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Drug Delivery using Polymeric and other Carrier Systems

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1.1 Introduction

Before advent of novel delivery systems, conventional delivery systems were mainstay in delivering drugs for treatment and prevention of diseases. The introduction of nanotechnology has revolutionized all scientific fields including medical, pharmaceutical and delivery systems. There are inherent problems associated with drug discovery including intricate and tedious research and in particular high cost associated in carrying such work. Further, the high cost is a strong deterrent especially for developing countries that cannot afford huge expenditure on such research. Nanotechnology has provided an excellent weapon to pharmaceutical scientist which is supportive in developing novel formulations of existing problematic drugs. Majority of drug used for treatment of dreaded diseases such as cancer and leishmaniasis have poor selectivity and profound toxicity towards normal body cells. The toxicity of such drugs can be substantially reduced by incorporation inside nano sized carrier systems. These polymeric nanoparticulate systems can deliver the entrapped therapeutic moiety in the vicinity of target area thereby reducing potential unwanted toxic effect with maximized efficacy. The prerequisite for polymeric carrier is biodegradability and

biocompatibility, the qualities that are needed to get approval of regulatory agencies. The polymers belonging to synthetic and natural category including, poly lactic acid (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL), chitosan and bovine serum albumin (BSA) have been widely used for formulation and development of nanoparticles incorporating drugs having diverse physicochemical characteristics. Various methods for preparation of polymeric nanoparticles are available in literature and the choice depends on property of drug to be incorporated and nature of polymeric carrier. This chapter sketches out an overview of formulation and development of nano sized carriers including polymeric nanoparticles, nanocrystals, nanofibers and nanorods and their applicability in drug delivery and therapeutics.

1.2 Basic Methods of Polymeric Nanoparticle Production

Polymeric Nanoparticles (PNPs) can be prepared either by dispersing preformed polymers into nano sized particles or by inducing polymerization reactions in monomers during the process of nanoparticle manufacture itself (Fig. 1.1). Methods like solvent evaporation, nano-precipitation, salting out, dialysis, ionic gelation and supercritical fluid technology can be conveniently utilized to form PNPs out of preformed polymers. Polymerization techniques such as emulsion polymerization and its various sub techniques which employ a monomer and a suitable initiator are also used to form PNPs.

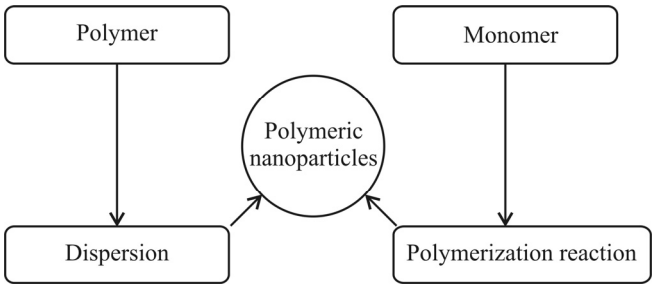

Fig. 1.1 Basic methods for production of polymeric nanoparticles.

The choice of preparation method is made on the basis of a number of factors such as type of polymeric system, area of application, size requirement, etc. For instance, a polymeric system developed for pharmaceutical field should be absolutely free of any organic solvents,

and therefore would employ methods which either refrain altogether from organic solvents or utilize trace quantities. Another important factor which plays a vital role in method selection is the nature of drug molecule. For example solubility of the active molecule in different solvents, thermal and chemical stability of the active agent, reproducibility of the release kinetic profiles of drug (Natural polymers generally may not provide the batch-to-batch reproducibility), stability of the final product and residual impurities associated with the final product.

1.2.1 Dispersion of Preformed Polymers to Form PNPs

1.2.1.1 Emulsification/Solvent Evaporation

Emulsification-solvent evaporation involves two steps. The first step requires emulsification of the polymer solution into an aqueous phase. During the second step polymer solvent is evaporated, inducing polymer precipitation as nanospheres. A polymer organic solution containing the dissolved drug is dispersed into nanodroplets, in a non-solvent or suspension medium such as chloroform or ethyl acetate. The polymer precipitates in the form of nanospheres in which the drug is finely dispersed in the polymer matrix network. The solvent is subsequently evaporated by increasing the temperature under pressure or by continuous stirring. The size can be controlled by adjusting the stir rate, type and amount of dispersing agent, viscosity of organic and aqueous phases, and temperature.

Solvent evaporation is the most widely employed technique to prepare nanoparticles of polymers. In the conventional methods, two main strategies are used for the formation of emulsions: the preparation of single-emulsions, e.g., [oil-in-water (O/W)] to entrap lipophilic drugs or double-emulsions, for hydrophilic drugs e.g., [(water-in-oil)-in-water, (W/O)/W] (Fig. 1.2 and 1.3). The drug in this case may either be finely dispersed through the polymeric core or may be dissolved in the internal aqueous phase which is stored inside the polymeric shell, forming a nanocapsule.

These methods utilize high-speed homogenization or ultrasonication. Afterwards, the solidified nanoparticles can be collected by ultracentrifugation and washed with distilled water to remove additives such as surfactants. Finally, the product is lyophilized. Generally, a polymer dissolved in an organic solvent forms the oil phase, whereas the aqueous phase containing the stabilizer forms the water phase.

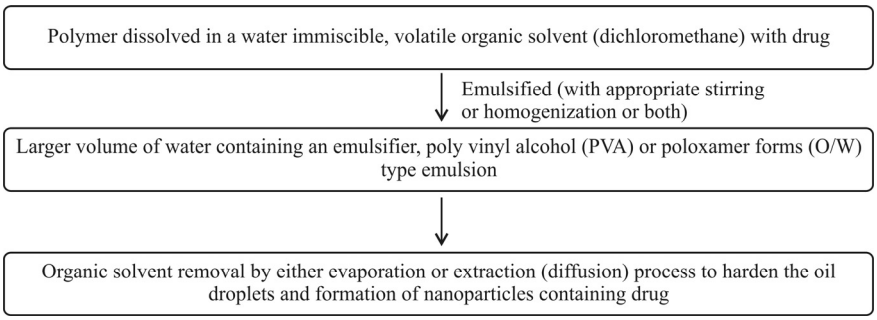

Fig. 1.2 Single emulsion method for lipophilic drugs.

Matsumoto *et al.,* (1999) described the preparation and the evaluation of biodegradable poly(L-lactide)-poly(ethylene glycol)-poly(L-lactide) copolymer (PLA-PEG-PLA) nanoparticles containing progesterone as a model drug by the above described single emulsion method. In another study, Ahlin *et al.,* (2002) reported the design and characterization of poly-(lactide-co-glycolide) (PLGA) and polymethylmethacrylate (PMMA) nanoparticles containing enalaprilat and evaluated the potential of these colloidal carriers for the transport of drugs through the intestinal mucosa. Nicoli *et al.,* (2001) prepared triptorelin loaded nanospheres for transdermal iontophoretic application using double emulsion method. In another study, nanoparticles were loaded with both a hydrophilic and a low molecular weight drug such as propranolol-HCl (Ubrich *et al.,* 2004).

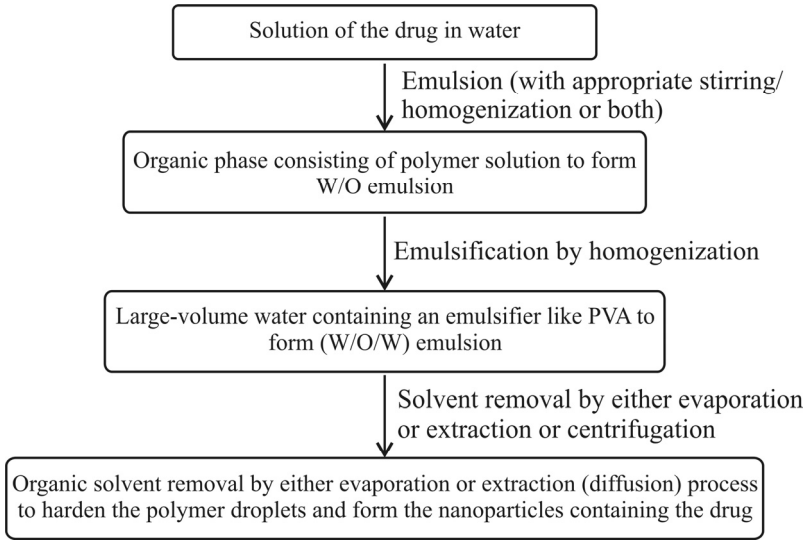

Fig. 1.3 Double emulsion process.

However, limitations are imposed by the scale-up of the high energy requirements in homogenization. Frequently used polymers are PLA, PLGA, ethylcellulose (EC), cellulose acetate phthalate, PCL, and poly (h-hydroxybutyrate) (PHB). Drugs or model drugs that have been encapsulated include albumin, tetanus toxoid, testosterone, loperamide, praziquantel, cyclosporin A, and indomethacin.

Major variables affecting the outcome of this process are the preparation temperature, solvent evaporation method, internal aqueous phase volume, surfactant concentration, and the influence of the molecular mass of the polymer on the particle size, the zeta potential, the residual surfactant percentage, and the polydispersity index. Because the process is governed by emulsification, utilizing a higher concentration of surfactant not only reduces the size of oil droplets, but also prevents their agglomeration. The concentrations of polymer and solvent used in the preparation of the emulsion also affect the final properties of the PNPs prepared by the solvent evaporation method. The mixing technique is also important in the preparation of PNPs by the solvent evaporation method. It was demonstrated that the duration of the second mixing step, which leads to the W/O/W emulsion, has a greater influence on the final mean particle size than the first step for the W/O emulsion.

1.2.1.2 Nanoprecipitation

The nanoprecipitation method was developed by Fessi *et al.,* (1989) for the preparation of PNPs. It is also called as solvent displacement method. The basic principle of this technique is based on the interfacial deposition of a polymer after displacement of a semipolar solvent, miscible with water, from a lipophilic solution acting as the oil phase for an infinitesimally small period of time. The basic difference from solvent evaporation lies with the fact that there is no apparent emulsification step, i.e., the formation of nanoparticles is instantaneous. Also it is a low energy method, i.e., moderate stirring is adequate, whereas high powered mechanized stirring is sometimes required in solvent evaporation to formulate the initial emulsion.

The process dynamics and the outcome are driven by the diffusion of solvent phase into the non-solvent water for most of the cases. Rapid diffusion of the solvent into non-solvent phase results in the decrease of interfacial tension between the two phases, which increases the surface area and leads to the formation of small droplets of organic solvent. The polymer and drug is consequently precipitated out in the non-solvent due to lack of solubility, forming a milky suspension of nano sized particles, however the overall colour of the suspension is dictated by the colour of

drug and its percentage entrapment and can act as a crude visual indicator of its quality. A suspension of a coloured drug may turn out to be whitish if it is properly entrapped in the polymeric matrix. The drug as in solvent evaporation is dispersed throughout the polymeric matrix or adsorbed onto the surface; some amount of drug may also be dispersed throughout the solution in nano sized form, or may precipitate out (Fig. 1.4).

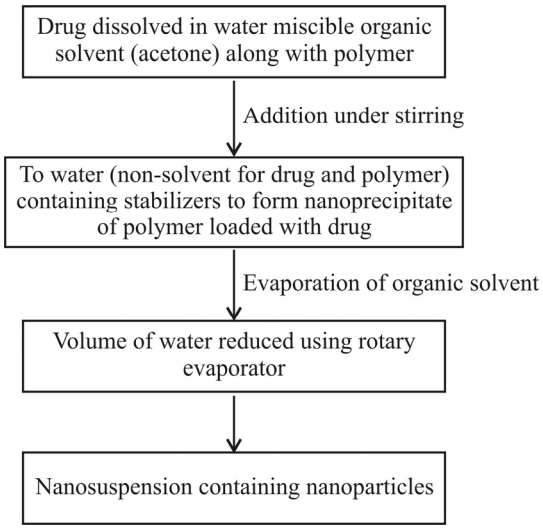

Fig. 1.4 Steps in nanoprecipitation method.

Nanoprecipitation system consists of four basic components: the polymer (synthetic, semi synthetic or natural), drug (to be entrapped), the polymer solvent and the non-solvent of the polymer with the optional presence of surfactants for example poloxamers, PVA, tweens, cetyl trimethyl ammonium bromide, Aerosol-AT etc. Organic solvent (i.e., ethanol, acetone, or dioxane) which is miscible in water and easy to remove by evaporation is chosen as polymer solvent. Due to this reason, acetone is the most frequently employed polymer solvent in this method. Sometimes, it consists of binary solvent blends, acetone with small amount of water, blends of acetone with ethanol and methanol. On the other hand, the non-solvent phase consisting of a non-solvent or a mixture of non-solvents is admixed with one or more naturally occurring or synthetic surfactants. Table 1.1 shows various examples of polymers, solvents, non-solvents and stabilizing agents used in the nanoprecipitation formulations and particle size achieved. Notice that, although an extensive range of polymers can be used theoretically, in practice only few are used regularly.

Table 1.1 Preparation of polymeric nanoparticles by nanoprecipitation method

Polymer	Solvent	Non-solvent	Stabilizing agent	Drug	Particle size (nm)	Ref.
PLGA	Acetone	Water	PVA	Curcumin	95-560	Yallapu *et al.,* 2010
Allylic starch	Acetone	Water	-	-	270	Tan *et al.,* 2009
PLA	Acetone	Water	Poloxamer 188	Muramyl-tripeptide cholesterol	250 ± 50	Seyler *et al.,* 1999
PLA	THF	Water	-	-	100-300	Legrand *et al.,* 2007
PCL	Acetone	Water	Span 20	Griseofulvin	741-924	Zili *et al.,* 2005
PCL	Acetone	Water	PVA		365 ± 5	Moinard-Checot *et al.,* 2008

The polymers commonly used are biodegradable polyesters, especially PCL, PLA and PLGA. Eudragit and polyalkylcyanoacrylate (PACA) have also been used for formulation of nanoparticles. PCL nanospheres of isradipine (Leroueil-Le Verger *et al.,* 1998) and nanocapsules of griseofulvin (Zili *et al.,* 2005) (poorly soluble drug) have been prepared by nanoprecipitation method. Chen *et al.,* prepared and characterized oleanolic acid (Chen *et al.,* 2005) (poorly soluble drug) nanosuspensions by the nanoprecipitation method to enhance the oral bioavailability by increasing dissolution rate and solubility. Nanoparticles of isradipine (Leroueil-Le Verger *et al.,* 1998) (poorly soluble drug), an antihypertensive agent, was encapsulated by the nanoprecipitation method using polymers including PCL, PLA and PLGA.

Natural polymers such as allylic starch, dextran ester can also be used; though synthetic polymers have higher purity and better reproducibility than natural polymers. Polymer modification or surface functionalization for example PEGylation can also be done in order to escape recognition from reticulo endothelial system and massive clearance. PNPs are produced by slow addition of the organic phase to the aqueous phase under moderate stirring. Reversing this order by adding the aqueous phase to the organic phase also leads to the formation of PNPs. The nanoparticles with a well-defined size are characterized by a narrow distribution formed instantaneously during the rapid diffusion of the

polymer solution in the non-solvent phase. The presence of surfactant prevents agglomeration of these nanoparticles on standing by providing steric or electrostatic stabilization. The ratio of organic to aqueous phase and water solubility of the organic solvent has a strong effect on characteristics of PNPs.

The key variables determining the success of the method and affecting the physicochemical properties of PNPs include organic phase injection rate, aqueous phase agitation rate and the method of organic phase addition. Likewise, PNPs characteristics are influenced by the nature and concentration of their components for example drug to polymer ratio. Although, a surfactant is not required to ensure the formation of PNPs by nanoprecipitation, the particle size is influenced by the surfactant nature and concentration. Lince *et al.,* (2008) indicated that the process of particle formation in the nanoprecipitation method comprises three stages: nucleation, growth and aggregation. The rate of each step determines the particle size. The separation between the nucleation and the growth stages is the key factor for uniform particle formation. Ideally, operating conditions should allow a high nucleation rate so that a large number of evenly sized nanoparticles are formed. Even if growth stage cannot be retarded completely it should not fluctuate abruptly in certain areas of the suspension. Nanoprecipitation is a simple, fast and reproducible method which is widely used for the preparation of both nanospheres and nanocapsules. Till now it has been predominantly employed to entrap lipophilic drugs, with low entrapment efficiency obtained for hydrophilic drugs.

1.2.1.3 Salting-Out

The methods discussed in the previous sections require the use of organic solvents, which are hazardous to the environment as well as to physiological systems. As an alternative Ibrahim *et al.,* (1992) first developed a modified version of emulsion process that involves a salting-out process, which avoids surfactants and chlorinated solvents. The emulsion is formulated with a polymer solvent which is normally totally miscible with water, i.e., acetone, followed by emulsification of the polymer solution in the aqueous phase. This emulsification is a spontaneous no energy step and is achieved without employing any high-shear forces, by dissolving high concentration of salt or sucrose chosen for a strong salting-out effect in the aqueous phase. Magnesium chloride, calcium chloride and magnesium acetate are usually employed as the salting out agents. The miscibility properties of water with other solvents

are modified as these components dissolve in the water. A reverse salting out effect, obtained by dilution of the emulsion with a large excess of water, leads to the precipitation of the polymer dissolved in the droplets of the emulsion. In fact, upon dilution, migration of the solvent for the polymer from the emulsion droplets is induced due to the reduction of the salt or sucrose concentration in the continuous phase of the emulsion (Fig. 1.5). PLA, Poly(alkylmethacrylate) (PMA) and EC have been used to produce nanoparticles in the size range of 1000 nm by the salting out method. A compilation of the polymer nanoparticles prepared by employing the salting-out method is given in (Table 1.2).

Table 1.2 Examples of polymer nanoparticles prepared by the salting-out method

Polymer	Salting-out agent	Organic solvent	Drug	Particle size (nm)	Ref.
EUDRAGIT L100-55	$MgCl_2 \cdot 6H_2O$	Acetone	Ibuprofen	174-557	Galindo-Rodriguez *et al.,* 2005
PMA	NaCl	Dilute HCl	Insulin	100-250	Fan *et al.,* 2006
PLGA	PVA	Acetone/ DCM	Dexamethasone	111.4 ± 2.3	Zhang *et al.,* 2006a
PLGA	$MgCl_2.6H_2O$	THF	Sunscreen agent	> 200	Perugini *et al.,* 2002
PLGA	PVA	Acetone/ DCM	Vincristine	60-120	Song *et al.,* 2008
PLA	PVA, $MgCl_2.6H_2O$	THF	Blank	< 200	Konan *et al.,* 2002
PLGA	PVA, $MgCl_2.6H_2O$	THF	Verteporfin	160-370	Konan-Kouakou *et al.,* 2005
DMAB* coated PLGA	$MgCl_2 \cdot 6H_2O$	DCM	Gene	180-237	Fay *et al.,* 2010
Poly (trimethylene carbonate)	PVA, $MgCl_2 \cdot 6H_2O$	THF	Dexamethasone	181 ± 1 - 214 ± 4	Zhang *et al.,* 2006b
PLGA,PEO	$MgCl_2 \cdot 6H_2O$	Acetone	-	139-250	Zweers *et al.,* 2004

*dimethyl didodecyl ammonium bromide

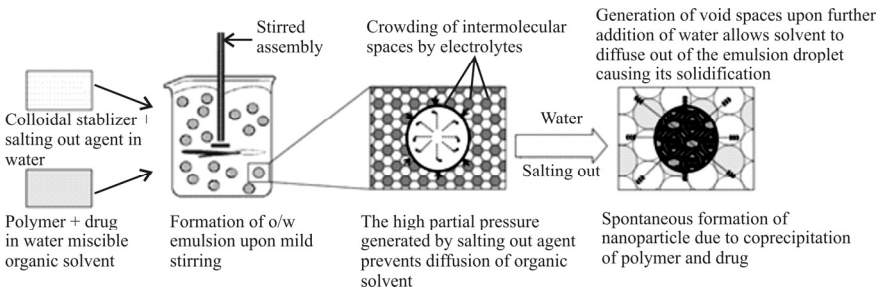

Fig. 1.5 Mechanism of salting out phenomenon in production of nanoparticles.

Salting-out procedure can be considered as a modification of the emulsification/solvent diffusion or nanoprecipitation method. Polymer and drug are initially dissolved in a solvent such as acetone, which is subsequently emulsified either spontaneously or upon mild agitation into an aqueous gel (so called due to its consistency because of high salt concentration) containing the salting-out agent and a colloidal stabilizer such as polyvinylpyrrolidone (PVP) or hydroxyethylcellulose (HEC). The system here exists in transition state as in nanoprecipitation for an extended period of time, only difference being that organic solvent cannot diffuse into bulk analogous to nanoprecipitation due to high concentration of salting out agent (Fig. 1.5). This oil/water emulsion is diluted with a sufficient volume of water or aqueous solution to enhance the diffusion of acetone into the aqueous phase, thus inducing the formation of nanospheres. The selection of the salting out agent is important, because it can play an important role in the encapsulation efficiency of the drug. Both the solvent and the salting-out agent are then eliminated by cross-flow filtration. Salting out does not require an increase of temperature and, therefore, may be useful when heat sensitive substances have to be processed. The greatest disadvantages are exclusive application to lipophilic drugs and the extensive nanoparticles washing steps.

1.2.1.4 Dialysis

Dialysis offers a simple and effective method for the preparation of small, narrow-distributed PNPs. Polymer and drug are dissolved in an organic solvent and the organic solution is placed inside a dialysis tube with proper molecular weight cut off (MWCO). Dialysis is performed against a non-solvent (for the polymer and drug); for example water which is freely miscible with the organic solvent present inside the dialysis tube (Fig. 1.6). The displacement of the solvent inside the membrane which

moves towards the bulk to attain overall equilibrium is followed by the progressive aggregation of polymer due to a loss of solubility in the non-solvent and the formation of homogeneous suspensions of nanoparticles which are loaded with the drug. The mechanism of PNPs formation by dialysis method is not fully understood at present. It is thought that it may be based on a mechanism similar to that of nanoprecipitation as solvent displacement is followed by instantaneous precipitation of PNPs.

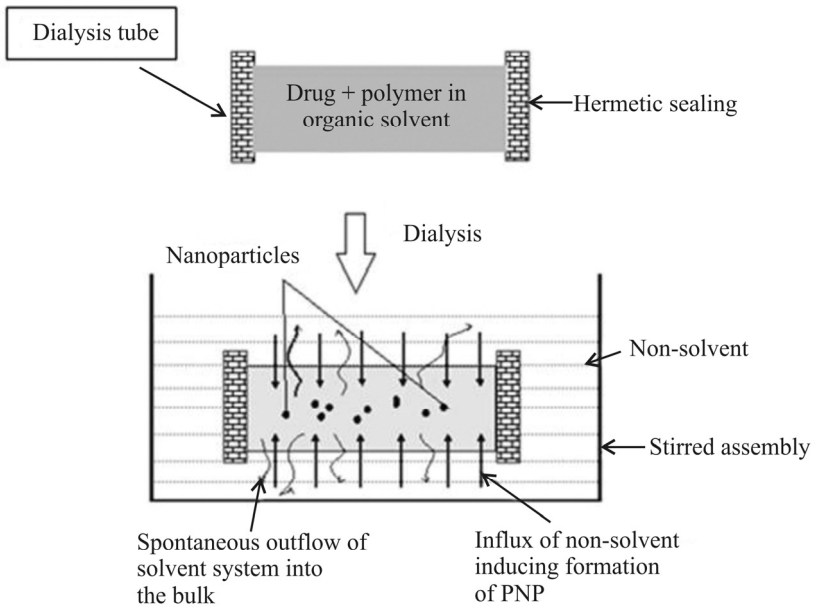

Fig. 1.6 Production of nanoparticles using dialysis method.

A number of polymer and copolymer nanoparticles have been obtained in this system. Careful selection of the lower MWCO membrane plays an important role in determining the size range of the PNPs. For example a low MWCO will exclude any loss of PNPs above that MWCO. Due to progressive dilution of solvent the washing step is simultaneously carried out. The time duration for which dialysis is carried out has a bearing on the process. Usually a period of 24 hours is sufficient to ensure complete diffusion of solvent system. Table 1.3 depicts the summary of the ingredients used and the results obtained with dialysis method.

Table 1.3 Examples of nanoparticles developed by dialysis method

Polymer	Solvent	MWCO (kg/mol)	Dialysis time (hr)	Drug	Particle size (nm)	Ref.
PBG-PEO	DMF	-	24	Norfloxacin	250-362	Jeon *et al.,* 2000b
PLGA	DMSO	12-14	48	Retionic acid	635 ± 102	Errico *et al.,* 2009
PCL-PVA	DMSO	03.5	96	Technitium^{99}	87	Park *et al.,* 2007
PLA	DMSO	15	6	HIV-1 p 24 antigen	300-600	Ataman-Önal *et al.,* 2006
PLGA	DMSO	12	24	Norfloxacin	248.9 ± 3.9	Jeon *et al.,* 2000a
PGA*	DMF	50	72	Ovalbumin	152 ± 44	Akagi *et al.,* 2005
PEG–PLGA	DMF	6-8	12	Estrogen	43.5 ± 2.3	Choi and Kim, 2007
PBG-PEO	DMF	-	24	Adriamycin	250-362	Oh *et al.,* 1999
PEG-PTMC**-PEG	DMF	-	24	Methotrexate	51.0-158.9	Zhang and Zhuo, 2005
Starch	DMSO	12	12	-	300-500	Namazi *et al.,* 2011

*Poly glutamic acid **Poly trimethylene carbonate

1.2.1.5 Ionic Gelation Method

This method utilizes the polycondensation reaction between oppositely charged cations and polyanions leading to formation of neutral particles which are insoluble in reaction media and can be separated by simple filtration methods. It is conceived that drug is also simultaneously fixated or trapped in the condensing polymeric matrix. The reaction is carried out in such a way that there is an associated loss of solubility (induced by change in pH of drug environment) of drug at the very instance this condensation reaction takes place. This method can be utilized for both hydrophilic as well as lipophilic drugs. Hydrophilic drugs are dissolved in any one of the electrolyte solutions whereas entrapment of lipophilic drug requires its dispersion in an oil emulsion or co-solublization in water miscible organic solvent. For example a solution of sodium aliginate

(possessing aliginate anion) when injected drop wise into calcium chloride solution leads to cross linking of aliginate by calcium forming calcium alginate beads. This crude experiment can be suitably modulated to obtain particles of micro and nano size. There have been instances where more than one polyelectrolyte has been utilized in the same reaction to obtain reinforced nanoparticles or to impart suitable functionalization properties on the PNPs.

The ionic gelation method is principally employed for production of chitosan nanoparticles (Fig. 1.7). Chitosan nanoparticles have been developed to encapsulate proteins such as bovine serum albumin, tetanus and diphtheria toxoid, vaccines, anticancer agents, insulin and nucleic acids. Chitosan considerably enhances the absorption of peptides such as insulin and calcitonin across the nasal epithelium. Chitosan nanoparticles obtained by formation of a spontaneous complex between chitosan and polyanions such as tripolyphosphate (TPP) have small diameters (200-500 nm). The principal factors affecting characters of formed PNPs include those associated with addition of one ionic phase into other such as dropping rate, stirring rate, the viscosity of solution and also pH of the solution which dictates the state of ionization of participating polyelectrolytes and the drug.

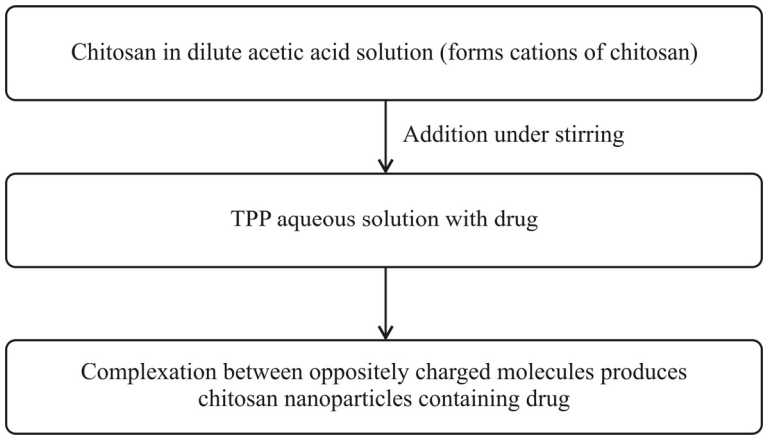

Fig. 1.7 Steps involved in ionic gelation method.

1.2.1.6 Preparation of Nanoparticles with a Membrane Contactor

Despite the numerous methods available to produce nanoparticles on a lab scale, there are still problems in establishment of large scale production methods. This is considered to be one of the major stumbling blocks in successful introduction of the nanoparticles to the clinic and the

pharmaceutical market. Charcosset and Fessi (2005) have developed a method for mass production of nanoparticles using a specialized device known as membrane contactor shown in the Fig. 1.8. The organic phase is pressed through the membrane pores allowing the formation of small droplets. The reaction occurs between the droplets of the organic phase and the aqueous phase flowing tangentially to the membrane surface. Large scale industrial pumps, membrane contactor and automated process control can be employed to obtain massive yields with fairly uniform properties.

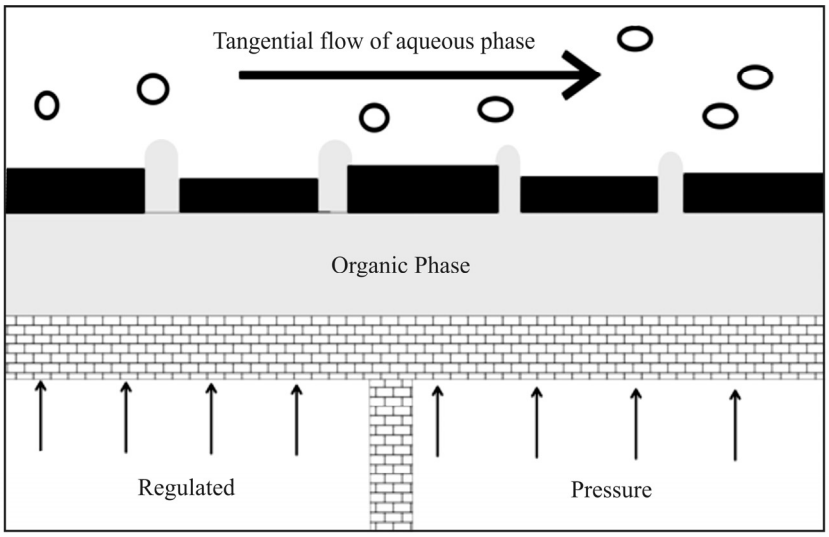

Fig. 1.8 Membrane reactor assembly.

This process may be compared to the membrane emulsification or the dialysis process, where the oil (or the water phase) permeates through the membrane pores to form droplets in water (or oil phase) for the preparation of O/W or W/O emulsions, respectively. The tangentially flowing aqueous phase sweeps along the formed nanostructures and is collected in a compartment where nanoparticles can be washed, purified and collected. The two main parameters of the process are the aqueous phase cross-flow velocity and the organic phase pressure. Another advantage of this membrane reactor is its versatility for the preparation of either nanocapsules or nanospheres, by methods involving a polymerization of dispersed monomers or a dispersion of preformed polymers, and the control of the average nanoparticles size by an appropriate choice of the membrane.

1.2.1.7 New Techniques based on Supercritical or Compressed Fluids

Some of the techniques described above are complex, and the products may often be characterized by high residual solvent content, low drug loading, drug degradation or denaturation, ineffective drug release, or unsuitable physical properties. Techniques based on supercritical or compressed fluid have come up as suitable alternatives which have huge potential to be exploited industrially. Supercritical fluid is any substance at a temperature and pressure above its critical point, where distinct liquid and gas phases do not exist. It can diffuse through solids like a gas, and dissolve materials like a liquid. In addition, close to the critical point, small changes in pressure or temperature results in large changes in density, allowing many properties of a supercritical fluid to be fabricated as per requirement. Supercritical fluids are suitable as a substitute for organic solvents in a range of industrial and laboratory processes. Carbon dioxide and water are the most commonly used supercritical fluids. Supercritical fluids provide a number of ways of achieving this by rapidly exceeding the saturation point of a solute by dilution, depressurization or a combination of these. These processes occur faster in supercritical fluids than in liquids, promoting nucleation over crystal growth and yielding very small and regularly sized particles.

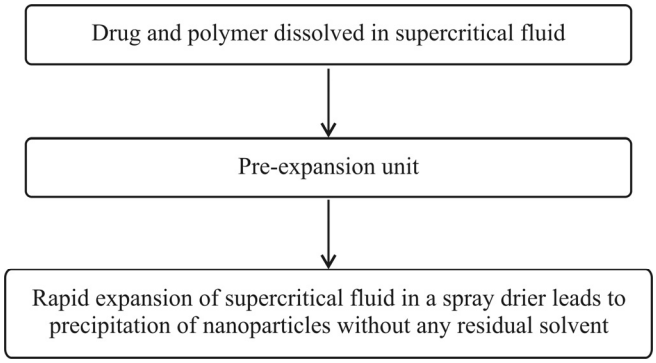

Fig. 1.9 Flowchart illustrating the basic steps in supercritical fluid expansion producing nanoparticles.

Recent supercritical fluids have shown the capability to reduce particles up to a range of 5-2000 nm. In this technique, the drug and the polymer are solubilized in a supercritical fluid and the solution is expanded through a nozzle either into ambient air or an aqueous non solvent system with additional colloidal stabilizers (Fig. 1.9). The

supercritical fluid is evaporated in the spraying process, and the solute particles eventually precipitate. The polymer concentration in the pre-expansion supercritical solution plays a vital role in determining the product morphology. This technique is clean, because the precipitated solute is free of solvent. It also provides advantages such as suitable technological and biopharmaceutical properties and high quality. It has been demonstrated for numerous applications involving protein drug delivery systems. Protein drugs such as insulin (Elvassore *et al.,* 2001) has been encapsulated in (PEG/PLA) nanoparticles by this technique. Other drugs whose nanoparticles have been prepared using this technique include Coenzyme Q-10, curcumin, fluorouracil, atorvastatin, methotrexate, etc. However, this new process requires a high initial capital investment for equipment, and elevated operating pressures requiring high pressure equipment. In addition, compressed supercritical fluids require elaborate recycling measures to reduce energy costs. Finally, it is very difficult to dissolve strong polar substances in supercritical CO_2. In fact, supercritical CO_2 has solvating properties characteristic of both fluorocarbons and hydrocarbons. However, the use of co-solvents and/or surfactants to form microemulsions makes it possible to dissolve polar and ionic species.

1.2.2 Polymerization of Monomers

The techniques discussed previously involve the production of PNPs from preformed polymers and did not involve any polymerization processes. To attain the desired properties for a particular application, suitable polymer nanoparticles must be designed, which can be done during the polymerization of monomers. The point here is that sometimes the properties of preformed polymers are too robust to be modified or manipulated; consequently they do not yield PNPs suited for the desired purpose. In this particular process polymerization starts from a monomer and can be processed to obtain the properties as per our requirements. Additionally, the advantage of obtaining nanoparticles by this method is that the polymer is formed *in situ*, allowing the polymer membrane to follow the contours of the inner phase of an oil/water or water/oil emulsion. But like every coin there is a flip side to this technique as well, wherein batch to batch reproducibility in the polymerization process is a difficult task. The polymer so synthesized can be difficult to characterize sometimes, and in these conditions, using commercially standardized preformed polymers is a better option.

1.2.2.1 Emulsion Polymerization

Emulsion polymerization is the most common method used for the production of a wide range of polymers which in turn form PNPs. Emulsion polymerization is one of the fastest methods for nanoparticles preparation and is readily scalable. In the conventional system, the ingredients like any polymerization reaction consists of a monomer of low water solubility, water-soluble initiator along with added drug molecule, surfactant and the most suited reaction media water. At the end of these actions, PNPs are typically nanosized, each containing many polymer chains which entangle amongst each other encapsulating the drug. The procedure is visualized in Fig. 1.10. As is evident from figure the small emulsion droplets formed initially on dispersion of oil phase into water function as minute nanoreactors and it is inside these the basic polymerization reaction takes place. So the sizes of nanoreactors directly determine the particle size of the eventual PNPs formed. The surfactant molecule ensures that the nanoreactor assemblies do not coalesce whilst the reaction is still taking place or before the oil droplets have hardened into PNPs. Colloidal stabilizers may be electrostatic, steric or electrosteric, displaying both stabilizing mechanisms. Initiation occurs when a monomer molecule dissolved in the aqueous phase collides with an initiator molecule. The monomer might also function as an auto initiator, when induced by photostimulation or λ-radiation, colliding with further monomer molecules carrying on the polymerization to its completion. Phase separation and formation of solid particles can take place before or after the termination of the polymerization reaction as shown in the figure. Polystyrene (PS), poly(methylmethacrylate) (PMMA), poly(vinyl-carbazole), poly(ethylcyanoacrylate) (PECA) and poly(butylcyanoacrylate) nanoparticles have been produced by dispersion *via* surfactants into solvents, such as cyclohexane, n-pentane, and toluene. These polymers have been used to entrap variety of drugs for example doxorubicin, ampicillin, dexamethasone, triamcinolone, insulin, vinblastine, etc.

Conventional emulsion polymerization systems require surfactants that need to be eliminated from the final product. But even after sustained efforts their complete removal cannot be guaranteed. In order to circumvent this drawback emulsion polymerization has been performed in the absence of any added emulsifier. The reagents used in an emulsifier free system include deionized water, a water-soluble initiator (i.e., KPS, potassium persulfate) and monomers, more commonly vinyl or acryl monomers. In such polymerization systems, stabilization of PNPs occurs through the use of ionizable initiators or ionic co-monomers. In such

PNPs the initiator molecule preferentially deposits on the surface and prevents agglomeration due to electrostatic repulsion.

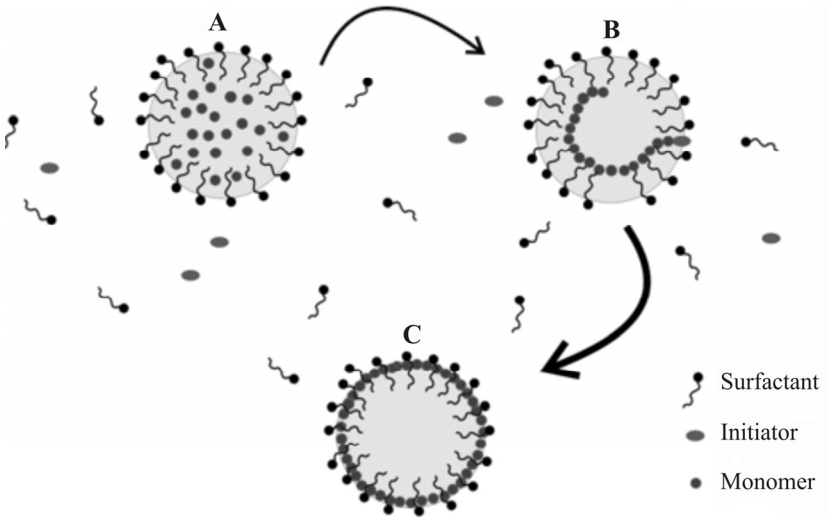

Fig. 1.10 Basic steps taking place in emulsion polymerization ultimately leading to formation of PNPs. A: Oil droplet with solubilized drug and monomer, dispersed randomly in aqueous phase consisting of surfactant and water. B: The initiator makes contact with monomer at oil-water interface to initiate polymerization reaction. C: Polymerization continues until a solid interfacial polymeric film is deposited, containing trapped drug.

1.3 Nanocrystals

The number of poorly soluble drugs is constantly on an upswing. With the pharmaceutical industry having already exhausted most of the simple molecules as potential drug candidates it has to look into complex bulky structures which are usually poorly water soluble and consequently have a very low bioavailability. The oral route is the most preferred for administration of drugs. However poor aqueous solubility of drugs with consequent low bioavailability makes oral administration unviable. The need of the hour requires the pharmaceutical scientist to work around these severe limitations and come up with ideas to overcome the low bioavailability of these new drug candidates as well as other existing BCS class II and class IV drugs.

Solubility is the property of a solid, liquid, or gaseous chemical substance called solute to dissolve in a solid, liquid, or gaseous solvent to

form a homogeneous solution of the solute in the solvent. The solubility of a substance fundamentally depends on the used solvent as well as on temperature and pressure. The extent of the solubility of a substance in a specific solvent is measured as the saturation solubility at that defined condition of temperature and pressure, where adding more solute does not increase the concentration of the solution. Solubility of a drug substance in water tends to dictate its bioavailability as drugs are available for absorption in systemic circulation only in solution form. Therefore any method which increases the solubility of drug substance will tend to increase its bioavailability.

One such universal approach to enhance the solubility and in turn the bioavailability of drugs is reduction of particle size such as micronization. Reduction in particle size increases the surface area exponentially, yielding higher dissolution rates according to Noyes-Whitney's equation. This technique has worked well for a few drugs such as griseofulvin whose bioavailability has been considerably enhanced, but has failed on most other drugs which are either more poorly water soluble or are very hydrophobic. The next obvious step to enhance the solubility of these insoluble drugs is to further reduce the particle size so as to move from micronization to nanoniztion of drug powder. Below a certain critical size, around 1 μm, saturation solubility becomes dependent on particle size, i.e., smaller particles have a higher solubility than larger ones. Such a state when the particle size of the drug powder is reduced to nanometer scale that is below 1000 nm, it is said to have assumed a nanocrystal structure. Thus nanocrystals are nanoparticles of drug molecules in crystalline form without any associated coating or dispersion of any form. They are composed totally of drug and do not possess any carrier moiety such as polymeric matrix or lipoidal structure.

Dispersion of nanocrystals in liquid media yields nanosuspension. Generally nanosuspensions need to be stabilized by surfactants, colloidal stabilizers or viscosity enhancers, so as to prevent any flocculation, clumping, gradual coalescence, particle size growth and ultimately phase separation. The dispersion media could be aqueous, or non-aqueous such as low viscosity poly-ethylene glycol. The nanocrystals display properties in between those of crystals and amorphous structures. They have higher solubility compared to their crystalline counterparts but also show extended stability which is non-existent in their amorphous analogues, amalgamating advantages of the two states.

1.3.1 Approaches to Formulating Nanocrystals

Nanocrystals can be obtained directly through 'bottom up' by modified crystallization/antisolvent precipitation or indirectly 'top down' by mechanical breakage/attrition of a crystalline powder. The production of nanosized particle by direct crystallization/precipitation can be carried out using extreme super saturation conditions in order to favor nucleation over growth. The stability upon agglomeration during rapid crystallization needs to be assessed for developmental feasibility. Another way to generate nanocrystals is to limit the amount of material available for crystallization by reducing the working volume. Microfluidics set-up or emulsion crystallization can be the method of choice to generate microcrystalline material, but the scalability and the energy input is a major limiting factor.

1.3.1.1 Precipitation Method

The intellectual property of this technique is owned by pharmaceutical giant Novartis. It is basically similar to the classical nanoprecipitation method utilizing solvent non-solvent interaction. The drug is dissolved in a solvent and the solution is poured into a non-solvent system for the drug but having affinity for the drug solvent. The solvent immediately diffuses throughout the non-solvent system leaving back minute nanoprecipitates of drug.

Problem arises in maintaining the particle size in nano range and preventing further aggregation. This requires introduction of a suitable steric, electronic or colloidal stabilizer. Viscosity enhancing agent can also be used which impedes any sedimentation or brownian motion of suspended nanocrystals and reduces the probability of particle-particle contact which can bring about growth. Additionally drug should be soluble in some solvent which in turn should have affinity for a non-solvent for drug. The probability of these conditions happening together is extremely low for modern day drugs which have solubility issues both in aqueous and non-aqueous media.

1.3.1.2 Homogenization Method

1.3.1.2.1 Drug Nanocrystals Produced by High-Pressure Homogenization

The Microfluidizer (Microfluidics^{TM} Inc., U.S.A.) is based on the jet-stream principle (Fig. 1.11); seen in regular fluid energy mills. Two streams of liquid collide, diminution of droplets or crystals is achieved mainly by particle collision, but occurrence of cavitation is also considered.

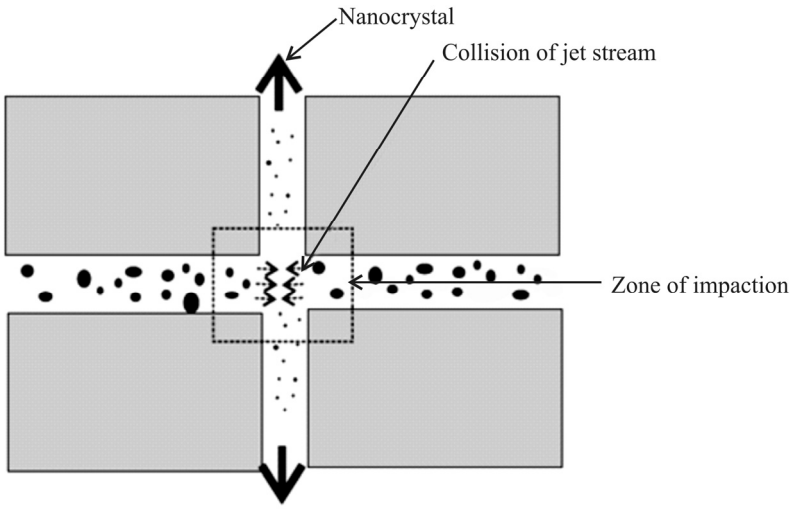

Fig. 1.11 Jet stream homogenization

Typical pressures for the production of drug nanosuspensions are 1000-1500 bar (corresponding to 100-150 Mpa, 14504-21756 psi); the number of required homogenization cycles varies from 10 to 20 depending on the properties of the drug i.e., its crystal strength. Sometimes upto 100 cycles might be required to induce desired particle size reduction. The particles breakdown due to high energy of impaction which also dissipates in form of heat requires cooling. The heating problem is also reduced to some extent by using water as the dispersion medium. The biggest advantage of this highly efficient process apart from scalability is zero contamination of feed material as the reduction is being effected by the particles themselves.

1.3.1.2.2 Piston Gap Homogenizer

The piston gap homogenizers work on the principle of colloid mills. The drug is made to pass through a narrow gap (of dimension less than 10 μm) between a fixed stator and a rapidly moving rotor. Size reduction is caused due to high shear, stress and grinding forces generated between rotor and stator. The upper ceiling of particle size can be ascertained by fixing the dissipation gap to required size. This means that yield will not be obtained unless and until the particles are ground down to a size which is equal or lower to that of the gap between rotor and stator. A basic visualization is displayed in Fig. 1.12.

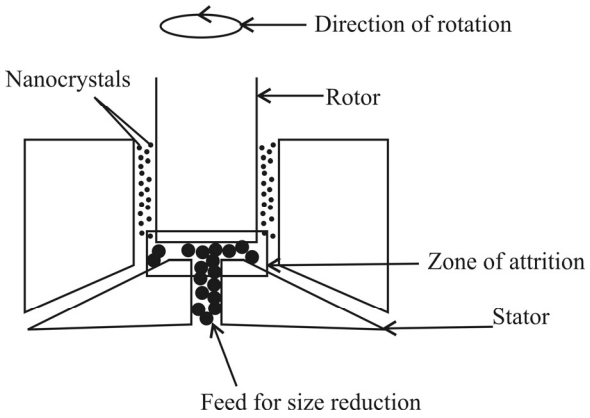

Fig. 1.12 Piston gap homogenizer.

1.3.1.3 Pearl/Ball-Milling Technology for the Production of Drug Nanocrystals

These mills consist of a milling container filled with fine milling pearls or larger-sized balls. The container can be static and the milling material is moved by a stirrer; alternatively, the complete container is moved in a complex movement leading to movement of the milling pearls. The basic difference between a ball and a pearl mill is related to the size of the grinding material, larger in case of ball mill. The principle of size reduction is combined impact and attrition. Since there are more dead spaces in a ball mill, the particle size distribution is broader. There are different milling materials available, traditionally steel, glass, and zircon oxide are used. New materials are special polymers, i.e., hard polystyrene. A problem associated with the pearl milling technology is the erosion from the milling material during the milling process introducing a chance of contamination. Apart from the milling material, the erosion from the container also needs to be considered. These factors play an important role in selection of milling material and type of mill. Normally, product containers are made of steel and can be covered with various materials to fulfill the required quality specifications of the formulation. Surfactants or stabilizers have to be added for the physical stability of the produced nanosuspensions. In the production process the coarse drug powder is dispersed by high-speed stirring in a surfactant/stabilizer solution to yield a macrosuspension which then is subjected to milling operations.

1.3.1.4 Production of Drug Nanocrystal Compounds by Spray-Drying

Spray drying is an excellent technique which has industrial feasibility. Starting from an aqueous macrosuspension containing the original coarse drug powder, surfactant, and water-soluble excipient, the homogenization process can be performed in an easy one step yielding a fine aqueous nanosuspension. In a subsequent step the water has to be removed from the suspension to obtain a dry powder. One method of removing the water from the formulation is freeze drying, but it is complex and cost-intensive leading to increase in the cost of the product. An alternative method for the industrial production is spray drying. The drug nanosuspension can directly be produced by high-pressure homogenization in aqueous solutions of water-soluble matrix materials, e.g., polymers [PVP, PVA or long chained PEG, sugars (saccharose, lactose), or sugar alcohols (mannitol, sorbitol)]. Afterward the aqueous drug nanosuspension can be spray dried under adequate conditions; the resulting dry powder is composed of drug nanocrystals embedded in a water-soluble matrix of drug nanocrystal-loaded spray-dried compounds.

1.4 Nanocapsules

Theoretically, polymeric nanocapsules are vesicular particles smaller than 1 μm composed of an oily core surrounded by an ultrathin polymeric wall. These devices are stabilized by surfactants and/or steric agents (Fig. 1.13).

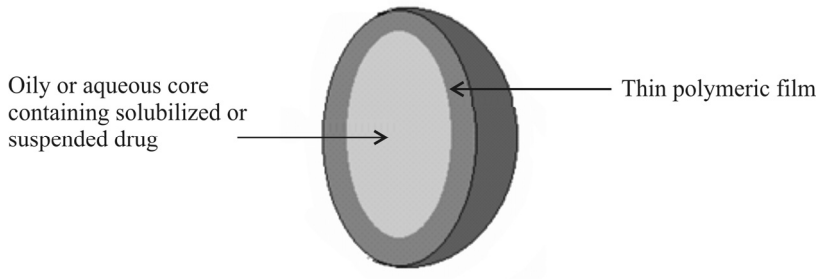

Fig. 1.13 Cross section of a polymeric nanocapsule

The structure differs from a nanoparticle or nanosphere in the way represented in Fig. 1.14. Nanospheres are matrix systems in which the drug is dispersed within the polymeric network throughout the particle. Contrarily, nanocapsules are vesicular or "reservoir" (heterogeneous) systems, in which the drug is essentially confined to a cavity surrounded

by a tiny polymeric membrane. The most advantageous feature of developing a nanocapsule has been the high payload or entrapment efficiency especially for lipophilic drug obtained in these systems expending extremely low polymer content, when compared to nanoparticles.

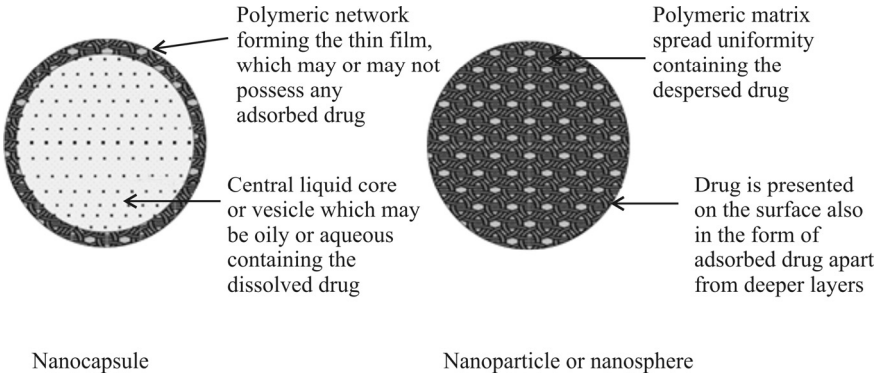

Fig. 1.14 Basic difference between a nanoparticle and nanocapsule.

The chances of burst effect are reduced since there is little or no surface adsorbed drug. Moreover any irritation reaction produced by drug at the site of administration is also absent due to avoidance of direct contact.

As far as formulation of nanocapsules is concerned, it is composed of polymer which forms the film, a solubilizing material such as oil, water or an organic solvent along with drug, a dispersion media (might be aqueous or non-aqueous) and stabilizer to prevent capsule growth. In general, polymeric nanocapsules have an oily core composed of triglycerides, the liquid active ingredient and, in addition, some cases have a mixture of chemical substances as the core, such as capric/caprylic triglycerides or vegetable oils and sorbitan monostearate. The basic strategies of production remain similar to that of nanoparticles, due to structural similarity, the only prerequisite being the hardening of the thin polymeric film, and the utilization of a solvent to dissolve the drug. The polymer utilized to encapsulate should not have any tendency to dissolve into the core solvent.

Nanocapsules can either be obtained by interfacial polymerization of monomers or from preformed polymers. In the former, the molar mass of the coating polymer will depend on the preparation conditions and even on the drug used, whereas in the later, it is determined at the outset.

Polymerization of monomers may lead to a covalent linkage between the polymer and the drug. To date, all the methodologies described for preparing nanocapsules involve the preparation of emulsions. (O/W) emulsions lead to the formation of nanocapsules with an oily core, suspended in water whereas (W/O) emulsions lead to nanocapsules with an aqueous core, suspended in oil.

1.4.1 Preparation

1.4.1.1 Nanocapsules Formulation by Interfacial Polymerization

The technique of obtaining nanocapsules by interfacial polymerization allows the polymer membrane to follow the contours of the internal phase of an O/W or W/O emulsion, minimizing drug escape by ensuring proper trapping, although there is always a slight chance of drug also undergoing some sort of chemical transformation. For a polymerization reaction to qualify as a fabrication tool for nanocapsules, it should be rapid and proceed preferentially along the interface. Alkylcyanoacrylates are excellent candidates for undergoing rapid polymerization within seconds, and have consequently been heavily utilized for the preparation of both oil- and water-containing nanocapsules.

1.4.1.1.1 Oil-Containing Nanocapsules

Nanocapsules which harbor oil in their core are adept at encapsulating lipophilic drugs. Most routine method of fabrication is interfacial polymerization of alkylcyanoacrylates. Initially O/W emulsion is prepared in an aqueous solution supplemented with ethanol/acetone and surfactant. Ethanol/acetone is necessary to disperse oil droplets uniformly in the aqueous phase. To initiate polymerization specific initiator, viz. ion or free radical is added to water. In typical method (Fig. 1.15) oil, monomer, and drug are dissolved to form a homogenous organic phase. Organic phase is then injected into aqueous phase under stirring. After formation of nanocapsules the residual organic phase is removed under reduced pressure. The excipient profile of nanocapsules, includes vegetable, mineral oils and derived compounds such as ethyl oleate and benzyl benzoate, Miglyol®, Lipiodol® (al Khouri *et al.,* 1986), benzyl benzoate Poloxamers, Triton X-100 and Tween 80. The only drawback of his method is that, simultaneous formation of small amount of nanospheres cannot be precluded. Solvents such as acetone, ethanol, n-butanol and acetonitrile lead to high-quality nanocapsule formation.

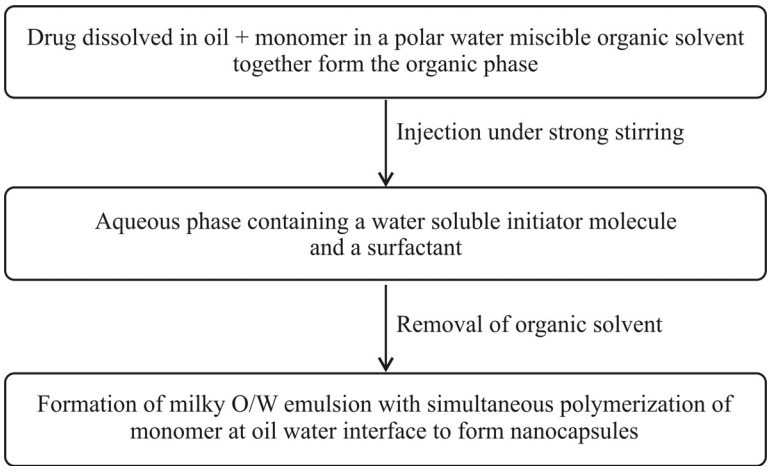

Fig. 1.15 General sequence of steps leading up to formation of oil containing nanocapsules.

1.4.1.1.2 Nanocapsules Containing an Aqueous Core

Considering the mechanism of nanocapsule formation explained above, it is difficult to comprehend the inclusion of water soluble compounds, like proteins, peptides within nanocapsules. Yet water soluble compounds have been captured in aqueous core. Interfacial polymerization, is the predominant pathway here too and the alkylcyanoacrylates monomers are added to external phase of a W/O emulsion. Polymerization of the monomer in the oily phase is initiated at the interface by initiators such as hydroxyl ions in the aqueous phase, leading to the formation of nanocapsules with an aqueous core. In a sample procedure, an aqueous phase consisting of ethanol and water is prepared. This solution is emulsified in an organic phase made up of Miglyol® or Montane® 80. Following this the monomer is slowly added to the external medium under stirring, leading to formation of aqueous nanocapsules. These nanocapsules are very useful for the encapsulation of hydrophilic compounds such as oligonucleotides and peptides. A basic illustration of the steps involved in formation of these nanocapsules is depicted in Fig. 1.16.

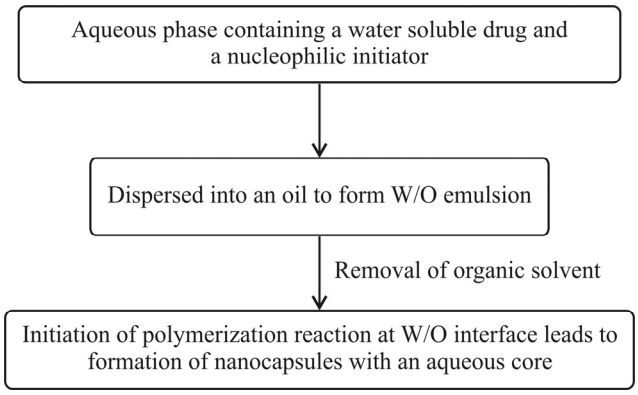

Fig. 1.16 Steps leading up to formation of aqueous core containing nanocapsules.

1.4.1.2 Nanocapsules Obtained from Preformed Polymers

Nanocapsules from preformed polymers are preferred in cases where the batch to batch variability of, interfacial polymerization is a cause of concern. The lack of control on polymeric mass, non-uniformity of size distribution and presence of trace amount of residual monomers all drawbacks, which can be circumvented by using pre formed polymers with standardized physic-chemical properties. Polymers are usually in soluble in both oil and water and consequently require an organic solvent to undergo solubilization in oil phase. Once formed this homogenous organic phase is added to aqueous phase. Due to the tremendous amount of turbulent forces generated, by the fluctuation in surface free energy of the system, the polymer diffuses with organic solvent towards the aqueous phase, only to be precipitated at the interface of oil and water. This leads to surrounding of oil phase with polymeric shell, forming a nanocapsule. If the organic solvent is not soluble in water as is the case with dichloromethane, an emulsion is formed upon stirring, in which the solvent droplets are distributed throughout the aqueous media. In such cases evaporation of the organic solvent under reduced pressure or otherwise is responsible for precipitation of polymer around the oily core. The basic outline of nanocapsule formation is described in Fig. 1.17. Synthetic polymers such as PLA, PLGA, PCL and poly (alkylcyano-acrylate) are most frequently employed. The size of nanocapsules is usually found between 100 and 500 nm, and depends on several factors, namely, the chemical nature and the concentration of the polymer and the encapsulated drug, the amount of surfactants, the ratio of organic solvent to water, the concentration of oil in the organic solution, and the speed of diffusion of the organic phase in the aqueous phase.

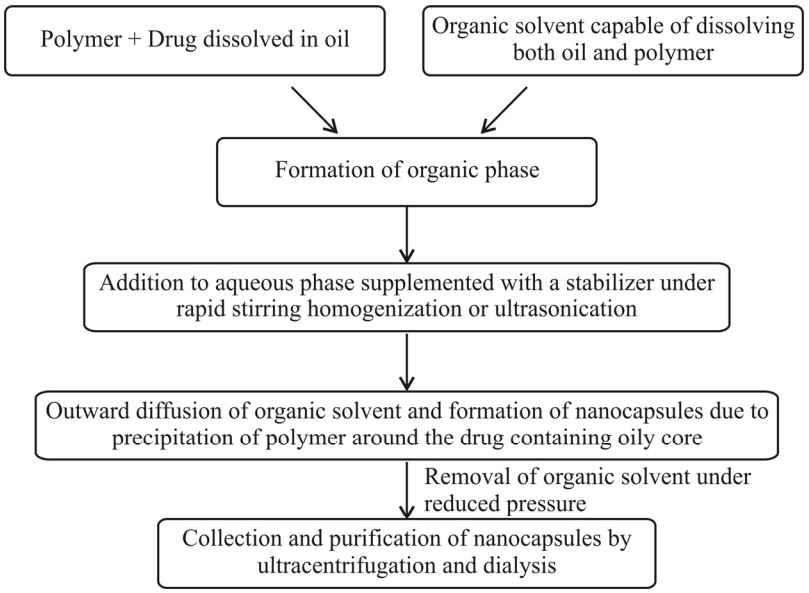

Fig. 1.17 Basic steps involved in formation of nanocapsules from preformed polymer.

1.5 Nanofibers

Nanofibers are porous polymeric thread like structures, entangled with each other, each individual fiber having a diameter in the range of 2 nm to several micrometers. These are classified as fibers due to their structural similarity to the fibrous structures such as silk found naturally or those synthesized, only difference being the size. The special nanometric size of these fibers accords them several specialized properties non-existent in their parent materials normally. Nanofibers have a very large surface-area-to-volume ratio, as large as 1000 times that of a microfiber. High porosity, interconnectivity, micro scale interstitial space, and a high aspect ratio means that nanofiber meshes are an excellent material for biomembrane replication, especially in biotechnology and environmental engineering applications. Nanofibers can form an effective size exclusion membrane for particulate removal from waste water.

Nanofibers can entrap drugs both within their polymeric structures and within the minute interstitial spaces due to surface adsorption or drug locking. The drug so dispersed in the nanofibrous structure is consequently even smaller in size, and becomes faster dissolving. The dispersion of drug in the nanomeric fibers controls drug release like all

polymeric network structures either by producing a rate controlling barrier, or by providing a tortuous pathway, limiting entry and exit of dissolving media. Polymers which hydrate or swell but are insoluble can also be used to create sustained-release nanofibers. Xiao-Mei Wu *et al.,* (2011) prepared core-shell PAN nanofibers encapsulated α-tocopherol acetate and ascorbic acid 2-phosphate for photoprotection. The results showed that core-shell nanofibers alleviated the initial burst release and gave better sustainability. Maedeh Zamani *et al.,* (2010) have developed biodegradable PCL nanofibers of metronidazole benzoate for treatment of periodontal infections prolonging drug release upto 19 days. These examples just elucidate the capability of nanofibres to modulate release rate of trapped drug as per requirement.

Dissolution rate can be accelerated by dispersing the drug in a nanofiber made out of water soluble polymer such as polyethylene glycol. The fiber instantaneously dissolves when in contact with water leaving behind nanomerized drug subject to rapid dissolution as in case of nanocrystals. Fibers have high encapsulation efficiency as there is no loss during the preparation. Nanofibres could pave the way for the development of a 'smart' polymeric drug delivery system. For example, a drug-loaded, pH-sensitive polymer is targeted to diseased cells through cell receptor binding of a ligand. It is subsequently endocytosed. In the low pH environment of the endosome, the polymer backbone detaches from the drug, disrupting the endosomal membrane, and releases the drug into the cytoplasmic compartment of the cells. One of the outstanding features of nanofibers is that they mimic the extracellular matrix developed by variety of proteins present in the human body to such an extent that they even stimulate cell proliferation when placed inside the body. This proves to be doorstep of huge possibilities of utilizing nanofibers in field of organ-transplant, tissue regeneration and bone reconstruction.

1.5.1 Polymers used for Development of Nanofibers

There are a wide range of polymers that are used in electrospinning and are able to form fine nanofibers within the submicron range. Nanofibers can be prepared from various materials including synthetic polymers, natural polymers or a blend including proteins, nucleic acids and even polysaccharides. Typical natural polymers include collagen, chitosan, gelatin, casein, cellulose acetate, silk protein, chitin and fibrinogen. Scaffolds fabricated from natural polymers promise better clinical functionality. Over the years, more than 200 polymers have been used. Enteric-release nanofibers can be created by enteric polymers such as

methacrylic acid copolymers, and sustained-release nanofibers can be created by PLA, PLGA or any other suitable polymers. Currently, there are three techniques available for the synthesis of nanofibers: electrospinning, self-assembly, and phase separation and out of these, electrospinning is the most widely studied technique.

1.5.2 Electrospinning

Electrospinning, an electrostatic fiber fabrication technique is most popular technique used for development of nanofibers. Electrospinning uses an electrical charge to draw very fine (typically on the micro or nano scale) fibres from a liquid. Electrospinning from molten precursors is also practised; this method ensures that no solvent can be carried over into the final product. Currently, there are two standard electrospinning setups, vertical and horizontal. Electrospinning is conducted at room temperature with atmosphere conditions. The typical set up of electrospinning apparatus is shown in Fig. 1.18. Basically, an electrospinning system consists of three major components: a high voltage power supply, an injector (usually a hypodermic syringe) and a grounded collector and utilizes a high voltage source to inject charge of a certain polarity into a polymer solution or melt, which is then accelerated towards a collector of opposite polarity. Most of the polymers are dissolved in some solvents before electrospinning, and when it completely dissolves, forms polymer solution. The polymer fluid is then introduced into the capillary tube for electrospinning.

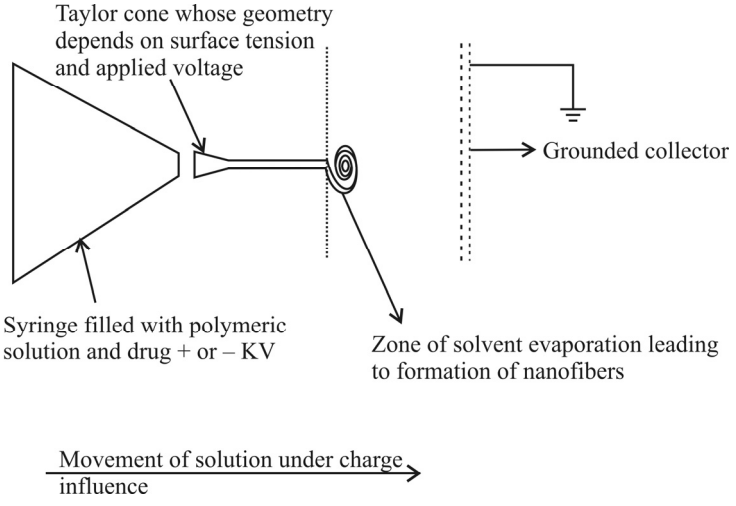

Fig. 1.18 Horizontal set up of electrospinning apparatus.

When a sufficiently high voltage is applied to a liquid droplet, the body of the liquid becomes charged. This charge creates an electrostatic repulsion within the solution which starts opposing the surface tension acting on the liquid and consequently causes surface disruptions and stretching of the polymeric solution; at a critical voltage, stream of liquid erupts from the surface. This point of eruption is known as the Taylor cone as the shape acquired by the liquid is that of a cone. If the molecular cohesion of the liquid is sufficiently high, stream does not breakup into tiny droplets (if it does, droplets are said to be electrosprayed instead of electrospinning) and a charged liquid jet is formed. As the jet of polymeric solution dries in flight due to evaporation of volatile solvent it is also elongated by a whipping process caused by electrostatic repulsion initiated at small bends in the fiber, until it is finally deposited on the grounded collector. Elongation of the fiber resulting from this bending instability leads to the formation of uniform fibers with nanometer-scale diameters. A stable jet is formed when the charge is increased above a critical voltage, and there is a balance between the surface tension of the fluid and the repulsive nature of the charge. The presence of molecular entanglements in the polymer solution prevents the jet from breaking into droplets. When more volatile solvents are used, solvent-rich regions begin to form during electrospinning that transform into pores (Bognitzki *et al.,* 2001). Drugs which are not thermolabile can be directly woven into fibers by employing polymeric melts. This avoids use of any organic solvents, which are always a concern. The electrospinning process is affected by many parameters, classified roughly into solution parameters, process parameters, and ambient parameters. Solution parameters include concentration, viscosity, conductivity, molecular weight, and surface tension, process parameters include applied electric field, tip to collector distance and feeding or flow rate. Orifice diameter, flow rate of polymer, and electric potential are influences fiber diameter. Process parameters such as distance between capillary and metal collector determine the extent of evaporation of solvent from the nanofibers, and deposition on the collector, whereas motion of collector determines the pattern formation during fiber deposition. The above stated parameters thus affect the fibers morphology obtained as a result of electrospinning, and by proper manipulation of these parameters we can get nanofibers of desired morphology and diameters (Chong *et al.,* 2007). In addition to

these variables, working parameters such as the humidity and temperature of the surroundings also play a significant role in determining the morphology and diameter of electrospun nanofibers (Li and Xia, 2004).

1.5.3 Spontaneous Assembly

It is also known as molecular assembly. It is the spontaneous organization of individual molecules into structurally-defined stable arrangements through preprogrammed non-covalent interactions, such as hydrogen bonds, van der Waals forces, hydrophobic interactions, London forces, dipole-dipole interaction, dipole induced dipole interaction and other electrostatic interactions. Self-assembly is a bottom up technique utilizing small building blocks such as peptides, small chain amino acids, polymeric segments and even monomers. A special class of compounds known as polymeric amphiphiles have been utilized in fabrication of nanofibers used to mimic bioactive structures such as collagen. In such cases the lipophilic tail much like as in micelles undergo spontaneous association (Fig. 1.19) due to hydrophobic bonding. The association is brought about by a triggering stimulus such as pH change, temperature fluctuation, introduction of a catalyst, or a bivalent cation such as calcium which neutralize the electrostatic repulsion between molecules to allow clustering of the hydrophobic tails in the core.

Under similar principles, synthetic diblock/triblock polypeptides and dendrimers can also self-assemble into nanofibrous structures. Ionic self-complementary oligopeptides (consisting of repeating ionic hydrophilic and hydrophobic amino acids) can also serve as building blocks to self-assemble into nanofibers.

The advantages of self-assembly for nanofiber fabrication include the simple fabrication process and easy drug encapsulation. It also paves the way for development of injectable form of nanofibers which undergo *in situ* scaffold formation in response to a triggering stimulus like temperature fluctuation, pH change, etc. Although the mechanical properties of nanofibers formed by self-assembly can be adjusted to some extent, they are often insufficient to provide a stable structure. There are also other shortcomings such as the limited choice of self-assembly molecules.

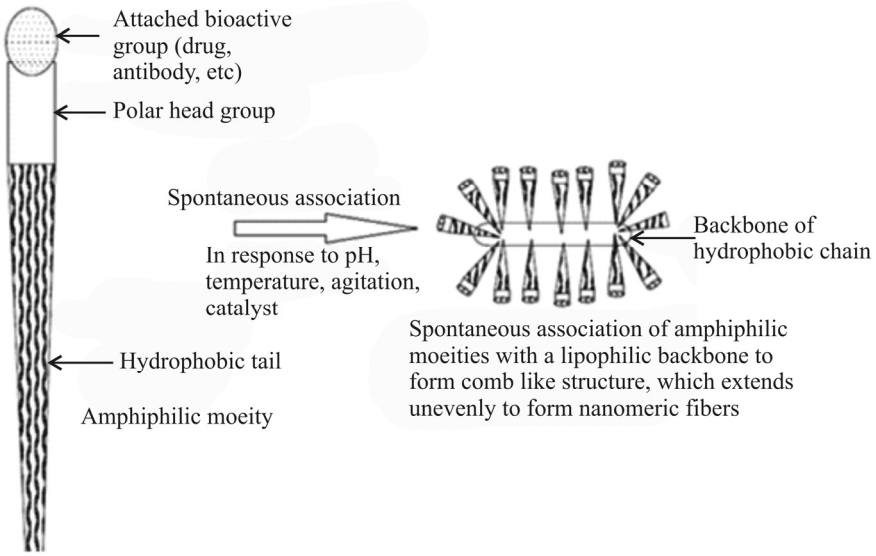

Fig. 1.19 Self-assembly for fabrication of nanomeric fibers.

1.5.4 Temperature Induced Phase Separation

In order to mimic the structure of collagen present in natural extra cellular matrix, developed a new technique called thermally induced liquid-liquid phase separation for the formation of nanofibrous foam materials has been developed (Ma and Zhang, 1999). Nano-fibrous matrices are prepared from the polymer solutions (such as PLA, PCL & PLGA) by a procedure involving thermally induced gelation, solvent exchange, and freeze-drying.The five basic steps of this method include:

1. Dissolution of polymer.
2. Liquid-liquid phase separation.
3. Polymer gelation (controls the porosity of nanoscale scaffolds at low temperature).
4. Extraction of solvent from the gel with water.
5. Freezing and freeze-drying under vacuum.

Gelation is the most critical step that controls the porous morphology of the nanofibrous foams. Other steps that can control the outcome include operating parameters such as duration and temperature of cooling, each and every step involved in process of freeze drying, etc. The duration of gelation varies with polymer concentration, solvent behaviour, gelation temperature and nature of drug molecule. The

procedure needs to be evaluated carefully since the system has to undergo freeze drying, which leads to very sensitive products and raises the operating cost aswell. A typical procedure is depicted in Fig. 1.20.

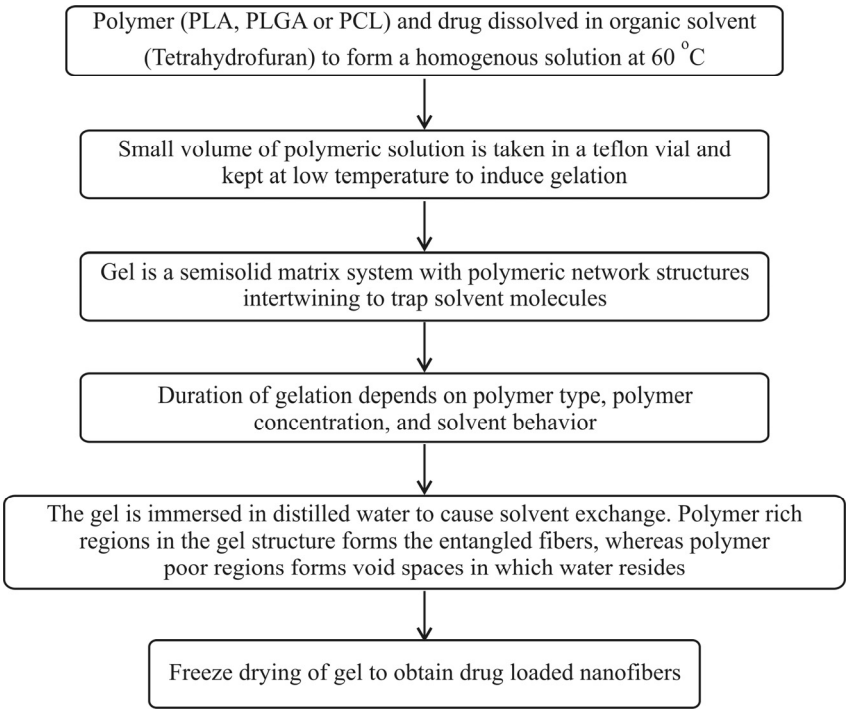

Fig. 1.20 Temperature induced phase separation/gelation causes formation of nanofibers.

1.6 Nanorods

In nanotechnology, nanorods are one class of nanoscale objects. They differ from the structures discussed above as each of their dimensions range from 1-100 nm which might not be the case in nanofibers. They may be synthesized from metals or semiconducting materials. Thus nanorods may be defined as shape anisotropic nanoparticles whose standard aspect ratios (length divided by width) are 3-25. Nanorods are produced by direct chemical synthesis. A large number of ligands act as shape control agents and bond to different facets of the nanorods with different strengths. Ligands provide:

(i) Control over nanorods shape during their synthesis,

(ii) The enhancement of colloidal stability of nanorods in solutions, and

(iii) The realization of specific properties and functions of nanorods.

This allows different faces of the nanorods to grow at different rates, producing an elongated object. In comparison to spherical particles, asymmetric particles offer addition of freedom in self-assembly due to their inherent shape anisotropy. Furthermore, the ability to synthesize nanorods with different sections provides the opportunity to introduce multiple chemical functionalities by exploiting the selective binding of different ligands to the different portions (Sun *et al.,* 2002). This facility to engineer nanorods with multiple functionalities in spatially defined region offers the potential for increased efficacy for drug and gene delivery systems.

1.6.1 Synthesis of Nanorods

1.6.1.1 Seed-Mediated Growth

Seed-mediated synthesis is the most frequently used method for producing metal nanorods such as gold (Au), silver (Ag), palladium (Pd), or copper (Cu). In order to synthesize rods with a narrow size distribution, the formation of seeds and their growth from these seeds has to be separated into two distinct stages, so that only growth of these seeds takes place in the second step and no new seeds are formed. For example, in the case of the synthesis of Au nanorods, seeds with dimensions in the range from 1 to 5 nm are prepared by reducing Au (III) to Au (0) with a strong reducing agent (sodium borohydride), while further formation of seeds in the second stage is inhibited by using a weak reducing agent (ascorbic acid) that can only reduce Au (III) to Au (I) in the presence of surfactant cetyltrimethylammonium bromide (CTAB) and silver ions. Longer nanorods (up to an aspect ratio of 25) can be obtained in the absence of silver nitrate by use of a three-step addition procedure. Elemental silver, has a lower reduction potential (less negative) than gold i.e., it has a higher affinity for electron and undergoes reduction more rapidly than gold; silver gets more rapidly deposited on the crystal structure than gold, thereby retarding the growth rate of specific crystal facets, allowing for one-directional growth and rod formation. The formation of Au nanorods from the Au seeds is achieved by the selective binding of CTAB to distinct Au crystal facets, which controls the growth rates of the different facets of the particles (Pérez-Juste *et al.,* 2005).

1.6.1.2 Synthesis of Nanorods at High Temperatures in Organic Solvents

Thermal decomposition of organometallic precursors and metal-surfactant complexes in high-boiling-point organic solvents is used to synthesize semiconductor, metal oxide, and metal nanorods with a diameter of 3-50 nm and the length 9-200 nm. The synthesis is performed *via* kinetically controlled anisotropic growth of inorganic crystals using various capping agents such as alkylamines, alkyl acids, alkylphosphonic acids, or trioctylphosphine oxide (TOPO). Generally, this method yields nanorods with a high crystallinity and a narrow size distribution. The method enables the synthesis of nanorods with a reduced number of internal defects and a uniform surface reconstruction. Both features lead to well defined physical properties of the nanorods.

1.6.1.3 Template Method

The template method for the synthesis of gold nanorods was first introduced by Martin and co-workers (Foss *et al.,* 1992); the method is based on the electrochemical deposition of gold within the pores of nanoporous polycarbonate or alumina template membranes. The method can be explained as follows: initially a small amount of silver or copper is sputtered onto the alumina template membrane to provide a conductive film for electrode position. This is then used as a foundation onto which the gold nanoparticles can be electrochemically grown. Subsequently, gold is electrodeposited within the nanopores of alumina (stage II). The next stage involves the selective dissolution of both the alumina membrane and the copper or silver film, in the presence of a polymeric stabilizer such as PVP. In the last stage, the rods are dispersed either in water or in organic solvents by means of sonication or agitation. The diameter of the gold nanoparticles thus synthesized coincides with the pore diameter of the alumina membrane. This means, that the gold nanorods with different diameters can be prepared by controlling the pore diameter of the template. The length of the nanorods can be controlled through the amount of gold deposited within the pores of the membrane.

1.6.1.4 Electrochemical Method

This method was demonstrated by Wang (1997). The synthesis is conducted within a simple two-electrode type electrochemical cell. Gold metal plate (typically 3.0 cm × 1.0 cm × 0.05 cm) is used as anode while the cathode is a platinum plate with similar dimensions. Both electrodes are immersed in an electrolytic solution containing a cationic surfactant, CTAB. CTAB not only functions as a stabilizer but also as a co-

electrolyte. During the synthesis, the bulk gold metal anode is initially consumed, forming $AuBr_4^-$. These anions are complexed to the cationic surfactants and migrate to the cathode where reduction occurs. Sonication is needed to shear the resultant rods as they form away from the surface or possibly to break the rod off the cathode surface.

1.6.1.5 Zinc Oxide (ZnO) Nanorods

In the thermal evaporation method, commercial ZnO powder is mixed with SnO_2 and evaporated by heating the mixture at elevated temperature. In the chemical reduction method, zinc vapor, generated by the reduction of ZnO, is transferred to the growth zone, followed by reoxidation to ZnO. ZnO nanorods can be prepared by following procedure reported by Xiaowang *et al.,* (Liu *et al.,* 2009). Aligned ZnO nanorod films on ITO (Indium tin oxide) glass are synthesized *via* a two-step solution approach. First, a layer of ZnO nanoparticles is formed on the surface of ITO glass by thermally decomposing of zinc acetate at 320 °C for 20 min. Then the treated ITO glass is suspended in an aqueous solution containing zinc nitrate hydrate (0.025M) and methenamine (0.025M) at 90 °C for 3 hr. On completion of the reaction, the ITO glass is removed from the solution and rinsed with deionized water.

References

Ahlin P, Kristl J, Kristl A, and Vrecer F (2002). Investigation of polymeric nanoparticles as carriers of enalaprilat for oral administration. *International Journal of Pharmaceutics* **239:** 113-120.

Akagi T, Kaneko T, Kida T, and Akashi M (2005). Preparation and characterization of biodegradable nanoparticles based on poly(γ-glutamic acid) with l-phenylalanine as a protein carrier. *Journal of Controlled Release* **108:** 226-236.

al Khouri N, Fessi H, Roblot-Treupel L, Devissaguet J.P, and Puisieux F (1986). [An original procedure for preparing nanocapsules of polyalkylcyanoacrylates for interfacial polymerization]. *Pharmaceutica Acta Helvetiae* **61:** 274-281.

Ataman-Önal Y, Munier S, Ganée A, Terrat C, Durand P.Y, Battail N, Martinon F, Le Grand R, Charles M.H, Delair T*, et al.,* (2006). Surfactant-free anionic PLA nanoparticles coated with HIV-1 p24 protein induced enhanced cellular and humoral immune responses in various animal models. *Journal of Controlled Release* **112:** 175-185.

Bognitzki M, Frese T, Steinhart M, Greiner A, Wendorff J.H, Schaper A, and Hellwig M (2001). Preparation of fibers with nanoscaled morphologies: Electrospinning of polymer blends. *Polymer Engineering & Science* **41:** 982-989.

Charcosset C, and Fessi H (2005). Preparation of nanoparticles with a membrane contactor. *Journal of Membrane Science* **266:** 115-120.

Chen Y, Liu J, Yang X, Zhao X, and Xu H (2005). Oleanolic acid nanosuspensions: preparation, *in-vitro* characterization and enhanced hepatoprotective effect. *Journal of Pharmacy and Pharmacology* **57:** 259-264.

Choi S.W, and Kim J.-H (2007). Design of surface-modified poly(d,l-lactide-co-glycolide) nanoparticles for targeted drug delivery to bone. *Journal of Controlled Release* **122:** 24-30.

Chong E.J, Phan T.T, Lim I.J, Zhang Y.Z, Bay B.H, Ramakrishna S, and Lim C.T (2007). Evaluation of electrospun PCL/gelatin nanofibrous scaffold for wound healing and layered dermal reconstitution. *Acta Biomaterialia* **3:** 321-330.

Elvassore N, Bertucco A, and Caliceti P (2001). Production of insulin-loaded poly(ethylene glycol)/poly(l-lactide) (PEG/PLA) nanoparticles by gas antisolvent techniques. *Journal of Pharmaceutical Sciences* **90:** 1628-1636.

Errico C, Bartoli C, Chiellini F, and Chiellini E (2009). Poly(hydroxyalkanoates)-based polymeric nanoparticles for drug delivery. *Journal of Biomedicine & Biotechnology* **2009:** 571-702.

Fan Y.F, Wang Y.N, Fan Y.G, and Ma J.B (2006). Preparation of insulin nanoparticles and their encapsulation with biodegradable polyelectrolytes *via* the layer-by-layer adsorption. *International Journal of Pharmaceutics* **324:** 158-167.

Fay F, Quinn D.J, Gilmore B.F, McCarron P.A, and Scott C.J (2010). Gene delivery using dimethyldidodecylammonium bromide-coated PLGA nanoparticles. *Biomaterials* **31:** 4214-4222.

Fessi H, Puisieux F, Devissaguet J.P, Ammoury N, and Benita S (1989). Nanocapsule formation by interfacial polymer deposition following solvent displacement. *International Journal of Pharmaceutics* **55:** R1-R4.

Foss C.A, Hornyak G.L, Stockert J.A, and Martin C.R (1992). Optical properties of composite membranes containing arrays of nanoscopic gold cylinders. *The Journal of Physical Chemistry* **96:** 7497-7499.

Galindo-Rodriguez S.A, Puel F, Briancon S, Allemann E, Doelker E, and Fessi H (2005). Comparative scale-up of three methods for producing ibuprofen-loaded nanoparticles. *European Journal of Pharmaceutical Sciences : Official Journal of the European Federation for Pharmaceutical Sciences* **25:** 357-367.

Ibrahim H, Bindschaedler C, Doelker E, Buri P, and Gurny R (1992). Aqueous nanodispersions prepared by a salting-out process. *International Journal of Pharmaceutics* **87:** 239-246.

Jeon H.J, Jeong Y.I, Jang M.K, Park Y.H, and Nah J.W (2000a). Effect of solvent on the preparation of surfactant-free poly(dl-lactide-co-glycolide) nanoparticles and norfloxacin release characteristics. *International Journal of Pharmaceutics* **207:** 99-108.

Jeon H.J, Jeong Y.I, Jang M.K, Park Y.H, and Nah J.W (2000b). Effect of solvent on the preparation of surfactant-free poly(DL-lactide-co-glycolide) nanoparticles and norfloxacin release characteristics. *International Journal of Pharmaceutics* **207:** 99-108.

Konan-Kouakou Y.N, Boch R, Gurny R, and Allémann E (2005). *In vitro* and *in vivo* activities of verteporfin-loaded nanoparticles. *Journal of Controlled Release* **103:** 83-91.

Konan Y.N, Gurny R, and Allémann E (2002). Preparation and characterization of sterile and freeze-dried sub-200 nm nanoparticles. *International Journal of Pharmaceutics* **233:** 239-252.

Legrand P, Lesieur S, Bochot A, Gref R, Raatjes W, Barratt G, and Vauthier C (2007). Influence of polymer behaviour in organic solution on the production of polylactide nanoparticles by nanoprecipitation. *International Journal of Pharmaceutics* **344:** 33-43.

Leroueil-Le Verger M, Fluckiger L, Kim Y.I, Hoffman M, and Maincent P (1998). Preparation and characterization of nanoparticles containing an antihypertensive agent. *European Journal of Pharmaceutics and Biopharmaceutics : Official Journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV* **46:** 137-143.

Li D, and Xia Y (2004). Electrospinning of Nanofibers: Reinventing the Wheel? *Advanced Materials* **16:** 1151-1170.

Lince F, Marchisio D.L, and Barresi A.A (2008). Strategies to control the particle size distribution of poly-epsilon-caprolactone nanoparticles for pharmaceutical applications. *Journal of Colloid and Interface Science* **322:** 505-515.

Liu X, Hu Q, Wu Q, Zhang W, Fang Z, and Xie Q (2009). Aligned ZnO nanorods: A useful Film to Fabricate Amperometric Glucose Biosensor. *Colloids and Surfaces B, Biointerfaces* **74:** 154-158.

Ma P.X, and Zhang R (1999). Synthetic nano-scale fibrous extracellular matrix. *Journal of Biomedical Materials Research* **46:** 60-72.

Matsumoto J, Nakada Y, Sakurai K, Nakamura T, and Takahashi Y (1999). Preparation of nanoparticles consisted of poly(L-lactide)-poly(ethylene glycol)-poly(L-lactide) and their evaluation *in vitro*. *International Journal of Pharmaceutics* **185:** 93-101.

Moinard-Checot D, Chevalier Y, Briancon S, Beney L, and Fessi H (2008). Mechanism of nanocapsules formation by the emulsion-diffusion process. *Journal of Colloid and Interface Science* **317:** 458-468.

Namazi H, Fathi F, and Dadkhah A (2011). Hydrophobically modified starch using long-chain fatty acids for preparation of nanosized starch particles. *Scientia Iranica* **18:** 439-445.

Nicoli S, Santi P, Couvreur P, Couarraze G, Colombo P, and Fattal E (2001). Design of triptorelin loaded nanospheres for transdermal iontophoretic administration. *International Journal of Pharmaceutics* **214:** 31-35.

Oh I, Lee K, Kwon H.Y, Lee Y.B, Shin S.C, Cho C.S, and Kim C.K (1999). Release of adriamycin from poly(γ-benzyl-l-glutamate)/ poly(ethylene oxide) nanoparticles. *International Journal of Pharmaceutics* **181:** 107-115.

Park K.H, Song H.C, Na K, Bom H.S, Lee K.H, Kim S, Kang D, and Lee D.H (2007). Ionic strength-sensitive pullulan acetate nanoparticles (PAN) for intratumoral administration of radioisotope: ionic strength-dependent aggregation behavior and (99m) Technetium retention property. *Colloids and Surfaces B, Biointerfaces* **59:** 16-23.

Pérez-Juste J, Pastoriza-Santos I, Liz-Marzán L.M, and Mulvaney P (2005). Gold nanorods: Synthesis, characterization and applications. *Coordination Chemistry Reviews* **249:** 1870-1901.

Perugini P, Simeoni S, Scalia S, Genta I, Modena T, Conti B, and Pavanetto F (2002). Effect of nanoparticle encapsulation on the photostability of the sunscreen agent, 2-ethylhexyl-p-methoxycinnamate. *International Journal of Pharmaceutics* **246:** 37-45.

Seyler I, Appel M, Devissaguet J.P, Legrand P, and Barratt G (1999). Macrophage Activation by a Lipophilic Derivative of Muramyldipeptide within Nanocapsules: Investigation of the Mechanism of Drug Delivery. *Journal of Nanoparticle Research* **1:** 91-97.

Song X, Zhao Y, Wu W, Bi Y, Cai Z, Chen Q, Li Y, and Hou S (2008). PLGA nanoparticles simultaneously loaded with vincristine sulfate and verapamil hydrochloride: Systematic study of particle size and drug entrapment efficiency. *International Journal of Pharmaceutics* **350:** 320-329.

Sun Y.P, Fu K, Lin Y, and Huang W (2002). Functionalized Carbon Nanotubes: Properties and Applications. *Accounts of Chemical Research* **35:** 1096-1104.

Tan Y, Wang P, Xu K, Li W, An H, Li L, Liu C, and Dong L (2009). Designing Starch-Based Nanospheres to Make Hydrogels with High Mechanical Strength. *Macromolecular Materials and Engineering* **294:** 855-859.

Ubrich N, Bouillot P, Pellerin C, Hoffman M, and Maincent P (2004). Preparation and characterization of propranolol hydrochloride nanoparticles: a comparative study. *Journal of Controlled Release : Official Journal of the Controlled Release Society* **97:** 291-300.

Wu X.M, Branford-White C.J, Yu D.G, Chatterton N.P, and Zhu L.M (2011). Preparation of core-shell PAN nanofibers encapsulated alpha-tocopherol acetate and ascorbic acid 2-phosphate for photoprotection. *Colloids and Surfaces B, Biointerfaces* **82:** 247-252.

Yallapu M.M, Gupta B.K, Jaggi M, and Chauhan S.C (2010). Fabrication of curcumin encapsulated PLGA nanoparticles for improved therapeutic effects in metastatic cancer cells. *Journal of Colloid and Interface Science* **351:** 19-29.

Yu Chang S.S, Lee C.L, and Wang C.R.C (1997). Gold Nanorods: Electrochemical Synthesis and Optical Properties. *The Journal of Physical Chemistry B* **101:** 6661-6664.

Zamani M, Morshed M, Varshosaz J, and Jannesari M (2010). Controlled release of metronidazole benzoate from poly epsilon-caprolactone electrospun nanofibers for periodontal diseases. *European Journal of Pharmaceutics and Biopharmaceutics : Official Journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV* **75:** 179-185.

Zhang Y, and Zhuo R.X (2005). Synthesis and drug release behavior of poly (trimethylene carbonate)–poly (ethylene glycol)–poly (trimethylene carbonate) nanoparticles. *Biomaterials* **26:** 2089-2094.

Zhang Z, Grijpma D.W, and Feijen J (2006a). Poly(trimethylene carbonate) and monomethoxy poly(ethylene glycol)-block-poly(trimethylene carbonate) nanoparticles for the controlled release of dexamethasone. *Journal of Controlled Release : Official Journal of the Controlled Release Society* **111:** 263-270.

Zhang Z, Grijpma D.W, and Feijen J (2006b). Poly(trimethylene carbonate) and monomethoxy poly(ethylene glycol)-block-poly(trimethylene carbonate) nanoparticles for the controlled release of dexamethasone. *Journal of Controlled Release* **111:** 263-270.

Zili Z, Sfar S, and Fessi H (2005). Preparation and characterization of poly-epsilon-caprolactone nanoparticles containing griseofulvin. *International Journal of Pharmaceutics* **294:** 261-267.

Zweers M.L.T, Engbers G.H.M, Grijpma D.W, and Feijen J (2004). *In vitro* degradation of nanoparticles prepared from polymers based on dl-lactide, glycolide and poly(ethylene oxide). *Journal of Controlled Release* **100:** 347-356.