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RESEARCH ARTICLE

Design and evaluation of gamma-sterilized vancomycin hydrochloride-loaded poly(ε-caprolactone) microspheres for the treatment of biofilm-based medical device-related osteomyelitis

Elif Sarıgöl[1], Sibel Bozdağ Pehlivan[1], Melike Ekizoğlu[2], Meral Sağıroğlu[2], and Sema Çalış[1]

[1]Department of Pharmaceutical Technology and [2]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey

Abstract

Context: There is a great necessity to find and use accomplished terminal sterilization technique for industrial manufacturing, research and development studies. Gamma (γ)-sterilization has been commonly employed for wide range of products as indicated by the pharmacopoeias. However, carefully examination should be performed prior to administration since γ-radiation can cause changes in drug and polymer excipients. No information is available in literature about γ-sterilization effects on vancomycin HCl-loaded poly (ε-caprolactone) (PCL) microspheres.

Objective: Formulations were developed using a different preparation approach for the treatment of medical device-related osteomyelitis, and γ-sterilization effects on the physicochemical characterization of the formulations were examined.

Methods: Water-in-oil-in-water (w/o/w) emulsion technique using polyvinyl alcohol (PVA) in inner and outer phase was applied to prepare formulations. Physicochemical properties of the formulations were investigated before and after γ-sterilization and the antibacterial activity against *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*) were measured.

Results: The particle size of the nonsterilized formulations were between 58 and 134 μm. 60% or 20% of vancomycin HCl were released from 42.500 Mn or 70.000–90.000 Mn PCL microspheres, respectively, in 24 h. No difference was observed in the particle size, drug-loading efficiency, morphology, *in vitro* release and antimicrobial activity of the formulations after γ-sterilization ($p > 0.05$).

Keywords

Biodegradable polymers, drug delivery, drug release, gamma sterilization, microparticles, osteomyelitis

History

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Introduction

Osteomyelitis is described briefly as an infection of the bone or bone marrow caused by microorganisms, which are migrated to the bone by bloodstream, contiguously from local areas of infection, trauma and medical devices[1]. Although precautions to provide aseptic conditions and progression in surgical techniques, medical device-related osteomyelitis (MDRO) has not been obstructed because of the tissue damage and inflammation during implantation procedure, device dysfunction and systemic infections[2]. MDRO is a critical complication of orthopedic surgery, its treatment is difficult, time-consuming and expensive procedure. In order to treat MDRO, firstly, the medical device should be removed with surgical procedure and then high-dose systemic antibiotic therapy is applied to the patient for 4–6 weeks due to avascular structure of bone. Furthermore, infected necrotic area in the bone is a suitable environment for the formation of biofilm which is highly resistant to both the immune system and antibiotics. This treatment method brings about the occurrence of serious side effects such as nephrotoxicity or ototoxicity and patient incompliance. After systemic antibiotic therapy, treatment is completed by the new surgical procedure for the placement of new device[3,4]. Therefore, for the management of MDRO, it is highly needed to develop antibiotic delivery system that can provide high local antibiotic concentrations for extended periods of time.

Many implantable systems including antibiotics, such as collagen, plaster of Paris, tricalcium phosphate, D, L-lactic acid oligomer, hydrogel implants, fibrin clots and cancellous homograft and fibrin adhesion[5] were examined for the local treatment of osteomyelitis. Although promising results have been obtained in these studies, more research is still required to attain more effective therapy.

Vancomycin is of the glycopeptide antibiotic class and is effective mostly against Gram-positive bacteria. It is suggested by the Infectious Disease Society of America as a first-line treatment for bone and joint infections, complicated skin infections, bloodstream infections, endocarditis and meningitis infections caused by methicillin-resistant *S. aureus*[6]. Previously, our group

Address for correspondence: Sibel Bozdağ Pehlivan, Faculty of Pharmacy, Department of Pharmaceutical Technology, Hacettepe University, 06100, Ankara, Turkey. Tel: + 90 312 305 12 41. Fax: + 90 312 310 09 06. E-mail: sbozdag@hacettepe.edu.tr

administered vancomycin-loaded poly-lactide-co-glycolide (PLGA) microspheres with vancomycin-impregnated human/rabbit bone grafts to the rabbit knee joint and an elevated vancomycin concentration in the local infection region were achieved[7]. Also, vancomycin-loaded chitosan and silk-coated poly (ε-caprolactone) (PCL) microspheres were evaluated in the experimental osteomyelitis in other related studies[8,9].

Due to hydrophilic nature of vancomycin, the double emulsion (W/O/W) solvent evaporation/extraction method employing emulsion stabilizers (PVA or Methocel®) in the external aqueous phase was commonly utilized for the preparation of drug-loaded PCL microspheres[8,9]. In the present study, PCL microspheres were prepared by using a different approach that is performed by adding polyvinyl alcohol (PVA) to both internal and external aqueous phase in order to evaluate whether this approach provide an improvement of the formulations.

Sterility is crucial quality attribute and implantable drug delivery systems should be sterile. Pharmaceutical industry has been commonly preferred γ-irradiation for the terminal sterilization of pharmaceutical products as stated by its acceptance in the European Pharmacopeia[10,11]. Gamma sterilization is an effective sterilization technique due to its capability for reaching 10^{-6} probability of microbial survival without excessive heating of the product or exposure to toxic chemicals[10]. However, γ-sterilization has some potential disadvantages such as radiolytic degradation of incorporating drug and polymer matrix[12]. In literature, different results have been reported about the effects of γ-sterilization on microparticulate systems; increased[12–15] or decreased[13,14] drug release rate were observed after γ-sterilization. Furthermore, Bartolotta et al.[16] investigated the γ-irradiation effects on trehalose–hydroxyethylcellulose microspheres containing vancomycin. Up to date, no information is available in literature about γ-sterilization effects on vancomycin HCl-loaded PCL microspheres.

In light of the above information, the purpose of study is to develop PCL microspheres containing vancomycin HCl using alternative preparation approach to treat MDRO and to evaluate the γ-irradiation effects on the physicochemical characterization of the formulations since they are planned for using implantation/injection route of drug administration for our future studies.

Materials and methods

Materials

Vancomycin HCl was kindly provided by Sandoz Pharma Co (Istanbul, Turkey). Poly-ε-caprolactone (PCL) Mw 14.000/Mn 10.000; Mw 65.000/Mn 42.500; Mn 70.000–90.000, poly(vinylalcohol) (PVA; MW 30 000–70 000) and dichloromethane were purchased from Sigma Aldrich (Munich, Germany). Distilled and deionized water (DI) (Millipore, MA) were used throughout the study in the preparation of buffers and solutions. All culture media were purchased from Difco Laboratories (Detroit, MI). All other chemicals and reagents used were of analytical grade or high-performance liquid chromatography (HPLC) grade.

Methods

Assay of vancomycin HCl

The quantitative determination of vancomycin HCl was performed by a validated HPLC method. The HPLC analyzes were performed by using Agilent HPLC system (Agilent 1100) with ultraviolet (UV) detector (322 nm) and a column (μBondapak, C18, 10 μm 125A, 3.9 × 300 mm, Waters, London, UK). The mobile phase (solution A; triethylamine solution (pH 3.2):acetonitrile:tetrahydrofuran (92:7:1) and solution B; triethylamine solution (pH 3.2):acetonitrile:tetrahydrofuran (70:29:1) and the flow rate (1 mL/min) used obeyed the USP 30-NF 25 (2007). Vancomycin HCl was detected at 280 nm at room temperature. The peak area used throughout this study and the chromatographic method were validated by linearity, sensitivity, precision, accuracy and specificity.

Preparation of microspheres

The microspheres were prepared based on the w/o/w emulsification/solvent evaporation method[17,18]. In order to prepare organic phase, 600 mg of PCL polymer (Mn 10.000, 42.500 or 70.000–90.000) was dissolved in 3 mL dichloromethane. Aqueous phase (inner) was prepared by dissolving PVA (0.05 or 0.1%, w/v) and vancomycin HCl (10% of polymer amount) in deionized water and was added to the organic phase and vortexed for 2 min. The resulting W/O emulsion was dispersed in 150 mL of the first outer phase, a 0.1% w/v PVA stabilizer solution and mixed for 1 h using a laboratory mixer (Silverson L4RT, East Longmeadow, MA) at 1000 rpm in order to obtain a multiple W/O/W emulsion. The emulsion was then diluted in second outer phase containing aqueous PVA solution (0.05%, w/v) in order to minimize particle aggregation and was stirred for 4 h at room temperature for evaporating dichloromethane. Microspheres were filtered through 0.22-μm filter, washed 2 times with deionized water. Following filtration and rinsing, the microspheres were transferred from the filter to centrifuge tubes, washed once with deionized water, centrifuged at 3000 rpm for 5 min, frozen at −80 °C and lyophilized (Heto PowerDry PL 3000, Waltham, MA) for 48 h.

Gamma irradiation of microspheres

The microspheres were γ-irradiated at 25 °C, 60% relative humidity in dark condition. A ^{60}Co gamma cell (4523 Ci, Hungary) supplying a dose rate of 1.28 kGy·h^{-1} was used as an ionizing radiation source at the Sarayköy Gamma Irradiation Facility of Turkish Atomic Energy Agency in Ankara. Samples were irradiated according to European Pharmacopeia[19], with a dose of 25 kGy which is adequate for the purpose of sterilizing pharmaceutical products when the bioburden is not known.

Characterization of the microspheres

Particle size analysis. The nonsterilized or sterilized (lyophilized) microspheres were suspended in distilled water and stirred gently for 1 min before the measurement to avoid particle aggregation. Particle size analysis was performed in a laser diffractometer (Malvern Mastersizer, Hydro 2000s, Worcestershire, UK). Results are expressed as volume–density mean diameter.

Drug content. Fifty milligrams of the nonsterilized or γ-sterilized microspheres were suspended in 1 mL deionized water and vortexed for 2 min. Suspensions were centrifuged at 13 500 rpm for 10 min and supernatants were filtered through 0.22-μm filter and injected to HPLC column to calculate the drug existing at the surface of the microspheres (solution 1). Then, microspheres were dried and dissolved by adding 1 mL of methylene chloride (solution 2). The amount of entrapped vancomycin HCl was measured by HPLC. Also, certain portions of solution 1 and solution 2 were used in antimicrobial activity studies.

Surface morphology. Surface morphology of nonsterilized or γ-sterilized microspheres were examined by scanning electron microscopy (SEM). Samples were fixed on metal plates by

two-sided adhesive tape followed by coating with 100 A° thick gold in brand coating device (Bio-Rad Laboratories Inc., Hercules, CA). After the coating process, samples were investigated by SEM (Nova™ NanoSEM 430, FEI, Muntinlupa City, Philippines).

In vitro *release studies.* *In vitro* release experiments under sink conditions were performed by dialysis bag method[20]. One hundred milligrams of nonsterilized or γ-sterilized microspheres were suspended in 0.5 mL saline phosphate buffered pH 7.4 (PBS). Suspensions were placed into the cellophane membrane (MWCO 300 000 Da) and the bags were fitted into the tube including 2 mL release media. The tubes were introduced into a water bath (37 ± 0.5 °C) stirred magnetically at 100 rpm (Heidolph, Schwabach, Germany). The tubes prepared for each sampling time. At specific time intervals, whole release medium were taken and immediately replaced with fresh medium. The drug amount in samples was assayed by HPLC methods and release studies proceeded until vancomycin HCl release terminated. The drug release profiles were evaluated by the zero- and the first-order model ($k0$, $k1$ are the release rate constants), the Hixson–Crowell model (kHC is the dissolution rate calculated from the Hixson–Crowell plot) and the Higuchi model (kH). Also, chemical stability of vancomycin HCl released from microspheres was confirmed using the HPLC method.

Differential scanning calorimetry. Glass transition temperatures (T_g) of polymers, nonsterilized or γ-sterilized microspheres were measured by a Perkin Elmer Diamond differential scanning calorimeter. Ten milligrams of samples sealed in aluminum hermetic pans and thermograms were determined first by cooling the sample to −65 °C, then heating to 150 °C at a scanning rate of 10 °C/min.

FT-IR spectroscopy. Fourier transform-Infrared (FTIR) spectra of polymers, nonsterilized or γ-sterilized microspheres were recorded on a FTIR spectrometer (FT-Raman, Bruker IFS 66V/S). The samples were previously mixed thoroughly with potassium bromide at 1/100 (sample: KBr ratio). The KBr discs were prepared by compressing the powders. Then, scans were obtained in the range of 4000–400 cm^{-1}.

Sterility test. For the sterility test, fluid thioglycolate medium (FTM) and Tryptic Soy Broth (TSB) media were used. Gamma-sterilized formulation samples were suspended in sterile-distilled water and 1000 µL of the suspension was inoculated to 15 mL of FTM and TSB. They were incubated 14 days, at 35 °C for FTM and 25 °C for TSB. After 14 days, the cloudy tubes were considered as nonsterile and the clear tubes were considered as sterile.

Determination of the antibacterial activity of the vancomycin HCl loaded PCL microspheres. The antimicrobial effectiveness of samples extracted from blank or drug containing PCL microspheres (please see section ''Drug content'') before and after γ-sterilization were evaluated in comparison with an aqueous vancomycin HCl solution (64–0.0625 µg/mL) by measuring minimal inhibitory concentrations (MIC) against *S. aureus* (ATCC 29213) and *S. epidermidis* (ATCC 35984) bacterial strains. Drug-loaded microspheres were compared to an equivalent drug solution. Blank microspheres were also evaluated whether or not showed any antibiotic effect that could interfere with the activity experiment. Broth microdilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) was used to determine the antimicrobial activity[21]. Antibacterial activity test was performed in Mueller–Hinton broth (MHB, Difco Lab., Detroit, MI). The inoculums densities were approximately 5×10^5 cfu/mL. Final twofold dilutions of the drug solution or extraction samples were prepared in the wells of the microtiter plates. Microtiter plates were incubated at 35 °C for 18–24 h. After the incubation period, MIC values (µg/mL) were defined as the lowest concentration of the tested samples that inhibits the visible growth of the microorganisms.

Statistical analysis. Statistical analysis was carried out using a software (Statistical Package for Social Sciences, version 18.0, SPSS Inc., Chicago, IL). One-way analysis of variance (ANOVA) was employed for the statistical evaluation of the results. Tukey's HSD test was performed to investigate the difference between the groups. Statistical level of significance was defined as $p < 0.05$.

Results and discussion

Although there are studies to evaluate the gamma sterilization effects on the formulations[12–15], each developed formulation that must be sterile has to be examined whether this beneficial sterilization procedure causes any changes in its characteristics. Also, it has been reported that the physicochemical characteristics of the formulations have an effect on their efficiency in drug delivery[22]. Therefore, in the present study, the impact of γ-sterilization on physicochemical properties of vancomycin HCl-loaded PCL microspheres, which were developed by using alternative preparation approach, for the treatment of MDRO was investigated.

The particle sizes of γ-sterilized and nonsterilized microspheres are presented in Table 1. As for nonirradiated formulations, increasing the weight of polymer from Mn 10.000 to

Table 1. The mean particle sizes and entrapment efficiencies values of vancomycin HCl-loaded PCL microspheres before and after γ-sterilization ($n = 6$; mean ± SD).

Formulation codes	Polymer molecular weight (Mn)	PVA concentration in inner phase (%)	Mean Particle size (µm) ± SD (before γ-sterilization)	Mean Particle size (µm) ± SD (after γ-sterilization)	Entrapment efficiency (%) ± SD (before γ-sterilization)	Entrapment efficiency (%) ± SD (after γ-sterilization)	T_g (°C) ± SD (before γ-sterilization)	T_g (°C) ± SD (after γ-sterilization)
F1	10.000	0.05	58.05 ± 0.09	61.67 ± 0.12	4.70 ± 0.01	4.40 ± 0.03	55.68 ± 0.19	55.39 ± 0.24
F3	10.000	0.1	58.08 ± 0.10	62.23 ± 0.13	4.10 ± 0.08	5.10 ± 0.01	55.66 ± 0.09	55.16 ± 0.26
F5	42.500	0.05	71.84 ± 0.62	77.71 ± 0.57	40.60 ± 0.21	39.50 ± 0.42	56.12 ± 0.23	56.05 ± 0.37
F6	42.500	0.1	76.36 ± 1.93	74.99 ± 1.47	47.10 ± 0.27	45.70 ± 0.39	55.98 ± 0.17	55.93 ± 0.25
F7	70.000–90.000	0.05	134.12 ± 1.98	130.26 ± 1.86	54.80 ± 0.16	54.10 ± 1.04	56.58 ± 0.32	56.34 ± 0.41
F8	70.000–90.000	0.1	120.15 ± 1.43	119.35 ± 1.32	58.40 ± 0.24	52.10 ± 0.97	56.85 ± 0.52	56.36 ± 0.48

70.000–90.000 in a constant volume of organic phase resulted in an increase in mean particle size from 58.05 ± 0.09 µm and 134.12 ± 1.98 µm. This is in agreement with the data of Benoit et al.[23], reporting that the higher polymer concentration in the medium can increase the bumping of the droplets into each other, leading coalesce into larger droplets and forming the larger particles. Another possible reason could be the increase in the viscosity of polymer solution (from 25.83 cP to 236 144.01 cP)

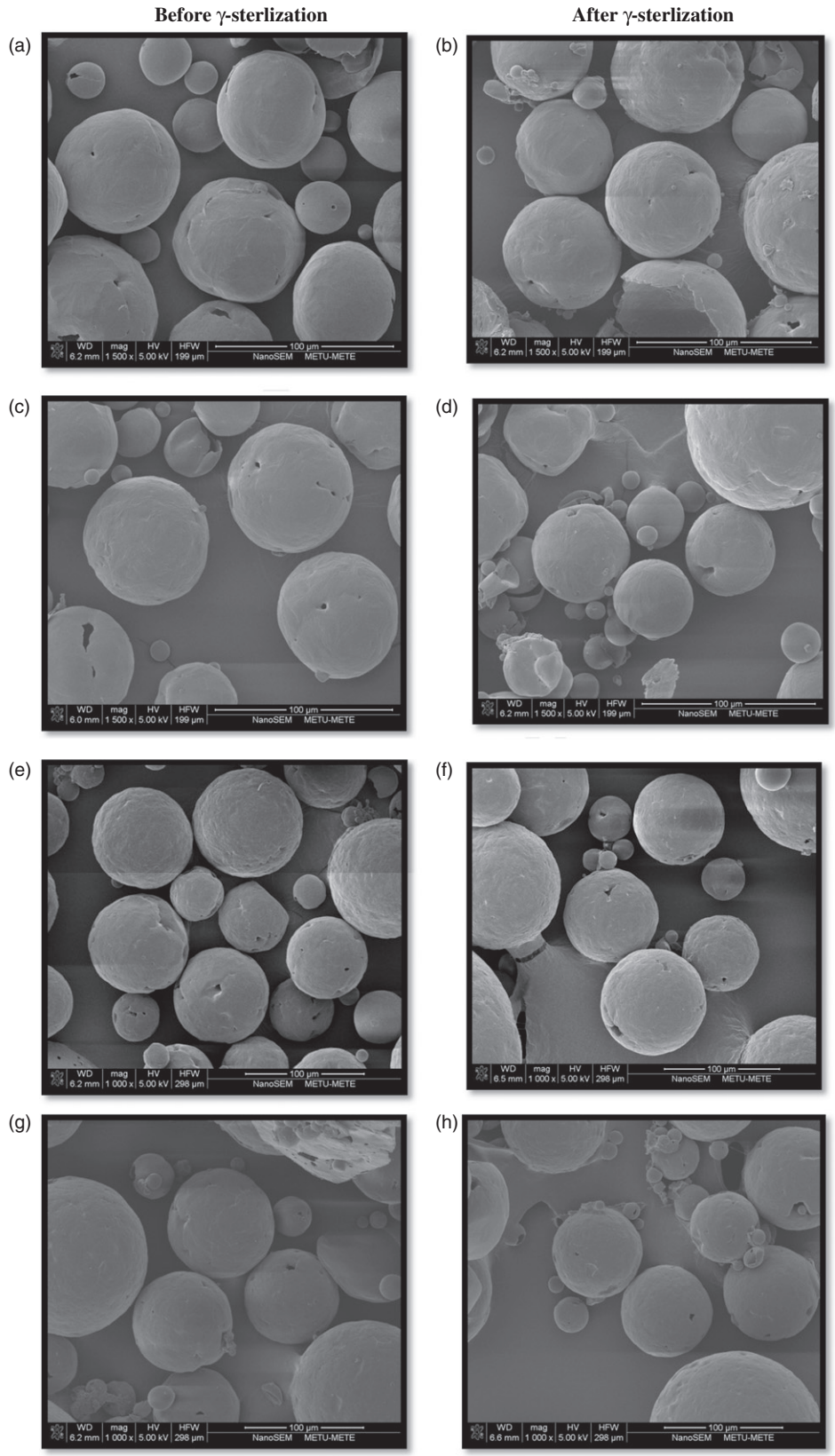

Figure 1. Scanning electron microscopy (SEM) photographs of PCL microspheres containing vancomycin HCl before and after gamma sterilization. (formulation codes: F1, F3, F5, F6, F7 and F8 before (a, c, e, g, i and k; respectively) and after (b, d, f, h, j and l; respectively) γ-sterilization (please see Table 1 for formulation codes).

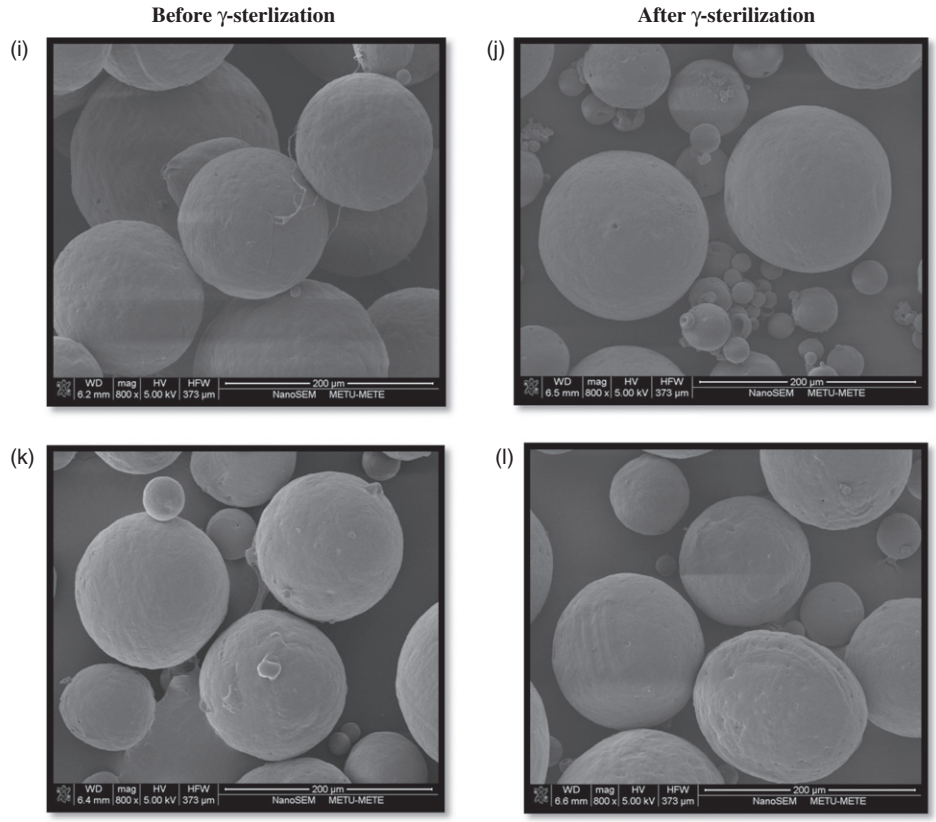

Figure 1. Continued.

due to increase in molecular weight, which causes the decrease in stirring efficiency compared to low-molecular-weight polymers[24]. Furthermore, no statistically significant changes were obtained between the particle sizes of the γ-sterilized and nonsterilized microspheres for all the formulations ($p > 0.05$).

The entrapment efficiency of non- and γ-sterilized formulations are presented in Table 1. An increase in the concentration of PVA from 0.05% to 0.1% (w/v) in inner emulsion phase led to an increase in the encapsulation efficiency for all the nonirradiated formulations except for formulation F3. In general, this could be attributed to increased inner emulsion phase viscosity (from 1.015 cP to 1.098 cP) which was associated with a reduction in the partitioning of the drug in to the outer aqueous phase, produced increase in vancomycin HCl entrapment. Similarly, another case which increased the inner emulsion phase viscosity and provided higher entrapment efficiency was the usage of higher molecular weight PCL in the formulations. When molecular weight of PCL increased, drug entrapment efficiency values increased from 4.10% (w/w) to 58.40% (w/w) for nonirradiated formulations. These findings are consistent with the results of Jeong et al.[25]. Furthermore, when compared to previous studies in which PVA was used in external phase[8,9], comparable and satisfactory drug entrapment efficiency values were obtained in the present study using new preparation approach and γ-sterilization did not have an effect on drug loading. Erdemli et al.[26] also reported no influence of γ-sterilization on the drug content of PLC microspheres containing immunoglobulin G.

SEM images of nonirradiated and sterilized formulations are shown in Figure 1. Generally, all the formulations were spherical in shape without drug crystals on their surface and had nearly homogenous distribution before and after γ-sterilization. Before γ-irradiation, no difference in microsphere morphology was observed between the formulations containing PVA in the concentration at 0.05% or 0.1% (w/v) in inner emulsion phase. While pores were detected on the surfaces of the microspheres containing Mn 10.000 or 42.500 of PCL, nonporous structure was observed for 70.000–90.000 Mn PCL microspheres. Similar results were acquired by Ravivarapu et al.[27] who stated that the lower-molecular-weight PLGA resulted in more porous microspheres whereas the higher-molecular-weight formulation had no porous morphology due to its dense structure. Furthermore, γ-sterilization did not influence on the morphology of the all PCL formulations which is in accordance with the study of Erdemli et al.[26].

The *in vitro* release profiles of nonirradiated and irradiated PCL microsphere formulations are shown in Figure 2. As for nonsterilized microspheres, it can be seen that PCL molecular weight plays important role in drug release behavior of the formulations (Figure 2a). When molecular weight of PCL decreased, increased drug release was observed. One of the explanations for this result could be the difference in the particle sizes of the microspheres; lower polymer molecular weight of PCL at a constant solvent volume led to smaller particle size of the microspheres which caused increased drug release due to the increased surface area-to-volume ratio of the formulations[28]. In addition, water penetration into smaller particles may be faster because of the shorter distance from the surface to the center of the microspheres. Nearly, 60% or 20% of vancomycin HCl released from 42.500 Mn PCL (71 or 76 µm) or 70.000–90.000 Mn PCL (120 or 134 µm) microspheres, respectively in 24 h, while the drug release ended up in the same period for formulations with a lower Mn (10.000) PCL (58 µm) (Table 1 and Figure 2a). Another explanation could be the porous structure of microspheres composed by lower-molecular-weight PCL as mentioned previously. The drug diffusion from the porous

polymer matrix is faster and higher than the dense and nonporous matrix[28,29]. The results are consistent with earlier research[29,30]. As indicated in Figure 2a, decrease in molecular weight of PCL in formulations caused increased initial release rates, which probably due to increased surface area for drug diffusion[29]. Different PVA concentrations in inner emulsion phase (0.05% or 0.1% (w/v)) of the formulations did not affect the release profiles of the microspheres. Furthermore, it was reported that *in vitro* vancomycin release was sustained for 7 days or 33 days in previous studies[8,9] in which PVA was used only in external aqueous phase; however, using PCL with similar Mn, more extended drug release was observed (up to 50 days) in present study, which provides superiority for the treatment of MDRO. As illustrated in Figure 2b, no significant changes in release profiles of all γ-irradiated formulations compared to nonsterilized formulations ($p>0.05$). In literature, there are several opponent studies on the effects on the release behavior of microparticle drug delivery systems; unchanged[12,31], decreased[13,14] or increased[13–15,32] release rates were acquired after γ-sterilization. In our study, similar drug release patterns were observed before and after irradiation, which can be attributed to the diffusion-based release of vancomycin HCl from the formulations due to its hydrophilic nature. Volland et al.[14] also evaluated captopril release from PLGA microspheres with increasing irradiation doses and stated that diffusion-based drug release occurred in all formulations because of hydrophilic property of the drug molecule. Although several studies have addressed the effect of γ-sterilization on PCL as pure polymer or blank nanospheres[33–36], little is known for PCL microspheres containing drug molecule. It has been reported that γ-irradiation process induced to chain scission and crosslinking in the non-ordered regions of PCL and flexible structure of the polymer converted into brittle materials in low irradiation doses[33–36]. Influence of γ-irradiation on IgG-loaded PCL microspheres were investigated by Erdemli et al.[26] and no significant changes were observed in particle size and drug-loading values of the formulations with or without excipients (i.e. poly vinyl alcohol, glucose, starch, heparin) after γ-irradiation. However, the authors reported that significant differences in the release profiles of formulations without excipients were detected after irradiation and this could be explained by the decreased Mn of PCL as revealed in their previous study[37]. They also reported that drug release profiles were not affected by γ-irradiation in excipient containing formulations due to their protective effects. In accordance with this study, we also determined to the similar vancomycin HCl release profiles after γ-sterilization.

Gamma irradiation has been shown not to affect the stability of vancomycin[16,38]. In the present study, Higuchi's square-root equation showed a significantly better fit than zero-order, first-order and cube-root equations, as determined by the *F* test (data not shown), confirming the drug release was occurred by diffusion.

DSC results revealed that T_g values of irradiated microspheres were similar to nonirradiated formulations (Table 1) and γ-sterilization had no effect on thermal behaviors of the PCL microspheres (Figure 3). Geraldes et al.[39] also reported that γ-irradiation at 25 and 50 kGy doses had no effect on the DSC curves of Holmium-165-loaded PCL microspheres.

Sample FTIR spectrums of non- or γ-sterilized microspheres are presented in Figure 4. Before γ-sterilization, vancomycin HCl-loaded PCL microspheres has a characteristic absorption band at strong bands such as the carbonyl stretching mode at 1720.12 cm^{-1} (C=O), asymmetric stretching 2943.56 cm^{-1} (CH_2) symmetric stretching 2865.55 cm^{-1} (Figure 4a). No differences in the positions of the absorption bands were observed in spectra of the drug-loaded PCL microspheres after γ-irradiation (C=O: 1719.77 cm^{-1}; CH2: 2943.57 cm^{-1}; 2865.42 cm^{-1}, Figure 4b).

Sterility tests demonstrated that all formulations were determined to be sterile after γ-irradiation. The antibacterial effectiveness of extraction samples obtained from vancomycin HCl containing non- or γ-sterilized microspheres were examined in comparison with drug solution and blank microspheres using a microbial method. A general view of the results is presented in Table 2. MIC values of non- or γ-sterilized microspheres were comparable for both bacterial strains with an equivalent vancomycin HCl solution and the values were in the range of 0.5–2 μg/mL and 0.6–4.5 μg/mL for drug solution and for all formulations, respectively. The results indicated that incorporation of vancomycin HCl into the PCL microspheres did not alter the MIC values, showing the drug was still effective to kill microorganisms after loading and γ-sterilization procedures. Blank microspheres have no antimicrobial activity.

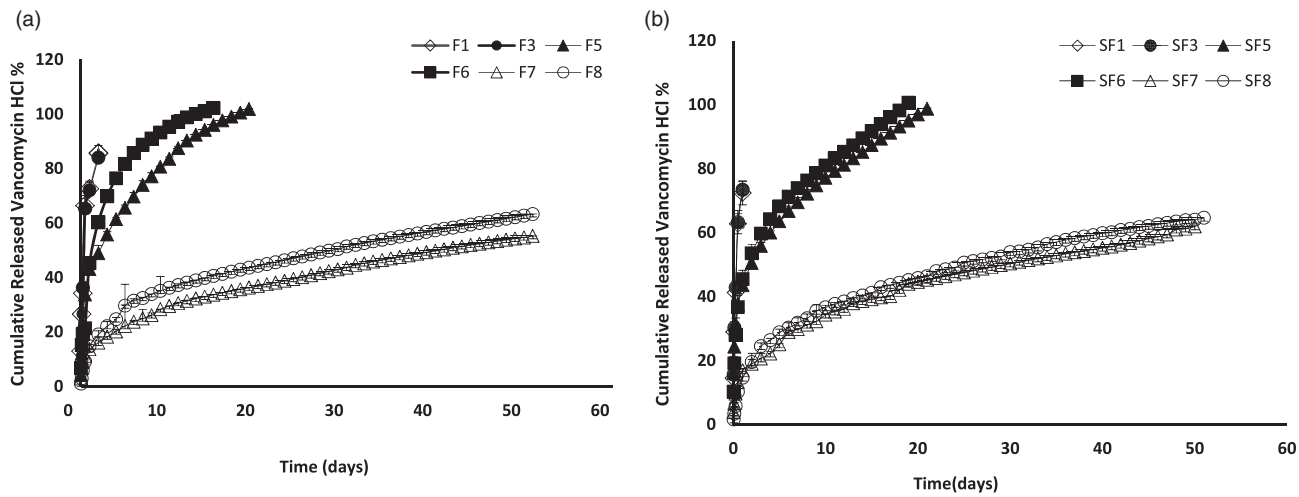

Figure 2. *In vitro* release profiles of vancomycin HCl-loaded PCL microspheres; (a) nonsterilized (b) sterilized formulations (Error bars represent standard deviation; $n=6$ for each data point).

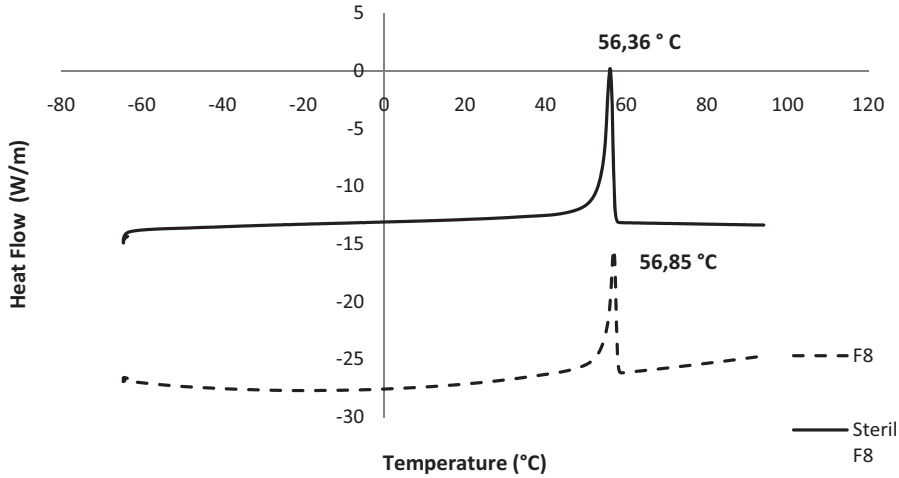

Figure 3. Sample DSC thermograms of non- or irradiated drug containing PCL microspheres (formulation F8).

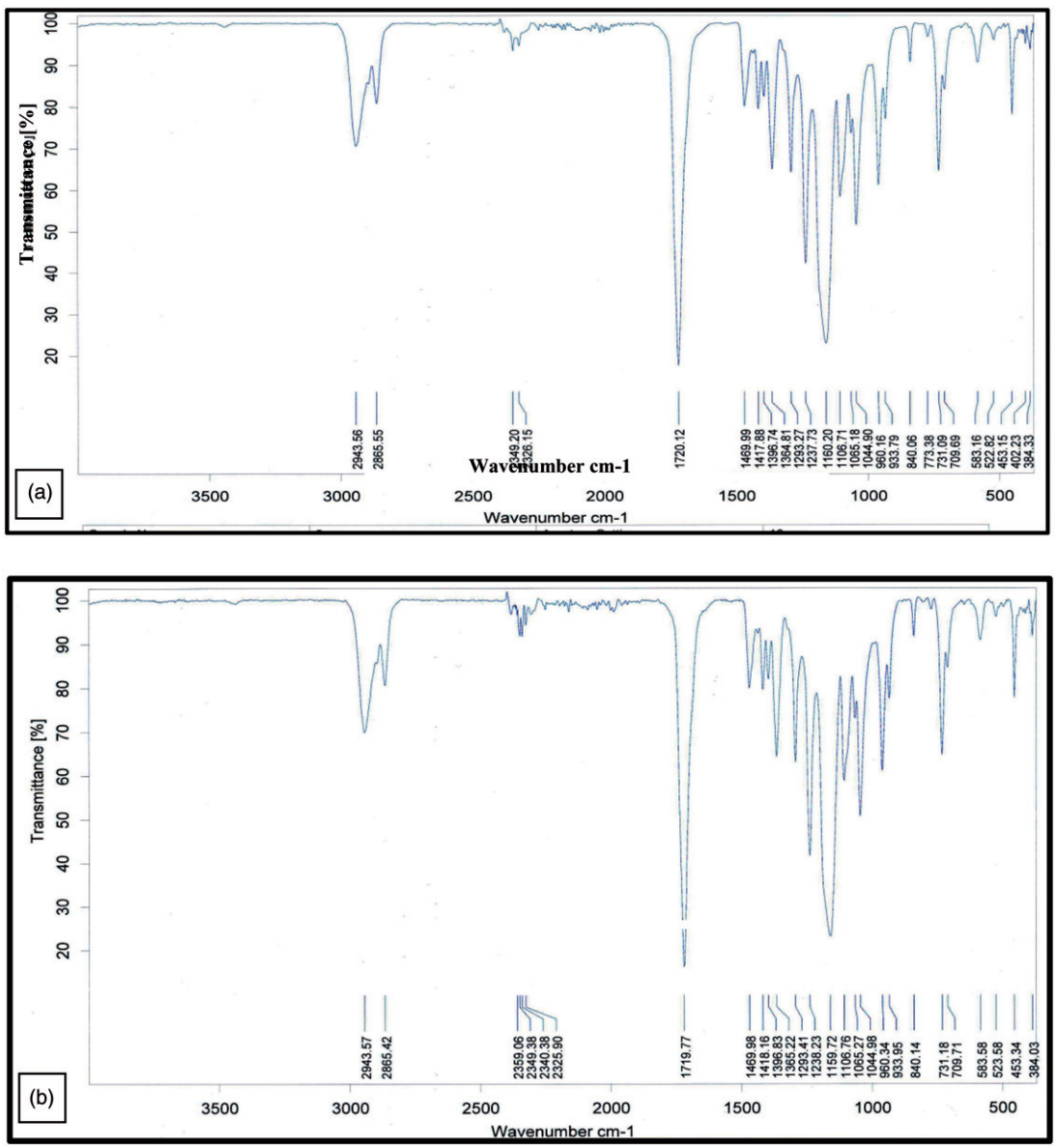

Figure 4. Sample FTIR spectrums of non- (a) or γ- (b) sterilized drug-loaded formulations (formulation F8).

Table 2. MIC values of the vancomycin HCl solution and samples extracted from the microspheres during drug-loading efficiency studies against *Staphylococcus aureus or Staphylococcus epidermidis* ($n = 3$; SD within 10%).

Formulation	*Staphylococcus aureus*		*Staphylococcus epidermidis*	
	Before γ-sterilization MIC (μg/mL)	After γ-sterilization MIC (μg/mL)	Before γ-sterilization MIC (μg/mL)	After γ-sterilization MIC (μg/mL)
Vancomycin HCl solution	0.5–2	1.0	1.0–2.0	2.0
F1	1.34	1.56	3.00	3.12
F3	1.78	2	3.52	4.0
F5	1.44	1.87	3.14	3.75
F6	2.0	2.25	3.44	4.5
F7	0.60	0.62	1.89	2.5
F8	1.12	1.25	1.92	2.5

Conclusions

In the present study, vancomycin HCl containing PCL microspheres were developed by employing new preparation approach and sterilized by γ-irradiation to treat MDRO using implantation/injection route of administration. Furthermore, possible effects of γ-sterilization on *in vitro* characteristics and antibacterial activity of the formulations were investigated. New preparation approach led to more extended drug release and satisfactory drug loading results and no difference were observed in the particle size, drug loading efficiency, morphology, *in vitro* release and antimicrobial activity of the formulations after γ-sterilization.

It can be concluded that when compared to other formulations, F8-coded formulation was the selected formulation for future *in vivo* studies due to its higher drug-loading value and prolonged drug release properties. Additionally, it is well understood that γ-irradiation can be used successfully and safely for the sterilization of the formulations developed.

Declaration of interest

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