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Review

Protein Formulations for Emulsions and Solid-in-Oil Dispersions

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Needs from medical and cosmetic areas have led to the design of novel nanosized emulsions and solid-in-oil dispersions of proteins. Here, we describe the production of those emulsions and dispersions using high-energy methodologies such as high-pressure homogenization or ultrasound. Recent work has resulted in new mechanistic insights related to the formation of protein emulsions and dispersions. The production method and composition of these formulations can determine major parameters such as size, stability, and functionality, and therefore their final application. Aqueous nanoemulsions of proteins can be used for drug delivery, while solid-in-oil dispersions are often used in transdermal applications.

Trends in the Production of Protein Emulsions and Dispersions

Protein formulations based on emulsions and dispersions are one of the most researched areas in biomedical and pharmaceutical/cosmetic agents. The production technology of such formulations can be found in applications ranging from targeted drug delivery to transdermal perfusion patches and cosmetics [1–3]. In this context, various methods have been used to formulate proteins, such as **coacervation/desolvation** (see Glossary), **thermal gelation**, **emulsification**, **self-assembly**, and **solid-in-oil dispersions** [4–7].

Nanoemulsions are emulsions with uniform and extremely small droplet size, which have attracted growing interest as colloidal drug carriers for pharmaceutical applications [8,9]. High-energy emulsification methods, such as sonication and high-pressure homogenization, are widely reported for preparing nanoemulsions [10,11]. The formation and stabilization of the droplets determines the preparation of a finely dispersed emulsion [12], where the high-energy methods are important through the promotion of efficient mass transfer. This phenomenon is mainly due to the formation of high turbulence that creates molecular agitation. The stabilization of the newly formed droplets against coalescence is the second step; **emulsifiers** are added to the system for this purpose [11,13]. The emulsifiers can lower the interfacial tension and prevent the agglomeration and coalescence of the droplets by increasing repulsion forces between droplets [13–15]. A wide variety of synthetic and natural emulsifiers can be used, such as surfactants, phospholipids, proteins, and polysaccharides [15]. Surfactants play a greater role in selecting emulsifiers due to their amphiphilic molecules, which can be applied in oil/water interface emulsions, resulting in the dispersion of one phase into another immiscible phase.

Solid-in-oil (S/O) dispersions consist of nanosized protein–surfactant complexes dispersed in an oil vehicle. S/O dispersions are attractive formulations for improving the dispersibility (or solubilization) of **hydrophilic** biomolecules such as **globular proteins** and peptides into the oil phase [16,17]. The method used to produce S/O dispersion is based on a combined high-energy

Trends

The design of protein-based templates of emulsions and solid-in-oil dispersions for drug delivery applications constitutes an immense platform for the delivery of active components intravenously or by skin permeation.

High-energy methodologies such as high-pressure homogenization and ultrasound play important roles in the formation and stabilization of emulsions and dispersions.

Emulsification and dispersibility methods are the most promising technologies to achieve controlled transport and delivery of active compounds.

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approach. This method involves the use of equipment, such as high-pressure homogenizer and ultrasonic probe, which are capable to generate huge amounts of energy for emulsification and dispersion of the protein in an appropriate oil. This technique is an effective alternative method to formulate poorly water-soluble compounds [18]. S/O dispersion is a type of colloid with a material in a solid state dispersed in a media in liquid state, and this is generally designated as sol or suspension [6,18–20]. Nanodispersions are biphasic submicron colloidal dispersion formulations [17].

Here, we focus on the emulsification and dispersibility methods that are the most widely used approaches to obtain stable microscale and/or nanoscale protein formulations (Figure 1) as promising systems enabling controlled transport and delivery. We review recent works related to the production of both emulsions and S/O dispersions using high-energy methodologies. These protein formulations can be used for delivering active components intravenously or in skin permeation.

The Influence of Emulsifiers in Producing Emulsions and Dispersions

Protein microemulsions and nanoemulsions have been used to stabilize, protect, and deliver active components in different formulations. Several factors are known to influence the behavior of these formulations, including particle size and distribution, emulsifier type and concentration, aqueous solubility of the dispersed phase, temperature, surface tension, and ionic strength [21]. The nature of the emulsifier is crucial in the formation of an emulsion. The most effective emulsifiers are nonionic or mixtures (e.g., ionic and nonionic, or mixtures of nonionic surfactants). They can be more effective in emulsification for lowering the interfacial tension and stabilizing the emulsion against flocculation and coalescence. The emulsifier concentration has a direct effect on emulsion production, especially for obtaining homogeneous and small emulsions.

Protein molecules demonstrate greater mobilities at emulsion interfaces with the microsphere walls having spherical and regular surfaces, as shown in the schematic illustrations in Figure 2.

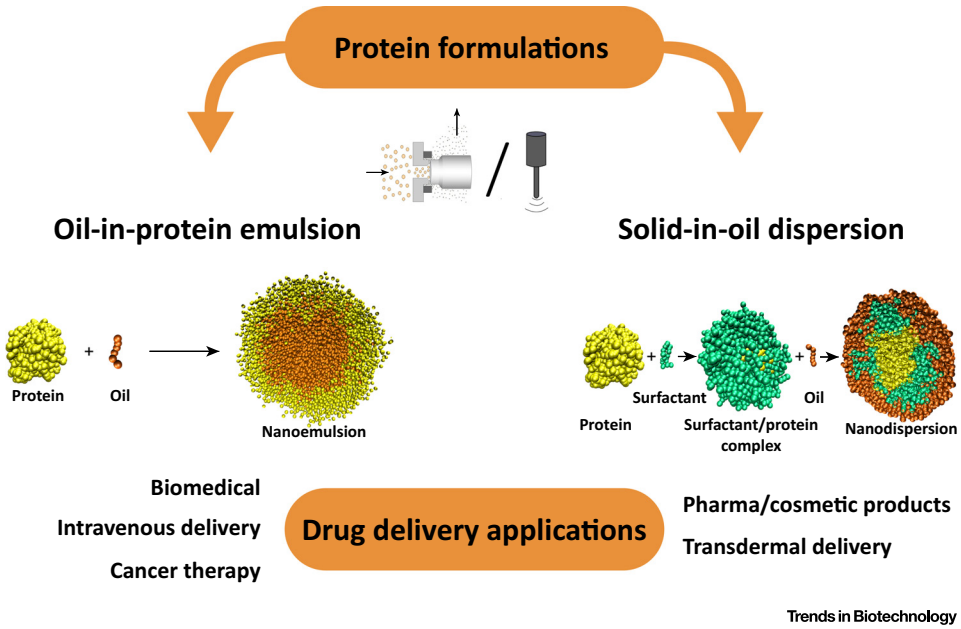

Figure 1. Schematic Illustration of Protein Formulations Based on Emulsification and Dispersibility Methods to obtain Microemulsions and/or Nanoemulsions and Dispersions, under Mechanical High-Energy Methodology for Drug Delivery Applications.

Glossary

Coacervation/desolvation: a thermodynamically driven self-assembly process for nanoparticle preparation, where a desolvating agent (acetone or ethanol) is added dropwise continuously to an aqueous protein solution under continuous stirring. In some cases, the addition of crosslinking agents is necessary to promote the nanoparticle stabilization.

Emulsification: a method widely used for preparing nanoparticles, in which an aqueous protein solution is emulsified in a nonaqueous medium, such as oil. There are different emulsification processes, including high-energy methods, which use mechanical devices such as high-pressure homogenizers or ultrasound generators; and low-energy methods, which use the stored chemical energy of the system. For stabilizing the nanoparticles, chemical or physical (thermal) crosslinking is used.

Emulsifier: an agent that helps emulsions become more stable. The chemical structures of emulsifiers contain a hydrophilic and hydrophobic part, and they act by reducing the interfacial tension between the oil and water phases.

Globular proteins: the most water-soluble proteins, which have polypeptide chains coiled into a compact shape and a tightly packed core of hydrophobic amino acids.

Hydrophilic: molecules that tend to interact with water (either polar or charged). Hydrophilic side chains tend to associate with water molecules or with other hydrophilic side chains.

Hydrophobic: molecules that tend to avoid water (nonpolar and uncharged). Hydrophobic side chains interact with each other due to their tendency to minimize their contact with water or polar side chains.

Nanocarriers: nanoscale drug delivery systems that transport drugs or biomolecules; an important objective is to improve their longevity in the blood, allowing their accumulation in pathological areas.

Self-assembly: a nanoparticle preparation method involving increasing the hydrophobicity of a protein; a protein can be made to self-assemble by breaking disulfide bonds or decreasing primary amine