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## Scaling-up the nano-fabrication and stability study of hydrocortisone and hydroxytyrosol co-loaded chitosan nanoparticles

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Topical application of aqueous cream (AQ) containing hydrocortisone/hydroxytyrosol (HC/HT) loaded chitosan nanoparticles (NPs) was reported to reduce signs of atopic dermatitis (AD). Despite development on large scale and its extended stability scars. Different methods of nano-fabrication of polymeric NPs are available, ionic gelation method is the simplest and non-destructive method for scaling-up. In this respect, an overhead spinning disc Silverson® mixer at various speeds (300–3000 rpm) was introduced for nanofabrication with an increased volume up to 10-fold that of the laboratory scale. Size of NPs was analyzed by photon correlation spectroscopy and transmission electron microscopy. HC/HT NPs AQ-cream was stored at 4, 25 and 45°C for a period of one year to assess its stability. According to the results obtained by laser Doppler anemometry, the mean particle sizes of both small and large scale CSNPs were not statistically significant. Mean zeta potential for small and large scale HC/HT CSNPs were  $+35.2 \pm 0.32$  mV and  $+31.7 \pm 2.65$  mV, respectively. HC/HT NPs AQ-cream had an initial pH of 6.2 which was suitable for topical application on human skin. At 25°C, pH of NPs formulation insignificantly decreased to 5.8 at the end of the 6th month. On the other hand, at 40°C, pH significantly decreased to 4.3. At 4°C, pH of formulation was stable as pH was insignificantly different from the initial pH (month 0). Measured particle size of HC/HT NPs was <250 nm whereas AQ-cream stored at 25 °C was comparatively stable. Taken together, these findings suggest that HC/HT NPs were relatively stable. Storage condition at 25°C was recommended for the NPs formulation as minimal changes in terms of rheology, pH and morphology were observed.

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