Melvin X. L. Tan
Michael K. Danquah

Monash University, Chemical Engineering Department, Bio Engineering Laboratory, Clayton, Victoria, Australia.

Review

Drug and Protein Encapsulation by Emulsification: Technology Enhancement Using Foam Formulations

Significant progress has been made in developing formulations for protein and drug encapsulation and delivery. The most frequently used method is the emulsion/solvent removal technology, where microencapsulation of proteins in polymeric matrices can be easily achieved with a simple stirrer setup. However, it remains a challenge to produce protein-encapsulated formulations with high encapsulation efficiencies. The emulsion/solvent removal technique and the relevant formulation and process parameters that govern the protein encapsulation processes are reviewed. A new encapsulation method of using foam as a delivery medium during the preparation of protein-loaded microparticles is proposed. The foam characteristics of direct relevance to protein encapsulation are discussed. The unique properties of foam could enhance intermolecular interactions and access to internal pore surfaces, thus making them superior compared to traditional methods employing aqueous liquid phases during protein encapsulation.

Keywords: Drug encapsulation, Emulsification, Foam formulation, Protein encapsulation

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1 Introduction

Improvement in protein encapsulation and delivery methodologies is a major research endeavor owing to the numerous biomedical and biochemical applications. A wealth of literature dedicated to protein delivery is available, with a strong focus on the synthesis of depot formulations in which the protein is entrapped by a carrier. Other applications, such as chromatography and immunoassays, involve binding protein molecules with sorbents for separation and/or purification purposes. Chromatography is also used to create protein-loaded particles for biopharmaceutical applications. All these applications hinge on the ability of protein molecules to bind onto a solid platform.

The extent of protein entrapment, which can be described by encapsulation efficiency or adsorption capacity, is one of the key performance indicating parameters of the encapsulation process. A large number of protein-encapsulated formulations have been successfully developed, but it remains a practical challenge to produce such formulations with high encapsulation efficiencies. Conventional encapsulation methodologies routinely achieve low encapsulation efficiencies usually in the range of 10–50 % [1–4].

For an effective protein encapsulation or adsorption process, the encapsulate or adsorbent should provide the ability to bind protein molecules without compromising bioactivity and release the molecules under suitable process conditions and with the appropriate release kinetics [5, 6]. In therapeutic protein delivery, poorly encapsulated proteins produce large initial burst due to poor control over the diffusional release of proteins during the initial release stage. A burst release of proteins is often difficult to predict and control, and generally results in poor pharmacokinetics with unattractive release economics in relation to effective dose cost analysis [7, 8]. An undesirable high initial release rate may completely release encapsulated proteins too rapidly, thus causing toxicity problems and stressing cellular uptake mechanisms. Effective protein binding is, therefore, important to control the initial release rate. However, high initial rates of release can be useful in other cases, such as dermal applications and wound treatment [7–9].

Various methods, such as emulsion/solvent evaporation or extraction, coacervation, and spray-drying, have been reported to develop depot formulations for protein delivery [10]. Among these methods, emulsion/solvent evaporation or extraction is widely used to prepare protein-loaded formulations [11]. This article focuses on the incorporation of protein into polymeric delivery systems by emulsion/solvent evapora-

Correspondence: Dr. M. Danquah (michael.danquah@monash.edu), Monash University, Chemical Engineering Department, Bio Engineering Laboratory, Wellington Road, Clayton, Victoria 3800, Australia.

tion or extraction. The formulation and process parameters that affect encapsulation efficiency are reviewed. A novel foam-based method to enhance protein encapsulation efficiency is discussed.

2 Emulsion/Solvent Removal Methods

The process for preparing microparticles by emulsion/solvent removal can be divided into four major steps: (i) incorporation of the protein or active biomolecular phase, (ii) droplet formation, (iii) solvent removal, and (iv) harvest and drying [12]. To incorporate the protein, it is dissolved or dispersed into an organic solvent containing the polymer matrix (dispersed phase). The dispersed phase is usually emulsified with the external continuous phase – often an aqueous phase which is immiscible with the dispersed phase – forming the emulsion droplets. Subsequent evaporation at elevated temperature or reduced pressure conditions or extraction of the solvent from the dispersed phase by the continuous phase leads to the precipitation of protein-encapsulated micro/nanoparticles. In the harvesting and drying steps, the solidified microparticles are filtrated or centrifuged and dried either at ambient or under reduced pressure conditions, by heating or lyophilization (see Fig. 1).

Depending on the number of emulsions formed, the methods can be classified as single or double emulsion. For proteins dispersed in a polymer organic solution, a single emulsion of water-in-oil (W/O) forms. For a W/O emulsion dispersed in an external phase, which can be mineral oil or aqueous solution, a double emulsion of water-in-oil-in-water (W/O/W) or of water-in-oil-in-oil (W/O/O) forms. Depending on the application, other complex emulsions can be generated, such as solid-in-oil-in-oil-in-water (S/O/O/W) emulsion [13] or water-in-oil-in-oil-in-water (W/O/O/W) emulsion [14].

The incorporation of protein can be achieved by either dispersing the solid-state protein in the organic polymer solution or emulsification of aqueous protein solution with the organic polymer solution [12, 15]. The state of protein incorporated is likely to influence the encapsulation efficiency, as aqueous and solid proteins exhibit different affinity, properties, and interactions when they bind to the polymer. The aqueous and non-aqueous encapsulation approaches are discussed below.

2.1 W/O/W Emulsion Methods

In W/O/W double-emulsion methods, the aqueous protein solution is first dispersed in a polymer organic solvent to form a primary W/O emulsion. Commonly used organic solvents include dichloromethane (DCM) and ethyl acetate (EtAc). A W/O/W emulsion is formed by further dispersing the primary emulsion in an external aqueous phase usually containing an emulsifier, such as poly(vinyl alcohol) (PVA) or poly(vinyl pyrrolidone) (PVP). Subsequent solvent removal by evaporation or extraction leads to solidification and hardening of microparticles. Solvent evaporation is usually carried out by increasing the temperature under reduced pressure whilst solvent extraction is achieved by exposing the W/O/W emulsion into a large volume of water or other extraction agents, such as methanol or isopropyl alcohol, to absorb the entire solvent leaching out of the solidifying microspheres.

The W/O/W emulsion method has been widely used, due to its simplicity, to prepare protein-encapsulated formulations, however, it is difficult to control the properties of the formulated microparticles. One of the critical factors is the risk of protein loss due to premature diffusion when the emulsion droplets are exposed to the external phase. Protein molecules can easily leach from the emulsion droplets into the external phase due to resistance to resolubilization and exposure to large the interface between internal and external phases, resulting in a lower loading capacity and encapsulation efficiency [16]. It has also been reported that proteins are prone to inactivation and aggregation at the aqueous/organic solvent interface during the formation of the W/O emulsion [17, 18]. For this reason, S/O/W or S/O/O emulsion methods, where proteins are dispersed as solid particles, have been developed to avoid the oil-water interface.

2.2 W/O/O Emulsion Methods (Coacervation Method)

In W/O/O emulsion methods, the primary emulsion (W/O), achieved by emulsifying the aqueous protein solution in an organic polymer system, is added into a nonsolvent (coacervating) agent in which the polymer has negligible solubility. For poly(lactide-*co*-glycolide) (PLGA), commonly used coacer-

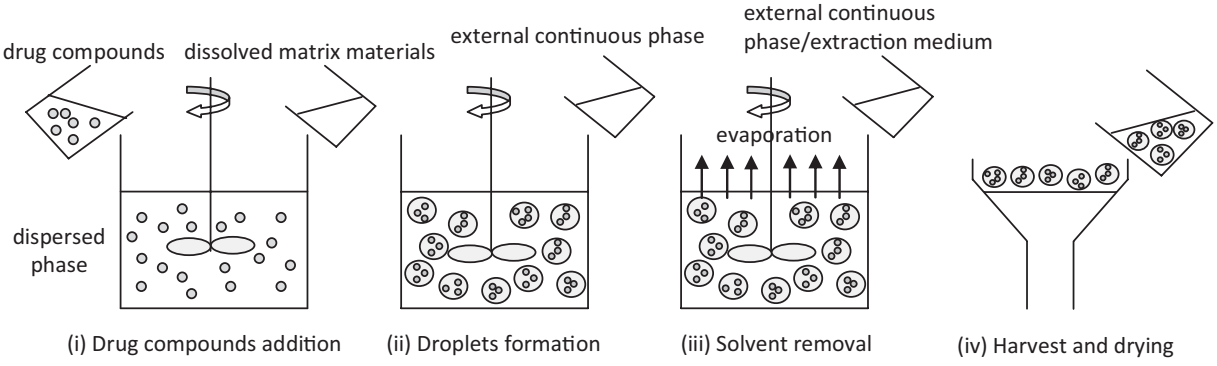

Figure 1. Preparation steps for microsphere formation by emulsion/solvent removal methods (reproduced from [12]).

vating agents include poly(dimethyl siloxane) (PDMS), known as silicon oil [19], and petroleum ether [20]. The addition of the nonsolvent induces the polymer to phase-separate and form coacervate droplets that contain the protein. A large volume of a second nonsolvent, which is the hardening agent, is added to harden the microparticles. Commonly used hardening agents include hexane and octamethylcyclote-trasiloxane.

W/O/O emulsion methods are widely used to encapsulate water-soluble drugs, such as proteins and peptides, since the drug-polymer mixtures are not exposed to the continuous aqueous phase. High encapsulation efficiencies are usually achieved for microparticles prepared with the W/O/O emulsion method because the loss of water-soluble drugs to the external phase is minimized [20, 21].

2.3 S/O/W Emulsion Methods

In S/O/W emulsion methods, the proteins are dispersed as solid particles in the polymer organic solution to form the primary emulsion (S/O). The dispersion of a solid-state protein eliminates the need for an internal aqueous phase, which is believed to enable a reduction in protein conformational mobility in comparison to the large structural change observed in the dissolved state and enhance protein stability [1, 22]. Similar to W/O/W emulsion methods, the primary emulsion is further dispersed into the external phase containing an emulsifying agent, such as poly(vinyl) alcohol (PVA) and poly(ethylene glycol) (PEG). Hardening of protein-loaded microparticles is obtained by solvent evaporation/extraction.

S/O/W emulsion methods are generally considered to be advantageous in avoiding protein aggregation and denaturation, but the method has often been associated with low encapsulation efficiencies, especially for encapsulating hydrophilic drugs, as a result of drug partitioning from the internal suspension phase to the external phase in the O/W emulsification step [3, 4, 23, 24]. Another critical challenge for S/O/W emulsion is the difficulty in creating a dispersion of protein particles in organic solvents, which is commonly addressed by using protein micronization methods such as lyophilization [25] or spray freeze-drying [26].

2.4 S/O/O Emulsion Methods

In S/O/O emulsion methods, the solid-state proteins are dispersed in the polymer organic solution to form the primary emulsion (S/O). Similar to the coacervation methods, addition of coacervating and hardening agents induce the polymer to phase-separate and harden the coacervates, resulting in the solidification of the microparticles.

The nonaqueous encapsulation approach to prepare microparticles avoids protein aggregation at the water/organic interface and holds promise for stabilizing proteins. By dispersing protein powders into an organic solvent and excluding water in the whole manufacturing process, the conformational rigidity of proteins is restricted and the protein bioactivity can be retained [26]. However, some drawbacks exist, particularly with the substantial initial burst release of protein molecules that have access to the solvent upon hydration, such as those on the microparticle surfaces or those with the ability to diffuse via the very porous microparticles [27, 28]. Some studies demonstrate that decreasing the size of the protein particles reduces the surface-exposed proteins and minimizes the initial release. Modified methods, such as the spinning oil film (SOF) method [29], spray freeze-drying, and the addition of poloxamer in the polymer phase [26, 28], have been applied to produce small protein particles [30]. These techniques have demonstrated to successfully reduce the initial release following microencapsulation.

3 Advantages and Limitations of the Emulsion Method

The emulsion method represents one of the widely used methods in preparing microparticles for biomolecules and drug delivery, as the process is relatively simple, convenient in controlling process parameters, and can be performed with inexpensive instrument with scale-up potential [10, 31]. The method can be used to encapsulate both hydrophilic and lipophilic drugs [32, 33]. Many studies have demonstrated that the size of the microparticles and the release kinetics of drugs can be controlled by adjusting the stirring rate, type and amount of dispersing agent, properties of organic and aqueous components, and temperature [20, 34–39]. By optimizing the formulation and processing conditions, some studies have reported high yield and encapsulation efficiencies achieved by the emulsion method [16, 40].

While emulsion methods have been widely used for protein drug encapsulation, their success in protein drug encapsulation is limited due to the harsh manufacturing conditions involved. The preparation procedures often involve high shear stresses, exposure of protein drugs to organic solvents, and extreme temperatures that can denature the protein drugs [11, 41]. When an aqueous protein solution is introduced into an organic solvent containing the dissolved polymer during the formation of the first emulsion, the protein drugs are prone to denaturation and structural perturbations at the water/organic solvent interface. Proteins are surface-active and tend to adsorb at water/organic solvent interfaces, which can lead to protein unfolding, inactivation, and irreversible aggregation [42–44]. In addition, mechanical forces employed in the creation of the emulsion, such as homogenization and sonication, may also cause protein degradation and loss of bioactivity due to shearing [11, 41].

As mentioned, nonaqueous encapsulation approaches have been developed to minimize protein exposure to a large interface between the water/organic solvent phase. Dispersion of dehydrated protein powder in an organic solvent may be suitable for protein drug encapsulation since there is no potentially destabilizing primary W/O emulsion. It is believed that proteins in the solid state are restricted in their conformational mobility in comparison to the large structural change found in the dissolved state [24, 27, 45]. While the nonaqueous encapsulation approach is beneficial to maintaining protein stability, low efficiencies are observed when hydrophilic drugs are encapsulated due to protein partitioning from the inner oil

phase suspension to the outer aqueous phase in the O/W emulsion step [23, 41, 45]. Similarly, the W/O/W emulsion methods are prone to protein drug loss. During generation of a secondary emulsion, W/O emulsion droplets are exposed to a high volume of water, leading to leakage of water-soluble drug into the external aqueous phase and resulting in low encapsulation efficiency [31]. These issues are discussed in Section 4.

4 Parameters Relevant to Encapsulation Efficiency

The difficulty in developing drug-loaded formulations with high encapsulation efficiencies is mainly due to the diffusional loss of drug into the external phase prior to the precipitation of polymer around the drug-contained droplets. The drug partitions into the external phase as long as the dispersed phase stays in the nonprecipitated state. When the polymer precipitation step is delayed, more drug molecules diffuse into the continuous phase, resulting in low encapsulation efficiencies [46]. A fast precipitation of the polymer is generally considered to be advantageous for achieving high encapsulation efficiencies because the solid polymer film acts as an efficient barrier to prevent diffusion of drug molecules into the external phase [47]. However, rapid precipitation methods could result in the generation of heterogeneous microparticles in terms of particle size and drug distributions. Many attempts to increase encapsulation efficiency, therefore, generally focus on reducing the outward transport of drug into the external phase and increasing the rate of polymer precipitation by modifying either the formulation or process conditions without compromising on the homogeneity of microparticle size and drug distributions.

4.1 Drug Properties

Water-soluble drug molecules can easily leach into the external phase and result in extensive drug loss during the encapsulation process. If the protein solubility in the external phase is higher than in the dispersed phase, diffusion of protein into the external phase will easily occur and result in a reduction of protein retention in the dispersed phase. In the microencapsulation of hydrochloride salt anesthetics into polylactic acid (PLA), low loading efficiencies were obtained at pH 5 of the external aqueous phase, as the salts were in anionic form and exhibited high solubilities in the external phase. Increasing the pH of the external phase by using an alkaline buffer solution increased the loading efficiency significantly [48]. Similarly, Bodmeier and McGinity [46] showed that reducing the drug solubility in the external phase through a pH change improved the encapsulation of quinidine sulfate within poly(*D*,*L*-lactide) microspheres [46].

Several other investigators also reported that highly hydrophilic drugs, such as procaine hydrochloride and henobarbital, suffered from low affinity with the hydrophobic polymer, leading to drug loss from the organic phase to the external phase [32, 49]. For example, Govender et al. [49] found that the procaine hydrochloride incorporation efficiency of PLGA nanoparticles increased significantly from 11 % to 58 % when the aqueous-phase pH increased from 5.8 to 9.3. The increase of incorporation efficiency at higher aqueous-phase pH is attributed to the reduction of the degree of drug ionization. Modifications of standard methods to influence the ionization of drug molecules and alter drug solubility can be achieved by either incorporating salt additives or using alternative solvents, and this will consequently lead to significant improvements in the encapsulation efficiency [48, 50–53]. Tab. 1 presents some examples of process modifications via drug ionization and solubility to enhance encapsulation efficiencies.

When the proteins are dispersed as solid particles, the protein particle size plays an important role in encapsulation efficiency. Incorporating fine protein particles usually results in high encapsulation efficiencies [25, 54, 55]. However, micronization of solid protein particles can cause undesirable molecular stress to the protein molecules. Micronization methods such as spray-drying and spray freeze-drying are complex and may cause low

Table 1. Improvements in encapsulation efficiencies via drug ionization and solubility modifications.

Methods	Polymer	Protein/drugs	Encapsulation efficiency (EE)	Ref.
W/O/W	PLGA	Lyozyme/r-met-HuGdNF[a]	Increased EE due to the increase of drug solubility in the solvent upon complexation of the proteins with ionic surfactants.	[40]
W/O/W	PLGA/CPP:SA[b]	Rhodium(II) citrate	Increased EE due to the increase of drug hydrophobicity upon complexation of the proteins with hydroxypropyl-β-cyclodextrin	[41]
W/O/W,O/W	PLGA	Dexamethasone	Increased EE due to the decrease of drug solubility in the aqueous phase upon addition of NaCl to the aqueous phase	[36, 38]
O/W	PLGA	Leuprolide acetate	Increased EE due to the increase of osmotic pressure of the external phase upon addition of NaCl.	[9]
W/O/W, O/W	PCL[c]	Propranolol HCl	Increased EE due to increase in drug solubility by increasing the pH of the external phase above the pK_a of propranolol HCl.	[42]

a) r-met-HuGdNF: recombinant methionyl human glial-cell line derived neurotrophic factor; b) CPP:SA: poly(1,3-bis(*p*-carboxyphenoxy)-propane-*co*-sebacic acid); c) PCL: poly(ε-caprolactone).

protein recovery and protein denaturation [22, 27, 30]. Another problem associated with fine protein particles relates to the homogeneity of the dispersed protein particles [31].

4.2 Polymer Properties

Drug loss due to migration of drug molecules to the external phase can also be prevented by modifying the polymer properties. Increasing the polymer concentration will reduce drug loss and increase the encapsulation efficiency, as drug diffusion is restricted in a more viscous medium. In addition, precipitation occurred faster due to a lower amount of solvent, thus inhibiting drug diffusion across the phase boundary. Rafati et al. [56] demonstrated that increasing the PLGA concentration from 1 % to 6 % in 3-% steps increased the bovine serum albumin (BSA) protein encapsulation efficiencies [56]. Yan et al. [56] also showed that the encapsulation of fluorescein isothiocyanate-BSA (FITC-BSA) into PLGA microparticles was enhanced when the PLGA concentration increased from 5 % to 15 %. The encapsulation efficiencies were maintained in the range between 90 % and 94 % with further increase of PLGA concentration from 15 % to 35 % in 5-% steps. It has been indicated in several other studies that increasing the polymer concentration is associated with a rise in encapsulation efficiency [46, 57–60].

For hydrophobic drug encapsulation with PLGA, polymers increasing the lactide content or using polymers with ester end groups (compared to carboxyl end groups) increase the hydrophobicity of the polymer, resulting in better solid-state solubility of the hydrophobic drug in the hydrophobic polymer matrix. Increasing the molecular weight of the polymer exhibits an equivalent effect in raising the solid-state solubility of the drug [61]. High solid-state drug-polymer solubility enhances drug retention in the polymer phase, leading to a reduction in drug diffusion to the external phase and an increase of drug loading.

4.3 Internal Aqueous, Organic, and External Phase

Attempts to reduce drug loss have also been made by changing the amount of internal water, organic and external phase. Alex et al. [62] obtained a reduction in protein encapsulation efficiency when the amount of internal water increased. Increasing the volume of the internal aqueous phase enhances droplet coalescence and the contact between internal drug solution and external aqueous phase. A larger volume of the internal aqueous phase is also associated with a thinner polymer layer, which give rise to larger diffusion through this barrier to the external phase. Both of these effects would result in higher drug loss, which have been also reported in other studies [16, 20]. In this case, raising the concentration of polymer organic solvent either by increasing the amount of polymer weight or decreasing the amount of organic solvent would assist in the formation of a thicker polymer film around the drug-contained droplets, reducing the possible coalescence of the internal phase and preventing drug loss into the external aqueous phase [62].

Increasing the amount of the external aqueous phase produces a similar effect like decreasing the amount of the internal phase. Mehta et al. [63] demonstrated that increasing the ratio of the dispersed phase to the continuous phase from 1/50 to 1/300 doubled the encapsulation efficiency. The authors attributed this effect to the rapid solidification of the microparticles, as the large volume of the continuous phase provided a sink condition to extract the solvent instantly [63]. In addition, there is an associated increase in the microparticle size with a larger external phase volume. Increasing the volumes of the external aqueous phase will decrease the mixing efficiency, resulting in the formation of larger microparticles. These larger microparticles provide an increase in the particle volume, which enables more drugs to be incorporated into the microparticles and leads to higher encapsulation efficiency [58, 64]. In several other studies, encapsulation efficiencies increased with increasing the volume of the external continuous phase [58, 62, 65].

4.4 Homogenization

Homogenization is one of the preparation steps that can affect the encapsulation efficiency. For emulsion/solvent removal methods it can be carried out by agitation, high-pressure homogenization, or sonication. In the encapsulation of FTIR-BSA, Wischke and Borchert [66] demonstrated that the use of ultrasound and high-pressure homogenization to homogenize the W/O emulsions produced higher encapsulation efficiencies compared to mechanical homogenization. For high-pressure homogenization, the encapsulation efficiency decreased with increasing pressure due to protein precipitation within the device, increased temperature, and fluid cavitation during the homogenization process [66].

In the encapsulation of an oligonucleotide drug within PLGA microparticles, Freytag et al. [16] investigated the stirring effect during emulsion homogenization. Using a propeller stirrer, the primary emulsion W/O was homogenized into an external aqueous phase to form a W/O/W emulsion. The encapsulation efficiency was found to decrease with increasing stirring time, as more drugs partition into the external phase due to the agitation effect and shear forces acting upon the drug-polymer emulsion droplets [16]. A short homogenization period is, therefore, desired to obtain high encapsulation efficiency. Note that the residual organic solvent also has to be removed sufficiently, desirably at longer stirring time, to comply with the residual solvent limits (in the final product).

Stirring speed is another factor that can influence the encapsulation process. Increasing the stirring speed produced an equivalent effect like increasing stirring time in reducing the encapsulation efficiency. In the encapsulation of phenylalanine ammonia lyase (PAL) in cellulose nitrate microcapsules, Shah et al. [67] described that reducing the stirring speed from 250 to 155 rpm increased the encapsulation efficiency from 45 % to 54 %. Similar results were also observed in the manufacture of carboplatin-loaded PLGA microspheres, where decreasing the stirring speed significantly improved the encapsulation efficiency [68]. Clearly, decreasing the stirring speed produces larger emulsion droplets due to weaker shear forces and de-

creased turbulence [12, 69]. This could provide a larger amount of polymer per unit area of the emulsion droplet available for encapsulating the drug although an increase in the droplet size does not necessarily lead to higher encapsulation efficiency.

4.5 Solvent Removal

Solvent removal can be achieved either by evaporation or extraction methods. Evaporation is commonly carried out at elevated temperature or reduced pressure conditions. Extraction is achieved by adding a continuous phase or extraction agents to absorb the entire solvent leaching from the solidifying microparticles.

4.5.1 Evaporation

In evaporation methods, high-temperature conditions will facilitate the evaporation of solvent from the continuous phase. This was demonstrated in the studies of Mehta et al. [70] and Li et al. [71], where the solvent removal profiles indicated that increasing the temperature decreased the solvent residual. In the encapsulation of solid theophylline (TH) in hydrophobic dextran derivate (PDME) dissolved in acetone solvent, Miyazaki et al. [72] investigated the evaporation kinetics of acetone following emulsion with the continuous phase containing liquid paraffin and sucrose-ester (DKF-10) as a function of the temperature increase rate. More than 60 % of acetone was evaporated within the initial few minutes regardless of the temperature increase rate, as acetone is soluble in liquid paraffin to a limited extent. The residual acetone was slowly evaporated until 30 min. The increasing temperature increase rate increased the percent of acetone evaporated. The encapsulation efficiencies were above 110 % in all cases and exhibited a slight increase from 112 % to 132 % with the higher evaporation rate [72].

The relation of the evaporation temperature to encapsulation efficiency depends on the mechanisms governing the encapsulation process. Yang et al. [35] reported a nonlinear trend between temperature and encapsulation efficiency of BSA in the blends of PLGA and PEG. The highest encapsulation efficiencies were obtained at the lowest (4 °C) and highest temperature (38 °C) tested. At lower temperature, an increased solubility of the solvent in water allowed relatively fast mass transfer between the dispersed and the continuous phases and led to fast polymer precipitation and sphere wall hardening. An increased rate of solvent evaporation due to higher temperatures also resulted in a rapid hardening of the sphere. In this case, the lower solubility of the solvent in the continuous phase at high temperature is compensated by a high evaporation rate. Rapid hardening at the sphere wall due to rapid solvent removal either at high evaporation rate or increased solvent mass transfer to the continuous phase contributed to the increase of encapsulation efficiency.

On the other hand, an increased solubility of protein in the external phase and protein denaturation at high temperatures may also increase the amount of protein leaving the dispersed phase and result in less (active) proteins encapsulated. These factors may compromise the increased encapsulation efficiency through rapid hardening of the sphere wall upon raising the temperature. Likewise, lower temperatures result in lower solubility and mass transfer of protein to the external phase, which may compensate for the relative ease of diffusing through the softer and less dense polymer film as a result of slow precipitation. In this case, the dependence of solvent removal temperature for encapsulation efficiency is insensitive, as demonstrated in several studies [34, 73].

Solvent evaporation can also be accelerated at reduced pressure conditions. Clearly, rapid solvent removal at reduced pressure will lead to rapid solidification. In the preparation of bovine hemoglobin encapsulated poly(lactic acid)-*co*-PEG (PELA) microspheres using the W/O/W emulsion method, the solidification time was reduced from 240 to 40 min when the emulsion was subjected to evaporation under reduced pressure conditions. By applying a reduced pressure for solvent evaporation, the shortened solidification time also led to a slight increase in the encapsulation efficiency [74]. In the work of Izumikawa et al. [75], the encapsulation efficiencies of progesterone in PLA were larger when the solvent was removed by stirring for 3 h under 200 mmHg compared to 18 h under 760 mmHg.

Opposite trends were reported for albumin/lidocaine encapsulation in poly(*L*-lactic acid) (PLLA) and poly(*D*,*L*-lactic acid) (PDLLA) microspheres [76, 77]. In both cases, the encapsulation efficiencies were higher for solvent evaporation carried out at 760 mmHg compared to 160 mmHg. In contrast to the study of Izumikawa et al. [75], Chung et al. [77] hypothesized that the conflicting results were due to the different droplet formation methods and size of microspheres.

4.5.2 Extraction

In solvent extraction methods, the solvent is removed by partitioning into the extraction medium. Proper control of the continuous-phase addition rate, amount and composition of the extraction medium is required to remove the entire solvent efficiently and ensure a minimum drug loss into the extraction medium.

In the system composed of aqueous BSA dispersed in a solution of PDLLA in methylene chloride and 0.05 % w/v PVA, Yang et al. [35] found that further addition of the continuous phase at constant rate ranging from 1.5 to 6 mL min^{-1} increased the encapsulation efficiency from 54 % to 62 % due to rapid solidification and reduced BSA migration. However, adding the continuous phase at 9 mL min^{-1} led to a reduction of the encapsulation efficiency to 56 %. The authors' hypothesis for the reduced encapsulation efficiency was an increased mass transfer and dissolution of BSA from the interior of the microspheres to the continuous water phase [35].

In the study of Jeyanthi et al. [73], adding different volumes of the aqueous continuous phase consisting of 0.4 % w/v sodium oleate to extract the methylene chloride and methanol co-solvent produced no significant influence on the salmon calcitonin (sCT) load. A similar observation was also made in the encapsulation of leuprolide acetate-containing PLGA microparticles [9]. Clearly, a sufficient amount of the continuous phase must be present to at least dissolve the dispersed

phase solvent during the droplet formation steps (see Section 2). Rice and Gilley [78] suggested the use of tenfold the amount of fluid necessary to extract all the disperse phase solvent for successful encapsulation of substances that are soluble in the continuous phase.

A number of studies investigated the effect of salt addition to the continuous phase during the preparation of drug-loaded microspheres [9, 16, 48, 50]. The presence of salts in the continuous phase will change the ionic strength, pH, polarity, and the osmotic pressure gradient of the continuous phase and thus the solubility of the drug and organic solvent in the continuous phase. A high solubility of organic solvent in the external aqueous phase is desired to promote rapid polymer precipitation, which is advantageous to the solvent extraction process and encapsulation efficiency. Uchida et al. [48] reported that the addition of sodium chloride to the external aqueous phase significantly enhanced the phenobarbital sodium loading. For hydrochloride salts-loaded microspheres, the encapsulation efficiencies were comparatively poor upon addition of sodium chloride. The authors explained that the poor loadings were due to the fact that hydrochloride salts are highly soluble under the pH conditions of the external aqueous phase containing sodium chloride [48]. A higher encapsulation efficiency upon addition of sodium chloride to the external continuous phase was also observed in other studies [9, 50].

5 Use of Foam Medium for Protein Encapsulation

Foam has been employed in many industrial applications including detergent, cosmetic, food, and pharmaceutical manufacturing. Foam possesses properties which make them superior in a wide range of industrial processes over the traditional application of aqueous liquid systems. In the pharmaceutical sector, foam finds use in drug delivery applications as a carrier for active substances in topical formulations [79–81]. For effective topical administration, the applied agents must penetrate and permeate from one skin layer to another functionally and structurally different skin layer [82]. The use of foam as a vehicle for delivering therapeutic agents through the skin offers a number of dermatological advantages. Foam formulations provide emollient or drying functions to the skin [83]. They are less dense and spread easily onto large areas of skin, with fewer residues remaining on the skin after application [84, 85]. More importantly, foam formulations have the ability to deliver a greater amount of drug with enhanced drug penetration and permeation through the skin layer, which also leads to an increase in efficacy [81, 82]. Foam has also become a prominent delivery system for cosmetically active substances [86, 87]. Foam characteristics such as large volume, good stability, and slow drainage are critical for the functionality of foam cosmetic formulations. Some common products include shampoo, shaving foam, toothpaste, and hair mousse. These products make use of foam to distribute the substances in order to enhance product application as required, e.g., keeping the skin moistened by providing enough rigidity to hold the fluid in place while using a relatively small amount of fluid [86, 88]. In pharmaceutical granulation processes, the carrying ability of foamed binder solution allows low levels of water-soluble drug to be uniformly distributed throughout the powder bed during high-shear wet granulation [89]. Foams take advantage of the tremendous increase in the liquid surface area and volume of binder foams to enable rapid spreading and efficient particle coverage as compared to a drop of binder solution [90–92]. These inherent foam properties proved the foam technology to be invaluable in the pharmaceutical sector. We propose the use of foam as a vehicle for protein encapsulation by introducing foamed protein, foamed polymer solution, or foamed external phase. Foaming can be achieved by mechanical agitation of a solution, dispersing a stream of gas into a liquid, rapidly reducing the pressure of a solution, and co-injecting gas and liquid into a chamber [86, 93, 94]. Surface-active agents such as surfactants can be used to stabilize the foam, and it should be noted that some protein drugs and hardening agents used in the emulsion formation step are also able to act as foaming agents.

Foams possess unique properties which make them superior compared to traditional applications of aqueous liquids in a wide range of industrial processes. These inherent properties shall prove to be valuable for the preparation of protein/drug-loaded microspheres. One common expression used to characterize foam is given as foam quality (FQ), which defines the volume ratio of gas to foam:

$$\mathrm{FQ} = \frac{V_{\mathrm{gas}}}{V_{\mathrm{gas}} + V_{\mathrm{liquid}}} \tag{1}$$

In the context of foam flow in porous media, three fundamental pore-level in situ foam generation mechanisms have been derived: snap-off, lamella division, and leave-behind mechanisms [95]. The mechanisms illustrated in Fig. 2 emphasize the capability of foam to create new bridging lamellae between particles. The particles can act as a branch point for bubble regeneration when the lamellae are contacted by free gas or foam. The bubbles in foam will keep flowing on the wet surface until it reaches a dry surface, and then collapse [96]. Combining these aspects, we believe that foam would be able to spread through the surfaces-of-interest efficiently, leading to large surface coverage and interactions between the protein/drug molecules and the carrier.

There are several characteristics that make foam systems a promising medium for protein encapsulation: (i) large gas-liquid interfacial area, (ii) low liquid holdup, (iii) strongly adhesive/excellent carrying capability, (iv) controlled drainage and decaying kinetics, and (v) highly porous structure. The large surface area and volume of foam would induce a larger and more uniform coverage with less material, leading to strong interactions between the protein/drug molecules and the carrier. Through the adhesive and carrying properties of foam, the protein/drug molecules can be strongly attached with the carrier to reduce protein/drug loss. Moreover, the highly porous structure of foam would support protein attachment and hold/release the protein molecules in a controllable manner. The controlled drainage and decaying kinetics of foams would also allow protein release in a reproducible mode. Due to the low liquid content in foamed systems, rapid extraction/evaporation, fast precipitation, and reduced drying time are enabled. These desirable properties would allow encapsulation

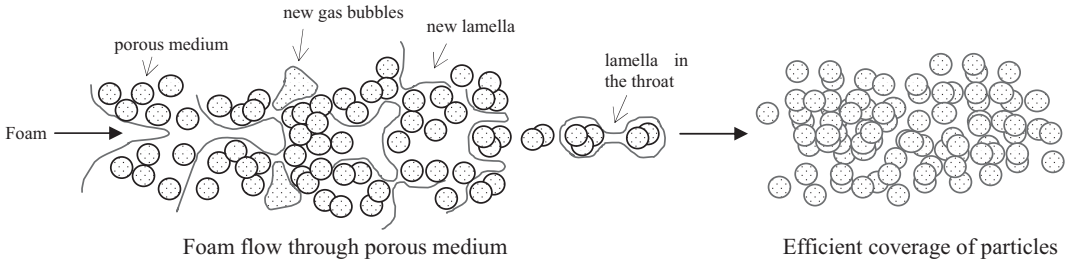

Figure 2. Foam generation mechanisms in a porous medium. Snap-off: a new gas bubble separated from the original gas by a liquid lamella is created when gas enters an initially liquid-filled pore. Lamellae division: a foam lamella is divided into two separate lamellae when the lamella meets a flow branch point. Leave-behind: a foam lamella is created as two gas menisci pass through adjacent liquid-filled pore bodies

or adsorption of protein/drug molecules and release kinetics in a more predictable manner.

6 Conclusions

This review focuses on the emulsion/solvent removal-based technology for producing protein-loaded microparticles and describes the relevant parameters affecting protein encapsulation. The properties of the protein molecules, polymers, and solvents are essential to optimize the protein-loaded microparticles formation process in order to ensure effective encapsulation and delivery. A new method using foam as a delivery medium for protein encapsulation is discussed. The unique characteristics of foam, namely large contact surface area, low liquid holdup, adhesive and carrying capability, controlled decaying rate, and highly porous structure would prove to be useful in developing an efficient process of protein encapsulation and delivery.

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