Int J Pharm Bio Sci 2012 Oct; 3(4): (P) 573 - 590

Review Article **Pharmaceutics**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

DEVELOPMENT OF SUSTAINED RELEASE MICRO/NANO PARTICLES USING DIFFERENT SOLVENT EMULSIFICATION TECHNIQUES: A REVIEW

J.B. NAIK*, A.B. LOKHANDE, S. MISHRA AND R.D. KULKARNI

Department of Pharmaceutical Technology, School of Chemical Technology, North Maharashtra University, Jalgaon- 425 001, Maharashtra, India.

ABSTRACT

Solvent extraction/evaporation, solvent diffusion/evaporation or any modification in the basic principle of the emulsification technique produces the drug loaded sustained release micro or nanoparticles of desired properties. For understanding the basic principles of these techniques, it is essential for the development of desired objectives. Emulsification is the only step where we can disrupt the size of droplets by applying external energies like high pressure, high speed, and sonication individually or alternatively. Solidification of the drop can be controlled by rate of evaporation, extraction or diffusion. Physical as well as chemical factors can affect final results of size, encapsulation efficiency and drug release pattern. These techniques are simple at lab level, economic, robust, and well controlled methods which also give preliminary idea about the controlled factors and parameters required for the production at large scale.

KEYWORDS: **Emulsification, external energies, multiple-emulsion, factors affecting, application.**

J.B. NAIK
Department of Pharmaceutical Technology, School of Chemical Technology, North Maharashtra University, Jalgaon- 425 001, Maharashtra, India.

INTRODUCTION

The three important parameters on which the selection of the most suitable drug delivery system is based are the drug, the disease state, and the latter's location in the body. Now days, small drug molecules continue to dominate the pharmaceutical market because it enjoys the advantages of small molecular size, solubility, and permeability, which are favorable for passive membrane diffusion. Researchers have succeed in part in controlling the drug absorption process to sustain adequate, effective plasma drug levels and better cellular uptake or drug targeting to specific tissues for those drugs with poor bioavailability over a prolonged period of time by designing delayed or controlled release micro and/or nanoparticulate delivery systems intended for either oral or parenteral administration [1]. The ultimate objective of microparticulate delivery system (size from 1 to 1000 μm) is to extend and control the release of the active molecule from the coated particle without attempting to modify the normal biofate of the active molecules in the body after administration and absorption [2]. On the other hand, nanoparticulate delivery systems (size from 10-100 nm) are usually intended for oral**,** parenteral**,** ocular**,** and topical use, with the ultimate objective being the alteration of pharmacokinetic profile of the active molecule[3]. Numbers of techniques are widely used in development and production of micro and/or nanoparticulate drug delivery system like emulsion polymerization, interfacial polymerization, interfacial polycondensation, polymerization of monomer and from linear polymers, solvent displacement and interfacial deposition, salting out with synthetic polymers, from natural macromolecules and spray drying [(4-6)]. The comparative studies of all these methods reveal that, emulsification solvent evaporation/extraction/diffusion is simple beaker-stirrer lab scale, economic, robust, and well controlled method which also gives preliminary idea about the controlled factors and parameters required for the production in large scale. The solvent evaporation technique is fully developed at the end of 1970s [7]. This classic technique convoluted by Bodmeier and Mc Ginity [8], Ogawa et al. [9] , Jeffery et al. [10], Iwata and Mc Ginity [11] and different recent variations are commonly used for encapsulation of various substances from simple pharmaceutical products to protein and DNA. In this review we mainly focused on the preparative steps of single, double, and multiple emulsification techniques, mechanics of mass transfer during solidification, factors influencing on the properties of particles, of some referred compounds.

EMULSIFICATION SOLVENT EVAPORATION /EXTRACTION /DIFFUSION METHOD

This technique is based on the evaporation of internal phase of an emulsion by agitation, therefore neither requires elevated temperatures nor phase separation inducing agents. Basically following main steps are involved in this method for the preparation of drug loaded micro and/or nanoparticles. All these steps are also summaries in Fig.1.

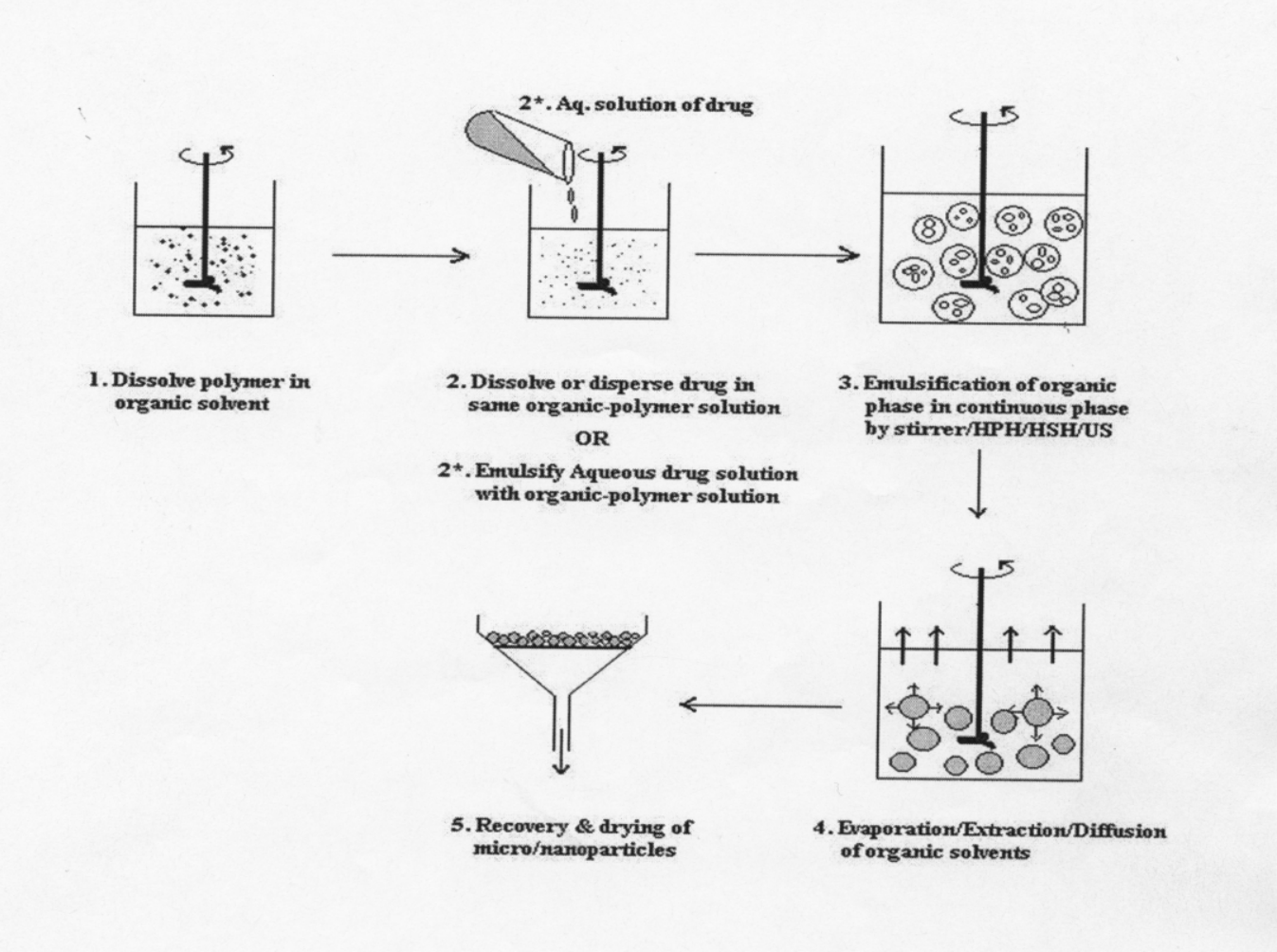

Figure 1.
Schematic diagram of solvent emulsification technique for the preparation of drug loaded micro/nano particles

(i) Dissolution of biodegradable polymers in organic solvent.

Select the appropriate polymer depending on molecular weight, copolymer blend ratio, and the degradation or erosion rate to achieve desired results. Dissolve the selected polymer with maintaining constant volume of organic solvent. The viscosity of the solution may change due to amount and properties of the polymer. The common biodegradable polymers used in drug delivery include (i) polyesters, such as lactide and glycolide copolymers, polycaprolactones [12], poly(β-hydroxybutarates), (ii) polyamides, which include natural polymers such as collagen, gelatin, albumin, and semisynthetic pseudo-poly(amino acids) such as poly(N-palmitoyl hydroxyproline ester), (iii) polyurethanes, (iv) polyphosphazenes, (v) polyorthoesters, (vi) polyanhydrides, and (vii) poly(alkyl cyanoacrylates) [13,14].

(ii) Dissolution or dispersion of drug in organic-polymers solution.

The drug to be encapsulated may be added to the above polymer solution by either codissolution in a common solvent, dispersion of finely pulverized solid material or emulsification of an aqueous solution of the drug immiscible with the above polymer

solution. The solvation of medicinal drug in organic solution of polymers can be achieved by addition of hydrophilic cosolvent [15]. The advantage of solubilizing the active drug is related to the flexibility of producing particles of extremely small sizes whose internal structure are more homogeneous irrespective of the initial drug particle size. Dispersion of the solid or dissolved drug in the polymer containing solution may be achieved by ultrasonication**,** impeller or static mixing, high speed rotor-stator mixing, or microfluidization**.** This step may primary emulsion formation step for the formation of nanoparticles by double/multiple emulsion technique. Therefore nanodroplets can be created by applying external energy like sonication or high pressure homogenization at this step [16].

(iii) Emulsification of organic phase in second continuous phase.

In this step, the organic phase emulsified under agitation in a continuous phase consisting of nonsolvent of the polymer, which is immiscible with organic solvent, which contain an appropriate emulsifier like poly (vinyl alcohol), polysorbate 80, poloxomer 188, Tween 80, Span 80. Emulsification is droplet formation step which determines the size and size distribution of the resulting microsphere. Therefore this step may affect drug encapsulation efficiency, rate of drug release, percentage yield. Stirring is basic method to generate droplet of the drug/polymer dispersion in the continuous extraction phase for subsequent solvent removal. In the simplest approach, extraction phase is filled in to a vessel and agitated by an impeller. The drug/matrix dispersion is then added, dropwise or all at once, under agitation at a speed sufficient to reach the desired droplet size. Currently different advanced methods are used for droplet formation in microsphere production but all that are not simple like stirring. The basic difference between the micropaticulate and nanoparticulate system is of size which is to achieve at this step only. For the production of nanoparticle the prepared emulsion is broken down into nanodroplets by applying external energy like high speed homogenization [17], high pressure homogenization [18], sonication[19] and these nanodroplet forms nanoparticle upon solvent evaporation/extraction. The aim of emulsification through these external energies is frequently to obtain droplets that are as small as possible (d < 1µm). Before introducing the mixture for nanoemulsification step it may be pre-emulsified through high shear or sonication for few seconds or minutes. This resulted coarse pre-emulsion then immediately passed through HPH or sonicator.

(a) High pressure homogenization

In order to produce emulsions, the dispersed phase must be distributed in finely divided form throughout the continuous phase. High-pressure homogenisers are particularly suitable for the production of finely dispersed emulsions [20]. An important as well as critical step in emulsification is droplet disruption. It is not possible to specify a single overall disruption mechanism for a certain type of device without taking the device parameter (eg. valve geometry), operating parameter (eg. volume flow rate, temperature), product parameters (viscosity of the two phases, interfacial tension) into consideration. In case of laminar flow drop can deformed either by shearing or elongation while in turbulent flow both shear forces and inertial forces come into action. Accordingly, a lot of controversies exist in the literature as regard the exact cause of the droplet disruption in the HPH. Different disruption mechanisms are generally emphasized. Thus in most cases, with the standard valve geometry, turbulence is said to be the predominant mechanism. Depending on the mode of operation, laminar shear and cavitation could also be effective disruption mechanism [21]. Basically a high-pressure homogeniser consists of a high-pressure generator and a homogenising valve assembly designed for this specific high-pressure application. The processed solution in any type of homogenizer valve passes under high

pressure and low velocity through a convergent section called “homogenizing gap” and then expand to generate nanoscaled nano-emulsion droplets [22]. During product enters the adjustable, close clearance area between the valve and seat, there is rapid increase in velocity with a corresponding decrease in pressure. The intense energy release causes turbulence and localized pressure difference, which will tear apart the particles. In cavitation theory the liquid encounters intense cavitation because of the large pressure drop through the valve. When the pressure drop is large enough, the vapor pressure of the liquid exceeds the ambient pressure causing formation of vapor bubbles (cavities in the liquid). When the cavitation bubbles implode (collapse of the cavities), shock waves are generated in the liquid. These shock waves break apart the dispersed droplets. The second homogenization theory of turbulence, suggests that the energy dissipating in the liquid generates intense turbulent eddies. Droplets passing through the turbulent jet at the discharge from the gap are immediately disrupted by intense turbulent flow [23]. If a narrow particle size distribution required, it may be necessary to homogenized the product more than once. This can be done by two or more homogenizers in series, which ensures discrete passes, or by re-circulating the product through a single unit. But all physical processes, i.e. pressure drop, fluid shear, turbulence, impact or cavitation, could play a role during disruption [24]. Thus high pressure homogenizer appears to be an efffective instrument to produce micro- and nanoparticles with a narrow size distribution and high encapsulation efficiency by controlling the pressure on regulator.

(b) Ultrasonication

Nano-emulsions generated by sonifiers are generally attributed to a mechanism of cavitation [25]. The ultrasound waves in liquid macroscopic dispersion, result in a sequence of mechanical depressions and compressions, generating cavitation bubbles, which tend permanent to implode**.** Consequently, this shock provides sufficient energy locally to increase surface area equivalent to nanometric-scaled droplets. Efficiency of nanoemulsification by sonication depends both on the composition of the emulsion and the power device (amplitude). Ultrasonic emulsification was described as two step process [26]. First instable interfacial waves form at the oil-water interface, which results in the eruption of rather large oil droplets into the water phase. Second the shock waves of cavitation events in the close vicinity of the course oil droplets will cause their disruption into much finer droplets.

(c) High speed homogenization

High speed homogenizer produces smaller emulsion droplets through stronger shear forces and increased turbulence. The shear forces acting upon the drug/matrix dispersion droplets and thus minimizing their size [27]. This homogenizer assembly makes up the rotor portion of the rotor-stator generator probe. The tube and collar assembly attached to the motor housing, but dies not spin. This is the stator portion of the rotor-stator generator probe. As the rotor knife spins within the tube and collar assembly, it creates a pumping action, pulling the sample into the open end of the generator probe and forcing the sample out through the windows in the tube. The interaction of the rotor knife with these windows sets up a shearing action, reducing the particle size of sample. The speed differential between the rapidly moving portion and the relatively stationary of the sample sets up a second force called cavitation pulls the sample apart, further reducing the particle size.

(iv) Evaporation/ extraction/ diffusion of organic solvents

To harden the micro and/or nanoemulsion droplet into solid particles, the organic solvent is evaporated or extracted from the system after it diffuses into the continuous phase by maintaining agitation. In the emulsification-evaporation method, the organic solvent of

dispersed phase of emulsion is eliminated in two stages- diffusion of solvent in the continuous phase (solvent extraction) and elimination of the solvent at the continuous phase-air interface (solvent evaporation) [28]. During the solvent evaporation process, there is the gradual decrease of the dispersion in the aqueous phase. Therefore the local concentration of the oil droplets in the aqueous phase is decreased and the diffusion rate is higher, thus resulting in smaller particle [29]. Elevated temperature or reduced pressure and stirring speed will facilitate the evaporation of the solvent from the continuous phase and thereby maintain a high concentration gradient for the solvent between the microspheres and the continuous phase**.** In solvent evaporation, the capacity of continuous phase is insufficient to dissolve the entire volume of the dispersed phase solvent. While in solvent extraction the amount and composition of the continuous phase are chosen so that the entire volume of the disperse phase solvent can be dissolved. Continuous phase will immediately extract the solvent(s) of dispersed phase so the evaporation stage will no longer necessary in the formation of microsphere. This can be achieved by large volume of continuous phase with respect to dispersed phase [30] or by choosing a dispersed phase consisting of cosolvents, of which at least one has a great affinity for the continuous phase or formulate a continuous phase with two solvents in which one acts as a solvent extractor of the dispersed phase [31]. In solvent extraction, first, the drug/polymer dispersion is mixed with a small amount of continuous phase to yield an emulsion of desired droplet size. Then a further continuous phase and/or additional extraction agents are added in sufficient to absorb the entire solvent leaching from the solidifying microsphere. Since the solvent extraction is normally faster than the evaporation rate (the latter depends on the boiling point of the solvent), the resultant porosity of nanoparticle matrix prepared by solvent extraction method is usually greater than nanoparticles prepared by using evaporation method [32]. Microspheres prepared by solvent extraction are more regular in shape, smaller, with a narrower size distribution and greater porosity. A combination of solvent evaporation and extraction is suggested to improve the economic efficiency of the microencapsulation process [33]. After emulsion formation, a sufficient quantity of an extraction fluid is added to induce the skin formation on the microsphere's periphery while the remaining solvent is removed by evaporation. The brief skin forming extraction step prior to evaporation minimizes the loss of drug during following evaporation step, while the volume of extraction fluid consumed is reduced as compared to an extraction process alone. The two steps of solvent extraction and evaporation may be combined by using a mixed solvent system [34].

All organic solvents may evaporate during extraction or diffusion with respect to speed of agitation or homogenization. But removal of total organic solvent through only evaporation may crystallize or leakage or deteriorate the active principle due to more time in contact with continuous phase. Therefore there was modification with or without evaporation by using organic solvents which are miscible with continuous aqueous phase to remove solvent fast eg. ethyl acetate, acetone, alcohol. Here not only miscibility but also volume of continuous phase plays important role. If the to be extracted solvent removed not by increasing external phase volume (extraction) but by continuous agitation still formation of microsphere then this method of preparation will called solvent evaporation indeed it having water miscible solvent. In case of diffusion during solidification the polymer deposition are conducted almost instantaneously and spontaneously and the uniform nanoparticle dispersion can always be attained even by mild agitation. Emulsion solvent diffusion method is relying on the concept of "solvent extraction" [35]. Same organic solvents may use for extraction as well as diffusion principle [36]. In solvent diffusion method, the oil phase consists of water-miscible organic solvents such as methanol or

acetone together with water-immiscible chlorinated organic solvents. During the formation of an o/w emulsion, acetone/methanol rapidly diffuses into the outer water phase and causes an interfacial turbulence between the two phases, thus resulting in the formation of smaller particles[37].

(v) Recovery and drying

Recovery or separation of the formulated micro or nanoparticles after evaporation of organic solvent was done by centrifugation or simple filtration using Whatmann filter paper or ultrafiltration. The residue is washed with 4-5 times in petroleum ether or n-hexane and dried at room temperature for 24 hrs [38, 39]. Rinsing may involve elevated temperatures or use of extraction agents to reduce the amount of residual solvent in the microsphere [40]. Finally the nano/microspheres are dried either at ambient condition or under reduced pressure, heat or by lyophilization to yield a free flowing powder.

SINGLE EMULSION AND MULTIPLE EMULSION TECHNIQUES

O/W:

This technique is generally preferred when water as nonsolvent to the polymer [41]. Particularly those drugs which are hydrophobic in nature and soluble in water immiscible organic solvents can be encapsulated by the oil-in water (o/w) emulsion solvent evaporation technique. By this method polymer is dissolved in an organic solvent, active drug dissolve or dispersed in the same medium, and then the resulting oil organic phase is emulsified in an aqueous solution containing an appropriate emulsifier. This continuous phase should have a low dissolving power for the active principle. The volatile solvent removed from such emulsion either by evaporation or by extraction to continuous phase or by combination of both the methods.

O/O

This technique is non-aqueous emulsion technique. Obviously the dispersed phase which is an oily in nature must be totally immiscible with continuous phase. Some methods expected to low encapsulation efficiency due to fluctuation of the active principle from the dispersed phase to continuous phase. To overcome this problem, O/O emulsion method can be used. This includes extraction of O_1- phase solvent, e.g. Acetonitrile or mixture of organic solvents, by solution of emulsifier in oil e.g. mineral oil or cotton seed oil or triglyceride which should be non solvent for both the polymer and drug[42]. If drug is not totally soluble in first organic phase then it suspended in form of extremely small (i.e. submicron) particle size to assure minimization of initial release. This may called Solid-in-oil-in-oil (S/O/O) which can almost protect proteins from forming aggregates, but burst release is severe problem [43]. Also an $O_1/O_2/O_3$ technique has been exemplified that may be applicable for certain hydrophobic drugs, which are soluble in flurosilicone oil (O_1-phase) but not in PLGA solvent like acetone (O_2- phase). The oil will removed from the particle by washing with hexane or petroleum ether [44].

W/O/W

The W/O/W emulsion technique is the most popular method, as developed by Ogawa et al [9]. The typical W/O/W double emulsion process consist of four steps [45]: (1) Primary emulsification: An aqueous solution of active agent (internal water phase, W_1) is emulsified into an organic solution containing the biodegradable polymer (oil phase, O); (2) re-emulsification: the primary emulsion (W /O) is further emulsified into a second aqueous phase containing a stabilizer (external water phase, W_2) to form a W/O/W double emulsion; (3) solidification: the organic solvent is removed by evaporation or extraction and then solid microparticles are formed; and (4) separation and purification: microparticles are collected by centrifugation or filtration and subsequently lyophilized. The organic phase acts as a barrier between the two aqueous compartments preventing the diffusion of the active principle toward the aqueous external phase. A W/O/W double emulsion is a

thermodynamically unstable system, thus a shorter procedure time in this stage, that is, a rapid solidification of the double emulsion droplets, will undoubtedly favor higher microentrapment efficiency. Currently this methodology is one of the most commonly used for protein and peptide encapsulation.

W/O/O

To enhance the encapsulation efficiency of hydrophilic drugs one more method of double emulsion is used [46]. In this method, drug and polymer is dissolved in organic solvent or mixture of organic solvents followed by addition of aqueous phase containing surfactant. The drug could also be dissolved or dispersed in internal water phase and then emulsified in the polymer phase. The initial W/O emulsion will be prepared by stirring or sonicating the mixture for specific time. This primary emulsion slowly added to second oil phase containing surfactant with continuous stirring for particular period of time to form W/O/O double emulsion. After removal of organic solvent by evaporation/extraction recover solid particles and dry it.

W/O/W/O [47]

Aqueous solution of active principle emulsified with organic solvent like DCM containing polymer to provide primary emulsion W/O. The resulting emulsion emulsified for specific period of time with aqueous phase containing surfactant to form W/O/W double emulsion. The resulting W/O/W emulsion slowly added to organic solvent like methanol as oil phase containing surfactant to present W/O/W/O multiple emulsion. This emulsion stirred at low speed under ambient conditions. The obtained small size solid particles are separate and dry after evaporation/extraction of the organic solvents.

S/O/O/W

W.Yuan et al suggest a novel method of multi-emulsion solid-in-oil phase1-in-oil phase2-in water (S/O/O/W) to prepare microsphere [48], in which solid phase is protein loaded microparticles of size is about 1-5 μm which prepared by low temperature induced phase separation. These microparticles were suspended in PLGA dichloromethane solution forming oil phase 1. After vigorous stirring for 1 min, the suspension was emulsified into hydrophilic oil phase 2 containing surfactant and stir again to form PLGA microsphere. This hydrophilic oil phase dissolved in water, so the oil phase is easily removed. This emulsion containing microsphere was immediately transfer into aqueous phase W containing sodium chloride to harden the microsphere. The PLGA microspheres were ageing in the water at gentle stirring to extract the organic solvent, and then system was warmed up to room temperature. The harden microsphere were rinsed with pure water for three times and subjected to lyophilization again prior to storage. The present method may decrease protein aggregation and improve protein complete release. Schematic representation of all methods shown in Fig.2**.** Selection of emulsification technique is depending on the physicochemical properties of the active principle.

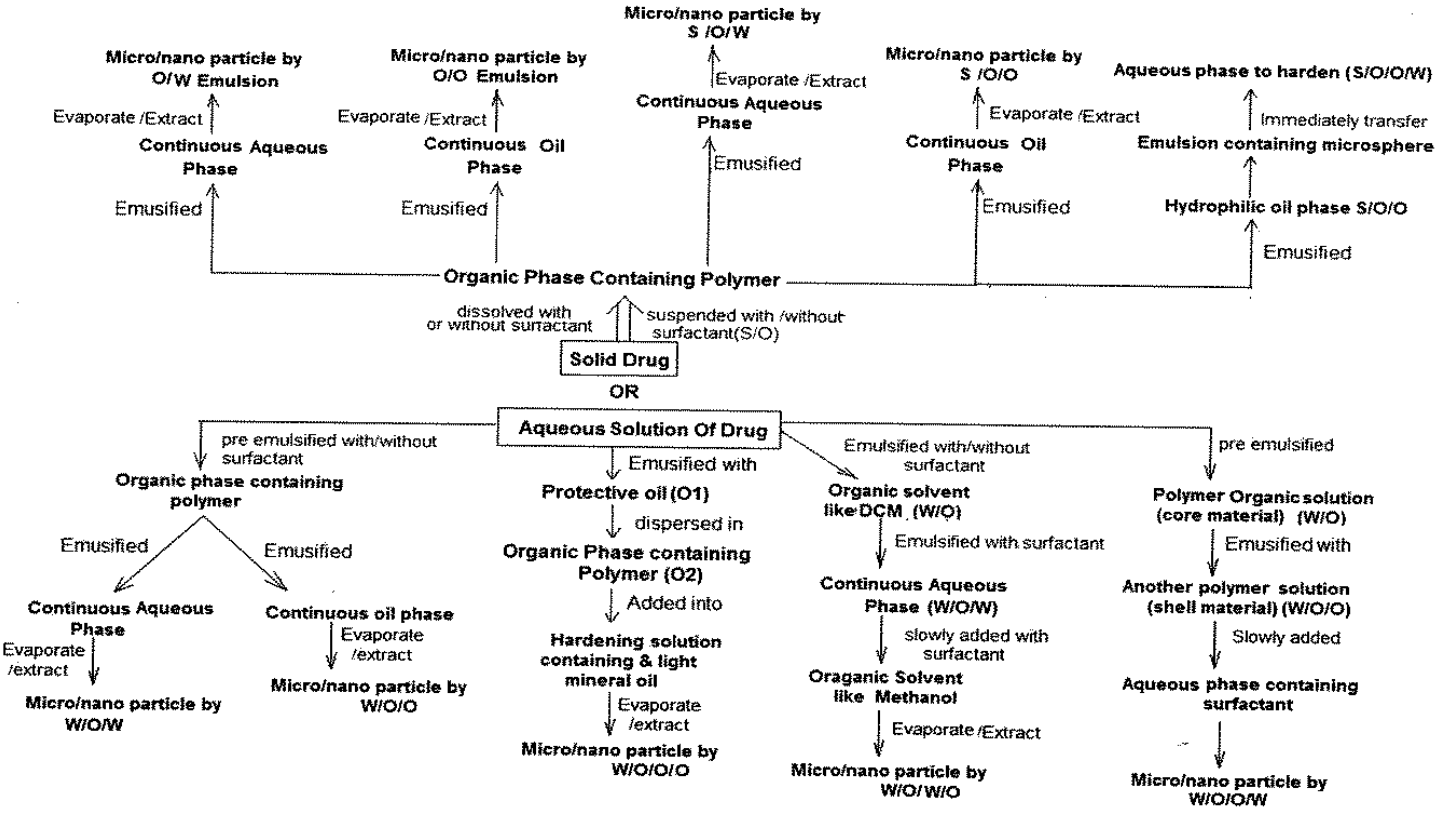

Figure 2.
Flow chart for the preparation of micro/nanoparticles using different solvent emulsification techniques

FACTORS AFFECTING

Various factors affect to yield micro and/or nanoparticles of desired physicochemical characteristics, drug entrapment efficiency, and drug release rate properties.

A) Chemical Factors:

(i) Solvents

When preparing microspheres or nanospheres containing a pharmaceutical agent, the choice of organic solvent is critical in developing a successful formulation. Apart from its ability to dissolve the polymer and its miscibility in the external phase, its toxicity should also be taken into account, since there will always be trace amounts of residual solvent in the final microsphere formulation. The organic solvents with high interfacial tension in the water phase are used for the preparation of polymer particles. Fuminori Ito et al studied the effect of types and mixture of solvents on the characteristics of refampicin loaded PLGA microsphere [49]. The drug loading efficiency and particle size of the microspheres prepared by using different solvents like acetone (0) <ethyl acetate (6.78) <dichloromethane (20.4) <chloroform (31.4) was increasing in order of the same sequence. Bracketed values define the interfacial tension between solvent and water (mN/min). The low drug loading efficiency of the microspheres was a result of further transition of the drug to the water phase because of the better miscibility of water and the oil phase. The common class 3 solvent, acetone, produces highly porous particles [50]. The effect of solvent composition was also studied on microspheres of water soluble drug

prepared by W/O/O double emulsion solvent diffusion method. From ethanol/DCM mixture, when the volume of DCM was greater than ethanol, large microspheres were formed in a non-spherical shape and if proportion of ethanol was greater, the size of microspheres decreased [46]. According to literature, the effect of co-solvents has only been studied for hydrophilic drugs in an attempt to increase their drug loading capacity. Porogen like hexane, n-heptane is used to generate the pores inside the microspheres to increase the degradation rate of polymer and improves drug release rate [51]. The relatively high solubility of ethyl acetate in water usually gives rise to a fast diffusion of EA from oil droplets into the outer aqueous phase which easily leads to polymer precipitation rather than the formation of microparticle. Indeed the fast diffusion rate of EA from oil phase into outer aqueous phase benefits rapid solidification of microparticles , which results in improved protein entrapment efficiency [52]. A study by Sani et al. reported the use of EA (a class 3 solvent) for PEG-PLGA that produced uniform small size range of nanoparticles [53].

(ii) Polymer

The biodegradability or biocompatibility is an essential property for the polymer used for pharmaceutical application. The choice of polymer used as drug carrier depends on the desired drug release rate, which is essentially determined by the polymer's physical properties. If one polymer cannot present a satisfying drug release, a single polymer, called co-polymer, can be synthesized from two different polymers. The common biodegradable polymer used for the preparation of micro and nanoparticulate DDS given in the above. Non-biodegradable polymers with good biocompatibility are also used as drug carriers, such as ethyl cellulose is degradable but not biodegradable and polymethyl methaacrylate is biocompatible but non-degradable.

It has been found that poly(lactide-co-ethylene glycol) (PELA) microspheres were slightly smaller in size more porous surface and internal structure, higher encapsulation efficiency and more rapid in vitro release rate compared with PLGA ones if prepared at identical emulsification strength due to change in hydrophobicity[54]. In case of PLA slight decrease in particle size and polydispersity index with increasing PLA molecular weight. Likewise zeta potential increases slightly when molecular weight of PLA increases which is related with carboxylate end groups of PLA [55]. Increasing the amount of PLGA resulted in the significant increase of particle size. This may be caused due to increasing the viscosity of internal phase, resulting not appropriate dispersability of the internal phase into the aqueous phase. Bigger particles are formed as a result of higher polymer concentrations due to a high viscous resistance against the shear forces during the emulsification. In microspheres made with low and high molecular weight blends of PLLA, PLGA [56] the glass transition temperature (Tg), crystillinity, and melting temperature (Tm), all changed with an increasing proportion of low molecular weight polymer in the blend. So, morphology and drug release from these microspheres may be modulated by the addition of low molecular weight polymer at different proportion. The higher concentration of polymer increases in drug–polymer phase viscosity, which could restrict the migration of the drug to the continuous phase and thus improve its entrapment [57].

(iii)Surfactant

The amount of surfactant plays an important role in the emulsification- solvent evaporation/ extraction process for the protection of droplets against coalescence. Polyvinyl alcohol (PVA), sodium dodecyl sulphate (SDS), methyl cellulose, gelatin, cetrimethyl ammonium bromide (CTAB) all give the successful preparation of micro and nanoparticles. The properties of microspheres are affected by both HLB and surfactants. At the same HLB, surfactants consist of more fatty acid chains fabricate microspheres of larger and have

higher drug contents. In contrast, surfactants with longer polyoxyethylene chain produce smaller microspheres. Within same blend of surfactants, HLB produces marked variation in sizes of the microspheres but, drug content is, however, not definite. The rate of drug release generally retard by surfactants with low HLB. Addition of hydrophilic surfactants to aqueous phase containing the drug produces larger microspheres having lower drug encapsulation efficiency [58]. Increasing the PVA concentration in the external aqueous phase ensures good emulsification process and results in both size reduction and a lower polydispersity index. During the preparation of microspheres of ovalbumin from blends of PLG and PEG by w/o/w method, decrease in microparticle size was obtained by increasing the concentration of PVP as surfactant in the continuous phase, but no significant effect on size or protein entrapment of ovalbumin by w/o/o method at same conditions [59].

The entrapped percentage of paclitaxel was lowered by using PVA, but emulsifier D-α-tocopheryl polyethylene glycol 1000 succinate (vitamin E TPGS) found significantly improved as high as 100%. This is due to shortage of PVA as emulsifier to fabricate polymeric nanosphere[60]. Higher concentration of surfactant may result in a more stable emulsion which hinders the mass transfer of insulin with surrounding leading to a more even and homogenous distribution of protein within the interior of PLGA microspheres.

(iv) Drug loading

An increase in initial loading of the drug increases nanoparticles mean diameter and polydispersity index. Greater amount of drug results in more viscous dispersed phase, making difficult the mutual dispersion of the phases and forming larger particle although encapsulation efficiency was practically same [61].

B) Physical Factors:

(i) Agitation speed, homogenization pressure and sonication time

Particle size and polydisperse index of micro and nanoparticles decreases with increase in agitation speed [11]. Other factors should also be considered with agitation like the geometry of the reactor, the number of impellers, and there position and the ratio of impellers diameter compare to reactors diameter, viscosity of disperse and continuous phases etc. Microspheres made with side baffles gives better yield, double recovery, small particle size than microspheres made without baffles. The nanoparticles prepared by a double emulsion pressure homogenization technique showed no significant change in size but decrease polydispersity index before 3 min of shear stress of the homogenization device, but after 3 min the stability of double emulsion seemed to be decreased gives uncontrolled coalescence of the droplets leading to an increase both size and polydispersity [62]. With increases in the sonication time leads to the reduction in nanoparticles mean diameter and also narrower granulometric distribution.

(ii) Ratio between external and internal phases

The ratio between external and internal phases of emulsion is of great importance to its stability and influences the size of globules. Mainardes and Evangelista [25] carried a study on praziquantel PLGA nanoparticles by oil-in-water solvent evaporation method and conclude that increase in internal phase volume leads to slight decrease of the nanoparticle's average size for a given polymer concentration. This conclusion was also similar for microparticulate DDS. This occurs because the coalescence of the droplets can be prevented by a large amount of organic solvent available for diffusion in the O/W emulsion. Besides, applying 25 ml of external aqueous phase resulted in lower mean sizes of nanoparticles among to those formulated with 50 ml of aqueous phase [27]. The encapsulation efficiency increased with decreasing amount of organic solvent. This is due to the increased viscosity of the organic/drug polymer solution at smaller amount of organic solvent, which

slow down the diffusion of the active into the external aqueous phase [63]. In case of w/o/o double emulsion the volume of internal water phase was directly proportional to the size of microspheres. For continuous phase as the volume increased the size of microsphere decreased because more volume had fewer chances for emulsifying globules to coalesce/aggregate with each other [46]. The rate of addition of organic phase to aqueous phase also affects on the drug entrapment efficiency.

(iii) Solvent evaporation rate

Effects of the rate of solvent evaporation on the characteristics of drug loaded microspheres were studied by Mainardes and Tze- Wen Chung et al [25, 64]. They reported that microspheres obtained by fast rate of solvent evaporation (reducing ambient pressure) shows smooth surface with smaller particle size and lower drug encapsulation efficiency than microspheres by normal rate of solvent evaporation.

(iv) Processing Temperature

Gradually increasing preparation temperature leads to decrease in particle size. This is due to the emulsion at high temperature is less viscous, thus it is much easier for the emulsion to be broken up into smaller droplets at the same power of mixing input. However higher temperature yield larger size of microsphere, probably due to rapid solvent evaporation [65]. In one more study of encapsulation of BSA in PLGA microspheres Yang et al revealed that low preparation temperature gives the fastest initial but the lowest overall shrinking rate, uniform internal pore distribution, and a very thin dense skin layer, while at high temperature the lowest initial yet the highest overall shrinking rate, thicker but porous skin layer and bigger pores at the middle of the sphere. In terms of in-vitro release, microsphere fabricated at low temperatures (5^0C, 15^0C, 20^0C) exhibit similar, steady rate but microspheres formed at higher temperature give very low release rates after their initial release [66].

(v) pH of processing environment

The droplet size of the primary emulsion could be influenced by the type of buffer used; this could also affect the microstructure of the microspheres. Increase in pH of external aqueous phase decreases the degree of ionization and solubility of drug which results in increasing the drug entrapment. Recovery of nanoparticles was also higher at high pH [67]. The addition of various buffers or salts to the internal aqueous or external aqueous phase affect the osmotic pressure gradient between the two aqueous phases and the organic solvent/ water flux during the microsphere formation. High encapsulation efficiencies were obtained when the salts were added to both the internal and external aqueous phases while encapsulation efficiencies were significantly lower when salt being added to only the internal aqueous phase [68].

(vi) Effect of pressure

The microspheres made by reducing pressure have an apparent smooth surface and smaller size than made at atmospheric pressure. Reduced pressure can improve the drug encapsulation efficiency in most cases. Izumikawa et al. revealed that drug encapsulation efficiency was greater for microspheres that have been prepared using solvent evaporation at a reduced pressure than for those prepared at atmospheric pressure [28]. In order to verify the influence of organic solvent evaporation rate a vacuum rotary evaporator presented nanoparticles of small diameter than the particles obtained by magnetic stirring method. The reason for the formation of smaller particle is the higher solvent front kinetic energy [25, 55].

Table 1.

Summary of drug loaded micro and nanoparticles prepared by different methods of emulsification.

Active Drug	Method of preparation	Polymer	Dispersed-Continuous Phase	Size	Application	Ref.
Paclitaxel	O/W	Methoxy PEG-poly(lactide)copolymer	Acetonitrile- Pluronic F68 Aq. Solution	89-90nm	Anticancer	69
Insulin	O/O	Poly(lactic-co-glycolic acid) (PLGA)	Acetonitrile-Mineral oil	1.04-8.47µm	In treatment of diabetes	57
Ganciclovir	S/O/O	Poly(lactic-co-glycolic acid) (PLGA)	Acetonitrile- Light mineral oil	30-110 µm	Antiviral	43
Ovalbumin	W/O/W	Poly(d,l-lactide-*co*-glycolide) PLG	OVA Aq. Solution-Dichloromethane-PVA aqueous solution	2.86-8.63 µm	Protein for immunization	70
Ciprofloxacin	W/O/W High-pressure emulsification	poly (lactic-co-glycolic acid) (PLGA)	Drug aq. Solution-DCM-PVA aq. Solution	130 nm - 353 nm	Antibacterial	71
Ovalbumin	W/O/O	Poly(d,l-lactide-*co*-glycolide) PLG	OVA Aq. Solution-Dichloromethane- Methanol	11.05-11.08µm	Protein for immunization	70
Bovin Serum Albumin	S/O/O/W	Poly (lactic-co-glycolic acid) (PLGA)	BSA-DCM-Hydrophilic oil-PVA aq. solution	40-100 µm	Protein for immunization	48
Ovalbumin	W/O/W/O	Poly(d,l-lactide-*co*-glycolide) PLG	OVA Aq. Solution-Dichloromethane-PVA Aq. solution- Methanol	1.98-2.03 µm	Protein for Immunization	70

CONCLUSION

Solvent evaporation / extraction, evaporation / diffusion, modified spontaneous emulsification diffusion are the techniques which start to grow from batch to continuous production of micro and/or nanoparticulate drug delivery systems. The processing conditions and contents of particular method mainly affects on the desired properties of system where each trial is with some error. The knowledge of solidification mechanics or mass transfer clears the basics of required kinetics of solvent removal. Emulsification is the only centered process where we can achieve the required size of droplets having high encapsulation efficiency of drug. It has been shown that those drugs which are unstable in single emulsion method can delivered with good stability and higher encapsulation efficiency by multiple emulsion techniques. Now application area of solvent emulsification is also becoming very vast. In response to these concerns, the modification in basic concepts, research and development in biodegradable polymer technology and new techniques for large scale production is essential for future studies.

ACKNOWLEDGMENT

Authors are very much thankful to Nano Mission, DST, New Delhi for providing financial assistance to carry out this precious work.

REFERENCES

1. Nordstierna L, Abdalla AA, Nordin M, Nydén M, Comparison of release behaviour from microcapsules and microspheres, Progress in Organic Coatings, 69: 49–51, (2010).
2. Hazedar S, Dortunc B, Preparation and in vitro evaluation of eudragit microspheres containing acetazolamide, Int J Pharm, 269:131-140, (2004).
3. Mahanraj VJ and chen Y, Nanoparticle-A review, Tropical Journal of Pharmaceutical Research, 5 (1): 561-573, (2006).
4. Kiminta DMO, Braithwaite G, Luckham PF. Colloidal dispersions, nanogels: In: Salamone JC, (ed), Polymer Materials Encyclopedia. CRC Press, Boca Raton, 1996, pp. 1298–1309.
5. Ganachaud F, Katz JL, Nanoparticles and nanocapsules created using the ouzo effect: Spontaneous emulsification as an alternative to ultrasonic and high-shear devices, Chem Phys Chem, 6: 209- 16, (2005).
6. Lambert G, Fattal E, Couvreur P, Nanoparticulate system for the delivery of antisense oligonucleotides, Adv Drug Deliv Rev, 47: 99 – 112, (2001).
7. Beck LR, Cowsar DR, Lewis DH, Cosgrove RJ, Riddle CT, Lowry SL, et al. A new long-acting injectable microcapsule system for the administration of progesterone, Fertil Steril, 31(5): 545-55, (1979).
8. Bodmeier R, Mc Ginity RW, The preparation and evaluation of drug-containing poly(dl-lactide) microspheres formed by the solvent evaporation method, Pharm Res, 4: 465–471, (1987).
9. 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, 36:1095– 1103, (1988).
10. Jeffery H, Davis SS, O'Hagan DT, The preparation and characterization of poly(lactide-co-glycolide) microparticles: I. Oil-in-water emulsion solvent evaporation, Int J Pharm, 77: 169– 175, (1991).

11. Iwata M, Mc Ginity JW, Preparation of multi-phase microspheres of poly(d,l-lactic acid) and poly(d,l-lactic-co-glycolicacid) containing a W/O emulsion by a multiple emulsion solvent evaporation technique, J Microencapsul, 9: 201–214, (1992).
12. Molpeceres J, Guzman M, Aberturas MR, Chacon M, Berges L, Application of central composite designs to the preparation of polycaprolactone nanoparticles by solvent displacement, J Pharm Sci, 85: 206 – 13, (1996).
13. El-Samaligy MS, Rohdewald P, Mahmoud HA, Polyalkylcyanoacrylate nanocapsules, J Pharm Pharmacol, 38: 216 -228, (1986).
14. Krause HJ, Schwarz A, Rohdewald P, Interfacial polymerization, an useful method for the preparation of polymethylcyanoacrylate nanoparticles, Drug Dev Ind Pharm, 12: 527- 52, (1986).
15. Wada R, Hyon SH, Ikada Y, Lactic acid oligomer microspheres containing hydrophilic drugs, J Pharm Sci, 10: 919-924, (1990).
16. Dillen K, Vandervoort J, den Mooter GV, Ludwig A, Evaluation of ciprofloxacin-loaded Eudragit® RS100 or RL100/PLGA nanoparticles, Int J Pharm, 314: 72–82, (2006).
17. Lemos-Senna E, Wouessidjewe D, Lesieur S, Duchene D, Preparation of amphiphilic cyclodextrin nanospheres using the emulsification solvent evaporation method. Influence of the surfactant on preparation and hydrophobic drug loading, Int J Pharm, 170:119–128, (1998).
18. Lamprecht A, Ubrich N, Hombreiro Pe´rez M, Lehr C.-M, Hoffman M., Maincent P, Biodegradable monodispersed nanoparticles prepared by pressure homogenization emulsification, Int J Pharm, 184: 97–105, (1999).
19. Chognot D, Leonard M, Six J-L, Dellacherie E, Surfactive water-soluble copolymers for the preparation of controlled surface nanoparticles by double emulsion/solvent evaporation, Colloids Surf B Biointerfaces, 51: 86–92, (2006).
20. Stang M., Schumann H, Schubert H, Emulsification in high-pressure homogenisers, Engineering in Life Science, 1 (4): 151–157, (2001).
21. Karbstein H, Schubert H, Developments in the continuous mechanical production of oil-in- water macro-emulsions, Chem Eng Process, 34: 205, (1995).
22. Middelberg APJ, Process-scale disruption of microorganisms, Biotechnology Advance, 13: 491–550, (1995).
23. APV Homogenizer Handbook. Processing of emulsions and dispersions, SPX Corporation 2009.
24. Pandolfe WD. Homogenisers. In: Franci FJ (ed), Encyclopedia of Food Science and Technology, 2nd ed. Wiley, New York, 1999, pp. 1289–1294.
25. Mainardes RM, Evangelista RC, PLGA nanoparticles containing praziquantel: effect of formulation variables on size distribution, Int J Pharm, 290:137–144, (2005).
26. Li MK, Fogler HS, Acoustic emulsification. Part I. The instability of oil–water interface to form the initial droplets, J Fluid Mech, 88: 499–511, (1978).
27. Javadzadeh Y, Ahadi F, Davaran S, Mohammadi G, Sabzevari A, Adibkia K, Preparation and physicochemical characterization of naproxen–PLGA Nanoparticles, Colloids Surf B Biointerfaces, 81: 498–502, (2010).
28. Izumikawa S, Yoshioka S, Aso Y, Takeda Y, Preparation of poly (l-lactide) microspheres of different crystalline morphology and effect of crystalline

morphology on drug release rate, J Control Release, 15: 133–140, (1991).
29. Jung T, Breitenbach A, Kissel T, Sulfobutylated poly(vinyl alcohol)-graft-poly(lactide-co- glycolide) facilitates the preparation of small negatively charged biodegradable nanospheres, J Control Release, 67:157–169, (2000).
30. Boisdron-Celle M, Menei P, Benoit JP, Preparation and characterization of 5-fluorouracil- loaded microparticles as biodegradable anticancer drug carriers, J Pharm Pharmacol, 47: 108-114, (1995).
31. Singh M, Singh A, Talwar GP, Controlled delivery of diphtheria toxoid using biodegradable poly(D,L-lactide) microcapsules, Pharm Res, 8(7): 958-961, (1991).
32. Arshady R, Preparation of biodegradable microspheres and microcapsules; part Polylactides and related polyesters, J Control Release, 17: 1–22, (1991).
33. Gibson JW, Holl RJ, Tipton AJ. Emulsion-based processes for making microparticles, U.S. Patent. 6,291,013, 2001.
34. Badri Viswanathan N, 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 Control Release, 58: 9 –20, (1999).
35. Weia XW, Gong CY, Gou M, Fu SZ, Guo QF, Shi S.et.al. Biodegradable poly(ε- caprolactone)–poly(ethylene glycol) copolymers as drug delivery system, Int J Pharm, 381: 1–18, (2009).
36. Sah H, Microencapsulation techniques using ethyl acetate as a dispersed solvent: effects of its extraction rate on the characteristics of PLGA microspheres, J Control Release, 47: 233–245, (1997).
37. Niwa T, Takeuchi H, Hino T, Preparations of biodegradable nanospheres of water soluble and insoluble drugs with d,l-lactide/glycolide copolymer by a novel spontaneous emulsification solvent diffusion method and the drug release behavior, J Control Release, 25: 89–98, (1993)
38. Sahoo SK, Mallick AA, Barik BB, Senapati PC, Formulation and in vitro Evaluation of Eudragit® Microspheres of Stavudine, Tropical Journal of Pharmaceutical Research, 4 (1) : 369-375, (2005).
39. Behera BC, Sahoo SK, Dhal S, Barik BB, Gupta BK, Characterization Of Glipizide-Loaded Polymethacrylate Microspheres Prepared By An Emulsion Solvent Evaporation Method, Tropical Journal of Pharmaceutical Research, 7 (1): 879-885, (2008).
40. Rickey ME, Ramstack JM, Lewis DH. Preparation of biodegradable, biocompatible microparticles containing a biologically active agent, U.S. Patent 6290983, 2001.
41. Fong JW. Microencapsulation by solvent evaporation and organic phase separation processes. In: Hsieh DST (ed) *Controlled Release Systems*, FL: CRC Press, Boca Raton,1988, pp. 81-108.
42. Patrick B, O'Donnell, Mcginity JW, Influence of processing on the stability and release properties of biodegradable microspheres containing thioridazine hydrochloride, Eur J Pharm Biopharm, 45: 83-94, (1998).
43. Janoria KG, Mitra AK, Effect of lactide/glycolide ratio on the in vitro release of ganciclovir and its lipophilic prodrug (GCV-monobutyrate) from PLGA microspheres, Int J Pharm, 338: 133–141, (2007).
44. Herrero-Vanell R, Ramirez L, Fernandez-Carballido A, Refolo MF, Biodegradable PLGA microspheres loaded with ganciclovir for intraocular administration. Encapsulation technique, in vitro release profiles and sterilization process, Pharm Res, 17:1323–1328, (2000).

45. Meng FT, Ma GH, Qiu W, Su ZG, W/O/W double emulsion technique using ethyl acetate as organic solvent: effects of its diffusion rate on the characteristics of microparticles, J Control Release, 91: 407–416, (2003).
46. Lee J-H, Park TG, Choi H-K, Effect of formulation and processing variables on the characteristics of microspheres for water-soluble drugs prepared by w/o/o double emulsion solvent diffusion method, Int J Pharm, 196 : 75–83, (2000).
47. Yeh M-K, Coombes AGA, Jenkins PG, Davis SS, A novel emulsification-solvent extraction technique for production of protein loaded biodegradable microparticles for vaccine and drug delivery, J Control Release, 33: 437-445, (1995).
48. Yuan W, Wu F, Guo M, Jin T, Development of protein delivery microsphere system by a novel S/O/O/W multi-emulsion, Eur J Pharm Sci, 36 : 212–218, (2009).
49. Ito F, Fujimori H, Honnami H, Kawakami H, Kanamura K, Makino K, Study of types and mixture ratio of organic solvent used to dissolve polymers for preparation of drug-containing PLGA microspheres, Eur Polym J, 45 : 658–667, (2009).
50. Ruan G, Feng SS, Preparation and characterization of poly(lactic acid)-poly(ethylene glycol)-poly(lactic acid) (PLA-PEG-PLA) microspheres for controlled release of paclitaxel, Biomaterials, 24: 5037–5044, (2003).
51. Spenlehauer G, Veillard M, Benoit JP, Formation and characterization of cisplatin loaded poly(d,l-lactide) microspheres for chemoembolization, J Pharm Sci, 75: 750, (1986).
52. Tinsley-Bown AM, Fretwell R, Dowsett AB, Davis SL, Farrar GH, Formulation of poly(D,Llactic-co-glycolic acid) microparticles for rapid plasmid DNA delivery, J. Control.Release, 66: 229–241, (2000).
53. Sani SN, Das NG, Das SK, Effect of microfluidization parameters on the physical properties of PEG-PLGA nanoparticles prepared using high pressure microfluidization, J Microencap, doi:10.1080/02652040802500655, (2008).
54. Ruan G, Feng S-S, Li Q-T, Effects of material hydrophobicity on physical properties of polymeric microspheres formed by double emulsion process, J Control Release, 84 :151–160, (2002).
55. Zambaux MF, Bonneaux F, Gref R, Maincent P, Dellacherie E, Alonso MJ, Labrude P, et.al. Influence of experimental parameters on the characteristics of poly(lactic acid) nanoparticles prepared by a double emulsion method, J Control Release, 50: 31–40, (1998).
56. Ravivarapu HB, Burton K, DeLuca PP, Polymer and microsphere blending to alter the release of a peptide from PLGA microspheres, Eur J Pharm Biopharm, 50:263-270, (2000).
57. Hamishehkar H, Emami J, Najafabadi AR, Gilani K, Minaiyan, H. Mahdavi M, Nokhodchi A, The effect of formulation variables on the characteristics of insulin-loaded poly(lactic-co-glycolic acid) microspheres prepared by a single phase oil in oil solvent evaporation method, Colloids Surf B Biointerfaces, 74 : 340–349, (2009).
58. Wan Lucy SC, Heng Paul WS, Chan LW, Surfactant effects on alginate microspheres, Int J Pharm, 103: 267-275, (1994).
59. Yeh M-K, Jenkins PG, Davis SS, Coombes AGA, Improving the delivery capacity of microparticle systems using blends of poly (DL-lactide-coglycolide) and poly (ethylene glycol), J Control Release, 37:1-9, (1995).

60. Mu L, Feng SS, Vitamin E TPGS used as emulsifier in the solvent evaporation /extraction technique for fabrication of polymeric nanospheres for controlled release of paclitaxel (TaxolÒ), J Control Release, 80 : 129–144, (2002).
61. Ravi Kumar MNV, Bakowsky U, Lehr CM, Preparation and characterization of cationic PLGA nanospheres as DNA carriers, Biomaterials, 25: 1771–1777, (2004).
62. Lamprecht A, Ubrich N, Hombreiro Pe´rez M, Lehr C.-M, Hoffman M, Maincent P, Influences of process parameters on nanoparticle preparation performed by a double emulsion pressure homogenization technique, Int J Pharm, 196: 177–182, (2000).
63. Herrmann J, Bodmeier R, Biodegradable somatostatin acetate containing microspheres prepared by various aqueous and non-aqueous solvent evaporation methods, Eur J Pharm Biopharm, 45: 75–82, (1998).
64. Chung T-W, Huang Y-Y, Liu Y-Z, Effects of the rate of solvent evaporation on the characteristics of drug loaded PLLA and PDLLA microspheres, Int J Pharm, 212: 161–169, (2001).
65. Yang Y-Y, Chung T-S, Bai X-L, Chan W-K, Effect of preparation conditions on morphology and release profiles of biodegradable polymeric microspheres containing protein fabricated by double-emulsion method, Chem Eng Sci, 55: 2223-2236, (2000).
66. Yang Y-Y, Chia H-H, Chung T-S, 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, 69:81–96, (2000).
67. Govender T, Stolnik S, Garnett MC, Illum L, Davis SS, PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug, J Control Release, 57: 171–185, (1999).
68. Herrmann J, Bodmeier R, The effect of particle microstructure on the somatostatin release from poly(lactide) microspheres prepared by a W/O/W solvent evaporation method, J Control Release, 36: 63-71, (1995).
69. Dong Y, Feng S-S, Methoxy poly(ethylene glycol)-poly(lactide) (MPEG-PLA) nanoparticles for controlled delivery of anticancer drugs, Biomaterials, 25: 2843–2849, (2004).
70. Yeh MK, Coombes AG, Jenkins PG, Davis SS, A novel emulsification–solvent extraction technique for production of protein loaded biodegradable microparticles for vaccine and drug delivery, J Control Release, 33: 437–445, (1995).
71. Jeong Y-I, Na H-S, Seo D-H, Kim D-G, Lee H-C, Jang M-K. et al. Ciprofloxacin-encapsulated poly(dl-lactide-*co*-glycolide) nanoparticles and its antibacterial activity, Int J Pharm, 352: 317–323, (2008).