Validation of a titration method to determine chondroitin sulfate loaded to solid lipid nanoparticles in an experimental factorial design.

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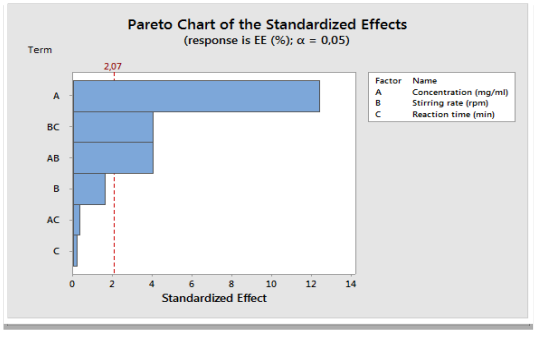
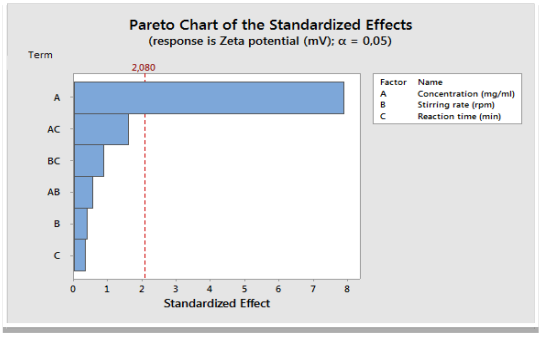
Abstract

Previous efforts at the Faculty of Pharmacy and Food Sciences of the University of Barcelona, have achieved to obtain cationic solid lipid nanoparticles (cSLN), with an average size of less than 200 nm, by the hot microemulsion method, which have been tested as a vehicle for pDNA and siRNA, in the transfection of cell lines [1,2]. It is of scientific interest to evaluate the capacity of SLN transporting different types of biomolecules with pharmacological potential. Chondroitin sulfate (CHON) is a major component of the extracellular matrix of several connective tissues, including skin, bone, ligaments, tendons and cartilage. For that reason, CHON is a potential therapeutical agent in Osteoarthritis (OA), which is characterized by progressive structural and metabolic changes in joint tissues [3]. Studies recommend topical administration in treating OA as first line therapy, and the development of topical systems with nanotechnology may introduce a new perspective for future treatment of OA [4].

An experimental factorial design, to optimize the production of SLN of CHON, was employed. The variables were defined as Concentration (mg/ml), Stirring rate (rpm) and Reaction time (min). Different properties were tested, including entrapment efficiency of CHON, zeta potential and particle size. A titration method was validated to test entrapment efficiency of CHON. A calibration curve was obtained from 0.10 to 1.20 mg mL^{-1} ($r > 0.9994$). Within-day % RSD was 0.7 and between-day % RSD was 1.11. Specificity/ selectivity experiments revealed the absence of important interference from excipients, mean recovery from spiked samples for CHON was 93.6 %.





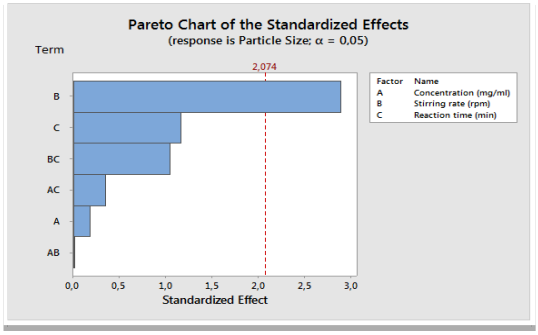


Fig 1. Pareto Chart to determine the magnitude and the importance of the effects in the responses. Bars that cross the reference line are statistically significant at the 0.05 level with the current model terms. The EE is mainly influenced by the Concentration of CHON. The combination of factors such as: Stirring rate with the Concentration and Stirring rate with the Reaction time have an important effect, and they should be considered in the production of SLN (A). Only the factor Concentration is statistically significant for

The optimal factors were attained by Minitab® program, using design of experiment (DOE) and pareto chart, see Figures 1 and 2.

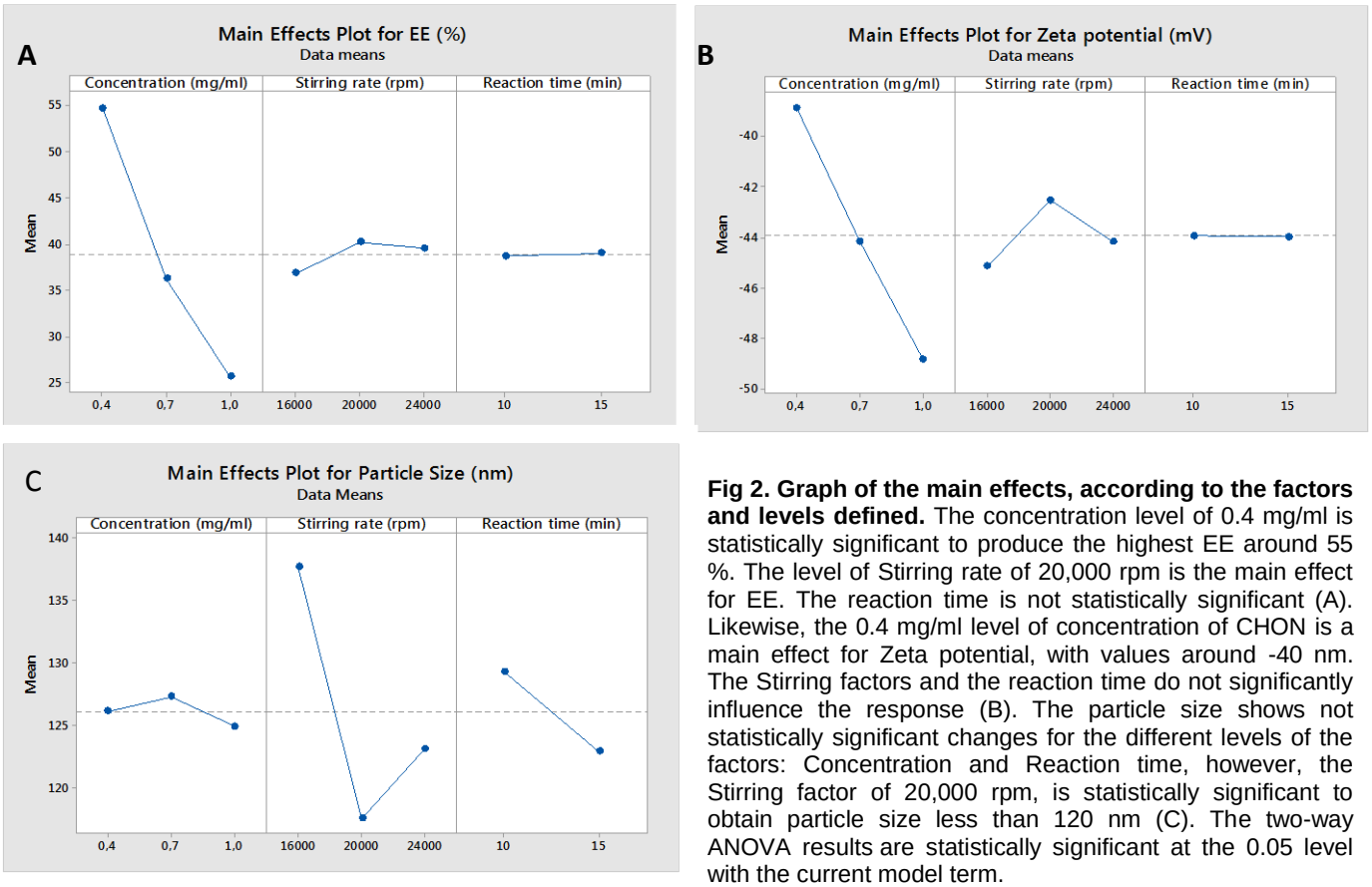


Fig 2. Graph of the main effects, according to the factors and levels defined. The concentration level of 0.4 mg/ml is statistically significant to produce the highest EE around 55 %. The level of Stirring rate of 20,000 rpm is the main effect for EE. The reaction time is not statistically significant (A). Likewise, the 0.4 mg/ml level of concentration of CHON is a main effect for Zeta potential, with values around -40 nm. The Stirring factors and the reaction time do not significantly influence the response (B). The particle size shows not statistically significant changes for the different levels of the factors: Concentration and Reaction time, however, the Stirring factor of 20,000 rpm, is statistically significant to obtain particle size less than 120 nm (C). The two-way ANOVA results are statistically significant at the 0.05 level with the current model term.

In conclusion, the titration method is a simple, rapid and reliable method for the determination of chondroitin sulfate loaded to SLN. The DOE revealed that reaction time does not have a significant impact in the evaluated responses. However, concentration (0.4 mg/ml) and stirring rate (20 000 rpm) were determinant to maximize entrapment efficiency of CHON in SLN and to get the optimum size and zeta potential of SLN.

References

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