted in Figures 3 to 5. Figure 3 shows the images of system surfaces.

As seen in figures, since the ratio of drugs loaded through nanoparticles to released drug increases, the implant surface becomes smoother and its cavities become smaller. Figure "A" has no nanoparticles and "C" has the highest nanoparticles. Reducing cavities on the surface by reducing the number of nanoparticles in the system can reduce burst release.

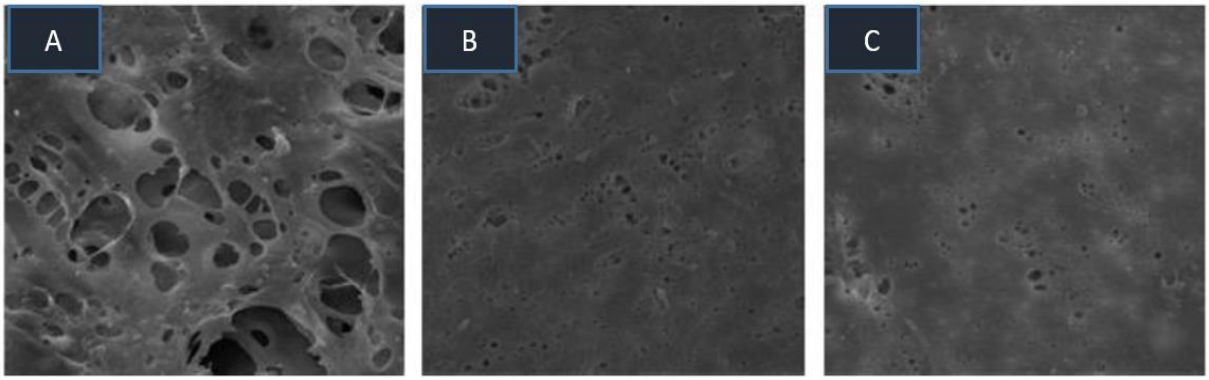


**FIGURE 3** Images of the system surface with 1 magnification of 1000 and the different loading ratio of released drug to drug loaded through nanoparticle: Figure “A” H33 (0:100), Figure “B” H4N1 (20:80), and Figure “C” H1N1 (49:51)

Figure 4 displays the created channels at the boundary of implant surface and cross-section. As seen, the created channels created are due to the rapid release of the drug, and with increasing nanoparticles, the size of implant porosity is reduced, which can decline the burst release and the total amount of drug release

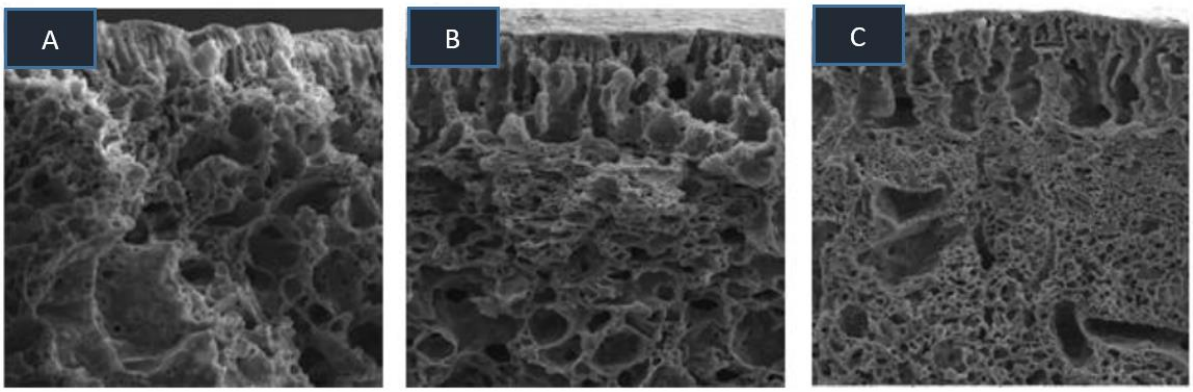


**FIGURE 4** Images of the boundary between the surface and cross-section of systems with magnification of 500 and with different ratios of released drug to loaded drug through nanoparticles: Figure "A" H33 (0:100), Figure "B" H4N1 (20:80), and Figure "C" H1N1 (49:51)

Figure 5 also demonstrates the cross-section of implants. Figure "A", which contains 100% released drug, has more porosity and larger cavities. By reducing the ratio of released drug to loaded drug through nanoparticles, the system porosity is reduced and Figure "C" shows that cavities are completely fine and spongy. It may be due to the slow exit of the solvent from the implant due to the large amount of nanoparticles loaded in the system. Figure 5 illustrates the results of examining drug release from systems with a combination of different percentages of released drug and loaded drug through nanoparticles, the profile of cumulative release of the drug released from the combination of different percentages of the released drug to the drug-containing nanoparticles.

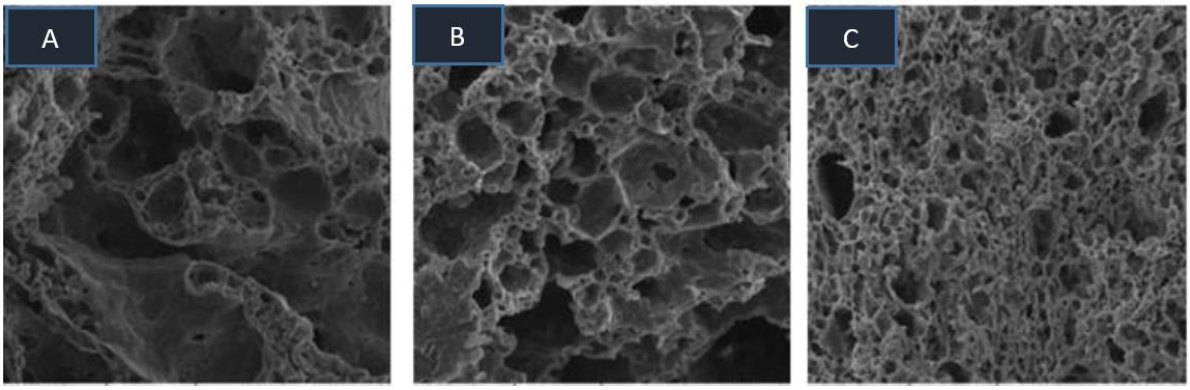


**FIGURE 5** Images of the cross-section of the systems with magnification of 500 and with different ratios of released drug to the loaded drug through nanoparticles: Figure "A" H33 (0:100), Figure "B" H4N1 (20:80), and Figure "C" H1N1 (49:51)

As demonstrated in Figure 6, the release of vancomycin hydrochloride from the H33 system has two stages of burst release and the release at a slower rate over a long period. In this implant, 87% of the drug is released during the first 24 hours, which can be due to the high hydrophilicity of the solvent and the drug. This high burst release is not desirable and the release from the controlled system will not be controlled. As shown in Figure 6, the release of drug-containing nanoparticles into the burst release system has decreased and the release time has become longer.

To change the percentage of released drug and drug-containing nanoparticles in the system, different amounts of nanoparticles need be loaded into the system. Thus, by increasing the percentage of nanoparticles, and thus increasing the system weight, the implant is solidified more slowly and the solvent exits slowly and the rate of burst release decreased significantly. Likewise, due to encapsulation of the released drug in the implant due to the increase in the number of nanoparticles and the reduction of pores in the system, the total release rate decreases. Increase in release time is further due to the release of drug inside the system and the drug released from the nanoparticles, which is very slow and has reduced the slope of the drug release profile. Table 3 presents the initial amount of drug release, total amount of drug release, and time of drug release from H33, H4N1, H1N1, and H2N3 systems.

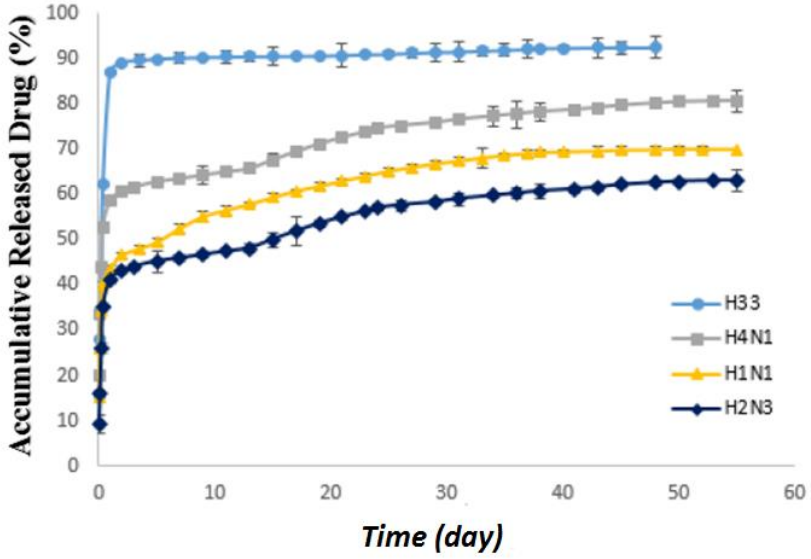


**FIGURE 6** Comparison of cumulative release of the drug released from the system with different ratios of released drug and loaded drug through nanoparticles: H33 (0:100), H4N1 (20:80), H1N1 (49:51), and H2N1 (60:40) (N=3))

**TABLE 3** Drug release time and percentage of the total release and initial drug release from the system with different loading ratios of released drug to loaded drug through nanoparticles: H33 (0:100), H4N1 (20:80), H1N1 (49:51), and H2N3 (60:40) (n=3)

| Formulation | Release in the first 24 hours (%) | Total release (%) | Release time (day) |
|---|---|---|---|
| **H33** | **87.0±2.2** | **92.4±1.2** | **45** |
| **H4N1** | **57.8±3.1** | **80.7±2.8** | **55** |
| **H1N1** | **43.6±0.9** | **70.0±2.4** | **55** |
| **H2N3** | **41.1±1.1** | **63.0±1.3** | **55** |

Based on the obtained release profiles and examination of nanoparticles effect on drug release from the system, the total amount of drug release decreases significantly by adding nanoparticles, due to less water penetration into the system.

## Discussion

Common treatments for chronic osteomyelitis include oral and injectable treatments. The oral method has many side effects due to the high dose of the drug, and the injectable method is not patient-friendly due to its long

hospital stay and high cost. Nowadays, new drug delivery systems such as *in situ* forming implants are used to treat this disease [6,7]. Biodegradable implants require reoperation, and biodegradable implants have high burst release, which reduces the release period of the drug.

In previous studies, the effect of different compositions of these solvents on the release profile of hydrochloride and free base forms of vancomycin was investigated [25]. To this end, several formulations with vancomycin (either hydrochloride or free base form) and different proportions of NMP and acetone were prepared. The cumulative drug release at specified time was determined and tested against conventional kinetic models. The surface and cross-sectional morphology of implants were investigated by SEM. In the study by Darestani-Farahani *et al.*, the effect of drug hydrophilicity and composition of mixed solvents on the *in vitro* vancomycin release and morphology of implants were investigated. Among all formulations which were prepared with different proportions of acetone and NMP, depots with lower viscosity exhibited a lower IBR. Furthermore, as the water solubility of vancomycin decreased, so did the IBR, but the rate of drug release during the following 42 days increased. From these results, it can be concluded that the release kinetic of vancomycin from a polymeric solution of PLGA in the mixed solvent of NMP and acetone, placed in an aqueous environment, is controlled by the solvent and drug hydrophilicity [26].

The aim of this study was long-term release of vancomycin from the *in situ* forming PLGA system by loading the drug-containing chitosan nanoparticles. PLGA 504H polymer and PEG 250DME solvent with a polymer to solvent ratio of 3:1 were used to prepare the *in situ* forming system. By designing the test, chitosan nanoparticles were prepared by ionization method with encapsulation efficiency of 25% and drug loading of 51%. Then, by adding different ratios of released drug to the loaded drug through nanoparticles in the system, their release profile was examined. Studies have indicated that adding nanoparticles reduces burst release by up to 44% and increases release time**.** Due to the controlled and long-term drug delivery as well as the reduction of side effects owing to reduced initial burst release, this system can be used for loading a variety of released drugs and loaded drugs in nanoparticles to treat infectious diseases, including chronic osteomyelitis. This drug formulation is in the form of *in situ* forming implant and can be used for long-term drug delivery in the treatment of chronic osteomyelitis**.**

## Conclusion

The *in situ* formulations formed in this study were composed of three main components: polymer, water-miscible solvent, and active pharmaceutical ingredient. Loading of nanoparticles in the system reduced burst release and increased release duration. In this system, the drug can be added to the polymer solution in different proportions of the free form and the drug-containing nanoparticle. Furthermore, in this system, it is possible to use the combination of different drugs in free form or loaded in nanoparticles to improve treatment process in the system. The use of biodegradable polymers eliminates the need for surgery in the use of this medicinal system. Moreover, due to the non-use of organic solvent in the system preparation and the use of PEG 250DME solvent, this system is biocompatible and non-toxic.

The most important benefits for this project are the reduction of burst drug release from the *in situ* forming system due to the loading of chitosan nanoparticles, increase of drug release time from the system, controlled drug release due to the optimal release profile, possibility of commercialization of the product according to its manufacturing method, reduction of side effects due to initial drug release, non-use of organic solvents in

the preparation of the system and as a result its non-toxicity and biocompatibility and the use of the system for the delivery of antibiotics for the treatment of infectious diseases such as osteomyelitis.

## Abbreviations

(H): Vancomycin hydrochloride; (N): Nanoparticles containing the drug.

## Innovation and originality of this study

The corresponding author of this study declares that the patent of this project has been registered in the Intellectual Property and Inventions Office of Iran under number **105935** and under the name of Bone Infection Drug Delivery System in the name of the corresponding author of this project and has received its scientific and industrial approval from the special headquarters for the Iran Nanotechnology Innovation Council (INIC) and Iranian Research Organization for Science and Technology (IROST) have received the code **DOI: 10.22104/IROST.1401.44**

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## Conflict of interest

The authors declare no conflict of interest in publishing this paper. This study was not supported by any grant money from a pharmaceutical company or for-profit organization.

## Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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