Characterization of a Water-In-Oil-In-Water Multiple Emulsion Integrating Biomimetic Aqueous-Core Lipid Nanoballoons Housing Protein Entities

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INTRODUCTION

Due to the increasing awareness related to the worlwide appearance of multiple bacterial resistance to conventional chemical antibiotics, bacteriophage particles are being re-discovered as high-potential candidates for biopharmaceutical applications.[1] However, phage particles are fragile, and therefore their full structural and functional stabilization is required.[2] Solubilization of these protein-like entities in the aqueous-core of lipid nanoballoons integrating multiple emulsions of the type water-in-oil-in-water, will protect them.[1] The aim of the study entertained herein was to optimize a water-in-oil-in-water (W/O/W) multiple emulsion formulation integrating aqueous-core lipid nanoballoons encasing a macromolecular protein entity and entail its thorough physicochemical characterization.

EXPERIMENTAL/THEORETICAL STUDY

Two emulsions were prepared sequentially, a primary emulsion (W/O), followed by emulsification of this emulsion in another (external) aqueous phase (W), thus forming a multiple emulsion (W/O/W). The inner aqueous phase was constituted by HCl 10 mM, Tween 80 and pure protein entity; the intermediate oily phase encompassed glycerol, Softisan 100™ and soybean phosphatidylcholine; the outer aqueous phase encompassed poloxamer 188 and UP water. W/O/W multiple emulsion formulation was characterized via DLS by measuring particle hydrodynamic size, size distribution and particle charge via Zeta potential analysis, surface morphology and diffusion coefficient via Nanoparticle Tracking Analysis (NTA), thermal analyses via Thermogravimetry (TGA) and Differential Scanning Calorimetry (DSC), infrared spectrophotometry with Fourier transform (FTIR), and X-ray diffraction (XRD).

RESULTS AND DISCUSSION

Two homogenization cycles of 10 min at 12500 rpm, 0.015% (w/w) protein, 0.75%, (w/w) lecithin and 0.50% (w/w) poloxamer 188, were found to be critical variables for producing stable (aqueous-core) lipid nanoballoon dispersions with average hydrodynamic diameters ranging from 184 nm to 189 nm, average polydispersity index ranging from 0.192 to 0.220 and average Zeta potential values ranging from -37.38 mV to -35.52 mV. No phase separation whatsoever could be found for the optimized multiple emulsion system, kept stable over a storage timeframe of 120 days. Figure 1 displays a frozen high-resolution frame of the lipid nanoballoons integrating the optimized W/O/W multiple emulsion housing protein entities, allowing to observe the spherical shape of the particles and the absence of aggregation. The analysis performed allowed to determine the diffusion coefficient of the lipid nanoballoons as 2.64879×10^{-12} $m^2.s^{-1}$, using the Stokes-Einstein equation. Diffusion coefficients for nanoparticles are typically of the order of magnitude of 10^{-12} $m^2.s^{-1}$. The lower the diffusion coefficient, the more stable the multiple emulsion is, and this was indeed observed during the long-term storage of our statistically optimized W/O/W multiple emulsion housing protein entities.

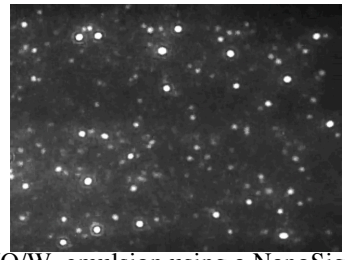

Figure 1. W/O/W emulsion using a NanoSight NS300.

A comparison between XRD, TGA, DSC and FTIR spectra of optimized multiple emulsions without and with encapsulated protein, allowed to conclude that there was no chemical interaction between the protein and the chemical components of the emulsion.

CONCLUSION

The multiple emulsion proved to be stable for 120 days and was able to encapsulate protein entities.

REFERENCES

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