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REVIEW

Microencapsulation techniques, factors influencing encapsulation efficiency

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Abstract

Microencapsulation is one of the quality preservation techniques of sensitive substances and a method for production of materials with new valuable properties. Microencapsulation is a process of enclosing micron-sized particles in a polymeric shell. There are different techniques available for the encapsulation of drug entities. The encapsulation efficiency of the microparticle or microsphere or microcapsule depends upon different factors like concentration of the polymer, solubility of polymer in solvent, rate of solvent removal, solubility of organic solvent in water, etc. The present article provides a literature review of different microencapsulation techniques and different factors influencing the encapsulation efficiency of the microencapsulation technique.

Key words: *Microencapsulation; encapsulation efficiency; coacervation; supercritical fluids*

Introduction

Microencapsulation is described as a process of enclosing micron-sized particles of solids or droplets of liquids or gasses in an inert shell, which in turn isolates and protects them from the external environment (Ghosh 2006). The products obtained by this process are called microparticles, microcapsules and microspheres which differentiate in morphology and internal structure. When the particle size is below 1 μm they are known as nanoparticles, nanocapsules, nanospheres, respectively (Remuñán and Alonso 1997), and particles having diameter between 3–800 μm are known as microparticles, microcapsules or microspheres. Particles larger than 1000 μm are known as macroparticles (Thies 1996).

Microencapsulation can be done (i) to protect the sensitive substances from the external environment, (ii) to mask the organoleptic properties like colour, taste, odour of the substance, (iii) to obtain controlled release of the drug substance, (iv) for safe handling of the toxic materials, (v) to get targeted release of the drug and (vi) to avoid adverse effects like gastric irritation of the

drug, e.g. aspirin is the first drug which is used to avoid gastric irritation.

Microparticles or microcapsules consist of two components, namely core material and coat or shell material. Core material contains an active ingredient while coat or shell material covers or protects the core material. Different types of materials like active pharmaceutical ingredients, proteins, peptides, volatile oils, food materials, pigments, dyes, monomers, catalysts, pesticides, etc. can be encapsulated with different types of coat or shell materials like ethylcellulose, hydroxyl propylmethyl cellulose, sodium carboxy methyl cellulose, sodium alginate, PLGA, gelatine, polyesters, chitosans, etc.

Microencapsulation techniques

Various techniques are available for the encapsulation of core materials. Broadly the methods are divided into three types. Different types of microencapsulation techniques are listed in table 1.

1. Chemical methods;
2. Physico-chemical methods; and

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Table 1. Different techniques used for microencapsulation (Ghosh 2006).

Chemical processes	Physico-chemical processes	Physico-mechanical process
Interfacial polymerization	Coacervation and phase separation	Spray drying and congealing
<i>In situ</i> polymerization	Sol-gel encapsulation	Fluid bed coating
Poly condensation	Supercritical CO ₂ assisted microencapsulation	Pan coating
		Solvent evaporation

Table 2. Microencapsulation processes and their applicability.

Microencapsulation process	Nature of the core material	Approximate particle size (µm)
Air suspension	Solids	5-5000*
Coacervation and phase separation	Solids and liquids	2-5000*
Multi-orifice centrifugation pan coating	Solids and liquids	1-5000*
Spray drying and congealing	Solids	600-5000*
Solvent evaporation	Solids and liquids	600
	Solids and liquids	5-5000*

*The 5000 µm size is not a particle size limitation. The methods are also applicable for macrocoating (Bakan 1991).

3. Physico-mechanical methods.

The above-mentioned techniques are widely used for microencapsulation of several pharmaceuticals. Among these techniques, fluidized bed or air suspension method, coacervation and phase separation, spray-drying and spray-congealing, pan coating and solvent evaporation methods are widely used. Depending on the physical nature of the core substance to be encapsulated the technique used will be varied. Table 2 illustrates microencapsulation processes and their applicabilities.

Chemical methods

Interfacial polymerization (IFP). In this technique the capsule shell will be formed at or on the surface of the droplet or particle by polymerization of the reactive monomers. The substances used are multifunctional monomers. Generally used monomers include multifunctional isocyanates and multifunctional acid chlorides. These will be used either individually or in combination. The multifunctional monomer dissolved in liquid core material and it will be dispersed in aqueous phase containing dispersing agent. A co-reactant multifunctional amine will be added to the mixture. This results in rapid polymerization at interface and generation of capsule shell takes place (Scher 1983). A polyurea shell will be formed when isocyanate reacts with amine, polynylon or polyamide shell will be formed when acid chloride reacts with amine. When isocyanate reacts with hydroxyl containing monomer it produces a polyurethane shell. For example, Saihi et al. (2006) encapsulated di-ammonium hydrogen phosphate (DAHP) by polyurethane-urea membrane using an

interfacial polymerization method. An elevated yield of synthesis (22%) of a powder of microcapsules was produced with a fill content of 62 wt% of DAHP as determined by elementary analysis. The mean size of DAHP microcapsules is 13.35 µm. Besides, 95% of the sized particles have a diameter lower than 30.1 µm.

In situ polymerization

Like IFP the capsule shell formation occurs because of polymerization monomers added to the encapsulation reactor. In this process no reactive agents are added to the core material, polymerization occurs exclusively in the continuous phase and on the continuous phase side of the interface formed by the dispersed core material and continuous phase. Initially a low molecular weight prepolymer will be formed, as time goes on the prepolymer grows in size, it deposits on the surface of the dispersed core material there by generating a solid capsule shell (e.g. encapsulation of various water-immiscible liquids with shells formed by the reaction at acidic pH of urea with formaldehyde in aqueous media (Cakhshae et al. 1985)). Wang et al. (2003) prepared Carboxyl-functionalized magnetic microspheres by *in situ* polymerization of styrene and methacrylic acid at 85°C in the presence of nano-Fe₃O₄ in styrene, using lauroyl peroxide as an initiator.

Physico-chemical methods

Coacervation and phase separation. Bungenberg de Jong and Kruyt (1929) and Bungenberg de Jong (1949)

defined this as partial desolvation of a homogeneous polymer solution into a polymer-rich phase (coacervate) and the poor polymer phase (coacervation medium). The term originated from the Latin 'acervus' meaning 'heap'. This was the first reported process to be adapted for the industrial production of microcapsules. Currently, two methods for coacervation are available, namely simple and complex processes. The mechanism of microcapsule formation for both processes is identical, except for the way in which the phase separation is carried out. In simple coacervation a desolvation agent is added for phase separation, whereas complex coacervation involves complexation between two oppositely charged polymers. The three basic steps in complex coacervation are: (i) formation of three immiscible phases; (ii) deposition of the coating; and (iii) rigidization of the coating.

The first step includes the formation of three immiscible phases; liquid manufacturing vehicle, core material and coating material. The core material is dispersed in a solution of the coating polymer. The coating material phase, an immiscible polymer in liquid state, is formed by (i) changing temperature of polymer solution, e.g. ethyl cellulose in cyclohexane¹² (N-acetyl P-amino phenol as core), (ii) addition of salt, e.g. addition of sodium sulphate solution to gelatine solution in vitamin encapsulation (Green 1960), (iii) addition of non-solvent, e.g. addition of isopropyl ether to methyl ethyl ketone solution of cellulose acetate butyrate (Heistand et al. 1966) (methylscopolamine hydrobromide is core), (iv) addition of incompatible polymer to the polymer solution, e.g. addition of polybutadiene to the solution of ethylcellulose in toluene (The National Cash Register Co. 1963) (methylene blue as core material) and (v) inducing polymer-polymer interaction, e.g. interaction of gum Arabic and gelatine at their iso-electric point (Brynko et al. 1967). The second step includes deposition of liquid polymer upon the core material. Finally, the prepared microcapsules are stabilized by cross-linking, desolvation or thermal treatment (Figure 1).

Cross-linking is the formation of chemical links between molecular chains to form a three-dimensional network of connected molecules. The vulcanization of rubber using elemental sulphur is an example of cross-linking, converting raw rubber from a weak plastic to a highly resilient elastomer. The strategy of covalent cross-linking is used in several other technologies of commercial and scientific interest to control and enhance the properties of the resulting polymer system or interface, such as thermosets and coatings (DeBord and Schick 1999, Stevens 1999, Wicks et al. 1999). Cross-linking has been employed in the synthesis of ion-exchange resins (Dyson 1987) and stimuli-responsive hydrogels (Lowe and McCormick 1999) made from polymer molecules containing polar groups. As polyelectrolytes, hydrogels are inherently water-soluble.

To make them insoluble, they are chemically cross-linked during manufacture or by a second reaction following that of polymerization of the starting monomers. The degree of cross-linking, quantified in terms of the cross-link density, together with the details of the molecular structure, have a profound impact on the swelling characteristics of the cross-linked system. For example, derivatives of ethylene glycol di(meth)acrylate like, ethylene glycol diacrylate, di(ethylene glycol) diacrylate, tetra(ethylene glycol) diacrylate, ethylene glycol dimethacrylate, di(ethylene glycol) dimethacrylate, tri(ethylene glycol) dimethacrylate; derivatives of methylenebisacrylamide like N,N- Methylenebisacrylamide, N,N-Methylenebisacrylamide, N,N-(1,2- Dihydroxyethylene) bisacrylamide (Klärner et al. 1999), glutaraldehyde, sodium tripolyphosphate, etc.

Yin and Stöver (2003) prepared microspheres by poly(styrene-alt-maleic anhydride) partially grafted with methoxy poly(ethylene glycol) (SMA-g-MPEG) were prepared by reacting poly(styrene-alt-maleic anhydride) with a substoichiometric amount of MPEG lithium alcoholate. Aqueous solutions of the resulting SMA-g-MPEG formed complex coacervates with poly(diallyldimethylammonium chloride) (PDADMAC). These phase-separated liquid polyelectrolyte complexes were subsequently cross-linked by the addition of two different polyamines to prepare cross-linked hydrogel microspheres. Chitosan served as an effective cross-linker at pH 7.0, while polyethylenimine (PEI) was used as a cross-linker under basic conditions (pH 10.5). The resulting coacervate microspheres swelled with increasing salinity, which was attributed mainly due to the shielding of the electrostatic association within the polyelectrolyte complex.

Huang et al. (2007) prepared microcapsules by using gelatine and gum Arabic by coacervation. The most frequently used cross-linking agent formaldehyde in the gelatin-acacia microencapsulation process was altered by glycerol in this study. They found that the yield of gelatin-acacia microcapsules decreases at surfactant concentrations above or below the optimum. Inhibition of coacervation due to high concentrations of surfactants and disturbance of microencapsulation due to high hydrophilic-lipophilic balance (HLB) values have been reported. In general, the concentration of a surfactant required to increase the yield of microcapsules is too low to produce regular-sized droplets. The analysis of the size distribution shows that the microcapsules are multi-dispersed. In the coacervation process, the pH value of a continuous gelatin phase would be adjusted above its iso-electric point to form negatively charged gelatin, which is able to create monodispersed droplets. The positively charged gelatin is attracted to the negatively charged acacia to form coacervate droplets when the pH value is adjusted to below its isoelectric point. Therefore, the

particle size distributions of emulsion droplets are effected by the factors of pH adjustment, especially the adding rate of the acidifying agent. The report shows the indomethacin microcapsules had the slowest release rate when the coacervation pH was adjusted to the electrical equivalence pH value and not to the pH of maximum coacervate yield. Gelatin is only stable at a pH value between 4–6 and this data shows that the alkalization caused the breaking of the wall of the microcapsule made by the cross-linking agent of glycerol. Not only is the purple-colour shikonin alkalinized into a blue colour, but the saponification effects may also be undergone by the solvent (sesame oil) of extract containing shikonin reacting with sodium hydride. However, this reaction would not be shown in the microcapsule made by the cross-linking agent of formaldehyde. This explains why the shell of the microcapsule made by formaldehyde is more rigid than that made by glycerol. In other words, the microcapsule made by glycerol has a more permeable shell than made by formaldehyde. The particle size of the microcapsule was not affected by the difference of cross-linking agents. Using the low concentration, 3% and 6% of plasticizer glycerol instead of formaldehyde, similar morphology results were obtained. Increasing the amount of cross-linking agent leads to an increase in the encapsulation ability. However, the results indicated that above 6% of glycerin, encapsulation ability decreases as the cross-linking agent increases due to the alteration of the mechanism and inability to integrate into the network even after the addition of an excess amount.

Polymer encapsulation by rapid expansion of supercritical fluids. Supercritical fluids are highly compressed gasses that possess several advantageous properties of both liquids and gases. The most widely used being supercritical CO₂, alkanes (C₂ to C₄) and nitrous oxide (N₂O). A small change in temperature or pressure causes a large change in the density of supercritical fluids near the critical point. Supercritical CO₂ is widely used for its low critical temperature value, in addition to its non-toxic and non-flammable properties; it is also readily available, highly pure and cost-effective.

The most widely used methods are as follows:

- Rapid expansion of supercritical solution (RESS);
- Gas anti-solvent (GAS); and
- Particles from gas-saturated solution (PGSS).

Rapid expansion of supercritical solution. In this process, supercritical fluid containing the active ingredient and the shell material are maintained at high pressure and then released at atmospheric pressure through a small nozzle. The sudden drop in pressure causes desolvation of the shell material, which is then deposited around the active ingredient (core) and forms a coating layer (Figure 2).

The disadvantage of this process is that both the active ingredient and the shell material must be very soluble in supercritical fluids. In general, very few polymers with low cohesive energy densities (e.g. polydimethylsiloxanes, polymethacrylates) are soluble in supercritical fluids such as CO₂. The solubility of polymers can be enhanced by using co-solvents. In some cases non-solvents are used; this increases the solubility in supercritical fluids, but the shell materials do not dissolve at atmospheric pressure. Kiyoshi et al. very recently carried out microencapsulation of TiO₂ nanoparticles with polymer by RESS using ethanol as a non-solvent for the polymer shell such as polyethylene glycol (PEG), poly(styrene)-b-(poly(methylmethacrylate))-copoly(glycidal methacrylate) copolymer (PS-b-(PMMA-co-PGMA) and poly(methyl methacrylate).

Gas anti-solvent (GAS) process. This process is also called supercritical fluid anti-solvent (SAS). Here, supercritical fluid is added to a solution of shell material and the active ingredients and maintained at high pressure. This leads to a volume expansion of the solution that causes supersaturation such that precipitation of the solute occurs. Thus, the solute must be soluble in the liquid solvent, but should not dissolve in the mixture of solvent and supercritical fluid. On the other hand, the liquid solvent must be miscible with the supercritical fluid. This process is unsuitable for the encapsulation of water-soluble ingredients, as water has low solubility in supercritical fluids. It is also possible to produce submicron particles using this method.

Particles from a gas-saturated solution (PGSS). This process is carried out by mixing core and shell materials in supercritical fluid at high pressure. During this process supercritical fluid penetrates the shell material, causing swelling. When the mixture is heated above the glass transition temperature (T_g), the polymer liquifies. Upon releasing the pressure, the shell material is allowed to deposit onto the active ingredient. In this process, the core and shell materials may not be soluble in the supercritical fluid.

Physico-mechanical process

Spray drying and congealing. Microencapsulation by spray-drying is a low-cost commercial process which is mostly used for the encapsulation of fragrances, oils and flavours. Core particles are dispersed in a polymer solution and sprayed into a hot chamber (Figure 3). The shell material solidifies onto the core particles as the solvent evaporates such that the microcapsules obtained are of polynuclear or matrix type. Chitosan microspheres cross-linked with three different cross-linking agents viz,

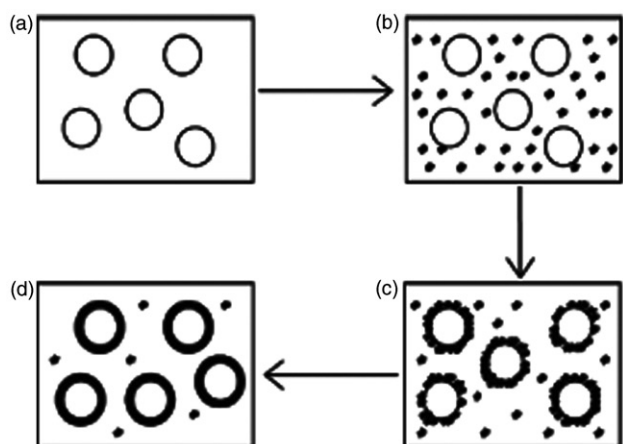


Figure 1. Schematic representation of the coacervation process. (a) Core material dispersion in solution of shell polymer; (b) separation of coacervate from solution; (c) coating of core material by microdroplets of coacervate; (d) coalescence of coacervate to form continuous shell around core particles. (Redrawn from Ghosh 2006.)

tripolyphosphate (TPP), formaldehyde (FA) and glutaraldehyde (GA) have been prepared by spray-drying technique. The influence of these cross-linking agents on the properties of spray-dried chitosan microspheres was extensively investigated. The particle size and encapsulation efficiencies of thus prepared chitosan microspheres ranged mainly between 4.1–4.7 μm and 95.12–99.17%, respectively. Surface morphology, percentage erosion, percentage water uptake and drug release properties of the spray-dried chitosan microspheres was remarkably influenced by the type (chemical or ionic) and extent (1 or 2% w/w) of cross-linking agents. Spray-dried chitosan microspheres cross-linked with TPP exhibited higher swelling capacity, percentage water uptake, percentage erosion and drug release rate at both the cross-linking extents (1 and 2% w/w) when compared to those cross-linked with FA and GA. The sphericity and surface smoothness of the spray-dried chitosan microspheres was lost when the cross-linking extent was increased from 1 to 2% w/w. Release rate of the drug from spray-dried chitosan microspheres decreased when the cross-linking extent was increased from 1 to 2% w/w. The physical state of the drug in chitosan-TPP, chitosan-FA and chitosan-GA matrices was confirmed by the X-ray diffraction (XRD) study and found that the drug remains in a crystalline state even after its encapsulation. Release of the drug from chitosan-TPP, chitosan-FA and chitosan-GA matrices followed Fick's law of diffusion (Desai and Park 2005).

Spray congealing can be done by spray-drying equipment where protective coating will be applied as a melt. Core material is dispersed in a coating material melt rather than a coating solution. Coating solidification is

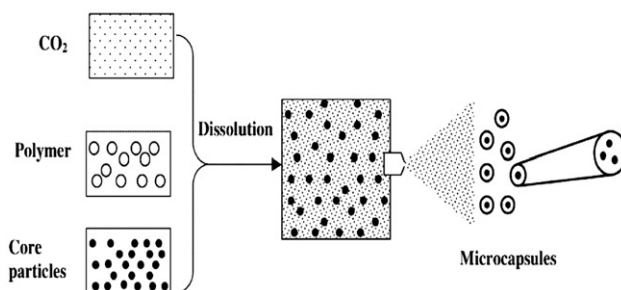


Figure 2. Microencapsulation by rapid expansion of supercritical solutions (RESS). (Redrawn from Ghosh 2006.)

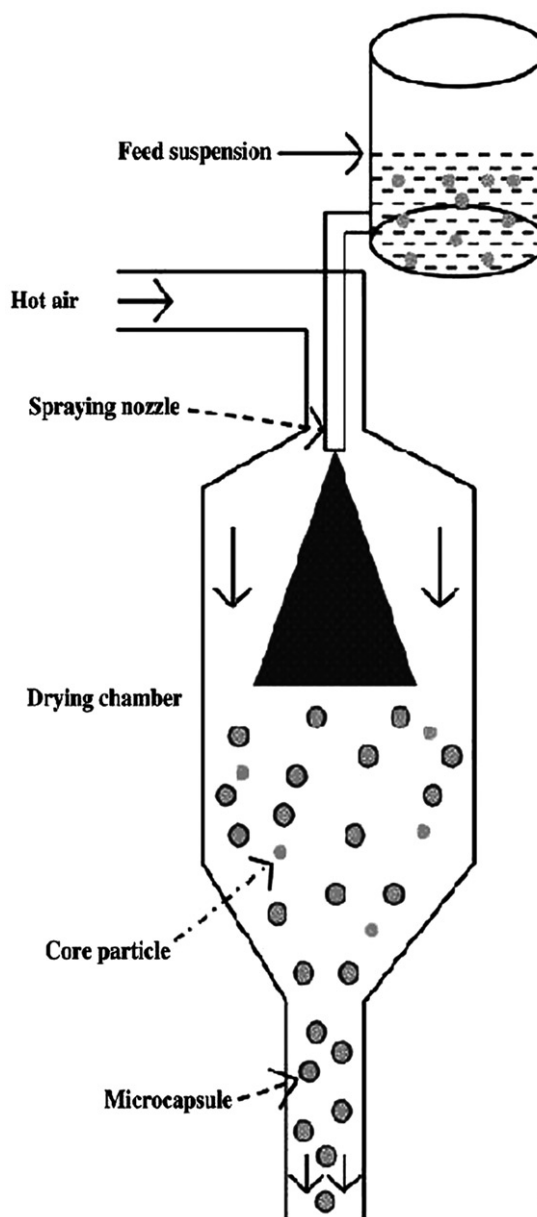


Figure 3. Schematic illustrating the process of micro-encapsulation by spray-drying. (Redrawn from Ghosh [1].)

accomplished by spraying the hot mixture into cool air stream. Waxes, fatty acids and alcohols, polymers which are solids at room temperature but meltable at reasonable temperature, are applicable to spray congealing. Albertini et al. (2008) prepared mucoadhesive microparticles and designed an innovative vaginal delivery system for econazole nitrate (ECN) to enhance the drug anti-fungal activity. Seven different formulations were prepared by spray-congealing, a lipid-hydrophilic matrix (Gelucire((R)) 53/10) was used as carrier and several mucoadhesive polymers such as chitosan, sodium carboxymethylcellulose and poloxamers (Lutrol((R)) F68 and F127) were added.

Fluidized-bed technology. The liquid coating is sprayed onto the particles and the rapid evaporation helps in the formation of an outer layer on the particles. The thickness and formulations of the coating can be obtained as desired. Different types of fluid-bed coaters include top spray, bottom spray and tangential spray (Figure 4).

In the top spray system the coating material is sprayed downwards on to the fluid bed such that as the solid or porous particles move to the coating region they become encapsulated. Increased encapsulation efficiency and the prevention of cluster formation is achieved by opposing flows of the coating materials and the particles. Dripping of the coated particles depends on the formulation of the coating material. Top spray fluid-bed coaters produce higher yields of encapsulated particles than either bottom or tangential sprays.

The bottom spray is also known as 'Wurster's coater' in recognition of its development by Wurster (1953). This technique uses a coating chamber that has a cylindrical nozzle and a perforated bottom plate. The cylindrical nozzle is used for spraying the coating material. As the particles move upwards through the perforated bottom plate and pass the nozzle area, they are encapsulated by the coating material. The coating material adheres to the particle surface by evaporation of the solvent or cooling of the encapsulated particle. This process is continued until

the desired thickness and weight is obtained. Although it is a time-consuming process, the multilayer coating procedure helps in reducing particle defects.

The tangential spray consists of a rotating disc at the bottom of the coating chamber, with the same diameter as the chamber. During the process the disc is raised to create a gap between the edge of the chamber and the disc. The tangential nozzle is placed above the rotating disc through which the coating material is released. The particles move through the gap into the spraying zone and are encapsulated. As they travel a minimum distance there is a higher yield of encapsulated particles.

Solvent evaporation. In solvent evaporation method three phases are present. They are core, coat material, liquid manufacturing vehicle (LMV). Initially coat material will be dissolved in a volatile solvent, which is not soluble in LMV phase. A core material to be encapsulated to be dissolved or dispersed in the coating polymer solution. This mixture is added to the liquid manufacturing vehicle phase with agitation, the mixture is heated to evaporate the solvent for polymer. Here the coat material shrinks around the core material and encapsulates the core. Microspheres of 5-fluorouracil have been prepared, using three grades of ethyl cellulose as wall forming materials, and utilizing a solvent evaporation technique under ambient conditions. An alcoholic solution of 5-fluorouracil and polymer was dispersed in liquid paraffin containing 33.3% *n*-heptane. The effect of stirring rate, time of stirring, drug loading and polymer grade on drug release in two different media were evaluated. The drug loaded particles were spherical in shape and had a diameter range of 25–200 μm and were suitable for incorporating into a gel base. Drug release studies in aqueous media showed that acidic media provide a faster release rate than neutral media. The drug release study from an aqueous gel base preparation at pH 7.0 through a synthetic membrane was found to be promising for formulation of

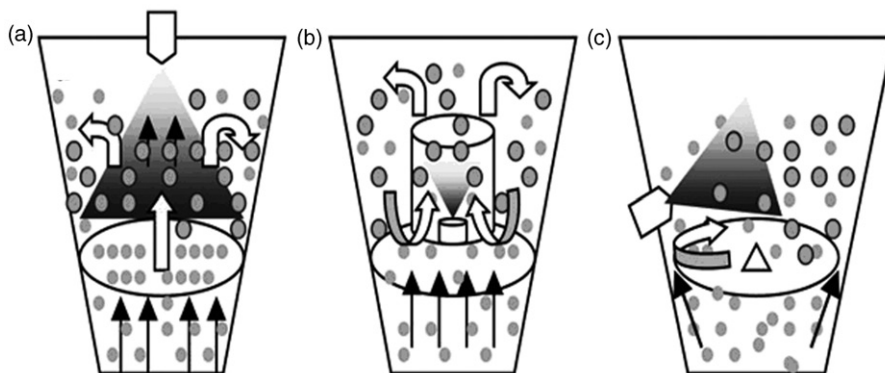


Figure 4. Schematics of a fluid-bed coater. (a) Top spray; (b) bottom spray; (c) tangential spray. (Redrawn from Ghosh [\[1\]](#)).

a gel-microsphere product for the treatment of skin lesions (Ghorab et al. 1990).

Pseudoephedrine HCl, a highly water-soluble drug, was entrapped within poly (methyl methacrylate) microspheres by a water/oil/water emulsification-solvent evaporation method. An aqueous drug solution was emulsified into a solution of the polymer in methylene chloride, followed by emulsification of this primary emulsion into an external aqueous phase to form a water/oil/water emulsion. The middle organic phase separated the internal drug-containing aqueous phase from the continuous phase. Microspheres were formed after solvent evaporation and polymer precipitation. The drug content of the microspheres increased with increasing theoretical drug loading, increasing amounts of organic solvent, polymer and polymeric stabilizer and decreased with increasing stirring time, increasing pH of the continuous phase and increased volume of the internal and external aqueous phase (Rainer and Bodmeier 1990).

Pan coating. The coating solution is applied as atomized spray to the solid core material in the coating pan. To remove the coating solvent warm air is passed over the coated material. By using this technique larger sized particles will be coated effectively.

Factors influencing encapsulation efficiency

The encapsulation efficiency of the microparticle or microcapsule or microsphere will be affected by different parameters. Figure 5 illustrates the factors influencing encapsulation efficiency.

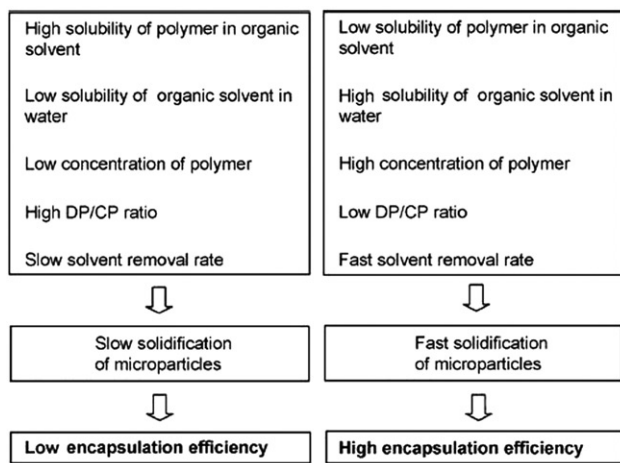


Figure 5. Factors influencing encapsulation efficiency. (Redrawn from Yeo and Park 2004).

Solubility of polymer in the organic solvent

Mehta et al. (1996) studied the effect of solubilities of different PLGAs polymers in methylene chloride, compared by measuring the methanol cloud point (Cs): Higher Cs meant that the polymer was more soluble in methylene chloride and, thus, required a greater amount of methanol to precipitate from the polymer solution. The PLGA polymer of a relatively high L/G ratio (75/25) had a higher solubility in methylene chloride than the other PLGA (L/G ratio = 50/50). A lower molecular weight polymer had a higher solubility in methylene chloride than a higher molecular weight polymer. End-capped polymers, which were more hydrophobic than non-end-capped polymers of the same molecular weight and component ratio, were more soluble in methylene chloride.

Diffusion of drugs into the continuous phase mostly occurred during the first 10 min of emulsification; therefore, as the time the polymer phase stayed in the non-solidified (semi-solid) state was extended, encapsulation efficiency became relatively low. In Mehta et al.'s (1996) study, polymers having relatively high solubilities in methylene chloride took longer to solidify and resulted in low encapsulation efficiencies, and vice versa. Particle size and bulk density also varied according to the polymer. Since polymers having higher solubilities in methylene chloride stayed longer in the semi-solid state, the dispersed phase became more concentrated before it completely solidified, resulting in denser microparticles.

Johansen et al. (1998) showed that the use of relatively hydrophilic PLGA which carried free carboxylic end groups resulted in a significantly higher encapsulation efficiency compared to that of an end-capped polymer. A similar explanation as above applies to this observation: Hydrophilic PLGA is relatively less soluble in the solvent, methylene chloride, and precipitates more quickly than the end-capped one. High solidification rate might have increased the encapsulation efficiency. On the other hand, the authors attribute the increase to the enhanced interaction between PLGA and the protein through hydrogen bonding and polar interactions (Johansen et al. 1998). Walter et al. (2001) also observed an increased encapsulation efficiency from using relatively hydrophilic PLGA in DNA microencapsulation. The hydrophilicity of the polymer enhanced the stability of the primary emulsion and it contributed to such an increase.

Solubility of organic solvent in water

Bodmeier and McGinity (1988) found that methylene chloride resulted in a higher encapsulation efficiency as compared with chloroform or benzene, even though methylene chloride was a better solvent for poly

(lactic acid) (PLA) than the others. Methylene chloride is more soluble in water than chloroform or benzene. The 'high' solubility allowed relatively fast mass-transfer between the dispersed and the continuous phases and led to fast precipitation of the polymer. The significance of solubility of the organic solvent in water was also confirmed by the fact that the addition of water-miscible co-solvents such as acetone, methanol, ethyl acetate or dimethyl sulphoxide (DMSO) contributed to increase of the encapsulation efficiency. Knowing that the methanol is a non-solvent for PLA and a water-miscible solvent, it can be assumed that methanol played a dual function in facilitating the polymer precipitation: First, the presence of methanol in the dispersed phase decreased the polymer solubility in the dispersed phase (Jeyanthi et al. 1997). Secondly, as a water-miscible solvent, methanol facilitated diffusion of water into the dispersed phase.

In order to explain the low encapsulation efficiency obtained with benzene, the authors mention that the benzene required a larger amount of water (non-solvent) than methylene chloride for precipitation of the polymer and the drug was lost due to the delayed solidification. However, given that benzene is a poorer solvent than methylene chloride for a PLA polymer, this argument does not agree with the widely spread idea that a poor solvent requires a smaller amount of non-solvent to precipitate a polymer. In fact, there could have been a better explanation if they had considered that the delayed solidification was due to the low solubility of benzene in water: As a poor solvent for a PLA polymer, benzene requires only a small amount of non-solvent for complete solidification of the polymer. However, since benzene can dissolve only a tiny fraction of water, it takes much longer to uptake water into the dispersed phase. That is, while solubility of a polymer in an organic solvent governs the quantity of a non-solvent required in precipitating a polymer, solubility of the organic solvent in the non-solvent limits diffusion of the non-solvent into the polymer phase. Thus, when a cosolvent system is involved, both solubility of a polymer in a solvent and solubility of the solvent in a non-solvent participate in determining the solidification rate of the dispersed phase.

Park et al. (1998) prepared lysozyme-loaded PLGA microparticles using the oil in water (o/w) single emulsion technique. Here, the authors used a co-solvent system, varying the ratio of the component solvents. DMSO was used for solubilization of lysozyme and PLGA and methylene chloride was used for generation of emulsion drops as well as solubilization of PLGA. Encapsulation efficiency increased and initial burst decreased as the volume fraction of DMSO in the co-solvent system increased. Particle size increased and density of the microparticle matrix decreased with increasing DMSO. Overall, these results indicate that the presence of DMSO increased the

hydrophilicity of the solvent system and allowed fast extraction of the solvent into the continuous phase, which led to higher encapsulation efficiency and larger particle size.

Concentration of the polymer

Encapsulation efficiency increases with increasing polymer concentration (Mehta et al. 1996, Rafati et al. 1997, Li et al. 1999). For example, the encapsulation efficiency increased from 53.1 to 70.9% when concentration of the polymer increased from 20.0 to 32.5% (Mehta et al. 1996). High viscosity and fast solidification of the dispersed phase contributed to reduce porosity of the microparticles as well (Schlicher et al. 1997). The contribution of a high polymer concentration to the encapsulation efficiency can be interpreted in two ways. First, when highly concentrated, the polymer precipitates faster on the surface of the dispersed phase and prevents drug diffusion across the phase boundary (Rafati et al. 1997). Secondly, the high concentration increases viscosity of the solution and delays the drug diffusion within the polymer droplets (Bodmeier and McGinity 1988).

Ratio of dispersed phase to continuous phase (DP/CP ratio)

Encapsulation efficiency and particle size increase as the volume of the continuous phase increases (Mehta et al. 1996, Li et al. 1999). For example, the encapsulation efficiency increased more than twice as the ratio of the dispersed phase to the continuous phase (DP/CP ratio) decreased from 1/50 to 1/300 (Mehta et al. 1996). It is likely that a large volume of continuous phase provides a high concentration gradient of the organic solvent across the phase boundary by diluting the solvent, leading to fast solidification of the microparticles. A relevant observation is described in the literature (Sah 1997). In this example, which utilized ethyl acetate as a solvent, the formation of microparticles was dependent on the volume of the continuous phase. When 8 mL of PLGA solution (o) was poured into 20 or 50 mL of water phase (w), the polymer solution was well disintegrated into dispersed droplets. On the other hand, when the continuous phase was 80 mL or more, the microspheres hardened quickly and formed irregular precipitates. This is because the large volume of continuous phase provided nearly a sink condition for ethyl acetate and extracted the solvent instantly. Due to the fast solidification of the polymer, particle size increased with increasing volume of the continuous phase. Microparticles generated from a low DP/CP ratio had a lower bulk density (0.561 g/cc at 1/50 vs 0.357 g/cc

at 1/300), which the authors interpret as an indication of higher porosity of the polymer matrix (Mehta et al. 1996). On the other hand, a different example shows that a higher DP/CP ratio resulted in increased porosity, providing a large specific surface area (measured by the BET method) and the scanning electron microscope (SEM) pictures as evidence (Jeyanthi et al. 1997). This apparent discrepancy can be explained by the fact that low bulk density (Mehta et al. 1996) is not a true reflection of porosity but a result of large particle size. In fact, porosity increases with increasing DP/CP ratio, i.e. decreasing rate of the polymer precipitation.

Rate of solvent removal

The method and rate of solvent removal influence the solidification rate of the dispersed phase as well as morphology of the resulting microparticles (Mehta et al. 1994). In the emulsion-solvent evaporation/extraction method, the solvent can be removed by (i) evaporation, in which the solvent is evaporated around its boiling point, or (ii) extraction into the continuous phase. The rate of solvent removal can be controlled by the temperature ramp or the evaporation temperature in the former and by the volume of the dilution medium in the latter. PLGA microparticles containing salmon calcitonin (sCT) were prepared by emulsification, followed by different solvent removal processes (Mehta et al. 1994, Jeyanthi et al. 1996). In the temperature-dependent solvent removal process, the solvent (methylene chloride) was removed by increasing the temperature from 15 to 40°C at different rates. The microparticles that resulted from this process had a hollow core and a porous wall. The core size and wall thickness were dependent on the temperature ramp. A rapid rise in temperature resulted in a thin wall and a large hollow core, whereas a stepwise temperature rise (15 to 25, then to 40°C) resulted in a reduced core size. It is believed that the hollow core was due to the rapid expansion of methylene chloride entrapped within the solidified microparticles. In controlled extraction of the solvent, the solvent was removed gradually and slowly by dilution of the continuous phase, which left the microparticles in the soft state for a longer period of time. The resulting microparticles showed a highly porous honeycomb-like internal structure without a hollow core. In the later study, it was noted that the porosity was a function of the amount of water diffused into the dispersed phase from the continuous phase, which could only be allowed before the dispersed phase solidified completely (Li et al. 1995). In other words, the high porosity of the microparticles was due to the slow solidification of the microparticles. Even though it is generally assumed that fast polymer solidification results in high encapsulation efficiency, this does not

apply to the observation of Yang et al. (2000). Here, the encapsulation efficiency was not affected by the solvent evaporation temperature. It may be due to the different processing temperatures influencing not only the rate of polymer solidification but also the diffusivity of the protein and its solubility in water. While the high temperature facilitated solidification of the dispersed phase, it enhanced diffusion of the protein into the continuous phase, compromising the positive effect from the fast solidification.

Interaction between drug and polymer

Interaction between protein and polymer contributes to increasing encapsulation efficiency (Boury et al. 1997). Generally, proteins are capable of ionic interactions and are better encapsulated within polymers that carry free carboxylic end groups than the end-capped polymers. On the other hand, if hydrophobic interaction is a dominant force between the protein and the polymer, relatively hydrophobic end-capped polymers are more advantageous in increasing encapsulation efficiency (Mehta et al. 1996). For example, encapsulation efficiencies of more than 60% were achieved for salmon calcitonin (sCT) microparticles despite the high solubility of sCT in the continuous phase (Jeyanthi et al. 1997). This is attributed to the strong affinity of sCT to hydrophobic polymers such as PLGA. On the other hand, such interactions between protein and polymer can limit protein release from the microparticles (Crotts and Park 1997, Park et al. 1998, Kim and Park 1999). In certain cases, a co-encapsulated excipient can mediate the interaction between protein and polymer ((Johansen et al. 1998). Encapsulation efficiency increased when gammahydroxypropylcyclodextrin (g-HPCD) were co-encapsulated with tetanus toxoid in PLGA microparticles. It is supposed that the g-HPCD increased the interaction by accommodating amino acid side groups of the toxoid into its cavity and simultaneously interacting with PLGA through van der Waals and hydrogen bonding forces.

Solubility of drug in continuous phase

Drug loss into the continuous phase occurs while the dispersed phase stays in a transitional, semi-solid state. If the solubility of the drug in the continuous phase is higher than in the dispersed phase, the drug will easily diffuse into the continuous phase during this stage. For example, the encapsulation efficiency of quinidine sulphate was 40-times higher in the alkaline continuous phase (pH 12, in which quinidine sulphate is insoluble) than in the neutral continuous phase (pH 7, in which quinidine sulphate is very soluble) (Bodmeier and McGinity 1988).

Molecular weight of the polymer

Fu et al. (2005) studied the effect of molecular weight of the polymer on encapsulation efficiency and developed a long-acting injectable huperzine A-PLGA microsphere for the chronic therapy of Alzheimer's disease, the microsphere was prepared by using o/w emulsion solvent extraction evaporation method. The morphology of the microspheres was observed by scanning electron microscopy. The distribution of the drug within microspheres was observed by a confocal laser scanning microscope. The results indicated that the PLGA 15 000 microspheres possessed a smooth and round appearance with average particle size of 50 μm or so. The encapsulation percentages of microspheres prepared from PLGA 15 000, 20 000 and 30 000 were 62.75, 27.52 and 16.63%, respectively. The drug release percentage during the first day decreased from 22.52% of PLGA 30 000 microspheres to 3.97% of PLGA 15 000 microspheres, the complete release could be prolonged to 3 weeks. The initial burst release of microspheres with higher molecular weight PLGA could be explained by the inhomogeneous distribution of drug within microspheres. The encapsulation efficiency of the microspheres improved as the polymer concentration increase in oil phase and PVA concentration decreased in aqueous phase. The burst release could be controlled by reducing the polymer concentration. Evaporation temperature had a large effect on the drug release profiles. It had better be controlled under 30°C. Within a certain range of particle size, encapsulation efficiency decreased and drug release rate increased with the reducing of the particle size (Fu et al. 2005).

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