

## A multiple emulsion formulation of bacteriophage encapsulated in lipid nanovesicles

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The emergence of antibiotic-resistant bacterial strains and the weak penetration of antibiotics in bacterial biofilms put an emphasis in the need for safe and effective alternatives for antimicrobial treatments. The application of strictly lytic bacteriophages (or phages) has been proposed as an alternative (or complement) to conventional antibiotics, allowing release of the natural predators of bacteria directly to the site of infection. Probably, the major advantage of phage-based therapy lies in the fact that phages replicate directly in the site of infection, becoming profusely available where they are most needed. When compared to antibiotics, phages present many relevant advantages: (i) permanently high concentrations at the infection site, increasing in the presence of (viable) bacterial host, with elimination occurring only after eradication of the later; (ii) total compatibility with antibiotics; (iii) specificity against target-bacteria; (iv) higher penetration in bacterial biofilms, by inducing production of enzymes that hydrolyze biofilm polymeric matrix; and (v) while bacteria can develop resistance to phages, isolation and large-scale production of new lytic phages is much simpler and economical than developing a new antibiotic. Water-in-oil-in-water (W/O/W) emulsions are examples of multiple emulsions, in which dispersions of small water droplets within larger oil droplets are themselves dispersed in a continuous aqueous phase. Due to their compartmentalized internal structure, multiple emulsions present advantages over simple O/W emulsions for encapsulation, such as the ability to carry both polar and non-polar molecules, and a better control over releasing of therapeutic molecules. In the present research work, the potential of nanoencapsulating a broad lytic spectrum phage able to infect enteric *Salmonella* and *E. coli* has been investigated. Phage phi-PVP-SE1 was entrapped within W/O/W multiple nanoemulsions, aiming at mimicking the multifunctional design of biology, with several lipid matrices, poloxamers and stabilizing layer compositions. Physicochemical characterization of the optimized phage-encasing nanovesicle formulations encompassed determination of particle size, size distribution and particle charge, via Zeta potential analysis, surface morphology via SEM, encapsulation efficiency, and thermal analysis via DSC. The antimicrobial activity of the nanoemulsions produced was also assessed *in vitro*, using several microbial strains.