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Protein microcapsules: Preparation and applications

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ABSTRACT

Liposomes and polymerosomes generally represent the two most widely used carriers for encapsulating compounds, in particular drugs for delivery. While these are well established carriers, recent applications in biomedicine and food industry have necessitated the use of proteins as robust carriers that are stable under extreme acidic and basic conditions, have practically no toxicity and are able to withstand high shear force. This review highlights the different methods for using proteins as encapsulating materials and lists some biomedical applications of the microcapsules. The advantages and limitations in the capsules from the different preparation routes are enumerated.

Contents

1. Introduction

Scientists have long been intrigued by the possibilities of controlling the release of an active ingredient by encapsulating it. The necessity to find biomacromolecular drugs has given rise to the development of new methods of encapsulating the drugs using hollow structures (capsules) of biomolecules. Liposomes traditionally from phospholipids represent the standard model for biological capsule [1] and are used widely in cancer therapy. However they need to be chemically modified by PEGylation to stabilize them against immune response [2]. Polymerosomes are another class of aggregated structures and are designed either from pure polymers or from polymer/peptide units grafted with methoxy polyethylene glycol [3]. All these systems have the common feature of amphiphilicity of the compounds involved.

These encapsulating systems find wide applications in pharmaceutical industry as controlled drug release devices, for therapy and for immobilizing other macromolecules [4]. In recent times, new microcapsules based on biopolymers like proteins are being introduced as viable alternative to the polymerosomes or liposomes. A protein has to be cross-linked or stabilized using various methods in order to achieve sustained or controlled release properties. Easily available and often biocompatible, biopolymers play an increasing role among the materials available to constitute the frame of microparticles. Furthermore, the functional groups of proteins are available for chemical modifications needed for encapsulation by chemical methods. However the different methods that are currently available have their own limitations with some of them suffering from toxicity issues. Recently, genome nucleic acids encapsulated in viral capsid proteins have been used for viral cell transformation [5].

This review highlights the different techniques used to form protein capsules that can act as vehicle for encapsulating drugs or even as templates to prepare new materials. In the preparation of protein microcapsules, a wide variety of protein aggregates are encountered ranging in

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size and characteristics (e.g., soluble or insoluble, covalent or noncovalent, reversible or irreversible) and span a broad size range, from small oligomers (nanometers) to insoluble micron-sized aggregates that can contain millions of monomer units. These aggregates result from various kinds of stress such as agitation and exposure to extremes of pH, temperature, ionic strength, or various interfaces (e.g., air–liquid interface). Therefore, the preparation of the microcapsules of protein needs to be carefully characterized and controlled during development, manufacture, and subsequent storage of a drug substance and formulated product.

Microcapsules in general refer to spherical microparticles and "microcapsules" happen to be a sub category of the microparticles which have a core surrounded by a material which is distinctly different from that of the core. The core may be solid, liquid, or even gas [6]. Till now several approaches have been used to design microcapsules and a number of applications have been reported [7–19].

2. Methods of preparation

In general, the term "microcapsule" is defined, as a spherical particle with the size varying between 50 nm to 2 mm containing a core substance. However, though the word capsule implies a core and shell structure, the term microcapsules can also include membrane enclosed particles or droplets but also dispersion in solid matrix lacking a distinctive external wall phase as well as intermediate types and depending on the method of preparation, the various types of capsules can be obtained (Fig. 1.). The general strategies for formation of protein capsules usually are from two different routes: covalent and noncovalent interactions.

Covalent interactions generally increase the mechanical strength of the capsules as has been demonstrated in the layer by layer method of preparation which uses glutaraldehyde [20–22]. However, the use of covalent interactions to form the capsules has its own limitations in that it results in permanent bonds which seem to trigger larger assemblies of the capsules and in some instances can trigger cytotoxicity [23–25].

A viable alternative is to use noncovalent interactions (e.g., with amphiphilic molecules, electrostatic interactions, hydrogen bonding and hydrophobic interactions). These include formation of hydrogels [26–30], particles formed between proteins and ligands, polymers and colloidal systems.

Using the hydrophobic/hydrophilic interface, Suslick et al. used ultrasonication on proteinaceous materials in which a water insoluble liquid was enclosed and demonstrated that high concentrations with narrow size distributions of the microcapsules can be formed. The scheme for the preparation of such assemblies is presented in Fig. 2. They also showed that this involves both emulsification and a chemical cross-linking of protein molecules through disulfide bond formation by sonochemically generated superoxide [31].

Interfacial tension between water and an organic phase was used by Liu et al. to prepare encapsulated assemblies of proteins on organic droplets [32]. These oil/water interfaces while stabilizing the protein at the interface, however have an inherent disadvantage in that the oil droplets are not suitable to dissolve water-soluble guest biopolymers. Morikawa et al. have used ionic liquid–water interface to encapsulate proteins in biopolymers [33]. Here surface-modified protein microcapsules spontaneously form at ambient temperature. The size and rate of formation depend on the type of protein and also on the charge density on the surface of the protein which can be regulated by suitable choice of the anions or cations of the ionic liquid. Following section pertains to use of LbL technique and modification of this technique in the preparation of protein capsules.

2.1. Capsules using covalent interactions

2.1.1. LbL and other modified techniques of LbL

Design of microcapsules based on electrostatic interactions has been carried out by layer by layer adsorption (LbL) of polyelectrolytes in many examples including polypeptides built on colloidal templates. This method developed by Decher et al. uses sequential deposition of oppositely charged layers on a template that can then form polyelectrolyte shells [34,35]. Here the shell thickness can be controlled and with suitable triggers can be used to release materials [36,37]. Capsules prepared using the LbL technique have been made using a variety of cargo molecules that include polymers, low molecular weight compounds, and enzymes [38–43].

Zelikin et al. have used a facile method to encapsulate both single as well as double stranded DNA in nanoengineered, degradable polymer

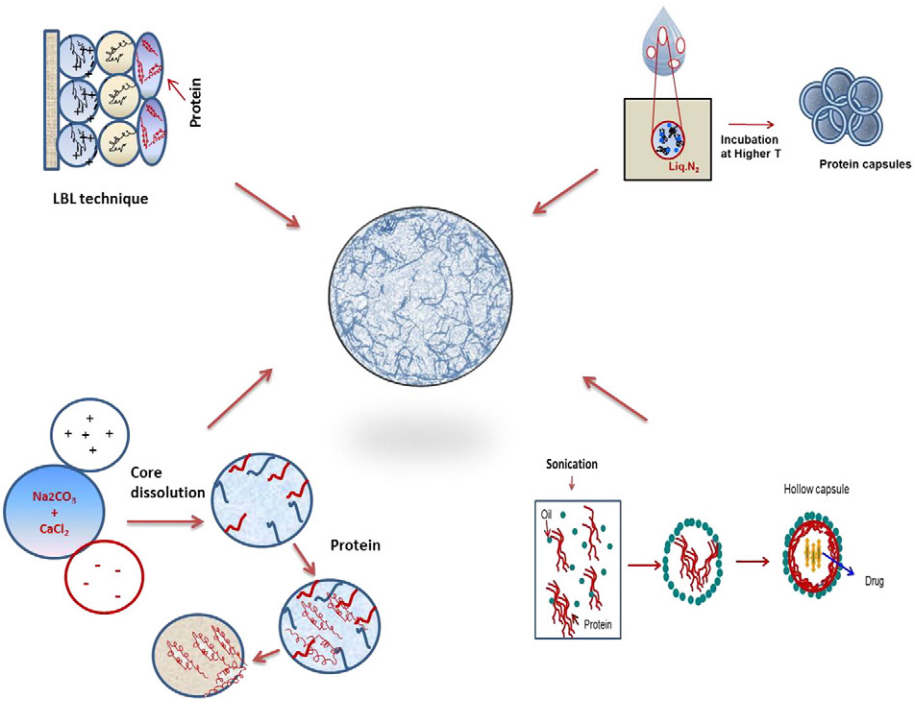

Fig. 1. Scheme showing different methods of preparation for protein microcapsules.