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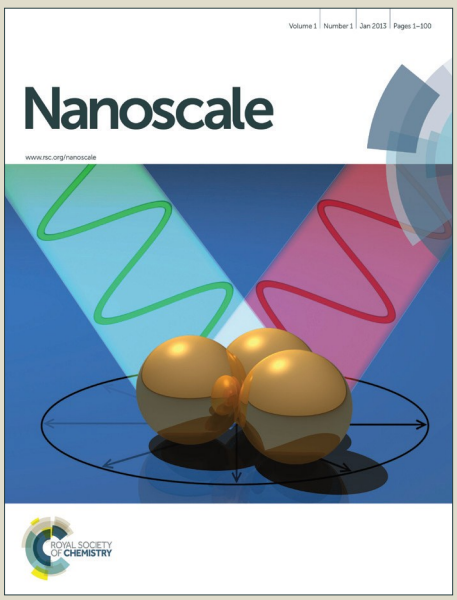

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Nanoformulation and encapsulation approaches for poorly water-soluble drug nanoparticles

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Abstract:

During the last decades the nanomedicine sector has emerged as a feasible and effective solution to the problems faced by the high percentage of poorly water-soluble drugs. Decreasing the size of such drug compounds to the nanoscale can significantly change its physical properties, which lays the foundation on the use of nanomedicine for pharmaceutical applications. Various techniques have been developed to produce poorly water-soluble drug nanoparticles, mainly to address the poor water-soluble issues but also for the efficient and targeted delivery of such drugs. These techniques can be generally categorized into top-down, bottom-up and encapsulation approaches. Among them, the top down approaches have been the main choice for industrial preparation of drug nanoparticles while other methods are actively investigated by researchers. In this review, we aim to give a comprehensive overview and latest progress of the top-down, bottom-up, and encapsulation methods for the preparation of poorly water-soluble drug nanoparticles and how solvents and additives can be selected for these methods. In addition to the more industrially applied top-down approaches, the review is more focused on bottom-up and encapsulation methods, particularly covering supercritical fluid-related methods, cryogenic techniques, and encapsulation with dendrimers and responsive block co-polymers. Some of the approved and mostly used nanodrug formulations on market are also covered to demonstrate the applications of poorly water-soluble drug nanoparticles. This review is completed

with perspective on the development and challenges of fabrication techniques for more effective nanomedicine.

1 Introduction

A survey done in 1988 over a period of 20 years demonstrated that 40% of all pharmaceutical drugs produced in major companies in the UK showed low bioavailabilty[1]. Bioavailability is defined as the fraction of an administered drug that reaches systematic circulation[2]. Drugs with low bioavailability are prone to either accumulate in tissue due to their high lipophilicity or be eliminated via a first-pass metabolism in the intestines or liver, because of low solubility and inability to pass the intestinal walls. Hence many water-insoluble drugs need to be administered intravenously and in high quantities to reach target sites in suitable concentrations, which often goes hand in hand with discomfort for patients. Estimation in 2000 put the cost of drug development, from first discovery to approval for a single drug at 800 million[3, 4], with one of the reasons being that only one in ten developed drugs achieve final approval[5]. Methods like high-throughput screening[6], computer-aided and structure-based drug design[7] as well as fragment-based lead discovery[8] increased the rate of success in pharmaceutical research. Due to the adoption of these methods, drugs showing low bioavailability may be identified early on. Hence the percentage of drugs not passing Phase I testing because of low bioavailability decreased from 40% to 10% between 1991 and 2000[5]. That involves the risk of highly efficient drugs being abandoned because of low solubility. As an example of this, a list of essential drugs compiled by the world health organisation in 2004 cited that only 23.6% of the listed drugs were the Biopharmaceutics Classification System (BCS) Class I drugs (high solubility and high permeability, Fig. 1), as defined by Amidon[9], and thus showing high bioavailability[10]. Over the past years, much research efforts went into tackling the poor water solubility problem. Various methods have been developed, for example, cyclodextrin complexation[11], crystal modification[12], solid dispersions[13], polymeric micelles[14], lipid-based delivery[15], and hot melt extrusion[16].

The prodrug design approach, where the drug is kept in an inactive state exhibiting better solubility or permeability, rely on specific metabolic mechanisms to change the inactive prodrug to an active one[17]. Prodrugs can either consist of a drug linked to a carrier or antibody that is cleaved off by a metabolic mechanism or be a precursor that is turned into the active form by hydrolyses, oxidation or other metabolic reactions[18-21]. In addition to the difficulty in synthesis and test, rodents and other animals show slightly different metabolistic conditions, *e.g.*, enzymes or level of transporters, metabolites from carriers, or metabolic intermediates during activation[22]. Another highly researched area is the formulation by nanotechnology, where the size of drug particles is decreased to the nanometre range. By downsizing the poorly water-soluble drug particles, thermodynamic and kinetic characteris-

tics change, giving rise to new attributes like enhanced water solubility and applications such as dry powder inhalation [23, 24]. Solubility is increased on the basis of the Oswald-Freundlich equation[25-27]:

$$\log \frac{c_S}{c_\infty} = \frac{2\sigma V}{2.303RT\rho r}$$

With c_S=saturation solubility, c_∞=solubility of large particles, σ=interfacial tension, V=atomic volume, R=gas constant, T=absolute temperature, ρ=density of the solid, r=radius.

The dissolution rate increases at the same time due to the decrease in size and subsequent increase in surface area as shown in the Noyes-Whitney equation[28]:

$$\frac{dc}{dt} = D \times A\left(\frac{c_s - c_x}{h}\right)$$

With D=diffusion coefficient, A=surface area of the particle, c_S=saturation solubility, c_x=bulk concentration and h=diffusional distance[29]. There are two major strategies in nanoparticle formulation: top-down and bottom-up approaches. That is, the drug material can either function in itself as a nanoparticle by being downsized from the large particles (top-down) or growing from molecules in solution (bottom-up). Drugs may be also encapsulated in nanoparticle forming material (*e.g.*, liposomes, dendrimers, polymers). In this review, we overview the working principles and recent development of various methods developed for both top-down and bottom-up techniques. Due to recent excellent reviews on top-down approaches[30, 31], we focus more on bottom-up techniques (particularly the novel cryogenic methods, supercritical fluid-related methods) and encapsulation approaches, discuss how solvents and additives may be selected to achieve stable and uniform nanoparticle dispersions, and also describe the applications of such poorly water-soluble drug nanoparticles. We conclude the review with a summary and perspective on the development and challenges of nanoformulation techniques.

2 Top-Down techniques

Starting with large particles and producing nanoparticles by mechanical means is known as a top-down process. In the following section the two main approaches are described and an overview of advantages and disadvantages are given in Table 1.

2.1 Wet Media Milling

In a wet media milling process, drug, stabilizer and dispersion media (*e.g.*, water) are mixed in a milling chamber with an agitator or by moving the whole container (Fig. 2) [32, 33]. As milling materials, small beads made of steel, glass or ceramics (*e.g.*, yttrium stabilized zirconium dioxide) are used. Since these materials are prone to abrading, polystyrene resin beads may provide a better option in terms of reducing abrading impurities into the drug formulation[34]. Size reduction is a consequence of

shear forces generated by the moving beads as well as particle collision. Final particle size is determined by bead size, concentration, dispersion media as well as hardness of the drug, temperature, and milling time (ranging from minutes up to several days)[30, 33]. Takatsuka *et. al.* needed 5 minutes to produce nanoparticles with a small amount of zirconia beads by using a rotation/revolution mixer[35]. By varying aforementioned conditions, fine tuning in size can be achieved, although limitations in the low nanometre range remain. The main drawbacks of wet medial milling are traces of solvent or milling material remaining after size reduction, as well as the need to use already micronized starting material[36]. Nanoparticles produced by milling have a wide variety of administrations.

Ye *et al.* could prepare niclosamide nanocrystals for parenteral administration[37], while Patel *et al.* produced nanosuspensions of efavirenz for improved oral bioavailability. Zhai and colleagues improved the media milling process of caffeine nanoparticles for dermal delivery by using Carbopol® 981 as a stabilizer in a medium of ethanol–propylene glycol (3:7)[38]. Subsequent stability tests showed the nanoparticles were stable at 4 and 25 °C for 60 days[39]. Krakan and colleagues reported in a study that quercetin nanocrystals produced by milling were stable as aqueous suspensions of 10 and 5 wt% at temperatures up to 40 °C for 180 days[40]. Further particle size reduction can be achieved by combining the media milling process with other top-down process. The combination technology (CT) could start with a low energy milling step, followed by a high pressure homogenization step. This improved homogeneity of the nanoparticle size and stability by preventing Oswald ripening[41]. It was utilized by Al Shaal *et al.* to produce apigenin nanocrystals and showed that anti-oxidant activity almost doubled in vitro[42].

2.2 High Pressure Homogenization

Depending on the homogenizer type or principle used, two basic techniques microfluidisation and piston-gap homogenisation have been utilized.

2.2.1 Microfluidisation

In the microfluidisation method, two drug suspensions are forced under high pressure (up to 1700 bar) to collide frontally[43]. The resulting shear forces, particle collisions as well as cavitation lead to size reduction[44]. Stabilizers are added to stabilize the resulting nanoparticles. Z-type or Y-type shaped collision chambers can be used. To generate nanoparticles a high number of cycles (up to 200 passes) may have to be performed[45]. In 2013, Strydom *et al.* synthesized an antibacterial and water insoluble drug silver sulfadiaziane nanoparticles by microfluidisation. By combining with poly(amidoamine) dendrimers, both high antibacterial properties and high drug delivery efficiency could be achieved[46]. Helgason *et al.* used microfluidisation to prepare nanoemulsions with droplet sizes ranging from 36-141 nm to demonstrate the change in absorbance by solidification of the nanosized droplets to nanoparticles[47].

2.2.2 Piston-Gap

Like microfluidisation, nanoparticle formation by piston-gap homogenization is also a consequence of particle collision, shear and cavitation forces. The starting suspension of drug microparticles and stabilizer in aqueous or non-aqueous media is forced with high velocity through a small nozzle. According to Bernoulli's equation, static pressure of a liquid decreases while passing through a narrow gap at high speed[48]. Nanosizing is a consequence of the sudden change in pressure from inside the piston-gap homogenizer, to atmospheric pressure. Due to the low pressure inside the piston-gap homogenizer the solvent begins to boil at room temperature[36]. With the sudden increase in pressure after the gap the formed gas bubbles implode, leading to cavitation and particle collision[49]. Final size is depending on pressure applied and number of cycles. With higher pressure more energy is available for the cracking of crystals[48]. At the highest pressure setting of 1500 bar the nozzle is minimizing to 25 µm. Particles bigger than 25 µm need to be pre-milled as to not block the nozzle. Size uniformity is a direct consequence of the number of cycles, although it should be noted that for each drug a different lower limit of size exists. Hence after a specific number of cycles, depending on the drug used, the particle size does not change, but size homogeneity of the batch gets more uniform[50]. Gao *et al.* showed that uniformity of oridonin nanoparticles was directly dependent on the surfactant used[48]. Möschwitzer *et al.* demonstrated the improved stability for the nanosuspension produced by piston-gap containing up to 60% more non-degraded omeprazole after one month at 4 °C than corresponding drug solution[50]. In recent years, the piston-gap technology has been mainly used as part of top-down bottom-up combination approaches. For example, Salazar *et al.* developed a technique named H96 combining a freeze-drying step followed by piston-gap homogenizing. The freeze drying step made the drug more brittle and fragile. As such it was easier broken into nanosized particles by homogenizing. Compared to standard high pressure homogenization methods, the H96 method gave generally smaller particles with less number of cycles[51]. Other combinative techniques including high pressure homogenization as a size reduction step are: NANOEDGE[52] (first step microprecipitation), H42[53] (first step spray drying) an CT[41] (first step pearl milling).

3 Bottom-up techniques

Methods starting with solutions and then producing nanoparticles are categorized as bottom-up techniques. In the following section different approaches are described and an overview of advantages and disadvantages is given in Table 2.

3.1 Nanoparticle formation by precipitation techniques

3.1.1 Solvent anti-solvent precipitation

Solvent anti-solvent (SAS) precipitation is the most straight forward bottom-up technique for producing nanoparticles. In the SAS process a water insoluble drug is dissolved in an organic solvent. The

solution is then mixed with a solvent in which the drug is not soluble (antisolvent), containing stabilizers. The chosen solvents need to be miscible with each other. Precipitation of nanoparticle sized drug is immediate[60]. Formation of nanoparticles is due to the Marangoni effect and directly dependent on interfacial turbulence of the phase interface and flow, diffusion and surface tension[61]. These are influenced by the speed of mixing, solvent, stabilizer, and temperature. Studies on mixing have shown that an increase of mixing speed reduces particle size. Higher mixing speed leads to greater micromixing, the mixing on molecular level, between two phases. As a result, mass transfer and diffusion from one phase to the other increases and a higher supersaturation of drug in the antisolvent phase is reached. This causes faster nucleation and subsequently a narrower particle size distribution (PSD)[62]. A study by Zhao *et al.* showed that a higher flow rate in a reactor led to smaller particles and that the ratio of antisolvent to solvent was inversely proportional to the size of the obtained particles [63]. Another study determined that the rate of diffusion was decreasing when the dielectric constant values of the solvents increased[61]. A study on cefuroxime without the use of stabilizers found that the state of the nanoparticles (crystalline or amorphous) was determined by the anti-solvent used. Crystalline particles were formed when water was used as anti-solvent, while organic anti-solvents gave amorphous particles.

Stabilizers prevent Oswald ripening by covering the surface of the formed nanoparticles. Surfactants can also be used as surface wetting agents for orally administered drugs to make them more available [64]. Generally an increase in surfactant concentration leads to a decrease in particle size, till a plateau is reached where the size does not change anymore. In certain cases it has been found that stabilizers were more effective when dissolved in the solvent phase. Although the particle size was slightly bigger it was possible to use less stabilizer since being dissolved in the same medium as the drug made the stabilizer more readily available[65]. Another factor determining size is temperature. Lower temperatures in general lead to smaller sizes. At lower temperatures the solubility of drug decreases and the metastable zone gets narrower. Simultaneously an increase in nucleation can be observed since nucleation is a process of free energy and heat release[62].

In the SAS process the obtained nanoparticles need to be dried immediately to prevent crystal growth. Due to this, research has focused on combining different bottom-up techniques. Hu *et al.* presented an industrially applicable process which combined the SAS precipitation followed by a freeze drying step for continuous and scalable nanosizing of fenofibrate. It was found that short stirring time in the SAS step and immediate freeze drying minimized the Oswald ripening and led to faster dissolution in water [66]. Homayouni and colleagues incorporated a high pressure homogenizing step between SAS precipitation and freeze drying. With soluplus as stabilizer, celecoxib nanoparticles around 440 nm were produced with a 4 times higher solubility than raw celecoxib. Unexpectedly, crystalline samples dissolved better than amorphous, which could be attributed to the devitrification of amorphous celecobix when in contact with water [67]. Using only the SAS technique, Zu *et al.* produced taxifolin

nanoparticles, a water insoluble antioxidant. By reducing the size to 24.6 nm, they found that the solubility increased two times compared to the raw material, as well as the improved bioavailability and antioxidative ability [68]. Another approach using ionic liquids as antisolvent and phosphate buffer as solvent was done by Viçosa *et al.*, producing amorphous rifamipicin particles with a mean size of 280-360 nm at room temperature[69].

A variation of the SAS precipitation process is high gravity reactive precipitation (HGRP). On the basics of the Higee technology[70], a rotating packed bed (RPB)[71, 72] is used to form nanoparticles. Solvent and antisolvent are added to the rotating RBP. Centrifugal forces and high gravity in the chamber generate a thin liquid film or fine droplets. This in turn intensifies micromixing as well as mass transfer (Fig. 3)[73, 74, 59]. Chen and colleagues showed that the size and size distribution were decreased for calcium carbonate and benzoic acid by increasing speed till a plateau was reached [75, 76]. The same effect was observed on intraconazole by Zhang *et al*. They argued that the decrease in size was a consequence of intensified splitting effects. The same decrease in size was observed when the solvent ratio and flow rate were altered. Higher anti-solvent concentration led to smaller particles, because of higher supersaturation[74].

3.1.2 Sonoprecipitation

Most SAS processes struggle with poor micromixing. This problem may be solved by the use of ultrasonic waves in a sonoprecipitation process. This process leads to rapid and more thorough mixing. As a consequence, maximal supersaturation and crystal growth arrest are reached faster. Initial cavitation and size reduction occur during the negative period of the sound wave, which generates cavitation bubbles. Implosion of bubbles results in localized spikes in temperature and pressure, leading to the creation of shock waves. The consequence for crystallisation is immediate formation of primary nucleation, reduction in crystal size, and inhibition of agglomeration[77, 78]. Particle size, particle size distribution, and particle morphology are dependent on duration of sonification. Dhumal and colleagues prepared amorphous cefuroxime axetil particles by sonoprecipitation. It was observed that by lowering the temperature, cefuroxime axetil precipitated faster with a higher yield and a smaller size. The same result was obtained when increasing the sonication amplitude. A comparison study for cefuroxime axetil nanoparticles produced by spray drying and sonoprecipitation demonstrated similarity in both lower yields, increased size and broader particle size distribution for both processes[79].Gawali *et al.* prepared amorphous ritonavir nanoparticles. The test sample was completely dissolved in 1h in 0.1 M HCl, while at the same time only 28% of crude material could be dissolved[80]. Jiang *et al.* downsized simvastatin particles to 360 nm and tested the dissolution rate and oral bioavailability. It was observed that not only the dissolution rate increased but that the area under the curve (AUC), drug blood plasma concentration against time, and c_{max} increased as well[81]. With sonoprecipitation it is possible to not only influence size but also morphology of particles, which has an impact on dissolu-

tion. Tran *et al.* studied the influence of three polymer types and ultra-sonication conditions on the shape and size of curcumin nanoparticles. It was found that by changing drug to polymer ratio and reaction conditions the molecular interaction of the curcumin hydroxyl groups changed and subsequent size and dissolution rate could be modulated[82]. The effect of morphology on dissolution rate was also investigated by Guo and colleagues on lovastatin. They produced rod-shaped drug nanoparticles via sonoprecipitation, while a milling process gave spherical particles. Although both particles had the same crystal state and diameter, rod-shaped lovastatin could be dissolved better and showed a higher oral bioavailability, yet the AUC was the same[83].

3.1.3 Supercritical fluid precipitation

Most drugs produced by precipitation techniques employing organic solvents show trace amounts of solvents even after purification. Using supercritical fluids as solvents could overcome this issue. Supercritical fluids, where both the temperature and pressure of the fluids are higher than the critical temperature and pressure respectively, exhibit the density of a liquid but the mass transfer ability between gas and liquid, making them ideal candidates to be used as solvents for precipitation processes[84]. The supercritical fluids can be removed from the nanoparticles simply by depressurisation. Due to its mild critical points, non-toxicity and non-flammability, CO_2 has been the most widely used supercritical fluid [85]. When a low temperature is favoured (e.g., < 31 ^oC), compressed CO_2 can be also used as solvent or medium for reaction or processing[86]. The major techniques to produce drug nanoparticles utilizing supercritical fluids are rapid expansion of supercritical solution (RESS), rapid expansion of supercritical solutions into a liquid solvent (RESOLV), supercritical antisolvent precipitation (SAS), gas antisolvent (GAS), and precipitation with a compressed fluid (PCA).

3.1.3.1 Supercritical fluid acting as a solvent

In the RESS and RESOLV processes, the supercritical fluid acts as a solvent. In the RESS process a supercritical liquid dissolving a drug pass through a thin nozzle and then release the presure[87]. The rapid expansion of the supercritical fluid via the reduction in pressure leads to a high supersaturation of drug in the fluid droplets and the subsequent homogenous nucleation. The size of the particles depends on expansion temperature, pressure, nozzle geometry and lengths, solute-solvent interaction, and solubility of compound in supercritical liquid. Most RESS processes employ supercritical CO_2 as solvent. Keshavarz and colleagues investigated the effect of extraction temperature and spraying distance for the preparation of raloxifene particles. It was found that at 50 °C, 17.7 MPa and 10 cm distance the size of raloxifene decreased from 45.28 µm to 18.93 nm and increased the dissolution rate seven folds[88]. This study was extended later by Keshmiri *et al.* who studied the influence of extraction pressure, pre-expansion temperature and co-solvent on the size of clobetasol propionate particles. It was found that higher pressure led to smaller particle sizes, while higher temperature and the addition of co-solvent resulted in an increase in particle size. The findings were explained by a higher concen-

tration of clobetasol propionate in the supercritical fluid which led to more particle collision and as such accumulation[89]. Although there are examples for nanosized particles, the RESS process produced mainly particles in the micrometre range[90]. To overcome this problem, the RESS process was extended by expansion of supercritical fluid into a liquid solvent rather than air. This process is called RESOLV. It is rationalized that the liquid solvent inhibits crystal growth in the expansion jet. The nanoparticles may be further prevented from accumulating by adding stabilizer to the solvent phase. For example, using the RESOLV process, Pathak *et al.* prepared ibuprofen nanoparticles at 40 nm[90]. A systematic screening of the influence of stabilizer in the liquid solvent on particle size was then performed. It was found that the critical factor impacting ibuprofen nanoparticles was the molecular weight rather than the concentration for the stabilizers of polyvinylpyrrolidone and poly(ethylene glycol). The particle size decreased with the increase of molecular weight[91].

3.1.3.2 Supercritical fluid as an antisolvent

Supercritical fluids can be introduced as anti-solvent for the SAS, GAS, and PCA processes. The common feature is that the drugs are dissolved in organic solvents (usually CO2-miscible) and then precipitate as a result of anti-solvent impact from the introduced compressed or supercritical fluid. For example, in the SAS process, the supercritical fluid is introduced into a high pressure vessel which already contains liquid solution. Like in the common SAS precipitation technique but better than the common anti-solvent, the high diffusion ability of the supercritical fluid leads to rapid diffusion/mixing at the interface and hence rapid supersaturation and precipitation. The supercritical fluid acts as an anti-solvent as well as a 'spray enhancer'. The homogeneity of the precipitated particles may be tuned by flow rate, temperature and pressure of the system. As an example, Mezzomo *et. al.* used the SAS technique to form ibuprofen nanoparticles of 380 nm in supercritical carbon dioxide ($scCO_2$)[92].

In contrast to the SAS process, a supercritical gas (the temperature is higher than the critical temperature but the pressure is lower than the critical pressure) instead of a supercritical fluid is introduced into the liquid solution in a GAS process. Quite often, the SAS and GAS process are not distinguished clearly during applications. Randolph *et al.* reported the production of poly-(L-lactic –acid) particles. It was found that similar sized particles were produced for batch and continuous method and that size was dependent on the density of $scCO_2$. The particle size was a consequence of mass transport and not of jet break-up or hydrodynamics of the droplets[93]. Yeo and colleagues utilized the GAS technique to downsize macromolecules, such as insulin, showing that the conditions of this process were mild enough to downsize complex biological structures[94]. The same was shown on insulin and catalase by Jean and co-workers. They micronized both proteins for the formation of drug-polymer microspheres for controlled release[95]. Due to the fast nature of this process, Elvassore and colleagues could capture drug molecules into a polymer matrix via simultaneous precipitation[96].

PCA process is similar to SAS in that a compressed fluid (usually higher than the critical pressure but lower than the critical temperature) is introduced into a drug solution. The solution may be also sprayed into the compressed fluid for the solute precipitation to occur. The compressed fluid is miscible with the solvent, but functions as an anti-solvent to the solute. It can diffuse into the solution and increase the supersaturation whereupon the solute precipitates [97-99]. Falk *et al.* showed the possibility to tune crystallinity of drug loaded poly-(L-lactide) microparticles by modulating the flow rate of CO_2 during precipitation. When the flow rate was increased after precipitation it was found that residual solvent could be removed[100]. Gentamicin-loaded bioadhesive microspheres were produced using compressed CO_2. In addition to high entrapment efficiency, homogeneous distribution of drug within polymer microspheres and continuous release of the drug were achieved[101]. In a recent study, aerogel silica particles with loaded drug were coated with polymer by the PCA process, which slowed down the release of the loaded drug [102]. The PCA process could also be applied to precipitate lysozyme in reverse micelles by introducing compressed CO_2 into the reverse micellar solution[103].

3.2 Spray drying

Spray drying is a one-step solvent evaporation process directly from liquid solution to powder. The process can be divided into four steps. In the first step a substance is dissolved in an organic solvent, which is then pumped through a nozzle and atomized into hot air in the second step. Thirdly the solvent evaporates and a dry powder forms. The last step is the separation of dried product from gas[104]. Atomization of solution is achieved by the use of pressure, rotating, or two fluids nozzle. Generally speaking, higher atomization pressure leads to smaller droplets and hence smaller particles. A high ratio of surface to volume favours rapid and effective drying. With regard to the spray from the nozzle, there are three different air current systems (Fig. 4). If hot air is flowing in the same direction of the feed, it is termed co-current. The advantages and disadvantages of co-current lie in the short contact between substance and hot air, since the air cools down rapidly further down in the chamber. Although not the whole chamber is used for drying, thermo sensitive substances have minimal heat contact. Most food powders are produced using co-current spray drying.

If air is let into the systems from the sides or from below it is called mixed flow and counter-current, respectively. In both processes the whole chamber is used for drying [105, 106]. Size is directly proportional to feed rate and not concentration, although higher concentrated solutions can lead to higher viscosity and slower feed rate. Particle morphology and size are dependent on the drying conditions and solvent evaporation rate. The Péclet number (Pe) is the ratio between convection time for drying and diffusion coefficient in the solid and can give a trend for morphology and size. For $Pe < 1$, small and dense particles can be expected. Fast drying can lead to large and hollow particles, while slow drying may result in small and dense particles [107]. In spite of a continuous, potentially easily scalable

and fast process, there are some drawbacks and limitations for the spray drying technique. One is that thermolabile substances are difficult to process, due to the potential of decomposition or change in property. Another factor is the poor thermal efficiency of the process which leads to a low cost efficiency[104, 108]. Despite these limitations spray drying is widely used in food production and in the pharmaceutical industry. This is mainly due to the possibility of adding additives to the feed mixture. Hence it is possible to produce encapsulated microparticles for release or better storage in one-step, just by adding polymer to the feed solution. Another possibility is adding agents to tune particle size, shape, or for ease of granulation[109].Another reason is the smaller particles produced in comparison to top-down processes and that spray dried particles tend to be amorphous, which is favourable for dissolution[110]. Möschwitzer and co-workers used spray drying as a first step in a two-step process with high pressure homogenization (HPH), calling the method H42. They tested glibenclamide in ethanol with surfactant. After spray drying and subsequent HPH, amorphous, smaller particles with a narrower size distribution were obtained, compared to the only HPH procedure[108]. The spray drying technique has been often used for encapsulation in particles. For example, Rizi and co-workers researched the encapsulation of three model drugs in the pH-responsive polymer Eudragit L100. It was found that a change in morphology could lead to the loss of drug release, even if it was the most stable morphology. Since changes in morphology are directly dependent on changes in solvent mixture and concentration as well as process conditions, it should be carefully considered when changing conditions for economic or environmental reasons without testing each parameter for specific substances first[111]. Similar drug encapsulation and drug release effects were observed by Kolakovic *et. al.*. They produced microparticles with nanofibre cellulose (NFC) and could form a tight fibre network with amorphous drug present in the matrix. Sustained drug release was dependent on the substance used and on its solubility and affinity for NFC for the same experimental conditions [112]. Another interesting study was done by Al-Qadi and co-workers, who made insulin-loaded chitosan nanoparticles for inhalation into deep lung tissue as an alternative absorption route into systematic circulation. *In vivo* tests in rats showed a higher and prolonged effect of hypoglycaemic effect [113].

3.3 Cryogenic Methods

3.3.1 Freeze drying

In a freeze drying process, a solution (or suspension) is frozen and the frozen solvent is sublimated under reduced pressure and low temperatures. Freeze drying alone is mainly used to achieve more stability in storage, since dry powder formulations tend to exhibit longer shelf lives and the freeze drying process does not cause shrinkage or toughening and leaves micropores in the material behind, which makes rehydration easier[114-117]. Nanosuspensions produced by other top-down or bottom-up can be freeze-dried into more stable powders without the risk of agglomeration as often encountered in other drying methods. Protective agents such as cryoprotectants like sugars can be added to avoid

possible decomposition or loss of activity during the freezing stage[118]. For example, fenofibrate was dissolved in tertiary butyl alcohol, mixed with water solution of mannitol. The resulting solution was immediately frozen and freeze-dried. Mannitol functioned as a nucleation seed in this case. After freeze drying, dispersions of the crystalline drug nanoparticles could be obtained, which showed an increase in dissolution behaviour [119]. In recent years, the ice crystals formed during the freezing stage has been explored as templates to produce porous polymer and ceramics[120, 121]. Particularly for porous polymer, the concentration of the polymer in the solution could have a very big impact on pore morphology. When diluted aqueous polymer solution was freeze-dried, polymer nanofibers could be readily formed[122], with the easy incorporation of small molecules or proteins by simply mixing them with the initial aqueous solution[123]. In a more complicated procedure, porous chitosan scaffolds with varied morphologies were incorporated with curcumin and curcumin-loaded silica microspheres via the freeze-drying method. A dual-tuned drug release profile was achieved with fast initial release and sustainable release at later stage[124]. The freeze-dried porous materials from polyvinyl alcohol, poly(ethylene glycol) and surfactant sodium dodecyl sulphate could be used as scaffold to form poorly water-soluble drug nanoparticles. By soaking the porous materials in organic drug solution and subsequent evaporation of the solvent, drug nanoparticles formed within the porous material, which could be re-dissolved in water to give aqueous nanoparticle dipsersion[125].

3.3.2 Spray freeze drying

In a spray freeze drying (SFD) process, a drug or more often a protein solution is sprayed through a thin nozzle into a vessel containing liquid nitrogen. The nozzle types used are the same as described in section of spray drying. Nucleation starts while the solution droplets are travelling through the cold nitrogen gas phase to the liquid phase, where they freeze. After recovery of the frozen droplets with a thin sieve, the solvent is removed by lyophilisation. Because of the freeze drying process, in contrast to air drying, the obtained particles are porous while maintaining the droplet size and shape. SFD is a popular method for downsizing protein powders for inhalation since the low processing temperature thwarts denaturation[23, 126]. As with other processes it is possible to add stabilizers, although a slight increase in size as well as some loss of activity for proteins can be the consequence[127, 128]. The main difference between spray drying and spray freeze drying is the drying process. A comparison study of the spray drying and spray freeze drying processes for the production of lipid-polymer hybrid nanoparticles made from poly(vinyl alcohol), lecithin and levofloxacin for inhalation was conducted. It was found that particles produced by SFD were superior in terms of flowability, physical handling and yield[129]. Although the particle size and shape mainly depended on droplet size and solution concentration, the use of additives during the process could also have an impact. This was improved by utilising a thermal-ink-jet printer as head of a spray freeze dry apparatus. Droplets with volumes as low as 2 –180 pL could be continuously formed. The resulting particles showed good stability in storage even without any excipients[130, 131]. Murugappan *et al.* reported the preparation of dry powder for

inhalation by immobilizing live influenza virus on insulin, dextran or dextran/trehalose. Activity tests showed that storage at 30 °C after the SFD for up to three months did not influence the receptor binding properties or biochemical integrity while the activity of unprocessed live vaccination decreased by 100-fold [132].

An extension of SFD is spraying into liquid nitrogen rather than across the cold nitrogen gas. In this process, an insulated nozzle is submerged into liquid nitrogen and the solution is sprayed directly in. The solution gets atomized by passing through a small nozzle at high pressure[133-135]. High atomization rate is furthermore a consequence of high Weber and Reynolds numbers at liquid-liquid collision [136]. The rapid freezing of solvent droplets lead to fast nucleation with arrested crystal growth. For proteins, the rapid freezing and hence fast passing of the critical temperature (the temperature between crystallisation and glass transition of the solution) can avoid the formation of large ice crystals which leads to denaturisation. Rapid freezing limits the time available for the drug molecules to crystallize, thus leading to the formation of amorphous drug particles.

3.3.3 Emulsion Freeze drying

Emulsions are mixtures of two immiscible liquids, with one droplet phase dispersed in a continuous phase, stabilized by a surfactant. Emulsions usually consist of a water phase and an oil phase. When the oil phase is emulsified as droplets in aqueous phase, the formed emulsion is called an oil-in-water (O/W) emulsion. Vice versa, a water-in-oil emulsion (W/O) can be formed [137]. In an emulsion-freeze-drying process to make drug nanoparticles (Fig. 5), a poorly water-soluble drug is dissolved in an organic solvent (oil phase) which is then dispersed into an aqueous polymer/surfactant solution (usually by stirring or homogenization) to form an O/W emulsion. The whole emulsion is frozen in liquid nitrogen and then freeze dried to remove both water and the organic solvent. During the freeze drying process, the drug nanoparticles are formed in situ in the porous polymer scaffold. Entrapment of nanoparticles in the porous polymer structure prevents nanoparticle aggregation, which makes it easy to handle, transport, and store the nanoparticles composites. More importantly, the highly porous nature of the hydrophilic polymer scaffold ensures the fast re-dissolution in water when required and produce stable aqueous nanoparticle dispersions[138]. In an emulsion, the number of droplets or the volume percentage can be varied. A high internal phase emulsion is formed when the volume percentage of the internal phase is greater than 74.05%, which can be used as templates to prepare highly interconnected porous polymers[137, 139]. Additives such as surfactants to stabilize the droplet phase can decrease the droplet size and hence pore size and narrower pore size distribution[140, 141]. An increase in polymer concentration generally leads to a decrease in porosity [142]. Pore morphology and pore size can also be tuned by varying the freezing rate. Rapid freezing in liquid nitrogen for instance produces smaller pores and higher order, whilst slower freezing rate favours disordered structures with larger pores[140]. Wang and co-workers used the emulsion freeze drying for emulsions containing sucrose in the aqueous phase and phospholipids and poly(ethylene glycol) in the oil phase. After lyophilisation

and re-suspension in water liposomes formed with a mean size smaller than 200 nm that showed high entrapment efficiency for selected water insoluble drugs [143]. Grant *et al.* produced amorphous indomethacin nanoparticles under 300 nm within porous poly(vinyl alcohol). The size and loading of indomethacin nanoparticles could be tuned by varying the emulsion compositions[144]. Furthermore, a one-step procedure was developed to generate protein-encapsulated poly(lactide-co-glycolide) microspheres in chitosan by freeze drying a W/O/W double emulsion, whereas a two-step method consisting of a microsphere preparation step followed by incorporating in a pre-formed scaffold was commonly used. Due to the mild processing conditions and non-chemistry specificity, this method may be applied to a wide variety of proteins and polymers. Porosity and morphology, both important factors for release profiles, could be easily tuned by changing stirring time and concentration[145]. Mc Donald *et al.* produced efavirenz particles with a size of 300 nm by the emulsion-freeze-drying approach. These particles showed reduced cytotoxicity , increased in vitro transport and a fourfold higher pharmacokinetic exposure in vivo[146]. Giardiello and colleagues presented the formation of two and three component nanoparticles. The two component particles were made up of FRET dyes and subsequently used for better cell imaging. For the three component particles it was possible to combine a polymer, drug and magnetic nanoparticles, giving rise to the possibility of tracing and sensing particles to enhance targeted drug delivery[147, 148].

3.4 Emulsion/Microemulsion

Emulsions are formed from two immiscible phases by energy input (e.g., stirring, homogenization) and the presence of surfactants to reduce surface tension and stabilize the droplets. Emulsions are thermodynamically unstable. As such phase separation occurs in an emulsion, giving back to two immiscible phases. This happens via flocculation, creaming, coalescence or Ostwald ripening[149, 150]. Surfactants and co-surfactant are added to increase kinetic stability. One way to prepare drug particles is by solvent extraction of an emulsion. Usually, a drug is dissolved in a non-polar solvent and is emulsified into aqueous continues phase containing polymers or surfactants (Fig. 6). The emulsion is then quenched with water. After stirring, the resulting particles can be filtered out. A second method is by single emulsion solvent evaporation. After an O/W emulsion is formed, the volatile oil phase is evaporated to produce aqueous nanoparticle dispersions. Final particle size may be controlled via droplet size and use of suitable surfactants[151, 152]. Microemulsions form a sub-class of emulsions with the droplets in the nanometer range (< 100 nm). Schuman and colleagues first observed the formation of microemulsions in 1943 by titrating a milky emulsion with hexanol and subsequently coined the term microemulsion in 1959 [153, 154]. Although many definitions of microemulsions can be found, the most commonly used is by Danielsson[155]. He defined a microemulsion as 'a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution.' Because of their liquid form microemulsions and their sub-micron droplet sizes are ideal candidates for the transdermal delivery of drugs[156-158]. Due to the nature of microemulsions, dermal and oral drug

deliveries are the two most favoured delivery routes. High solubility potential in the oil droplet phase resulting in high drug concentration in microemulsion can be beneficial for potential therapeutic treatment. Lastly, high concentration of surfactants and co-surfactants contained in the emulsion may enhance permeation by reducing the diffusional barrier of the stratum corneum[159-161]. For example, cyclosporin A, an immunosuppressant [162], docetaxel[163], an anti-cancer drug and biphenyl dimethyl dicarboxylate[164], a treatment drug for liver diseases, have been investigated for microemulsion delivery.

When coming to storage, microemulsion for oral delivery is usually encapsulated. Mostly this is done in gelatine capsules. However, due to the hydrophilic nature of gelatine, water containing formulations cannot be filled into a gelatine capsule, without change in emulsion composition[165]. Self-microemulsifying drug delivery systems (SMEDDS) present as a solution for this storage problem. The basic principle of SMEDDS is the ability of an oil, surfactant, co-surfactant and drug to form spontaneous emulsions under gentle agitation and dilution with water. The gastrointestinal tract provides aqueous medium and sufficient agitation[166, 167]. By virtue of high bioavailability, direct formation in the gastrointestinal tract, and good storage, current research in emulsion drug delivery focuses more on SMEDDS and SEDDS (Self-emulsifying drug delivery systems) for industrial production. SMEDDS is preferred over SEDDS, because of the higher interfacial surface area for drug absorption facilitated by the smaller droplet size. As such, very recently, Behati and colleagues formulated terbinafine by SMEDDS, while Benival *et al.* developed SMEDDS of doxorubicine hydrochloride[168, 169].

4 Encapsulation methods

The encapsulation methods are discussed here separately because of the unique use of functional polymers and the drug nanoparticles enclosed in polymer shell. Table 3 lists the advantages and disadvantages of the encapsulation methods.

4.1 Liposomes

Liposomes are stable microscopic vesicles of natural or synthetic lipids (usually phospholipids), first observed in 1964 by Bangham *et al.*[170-172]. Liposomes are formed when amphipathic lipids spontaneously assemble in layer form in aqueous medium[173]. Depending on the conditions multilayer or monolayer vesicles are formed (Fig. 7). In multilayer vesicles lipids are either ordered in circular rings with a head to tail structure with aqueous compartments in between or in a tail to tail arrangement with an aqueous internal department. The ability to enclose water soluble and entrap lipophilic drugs in the lipid layers as well as encompassing drugs with intermediate logP makes liposomes ideal candidates for drug delivery[174]. In drug delivery by liposome, cell entry is not happening by plasma clearance or tissue disposition, but by fusion or endocytosis, which increases the success rate of drug

delivery to cells [175].This trait makes liposomes highly attractive not only for drug delivery but also as carriers for enzymes and other proteins into the inner part of the cell[176-178]. Surface charge, hydrophobicity, size, fluidity and packing of lipid layers heavily influence the stability and type of proteins for binding of liposomes[179, 180]. Changes in these parameters can be used for fine tuning of liposomes properties. However, liposomes tend to be sensitive to fast elimination from the bloodstream as well as accumulation in tissue especially in the liver[181]. To prevent accumulation, different strategies may be applied. For example, liposomes could be formed with pH sensitive materials which passed into the cell as a whole and then release drug load because of the change in pH[182]. Liposomes were modified with suitable ligands for targeted delivery[183, 184]. Methods have been developed to circumvent fast clearance. One of the most important is the employment of 'stealth' liposomes. These liposomes were linked to poly(ethylene glycol) which decreased the aggregation between the liposomes and particle-protein interaction[185].

4.2 Dendrimers

Dendrimers belong to the family of polymers with a branched polymer arms radiating from the core, reminiscing of a tree. The first reports about dendrimers were published in the late 70s early 80s by the groups of Buhleier[186], Denkewalter[187], Newkome[188] and Tomalia[189]. Tomalia and co-workers reported the formation of polymers with 'controlled occupation of space in three-dimensions as a function of size, shape and disposition of desired organic functionality'. These differed from classic monomers and oligomers in a higher degree of symmetry, higher branching and a maximized density in reactive end groups. A dendrimer includes three structural components. An initiator core, an interior layer (made up of repeating units radially attached to the core and an exterior), functionalized end groups (attached to the outermost interior layer) (Fig. 8). By removing the central core, identical dendrons can be obtained. These branched polymer arms are again constructed from the above described three structural elements. The number of dendrons is dependent on the multiplicity of the central core. Further away from the core a dendron possesses more and more branch points. Every branch points represents one generation. Higher generations are more branched and have more end groups. Synthesis of dendrimers is a stepwise process, which can either start from the central core outwards, in which case the growth is exponentially [190], or by synthesizing dendrons, which are then coupled to a central core [191]. Both cases lead to a high degree of monodispersity in contrast to classical polymers, where growth is statistical and the end product is polydisperse[192]. Like classical polymers, a wide variety of materials can be used as starting materials, even DNA[193]. Dendrimers of different generations are commercially available. Polyamidoamine (PAMAM) is commonly selected as starting material[190]. With a size between 2 and 10 nm dendrimers possess the favourable characteristics of nanoparticles. Depending on the number of generations the shape of dendrimers can change from small and floppy disks (up to generation 4) to spherical, three dimensionally defined and densely packed particles (generation 5 and up). The dense packaging can be used to achieve more concentrated drug loading. End

groups can be changed to obtain hydrophilic or hydrophobic dendrimers with a hydrophobic or hydrophilic core respectively as well as to facilitate higher binding affinities to target sites. These attributes make dendrimers ideal carriers for drugs, DNA or other particles. As such much research has been done in these fields. Thomas and co-workers synthesized generation 5 dendrimer with folic acid (FA) and methotrexate that showed a 4300 fold higher affinity to the FA-receptor, a receptor commonly expressed on many cancer cells, than free methotrexate[194]. Shan and colleagues utilized dendrimer to trap gold nanoparticles with generation five PAMAM dendrimers for gene delivery. Non-viral gene transfection is usually inefficient. The obtained Au-dendrimers in comparison showed significantly higher delivery into the cell than previous studies [195]. Third generation PAMAM surface modified with lauryl chains were produced by Teow *et al.* in order to enhance the permeability of paclitaxel, which was conjugated to the dendrimer via glutaric anhydride linker. Permeability test were performed on human colon adenocarcinoma cell line (Caco-2) and porcine brain endothelial cells (PBECs). Both cell lines showed significantly improved uptake, with a higher uptake in the direction going in the cell, in comparison to free paclitaxel[196].Cell permeability of dendrimers can be used not only for pharmaceutical applications but also for imagining. As shown in a study carried out by Peng and co-workers, where gold nanoparticles entrapped in dendrimer enabled X-ray and computer tomography (CT) blood pool imaging and CT imaging of a xenograft tumour model[197].

4.3 Block copolymer micelles

Block copolymers are a type of macromolecules of chemically different segments connected via their terminal groups. There exist three basic structural types for block copolymers (Fig. 9). The first is the A-B structure, so called diblock, with A and B being two different repeating units. A block of repeating unit A is thereby connected to a block of unit B. The second type is an A-B-A or A-B-C structure, called triblock. A block of B is enclosed between a block on either side. The third type is a multiblock in the style of $(A\text{-}B)_n$, where blocks of A and B are repeated to form areas of repeating unit A and B. A special sub-group of block copolymers are radial block-copolymers. These are formed by two or more block copolymers radiating out from a central hub, forming star-shaped macromolecules with a pre-designed symmetry. In comparison to random copolymers, block copolymers exhibit well defined structures and their synthesis requires sequential architecture. Since the two repeating units are different in chemical and physical properties, a two-phase morphology on a micro-scale level can be observed for block copolymers[198]. This gives rise to the spontaneous formation of micelles in certain solvents, where one of the blocks is insoluble, while the other is soluble. The insoluble block forms a core, while the soluble block surrounds it as corona. This behaviour is further favoured by the repulsive forces between blocks A and B[199]. This attribute leads to manifold applications in cosmetics, stabilisation of insoluble materials, and drug delivery. Drugs can be incorporated in the core by chemical, physical or electrostatic interactions[200-202]. Size and steric hindrance from the hydrophilic

shell leads to a long circulation time in the bloodstream. With a size commonly between 10 to 100 nm block copolymers are small enough to bypass filtration, but too large for renal excretion [203]. In addition to long circulation, a long retention time of the drug can be reached. This is dependent on the strengths of the interaction between drug and polymer and has to be carefully considered to avoid immediate dissociation into the bloodstream as well as no release of drug at all. Synthetic, biodegradable block copolymers suffer from low drug loading and instability in vivo. Decomposition products may be toxic [204]. One solution is to form block copolymers containing natural occurring cholesterol, which can serve as a site for cell attachment, as a base for polymeric scaffold. Such block copolymers could show an improvement of blood compatibility, more efficient drug delivery, and no cytotoxicity. This was demonstrated by Lee et. al in 2012[205] and Yu an co-workers in 2013[206] on the anticancer drugs paclitaxel and docetaxel, respectively. However those cholesterol containing block copolymers tended to exhibit limited drug loading, low stability in vivo, and premature drug release.

Another solution to increasing stability of block copolymers is the formation of polymers with so called bottle brush structures (Fig. 9). In bottle brush block copolymers a central linear polymer is lined perpendicular with A and B blocks, forming a comb like structure. This leads to a large domain spacing of around 100 nm, which is difficult to achieve in linear block copolymers[207]. The distinctive topology and the large spacing further enhance many features already observed in liner block copolymers, for example the stabilization of drug nanoparticles[208]. Cambón and co-workers synthesised poly(butylene oxide)–poly(ethylene oxide)–poly(butylene oxide) block copolymers as drug carriers for doxorubicin and inhibitors to the P-glycoprotein (P-gp) efflux pump. Slower sustained release in comparison to free doxorubicin was observed[209]. Tran and colleagues formed brush-like block copolymers composed of polynorbonene-cholesterol/poly(ethylene glycol) loaded with doxorubicin, combining the advantages of cholesterol containing polymers with the favourable topology of bottle brush block copolymers. The micelles exhibited high drug loading (22.1 wt%), as well as increased internalization in human cervical cancer cells and a steady release profile in comparison to free doxorubicin. Furthermore no cytotoxicity for blank micelles could be observed and tissue imaging showed that doxorubicin nanoparticles were in fact accumulating preferentially in tumour cells and not in vital organs [210].

Owing to the unique possibility of functionalized end groups it is possible to synthesise block copolymers that respond to specific physiological changes. Typically, tumour microenvironment is more acidic (around pH 6.7) than in normal tissues (around pH 7.2) due to the formation of lactic acid and hydrolysis of adenosine triphosphate (ATP) in hypoxic regions of tumour. Studies suggested that acidic microenvironment may promote tumour metastasis. pH could be employed as stimuli to disassemble the drug loaded polymer micelles followed by release of drugs. For example, doxorubicin (DOX) loaded polymer micelles which could degrade at pH 5.0-5.5 has been developed by Kataoka and colleagues[211, 212]. Bae's group developed a pH-responsive polymer micelle system composed of

polyion complex. On approaching the acidic environment, the charge of the micelles was firstly neutralized, followed by destabilization of the micelle structures and release of cell-penetrating peptide which could penetrate into cells and increase the whole intracellular uptake[213]. Kim *et al.* formed block copolymers of poly(styreneboroxole) (PBOx) and poly(ethylene glycol) (PEG) exhibiting monosaccharide induced disassembly in neutral pH. They demonstrated the possibility of sugar-responsive delivery systems for insulin, which would only be released in physiological conditions in the presence of sugar [214].

For clinical need, conjugating polymer micelles/nano-materials with active-targeting moieties, which could strongly interact with receptors overexpressed on the target tissue or cells, can lead to preferential accumulation of drugs or bio-imaging agents in the targeted tissue and cells. Several excellent review papers have been published in this areas regarding the employment of active-targeting moieties in improving the targeting activities of the drug delivery systems[215-218]. The typical active targeting moieties lie in folate, transferrin, monoclonal antibodies, somatostatin analogs, arginine-glycine-aspartic (Arg-Gly-Asp or RGD), JK591 etc.[219-222]. Due to the scope of this review, we will not expand this part here. However, examples from conjugating polymer nanomaterials with targeting moieties such as folate did prove to be able to enhance the whole delivery system to bypass tumor cell multidrug-efflux pumps[223] and improve the intracellular drug delivery[224].

5 Solvents and additives used in the formulations

5.1 Solvents

Although the use of organic solvents in general is unfavourable, for some techniques the use of such solvents is inevitable. For the production of pharmaceutical drugs some consideration has to be made before choosing a solvent, *e.g.*, toxicity and production method. A guideline on toxicity of organic solvents and their residue limit was proposed at the second international conference on harmonization of technical requirements for registration of pharmaceuticals for human use (ICH) and subsequently published as Guidance for Industry Q3C[226]. In this guideline commonly used organic solvents are sorted into three categories. Class 1 solvents are known or strongly suspected of human carcinogens and environmental hazards and should be avoided. If avoidance is not possible their use needs to be strongly justified and solvent residue needs to be highly restricted. Examples are benzene (residue limit 2 ppm) and carbon tetrachloride (residue limit 4 ppm). Class 2 solvents are non-genotoxic animal carcinogens, or solvents that can possibly cause irreversible toxicity such as neurotoxicity or teratogenicity, as well as solvents with reversible toxicity. The use of these solvents should be limited. Residue limits can be calculated as either parts per million or as a permitted daily exposure (PDE) in gram per day (milligram per day), which takes the maximal administered mass of drug per day into

account. Examples for such solvents are chloroform (limit 0.6 mg/day and 60 ppm), methanol (limit 30 mg/day and 3000 ppm), acetonitrile (limit 4.1 mg/day or 410 ppm) and cyclohexane (limit 38.8 mg/day and 3880 ppm). Class 3 solvents have a low toxic potential and no exposure limit is needed. They generally have PDEs above 50 mg per day. Such solvents include acetone, DMSO, and ethanol. Complete classification of the most commonly used organic solvents can be found in the Guidance for Industry Q3C[226] and in specific Reviews[227, 228]. Other considerations, as already mentioned, are challenges related to the preparation methods. Many of these have already been described with the corresponding techniques, therefore only some general requirements are described here. Techniques such as SAS and HGRP require organic solvents to function as solvent to the drugs and as anti-solvent to water, another organic solvent or a supercritical fluid, while being simultaneously miscible with the antisolvent. Whereas in methods related to emulsion and emulsion-freeze-drying, solvents need to be immiscible with water, or at least partially immiscible for the formation of emulsions. If the solvent is removed by freeze-drying, the melting points of the selected solvents will be very important. The solution made from the solvents should have a melting point above the lowest temperature in the chamber of the freeze-dryer, although it is still possible to freeze dry a small volume frozen solution with a lower melting point with high capacity vacuum. When the melting point is low and the frozen sample melts in the freeze dryer, the porous structure and the quality of the nanoparticles can be compromised.

5.2 Additives

Nanoparticle formation is a process where nucleation is induced by a drastic increase of supersaturation or by downsizing microparticles with mechanical force. In both cases, particle agglomeration or further particle crystallisation needs to be suppressed. Additives, mainly surfactants, are usually added in the processing step to address the aggregation problem. Surfactants are amphiphilic molecules consisting of a hydrophobic part and a hydrophilic part. Surfactants can be classified according to the charge of the hydrophilic part of the molecule as anionic, cationic, zwitterionic, or non-ionic. Adsorption of surfactants on surface or surrounding droplets is driven by the decrease in free energy at the phase interface. Surfactants can form micelles with a hydrophobic core in aqueous medium. The hydrophilic part of the molecule interacts strongly with the surrounding water and hence well dispersed[229]. In emulsions, the oil droplets are enclosed by surfactant and stabilized in the surrounding aqueous medium. For hydrophobic solid drug nanoparticles, the surfactant molecules are adsorbed on nanoparticle surface and are prevented from aggregation and dispersed in aqueous medium. Faster adsorption of surfactants leads to smaller particles[230]. This layer of surfactant prevents further particle growth, due to hydrophilic groups on the exterior reducing hydrophobic interaction with the surrounding solution and agglomeration by steric hindrance between two particles[231]. An increase of surfactant concentration usually leads to a decrease in particle size due to enhanced adsorption on the surface, however if the concentration is above the critical micelle concentration, the molecules will exist in

micelle form [232]. Length and charge of surfactants play another important role for stabilization. Longer hydrophobic chains can cover more surface area and the required amount would be lower. Particles with charged surfactants on the other hand show greater electrical repulsion due to the resulting surface charge [231]. Besides stabilising nanoparticles, surfactants can also help to increase the dissolution in aqueous medium. One major concern in using surfactants is the impact that surfactants may have for biomedical application and on the environment. Studies show that surfactants can accumulate in water and may be toxic to marine life[233, 234]. High levels of surfactants are also known to lead to irritation of the human skin due to changes in the stratum corneum[235, 236].

5.3 Polymers

There are two main routes polymers can be used in nanoparticle formulation, either as stabilizers or in drug encapsulation. Some polymers can stabilize nanoparticles by adsorption on the surface in the same way as surfactants, although in contrast to surfactants polymers can agglomerate in the hydrodynamic layer between particles and prevent particle collision and agglomeration[237]. Depending on the techniques applied, polymers can form scaffolds in the dried state, which prolongs the shelf life of nanoparticles [125, 144]. When polymers are used for encapsulation they not only increase the solubility due to their amphiphilic character but can also respond to environmental changes simultaneously. It is possible to engineer polymer-drug nanoparticles for controlled drug release[238, 239], *e.g.*, pH-sensitive targeted release[240], or temperature dependent release from gels for dermal applications[241, 242]. Again, concerns about toxicology of polymers and their decomposition products arise. To solve this problem recent research has focused on the synthesis of biodegradable polymers and polymers made from materials native or similar to the human body, which may also increase the cellular uptake. Typical examples of such polymers include poly(lactic acid)[243], poly(lactide-*co*-glycolide)[244] and chitosan [245].

6 Applications

Table 4 shows a selection of approved nanodrug products on the market and the administrations achieved by drug nanoformulations[246-275]. Most of these nanomedicines are either produced by top-down processes or encapsulation. The only industrially applied bottom-up technique is spray drying, since it is possible to mix additives in the feed solution and achieve encapsulation in one-step. All the drugs produced by spray drying listed in Table 4 have utilized polymers. Encapsulation in liposomes and drug delivery *via* emulsions are well established methods especially in cancer and HIV treatment. Dendrimers are not as well established, which may in part be due to the difficulty in synthesizing such polymer and also the possible toxicity. To the best of our knowledge, the first dendrimer-nanomedicine containing the dendrimer SPL7013, an antiviral agent, is VivaGel™ by Starpharma. The drug may be applied for topical use or prevent infection with HIV and other sexual transmitted

diseases. The similar limited use has been noticed for block copolymer micelles. Genexol®-PM by Samyang Biopharmaceuticals, a polymeric micelle formulation of paclitaxel, is currently undergoing Phase II and III tests. Other cancer drugs, SP1049C (a doxorubicin olymer micelle conjugate) and NC-6004 (a cisplatin micelle conjugate) have also been tested [247, 248].

7 Summary and Perspective

Nanomedicine has shown to be a solution to many problems in pharmaceutical applications, particularly in addressing the poor water soluble problem. A wide range of techniques, including top-down methods, bottom-up methods, and various encapsulation approaches, have been developed to make drug nanoparticles. Reducing the drug particles to nanoscale can enhance dissolution rates and increase water solubility. Furthermore, the decrease in size and functionalisation of the particle surface can facilitates the sustainable, targeted, and responsive drug delivery and release. Due to the tendency of drug nanoparticle aggregation, the preparation methods have been improved to produce stable aqueous nanoparticle dispersions or prevent nanoparticles aggregation by efficient use of stabilizers or dry porous scaffolds.

Because of the easy control in fabrication process and scale-up potential, top-down approaches such as wet-milling and high pressure homogenization have been mostly used by industry for nanoformulated drugs. Spray drying is also a technique that has been employed industrially. It is generally very difficult to produce small, uniform, and non-aggregated nanoparticles by the top-down methods. Micron or sub-micron particles are often produced. To produce smaller nanoparticles, substantially longer processing time and increasing amount of stabilizers will be required. Also, the presence of soft stabilizers may negatively influence the milling efficiency. There are also limits for soft compounds or temperature-sensitive drug compounds to be processed by milling or homogenization. Various bottom-up processes have been developed and investigated to address different aspects of drug nanoformulations, for example, spray drying, sonoprecipitation, use of supercritical fluids, emulsions, cryogenic methods, and different encapsulation techniques. The results are highly promising. However, the cost of production, quality control in different production batches, and meeting the regulation requirements, have to be considered carefully before adopting these techniques for marketing nanodrug formulations.

For poorly water soluble drugs with different properties, a suitable preparation technique should be selected to match the compound property and the requirement for the targeted application. For example, hard and crystalline drug compounds, particularly when it is difficult to find a suitable solvent, are better to be processed by top-down approaches, whilst bottom-up methods should be employed for polymer or soft organic compounds. In terms of producing drug nanoparticles, the challenge is how to produce drug nanoparticles with controlled sizes, narrow particle size, and also the shape of the nanoparticles. For intravenous injection, drug nanoparticles in the region of 200 nm or smaller have

proven to be good, which also offers good stability. However, for the drug nanoparticles with cell-targeting groups and responsive cargo release, the sizes of nanoparticles in the region of 10-50 nm may be preferred depending on the cell environment. The shape control of the nanoparticles is important because certain shapes of nanoparticles may be engulfed easily by the cells. When the drug nanoformulations are used for potential treatment, a reasonably long shelf life with no aggregation is required, which can be normally met by the formation of nanoparticles < 300 nm and the use of surfactants and/stabilizer. However, in terms of treatment, a high ratio of drug to stabilizer is required for therapeutic efficacy, not to mention the potential toxicity or side effects of the stabilizers. To reduce potential toxicity, biocompatible polymers or biopolymers such as hydroxypropyl methylcellulose, polyethylene glycol, phospholipid and amphiphilic proteins are better to be used to stabilize drug nanoparticles. For optimal use of nanomedicine, the surface of the drug nanoparticles should be properly functionalized (e.g, stabilized by polyethylene glycol, responsive polymer shell, functional groups to specifically interact with the target cell surface), binding with multiple modalities (e.g., imaging modality such as iron oxide nanoparticles, Gd-containing polymer), to allow sufficient circulation time in bloodstream, to deliver to the targeted sites, and to release the drug payload on demand by triggers including pH, temperature, and light. Imaging techniques will be employed to track where the drug nanoparticles are and to better understand the treatment outcome and hence to help develop more efficient drug formulations. Overall, nanoformulations for poorly water soluble drugs are highly promising and fast growing in the pharmaceutical sector. Great efforts are required from researchers and industries to develop and optimize different nanoformulation techniques to achieve the best possible results in healthcare.

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Figure Captions

Table 1 Commercially used top-down methods with advantages and disadvantages.

Table 2 Bottom-up methods with advantages and disadvantages.

Table 3 Encapsulation methods with advantages and disadvantages.

Table 4 Selected marketed nanodrugs.

Fig. 1 The types of drugs by the Biopharmaceutics Classification System (BCS)

Fig. 2 Schematic representation of the media milling process. Batch or continuous mode is possible. Adapted with permission from ref. 33. © 2012 Elsevier Science B. V.

Fig. 3 Schematic representation of high-gravity process. 1-rotating packed bed; 2-packing; 3-motor; 4-liquid distributor; 5-flow meters; 6-outlet; 7-pump; 8,9-liquid storage tank. Adapted with permission from ref. 74. © 2012 Informa Healthcare USA.

Fig. 4 Schematic representation of spray drying apparatus with different currents.

Fig. 5 Schematic representation of the emulsion freeze drying process.

Fig. 6 Schematic representation of an emulsion droplet with dissolved drug molecules

Fig. 7 Schematic representation of a) monolayer liposome, b) bilayer liposome, and c) multilayer liposome.

Fig. 8 Schematic representation of a dendron and a dendrimer.

Fig. 9 Schematic representation of different types of block copolymers.

Table 1 Commercially used top-down methods with advantages and disadvantages

Method	Advantages	Disadvantages	Commercial name
Wet media milling	• Preparation of Nanosuspensions from 1 mg/ml to 400 mg/ml with low variations in batches • Wide distribution in nanometre range[30] • Energy-efficient	• Time consuming • Hard to scale-up[54] • Trace impurities in products • Pre downsizing step necessary	NanoCrystal™and Nanomill ™(élan Nanosystems) [32, 55]
Microfluidization	• small size distribution[56] • direct formation of Nanosuspensions	• high number of cycles[45] • Microparticles remain for hard drugs[54]	Microfluidizer®(Microfluidics International Corp.)[57]
Piston-Gap homogenizer	• drugs that are poorly soluble in aqueous or non-aqueous medium • easy scale up with small batch to batch • variation[49] • production of high or low concentrated Nanosuspensions with small size distribution[58]	• drug particles need to be in the micrometre range before processing	Dissocubes® (SkyePharma)[59]

Table 2 Bottom-up methods with advantages and disadvantages

Method	Advantages	Disadvantages
Solvent Antisolvent precipitation (S/AS)	• Cost and energy efficient • Straight forward production conditions[60]	• Particle agglomeration if not immediate worked-up[66] • Not under specific size plateau • High concentrations of surfactants needed[64] • Drugs needs to be soluble in one solvent • Solvent and anti-solvent need to be miscible • Not under specific size plateau • Organic solvent residue
High gravity Reactive precipitation (HGRP)	• Intensified micromixing[73] • Smaller particles and narrower size distribution	• Drugs needs to be soluble in one solvent • Solvent and anti-solvent need to be miscible • Not under specific size plateau[71] • Organic solvent residue • Additives
Rapid expansion of supercritical solution (RESS)/ Rapid expansion of supercritical solutions into a liquid solvent (RESOLV)	• Green chemistry[85] • Non-toxic, no solvent residue[85] • Formation of Nanoparticles possible in RESOLV[90]	• Expensive machinery and material • Many AIPs cannot dissolve in $scCO_2$ and need an organic co-solvent • Microparticles in RESS[90] • Additives
Supercritical Antisolvent precipitation (SAS)	• mild conditions • AIP does not need to be miscible with supercritical fluid, giving way to a broad range of materials • Recrystallization can be controlled leading to either smaller or larger particles of a desired morphology	• Solvent residue • Solvent needs to miscible with super critical fluid • Immediate work up required, otherwise particle can change morphology
Gas antisolvent (GAS)	• mild conditions allowing the handling of proteins[95] • AIP does not need to be miscible with	• Solvent residue, which is difficult to strip • Batch technique, difficult to scale-up

	supercritical fluid • Broad range of materials • Very small particles possible and easy particle size control[93] • Recrystallization can be controlled leading to either smaller or larger particles of a desired morphology	• Difficult separation of gas and solvent stream
Spray drying	• One –step processes[104] • Continuous and fast[104] • Additives can be mixed in with feed solution making encapsulation possible in one-step as well[109]	• Efficiency loss when milder conditions are needed[105, 106] • Energy and thermal inefficient[105, 106]
Spray freeze drying	• Mild conditions[23, 126] • No agglomeration, due to immediate freezing of particles • Lyophilisation leads to enhanced characteristics, like wettability, storage and handling[114-117]	• Expensive equipment • Freeze-drying process is time and energy consuming • Solvent residue[118]
Emulsion freeze-drying	• Mild conditions • Particles stabilized against agglomeration even after long storage[114-117] • Lyophilisation leads to enhanced characteristics, like wettability, storage and handling[114-117]	• Solvent residue[225] • Additives[140, 141] • Freeze-drying process is time and energy consuming • Expensive equipment
Emulsions /microemulsions	• Dermal application possible[156-158] • High solubility and high drug concentration[159-161] • High affinity to the internal emulsion phase, can be modified to increase transdermal flux[159-161] • high concentration of surfactants and cosurfactants enhance permeation to the stratum corneum[159-161] • SMEDDS make it possible to form emulsions directly in the intestines[166, 167]	• High concentrations of additives[159-161] • difficult to achieve long time stability[166, 167]

Table 3 Encapsulation methods with advantages and disadvantages

Method	Advantages	Disadvantages
Liposomes	• Drug delivery to inner cell parts due to biological similarities to membranes • Surface modification for targeted drug delivery possible[182-184] • Biodegradable • Applicable for all logP ranges[174]	• Need to be PEGylated to avoid fast blood stream clearance[185] • Too rapid or slow drug release while in the bloodstream[181]
Dendrimers	• High drug loading • Versatile in end group and core functionality as well as materials used for synthesis[189, 193] • Specific morphology design (spherical or floppy disk) possible [189]	• Circulation time in bloodstream still a challenge • Tissue localization difficult to predict[196] • Dendrimer density makes release rate difficult to predict and present a steric hindrance for enzyme cleaving for proteins • Decomposition products may be toxic[204]
Block Copolymer Micelles	• Highly functionalised due to end group modification[214] • Modifiable to be biodegradable[206] • Stabilisation of drug Nanoparticles[200-202]	• Difficult synthesis • Prediction of interaction of drug and polymer difficult leading to variations in release time and stabilization[210] • Interaction with proteins and subsequent clearance from blood stream [210]

Table 4 Selected marketed nanodrugs

Name	Active Ingredient	Dosage form	Application	Method	Company
Pletal	Cilostazol	oral	peripheral vascular disease	Wet pearl/ball milling (NanoCrystal™)	Otsuka Pharmaceutical Co.[249]
Rapamune®	Sirolimus	oral	Immunosuppressant	Wet pearl/ball milling (NanoCrystal™)	Pfizer[250]
Emend®	Aprepitant	oral	Suppresses nausea and vomiting	Wet pearl/ball milling (NanoCrystal™)	Merck[251]
Megace® ES	Megestrol acetate	oral	Breast cancer	Wet pearl/ball milling (NanoCrystal™)	Par Pharmaceuticals Inc.[252, 253]
Tricor®	Fenofibrate	oral	Reductionof cholesterol levels	Wet pearl/ball milling (NanoCrystal™)	Abbott Laboratories[254]
Avinza®	Morphine Sulphate	oral	Severe pain treatment	Wet pearl/ball milling (NanoCrystal™)	King Pharmaceuticals[255]
Focalin®	Dexmethylphenidate hydrochloride	oral	Attention deficit hyperactivity disorder (ADHD)	Wet pearl/ball milling (NanoCrystal™)	Novartis[256]
Ritalin LA®	Methylphenidate hydrochloride	oral	Attention deficit hyperactivity disorder (ADHD)	Wet pearl/ball milling (NanoCrystal™)	Novartis[257]
Triglide®	Fenofibrate	oral	Reduction of cholesterol levels	High pressure homogenization (IDD-P Skyepharma)	Sciele Pharma Inc.[258]
Abraxane®	Albumin-bound paclitaxel	i.v.	Cancer treatment	High pressure homogenization	Abraxis BioScience[259]
Sporanox®	Itraconazole	oral	Antifungal agent	Spray drying	Janssen Pharmaceutica[260]
Prograf®	Tacrolimus	oral	Immunosuppressant	Spray drying	Atellas[261]

Cesamet®	Nabilone	oral	Suppresses nausea and vomiting	Spray drying	Meda Pharmaceuticals[262]
Intelence®	Etravirine	oral	HIV treatment	Spray drying	Janssen Pharmaceutica[263]
Diprivan®	Propofol	i.v.	Anaesthetic	Microemulsion	Fresenius Kabi[264]
Neoral®	Cyclosporine	oral	Immunosuppressant	SMEDDS	Novartis[265]
Norvir®	Ritonavir	oral	HIV treatment	SMEDDS	Abbott Laboratories[266]
Fortovase®	Saquinavir	oral	HIV treatment	SMEDDS	Roche[267]
Restasis®	Cyclosporine A	Ophthalmic	Dry eye syndrome	Lipid emulsion	Allergan[268]
Durezol®	Difluprednate	Ophthalmic	Eye inflammation	Lipid emulsion	Alcon Pharmaceuticals[269]
Doxil®	Doxorubicin	i.v.	Treatment of Kaposi's sarcoma	PEGylated liposomes	Janssen Pharmaceutica N.V.[270, 271]
Myocet®	Doxorubicin	i.v.	Breast cancer	liposomes	Enzon Pharmaceuticals[272]
DepoCyt®	Cytarabine	i.v.	Leukaemia and non-Hodgkin lymphoma	liposomes	Pacira pharmaceuticals[273]
DaunoXome®	Daunorubicin citrate	i.v.	Leukaemia	liposomes	Galen[274]
LMX®-4	Lidocaine	topical	Local anesthetic	liposomes	Ferndale Laboratories
Epaxal	Inactivated hepatitis A virus	parenteral	Hepatitis A vaccine	liposomes	Crucell[275]

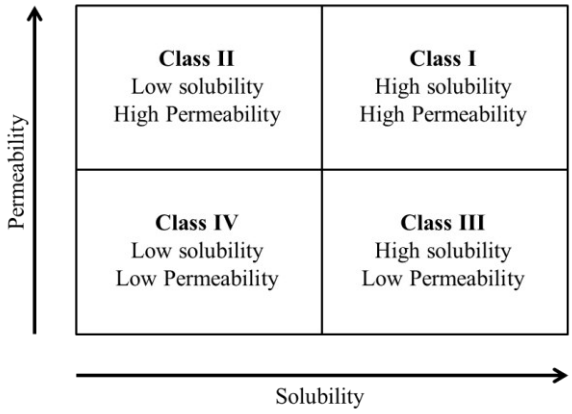
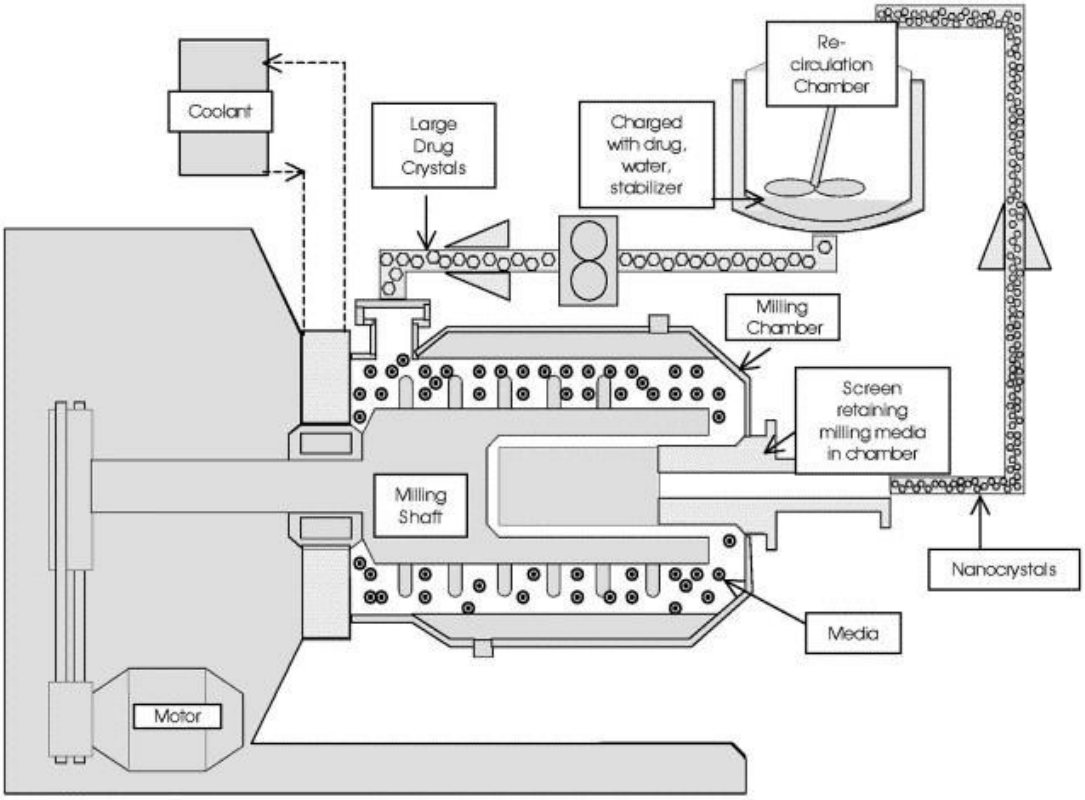


Fig. 1 The types of drugs by the Biopharmaceutics Classification System (BCS)



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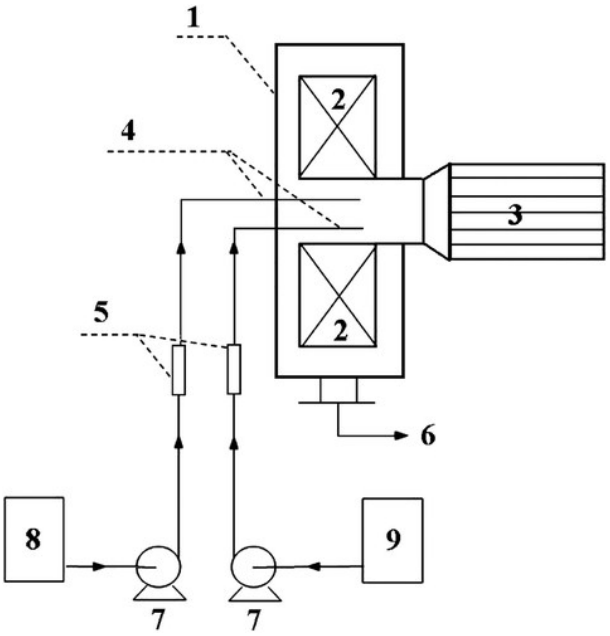

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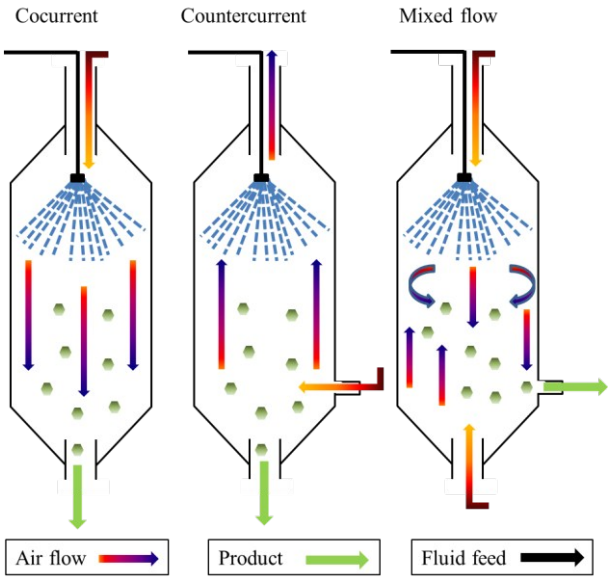


Fig. 4 Schematic representation of spray drying apparatus with different currents.

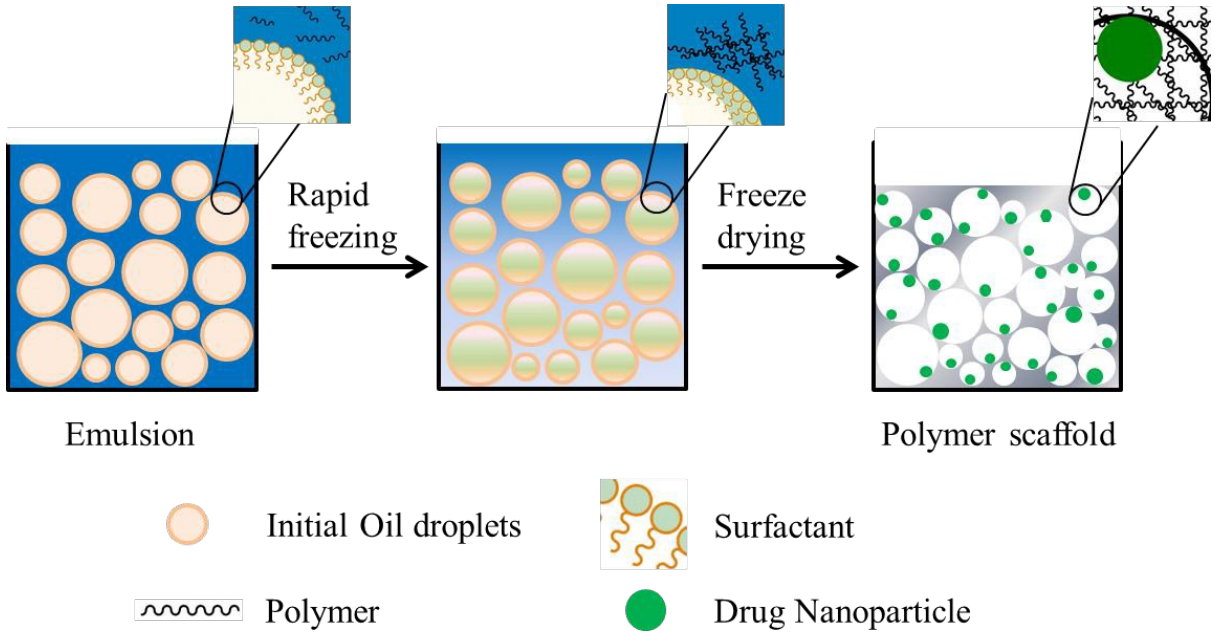


Fig. 5 Schematic representation of the emulsion freeze drying process.

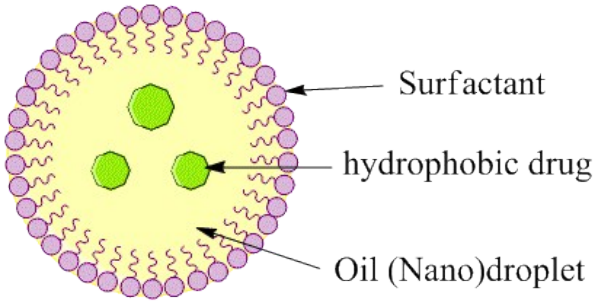
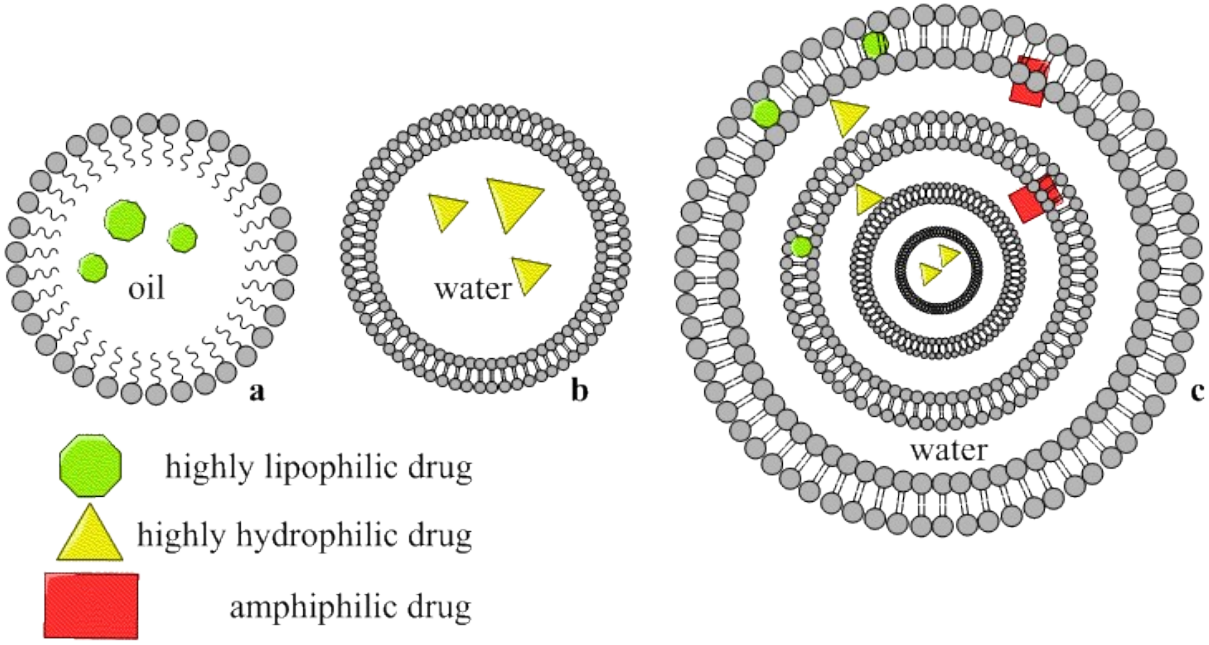


Fig. 6 Schematic representation of an emulsion droplet with dissolved drug molecules



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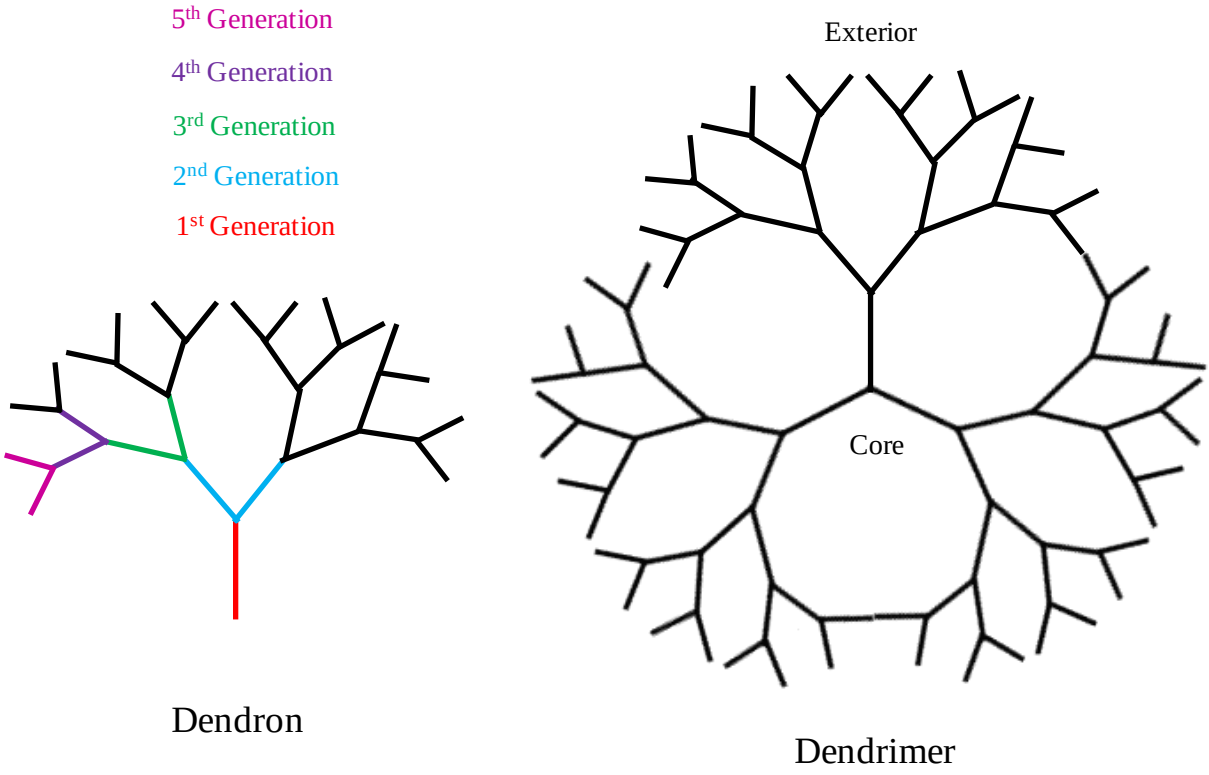

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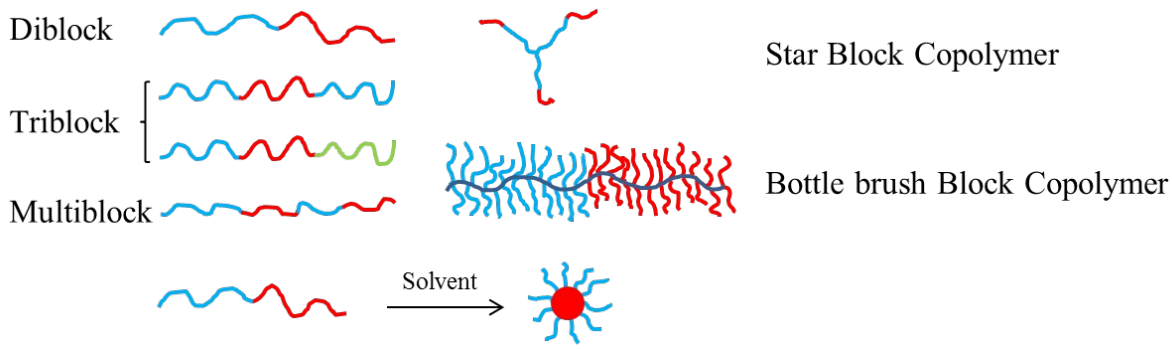

Fig. 9 Schematic representation of different types of block copolymers.