High-Pressure Homogenization Techniques for Nanoparticles

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Abstract

High-pressure homogenization (HPH) has been employed for unit operations like comminution, mixing, and stabilization of pharmaceutical solids and nanoparticles. With advancing nanotechnology, the HPH technique has undergone discernible evolution and has broadened the scope of its pharmaceutical applications by facilitating particle engineering. An in-depth understanding of fluid dynamics has helped the researchers devise innovative designs for high-pressure homogenizers with higher processing capacity and efficiency. The present chapter provides useful insights on the fundamentals involved in the process of HPH of colloidal dispersions, basic instrumentation of homogenizers, and theories on forces involved in homogenization. HPH has the distinct advantage of being one of the most versatile and scalable processing methods for the preparation of different vesicular and non-vesicular lipid-based nanosystems such as nanoemulsions, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), nanocrystals, as well as polymeric nanoparticles. The chapter has summarized the effect of various processing and product variables on characteristics of the aforementioned nanoparticle formulations. The chapter provides a comprehensive overview of the processing attributes of HPH that may facilitate the development of nanoparticles to attain desirable pharmaceutical attributes.

Keywords

Nanoparticles · Particle engineering · Homogenization pressure · Homogenization cycles · Fragmentation and disruption

1 Introduction

The concept of homogeneity and heterogeneity is derived on the basis of uniformity in a substance or a system, wherein the homogeneity signifies uniformity in a character or composition, while heterogeneity designates nonuniformity. The applicability of these notions is possible at a diverse level of intricacy from atoms or molecules to galaxies. The term "homogenization" denotes "to render uniformity throughout in terms of structure, composition, and character." Homogenizing is an umbrella word depicting multiple unit operations like mixing, blending,

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dispersing, disrupting, emulsifying, stirring, etc. (Dhankhar 2014). In 1899, Auguste Gaulin discovered and patented the process of homogenization of milk and pioneered the pressure homogenization method (Gaulin 1904). Gaulin's equipment was portrayed in 1900 at Paris world's fair having a three-piston thruster and filtration tubes working at pressures up to 30 MPa. Conventional homogenizers or standard homogenizers protracted about 50 MPa pressure range since then. Nowadays to enhance the proficiency and outcome of the process, high-pressure homogenizers operating at pressure as high as 100–500 MPa are developed.

HPH technique is exceedingly utilized in the sector of food and beverage preparation, pharmaceutical, chemical, cosmetics, and personal care industries. The major utilization of the HPH technique in the pharmaceutical arena is for numerous purposes like particle size reduction, preparation of highly stable emulsions or suspensions, mixing, increasing product stability and consistency, micronization, nanonization, etc. The process often ensues smaller and monodisperse particles that provide stability to the dispersions and increases its shelf-life. The HPH technique ensures drastic size reduction owing to the action of very high sheer, acceleration, pressure, turbulence, and impact forces on the subjected particles. The technique comes under top-down approach in which the particles subjected for size reduction are dispersed in nonsolvent media and passed through HPH. With the advent of nano-formulations in the pharmaceutical sector, the employment of the HPH technique for producing nanoparticles has increased. Nowadays, nanoparticle research has become a great interest to the scientific community because of well-known benefits in drug delivery approaches like controlled and targeted drug release, increased drug bioavailability, increased drug effectiveness, and stability. HPH technique allows the formulation of uniform and consistent nano-sized particles more efficiently owing to the combined effect of high pressure and mechanical forces (Yadav and Kale 2019). A detailed understanding of the effect of HPH processing parameters on nanoparticles size, stability, polydispersity index (PDI), and the surface charge will provide a benefit in formulating nanoparticles with desired characteristics. The chapter mainly focuses on the fundamentals of homogenization technique including its instrumentation, working principle, theories of homogenization, its application in various lipid-based nanoparticles, nanocrystals and polymeric nanoparticles, process analytical technique for HPH, scale-up aspect, and future perspectives.

2 Fundamentals of High-Pressure Homogenization

HPH is a process in which the fluid, i.e., the product to be homogenized (premix), is subjected under high pressure through homogenizing nozzle comprising a very narrow gap. The premix fed in the high-pressure homogenizer can be a suspension, emulsion or dispersion subjected for size reduction, droplet breakup, and homogenization. HPH technique provides enormous energy for breaking down particles efficiently to nanoscale. The commonly used units for pressure indication include bar, psi, and MPa. The instruments, operating pressure ranges from 50 to 500 MPa in which the instrument working at 200 MPa or above that, are termed as ultrahigh-pressure homogenizer (UHPH) (Dumay et al. 2013). Preliminarily, the premix under high pressure passes through a narrow gap or the homogenization valve from the inlet chamber to the outlet chamber of the homogenizer. A schematic representation of the HPH process is given in Fig. 11.1. According to Bernoulli's principle, the increase in velocity proportionally decreases the pressure; thus in the gap, the pressure decreases tremendously due to very high velocity. Moreover, the pressure applied through the pump should be such that the Laplace pressure imparting resistance against droplet deformation or breakup can be surmounted. The Laplace pressure increases to a certain level with the decrease in droplet diameter; thus the smaller the particle size, the higher is the pressure required for its breakup (Digby 2002; Yong et al. 2017).

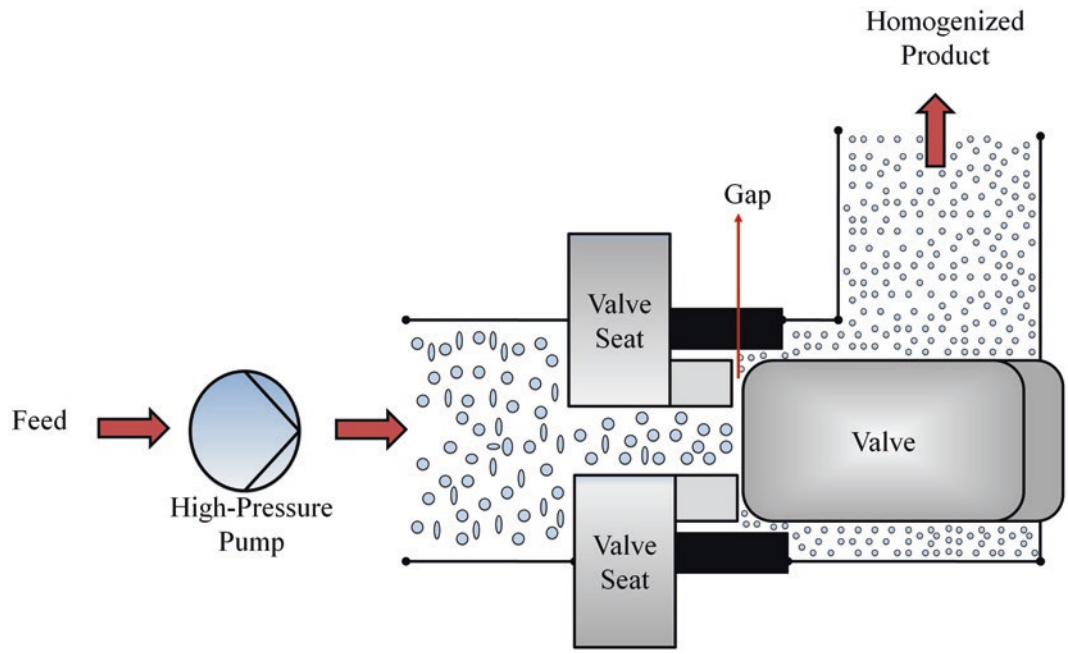

Fig. 11.1 Schematic representation of HPH technique

3 Instrumentation of High-Pressure Homogenizer

The high-pressure homogenizer instrument encompasses two main components responsible for homogenization and size reduction: (a) high-pressure pump and (b) homogenizing valve. The equipment used for pharmaceutical and food industries is usually fabricated using stainless steel of high-quality grade. The use of corrosion-resistant and wear-resistant material is a major concern for complying with the safety guidelines by the regulatory agencies. The modern homogenizing valves are usually constructed using tungsten carbide, nierite, and zirconium oxide for the purpose of inculcating corrosion-resistant characteristics and producing mechanically efficient valves that can withstand intense hydrodynamic forces. Modified equipments with soundproof or anti-vibration casing and facilities like steam in place (SIP) and clean in place (CIP) are also provided in certain new models (Yadav and Kale 2019).

There are basically two main categories of high-pressure homogenizers bifurcated on the basis of the geometry of the disruption unit and the flow pattern of the fluid: (1) piston gap homogenizer and (2) jet stream homogenizer. A piston gap homogenizer cannot achieve very high pressure ranges as that of Microfluidizer (jet stream homogenizer). The difference between both the assemblies is the valve design that is discussed further in Sects. 3.2.1 and 3.2.2. The main components of the homogenizer assembly affecting the homogenization process are high-pressure pump, valve assembly, impact ring, and O ring. The valve assembly can be considered as the heart of the instrument as the homogenization is completely dependent on the valve geometry and type of valve assembly. The integral parts of high-pressure homogenizer are discussed further in detail.

3.1 High-Pressure Pump

The integral part of high-pressure homogenizer equipment is high-pressure pumps that regulate the pressure under which the premix will be forced through the narrow gap. The largest part of

the homogenizer equipment in consideration of volume and weight includes the pumps and the motors for pressurizing the flow. Generally, laboratory-scale HPH encompasses 1 piston high-pressure pump, while production-scale or pilot-scale HPH has 3–5 piston high-pressure pump (Håkansson 2018b). The pump is usually pneumatically or electrically actuated and is made up of highly resistant materials. The diameter of the piston affects the attainable pressure and the capacity of the instrument. Piston with smaller diameter results in a high-pressure but moderate-capacity machine, while piston with large diameter confers moderate-pressure but a high-capacity machine. A piston pump often generates a pulsating flow that results in flow variations that causes acceleration and deceleration of the liquid in the downstream and upstream pipes of the equipment. These flow variations lead to vibrations and create possibilities of pipe breakage as well. The major effect due to pressure fluctuation occurs when there is a sudden pressure drop in the valve inlet. When the inlet temperature drops below the products boiling point, cavitation bubbles are generated that implodes on the valve. Dampers are often used to surmount the intense wear on valve walls owing to the cavitation effect. Heat exchangers are sometimes added to control the temperature increase that occurs due to increased pressure.

3.2 Homogenization Valve

The homogenization valve design and geometry play an indispensable role in the size reduction and droplet disruption of the premix. Among various parts of the homogenizer, the valve assembly is the most crucial component affecting the homogenization. The standard valve assembly generally comprises a valve rod and valve seat that forms a narrow gap in between. The fluid flow undergoes an intense increase in velocity and decrease in pressure while passing through the valve gap that leads to disruption of particles. After passing through the gap generally, the flow impinges on the impact ring and in turn gets deflected by a particular angle from where the homogenized product exits (Martínez-Monteagudo et al. 2017). On the basis of the number of valves used, high-pressure homogenizer can be categorized as one-stage homogenizer (single-valve assembly) and two-stage homogenizer (two-valve assembly). One-stage homogenizer is sufficient for most of the products, although, in cases where maximum size reduction with narrow particle size distribution is essential, a two-stage homogenizer is required. Usually, the products that require highly efficient homogenization, viz., high-fat content products, are subjected to the two-stage homogenizer. The second stage, where pressure equivalent to 10% of total pressure is applied, reduces the clumping and controls back pressure. Depending on the application, several types of homogenization valves are available commercially. Numerous patents have been granted to date for a variety of homogenization valves with different design and geometry. There are three main types of homogenization valves based on the design, flow, operating pressure, and scalability. The three main types are (1) radial diffuser valve, (2) counter jet valve, (3) and axial flow through orifice valve as represented in Fig. 11.2.

3.2.1 Radial Diffuser Valve

The radial diffuser valve is the most common type of homogenizing dispersion unit that consists of a mobile valve seat and an axial valve face. The mobile valve seat enables variation of homogenization pressure through adjusting upstream flow and the slit width. The flow enters through a nozzle and further gets deflected consecutively at a 90° into two coaxial annular chambers. The maximum pressure level up to 150 MPa can be obtained by this valve assembly. Gandini and Grandi studied the effect on homogenization efficiency by increasing the number of homogenization valves and reported that there was no significant improvement in homogenization efficiency (Gandini and Grandi 2006). The pressure variations while passing through a flat radial diffuser valve were studied by Phipps. While entering into the inlet, due to the intense increase in velocity, the pressure suddenly drops. Furthermore, the formation of vapor bubbles

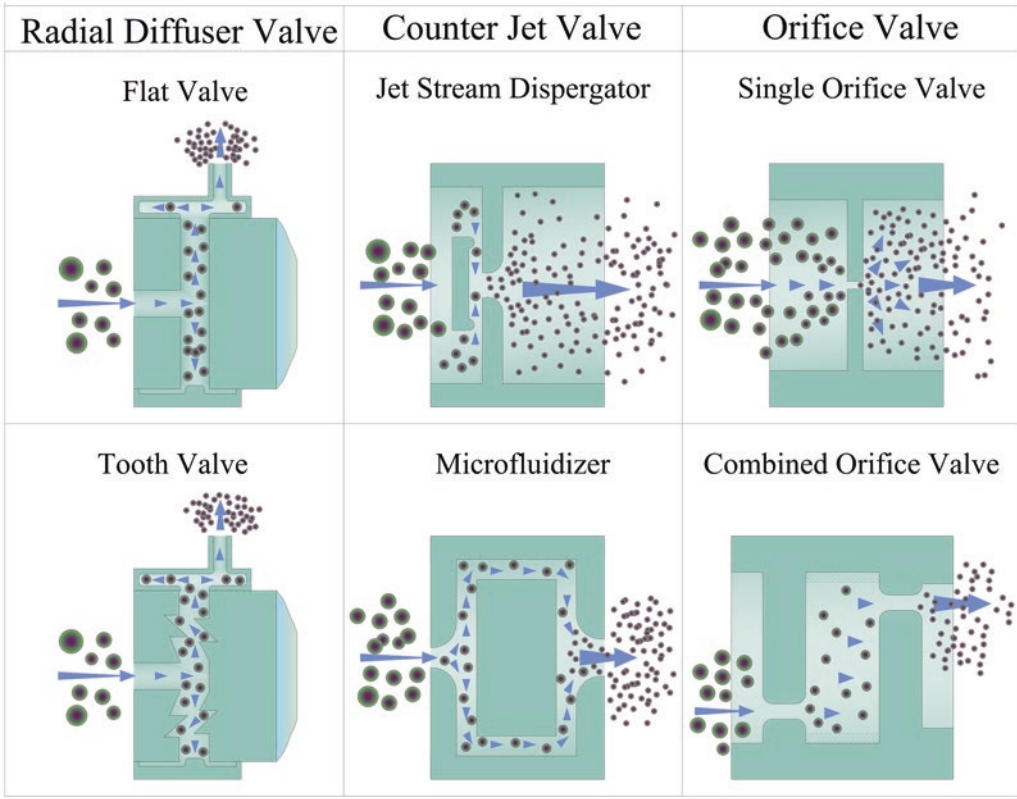

Fig. 11.2 Schematic representation of different valve designs for high-pressure homogenizer

converts the flow into two-phase flow, i.e., vapor/liquid flow. Thereafter due to compression shock, the vapor bubbles collapse due to a rise in pressure again. Based on the pressure variation study, Phipps explained that the disruption of droplet mainly occurs in the inlet of the homogenization slit (Phipps 1974). However, the exact phenomena described by various researchers remain contradictory as the accessibility to the very small dimensions and extreme conditions during homogenization is difficult. Also, many variables influence the homogenization process; thus complete theory is not established.

3.2.2 Counter Jet Valve

The counter jet dispergators involve impingement of jet streams from opposite directions leading to particle size reduction and homogenization. Alike radial diffusers, counter jet valves do not have movable parts and thus allow higher pressure range up to 300 MPa. Bayer AG patented a jet dispergator consisting of two orifices (axially opposing) and sharp edge inlets. On the basis of experimental results, the length/diameter optimal ratio reported is between 1.5 and 2 range and the optimal diameter of the bore is 0.3 mm to 1 mm. For attaining mean droplet diameter x by jet dispergator, Klinksiek and Koglin gave the following equation:

$$X = \frac{C \cdot d_B^{0.165} \cdot \gamma^{0.365} \cdot \eta_d^{0.495}}{\Delta p_H^{0.6} \cdot \eta_k^{0.025} \cdot \rho_d^{0.235}}$$

where C = constant dependent on product; Δp_H = inclined differential pressure; ɣ = interfacial tension; ρ_d = dynamic viscosity of disperse phase; η_d = dynamic viscosity of disperse phase; and η_k = dynamic viscosity of continuous phase. Stang studied the impact of collision of jet emulsion on mean droplet diameter. He concluded that there is no significant influence of collision because as per the experiment the laminar extension flow at the bore of the orifice leads to droplet

disruption. The experiment with setup of one orifice and two or four orifices resulted in the smallest mean droplet diameter with one orifice setup. The number of orifices does not impact on homogenization quality, but for scale-up, higher number of orifice is beneficial (Schultz et al. 2004).

Another widely used homogenization dispergator based on the collision of jet streams is Microfluidizer®. In Microfluidizer® the premix gets divided into two microchannels that are directed toward each other, and they collide in the interaction chamber. The fluid velocity gets increased around tenfold in the microchannels, and then under pressure, the opposite streams impinge. The outlet is ninefold bigger than the diameter of the microchannel; thus the pressure gets discharged in the outlet chamber. Similar to the case of jet dispergators, Stang does not consider collision as the only mechanism for reduction of size. As per experiments, he considered a combination of turbulent flow in the interaction chamber as well as the laminar extensional flow in the inlet as the mechanism of size reduction. The Microfluidizer showed an enhanced reduction in mean droplet diameter compared to radial diffusers, which was studied by Robin et al. Since the original design development, numerous modifications are reported in the jet dispergator valve assembly. To increase the volume stream, Y-chamber valve or jet to jet valve was developed as multi-slotted Y channels can also be incorporated for increasing the volume capacity. Z-chamber design or jet to wall valve assembly was developed to produce effective microemulsion and reduce the rise in temperature. It has been reported on the basis of experiments that the Y-chamber valve assembly gives more uniform droplet size distribution compared to the Z-chamber valve assembly. The counter jet valves commercially available are capable of handling large volume scale up to 1000 L h^{-1}, and 200 MPa pressure is the key advantage of counter jet valves. Contrarily when pressure level more than 200 MPa is desired, there is a need for extremely high flow rates, as the design of the valve is such that the flow rate controls the homogenization pressure. Thus, a great amount of energy input is required, which is the disadvantage of counter jet dispergators (Schultz et al. 2004).

3.2.3 Axial Flow Through Orifice Valve

Axial flow through orifice valve (also known as nozzle aggregate) has an orifice with sharp edge inlet and outlet through which the pressurized fluid enters axially. Like counter jet dispergators, nozzle aggregates also do not contain any movable parts and thus provide the advantage of working at very high-pressure ranges. Experiment using a high-speed video system has been done to study the mechanism of droplet disruption. The study showed that the droplet breaks up after passing through the nozzle bore when the laminar flow changes to turbulent flow in the core part of the open jet. F Hoffmann-La Roche AG patented a novel type of combined orifice valve for stronger breakdown and improved droplet stabilization. The valve design has an arrangement of three consecutive orifices, wherein the second orifice has a larger diameter than the first and third orifices. High turbulence is created in the second chamber having a larger diameter. Compared to simple orifice valves, combined orifice valves have the capability of producing smaller mean droplet diameters. The turbulence chamber in the combined orifice valve is responsible for achieving a smaller mean droplet diameter. The residence time in combined orifice valves is higher, so even the surfactants with low-medium adsorption velocities can successfully give highly stable products and smaller-sized products.

3.3 Impact Rings and "O" Rings

The high-velocity liquid passing out from the valve gap strikes firstly with the impact ring. The impact ring prevents the damaging of the chamber due to an annular high-pressure fan. The impact ring is also known as the breaker ring as the fluid after leaving the gap firstly strikes perpendicularly to the impact ring (Kelly and Muske 2004). Most of the poppet-type valves have a breaker ring in the valve assembly. In the valve

packing, an O ring is fitted to prevent the leakage and seal the interface. It is made up of an elastomer loop usually by ethylene propylene diene monomer. However, the O ring might sometimes get contaminated with the crevices or clefts and, in turn, lead to leakage. To avoid the chances of contamination, Avestin introduced oped emulsifiers where no O ring is required.

4 Theories of Homogenization

4.1 Shear

High shear is produced owing to the disruption of fluid motion while passing through the minute gap of the homogenization valve. The change in fluid motion, in turn, leads to enhanced shear effect within the fluid system as well as among the valve seat and the fluid system (Martínez-Monteagudo et al. 2017). High shear is produced mainly in the inlet chamber and boundary layers of the gap in the valve. In the case of solid particle, the velocity gradient will impart a force on the particle and cause rotation, while in the case of liquid droplet, the elongation and deformation of drop occur. The term "shear" specifically indicates the elongation of dispersed-phase droplets followed by droplet breakup due to the high-velocity gradient surrounding the droplet. The fluid acceleration in the inlet chamber gives rise to elongational stress G on the droplet. Deformation of drop from spherical to ellipsoidal shape increases with an increase in elongational stress. However, the interfacial tension counteracts the deformation due to shear stress. The deformation extent is usually expressed in terms of capillary number. The droplet breakup is expected when sufficiently large deformation takes place, i.e., capillary number exceeds the critical limit.

$$Ca = \frac{G\mu cD}{2\gamma}$$

Hydrodynamic modeling and experiments have represented that the maximum shear rate G depends on the gap height and velocity of fluid passing from the gap.

$$G \propto \frac{Ug}{h}$$

The shear rate G can be used in order to determine the fragmentation stress on the droplet by the following equation:

$$\sigma frag = \mu cG$$

Contrarily, this fragmentation stress is experienced by the droplets for a very short duration of time, i.e., before entering the gap. Thus, deformation timescale can be expressed as:

$$\tau def = \frac{\mu D}{\sigma frag}$$

Walstra argued that as the fragmentation stress experienced by droplet will be for a very short duration, only low-viscosity drops will get fragmented due to laminar shear. It was concluded that the laminar shear in the inlet chamber will be unable to fragment the highly viscous drops. In the high-pressure homogenizer, the second region exhibiting high laminar shear is laminar boundary layers of the gap. Maximum droplets pass through the center of the gap as per the flow profile; thus the boundary layer extending in the center of the gap has a significant impact on fragmentation. The boundary layer thickness δ and gap distance correlation, x, as per flat plate approximation, can be stated as:

$$\delta(x) = 5\frac{x\sqrt{vc}}{\sqrt{Ugx}}$$

The boundary layer merges only if the gap length is long enough to give $\delta(x = lg) = h/2$. Herein, the difference in laboratory HPH and production-scale HPH can be noticed as the boundary layer had an impact on small-scale homogenizers but not on production-scale homogenizers.

4.2 Turbulence

The passage of fluid gets abruptly reduced to about 100–1000-fold, which generates a high degree of the velocity gradient. The turbulence

created by the highly irregular motion is considered as the chief phenomena responsible for efficient mixing, homogenization, and emulsification. The turbulent flow causes different mixing zones in which fluid particles undergo complicated and unpredictable paths leading to enhanced mass transfer, heat, and momentum (Martínez-Monteagudo et al. 2017). Turbulent eddies (coherent structures formed at different length scales) and heat are generated due to the dissipative nature of turbulence that sequentially offers adequate energy for the disruption of particles. Kolmogorov theoretically described the interaction of turbulent eddies with the drops that lead to the fragmentation of drops. Furthermore, the Kolmogorov-Hinze model described two mechanisms: (1) turbulent inertial fragmentation and (2) turbulent viscous fragmentation. According to turbulent inertial fragmentation, the fragmentation occurs by small eddies, while in the case of turbulent viscous fragmentation, disruption occurs by large whirlpools (Steiner et al. 2006). Furthermore, the theory of maximum size (dmax) that a droplet withstands was established by linking Kolmogorov's theory with the Laplace pressure, which was mathematically expressed as:

$$\mathrm{dmax} \approx \varepsilon^{-0.4} \times \gamma^{0.6} \times \rho^{-0.2}$$

where ε expresses the average amount of energy dissipated per time, γ is the interfacial tension, and ρ indicated the density (Hinze 1955).

The Reynolds number is very high at the exit of the gap, which indicates the formation of a turbulent jet.

$$\mathrm{Re} = \frac{Q}{\pi rev}$$

According to Kelemen et al., at Re > 14,000, kinetic energy gets converted to turbulence, and a turbulent jet is formed (Kelemen et al. 2014). The fragmentation rate in turbulent flow can be estimated by the turbulent kinetic energy dissipation rate by using the following equation:

$$\eta = \left(\frac{V_C^3}{\varepsilon} \right)^{1/4}$$

where ε = rate of dissipation of turbulent kinetic energy and Vc = kinematic viscosity of continuous phase. Breakup visualization studies have reported that the size reduction occurs in the downstream near the outlet chamber (Innings and Trägårdh 2005). This can be attributed to the turbulent energy force. The large turbulent eddies get further broken up to smaller size until they can be damped out of the viscous fluid. The eddies having a similar size as that of the droplet are able to break them more efficiently. The visualization experiments have represented that the droplet breakup occurs at the position where the turbulent flow has transformed its energy to suitable length scales (Håkansson et al. 2013).

4.3 Cavitation

Another mechanism involved in droplet breakup is cavitation that occurs as a result of the large pressure drop encountered by the liquid while passing through the valve. The fluid velocity in the gap increases by several folds owing to the narrow gap size. Thus, considering the energy conservation, the high-velocity region will be having high dynamic pressure but a very low static pressure. Formation of cavities within the fluid and its subsequent collapse occur when the static pressure falls below the vapor pressure. The bubbles/cavities collapse and generate shock waves that cause particle size reduction in the fluid. Cavitation can be quantified by Thoma number (Th)/cavitation number (Nc).

$$Nc = \frac{P\infty - P_v(T)}{0.5\ \rho_c V_\infty^2}$$

where $P\infty$ = upstream static pressure; $P_v(T)$ = vapor pressure at temperature of fluid; ρ_c = velocity of continuous phase; V_∞^2 = characteristic fluid velocity; and Nc = cavitation number.

Several experiments performed by researchers have demonstrated cavitation phenomena ensuing in HPH. On the basis of cavitating flow properties including the light scattering of

vapor bubbles, ultrasonic emissions, wear, and free radical formation, cavitation phenomena have been studied. In the HPH valve, the cavitation occurs at the beginning and inside the gap due to high local velocity and has been confirmed by several gap visualization experiments. With an increase in homogenization pressure, the cavitation increases, and contrarily, employing the second homogenization stage, the cavitation bubbles decrease. Cavitation being the mechanism for droplet breakup is beneficial in HPH, but on the counterpart, it also leads to wear in the HPH valve when it occurs in close proximity to the walls. This, in turn, causes a decrease in HPH valve efficiency for suitable size reduction and homogenization (Innings et al. 2011).

5 Process Analytical Techniques for High-Pressure Homogenization

The efficiency or the outcome of high-pressure homogenizer can be known by studying the particle size or particle size distribution of the obtained product. However, the influence of pressure, geometry of the device, or formulation on the homogenization efficiency in detail cannot be known with particle size analysis. The final product analysis gives the combined effect of superimposed mechanisms, but the effect of each intermediate step cannot be justified. Understanding the influence of all intermediate steps allows a deeper understanding of the selection of proper design and processing parameters for formulating products with desired properties. For understanding the droplet/particle deformation or disruption, inline measurement is necessary. However, inline measurement is often challenging due to high velocity, complex flow patterns, and high-pressure ranges contributing to the HPH process. Recently, several optical measurement methods have been established to understand the inline parameters influencing homogenization efficiency. An overview of different optical measurement methods is given in Table 11.1 (Bisten and Schuchmann 2016).

6 Applications of High-Pressure Homogenization in Nanoparticles Development

In the pharmaceutical industries, the HPH technique can be employed for the preparation of stable emulsions, suspensions, colloidal dispersions, as well as various types of nanoparticles. The high pressure and intense energy produced during HPH allow efficient particle size reduction and also yield uniform monodisperse particles and highly stable product. Apparently, the known advantages of nano-sizing in the pharmaceutical field have increased research and development in the field of nanoparticles for drug delivery (Möschwitzer 2010). However, most of the techniques have a major issue of scalability due to complex processing and product parameters affecting nanoparticle formation. Applying quality by design approach can be beneficial to understand various interdependent parameters involved. HPH is a technique that can be used also at a larger scale, so it provides an advantage over other competing techniques of nanoparticle production. The issues of traditional size reduction techniques like polymorph transformation, metal contamination, and high amorphization do not prevail in the HPH technique. The technique is also suitable for both aqueous and nonaqueous systems. The factors affecting the HPH process for nanoparticle preparations should be considered during nanoparticle preparation. Basically, the processing variable and product variable affect homogenization efficiency. The process variables are homogenization pressure, the number of homogenization cycles, valve and impingement design, flow rate, and temperature. In the case of nanoparticles, the product variables like initial size range, the viscosity of the medium, sample concentration, and sample volume play an indispensable role affecting homogenization efficiency (Yadav and Kale 2019). Different nanoparticles require different processing conditions according to the product characteristics that need to be optimized while performing HPH for producing nano-formulations. The application of the HPH technique for different types of nanoparticles has been discussed further.

Table 11.1 Optical measurement techniques for process analysis of HPH (Bisten and Schuchmann 2016)

Optical measurement method	Description
High-speed image processing (HSIP)	The HSIP method is based on capturing images of inline events within a very short time duration. A high-speed camera with a light source and good resolution is connected to a computer for visualization. In the breakup of droplet visualization, capture in minimum time with minimum motion blur is necessary. Droplet deformation or breakup can be investigated by this method, but on the contrary, velocity profiles or local stresses cannot be calculated and are the limitations of the HSIP method
Particle image velocimetry (PIV)	The PIV method allows visualization of disruption as well as the measurement of local velocity fields. It involves the addition of tracer particles that follow the flow pattern. Pulsed laser light is used to illuminate the tracer particles. High-speed and high-resolution camera captures double images (two images at a particular time difference) of the tracer particles that are at a 90° to the light beam. From the displacement between double images, the velocity field is calculated. The cross-correlation or autocorrelation method is usually applied for further processing
Microparticle image velocimetry (μPIV)	The macroscopic PIV technique is modified to a microscale to characterize flow patterns in microfluidic devices. There is a difference in illumination in PIV and μPIV methods. In μPIV technique whole volume is illuminated. The laser light gets absorbed by fluorescent particles, and thereafter they emit light at a different wavelength. The further process includes the same steps as in the PIV technique. However, the visualization of small droplets is difficult, and temporal and spatial resolution limitations are the drawbacks of this method
Shadow graphic imaging	The method is employed for visualization of cavitation pattern. There is a light source in line with the camera. The cavitation forms gas bubbles that in turn block the light and reflect it back forming a shadow. The camera records the shadow and describes the flow pattern. Thus, the area where the cavitation occurs can be established by visualization of the shadow. However, the gas bubble collapse cannot be determined by this method
Sono-chemiluminescence (SCL)	Luminol is added to the fluid that emits light on the collapse of cavitation bubbles. This is due to the formation of free OH radicals that oxidizes luminol and forms an intermediate product. The intermediate product on decomposition emits light that is detected by a camera or a sensor. The method provides an advantage to measure the intensity of gas bubble collapse. However, velocity measurement is not possible by this method

6.1 High-Pressure Homogenization for Lipid-Based Nanoparticles

6.1.1 Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs)

SLNs and NLCs are a new generation of colloidal carriers that are derivative of o/w emulsion in which oil drops are replaced by solid lipids. Owing to their distinct advantages like controlled and targeted drug release, biocompatible and biodegradable nature of most of the lipids, increased drug stability, and easy scalability, SLNs and NLCs are attracting major attention in the pharmaceutical industry. SLNs are considered as first-generation lipid nanoparticles wherein drug-entrapped solid lipid nanospheres are dispersed in the surfactant-stabilized aqueous phase. NLCs were developed to overcome the drawbacks of SLNs like low drug loading and stability issues. NLCs comprise of liquid lipids along with solid lipids in the lipid core that enhances the drug loading capacity (Rawal and Patel 2018,

2019). In 1992, Siekmann and Wetesen and Muller et al. in 1993 introduced the HPH technique for formulating SLNs and NLCs. Furthermore, the HPH technique for lipid colloidal carriers was patented by Muller and Lucks in 1996 (Muller et al. 2011). Among various methods proposed for the preparation of SLNs and NLCs, HPH is most widely used. Researchers have developed numerous therapeutically effective SLNs and NLCs employing HPH technique (Sinhmar et al. 2018a, b; Mathur et al. 2019; Khatri et al. 2019; Chokshi et al. 2019). A summary of various experiments done for the preparation of SLNs and NLCs employing the HPH technique is given in Table 11.2.

For the preparation of SLNs and NLCs, there are two types of HPH techniques based on the process temperature: (1) hot homogenization and (2) cold homogenization. In both the techniques, initially, the drug is dissolved in lipid at 5–10 °C above the melting point of lipid.

1. Hot homogenization

In hot homogenization, the complete process is carried out at a temperature higher than the lipid's melting point; thus it can be considered as the homogenization of an emulsion. Primarily, using a high-shear device, a pre-emulsion of drug-entrapped lipid melt and aqueous surfactant phase is prepared. The lipid melt and aqueous phase subjected to emulsify are kept at the same temperature (5–10 °C above the melting point of lipid). Immediately after the pre-emulsion is formed, it is subjected to high-pressure homogenizer for further size reduction. The high temperature allows higher size reduction due to lower inner-phase viscosity. Contrarily, chances of drug degradation are higher at high temperatures. Also, the higher pressure leads to increased sample temperature (almost 10 °C at 500 bar). Homogenization requires at least 5 cycles at 500–1000 bar for sufficient size reduction that apparently increases sample temperature as well. After cooling of the product, the primary homogenized product, i.e., nanoemulsion, converts to colloidal dispersion (Mehnert and Mäder 2012; Ganesan and Narayanasamy 2017). Figure 11.3. represents the hot homogenization process for the preparation of SLNs/NLCs.

2. Cold homogenization

To overcome the drawbacks of hot homogenization such as drug degradation at high temperature and chances of drug expulsion in the aqueous phase during homogenization, cold homogenization technique has been established. In cold homogenization, the lipid remains in solid state; thus it can be correlated with high-pressure milling of suspension. The first step of solubilizing drugs in lipid melt remains the same as in hot homogenization, although, further steps are carried out at a lower temperature range. After the dissolution of the drug in lipid melt, it is rapidly cooled by liquid nitrogen or dry ice. Immediate cooling at very low temperatures leads to uniform distribution of the drug in the lipid phase. The drug-lipid solid mixture is further milled to form microparticles by mortar milling or ball milling. At lower temperatures the fragility of lipids increases so particle comminution occurs efficiently. The milled drug-loaded lipid microparticles are added to the cold aqueous surfactant solution and subjected to HPH. The cold homogenization avoids the higher temperature exposure but does not completely surmount it due to the dissolution of the drug in lipid melt in the preliminary step. Higher particle size and broader size distribution of nanocarriers are observed in cold homogenized samples compared to hot homogenization samples (Naseri et al. 2015). Figure 11.4 represents the cold homogenization process for the preparation of SLNs/NLCs.

6.1.2 Nanoemulsion

HPH technology has two main advantages that have made it very attractive for the formulation of nanoemulsion: (1) the intense energy and hydrodynamic stresses are beneficial for achieving small droplet sizes; (2) it is well-tested technology for large-scale continuous production. The formulation parameters like disperse-phase volume, emulsifiers, and surfactants and process parameters like homogenization pressure, number of cycles, and temperature have a major effect on nanoemulsion quality. The disperse-phase volume affects the process of fragmentation of droplets. The dispersed-phase droplets in the

Table 11.2 Summary of research findings related to SLNs and NLCs formulated by employing HPH technique

Type of nanocarrier	Drug/active moiety	Lipids used	Surfactant/ co-surfactant	HPH used	Homogenization pressure (bar)	Homogenization cycles	Particle size (nm)	PDI	References
1. Solid lipid nanoparticles (SLNs)	Rifampicin	Compritol 888 ATO	Polysorbate 80	Panda Plus 2000, GEA (Niro Soavi, Germany)	1000	12	456 ± 11	0.205 ± 0.03	Chokshi et al. (2018)
	Citral	Glyceryl monostearate (GMS)	Tween 80 and Span 80 (1:1)	AH100D, ATS Engineering Inc., Vancouver, Canada	500	2	194 ± 2.19	0.358 ± 0.04	Tian et al. (2018)
	Progesterone	Tristearin	Poloxamer 188	Panda Plus 2000/ GEA (Niro Soavi, Parma, Italy)	1000	1–3	181 ± 14	0.031	Esposito et al. (2017)
	Astaxanthin	Stearic acid	Tween 20	AH-basic, Shanghai, China	300	15	167 ± 18.1	0.19 ± 0.042	Li et al. (2016)
	Dibucaine	Myristyl myristate	Poloxamer 188	Panda homogenizer (Niro Soavi, Parma, Italy)	600	3	234.33 ± 42.87	0.32 ± 0.01	Barbosa et al. (2018)
	Dibucaine	Cetyl palmitate	Poloxamer 188	Panda homogenizer (Niro Soavi, Parma, Italy)	600	3	239.37 ± 18.31	0.18 ± 0.03	
	Voriconazole	Witepsol® W35	Polysorbate 80	Avestin Emulsiflex B15 instrument (Avestin Europe GmbH, Germany)	600	5	182 ± 4.1	0.269 ± 0.01	Füredi et al. (2017)
	Artemether	Glyceryl monostearate: Compritol (50:50)	Poloxamer 188	Panda Plus 2000 (Niro Soavi, Germany)	1000	10	419 ± 09 nm	0.235 ± 0.02	Khatri et al. (2018)

2. Nanostructured lipid carrier (NLCs)	Quercetin	Glyceryl monostearate (GMS), linseed oil	Polyglycerol-6 monostearate, Tween 80, 1,1-propylene glycol	FB-110, LiTu Mechanical Equipment Engineering Co., Ltd., Shanghai, China	800	6	95.6 ± 0.3	0.253 ± 0.010	Huang et al. (2017)
	Ondansetron hydrochloride	Tristearin, Phosal 53MCT	Tween 80	Emulsiflex C3, Avestin, ON, Canada	500	6	266 ± 10	0.280 ± 0.007	Duong et al. (2019)
	Minoxidil	Stearic acid, oleic acid	Tween 80, Span 80	M-110P Microfluidics, Newton, USA	1380	2	281.4 ± 7.4	0.207 ± 0.009	Wang et al. (2017b)
	Ibuprofen	Witepsol E85, Miglyol 812	Lutrol F68	Emulsiflex C3 high-pressure homogenizer (Avestin Europe GmbH, Mannheim, Germany)	600	5	107.47 ± 14.4	0.18 ± 0.3	Sütő et al. (2016)
	5-Fluorouracil	Precirol® ATO 5, Labrasol®	Poloxamer 188, Solutol® HS15	Emulsiflex C3; Avestin, Ottawa, ON, Canada	1500	5	208.32 ± 8.21	0.352 ± 0.060	Rajinikanth and Chellian (2016)
	Budesonide	Compritol 888 ATO, Labrafac WL 1349	Span® 80	Panda Plus, GEA Niro Soavi, Italy	700	5	284.0 ± 4.53	0.155 ± 0.06	Sinhmar et al. (2018a)
	Domperidone	Palmitic acid, oleic acid	Tween 80	Panda 2 K, NS1001L Spezial, GEA Niro Soavi, Lübeck, Germany	500	5	283.97 ± 2.25	0.176 ± 0.015	Tetyczka et al. (2017)
	Voriconazole	Compritol 888 ATO, Miglyol 812N	Tween 80, Solutol HS 15	Nano DeBEE; BEE International, Easton, MA, USA	1380	3	45.62 ± 0.53	0.18 ± 0.01	Tian et al. (2017)
	Citral	Hydrogenated palm oil (HPO), lipoid S-100 and olive oil	Tween 80	High-pressure homogenizer (Avestin, Ottawa, ON, Canada)	1000	15	54.12 ± 0.30	0.224 ± 0.005	Nordin et al. (2018)

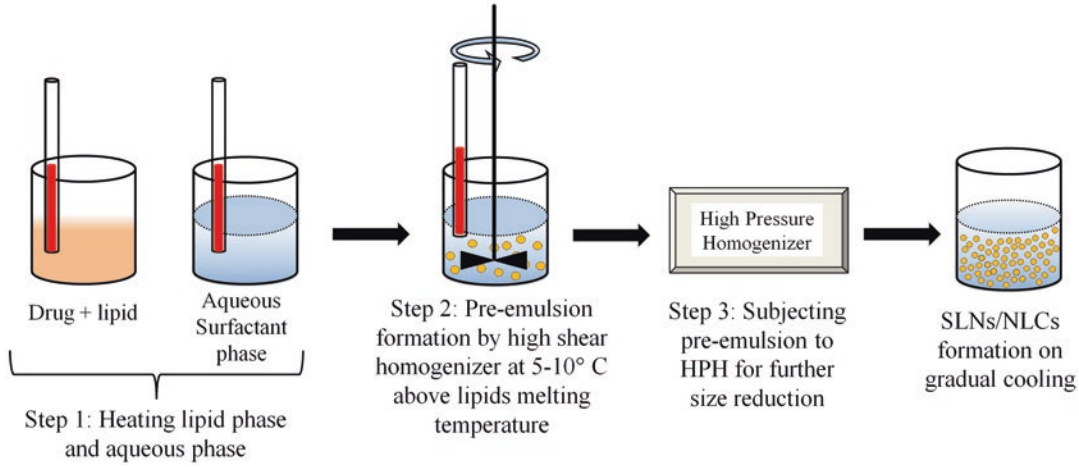

Fig. 11.3 Hot high-pressure homogenization process

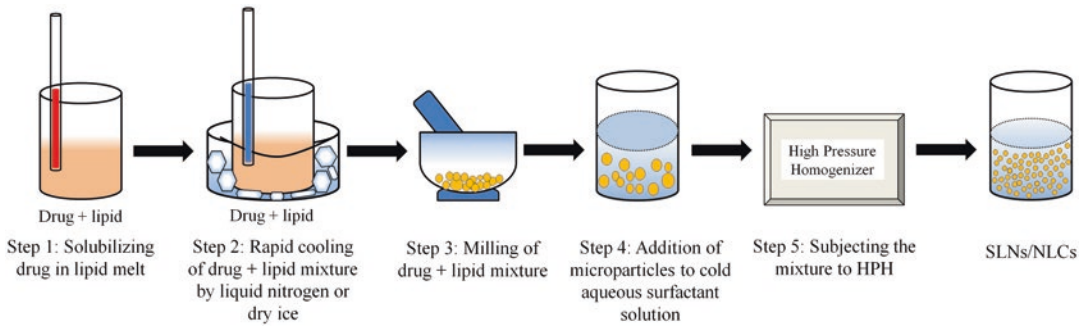

Fig. 11.4 Cold high-pressure homogenization process

flow lead to an increase in viscosity as well as viscous stresses. With increased viscosity, the drop formation and its fragmentation become complicated. However, the effect of higher viscosity of dispersed phase is less in orifice-type valves and jet dispergators (Håkansson 2018a). Stang reported laminar extension flow in jet dispergators and orifice valves leading to droplet disruption in spite of higher viscosity. There is also an effect of the disperse phase on the turbulent energy that on the basis of droplet size can lead to either attenuation or enhancement of turbulent stresses. Thus, the disperse-phase volume can significantly affect the homogenization mechanism (Stang et al. 2001). Surfactants and emulsifiers have the same role in nanoemulsion formulation by HPH as in the case of any emulsification process. They reduce the interfacial tension and thereby increase the fragmentation rate and decrease the chances of recoalescence. However, under HPH the emulsifier has a very short time to stabilize the new fragments as high-intensity passage time is very short. It takes only 10 μs for the emulsion to pass through the valve gap; thus the stabilization may be problematic in such a short duration. Also, the intense high pressure might cause changes or break covalent bonds and, in turn, may decrease the emulsifying efficiency of emulsifiers. Apart from the product parameters, process parameter like temperature also affects the emulsion quality. Experimental studies have investigated that the product temperature increases with homogenization pressure (19–23 °C temperature increase per 100 MPa

increase in pressure) (Mao et al. 2010; Benzaria et al. 2014).

Almost all the experiments done for preparing nanoemulsion by HPH have concluded that nanoemulsion prepared by HPH is more stable on storage and has desired characteristics and droplet size less than 500 nm. As per the trials, not all nanoemulsion requires the same homogenization pressure to attain a particular droplet size, viz., studies of nanoemulsion having the same droplet size (200 nm) have required different pressure (50 MPa to 200 MPa). Thus, no specific homogenization pressure can be concluded for nanoemulsion formation because the droplet size depends on several other factors as well. Another process parameter is HPH passages/HPH cycles. There is a requirement of at least 2–3 passages for nanoemulsion preparation. For laboratory scale, recirculation is possible, but in the case of industrial continuous processing, 2–3 homogenizers need to be connected in a series that in turn increases the production cost. After processing the nanoemulsion at a certain pressure and after many numbers of passages, a phenomenon of droplet size increase termed as "recoalescence" occurs. Various experimental studies have been done to justify the mechanism of recoalescence. Lee et al. in his experimental study observed that after five passages recoalescence occurred. He attributed the reason that due to temperature increase coalescence rate increased. However, an increase in temperature leads to a decrease in fluid viscosity, and thus his reason for a coalescence rate increase is not justifiable (Lee et al. 2014). Ali et al. suggested two mechanisms for recoalescence based on his experiment, viz., denaturation of emulsifier due to higher passages through the HPH valve and another aspect very less adsorption time (Ali et al. 2016). Various studies have reported such findings representing the denaturation of emulsifiers while passing through HPH. Floury et al. reported that during HPH methylcellulose underwent degradation (Floury et al. 2002). Conformational changes and degradation in barley wax even at low homogenization pressure 5–20 MPa were also reported (Floury et al. 2002). However, several studies have also shown no conformational changes or damage in emulsifiers in overprocessed nanoemulsion. Thus, to what extent the emulsifier deformation affects recoalescence still needs to be evaluated further. Several studies have reported various factors affecting the HPH process for nanoemulsion formulation. Mistry et al. studied the effect of stabilizers and HPH on chemical and physical properties of curcumin-containing chitosan/glycerol monooleate nanoemulsion. Polyvinyl alcohol and poloxamer 407 were the two stabilizers used for oil in water chitosan/GMO nanoemulsion stabilization. The results represented that three homogenization cycles reduced 50–65% droplet size; further increasing cycles did not significantly reduce the size (Mistry and Mohapatra 2012). Sharma et al. studied the effect of HPH on rutin- (active moiety) and TPGS (emulsifier)-loaded nanoemulsion. Comparison of the nanoemulsion characteristics like droplet size, zeta potential, and in vitro drug release was done for rutin-loaded nanoemulsion prepared by HPH method and spontaneous emulsion method. Compared to nanoemulsion prepared by spontaneous emulsification method, the nanoemulsion prepared by HPH represented increased in vitro release and smaller droplet size. The nanoemulsion by HPH also showed increased permeability in ex vivo studies compared to rutin suspension. The homogenization pressure of 200 MPa and 4 homogenization cycles was found to be optimum (Sharma et al. 2015). Another novel approach of using ultrasound along with HPH to reduce the energy requirement of individual processes was done by Calligaris et al. Tween 80 and Span 80 (1:1) blend and 15% (w/w) oil in water mixture were homogenized at 20–100 MPa prior to or after 20–60 s of ultra-sonification. While comparing nanoemulsion with individual method, nanoemulsion prepared by the combination was found to be more stable with a lower mean size (Calligaris et al. 2016).

6.1.3 Liposomes

Liposomes are lipid-based artificial nanovesicles of spherical geometry comprising of a phospholipid bilayer. The key advantage of liposomes is that they can entrap hydrophobic material within

the phospholipid bilayer and hydrophilic material in the internal aqueous core. The conventional methods for the preparation of liposomes are film hydration method, reverse-phase evaporation, freeze thawing, extrusion, ethanol injection method, etc. These traditional methods have a requirement of organic solvents and very complicated processing steps. HPH technique allows easy scale-up as well as does not require the use of toxic solvents. Wang et al. developed phytosterol- and phytosterol ester-encapsulated soy phospholipid liposomes employing HPH. The phospholipid and phytosterol dispersion in 3,4-morpholinopropanesulfonic acid (MOPS) buffer was initially pre-homogenized in an Ultra-Turrax homogenizer at 22,000 rpm for 5 min. Furthermore, the pre-emulsion was subjected to high-pressure homogenizer (Microfluidizer) at 690 bar pressure, 80 °C. The liposomes formulated by Microfluidizer were highly stable, and TEM images showed that most of the structures are unilamellar and some appeared multivesicular. The study provided an outlook for pharmaceutical and nutraceutical companies toward using Microfluidizer for the preparation of liposomes to deliver bioactive agents (Wang et al. 2017a). Another approach that encompasses the use of HPH in liposome manufacturing is preparing liposomes by conventional approaches and further reducing their size in a high-pressure homogenizer. The size distribution of the liposomes depends on processing parameters like the number of homogenizing cycles and homogenization pressure. Apart from that, the sample related factors like composition of the bulk medium, phospholipid concentration and composition, liposome lamellarity, and initial size distribution of the liposomes. Barnadas et al. studied the effect of homogenization process parameters like the effect of recirculation mode and non-recirculation mode of the Microfluidizer. Along with process parameters, the effect of sample parameters like phospholipid and ethanol concentration was also studied. In a non-recirculation mode, the study depicted a continuous effect of pressure on the liposome size (increase in pressure led to a decrease in liposome diameter). In the case of homogenization cycles, there was no significant effect on the mean diameter after seven cycles. While in a recirculation mode, at the homogenization outset, there is an increase in size distribution width initially because small quantities of suspension in the reservoir get mixed with the processed sample. But when liposomes attain a certain small size, the width of size distribution decreases. The study for determining the effect of ethanol concentration and phospholipid concentration represented that under fixed conditions the liposome diameter decreased and size distribution became narrower with increased concentration of ethanol (Barnadas-Rodríguez and Sabés 2001).

Kyun et al. studied the effect of HPH on the physicochemical properties of cationic polymer-coated liposomes. Non-coated liposomes, chitosan-coated liposomes, and Eudragit-coated liposomes were formulated employing the HPH method. There was an effect on homogenization pressure, the ratio of core material to lecithin, and the number of homogenization cycles on mean size, PDI, encapsulation efficiency, and surface charge of the non-coated liposomes. The experimental results depicted that three homogenizing cycles and 1000 bar pressure gave the optimal results. There was a decrease in particle size and PDI with increase in pressure and number of cycles. However, with an increase in homogenization pressure, the encapsulation efficiency decreased. At 500 bar, the encapsulation efficiency was highest, but the particle size and PDI were higher. Thus, 1000 bar was selected for preparation of liposomes with desired characteristics (Kyun et al. 2014).

6.2 High-Pressure Homogenization for Nanocrystals

Nanocrystals are basically drug particles of submicron sizes that have a semicrystalline state with a high surface area. HPH is one of the most important techniques for the production of nanocrystals. When nanocrystals are dispersed and stabilized in a dispersion medium, nanosuspension is formed (Keck and Müller 2006). Various

processes based on the HPH technique have been patented by several industries. DissoCube® technology was the first HPH-based technology that was granted a patent for pure aqueous homogenization of particles. Another HPH-based technology named as Nanopure® was patented by PharmaSol GmbH, Berlin, for non-aqueous media milling of drug particles to form nanosuspension. Herein, isotonic hydrophilic solvents such as aqueous solution of polyethylene glycol (PEG) or glycerol are used for the production of parenteral nanosuspensions. Yet another HPH-based method, used for the production of nanosuspensions, was patented under the name NANOEDGE® by Baxter pharmaceuticals to overcome the problem of uncontrolled crystal growth, associated with the traditional precipitation method by combining this bottom-up approach with the top-down process of HPH.

6.2.1 DissoCube®

This technology was based upon the fact that large cavitation forces are generated upon subjecting the drug dispersion in an aqueous medium to piston gap homogenization. Based on Bernoulli's principle, in a closed system, the flow volume of liquid is constant per section. Hence, upon passing an aqueous drug dispersion through a narrow orifice diameter, enormous dynamic pressure is generated with a simultaneous decrease in static pressure. Reduction in static pressure results in reduced vapor pressure, due to which, the liquid starts to boil and form gas bubbles that get imploded on leaving the homogenization gap. The resultant cavitational forces are believed to contribute to the size reduction of drug dispersion to nano-size and form nanocrystal/nanosuspension. The process of nanocrystal formation depends on the powder density of the homogenizer, temperature, and number of homogenization cycles. In addition to this, initial particle size should be <1 mm, and the pre-homogenized drug powder should be monodisperse to prevent physical destabilization.

6.2.2 Nanopure®

This specialized HPH technique also referred to as "deep-freeze homogenization" is used for the development of specialized nanocrystals/nanosuspensions in which the drug particles are dispersed in non-aqueous hydrophilic media such as PEG 400, PEG 600, or water-glycerol mixtures. Some examples of such pharmaceutical nanocrystals are nanosuspensions to be filled in capsules or hydrolytically unstable drug nanosuspensions that can be diluted prior to their administration with aqueous vehicle to have dry products with low moisture content. In contrast to cavitation being the driving force for size reduction of nanoparticles in the DissoCube® technology, the driving force for size reduction using the Nanopure® technology involves size reduction at zero or subzero temperatures (−20 °C). This technology provides a subtle method for the size reduction of thermolabile drugs and the drugs that have the aforementioned prerequisites. This method has also been employed to homogenize drug powder in melted solid/semi-solid PEG like PEG 1000 or PEG 6000 and to obtain nanocrystals that can be filled in hard gelatin capsule directly or after grinding the solidified PEG nanosuspension.

6.2.3 NANOEDGE®

As described earlier, this technique involves size reduction using HPH after the precipitation step of the bottom-up approach. The main advantage of this process is that it yields nanoparticles in a state wherein a balance of particle energy is achieved to attain good particle stability of the nanocrystals. Moreover, irrespective of the initial state of the material (amorphous, semicrystalline, or crystalline), the precipitated particles undergo "annealing" step when processed using this patented HPH technology and are transformed to a crystalline state. However, this technique has a prerequisite of having a predefined solvent and an anti-solvent for the chosen drug to facilitate drug precipitation. Despite these advantages, the method has drawbacks of using solvents that need to be removed from the product for regulatory approval and being more expensive. Some examples of nanocrystals/nanosuspensions produced using HPH have been described here in detail.

Solid concentration/solid content of nanosuspension is one of the major parameters affecting particle size reduction using HPH. Typically, the process of HPH is more efficient at solid concentrations<10%. The research work of Krause and Muller demonstrates the production of nanosuspensions at typically high solid contents (20 and 30%) with the use of different amounts of surfactants. The factors that led to an efficient nanonization of powders with high solid content were observed to be pre-milling, homogenizer design, and product viscosity. The pre-milling step typically involves operating HPH at 100 bar for 2 cycles, followed by 2 cycles at 500 bar and 2 cycles at 1000 bar. However, this step may be obviated if the initial powder size is to a fine degree. It was observed that there was a significant increase in the viscosity of suspension with an increase in the solid content to >40% and yield of paste. Processing such high-viscosity materials can be difficult with the lab-scale high-pressure homogenizers and demands large-scale piston gap homogenizers to be employed. It can be summarized from the undertaken research work that in order to achieve particle sizes similar to that for the low solid concentration suspensions (<10%), homogenization cycles can be increased (to achieve high total disintegration energy), and a homogenizer design that facilitates active transport of suspension should be used (Krause and Muller 2001).

In a research work of Karadag et al., quercetin nanocrystals were prepared using HPH technology to enhance its water solubility and bioavailability. The optimized quercetin nanocrystals were formed by dispersing (0.5%w/w) quercetin in aqueous solution of Tween 80 at 70 °C for 20 min. This coarse dispersion was subjected to further size reduction using the high-shear homogenizer (Ultra-Turrax T-25 basic, IKA Works Inc., Wilmington, NC, USA) at 24,000 rpm for 5 min. The dispersion thus obtained was subjected to filtration through Whatman cellulose filters (grade 3, 6 μm mesh). Further size reduction was carried out using a high-pressure homogenizer (Emulsiflex C3, 90 Avestin Inc., Ottowa, Canada) at 50 to 200 MPa and 40 cycles. It was observed that on increasing the homogenization pressure, there was a decrease in the particle size. Almost 50% size reduction was observed after two cycles. However, the rate of size reduction was reduced on the further increase and was found to get stagnant after 10 cycles. The particle size and PDI of nanocrystals thus obtained were observed to be ~430 nm and 0.2, respectively. Also, fluctuations in PDI have been observed in between the homogenization cycles, indicating the formation of reversible aggregates that get comminuted during subsequent cycles. The predominating size reduction force involved in the processing of the quercetin nanocrystals was observed to be cavitation (Karadag et al. 2014).

In a similar study performed by Sun et al., itraconazole (ITZ) nanosuspension was prepared using HPH and evaluated for the effect of stabilizer on particle size, zeta potential, and surface morphology. For the preparation of ITZ nanosuspension, the researchers subjected the coarse ITZ powder to homogenization in aqueous stabilizer solution using Ultra-Turrax T-25 (Jahnke & Kunkel, Staufen, Germany) at 8000 rpm for 1 min. The dispersion thus prepared was subjected to HPH using AH100D (ATS Engineering Inc., Shanghai, China). The dispersion was processed for two homogenization cycles at 150, 500, and 1000 bar and repeated at 1350 bar for several cycles to obtain the desired particle size. The ITZ nanosuspension was evaluated for deducing the effect of multiple stabilizer system (Lutrol F127 and sodium lauryl sulfate (SLS)) on particle size using central composite design. There was no alteration in the crystalline form of ITZ after being processed by HPH. However, the in vitro dissolution rate was observed to be directly proportional to the size of the ITZ nanocrystals. The in vivo pharmacokinetics in rats showed a significant enhancement of drug concentration-time curve and maximal plasma concentration (AUC) with the ITZ nanosuspensions ($n = 3$) (Sun et al. 2011).

In the past decade, several research works have been performed for facilitating the formation of solid dispersions of nanocrystals. In a research work performed by Ye et al., nanosuspension of Efavirenz (EFZ) were prepared by

HPH and converted to a solid dispersion by combining it with the hot-melt extrusion process. The EFZ nanodispersion was stabilized using Kollidon® 30 and sodium lauryl sulfate (SLS), followed by its blending in the extruder barrel with Soluplus®. Particle size reduction was significant with an increase in the homogenization cycles till an optimum, after which there was no further size reduction. The particle size of ~320 nm was achieved on subjecting 2% drug suspension to homogenization for 20 homogenization cycles at 1500 bar. While HPH was performed efficiently at % drug loading of 2% and 4%, the % drug loading of 8% was observed to block the HPH. There was a significant increase in the dissolution rate of EFZ with the nanocrystals, due to increased wetting ability and surface area (Ye et al. 2016).

6.3 High-Pressure Homogenization for Polymeric Nanoparticles

Polymeric nanoparticles are generally of two types, nanospheres and nanocapsules, wherein the active moiety is encapsulated within the polymeric material. Emulsification solvent evaporation, salting out method, precipitation method, supercritical fluid technology, and ionic gelation method are the general methods used for the preparation of polymeric nanoparticles. The HPH method is usually employed for further size reduction and producing uniform monodisperse particles. Various experimental studies done by researchers have proved the efficiency of the HPH technique to produce smaller and uniform particle sizes. Also, the redispersibility and stability of the nanoparticles are found to be increased by employing HPH for its production. Lamprecht et al. developed PLG/PCL 50:50 polymeric nanoparticles by double emulsion pressure homogenization technique and checked the influence of process parameters on the preparation of nanoparticles. The homogenization time, amount of polymer, and surfactant amount were influencing the particle size and particle size distribution. The experimental results depicted that the homogenization time up to 3 min was effective for maximum size reduction and size distribution. After 3 min, the particle size and polydispersity index increased which might be due to decreased stability of double emulsion leading to uncontrolled coalescence (Lamprecht et al. 2000). Another study includes the preparation of 5-fluorouracil-loaded PLGA nanoparticles by a high-pressure homogenization-emulsification method. The nanoparticle preparation owes a benefit of increasing bioavailability of 5-FU. As 5-FU is a hydrophilic drug, the w/o/w emulsion method is most appropriate. However, to achieve proper size reduction and monodisperse particles, HPH can be beneficial. Optimum cycles and homogenization pressure were found to be 3 cycles at 800 bar pressure as per the experimental trials. The particle size range obtained was 75 nm to 102 nm and the mean diameter was 85 nm. The polydispersity index was 0.10 to 0.18, which indicates that the HPH was effective enough to get uniform small-sized particles (Li et al. 2008). Aimin Shi et al. formulated starch nanoparticles by combined mini-emulsion cross-linking and HPH technique. The influence of processing parameters on stability and particle size was studied. The coarse emulsion when subjected to high-pressure homogenizer at 10 MPa to 60 MPa and 1–5 passes produced small-sized and uniform particles (Shi et al. 2011). Dong et al. prepared paclitaxel-incorporated PLGA nanoparticles employing the HPH technique. The author described three advantages of using HPH for particle size reduction, viz., excellent redispersibility of nanoparticles, uniform particle production, and easy scalability. The experiments for adjusting the homogenization pressure and cycles revealed that there was no significant influence of very high pressure on particle size. The pressure range of 86 MPa to 155 MPa gave a particle size range of 200–300 nm. A homogenization pressure of 86 MPa and 1 cycle also gave a sufficient nano-size. Also, the entrapment efficiency decreased with an increase in pressure and cycles; thus lower pressure and one cycle can be considered optimum for this formulation (Dong and Feng 2007).

7 Scale-Up and Industrialization Perspective

The key advantage of employing HPH techniques for nanoparticle preparation is that the high-pressure homogenizers of different sizes and capacities are available. Talking about other techniques of nanoparticle preparation, a major problem remains the scalability issue. Contrarily, HPH provides easy scale-up as various models, viz., benchtop models/laboratory-scale models with 10 L/h capacity, pilot-scale models with 100 L/h capacity, as well as large production scale 100,000 L/h, are available. Currently, high-pressure homogenizers are widely used in food and beverage preparation, cosmetics, nutraceuticals, as well as pharmaceuticals for industrial manufacturing of stable emulsions, suspensions, and colloidal dispersions. Thus, the fact that already the mature technique is well established at industrial scale manufacturing makes it more convenient for the manufacturing of nanoparticles. However, there are certain factors like change in geometry of valve, change in flow rate, gap height, as well as different fluid volume which have an effect on the homogenization results at laboratory scale and industrial scale. The optimization of the flow rate, homogenization pressure, and number of homogenizing cycles for nanoparticle production should be done for obtaining nanoparticles with desirable properties at an industrial scale as well. As a concluding remark, it can be said that compared to other competing manufacturing techniques of nanoparticles, HPH has an advantage as large-scale production is possible. Conversely, theoretical analysis of pressure loses, cavitation number, and Reynolds number suggests differences in the capacity of homogenization by different scale homogenizers. However, more experimental insights are necessary for studying the factors involved in different scale production in terms of homogenization efficiency as well as stability of homogenized products obtained.

8 Concluding Remark and Future Prospects

High-pressure homogenization is a versatile technique providing numerous advantages for the manufacturing of nanoparticles with desirable characteristics and stability. The technique utilizes mechanical action as well as pressure that provides a benefit to achieve nano-sized particles having uniform structures and lower PDI. The type of high-pressure homogenizer, valve design, geometry, type of pressure pump, and processing parameters have a prodigious influence on the final homogenized product. A deeper understanding of the forces acting on the size reduction, viz., shear, turbulence, and cavitation, will provide an outlook to achieve the desired product quality. Several factors affecting the therapeutic outcome of the nano-products like improved targetability, bioavailability, stability, etc. get influenced by the processing parameters of the HPH technique. The modifications and improvements were done in the HPH technology and allow flexibility and improved functionality in obtaining the nanoparticles with the desired pharmaceutical application. Newer valve designs and improved instrumentation enable the homogenizer to achieve higher durability and withstand higher pressure levels. At both industrial as well as research levels, the technique has a bright future and a broader impact on formulating highly stable nano-formulations with desired characteristics because of the key advantage of scalability.

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