Sterilization by Gamma Radiation of Poly-Epsilon-Caprolactone Microspheres Containing Vancomycin Hydrochloride
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**Purpose**
Conventional treatment of medical device-related osteomyelitis (MDRO) consists of removal of medical device with surgical procedure, application of high dose systemic antibiotic therapy for 4-6 weeks and new surgical procedure for the placement of new device. This high cost treatment method causes the occurrence of serious side effects and patient incompliance. Thus, it is highly necessary to develop implantable antibiotic delivery systems such as microspheres that can provide high local antibiotic concentrations for extended periods of time for the treatment of MDRO.

Implantable drug delivery systems should be sterile and gamma radiation has been commonly preferred for the sterilization of these systems. Although it is very effective technique, gamma irradiation has some potential disadvantages such as radiolytic degradation of incorporating drug and polymer matrix.

The purpose of this study is to develop Poly-Epsilon-Caprolactone (PCL) microspheres containing Vancomycin HCl to treat MDRO and to evaluate the gamma irradiation effects on the physicochemical characterization and the antimicrobial effectiveness of the formulations.

**Methods**
Vancomycin HCl was kindly provided by Sandoz Pharma Co (Turkey). PCL (Mw 14.000 Mn 10.000, Mn 60.000, Mn 70.000-90.000), poly(vinylalcohol) (PVA; MW 30000-70000) and dichloromethane were purchased from Sigma Aldrich (Germany). All culture media were purchased from Difco Laboratories (Detroit, MI, USA).

The microspheres were prepared based on w/o/w emulsification/solvent evaporation method. Briefly, aqueous solution of PVA and Vancomycin HCl was added to the organic phase including 600 mg of PCL in dichloromethane and vortexed. 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, USA). After dilution with second outer phase containing aqueous PVA solution, the dispersion was stirred for 4 h at room temperature for evaporating dichloromethane. The microspheres were filtered through 0.22 µm filter, washed 2 times with deionized water.

The microspheres gamma irradiated with a dose of 25 kGy using a 60Co gamma cell (4523 Ci, Hungary). Then, in vitro characteristics of non- or sterilized- formulations such as morphology, particle size, drug content and in vitro release behavior were examined. Also, the antimicrobial effectiveness of samples extracted from microspheres before and after sterilization were evaluated in comparison with an aqueous Vancomycin HCl solution (64–0.0625 µg/mL) by measuring minimal inhibitory concentrations (MIC) against against Staphylococcus aureus (ATCC 29213) and Staphylococcus epidermidis (ATCC 35984) bacterial strains.

**Results**
Particle size analysis indicated that increasing the weight of polymer from Mn 10000 to 70.000-90.000 in a constant volume of organic phase resulted in an increase in mean particle size of the non-sterilized microspheres from 58.05 ± 0.09 µm and 134.12 ± 1.98 µm. Similarly, when molecular weight of PCL increased, drug entrapment efficiency values increased from 4.10% (w/w) to 58.40% (w/w) for non-irradiated formulations.

Before gamma irradiation, no difference in microsphere morphology were 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 65.000 of PCL, the microspheres prepared with 70.000-90.000 molecular weight PCL surface was found to be a non- porous structure. Also, nearly, 60% or 20 % of Vancomycin HCl released from formulations made of Mn 65.000 or Mn 70.000-90.000 PCL, respectively in 24 h, while the drug release ended up in the same period for formulations with a lower Mn (10.000) PCL.

No significant differences in the particle size, drug content value, morphology and in vitro release pattern of the microspheres were observed after gamma sterilization.

MIC values of non- or gamma-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.

**Conclusion**
The results indicated that the particle size, drug loading efficiency, morphology, in vitro release and antimicrobial activity of the formulations were not affected by gamma irradiation and this technique can be used successfully and safely for the sterilization of the formulations developed.