

## Bioactive Substances-Loaded Water-in-Oil/Oil-in-Water Emulsions for Dietary Supplementation in the Elderly

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**Abstract :** Maintaining a bioactive substances dense diet is important for the elderly, especially to prevent diseases and to support healthy ageing. Adequate bioactive substances intake can reduce the risk of developing chronic diseases (e.g. cardiovascular, osteoporosis, neurodegenerative syndromes, diseases of the oral cavity, gastrointestinal (GI) disorders, diabetes, and cancer). This can be achieved by introducing a comprehensive supplementation of components necessary for the proper functioning of the ageing body. The paper proposes the multiple emulsions of the W1/O/W2 (water-in-oil-in-water) type as carriers for effective co-encapsulation and co-delivery of bioactive substances in supplementation of the elderly. Multiple emulsions are complex structured systems ("drops in drops"). The functional structure of the W1/O/W2 emulsion enables (i) incorporation of one or more bioactive components (lipophilic and hydrophilic); (ii) enhancement of stability and bioavailability of encapsulated substances; (iii) prevention of interactions between substances, as well as with the external environment, delivery to a specific location; and (iv) release in a controlled manner. The multiple emulsions were prepared by a one-step method in the Couette-Taylor flow (CTF) contactor in a continuous manner. In general, a two-step emulsification process is used to obtain multiple emulsions. The paper contains a proposal of emulsion functionalization by introducing pH-responsive biopolymer—carboxymethylcellulose sodium salt (CMC-Na) to the external phase, which made it possible to achieve a release of components controlled by the pH of the gastrointestinal environment. The membrane phase of emulsions was soybean oil. The W1/O/W2 emulsions were evaluated for their characteristics (drops size/drop size distribution, volume packing fraction), encapsulation efficiency and stability during storage (to 30 days) at 4°C and 25°C. Also, the in vitro multi-substance co-release process were investigated in a simulated gastrointestinal environment (different pH and composition of release medium). Three groups of stable multiple emulsions were obtained: emulsions I with co-encapsulated vitamins B12, B6 and resveratrol; emulsions II with vitamin A and  $\beta$ -carotene; and emulsions III with vitamins C, E and D3. The substances were encapsulated in the appropriate emulsion phases depending on the solubility. For all emulsions, high encapsulation efficiency (over 95%) and high volume packing fraction of internal droplets (0.54-0.76) were reached. In addition, due to the presence of a polymer (CMC-Na) with adhesive properties, high encapsulation stability during emulsions storage were achieved. The co-release study of encapsulated bioactive substances confirmed the possibility to modify the release profiles. It was found that the releasing process can be controlled through the composition, structure, physicochemical parameters of emulsions and pH of the release medium. The results showed that the obtained multiple emulsions might be used as potential liquid complex carriers for controlled/modified/site-specific co-delivery of bioactive substances in dietary supplementation in the elderly.

**Keywords :** bioactive substance co-release, co-encapsulation, elderly supplementation, multiple emulsion

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