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Production Techniques of Lipid Nanoparticles: A Review

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ABSTRACT

Different production methods have been developed for lipid nanoparticles and most of the methods use two basic steps; emulsification and size reduction to nano size. Homogenization techniques are most frequently employed using hot and cold homogenization or ultrasonication for the production. On the other hand, few methods based on emulsification are also applied used earlier for polymeric nanoparticle production. Hot high pressure homogenization and ultrasonication are most commonly used method with scale up feasibility but costly equipment is biggest drawback. Other methods used to produce lipid nanoparticles are possible in a laboratory setup with no expensive equipments are needed but scale up is still a problem with such method along with regulatory problems associated with high surfactants concentrations in these formulations. This review focus on different methods used for lipid nanoparticles production with their procedure, advantages and disadvantages associated with them. Furthermore, a comparison of the all commonly used methods is also summarized in this review.

Keywords: NLC, SLN, Lipid nanoparticles, HPH, Melt emulsification.

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INTRODUCTION

Carrier systems has been developed continuously ever since it was conceived in the beginning of 20th century that drugs can be delivered at the required site of action with controlled release. This was first made possible by using localized delivery with the use of polymer implants and reservoir system. These systems are capable of controlling the release rate of drug but targeting to specific site of action is still not possible. Moreover, their size is too not suitable for intravenous administration.

The large interest in nano structures results from their numerous potential applications in various segments of pharmaceutical and healthcare system. One such area of exiting application potential is lipid based nano-scale particulate system for drug delivery synthesized by the colloidal route.

In order to overcome the drawbacks associated to the traditional colloidal systems such as emulsions, liposomes and polymeric nanoparticles, lipid colloidal has been received considerable attention. Solid lipid nanoparticles (SLN) were developed at the beginning of the nineties [1, 2], it consist of a matrix composed of a physiologically tolerated lipid component having property of being solid at both room and body temperatures. They have a mean particle size ranging between 50 nm and 1000 nm [3]. The first patents for SLN were filed in 1991, one by Muller and Lucks [4] describing the production of SLN by high pressure homogenization (HPH), and another by Gasco[5] developed it via microemulsions method. Nanostructured lipid carriers (NLC) were introduced to overcome the problem associated with SLN.

During the last ten years different substances have been entrapped into lipid nanoparticles (SLN and NLC), including both lipophilic and hydrophilic compound, including labile substances, such as peptides and proteins. A comprehensive list of such active molecules loaded in SLN and NLC with method of production employed has been summarized in Table 1.

Methods of Manufacturing of NLC/SLN

Different methods of SLN/NLC formulation are described here-

1. Homogenization techniques
   i. Hot high pressure homogenization technique
   ii. Cold high pressure homogenization technique
   iii. Melt emulsification ultrasound (ultrrasonication) homogenization technique (High shear homogenization and/or ultrasound technique)
2. Microemulsion technique
3. Emulsification-solvent evaporation technique
4. Solvent displacement or injection technique
5. Emulsification-solvent diffusion technique
6. Phase inversion technique
7. Film ultrasonication dispersion technique
8. Multiple emulsion technique

9. Membrane contactor technique
10. Supercritical PGSS technique (Particle from gas saturated solution)

Table 1: Different methods of manufacturing, compounds loaded and applications of SLN/NLC

S. No.	Method	Compounds loaded	Application	Reference
1	Melt emulsification ultrasonication	Ketoprofen, Naproxen	Topical	6
2	Melt emulsification	Progesterone	-	7
3	Solvent Diffusion	Progesterone	-	7
4	HPH	Ratinol	Topical	8
5	Solvent Diffusion	Valproic Acid	Nasal to Brain	9
6	Microemulsion	Celecoxib	Topical	10
7	Melt emulsification ultrasonication	Nitrendipine	Oral (SLN)	11
8	Hot HPH	Clotrimazole	Topical	12
9	Microemulsion	Minoxidil	Topical	13
10	Hot HPH	Flurbiprofen	Transdermal	14
11	Solvent Diffusion	Clobetasol propionate	-	15
12	Hot HPH	Nile Red	Topical	16, 17
13	Melt emulsification ultrasonication	Thiolated PEG stearate	Ocular (NLC)	18
14	Solvent Diffusion	Tretinoin	Topical (SLN)	19
15	Microemulsion	Tretinoin	Topical (SLN)	20
16	Hot HPH	Coenzyme Q_{10}	Topical (NLC)	21 - 24
17	Microemulsion	Pepsin A, Pancreatin, Insulin	Oral (SLN)	25

High Pressure Homogenization Techniques

Since the fifties of the last century, high pressure homogenization is a well established technology for the production of emulsions for parenteral nutrition, such as Intralipid and Lipofundin, and it can also be adapted for scale-up production of lipid nanoparticles. The preparation of lipid nanoparticles applying the high pressure homogenization techniques has been developed and practiced extensively [4, 26, 27]. Hot as well as cold homogenization processes can be used for the preparation of lipid nanoparticles. In both processes the active compound is dissolved or dispersed in the melted lipid prior to the high pressure homogenization. High pressure homogenizers push a liquid with high pressure (100–2000 bar) through a narrow gap (having size of few microns). Particles formed are in submicron range due to very high shear stress and cavitation forces generated in the homogenizer. Large scale production of lipid nanoparticles are possible with this technique with regulatory acceptance as production lines are very much in use for manufacturing of parenteral lipid emulsions since long period. This is the main advantage of this method as compared with other available technique but high energy conditions of temperature and pressure questioned its applicability in certain conditions.

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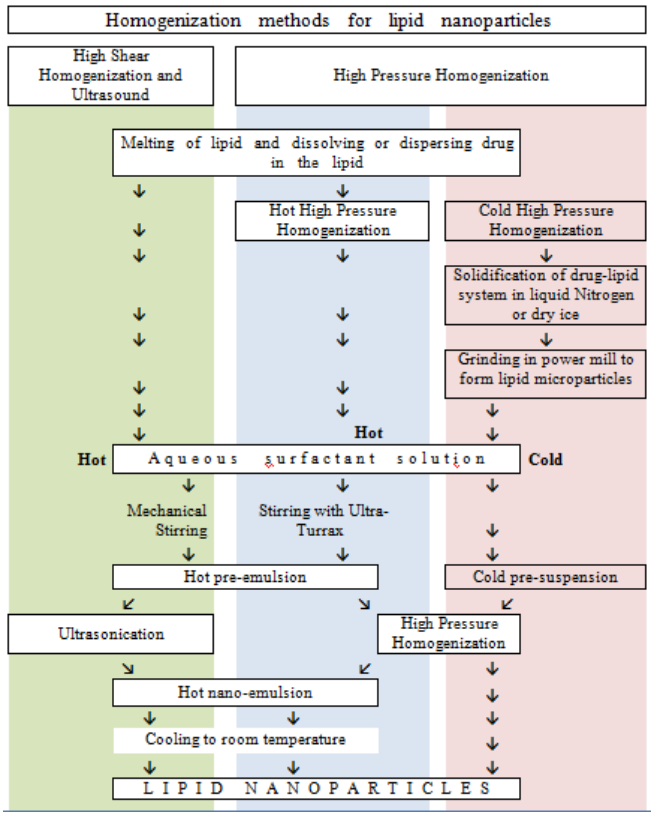
Hot High Pressure Homogenization Technique

For hot homogenization, a pre-emulsion of the drug loaded lipid melt and the emulsifier solution is prepared with a high-shear mixing device (such as Ultra-Turrax). Pre- emulsion is then passed through high pressure homogenization cycle at temperatures above the melting point of the lipid. Lipid nanoparticles are formed by the following cooling of the sample to room temperature or to temperatures below. The active compound-containing melted lipid is dispersed in the hot surfactant solution at the same temperature applying high-speed stirring. The obtained hot pre-emulsion is passed through a high pressure homogenizer applying number of homogenization cycles. A nanoemulsion is formed which is upon cooling yield aqueous dispersion of lipid nanoparticles.

Hot HPH technique is the most frequently applied. It can be used for the entrapment of lipophilic and insoluble drugs in the lipid. Temperature sensitive compounds can also be processed by hot HPH as exposure time to high temperatures is relatively short. However, for hydrophilic drugs this procedure is not the most appropriated one. During the homogenization of the melted lipid phase the drug will partition to the water phase resulting in a too low encapsulation rate. Figure 1 describes the schematic procedure for the preparation of lipid nanoparticles by this method.

Figure 1: Schematic representation of the different homogenization techniques for the production of lipid nanoparticles



Cold High Pressure Homogenization Technique

In contrast to hot homogenization, the cold homogenization is carried out with the solid lipid without melting as done in hot process. Drug along with lipid in solid state is milled to form microparticles, and further dispersed in a solution containing emulsifier. The pre-suspension formed is then subjected to high pressure homogenization at or below room temperature [28, 29].

In the cold HPH technique, lipid is melted above its melting point and drug is dissolved or dispersed in it. The system is cooled down by means of dry ice or liquid nitrogen. After solidification, the lipid mass is grounded using ball or mortar milling to yield lipid microparticles in a range between 50 and 100 µm. Then a microemulsion is formed by adding these microparticles into cold surfactant solution with stirring. This suspension is passed through a high pressure homogenizer at/or below room temperature and the microparticles are broken down to nanoparticles.

The cold HPH technique minimizes the thermal exposure to the drugs and active substances. Therefore, this technique may be applied for temperature sensitive compounds. Hydrophilic compounds can also be incorporated by this method which might partition from the liquid lipid phase to the water phase during the hot HPH. To further minimize the loss of hydrophilic compounds to the aqueous phase of the suspension, water can be replaced by liquids with low solubility for the drug, such as oils and polyethylene glycols of low molecular weight. Lipid particles prepared using the cold HPH technique possess a slightly higher PI and mean particle size compared to the ones obtained by hot HPH technique. Homogenization cycles can be increase to further reduce the particle size and to minimize the polydispersity. Figure 1 has shown the schematic chart of this procedure.

Melt Emulsification Ultrasound Homogenization Technique

Different techniques can be employed to prepare lipid nanoparticles. Hot homogenization technique is the most commonly applied method. However, it requires the use of appropriate devices which are not commonly available in research labs. Ultrasonication instead of high pressure homogenization has been employed to produce lipid nanoparticles [30, 31, 6, 32]. This technology is based on the extreme conditions generated within the collapsing cavitational bubbles of the inner phase leading to size reduction. This method employs same procedure as hot high pressure homogenization except using ultrasonication device in place of homogenizer. Ultrasonic processing is fast and highly reproducible if the operating parameters are optimized. These parameters are operating temperature, ultrasonication time and power. Ultrasound probes are very easy to clean; sample losses are negligible and can be used for high scale production. However, it is believed that when applying high-shear homogenizers and ultrasonication, inhomogeneous power distributions are most likely to occur as compared with high pressure homogenizers which are characterized by a homogeneous power distribution due

to the small size of the homogenizing gap. See Figure 1 for the schematic chart of this procedure.

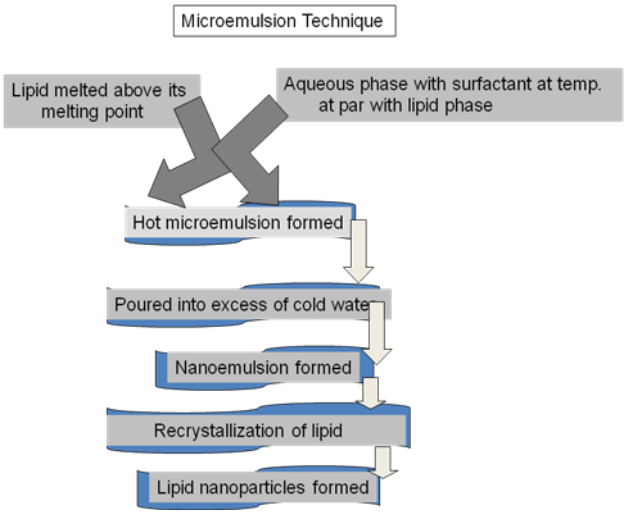
Microemulsion Technique

This method is adapted since the early start of lipid nanoparticle formulation by different research groups [5, 10, 33-39]. In the microemulsion method, when excess amount of outer phase in cooling conditions added to a hot microemulsion the system has broken down and converting it into nanoemulsion which recrystallize internal oil or lipid phase forming particles. Briefly, the melted lipid containing drug mixed with surfactant, cosurfactant containing aqueous phase prepared at the same temperature as of the lipid in such a ratio to form microemulsion. The hot microemulsion is then diluted into excess of cold water. Sudden reduction in temperature causes breaking of the microemulsion, converting it into nanoemulsion, which upon recrystallization of lipid phase produces lipid particles. Break in microemulsion is supposed to be due to the dilution with water and the reduction in temperature narrowing the microemulsion region. The process variables affecting size and structure are microemulsion composition, dispersing device for the microemulsion dilution to the cold water, temperature condition and lyophilization of the product.

This method has certain advantages which include no need for specialized equipment, energy for production is not required and scale-up production of lipid nanoparticles is possible.

Disadvantage of the microemulsion technique is the dilution of the particles suspension with water, thus removal of excess water need additional efforts. In addition, high concentrations of surfactants and co-surfactants, in the formulation raise regulatory concern. The removal of surfactants can further be performed using ultrafiltration, ultracentrifugation or dialysis adding one more step to the procedure which is time consuming and costly. Different steps of the method are schematically represented in Figure 2.

Figure 2: Schematic representation of the microemulsion technique for the production of lipid nanoparticles.



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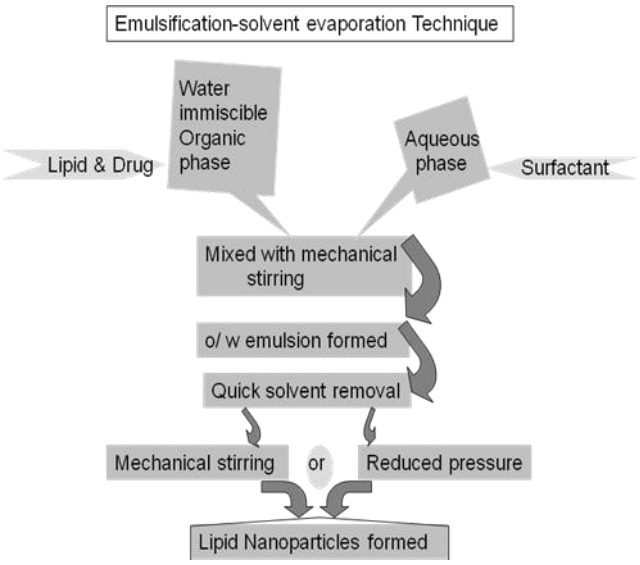
Emulsification-Solvent Evaporation Technique

This is a method analogous to the production of polymeric nanoparticles and microparticles by solvent evaporation in o/w emulsions [40, 41] via precipitation. In the solvent emulsification-evaporation the lipid is dissolved in a water-immiscible organic solvent (e.g. toluene, chloroform) which is then emulsified in an aqueous phase before evaporation of the solvent under condition of reduced pressure. The lipid precipitates upon evaporation of the solvent thus forming nanoparticles.

Firstly, an organic phase has produced containing the lipid material dissolved in a water-immiscible organic solvent, and then the drug is dissolved or dispersed in that solution. This organic phase is emulsified in an o/w surfactant containing aqueous phase by mechanical stirring. Subsequent quick removal of solvent by evaporation from the obtained o/w emulsion under mechanical stirring or reduced pressure nanoparticle dispersion is formed by precipitation of the lipid in the aqueous medium. The solvent evaporation step must be quickly in order to avoid particle aggregation.

This method is suitable for the incorporation of highly thermolabile drugs due to avoidance of heat during the preparation but presence of solvent residues in the final dispersion may create problems due to regulatory concern. Limited solubility of lipids in organic materials generally leads to dilute dispersions and need to concentrate by means of another process such as ultra-filtration, evaporation or lyophilization. On the other hand small particle size around 100 nm with narrow size distribution can be achieved by this method. This procedure has schematically depicted in Figure 3.

Figure 3: Schematic representation of the emulsification- solvent evaporation technique for the production of lipid nanoparticles.



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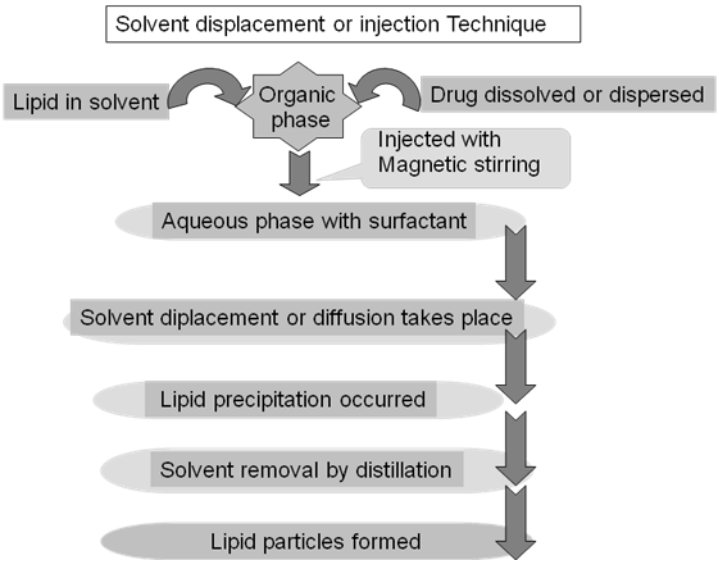
Solvent Displacement or Injection Technique

The solvent displacement technique was first described for the preparation of liposomes and polymeric nanoparticles from pre-formed polymers. Recently, this technique has also been used to prepare lipid nanoparticles [42, 43]. Precipitation of lipid dissolved in solution is the basis of this process. In this method, a solution of the lipid in a water-miscible solvent or a water-miscible solvent mixture is rapidly injected into an aqueous phase with or without surfactant. In this process, an o/w emulsion has been formed by injecting organic phase into the aqueous phase under magnetic stirring. The oil phase is a semi-polar water-miscible solvent, such as ethanol, acetone or methanol, lipid material is dissolved in it and then the active compound is dissolved or dispersed in this phase. Aqueous phase consists of surfactant. In this procedure solvent displacement of diffusion takes place and lipid precipitate has obtained. Solvent removal is necessary and can be performed by distillation. The lipid nanoparticles are formed after total evaporation of the water miscible organic solvent. Particle size is dependent on the preparation conditions such as amount to be injected, concentration of lipid and emulsifier.

This method offers clear advantages over the existing methods such as the use of organic solvents which is pharmaceutically accepted, high pressure homogenization not required, ease in handling and less time consuming without technically sophisticated equipment. Disadvantages clearly evident is use of organic solvent although they are pharmaceutically accepted excipients frequently used in formulations. Figure 4 schematically represents this production procedure.

Figure 4: Schematic representation of the solvent displacement or injection technique for the production of lipid nanoparticles.



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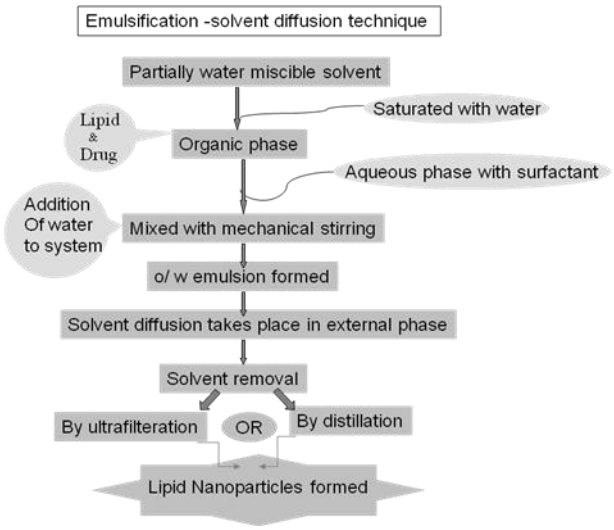
Emulsification-Solvent Diffusion Technique

The emulsification-solvent diffusion technique is usually used to produce polymeric nanoparticles based on synthetic polymers via precipitation. However, it has been recently applied to prepare lipid nanoparticles. In the solvent-diffusion technique, partially water miscible solvents (e.g. benzyl alcohol, ethyl formate, tetrahydrofuran) are used [15, 44-48]. Initially, they are mutually saturated with water to ensure initial thermodynamic equilibrium of both liquids. Then the lipid is dissolved in the water-saturated solvent and subsequently emulsified with solvent-saturated aqueous surfactant solution at elevated temperatures. The lipid nanoparticles precipitate after the addition of excess water (typical ratio: 1:5–1:10) due to diffusion of the organic solvent from the emulsion droplets to the continuous phase.

In this procedure an o/w emulsion is formed comprising organic phase of a partially water soluble solvent which is previously saturated with water to ensure the initial thermodynamic equilibrium between the two liquids (that is water and solvent). This saturated solution is used to dissolve lipid followed by the drug in the organic phase. This organic phase is then emulsified in an aqueous solution containing surfactant under mechanical stirring for the preparation of an o/w emulsion. The subsequent addition of excess water to the system causes solvent diffusion into the external phase and the lipid starts precipitating. The solvent can be eliminated by ultra-filtration or by distillation. After the complete removal of organic solvent, an aqueous dispersion of lipid nanoparticles is formed. The dispersions obtained in this procedure is fairly dilute similar to microemulsions method, and required to be concentrated by means of ultra-filtration or lyophilisation thus extra step is needed. Particle size is also small around 100 nm with narrow size distribution in this method. This procedure has been schematically represented in Figure 5.

Figure 5: Schematic representation of the emulsification-solvent diffusion technique for the production of lipid nanoparticles.



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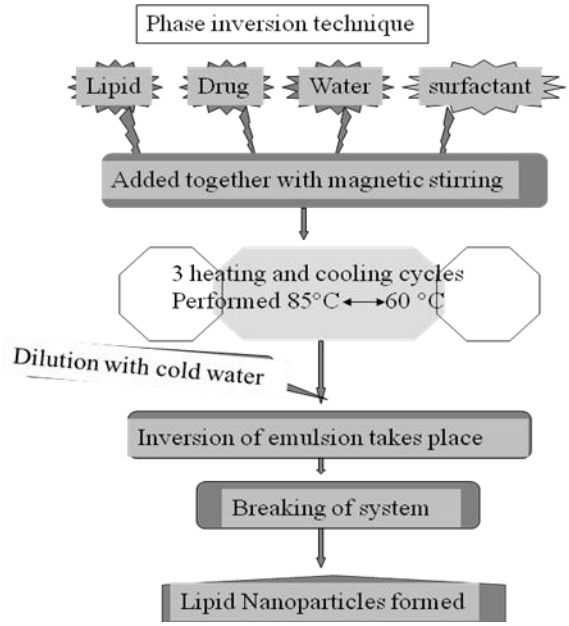
Phase Inversion Technique

Recently, a novel phase inversion-based technique has been described for the preparation of lipid nanoparticles [49]. It involves two basic steps, first is addition of formulation components with magnetic stirring and subsequent heating cooling cycles and second is dilution under cooling conditions. The general procedure consists of magnetic stirring of all the components (lipid, surfactant and water) in the correct proportions optimized previously. Three cycles of heating and cooling from room temperature to 85ºC and back to 60ºC are subsequently applied at a rate of 4ºC/min (2). This thermal treatment (85ºC-60ºC-85ºC-60ºC-85ºC) will cause the inversion of the emulsion. It is followed by dilution with cold water. The system will break down due to an irreversible shock induced by dilution with cold water to the mixture maintained at the elevated temperature. This fast cooling dilution process with cold water leads to lipid particles in the nanometer range. Afterwards, a slow magnetic stirring is applied to avoid particle aggregation. A general procedure has been schematically represented in Figure 6.

Figure 6: Schematic representation of the phase inversion-based technique for the production of lipid nanoparticles.



This technique has the advantage of the incorporation of thermolabile drugs as thermal degradation is not expected to occur as it has very short heating period. In addition, this technique also avoids the use of organic solvents. Different formulation components proportion influences the size parameters of the obtained particles thus need to be optimized.

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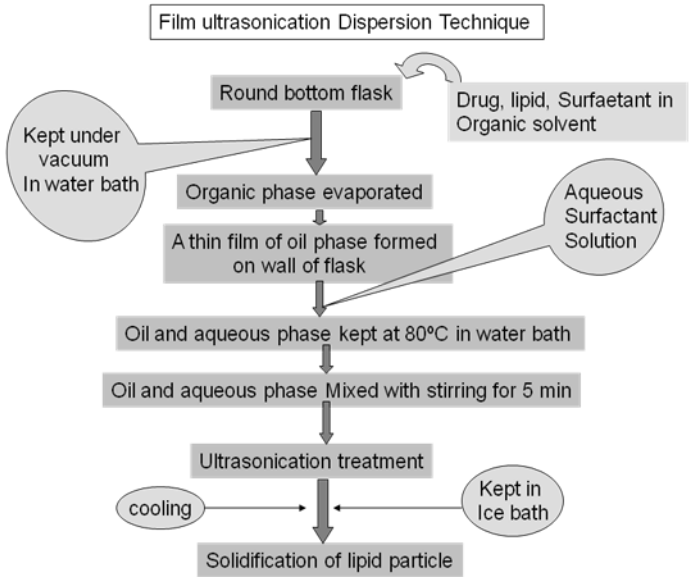
Film Ultrasonication Dispersion Technique

Lipid nanoparticles can also be prepared by high speed stirring or sonication [50, 51]. In this method a thin film of lipid phase has been formed upon evaporation of solvent followed by ultrasonic dispersion in the presence of aqueous surfactant solution at elevated temperature; subsequent cooling of the system lead to the formation of lipid nanoparticles. Method of preparation has been schematically represented in Figure 7.

Figure 7: Schematic representation of the film ultrasonication dispersion technique for the production of lipid nanoparticles.



The advantages of this method are use of common equipments that are available in every lab set up. Broader particle size distribution ranging into micrometer range is the biggest problem of this procedure and affects physical stability lead to particle growth upon storage. Contamination of metal during ultrasonication is also a major problem in this process.

Multiple Emulsion Technique

This is a modified solvent emulsification-evaporation method based on a w/o/w double emulsion. It applied emulsification followed by solvent evaporation for the preparation of hydrophilic drug substance loaded SLN. The drug is encapsulated with a stabilizer to prevent drug partitioning to external water phase during solvent evaporation in the external water phase of w/o/w double emulsion [25, 52]. A general procedure has been schematically represented in Figure 8.

It has advantages and limitation of previously described method of emulsification solvent evaporation technique but it can be applied for the incorporation of hydrophilic molecules such as peptides and proteins also.

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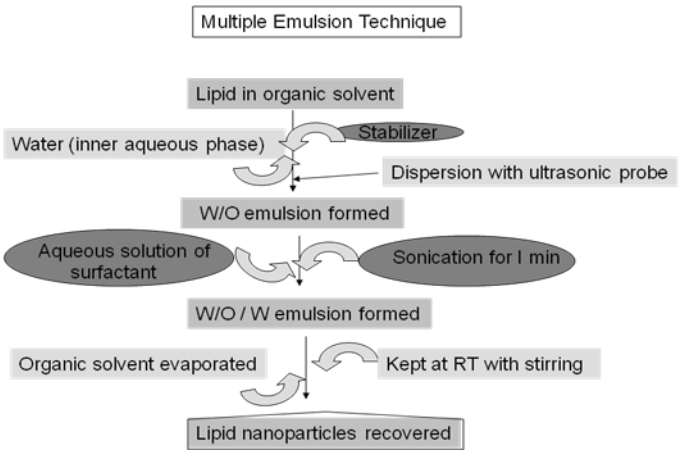
Figure 8: Schematic representation of the multiple emulsion technique for the production of lipid nanoparticles

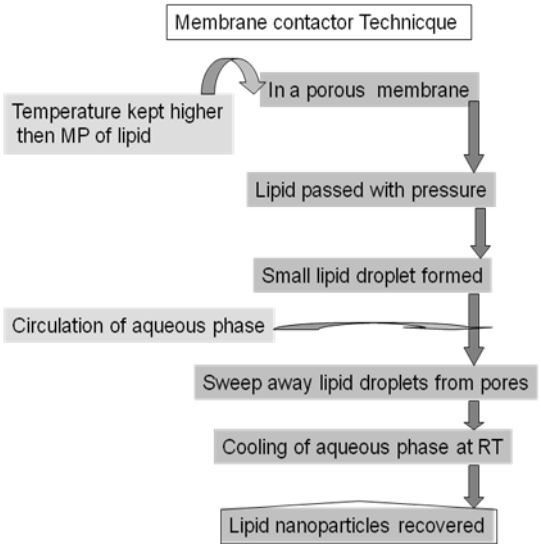


RT= Room Temperature

Membrane Contactor Technique

This method work on simple procedure of passing lipid from pores of a membrane with pressure keeping system above the melting temperature of lipid. The lipid phase is pressed through the membrane pores, at a temperature above the melting point of the lipid, lead to formation of small droplets. On the other hand aqueous phase is circulated inside the membrane module, and droplets formed at the pore outlets are sweeps along with this aqueous phase. SLN are formed by the cooling of the preparation to room temperature. The velocity of aqueous phase flow, temperature of lipid and aqueous phase, membrane pore size and lipid phase pressure is the process variables which affect size and lipid flux of SLN [53]. Procedure has been represented schematically in Figure 9.

Figure 9: Schematic representation of the membrane contactor technique for the production of lipid nanoparticles.



MP= Melting Point, RT= Room Temperature

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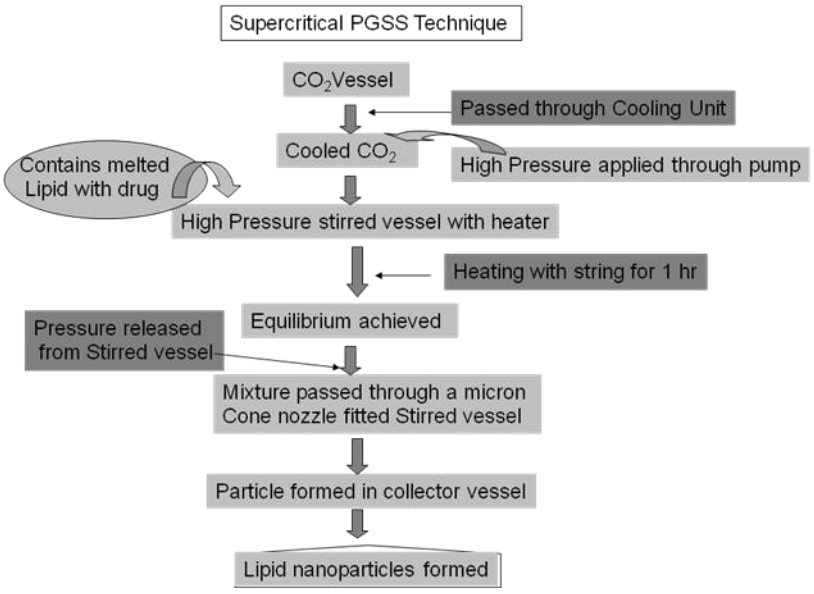
The advantages of this method are its simple methodology and equipment, the control of the SLN size by an appropriate choice of process parameters, and its scaling-up abilities.

Supercritical PGSS Technique (Particle from gas saturated solution)

This is a relatively new technique for lipid nanoparticle production based on the use of supercritical carbon dioxide ($scCO_2$). It has been described as a single step method capable of encapsulating drugs into organic solvent-free lipid particles. Carbon dioxide (99.99%) is a good choice as a solvent for this method.

In this method lipid is melted and drug is subsequently dissolved or dispersed in it. Then $scCO_2$ is dissolved in the bulk of a melted lipid phase, followed by quick expansion through a micron cone shaped nozzle upon pressure release. This event leads to the atomization of the melt, causes evaporation of the gas and the precipitation of the lipid nanoparticles [54, 55]. Schematic representation of the procedure depicted in Figure 10.

Figure 10: Schematic representation of the Supercritical PGSS technique for the production of lipid nanoparticles.



This method has advantages such as one step procedure, no need of organic solvent and low processing temperature conditions (lower melting temperatures of the lipids obtained from the dissolution of CO_2 in the bulk mixture). Disadvantages include frequent nozzle blockage with hydrophilic drug and machinery is costly.

A comparative chart of advantages and disadvantages of different methods of SLN/NLC production has been presented in tabular form at Table 2.

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In a literature study, 82 numbers of research papers are reviewed to assess the choice of the method amongst researchers and the result is summarized here under:

1. Homogenization techniques
   i. Hot high pressure homogenization 28 34%
   ii. Cold high pressure homogenization 00
   iii. Melt emulsification ultrasound homogenization 12 15%
2. Microemulsion 13 16%
3. Emulsification-solvent diffusion 12 15%
4. Emulsification-solvent evaporation 05 06%
5. Solvent displacement or injection 02 02%
6. Phase inversion 01 01%
7. Film ultrasonication dispersion 02 02%
8. Multiple emulsion 03 04%
9. Membrane contactor 01 01%
10. Supercritical PGSS 01 01%
    (Particle from gas saturated solution)

Table 2: Comparison of different methods of SLN/NLC production

S. No	Method	Advantages	Disadvantages	References
1a	Hot HPH	Versatile, avoid use of organic solvent, easy scalability, Short production time, instruments easily available and no regulatory problems	High temperature lead to degradation, confor-mational changes in protein, coalescence of particles, burst release due to high emulsifiers	6, 56, 15
1b	Cold HPH	Minimizes thermal exposure of the drug but does not avoid it completely. Useful in temperature labile drugs or hydrophilic drugs	Higher Polydispersity index	28, 29
1c	Melt Emulsificatio n ultrasound	No organic solvent residue, no burst release, high lipid concentration, Versatile, avoid use of organic solvent, better drug loading than HPH	Metallic particle contamination, broader particle size	7, 6
2	Emulsificatio n-solvent evaporation	Avoidance of heat during production thus useful for thermolabile drugs. Simple procedure	solvent residues	40, 41
3	Emulsificatio n-Solvent diffusion	Simple procedure, Fast drug release (drawback when slow release is required)	Low lipid content, Low EE and DL, organic solvent residue, Lack of scaleup	7, 15, 44 - 46
4	Micro emulsion	No need for specialized equipment and energy for production	high concentrations of surfactants and co-surfactants, presence of large amounts of water in system	33, 10
5	Membrane Contactor	Simple method, Control of particle size by selection of process parameters, and its scaling-up abilities	-	53

6	PGSS	one step procedure, no need of organic solvent, low processing temperature conditions	frequent nozzle blockage with hydro-philic drugs, machinery is costly	54, 55
7	Multiple emulsion	No need to melt lipids, high loading of hydrophilic drugs, useful for protein loading	Use of solvent and surfactant	52, 25
8	Solvent injection	no need for high pressure homogenization, easy handling, fast production process, No need for specialized equipment	Use of solvent and surfactant	43
9	film Ultra-sonication dispersion	Simple, No need for specialized equipment	Metallic particle contamination, broader particle size	50, 51
10	Phase inversion	Useful for thermolabile drugs, avoid organic solvent, No need for specialized equipment	-	49

PGSS- Particles in gas saturated solution, HPH- High Pressure Homogenization

CONCLUSION

Lipid nanoparticles were developed as colloidal carriers to avoid drawbacks associated with earlier colloidal carriers such as liposomes and nanoemulsions. Production technology of emulsions and parenteral nutrition was first employed for the manufacturing of lipid nanoparticles as set of equipment required was easily available and can be employed at both lab scale and industrial production. The scalability of formulation technique developed always lead to an ease of commercialization of a product. This is true in case of lipid nanoparticles as a number of formulations are available commercially in the market. However, there is still need for development of other methods which will be reliable, cost effective and can be comply with regulatory standards.

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