Emulsions and nanoparticles on innovative biocatalysis, design of drug delivery and cell-free protein expression systems

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INTRODUCTION

Microencapsulation and encapsulated products have played a very important role in numerous industries like agriculture, chemical, pharmaceutical, cosmetic and the food industry. In recent decades microencapsulation of bioproducts (bioencapsulation) has been also applied to biotechnology and medicine, including cell encapsulation of probiotics, generation of artificial implants, encapsulation of enzymes for biocatalysis, pharmaceutical compounds for drug delivery, and many others.

Successful application in these areas requires anyway a technology, which has the capability to produce mono-dispersed, homogenous shaped capsules, narrow size distribution, short production time and under simple condition, using many different materials, including highly viscous solutions.
The heterophase systems have shown to be suitable methods for producing materials matching the previous requirements of functionality and microencapsulation of active and sensible biomolecules. These systems, in particular emulsion technique, continue to be an exciting field for the development of new engineering technology, and new production procedures and applications.
This paper will be focus on emulsions in the form of micelles with only one lipid layer or liposomes with unilamellar lipid bilayers (Fig. 1) used named in the form of miniemulsions, micellar systems, solid-lipid nanoparticles (SLNs) and vesicles.

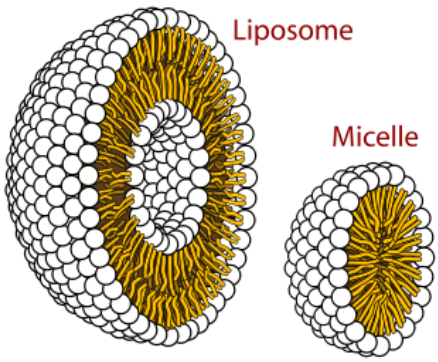

Figure 1 –Liposome (a closed bilayer) and the micelle (a closed monolayer) of tension-active compounds.

THE HETEROPHASE SYSTEMS

Microencapsulation of biological organelles, DNA, vaccines, proteins, chemical reagents and products, drugs, and others compounds in the heterophase systems has shown to provide excellent method on the protection of the biomaterial inside of the liquid or solidify core, improved handling properties, controlled release of the encapsulated bioproduct, among many others advantages.

A liquid heterophase system is described as a system of at least two immiscible components, for which one (dispersed phase) is finely distributed in the other (continuous) phase. Normally, surfactants, lipids, phospholipids and others tension-active compounds are used to stabilize these submicron range liquid heterophase systems also designed as colloidal systems. Emulsions are metastable colloidal dispersions consisting of two immiscible liquids.

Miniemulsion and micellar systems

For the formation of these heterophase systems and to disperse one phase it is necessary the application of shear forces like shaking, stirring or sonication. According to the energy load to the heterophase systems and based on the stability and the droplet size of the dispersed droplets, for example, the emulsions are classified into macro, micro- and miniemulsions.
When the dispersed phase is consisting of an oil and the continuous phase of water, the dispersion are called direct or oil-in-water emulsions (o/w-emulsions) like miniemulsion. In an inverse or water-in-oil emulsion (w/o), the water droplets are dispersed in a continuous phase of oil illustrated by the micellar system. The liposome can be described as a vesicle with a bubble of liquid within another liquid separated by a double lipid bilayer.

The miniemulsion is a special class of emulsion that consists of narrowly distributed droplets with a size ranging from 50 to 500 nm by the use of high energy homogenization such as ultrasound or high-pressure homogenization. Miniemulsions are stabilized against the two main destabilization processes that can lead to breaking of emulsions, coalescence and *Ostwald* ripening (Fig. 2).

Miniemulsion and micellar system showed to be excellent non-conventional medium for biocatalysis especially when the reagents and products have low solubility in aqueous phase or present strong inhibition or deactivation on the biocatalyst. Furthermore, miniemulsion showed also enhances the reaction kinetic and shift the thermodynamic equilibrium by expulsion of the water formed during the synthesis reactions to out of the interface where occurs the reaction (de Barros et al 2012).

Figure 2 - Homogeneity of the stable miniemulsion (right) in comparison to the reaction mixture prior to formation of the miniemulsion (left) from the separated phases water and substrate.

Solid lipid nanoparticles (SLNs)

Dispersions of the solid lipid nanoparticles (SLNs) are prepared using biodegradable materials, generally regarded as safe (GRAS) for pharmaceutical applications by melt-emulsify-chill (MEC) method. These are particulate systems ranging from 50 - 1000 nm in diameter and are composed of lipids that are in solid phase at the room temperature and include various fatty acids, triglycerides, cholesterol, partial and waxes (Fig. 3).

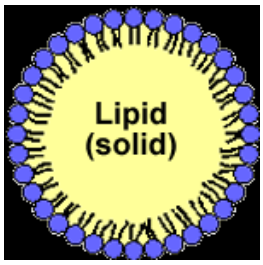

Figure 3 Solid lipid nanoparticle scheme.

Several types of surfactants are also used as emulsifiers to stabilize lipid dispersion. Lipid nanoparticles were originally developed for oral drug administration. SLN-based antimicrobial drug delivery systems though relatively new, are particularly attractive in overcoming antibiotic resistance in view of their potential for oral, parenteral, ocular, pulmonary and skin drug administration. Under optimized conditions, SLNs can be produced to incorporate hydrophobic or hydrophilic substances.

Storage stability and the problem of uptake and sequestration by the cells of the RES/ macrophage system have been resolved by coating SLNs with hydrophilic substances, e.g., polyoxypropylene block copolymers, chitosan and PEG. Important peptides (cyclosporine A, insulin, etc.) have been also incorporated into SLNs and are currently under investigation (Muller 2006).

Vesicles - Liposomes

Liposomes are a special case of microencapsulation. However, since the typical liposomal particle size is around 200 nm such systems should more rightly be named sub-microcapsules or nanocapsules. Liposomes are self-assembled spherical micro-structures of lipids and lipid-like amphiphilic molecules that are arranged in single or multilayered closed bilayers in aqueous media where the active compounds are trapped inside this fatty wall containing the aqueous core.

There are large numerous of biotechnology and medical applications but the most recent examples in this field are the development of nanoparticles that can be controllably triggered to synthesize proteins. These nanoparticles consist of lipid vesicles filled with the cellular machinery responsible for transcription and translation, including amino acids, ribosomes, and plasmid DNA. To create particles capable of synthesizing proteins, phospholipid vesicles were formed around a minimal bacterial extract and plasmid DNA template encoding a reporter protein (Fig. 4). These nanoparticles can serve as nanofactories capable of producing proteins spatially and temporally controllable *in vitro* and *in vivo* and designed as remotely activated drug delivery (Schroeder 2012).

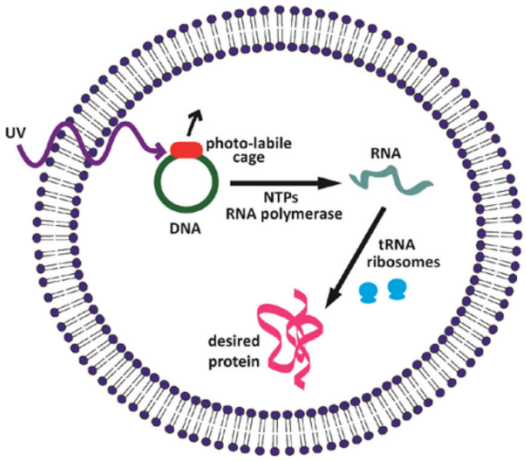

Figure 4 – Lipid vesicles filled with the cellular machinery for cell-free protein synthesis.

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