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Investigation of Formulation Parameters of PLGA Nanoparticles Prepared By Nanoprecipitation Technique

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Extended Abstract

Drug delivery applications by using polymers are becoming important field for pharmaceutical applications. To deliver drugs efficiently to target site a range of organic systems like micelles, liposomes, and polymeric nanoparticles have been designed. These novel drug delivery systems minimized drug degredation and loss, prevent harmful side effects and increase drug bioavailability. Among these systems polymeric nanoparticles made by biodegradable polymers are interesting options for controlled drug delivery and ideal vehicles for poor solubility drugs. Other advantages of polymeric nanoparticles include simple preparation methods with polymer. Poly (D,L Lactide-co-glycolide) (PLGA) has been widely used as a drug delivery systems due to their biodegredability, biocompatibility and low toxicity (Jawahar et al., 2009). PLGA nanoparticles prepared by solvent evaporation, monomer polymerisation, nanoprecipitation and the salting out procedure (Govender et al., 1999). Among these techniques, nanoprecipitation represents simple and easy method.

The various formulations on the PLGA nanoparticles which prepared by nanoprecipitation method effect the basic properties like particle size, drug release rate, drug loading capacity and cellular uptake. Particle size, drug release rate, drug loading capacity and cellular uptake of PNPs are affected by the various formulation parameters. Especially, nanoparticle size is controlled by modifiying PLGA and stabilizer ratio, the organic /aqueous phase (O/A) volume ratio and organic solvent type. In our study we prepared PLGA nanoparticles (NPs) by modified nanoprecipitation techniques using polymeric stabilizer Pluronic F-68 (Fessi et al,1988). The aim of this study is to set polymer/stabilizer ratio, (O/A) phase volume ratio and organic solvent type for optimizing the formulation in respect to particle size and examine selected formulations to get an optimum formulation of PLGA nanoparticles and then, the optimum formulation is being tested by cell culture studies to increase the cellular uptake.

PLGA (50:50) was dissolved in acetone (organic phase) at different concentrations and this solution was added drop wise into the 10 mL of ultra pure water containing Pluronic F-68 (aqueous phase) at different concentrations. Mixture was stirred magnetically for 15 min at room temperature. The organic solvents were evaporated to a final volume 10 mL at 26 °C and 550 rpm using rotary evaporation (Rotavapor R-124, Buchi, Switzerland). Subsequently, nanoparticle suspension was centrifuged for 3 min at 5000 rpm to remove the polymer aggregates and suspension was stored at +4 °C until analyzed. For preparation of fluorescent labeled nanoparticles, 100 µl of Nile red was added to the organic phase and formulation was carried out as described above. The labeled nanoparticles were stored in the dark at 4°C. The O/A phase volume ratio was exercised as 1:2, 1:3 and 1:5 and organic solvent content was also changed as acetone/ethanol mixture (5:1 and 2:1 Particle size, zeta potential and polydispersity index of

NPs were determined using Photon Correlation Spectroscopy with a Zetasizer 3000 (Malvern Instruments, Malvern, UK).

For cellular uptake studies of nanoparticles, MCF-7 cells were grown under standard cell culture conditions in DMEM media supplemented with 10% FBS and 1% penicillin/streptomycin. Cellular uptake of Nile red-labeled nanoparticles was determined using a fluorescence microscope (Leica, USA). For these experiments, cells were grown in 96 well culture plates and incubated overnight at 37°C . The cells were then exposed to nile red labeled nanoparticles for 4 hours and viewed under the microscope to determine uptake of nanoparticles.

Results showed that nanoparticle size, zeta potential and polydispersity index is effected by several variables (amount of polymer, O/A phase volume ratio). It was found that the increase in polymer concentration (%1) caused an increase in the particle size (131 nm) unlike, decrease in the zeta potential. This was probably caused by the increasing viscosity of polymer solution, resulting a poorer dispersibility of the organic phase into the aqueous phase and decrease in negative zeta potential is because of dispersed PLGA particles' carboxyl groups can be due to interaction with water molecules. The mean size decreased with the decrease in O/A volume ratio. When aqueous phase volume is increased (1:3, 1:5) particle size is decreased. Increasing amount of pluronic caused a reduction in the interfacial tension, as a result of this; particle size of nanoparticles was decreased. Variety of organic phase also affected the particle size. When acetone and acetone/ethanol volume ratio was evaluated, it was found that minimum particle size was obtained with acetone/ethanol (5:1) mixture. This could be due to the increasing solubility of PLGA in the organic solvent. Cellular uptake studies were performed with this selected formulation. Figure 1 shows the fluorescence microscope image of MCF-7 cells incubated with Nile red-labeled PLGA nanoparticles for 4 hours. Control cells without any nanoparticle treatment did not show any fluorescence (data not shown). The cells incubated with Nile red-labeled nanoparticles exhibited red fluorescence and accumulation of nanoparticles by the cancer cells.

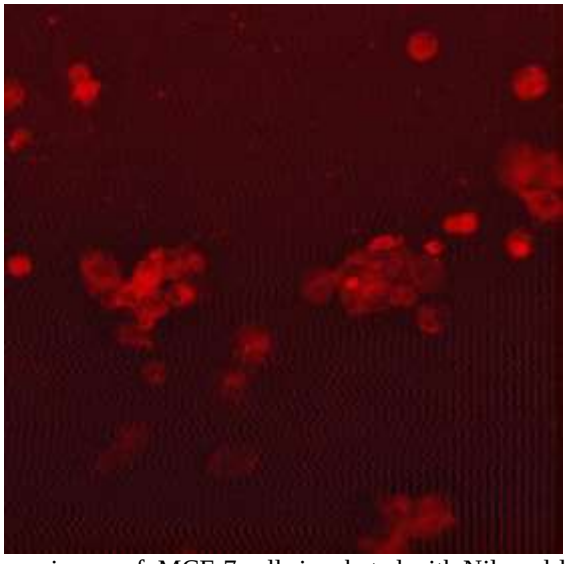

Fig. 1. Fluorescence microscope image of MCF-7 cells incubated with Nile red-labeled PLGA nanoparticles.

As a result of this optimization, the most appropriate formulation of PNPs has been selected as 0.5 % (polymer and pluronic concentration), 1:5 O/A phase volume ratio and 5:1 acetone/ethanol volume ratio. Particle size, polydispersity index and zeta potential of this formulation was found as 149 nm, 0.066, -8.44 mV respectively. Figure 1 shows the fluorescence microscope image of MCF-7cells incubated with Nile red-labeled PLGA nanoparticles for 4 hours. Control cells without any nanoparticle treatment did not show any fluorescence (data not shown). The cells incubated with Nile red-labeled nanoparticles exhibited red fluorescence and accumulation of nanoparticles by the cancer cells.

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