GELATIN NANOPARTICLE PRODUCTION: AN IN-PROCESS STUDY USING SIZE EXCLUSION CHROMATOGRAPHY

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Gelatin, a naturally occurring polypeptide, is a good candidate for the preparation of nanoparticles, and a method for reproducibly preparing nanoparticles from gelatin has been described [1]. The objective of this study was to carry out in-process development of the method by characterising the molecular weights of the species present in solution at various stages of the production using size exclusion HPLC.

Gelatin nanoparticles were prepared according to the method described by Farrugia and Groves [1]. Briefly, colloidal dispersions of gelatin were produced by desolvation of dilute aqueous solutions of B225 gelatin with 70% w/w ethanol at 37° C. The colloidal particles were then crosslinked with 1% w/w glutaraldehyde; excess glutaraldehyde was neutralised by the addition of sodium metabisulfite solution. Separation and purification of the nanoparticles was performed by ultrafiltration, using distilled water as the washing agent. Samples from various stages throughout the nanoparticle production were filtered through 0.2-µm filters, and analysed by HPLC on a Waters™ Ultrahydrogel Linear size exclusion column at 29°C, using phosphate-buffered saline mobile phase at a flow rate of 0.3 mL min⁻¹, and a tuneable absorbance detector set at 205 nm.

Addition of the non-solvent ethanol to the initial gelatin solution resulted in removal of all but the low molecular weight species, the original gelatin solution having a characteristic broad peak extending from approximately 22 to 36 minutes of elution time while the filtered desolvated solution contained a much lower concentration of gelatin species with retention times between 27 to 36 minutes. These results are consistent with those observed in earlier studies [1]. However, these residual soluble species were not present in the final nanoparticle dispersion, as filtrates of the nanoparticle dispersion did not exhibit any significant concentrations of eluted species, while the ultrafiltrate washings only contained species with retention times greater than approximately 34 minutes. The chromatogram of a water control taken through the nanoparticle production process was practically superimposable on that of the filtered nanoparticle dispersion, indicating that the soluble gelatin species present post-desolvation were effectively absent following cross-linking and neutralisation. An explanation for this observation is that the glutaraldehyde crosslinked both the desolvated and the soluble gelatin, a hypothesis supported by the fact that crosslinking of an undesolvated gelatin solution also did not have any residual detectable gelatin species. The residual gelatin species following desolvation thus appear to be crosslinked onto the surface of previously existing nanoparticles (nanoencapsulation), possibly establishing a gelatin ‘brush border’ and accounting for the dispersion stability of the nanoparticles [2].

Ultrafiltration appeared to be an effective method for separation and purification of the nanoparticles. The glutaraldehyde-metabisulfite addition product formed during the neutralisation process was present in the first ultrafiltrate of both the nanoparticle and control preparations, which exhibited a sharp absorption peak at high retention times. This peak was practically absent in the third ultrafiltrate and also in the filtered nanoparticle preparation, indicating its removal from the nanoparticle...
dispersion. The effectiveness of the ultrafiltration process at removing gelatin species should theoretically not have been of any direct concern, since nanoparticle dispersions did not appear to contain any significant amount of residual gelatin species, as described above, and dispersed gelatin nanoparticles incubated in aqueous media did not appear to undergo any hydrolysis to release soluble gelatin that could be detected by HPLC. Nevertheless, ultrafiltration of dilute gelatin solutions was shown to be effective at removing gelatin species of low to intermediate molecular weights, with medium to high molecular weight species being detected in the retentate.

We have therefore concluded that, during nanoparticle production, the crosslinking process not only crosslinks the colloidal gelatin particles but also removes residual soluble gelatin fractions from solution, probably by crosslinking to the surface of the existing nanoparticles. The ultrafiltration process is effective both at removing the addition reaction impurities and at removing low molecular weight gelatin species. However, the latter do not appear to be present in the nanoparticle dispersion prior to purification.

References:
