QbD optimization of hydrogels loading PLGA nanoparticles for wound healing

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Burns, chronic, and acute wounds represent a social and economic burden for many countries worldwide. Conventional treatment inludes the use of non-occlusive dressings with natural and synthetic polymers and may de used drug carriers to promote healing, but are still ineffective [1]. Hydrogels are hydrophilic molecular networks produced by the physical or chemical cross-linking of polymers and have been used for wound dressings [2]. The hydrogel potential in wound healing can be further explored with the loading of nanoparticles that allow controlled or sustained release of drugs. In this work, we propose the development of alginate hydrogels loaded with *poly (lactic-co-glycolic acid***)** (PLGA) nanoparticles prepared by cross-linking with calcium. The optimisation of the hydrogel was carried out by Quality by Design using a Design of Experiments approach for the delivery of growth factors to accelerate wound healing. PLGA nanoparticles (PLGA NP) were produced by a modified solvent evaporation water-in-oil-in-water emulsion method [3]. A Central Composite Design was used to define the sample size and formulation composition. A total of 4 factors were studied; sodium alginate, glycerine, calcium chloride, and chitosan concentrations. 16 formulations were generated with 3 central points. The particle size and polydispersity index (PdI) were assessed by Dynamic Light Scattering (DLS) prior gelification and rheology was assessed with a rheometer (Rheometrics, TA Instruments, USA). An increase in particle size (280-1300 nm) and PdI (0.1-0.3) were observed with the increase in chitosan concentrations, suggesting that the nanoparticles were efficiently coated. Furthermore, a shift in the zeta potential was also observed with from -20 mV to 25 mV, confirming the coating with chitosan. High zeta potential values and low PdI values contribute to higher staibility against aggregation and mitigate the Ostwald ripening effect. The moduli G', G'', and viscosity shows that calcium chloride and the interaction between calcium chloride and chitosan are the most relevant factors for the rheology properties of the hydrogels. Viscosity and G'' were significantly affected by the concentration of calcium chloride ($p=0,05$). A correlation value of 0.85 was found between the predicted values and the observed results, showing the adequacy of the model to predict the formulation behaviour. The gelification process to produce the hydrogel did not hamper the nanoparticles features, showing that the experimental design was suitable to load polymer nanoparticles into the hydrogel for the delivery of growth factors. The formulations will be further used to deliver growth factors and the *in vitro* and *in vivo* performance will be evaluated.

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