

Solvent Evaporation Technique of Microencapsulation: A Systemic Review

Chitra Singh^{*1}, S. Purohit², B. L. Pandey² and Madhu Singh²

¹Department of Pharmacology, IMS, BHU, Varanasi-221005, India

²Department of Pathology, Bundelkhand Govt. Ayurvedic college, Jhansi-284001, India

Abstract

Solvent evaporation is one of the most widely employed and investigated technique in pharmaceutical industries and research area for microencapsulation process. Microspheres are particles coated with a continuous film of polymeric material, having a diameter in range of 1 to 1000 μm and are widely used as drug carriers. This technique provides a controlled drug release, having various clinical benefits. While initial lab scale experiments are carried out in simple beaker, stirrer setups, clinical trials and market introduction needs more sophisticated technologies, permitting economic robust, well-controllable and aseptic production of microspheres. In this review article our aim is to review and compile recent research work on solvent evaporation technique while focusing on different methods of above said technique and various factors affecting microencapsulation prepared by solvent evaporation technique.

Keywords: microencapsulation, controlled drug release, drug carrier, polymer

1. Introduction

Microspheres are defined as homogeneous, monolithic particles in the size range of about 1–1000 μm and are widely used as drug carriers for controlled release. These systems have significant importance in biomedical applications. Administration of drugs in the form of microspheres usually improves the treatment by providing the localization of the active substance at the site of action and by prolonging release of drugs. Furthermore, sensitive drugs such as peptides and proteins may be protected against chemical and enzymatic degradation when entrapped in microspheres^{1,2}. In drug delivery applications, poly (DL-lactide-co-glycolide) microspheres are being considered as the pharmaceutical products of the future.

Controlled/targeted drug delivery using biodegradable polymeric carriers has gained increasing interest in the last two decades. In a majority of studies the lactide/glycolide homo- and copolymers have been used for drug delivery applications because they can be fabricated into a variety of morphologies, including films, rods, and microparticles, by compression molding, solvent casting, and solvent evaporation techniques³⁻⁸. For employment in the body, biodegradable polymers have an obvious advantage that after performing their function they degrade into nontoxic monomers, i.e., lactic and glycolic acids, thus avoiding the need for surgical removal. So, the risk of long-term toxicity or a probable immunological reaction when compared with nondegradable systems is also minimized. The release rate of an incorporated drug can be modulated by variation of the copolymer ratio and molecular weight. Gopferich reviewed polymer degradation mechanisms and mechanical properties extensively⁹⁻¹¹. An intelligent approach to therapeutics using drug carrier technology requires a detailed understanding of drug-carrier interactions with critical cellular and

organ systems as well as an understanding of the limitations of the system with respect to formulation procedures and stability.

A wide range of microencapsulation techniques have been developed to date¹². Among them, solvent evaporation technique has become more useful as compared to other methods. In this method of microencapsulation, controlled particle sizes in the nano- to microsphere range can achieve high encapsulation materials and various conditions in order to achieve high encapsulation efficiency and a low residual solvent content. However, several process variables had been identified by researcher, which could affect the formulation of microsphere by solvent evaporation method, such as, type of solvent, volume of solvent, drug to polymer ratio, rate of solvent removal, effect of internal aqueous phase volume in case of solvent evaporation followed by multiple emulsion, effect of addition of buffer or salts to the internal or external phase, which can affect the size of microcapsules and also the release pattern of drug from microcapsules¹³⁻¹⁶. The current review deals with the various methods, factors and other related aspects of solvent evaporation technique to formulate microencapsulation for the pharmaceutical use.

2. The techniques of microencapsulation

The technique of microencapsulation by solvent evaporation is widely applied in pharmaceutical industries to obtain the controlled release of drug. The obtained polymer microspheres with drug trapped inside can degrade and release the encapsulated drug slowly with a specific release profile. This controlled drug release has outstanding clinical benefits: reduction of dose frequency, more convenient and acceptable for patients and drug targeting to specific locations resulting in a higher efficiency^{17,18}. There are different methods to use

microencapsulation by solvent evaporation technique. The choice of the method that will give rise to an efficient drug encapsulation depends on the hydrophilicity or the hydrophobicity of drug. Basic steps involved in solvent evaporation techniques are:

2.1 Incorporation of pharmaceutical agents

The polymer is dissolved in a suitable water immiscible solvent, and the pharmaceutical agent is directly added into the solution of polymeric-matrix materials by dissolution/dispersion in suitable solvents, or emulsification of aqueous solution of the pharmaceutical agents immiscible with the matrix-material solution. For the preparation of solution or dispersion of pharmaceutical agents, impeller or static mixing, high speed-stator mixing or microfluidization techniques are generally used.

2.2 Droplet formation

This step determines the size of resulting microcapsules. The size of microcapsules affects the drug encapsulation efficiency and the rate of drug release. The following procedures are used in droplet formation:

2.2.1 Stirring: The external phase is filled into a vessel and agitated by an impeller. The drug/matrix dispersion is then added, drop wise or all at once, under agitation at a speed sufficient to reach the desired droplet size¹⁹.

2.2.2 Static mixing: Static mixer consists of baffles or other flow obstacles installed in a tube. The baffle arrangement repeatedly splits and recombines the stream of fluid passing through the tube.

2.2.3 Extrusion: It involves feeding of drug/matrix dispersion through single or multichannel pathways directly into the continuous phase. When drug/ matrix dispersion leaves the pathways, discrete droplets are formed within the slow flowing continuous phase. In extrusion, flow is laminar, the droplets are formed at the site of introduction of drug/matrix dispersion into continuous phase, due to which there is no effect on size of droplets formed thereafter. On the contrary, static mixing relies mainly on turbulent flow, which constantly acts on the disperse phase and thus causes

the size of the droplets to change over the whole length of the mixer. Therefore, extrusion is considered to allow for more uniform and better controlled microsphere sizes than static mixing.

2.3 Solvent removal.

Solvent removal can be achieved either by evaporation or by extraction. In both processes, the drug/matrix dispersion should be slightly soluble in the continuous phase, so that, partitioning into continuous phase can occur that leads to precipitation of the matrix material. The two ways of solvent removal can be performed:

2.3.1 Solvent evaporation: In this method, the capacity of the continuous phase is insufficient to dissolve the entire volume of disperse phase solvent. Thus, solvent evaporates from the surface of the dispersion to obtain hardened microcapsules.

2.3.2 Solvent extraction: This is a two-step process. Firstly, the drug/matrix dispersion is mixed with a small quantity of continuous phase to yield desired size of droplets. Then secondly, further more continuous phase and/or additional extraction agents are added at an amount sufficient to absorb the entire solvent leaching from droplets of drug/matrix. This results into formation of solid microcapsules.

2.3.3 Drying: Solidified microparticles from the continuous phase are by either filtration or centrifugation. Then the particles are rinsed with suitable liquids to remove adhering substance such as dispersion stabilizers or non-encapsulated drug. Finally, these microparticles are dried at elevated temperature or under reduced pressure to yield free flowing powder. The drying procedure removes not only continuous phase and wash fluid adhering to the microspheres' surface but also traces of solvents and continuous phase from the interior of the particles. Thus, the conditions and rate of drying influence the amount of solvent and moisture residue²⁰. Microsphere morphology and porosity as well as drug recrystallisation inside the spheres, and are therefore likely to affect the release behaviour of the final product.

Fig. 1: Parallel installation of several static mixers for scale-up of microsphere production⁴³..

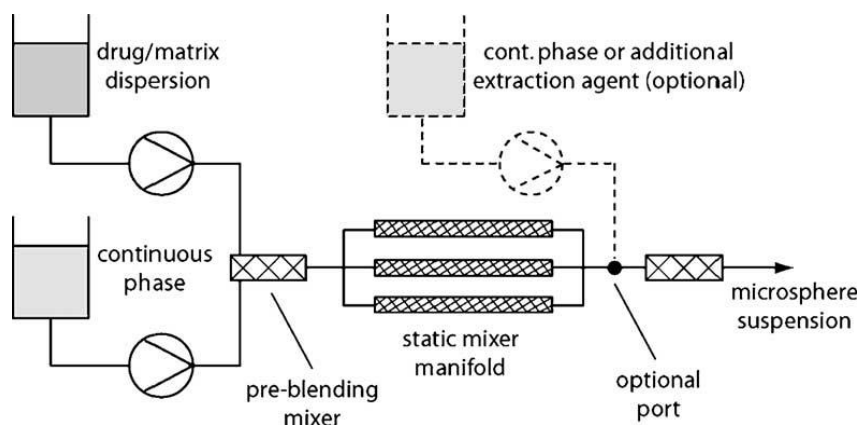


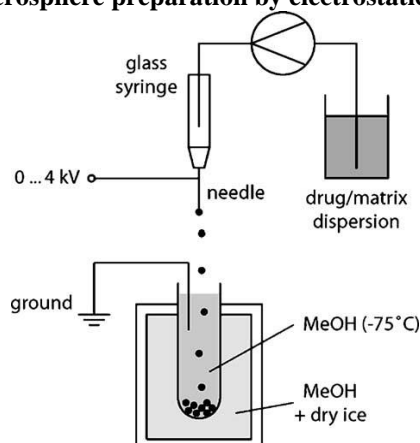
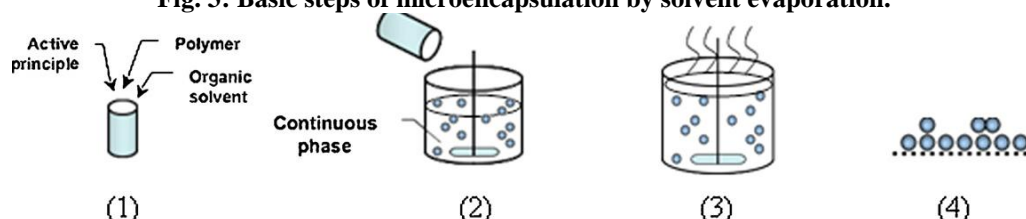
Fig. 2: Microsphere preparation by electrostatic dripping⁷¹

Fig. 3: Basic steps of microencapsulation by solvent evaporation.



3. Different Techniques of micro-encapsulation

3.1 Oil/Water emulsion followed by solvent evaporation:

The drug substance is either dispersed or dissolved in the polymer/solvent system. Then it is added to the aqueous phase by continuous agitation. Agitation of the system is continued until the solvent partitions into the aqueous phase and is removed by evaporation. This process results in hardened microsphere which contains the active moiety that is drug. Several methods have been utilized to achieve dispersion of the oil phase in the continuous phase. The most common method is the use of a propeller type blade attached to a variable speed motor. Since high shear is used to produce the emulsion, the resultant product has a much smaller particle size than the emulsion produced by conventional agitation.

Other method includes the use of a micro fluidizer to produce micro-emulsions, sonication and potentiometrics dispersion.

A major problem with this technique is a poor encapsulation efficiency of moderately water soluble and water soluble compounds, which partitioned out from the organic dispersed phase into the aqueous continuous phase. Successfully entrapment of drug within the microspheres is thus highly dependent on solubility in the aqueous phase. Water soluble drugs (e.g. theophylline, caffeine and salicylic acid) could not be entrapped within the poly (lactic acid) (PLA) microsphere using an Oil/Water emulsion method, while drugs with low water solubility, such as Diazepam, Hydrocortisone and Progesterone were successfully retained within the microspheres.

Table 1: Examples of hydrophobic and hydrophilic drugs encapsulated using solvent evaporation technique

Hydrophobic drugs	Name of drug	Polymer	References
Hydrophobic drugs	Cisplatin, 5-fluorouracil (anticancer agents)	PLGA	21,22
	Lidocaine (local anesthetics)	PLGA PLA	23,24
	Naltrexone, cyclazocine (narcotic antagonists)	PLA PLA	25,26,27
	Progesterone (hydrophobic steroids)	PELA (PLA+ PEG) PLGA PLA	28,29,30
Hydrophilic drugs	Insulin	O/O W/O/W O/W dispersion	31 32,33 34
	Proteins	O/O W/O/W	35 36,37,38
	Peptides	co-solvent O/W dispersion O/O	72,39 72,40 72
	Vaccine	W/O/W	41,42,43

3.2 Water-oil-water multiple emulsion system

This method for preparation of microsphere was reported to overcome the problem of low encapsulation efficiency of water-soluble drugs prepared by conventional w/o emulsion solvent evaporation method^{44,45}. In this technique, polymer is dissolved in a suitable organic phase. In this organic phase, aqueous drug solution is emulsified using high-speed homogenizer operating around 15000-20000 rpm for about 30 seconds to prepare w/o primary emulsion. This primary emulsion is added to external aqueous phase containing surfactant at homogenizer speed around 8000 rpm for 30 seconds and then stirred at 300 rpm for 3 hours at room temperature for permitting evaporation of organic solvent or it can be also performed under vacuum. The microcapsules obtained is collected by ultracentrifugation, filtration and then, lyophilized. Among them, lyophilisation decreases the burst effect. However, it may be change from drug to drug to obtain acceptable microcapsule size and drug release.

3.3 Multiple emulsion of the Water/Oil/Oil or Water/Oil/Oil/Oil type

Iwata and McGinity developed a multiple emulsion of the W/O/O/O type⁴⁶. Multiphase microcapsules of PLGA containing water-in oil (W/O) emulsions were prepared by a multiple emulsion solvent evaporation techniques. Acetonitrile was used as the solvent for the polymer, and light mineral oil comprised the continuous phase for the encapsulation procedure in this investigation. Drug loading efficiencies of model water-soluble compounds was found 80 to 100 %. Scanning electron microscopy of transverse cross sections of the multiphase microsphere of W/O/O/O type belonged to the class of reservoir-type drug delivery devices. Utilization of this type of multiple emulsion system allows the encapsulation of the primary water in oil emulsion within a polymeric microsphere. The oil in the primary emulsion prevents contact between the internalized protein and the polymer/solvent system prevents possible denaturation of the protein by the polymer or the solvent. Likewise, the possibility of polymeric degradation due to reactive proteins or drug compounds is also limited^{46,47}.

3.4 A modified water-in-oil-in-water (W/O/W) double emulsion solvent evaporation

Taek Kyoung Kim et al has developed Gas foamed open porous biodegradable polymeric microsphere. Highly opened porous biodegradable polymeric microspheres were fabricated for use as injectable scaffold micro carriers for cell delivery. They modified water-in-oil-in-water (W/O/W) double emulsion solvent evaporation method for producing the microspheres. When an effervescent salt, ammonium bicarbonate is added in to the primary W1 droplets, carbon dioxide and ammonia gas bubbles were spontaneously produced during the solvent evaporation process that stabilized the primary emulsion as well as created well inter-connected pores in the resultant microspheres. The porous

microspheres fabricated under various gas foaming conditions were characterized. The size of the surface pores formed under the various gas foaming conditions became as large as 20µm in diameter. As the concentration of ammonium bicarbonate increased, the diameter which was sufficient enough for cell infiltration and seeding. These porous scaffold microspheres could be potentially utilized for cultivating cells in a suspension manner and for delivering the seeded cells to the tissue defect site in an injectable manner.

3.1 Impact of various variables on microencapsulation via solvent evaporation technique

3.1.1 Effect of polymer: Various polymers have their inherent quality. Therefore, the quality of the microencapsulation varies depending upon the polymer used. In pharmaceutical industry, biodegradable polymers are used for microencapsulation. Polymers used for microencapsulation of pharmaceuticals are:

- Natural proteins like albumin, collagen, gelatin, fibrinogen, casein, fibrin, hayaluronic acid, *etc.*
- Natural polysaccharides like starch, dextrin, alginic acid, chitin, chitosan, *etc.*
- Semisynthetic polysaccharides like ethyl cellulose, hydroxyl ethylcellulose, hydroxyl propylcellulose, methyl cellulose, hydroxyl propyle ethylcellulose, *etc.*
- Synthetic polymers like poly (lactic acid), poly (lactic/glycolic acid), poly (L-hydroxybutyric acid), poly orthoester, poly alkyl cyanoarylate, various grades of Eudragit, *etc.*

Owing to the excellent biocompatibility property of the biodegradable polyesters poly (lactic \ acid) (PLA) and poly (lactic-co-glycolic acid) (PLGA), these are the most widely used biomaterials for the microencapsulation of therapeutics and antigens^{48,49}. Ethyl cellulose, cellulose acetate, cellulose acetate phthalate (CAP), cellulose acetate butyrate (CAB) and various grades of Eudragit are also used for the microencapsulation of various drugs using solvent evaporation technique and found suitable in terms of ease of preparation, size and better controlled release drug delivery⁵⁰⁻⁵⁷. Moreover, these polymers are comparatively low-cost than PLA and PLGA. The molecular weight of polymers used in microencapsulation using solvent evaporation technique is also another important factor. Fu *et al* have developed a long acting injectable Huperzine A-PLGA microsphere for the chronic therapy of Alzheimer's disease. These microspheres were prepared by solvent evaporation method. It was observed that the PLGA 15000 microspheres possessed a smooth and regular particle size of around 50 µm. The drug encapsulation percentage of microsphere prepared from PLGA 15000, 20,000, and 30,000 were 62.75 %, 27.52 % and 16.63 %, respectively⁵⁸. The drug encapsulation efficiency of these microspheres was found increased with the increment in molecular weight of polymer (here, PLGA) due to stronger barrier characteristics of high molecular weight polymers. In this study, they have

also found an increment of drug encapsulation efficiency in these microspheres as the polymer concentration was increased in oily phase, and poly vinyl alcohol (PVA) concentration decreased in aqueous phase. In another investigation, Uchida *et al.*, have used different PLGA and they have experienced the same as previous investigation stated above. They found highest ovalbumin loading in PLGA (85:15) than other PLGA grades⁵⁹.

3.1.2 Effect of drug to polymer ratio: For the entrapment of bovine serum albumin (BSA) into poly (methyl methacrylate)(PMMA) microspheres, a ratio of less than 1:10 was suggested to yield protein loadings of >80%. A higher load of bioactive material is likely to decrease the encapsulation efficiencies of proteins and peptides in PLGA and increase the 24 hrs (“burst”) drug release. In some cases, increase in drug content, increases the entrapment efficiency e.g. an increase in entrapment efficiency ovalbumin (ova) from 40% to 98% with an increase in actual ova content from 7% to 16%(w/w). Cavalier et al (1986) studied the preparation of PLA microcapsules containing hydrocortisone using polyvinyl alcohol (PVA) (partially hydrolyzed 88%) and methyl cellulose (400cps and 10cps grades) as colloidal emulsifiers. They reported that when the drug/polymer ratio was less than 0.4, microcapsules could be prepared using PVA (0.1% in water) only and in regular dimensions. But when this ratio exceeded 0.6, irregular and large aggregated microcapsules were formed due to the formation of unstable emulsion.

Effects of organic solvents. For the technique of microencapsulation by solvent evaporation, a suitable solvent should meet the following criteria:

- (1) Being able to dissolve the chosen polymer;
- (2) Being poorly soluble in the continuous phase;
- (3) Having a high volatility and a low boiling point;
- (4) Having low toxicity

Chloroform was frequently used before, but due to its toxicity and low vapour pressure, it is gradually replaced by methylene chloride. Methylene chloride is the most common solvent for the encapsulation using solvent evaporation technique because of its high volatility, low boiling point and high immiscibility with water. Its high saturated vapour pressure compared to other solvents (at least two times higher) promises a high solvent evaporation rate, which shortens the duration of fabrication of microspheres. However this solvent is confirmed carcinogenic according to EPA (Environmental Protection Agency) data and the researchers are making great efforts to find less toxic replacements. Ethyl acetate shows promising potential as a less toxic substitute of methylene chloride. But due to the partial miscibility of ethyl acetate in water (4.5 times higher than that of methylene chloride), microspheres cannot form if the dispersed phase is introduced directly into the continuous phase. The sudden extraction of a big quantity of ethyl acetate from the

dispersed phase makes the polymer precipitate into fibre-like agglomerates⁶⁰. To resolve this problem created by the miscibility of solvent with water, three methods can be used: the aqueous solution is pre-saturated with solvent⁶¹; the dispersed phase is first emulsified in a little quantity of aqueous solution; after the formation of drops this emulsion is poured into a large quantity of aqueous solution⁶⁰; the dispersed phase is emulsified in a little quantity of aqueous solution, the solution is agitated and the solvent evaporates leading to solidification of microspheres⁶². Using these methods, the microspheres are manufactured successfully with ethyl acetate. However, the microspheres prepared by methylene chloride are spherical and more uniform, while the use of ethyl acetate results in particles which appear to be partly collapsed⁶³.

The drug encapsulation efficiency reduces significantly compared to the microspheres made by methylene chloride. The author assumed that it is due to the high solubility of ethyl acetate in water, leading to the loss of drug. Based on this assumption, we make a further assumption that it is due to two main causes: (1) more drug is entrained into the continuous phase by the higher mass flux of solvent, which is driven by the diffusion from the dispersed phase into the continuous phase; (2) the big quantity of solvent present in the continuous phase increases the solubility of drug in the continuous phase, facilitating the diffusion of drug into the continuous phase. Ethyl formate shows also interesting results. Sah has succeeded in manufacturing microspheres of PLGA with ethyl formate⁶⁴. He observed that the evaporation rate of ethyl formate in water was 2.1 times faster than that of methylene chloride although ethyl formate possesses a lower vapour pressure and a higher boiling point according to the authors. This phenomenon is explained by the fact that more molecules of ethyl formate are exposed to the air-liquid interface because of its higher water solubility. His work proved that water immiscibility of a solvent is not an absolute prerequisite for making an emulsion. More experiments have to be carried out to confirm the promising use of ethyl formate. In summary, less toxic solvents have been tested and show a promising future. But there are not enough results to compare the quality of microspheres prepared by different solvents. Methylene chloride is still the most used solvent because it evaporates fast, shows high drug encapsulation efficiency and produces microspheres with spherical and more uniform form.

3.1.3 Effect of solubility of drug in continuous phase: Drug loss may occur to continuous phase while the dispersed phase remains in a transitional, semi-solid state. If the solubility of drug in the continuous phase is higher than in the dispersed phase, then the drug may easily diffuse into the continuous phase during this stage. It was observed that the encapsulation efficiency of quinidine sulphate was 40 times higher in alkaline (pH-12) continuous phase than in the neutral (pH-7) continuous phase

because quinidine sulphate is insoluble in alkaline pH whereas very soluble in neutral pH.

3.1.4 Rate of solvent removal: The rate of solvent removal from microsphere prepared by the solvent evaporation method has a great impact on the physiochemical properties of the microsphere. Izumikawa et al observed a significant difference in physical property and drug release profile between progesterone loaded PLA microsphere prepared by either reduced pressure solvent evaporation or a solvent evaporation method under atmospheric conditions. Encapsulation efficiency was greater for microsphere prepared by the reduced pressure solvent extraction method (RSE) than for those prepared by the atmospheric solvent evaporation (ASE). When surface morphology of these microspheres were studied under scanning electron microscopy, it indicated a porous and rough surface for RSE microsphere. The ASE microsphere exhibited peaks due to crystalline progesterone in addition to peaks due to crystalline poly (l-lactide) PLA. The RSE microspheres displayed no such peaks due to crystalline PLA which suggest that the PLA was present in the amorphous state. Additionally, the RSE microsphere exhibited no peaks corresponding to crystalline progesterone which indicated that drug was dispersed in an amorphous polymer network. It was assumed that the solvent removal under the reduced pressure occurred too rapidly for the polymer to crystallize. Drug release from the microsphere was found to be significantly influenced by the crystallinity of the polymer matrixes, the drug release rate increased with the drug loading for both types of microsphere. For the ASE microsphere, there was a rapid release in the initial stage, and the release rate was much greater than that of the RSE microspheres. The ideal rate of solvent removal depends on a variety of factors like the types of matrix material used, drug and solvent as well as the desired drug release profile of microsphere. Like, fast microsphere solidification will be preferred if the drug easily partition into the continuous phase, whereas slow solidification favours denser over more porous microsphere, affecting the drug release.

3.1.5 Effect of preparation temperature: Yang *et al.*, have studied the influence of preparation temperature on the various characteristics and release profile of PLGA microspheres, prepared using solvent evaporation technique⁶⁵. They have studied the formation of PLGA microspheres in varying temperature (4-42°C). The PLGA microspheres tend to be larger when prepared at higher temperature (38 and 42°C), showed wider size distributions, and decreased particle density as compared to microsphere prepared at lower temperature (4-33°C). The morphology of the particle interior (honeycomb-like) and drug encapsulation efficiency (53 % to 63 %) were unaffected by the preparation temperature. In case of PGLAPEG blend, drug encapsulation efficiency was unaffected with a minimum efficiency of (15 % to 63 %) were unaffected by the preparation temperature, whereas in case of PLGA-PEG blend,

drug encapsulation efficiency was affected with a minimum efficiency of 15 % at 22°C, which steadily got improved (around 52 %) for lower and higher temperatures. Microspheres prepared at high temperature were found to be a uniform internal pore distribution and a very thin dense skin layer, whereas microsphere prepared at lower temperature showed a thick but porous skin layer and bigger pores in the middle of the sphere. Microspheres formed at 33°C experienced the highest initial burst release. In term of *in vitro* drug release, microspheres fabricated at low temperature (5, 15, 22°C) exhibited similar steady drug release rates. However, microspheres formed at higher temperature exhibited low release rates after their initial drug release.

3.1.6 Effect of interaction between drug and polymer: The interaction between drug encapsulated and polymer can change in drug encapsulation efficiency. The interaction between drug and polymer may be hydrophilic or hydrophobic interaction. In case of hydrophilic or ionic interaction, the drug is best encapsulated in polymers containing free carboxylic end groups.

In case of hydrophobic interaction, relatively hydrophobic end capped polymers are more effective in increasing encapsulation efficiency⁶⁶. On the other side, such interaction between drug and polymer may limit the protein release from the microsphere⁶⁷. In certain cases, co-encapsulated excipients can mediate interaction between protein and polymer. Encapsulation efficiency of tetanus toxoid in PLGA increased, when gamma hydroxyl propyl cyclodextrin (g-HPCD) were incorporated. It is supposed that the g-HPCD increased the interaction by involving amino acid side group of the toxoid into its cavity and simultaneously interacting with PLGA through Vander Waal and hydrogen bonding forces⁶⁸.

3.1.7 Effect of buffer or added salt: On considering the properties of somatostatin acetate containing polylactide microsphere, Herrmann and Bodmeier prepared microsphere for encapsulation efficiency, drug release, and morphological properties. Addition of buffers or salts to the internal aqueous phase resulted in the formation of a dense and homogenous polymer matrix. Drug release profile consisted of a rapid drug release phase followed by a slow release phase. This pattern is consistent with many matrix-type drug delivery systems. In this the drug release profile can be divided into two phase; a rapid release phase representing the release of the peptide by diffusion through the polymer matrix. Lower encapsulation efficiencies were obtained with the more porous microspheres. Addition of buffer salts to the internal aqueous phase promoted an influx of water from the external phase due to a difference in osmotic pressure. This resulted in a more porous microsphere structure, faster drug release, and lower encapsulation efficiency. Ahmad Al-Maaieh and Douglas R. Flanagan found increase in microsphere drug loading by changing the aqueous solubility of both the drug and the organic solvent DCM. Quinidine sulfate solubility was depressed by either a

common ion effect (Na_2SO_4) or by formation of new, less soluble drug salts (e.g., bromide, perchlorate, and thiocyanate). Inorganic salts depress DCM aqueous solubility to different extents as described by the Hofmeister series. NaClO_4 and NaSCN depressed drug solubility to the highest extent, resulting in microspheres with high drug loading (e.g., >90%). Other salts such as Na_2SO_4 did not depress quinidine sulphate solubility to the same extent and did not improve loading. The use of a cosolvent (ethanol) in the organic phase was found to improve drug loading and make uniform drug distribution with smooth release profiles.

3.1.8 Effect of internal aqueous phase volume on loading capacity: Comparatively with large volume of internal aqueous phase (500 μl or 1000 μl), decrease in loading efficiency of ovalbumin was observed^{68,69}. While using smaller of internal phase of 50 μl , high drug loading efficiency was observed. With the increment of internal phase volume, thin layer of phase (methylene chloride) might be formed, which could act as a barrier for diffusion of drug to the external aqueous phase. With the thinner organic phase, the more diffusion and probability of diffusion from internal phase to external phase was observed, which could lower the drug loading efficiency. So, small internal phase volume was found to be beneficial to get high drug loading⁷⁰.

3.1.9 Effect of external aqueous phase volume on loading capacity: Parikh *et al.*, have found that an increase in the volume of the external phase of the secondary emulsion lead to a decrease in the particle size of microspheres⁶⁹. The droplet size of the secondary emulsion may decrease because of a decrease in the frequency of collision of droplets with an increase in the volume of the external phase of the secondary emulsion. The decrease in the particle size of microspheres associated with an increase in the volume of the external phase of the secondary emulsion may attribute to a decrease in the secondary emulsion.

3.1.10 Effect of stirring speed: During droplet formation step, the stirring of impeller or baffles used, determines the size of microsphere. Increasing the mixing speed generally, results in decreased microsphere mean size⁷¹. In the investigation by our group, we found that increased stirring speed produced smaller sized microparticles using emulsification solvent evaporation technique. This phenomenon strongly supports the idea that the high stirring speed could provide high shearing force needed to breakdown the drug-polymer droplets into smaller particles⁵³. The drug release from these smaller microparticles prepared with high stirring speed was found faster than that lower speed. The reduction of mean particle size of microparticles could facilitate higher rate of drug diffusion from larger surface area provided by the smaller microparticles.

4. Conclusion

Solvent evaporation techniques offer a versatile, easy, and practical method for the manufacture of biodegradable microspheres. This technique makes possible the entrapment of a wide range of drugs having different physical properties and solubility characteristics. The properties of materials and the processing aspects strongly influence the properties of microspheres and the resultant controlled release rate. Because of outstanding biocompatibility and biodegradability PLGA polymers are frequently used as most promising synthetic biodegradable polymeric carrier. Most widely used solvent is methylene chloride owing to its high volatility and capacity to dissolve most polymers. However due to its carcinogenic nature, efforts are being made to replace it and substitutes such as ethyl acetate and ethyl formate give promising results. The microspheres with the desired properties are obtained by various operating variables such as organic solvent, rate of solvent removal, and amount of organic solvent or drug solubility, drug to polymer ratio, partition coefficient, polymer composition and molecular weight, and method of manufacture etc. All these factors play an important role in the development of successful controlled release polymeric microspheres containing drugs. Based on previous studies it was found that during microencapsulation of peptides and proteins; mechanical processing, solvents interaction, pH of the medium must be taken into account while processing and storage. These are the conditions that can result in degradation, denaturation and conformational changes in peptides and proteins; rendering them inactive. Sometimes certain proteins may prematurely degrade the polymer used in microencapsulation. In response to these concerns, innovative methods of microsphere production, such as multiple emulsion following solvent evaporation techniques have been investigated. After reviewing different aspects of solvent evaporation technique; overall summary is that there is necessity of designing formulations with desired release rate and drug entrapment efficiency.

References

1. Okada H, Toguch H. Biodegradable microspheres in drug delivery. *Crit Rev Ther Drug Carrier Syst* 1995; 12:1-99.
2. Crofts G, Park T G. Protein delivery from poly (lactic-co-glycolic acid) biodegradable microspheres: release kinetics and stability issues. *J Microencap.*1998; 15: 699-713.
3. Gombotz W R, Pettit D K. Biodegradable polymers for protein and peptide drug delivery. *Bioconj Chem* 1995; 6:332-346.
4. Sinha VR, Kohosla L. Bioabsorbable polymers for implantable therapeutic systems. *Drug Dev In Pharm* 1998; 24: 1129-1138.
5. Athanasiou KA, Niederauer GG, Agrawal CM. Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. *Biomaterials* 1996; 17: 93-102.

6. Holland S J, Tighe BJ, Gould PL. Polymers for biodegradable medical devices. 1. The potential of polyesters as controlled macromolecular release systems. *J Control Rel* 1986; 4: 155–180.
7. Langer R, Moses M. Biocompatible controlled release polymers for delivery of polypeptides and growth factors. *J Cell Biochem* 1991; 45: 340–345.
8. Rafler G, Jobmann M. Controlled release systems of biodegradable polymers. *Pharm. Ind* 1994; 56: 565–570.
9. Gopferich A. Mechanism of polymer degradation and erosion. *Biomaterials* 1996; 17: 103–114.
10. Gopferich A. 1997. Erosion of composite polymer matrices. *Biomaterials* 1997; 18: 397–403.
11. Göpferich A. Polymer degradation and erosion: mechanisms and applications, *Eur J Pharm Biopharm* 1996; 42: 1–11.
12. Thies C. A survey of microencapsulation processes In: S. Benita (Ed.), *Microencapsulation* Marcel Dekker New York 1996. p. 1–21.
13. Sansdrap P, Moes AJ. Influence of manufacturing parameters on the size characteristics and the release profiles of nifedipine from poly (dl-lactide-co-glycolide) microspheres. *Int J Pharm* 1993; 98: 157–164.
14. Jeyanthi R, Mehta RC, Thanoo BC, Deluca PP. Effect of processing parameters on the properties of peptide-containing PLGA microspheres. *J Microencapsul* 1997; 14: 163–174.
15. Mao S, Xu J, Cai C, Germershaus O, Schaper A, Kissel T. Effect of w/o/w process parameters on morphology and burst release of fitc-dextran loaded PLGA microspheres. *Int J Pharm* 2007; 334: 137–148.
16. Hamishehkar H, Emami J, Najafabadi AR, Gilani K, Minaiyan M, Mahdavi H. The effect of formulation variables on the characteristics of insulin-loaded (polylactide-co-glycolic acid) microspheres prepared by a single phase oil in oil solvent evaporation method. *Coll Surf B: Biointerf* 2009; 74: 340–349.
17. Berkland C, King M, Cox A, Kim KK, Pack DW. Precise control of PLG microsphere size provides enhanced control of drug release rate. *J Contr Rel* 2002; 82, 137–147.
18. Freiberg S, Zhu XX. Polymer microspheres for controlled drug release. *Int J Pharm* 2004; 282, 1–18.
19. Freitas S, Merkle HP, Gander B. Microencapsulation by solvent extraction/ evaporation reviewing the state of the art of microsphere preparation process technology. *J Control Release* 2005; 102: 313–332.
20. Passerini N, Craig DQ. An investigation into the effects of residual water on the glass transition temperature of polylactide microspheres using modulated temperature DSC, *J Control Release* 2001; 73: 111 –115.
21. Verrijck R, Smolders IJH, Bosnie N, Begg AC. Reduction of systemic exposure and toxicity of cisplatin by encapsulation in poly (lactide-co-glycolide). *Cancer Res* 1992; 52: 6653–6656.
22. Boisdron-Celle M, Menei P, Benoit JP. Preparation and characterization of 5-fluorouracil-loaded microparticles as a biodegradable anticancer drug carrier. *J Pharm Pharmacol* 1995; 47: 108–114.
23. Lalla JK, Sapna K. Biodegradable microspheres of poly (dl-lactic acid) containing piroxicam as a model drug for controlled release via the parenteral route. *J Microencap* 1993; 10: 449–460.
24. Chung TW, Huang YY, Liu YZ. Effects of the rate of solvent evaporation on the characteristics of drug loaded PLLA and PDLLA microspheres. *Int. J. Pharm* 2001; 212: 161–169.
25. Yolles S, Leafe TD, Woodland JHR, Meyer FJ. Long acting delivery systems for narcotic antagonists. 2. Release rates of naltrexone from poly (lactic acid) composites. *J Pharm Sci* 1975; 64:348–349.
26. Mason N, Thies C, Cicero TJ. In vivo and in vitro evaluation of microencapsulated narcotic antagonists. *J Pharm Sci* 1976; 65:847–850.
27. Deng LiX, Yuan X, Xiong M, Huang C, Zhang Z, Jia Y, W. Investigation on process parameters involved in preparation of poly-dl-lactide-poly(ethylene glycol) microspheres containing *Leptospira Interrogans* antigens. *Int J Pharm* 1999; 178:245–255.
28. Cowsar DR, Tice TR, Gilley RM, English JP. Poly (lactide-co-glycolide) microspheres for controlled release of steroids. *Methods Enzymol.*1985; 112: 101–116.
29. Bums PJ, Steiner JV, Sertich PL, Pozor MA, Tice TR, Mason DW, Love DF. Evaluation of biodegradable microspheres for the controlled release of progesterone and estradiol in an ovulation control program for cycling mares. *J. Eq. Vet. Sci.*1993; 13: 521–524.
30. Aso Y, Yoshioka S, Li Wan Po A, Terao T. Effect of temperature on mechanisms of drug release and matrix degradation of poly(-lactide) microspheres. *J Cont. Rel* 1994. 31:33–39.
31. Mana Z, Pellequer Y, Lamprecht A. Oil-in-oil microencapsulation technique with an external perfluorohexane phase. *Int J Pharm* 2007; 338: 231–237.
32. Meinel L, Illi OE, Zapf J, Malfanti M, Merkle HP, Gander B. Stabilizing insulin next-like growth factor-I in poly (d,l-lactide-co-glycolide) microspheres. *J Contr Rel.* 2001; 70:193–202.
33. Singh M, Shirley B, Bajwa K, Samara E, Hora M, O'Hagan D. Controlled release of recombinant insulin-like growth factor from a novel formulation of polylactide-co-glycolide microparticles. *J Contr Rel* 2001; 70: 21–28.
34. Furtado S, Abramson D., Simhkay L, Wobbekind D, Mathiowitz E. Subcutaneous delivery of insulin loaded poly(fumaric-co-sebacic anhydride) microspheres to type 1 diabetic rats. *Eur J Pharm Biopharm* 2006; 63: 229–236.
35. Viswanathan NB, Thomas PA, Pandit JK, Kulkarni MG, Mashelkar RA. Preparation of non-porous microspheres with high entrapment efficiency of proteins by a (water-in-oil)-in-oil emulsion technique. *J Contr Rel* 1999; 58:9–20.
36. Li X, Zhang Y, Yan R, Jia W, Yuan M, Deng X, Huang Z. Influence of process parameters on the protein stability encapsulated in poly-dl-lactidepoly(ethylene glycol) microspheres. *J Contr Rel* 2000; 68: 41–52.
37. Diwan M, Park TG. Pegylation enhances protein stability during encapsulation in PLGA microspheres. *J Contr Rel* 2001; 73: 233–244.
38. Mana Z, Pellequer Y, Lamprecht A. Oil-in-oil microencapsulation technique with an external perfluorohexane phase. *Int J Pharm* 2007; 338: 231–237.
39. Luan X, Skupin M, Siepmann J, Bodmeier R. Key parameters affecting the initial release (burst) and encapsulation efficiency of peptide containing poly(lactide-co-glycolide) microparticles. *Int J Pharm* 2006; 324: 168–175.
40. Reithmeier H, Herrmann J, Göpferich A. Lipid microparticles as a parenteral controlled release device for peptide. *J Contr Rel* 2001; 73: 339–350.

41. Little SR, Lynn DM, Puram SV, Langer R. Formulation and characterization of poly (beta amino ester) microparticles for genetic vaccine delivery. *J Contr Rel* 2005; 107: 449–462.
42. Azevedo AF, Galhardas J, Cunha A, Cruz P, Goncalves LMD, Almeida AJ. Micro encapsulation of *Streptococcus equi* antigens in biodegradable microspheres and preliminary immunisation studies. *Eur J Pharm Biopharm* 2006; 64: 131–137.
43. Feng L, Qi XR, Zhou XJ, Maitani Y, Cong Wang S, Jiang Y, Nagai T. Pharmaceutical and immunological evaluation of a single-dose hepatitis B vaccine using PLGA microspheres. *J Contr Rel* 2006; 112: 35–42.
44. Ogawa Y, Yamamoto M, Okada H, Yashiki T, Shimamoto T. A new technique to efficiently entrap leuprolide acetate into microcapsules of polylactic acid or copoly (lactic/glycolic) acid. *Chem Pharm Bull* 1988; 36:1095-1103.
45. Leo E, Pecquet S, Rojas J, Couvreur P, Fatal E. Changing the pH of the external aqueous phase may modulate protein entrapment and delivery from poly (lactide-co-glycolide) microspheres prepared by a w/o/w solvent evaporation method. *J microencapsul* 1998; 15: 421-430.
46. Iwata M, McGinity JW. Preparation of multiphase microsphere of poly (D, L-Lactic acid) poly (D, L-lactic-coglycolic acid) containing a W/O emulsion by a multiple emulsion solvent evaporation technique. *J Microencapsul*. 1991; 9: 201-214.
47. Vishwanathan NB, Thomas PA, Pandit JK, Kulkarni MG, Mashelkar RA. Preparation of non-porous microspheres with high entrapment efficiency of proteins by a (water-in-oil) emulsion technique. *J Control Release* 1999; 58: 9-20.
48. Smith A, Hunney Ball LM. Evaluation of poly (lactic acid) as a biodegradable drug delivery system for parenteral administration. *Int J Pharm* 1986; 30: 215-220
49. Anderson JM, Shive MS. Biodegradation and biocompatibility of PLA & PGLA microsphere. *Adv Drug Deliv Rev* 1997; 28: 5-24.
50. Basu SK, Adhiyaman R. Preparation and characterization of nitrendipine-loaded Eudragit RL 100 microspheres by an emulsion-solvent evaporation method. *Trop J Pharm Res* 2008; 7: 1033–1041.
51. Dash V, Mishra SK, Singh M, Goyal AK, Rath G. Release kinetic studies of aspirin microcapsules from ethyl cellulose, cellulose acetate phthalate and their mixtures by emulsion solvent evaporation method. *Sci Pharm* 2010; 78: 93–101.
52. Sahoo SK, Mallick AA, Barik BB, Senapati PC. Preparation and in vitro evaluation of ethyl cellulose microspheres containing stavudine by the double emulsion method. *Pharmazie*, 2007; 62: 117–121.
53. Maji R, Ray S, Das B, Nayak AK. Ethyl cellulose microparticles containing metformin HCl by emulsification solvent evaporation technique: Effect of formulation variables. *ISRN Polym Sci* 2012: Article ID 801827.
54. Choudhury PK, Kar M. Controlled release metformin hydrochloride microspheres of ethyl cellulose prepared by different methods and study on the polymer affected parameters. *J Microencapsul* 2009; 26: 46-53.
55. Obedeidat WM, Price JC. Preparation and in vitro evaluation of polythiouracil microspheres made of ERL 100 and cellulose acetate butyrate polymers using the emulsion-solvent evaporation method. *J Microencapsulation* 2005; 3: 281-289.
56. Soppimath KS. Cellulose acetate microspheres prepared by o/w emulsification and solvent evaporation method. *J Microencapsul* 2001; 18: 811-817.
57. Kar M, Choudhury PK. Formulation and evaluation of ethyl cellulose microspheres prepared by the multiple emulsion technique. *Pharmazie* 2007; 62: 122–125.
58. Fu X, Ping Q, Gao Y. Effects of formulation factors on encapsulation efficiency & release behaviour in vitro of huperzine A-PLGA microspheres. *J Microencapsul* 2005; 22: 57-66.
59. Uchida T, Yoshida K, Ninomiya A, Goto S. Optimization of preparative conditions for polylactide (PLA) microspheres containing ovalbumin. *Chem Pharm bull* (Tokyo) 1995; 43: 1569-1573.
60. Freytag T, Dashevsky A, Tillman L, Hardee GE, Bodmeier R. Improvement of the encapsulation efficiency of oligonucleotide-containing biodegradable microspheres. *J Contr Rel* 2000; 69: 197–207.
61. Bahl Y, Sah H. Dynamic changes in size distribution of emulsion droplets during ethyl acetate-based microencapsulation process. *AAPS Pharm Sci Technol* 2000; 1.
62. Sah H. Microencapsulation techniques using ethyl acetate as a dispersed solvent: effects of its extraction rate on the characteristics of PLGA microspheres. *J Contr Rel* 1997; 47: 233–245.
63. Sah H. Ethyl formate—alternative dispersed solvent useful in preparing PLGA microspheres. *Int J Pharm* 2000; 195:103–113.
64. Yang YY, Chia HH, Chung TS. Effect of preparation temperature on the characteristics and release profiles of PLGA microspheres containing protein fabricated by double-emulsion solvent extraction/evaporation method. *J Control Release* 2000; 69: 81-96.
65. Park TG, Lee HY, Nam YS. A new preparation method for protein loaded poly (D,L-lactic-co-glycolic acid) microsphere and protein release mechanism study. *J Control Release* 1998; 55: 181-191.
66. Crotto G, Park TG. Stability and release of bovine serum albumin encapsulated within PLGA microencapsulation. *J Controlled Release* 1997; 44: 123-134.
67. Schlicher E, Postma NS, Zuidema J, Talsma H, Hennink WE. Preparation and characterization of poly (d, l-lactic-coglycolic acid) microspheres containing desferrioxannine. *Int J Pharm* 1997; 153: 235-245.
68. Parikh RH, Parikh JR, Dubey RR, Soni HN, Kapadia KN. Poly (d, l-lactide-co-glycolide) microspheres containing 5- flurouracil optimization of process parameters. *AAPS Pharm Sci Tech* 2003; 4: 14-21.
69. Smith A, Hunney Ball LM. Evaluation of poly (lactic acid) as a biodegradable drug delivery system for parenteral administration. *Int J Pharm* 1986; 30: 215-220.
70. Yang YY, Chung TS, Ng NP. Morphology drug distribution and in vitro release profile of biodegradable polymeric microsphere containing protein fabricated by double emulsion solvent extraction/evaporation method. *Biomaterials* 2001; 22: 221-241.
71. O'Donnell PB, Iwata M, McGinity JW. Properties of multiphase microspheres of poly (d,l-lactic-co-glycolic acid) prepared by a potentiometric dispersion technique. *J Microencapsulation* 1995; 12: 155– 163.
72. Herrmann J, Bodmeier R. Biodegradable somatostatin acetate containing microspheres prepared by various aqueous and non-aqueous solvent evaporation methods. *Eur J Pharm Biopharm* 1998; 45, 75–82.