pH-Driven Optimization of Chitosan–Genipin Nanogels Enables Efficient Cellular Uptake with Preserved Biocompatibility

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Chitosan–genipin nanogels have emerged as promising platforms for biomedical applications due to their biocompatibility and ability to form stable crosslinked networks. However, formulation parameters such as the pH of the chitosan solution can significantly influence their physicochemical properties and biological performance. In this work, nanogels were synthesized under different pH conditions (S1, S2, and S3) to evaluate the impact of this variable on system behavior.

Dynamic light scattering analysis revealed that S1 and S3 presented size distributions with dominant populations around 460 nm, whereas S2 exhibited a more favorable profile, with a main population around 205 nm and an additional fraction in the nanometric range. Electron microscopy showed that S2 displayed a more homogeneous morphology, while S1 and S3 exhibited more irregular structures. Fourier-transform infrared spectroscopy indicated the formation of chemical crosslinks between chitosan and genipin, with spectral features consistent with amide groups and Schiff base formation.

Cell viability was assessed by MTT assay in L929 cells. All formulations maintained viability above 90% within the concentration range of 5–100 µg/mL, while higher concentrations (≥125 µg/mL) resulted in a decrease below the 75% viability threshold defined by ISO 10993-5. The cellular uptake of the selected formulation (S2) was evaluated in ARPE-19 cells by fluorescence microscopy, showing a progressive increase in internalization over time, reaching maximum levels at 24 h, as confirmed by fluorescence quantification.

These results demonstrate that the pH of the chitosan solution plays a key role in defining nanogel properties. The S2 formulation exhibited a favorable size distribution, homogeneous morphology, evidence of chemical crosslinking, high cell viability within a defined range, and efficient cellular uptake.