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ReviewArticle

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A Descriptive review on various lipids and techniques used in formulation of solid lipid nanoparticles

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Abstract

Solid lipid nanopaticles (SLNs) emerged in early 1990s as a next-generation drug delivery system, an alternative to traditional colloidal carriers like liposome's, polymeric nanoparticles, emulsions etc. Their size range is between 1 to 1000 nm and their biodegradable and inacceptable nature make them less toxic and thus better suited to patients. SLNs have got potential applications in pharmaceutical field, cosmetics, clinical medicine and other allied sciences. Presently, formulation scientists have been focusing on SLNs as colloidal drug carriers for incorporating hydrophilic as well as lipophilic drugs. The ability to incorporate drugs into nanocarriers offers a new prototype in drug delivery which can be used for drug targeting. They hold great promise for reaching the goal of controlled and site specific drug delivery. Furthermore, SLNs have got advantage of being introduced in the body by oral, parenteral and topical routes. So the present review attempts to enlighten various lipids used in SLNs, manufacturing techniques as well as the potential applications through various routes for a variety of disorders. Furthermore, the manuscript also focuses on the fate of these lipids (constituents of SLNs) in the body and their way out (i.e. elimination).

Kewords: Lipid, Nan particles, Biodegradable, Sanitation, Homogenization

Introduction

The bioavailability of drugs has been the main issue from decades, because about 40% of the commercialized drugs are poorly water soluble in nature. These drugs pose difficulties in obtaining adequate and reproducible drug absorption from the gastrointestinal tract. To overcome these issues, some colloidal carriers like nanoemulsions, nanosuspensions, micellar solution etc. were introduced previously. However, physical stability, low drug loading, drug expulsion on storage, presence of residual organic solvents and polymer cytotoxicity are the major drawbacks which limit the use of these colloidal carriers. [1] Prof. Rainer Muller found out the solution to these drawbacks in solid lipid nanoparticles (SLNs). SLNs are small sized lipid nanoparticles composed of biocompatible and biodegradable solid lipids. Their matrix is compossed of physiological lipids that reduces/decreases the danger of acute & chronic toxicity. [2] Despite their small size (10-1000nm), they offer high drug loading capacity, larger surface area and thus enhanced bioavailability. These characteristics make SLNs an interesting drug delivery system. [3]

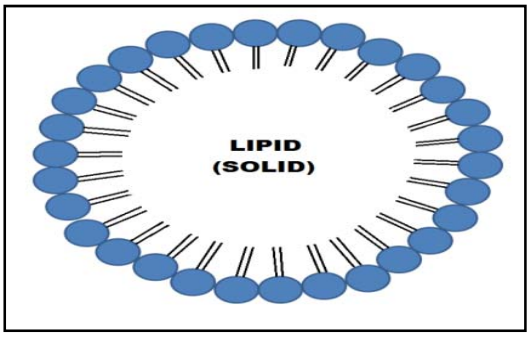

Figure 1. Solid Lipid Nanoparticle

SLNs have following advantages [4, 5, 6].

Enhancement of bioavailability of poorly water soluble drugs.
High drug loading capacity.
Site specific delivery of drugs, enhanced drug penetration.
High physical stability of product.
Possibility of scaling up.
Better control over release kinetics of encapsulated compound.

They can be administered through different routes such as oral, parenteral, transdermal and ocular.
The above stated characteristics make SLNs a better alternate over colloidal carrier [7].

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Various Lipids Used in SLNs

Fatty acids	• Stearic acid • Palmitic acid • Decanoic acid • Behenic acid
Triacylglycerols	• Trimyristin • Tristearin • Tricaprin • Trilaurin • Tripalmitin
Acylglycerols	• Glycerol monostearate • Glycerol behenate • Glycerol Palmitostearate
Waxes	• Cetyl palmitate
Cyclic complexes	• Cyclodextrin
Hard fat types	• Witepsol W 35 • Witepsol H 35
Others	• Cholesterol

Stearic Acid

Stearic acid is a hard, white or faintly yellow-colored, somewhat glossy, crystalline solid or a white or yellowish white powder. It has a slight odor (with an odor threshold of 20 ppm). Chemically its name is Octadecanoic acid. Its Molecular formula is $C_{18}H_{36}O_2$ and molecular weight is 284.47. [8]

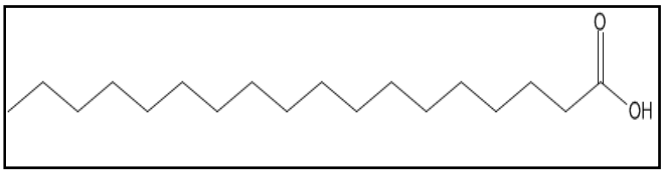


Figure 2. Structure of Stearic Acid

Soluble in benzene, carbon tetrachloride, chloroform, and ether; soluble in ethanol (95%), hexane, and propylene glycol; practically insoluble in water.
Acid value is 195–212.
Boiling point is 383 C.
Melting point is 69–70 C.
Contains practically no water.
Stearic acid is a stable material; an antioxidant may also be added to it. The bulk material should be stored in a well closed container in a cool, dry place.

Palmitic Acid

Palmitic acid occurs as white crystalline scales with a slight characteristic odor and taste. Chemically it is Hexadecanoic acid. Its molecular formula is $C_{16}H_{32}O_2$ and molecular weight is 256.42. [9]

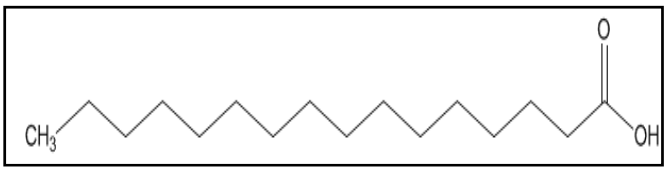


Figure 3. Structure of Palmitic Acid

Soluble in ethanol (95%); practically insoluble in water.
Melting point is 63–64 C.
Boiling point 351–352 C.
Acid value is 216–220.
The bulk material should be stored in a well-closed container in a cool, dry, place.

Decanoic Acid

Decanoic acid is a solid compound that belongs to the straight chain fatty acids. It is white crystalline solid with a rancid odor. Its molecular formula is $C_{22}H_{44}O_2$ and molecular weight is 340.58. [10]

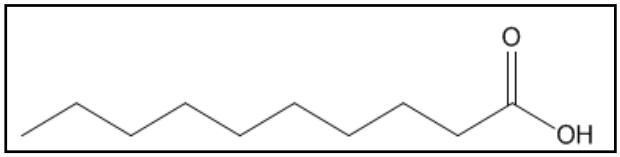


Figure 4 Structure of Decanoic acid

Melting point is 31.5 C
Practically insoluble in water.
Soluble in ethanol, ether, chloroform, benzene, carbon disulfide, dilute nitric acid

Behenic Acid

Behenic acid is white to cream color waxy crystals or powder. It is a carboxylic acid, the saturated fatty acid with formula $C_{21}H_{43}COOH$ and molecular weight of 340.59. [11]

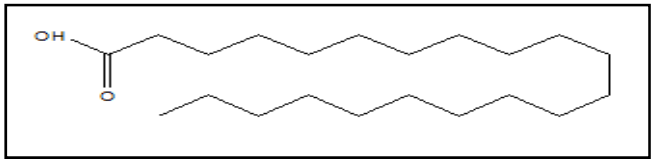


Figure 5. Structure of Behenic acid

Melting point is 79.95 C
Boiling point is 306 C
Slightly soluble in water, ethanol, ethyl ether.

Trimyristin

Trimyristin is a white to yellowish-gray solid saturated fat which is the triglyceride of myristic acid. Its molecular formula is $C_{45}H_{86}O_6$ and molecular weight is 723.18 g/mol. [12]

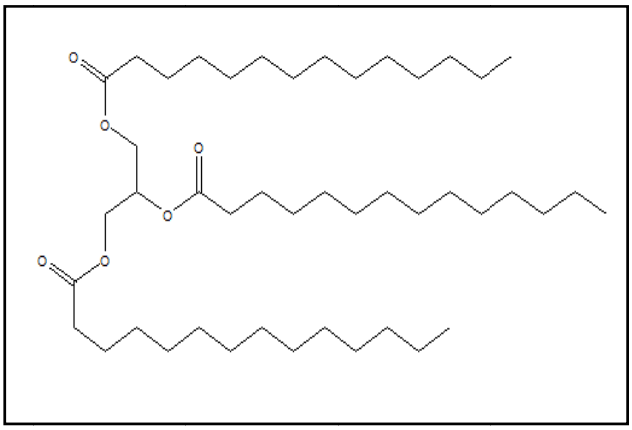


Figure 6. Structure of Trimyristin

Melting point is 56-57 C.
Soluble in acetone and chloroform.
Slightly soluble in alcohol.

Tristearin

Tristearin is a white colored, odorless, powder triglyceride derived from three units of stearic acid. Its molecular formula is $C_{57}H_{110}O_6$ and molecular weight is 891.47 g/mol. [13]

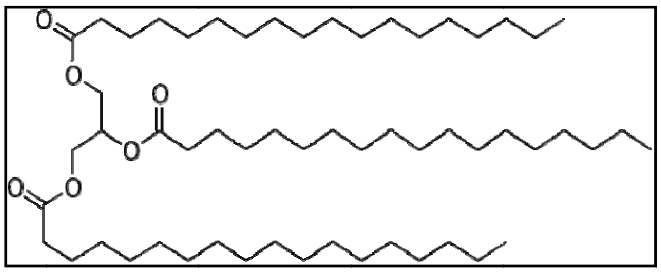


Figure 7. Structure of Tristearin

Melting point is 55-73 C.
Very soluble in acetone and benzene
Soluble in chloroform and carbon disulfide.
Insoluble in water.

Glyceryl Monostearate

Glyceryl monostearate is a white to cream-colored, wax-like solid in the form of beads, flakes, or powder. It is waxy to the touch and has a slight fatty odor and taste. Its molecular formula is $C_{21}H_{42}O_4$ and molecular weight is 358.57 g/mol. [14]

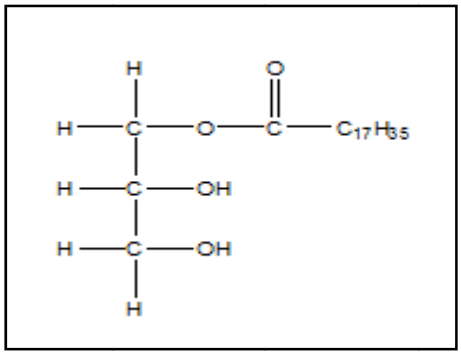


Figure 8. Structure of glyceryl monostearate

Melting point 55-60 C
Soluble in hot ethanol, ether, chloroform, hot acetone, mineral oil, fixed oils and practically insoluble in water.
it is incompatible with acid substances
When stored at warm temperature, there is an increase in the acid value upon aging owing to the sponification of ester with trace amount of water.

Glycerol behenate

Glycerol behenate is fine white powder made up of fat mixture of various esters of behenic acid and glycerol (glycerides). Its molecular formula is $C_{25}H_{50}O_4$ and molecular weight is 414.6621 g/mol. [15, 16]

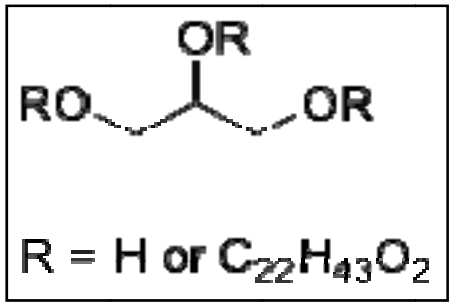


Figure 9. Structure of Glycerol behenate

Melting point is 65-77 C
Soluble, when heated, in chloroform and dichloromethane.
Slightly soluble in hot ethanol (96%)
Practically insoluble in cold ethanol (95%), hexane, mineral oil, and water.

Glyceryl palmitostearate

It consists of a mixture of mono, di and tri-glycerides of C_{16} and C_{18} fatty acids. It is fine white powder with a faint odour. [17]

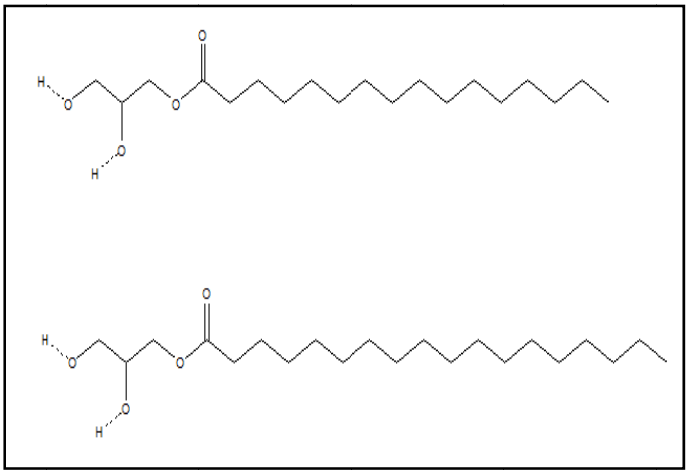


Figure 10. Structure of Glyceryl palmitostearate

Melting point 52-55 C
Freely soluble in chloroform and dichloromethane; practically insoluble in 95% ethanol, mineral oil and water
It should not be stored at temperature above 35 C, should be stored at temperature of 5-15 C in an airtight container, protected from light and moisture for a period over 1 month.

Cetyl palmitate

Cetyl palmitate is the colorless wax ester derived from palmitic acid and cetyl alcohol. Its molecular formula is $C_{32}H_{64}O_2$ and molecular weight is 480.84 g/mol. [18]

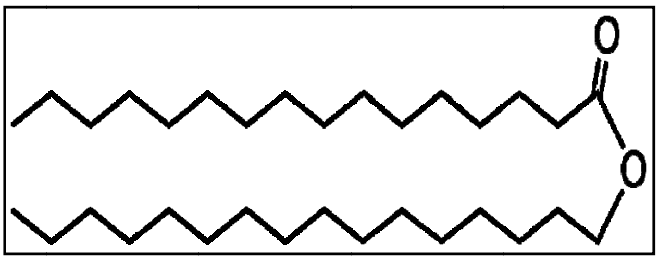


Figure 11. Structure of Cetyl palmitate

Melting point is 43-48 C.
Insoluble in water
Soluble in oils.

Cyclodextrin

It is a crystalzline , non-hygroscopic, cyclic oligosaccharide derived from starch with at least six D-(+)-glucopyranose units attached by (1 4) glycosidic linkage. Different types of cyclodextrin available are -cyclodextrin, β-cyclodextrin and γ-cyclodextrin which have glucose units of 6, 7 and 8 respectively. [19]

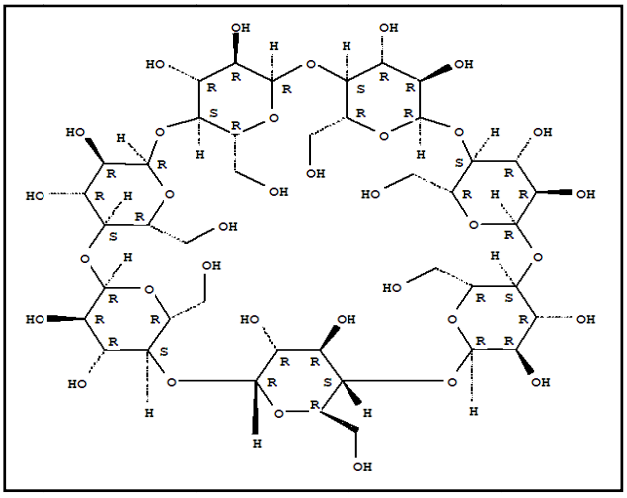


Figure 12. Structure of cyclodextrin

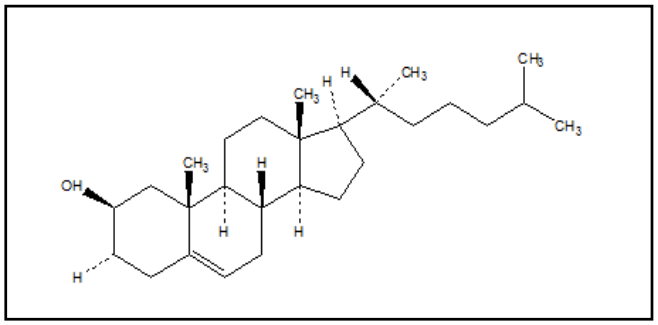
-cyclodextrin is mainly used in parenteral formulations. It has small cavity due to which it can form inclusion complex with small sized molecules.
β- cyclodextrin is most commonly used although it has least solubility. Due to its nephrotoxicity, it is not suitable for parenteral formulations. Hence it is primarily used in tablet and capsule formulations.
γ-cyclodextrin have large cavity due to which it can form inclusion complex with large molecules. It has less toxicity and more water solubility.

Witepsol

It consists of a mixture of triglyceride esters of the higher saturated fatty acids along with varying proportions of mono and diglycerides. It is manufactured by hydrolysis of natural vegetative oils such as coconut or palm kernel oil followed by the fractional distillation of free fatty acids produced. Under controlled conditions, C_8 to C_{18} fractions get hydrogenated and resterified by glycerine to form mono, di and tri-glycerides of required characteristic hydroxyl value. [20]
Witepsol H35:
Acid value 0.2-
Hydroxyl value 3
Iodine value 3
Melting point 33.5-35.5 C
Sponification value 240-250
Witepsol W35:
Acid value 0.3
Hydroxyl value 40-50
Iodine value 3
Melting point 33.5-35.5 C
Sponification value 225-235

Cholesterol

Cholesterol occurs as white or faintly yellow, almost odorless, pearly leaflets, needles, powder, or granules. On prolonged exposure to light and air, cholesterol acquires a yellow to tan color. Its molecular formula is $C_{27}H_{46}O$ and molecular weight is 386.67. [21]



Figur 13. Structure of Cholesterol

Melting point is 147-150 C.
Soluble in Acetone, Benzene, Chloroform, Ethanol, Methanol and Vegetable oils.
Practically insoluble in water.

Various Techniques Used For Preparation of SLNs

There are different methods for preparation of SLNs:
High Shear Homogenization Method.
Hot Homogenization Method.
Cold Homogenization Method.
Ultrasonication Method.
Microemulsion Based Method.
Solvent Emulsification/ Evaporation Method.
Solvent Emulsification/ Diffusion Method.
Double Emulsion Method.
Supercritical Fluid Method.
Solvent injection Method.
Spray Drying Method.

High Shear Homogenization method

This was the first technique for the preparation of SLNs. It is very powerful and reliable technique. With the high pressure of 100-2000 bar, homogenizer pushes the liquid through narrow gap of few microns. The fluid accelerates on a very short distance to very high velocity over 1000 km/h. Very high shear stress and cavitation forces disrupt the particles down to the submicron range. [21]

Hot Homogenization Method

This technique involves temperature higher than melting point of lipid. It contains about 5-10 % of lipid content. A pre-emulsion of the drug loaded lipid melt and the aqueous emulsifier phase (same temperature) is obtained by high-shear mixing device. High temperature results in small size of particle. [22]

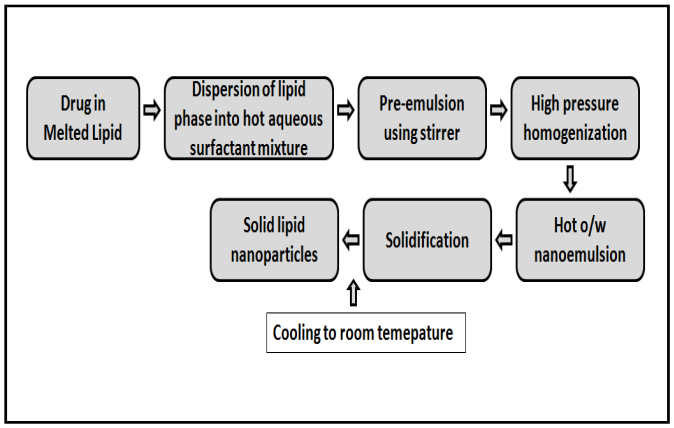


Figure 13. Hot homogenization method

Cold Homogenization

Cold homogenization technique overcomes disadvantages of hot homogenization technique like the temperature-induced drug degradation, drug distribution into the aqueous phase during homogenization and complexity of the crystallization step of the nanoemulsion leading to several modifications. Solubilization or dispersion of the drug in the melted lipid is same as hot homogenization technique. Then it is solidified with dry ice or liquid nitrogen. Size of solid is reduced by using mortar mill or ball mill to 50-100 microns. These microparticles are dispersed in aqueous phase as pre-emulsion. Then this pre-emulsion is homogenized at high speed below room temperature to obtain SLNs. [23]

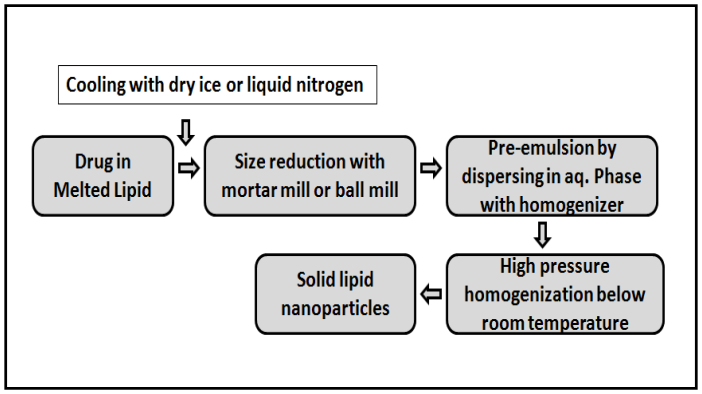


Figure 14. Cold homogenization method

Ultrasonication or High Speed Homogenization Method

Ultrasonication or High Speed Homogenization is another technique to prepare SLNs with ease. It is simple in comparison to hot and cold homogenization technique, because equipment used is very common. Firstly, the drug is added to melted lipid. Then heated aqueous phase (at same temperature) is added to this melted lipid and emulsified by probe sonicator. This pre-emulsion is ultrasonicated using probe sonicator. Keeping at least 5 C above melting point of lipid, it prevents recrystalization. Obtained SLNs

are filtered through 0.45µm membrane to remove impurities and stored at 4 C. [24]

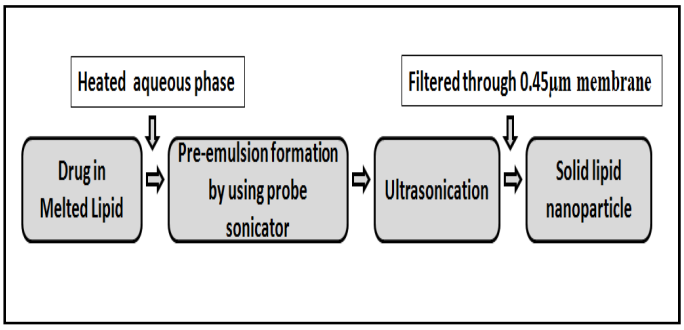


Figure 15. Ultrasonication or High speed homogenization method

Microemulsion based method

Dilution of microemulsions based method contains microemulsions having two-phase systems composed of an inner and outer phase (e.g. o/w microemulsions). In this technique, mixture of a low melting fatty acid (e.g. stearic acid), an emulsifier (e.g. polysorbate 20), co-emulsifiers (e.g. butanol) and water is prepared by stirring at temperature of 65-70 C until it becomes optically transparent. This hot microemulsion is dispersed in cold water (2-3 C) under stirring. The dilution process is critically determined by the composition of the micro emulsion. After dilution process, dispersion medium is washed by ultrafiltration system. [25]

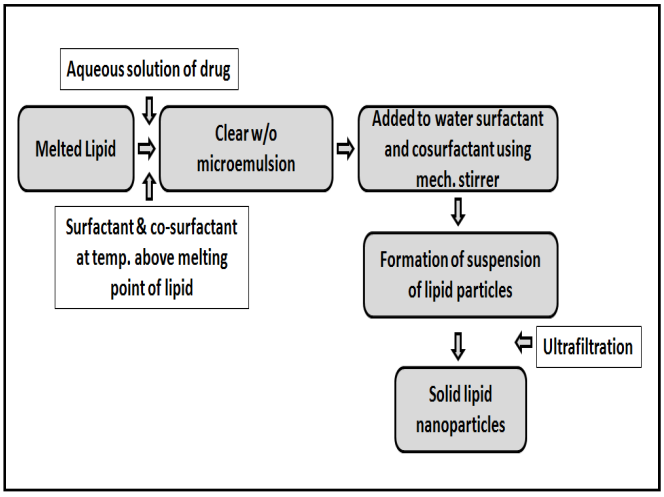


Figure 16. Microemulsion based method

Solvent emulsification-evaporation method

In this method, the lipid and hydrophobic drug is dissolved in a water immiscible organic solvent like cyclohexane, dichloromethane, toluene, chloroform, etc. Then emulsification is done in an aqueous phase by using high speed homogenizer. The micro fluidizer is used for coarse emulsion, which improves the efficiency of fine emulsification. Then the organic solvent is evaporated by rotary evaporator by maintaining its temperature at room temperature. SLNs of size 25nm can be obtained by using this method. [26]

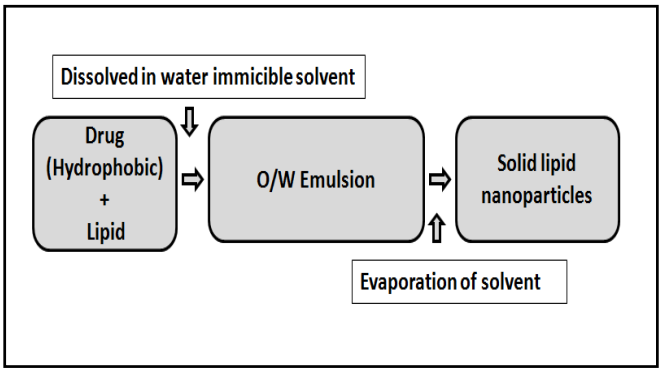


Figure 17. Solvent emulsification-evaporation method

Solvent emulsification- diffusion method

In solvent emulsification- diffusion method, partially water miscible solvents like benzyl alcohol, ethyl acetate, methyl acetate etc. are used. Solvent and water are saturated until they gain thermodynamic equilibrium. Saturation step requires heating if lipid requires heat to solubilisation. Drug and lipid is added to this saturated solution of solvent and water using mechanical stirrer. This will form o/w emulsion. Water in ratio of 1:5 to 1:10 is added for diffusion to continuous phase to form aggregation of lipid in nanoparticles. Temperature is maintained to room temperature or at temperature at which lipid is dissolved. After lyophilization or vaccume distillation, SLNs are obtained. [27]

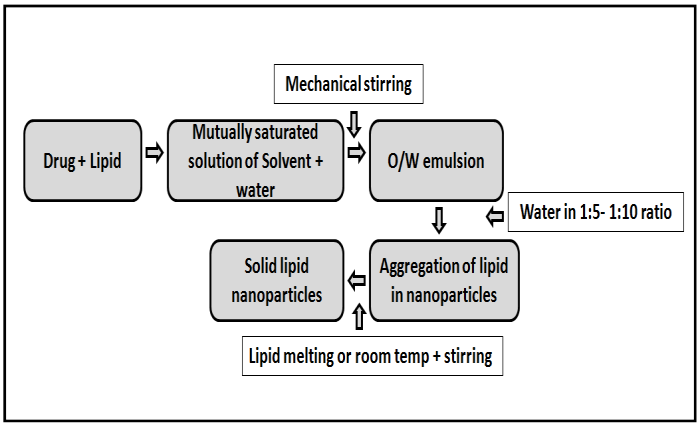


Figure 18. Solvent Emulsification- Diffusion method

Double emulsion method

In this technique, the hydrophilic drug is dissolved in aqueous solution and emulsified in melted lipid to form primary emulsion. The stabilizers like gelatine or poloxamer 407 are added in this primary emulsion to stabilize it. On the other side, the aqueous phase containing hydrophilic emulsifier by adding PVA is prepared

and primary emulsion is added to it. This double emulsion is stirred and is isolated by filtration. [28]

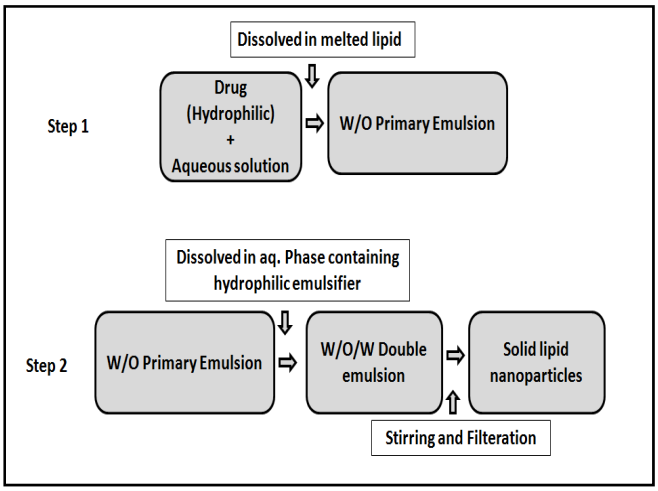


Figure 19. Double emulsion method

Supercritical Fluid Method

The supercritical fluid technology is a novel technique, which eliminates solvent processing steps. In this technique, water-insoluble drugs are combined with lipids and lipid nanosuspension is formed. The continuous supercritical fluid extraction of emulsions method is used for precipitation of solid lipid nanoparticles. The rapid expansion of supercritical carbon dioxide solutions (RESS method) produces SLNs. [29]

Solvent injection technique

This is the technique which is based on lipid precipitation from the dissolved lipid in solution. Then the solid lipid is dissolved in a water-miscible solvent like ethanol, acetone, isopropanol or their mixture. Then this organic solvent mixture is slowly injected through an injection needle in to stirred aqueous phase with surfactant. The dispersion so formed is sonicated for 12 minutes to obtain SLNs. [30]

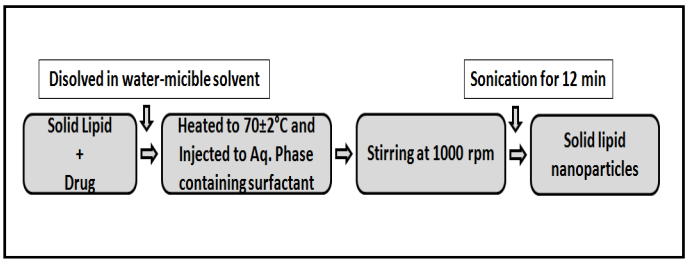


Figure 20. Solvent injection method

Spray Drying Method

Spray-drying is a process for preparing SLNs which converts a liquid feed to a dried particulate form. The feed is a solution, but it can also be a coarse or fine suspension or a colloidal dispersion (e.g., emulsions, liposomes, etc.). Firstly, this feed is atomised through various techniques (centrifugal, pneumatic, ultrasonic and electrostatic atomisation) to a spray form, which is put immediately into thermal contact with a hot gas, resulting in the rapid evaporation of the solvent to form dried solid particles. [31] The dried particles are then separated from the gas by means of a cyclone, an electrostatic precipitator or a bag filter.

Table 1. Various lipids used with different techniques for formulation of SLNs

Lipid	Drug	Technique	Reference
Glyceryl monostearate	Haloperidol	Emulsification–diffusion technique	Yasir *et al. 2014.* [32]
	Vinpocetine	Ultrasonic solvent emulsification	Luo *et al. 2006.* [33]
	Efavirenz	Hot homogenization method	Gaur *et. al. 2014.* [34]
	Dibenzoyl peroxide, Erythromycin base, and Triamcinolone acetonide	High shear hot homogenization	Gardouh *et.al 2013.* [35]
Trimyristin	Letrozole	Hot homogenization -ultrasonication	Nerella *et al. 2014.* [36]
Tristearin	Dithranol	Solvent emulsification evaporation	Khan *et. al. 2012.* [37]
	Clozapine	Modified hot homogenization	Venkateswarlu *et. al. 2004.* [38]
Glyceryl behenate	Valsartan	Solvent injection method	Parmar *et. al. 2011* [39]
Cetyl palmitate	Rifampin	Microemulsion-based method	Aboutaleb *et. al. 2012.* [40]
Stearic acid	Carbamazepine	Rapid Expansion of Supercritical Solution (Supercritical Fluid Method)	Akbari *et. al. 2014.* [41]
Tripalmitin	Paclitaxel	Microemulsion based method	Cavalli *et. al. 2000.* [42]
	Catalase	Double Emulsion Method	Ce Qi *et. al. 2011.* [43]
Palmitic acid	Moxifloxacin	Microemulsion based method	Silpa *et. al. 2012.* [44]
Cholesterol	Salbutamol sulfate	Spray Drying Method	Daman *et. al.* 2014. [45]

Applications of SLNs through various routes

Oral administration

For the drugs having low bioavailability through the oral route, SLNs are very useful approach for their delivery. These systems have controlled release behavior and bypass the gastric and intestinal degradation of the drug. [46] It provides transport of drug though GIT mucosa. The adhesive property of SLNs also reported to increase bioavailability and reduce or minimize erratic absorption. [47] Their firmness upon contact with gastrointestinal (GI) fluids since they are poised of biodegradable materials and having particle size in nanorange maximizes the surface area for enzymatic degradation. For the oral administration, lyophilized SLNs, powder can be placed into capsules, compressed into tablets or integrated into pellets.

Parenteral administration

SLNs consist of physiologically well-tolerated ingredients and have fine storage capabilities after lyophilization, which are very appropriate for systemic delivery. With parenteral administration, the approach of targeted gene therapy in the treatment of cancer can be executed by using cationic SLNs, which attach to genes via electrostatic interactions. [48] The plasma half life and steadiness of the SLNs can be increased by coating SLNs with polymers like PEG, which will reduce phagocytic uptake and increase bioavailability. [49]

Rectal administration

Rectal route is used for pediatric patients due to easy application. In some circumstances, when faster pharmacological action is needed, rectal route is preferred. With similar dose, rectal route reported superior plasma levels and therapeutic effectiveness than oral or intramuscular route. [50]

Nasal administration

Nasal route gives fasten absorption and rapid onset action of drug. It avoids degradation of drug by GIT fluids and poor transport across epithelial cell layers. Nasal route can also be used for targeting the brain. [51]

Respiratory delivery

Respiratory route for SLNs is a novel approach for delivery of drugs. This route through lungs avoids first pass effects by offering a high surface area for drug absorption. Solid lipid particles have effective carrying capacity for anti-tubercular drugs, anti-asthmatic drugs and anticancer drugs, enhancing their bioavailability and reducing the dosing frequency for better management of pulmonary action. [52]

Transdermal route

SLNs are having lipid contents, which are having great penetrating power across the skin. They are well suitable for use on inflamed or damaged skin because they are based on non-toxic and non-irritant lipids. [53]

Ocular route

Eyes have multiple barriers resisting penetration of drugs. Ocular delivery is considered as the most difficult thing to achieve. Drug absorption and bioavailability is poor by ocular route. To overcome these challenges, SLNs having muco-adhesive properties improve interaction with eye mucosa and prolong corneal resisting time of drug. It gives improved bioavailability and targeting effect. [54]

Fate of Lipids/Excretion of Lipids/Degradation Mechanism of Lipids *in-vivo*

The solid lipids in SLNs can be degraded by pancreatic lipases in the GI tracts when administrated orally. There are some *in-vitro* models which show process of enzymatic degradation. [55] There is some relation of degradation of lipid with the length of fatty acid chains. Degradation will be slow if the chains will be longer. The influence of surfactants in the formulation can be either degradation accelerating (e.g. sodium cholate) or hindering, degradation show down effect due to steric stabilization (e.g. Poloxamer 407). [56] Fast degradation was noticed when the size of SLNs were in renge of 180-300 nm, and size having 800 nm showed slow degradation. [57] SLNs which were having low crystallinity matrix (Dynasan 114 and 116, sodium cholate) show faster degradation than higher crystalline particles (all SLN with Poloxamer 407). [58] Little information is available till now for the fate of intravenously administered SLNs. Although the distribution of SLNs had been investigated by monitoring the drug level or the tracing elements such as fluorescent probes or nuclear substances, we know little about the metabolism and excretion of either the SLNs vehicle itself or the lipids comprising the vehicle. Most of the SLNs accumulate in the liver, spleen, or lungs, degraded at the place, when intravenously administered. The fatty alcohol dehydrogenase is present in the liver, which degrade fatty alcohol-based SLNs (e.g. cetyl or stearyl alcohol). *In-vitro* incubation of cetyl or stearyl alcohol-based SLNs with alcohol dehydrogenase enzymes showed 80-90% degradation after 15-24 h, which suggested similar degradation mechanisms of SLNs *in-vivo*. The release of drug molecule and disruption of SLNs can be increased by enzymatic degradation. [59] It is revealed by in-vivo studies that an ocular SLN system have increased residence time on the ocular surface and conjunctival sac as compared to an aqueous eye drops, which leads to the sustained release of the drug. SLNs have small particle size due to which it has increased adhesive ability and decreased clearance of the nasolachrymal duct. [60] The thiolated SLNs have found to increase the mucoadhesive properties and slowed down the rate of elimination from ocular surface. [61] SLNs also have their role in pulmonary delivery. SLNs of larger sizes get deposited in the throat and the smaller sizes get exhaled without any deposition in the end of bronchus. [62] SLNs that reach the pulmonary alveoli get eliminated by clearance route other than

respiration and also through the mucociliary escalator; the clearance shows the rapid initial elimination phase. [63] SLNs also work to target a lymphatic system because after few minutes, inhaled particles begin to translocate to regional lymph nodes. [64]

Conclusion

The selection of appropriate drug carrier, possessing non-toxicity and neutrality to our body system and, crucially, compatible with the active substance, is a very smart task, not an easy one. In recent years the solid lipid nanoparticles have proved to be the ideal solution to this problem and an alternative to traditional colloidal and vesicular systems. The SLN are exciting carrier systems for encapsulating bioactive substances with considerable potential for application. Along with a number of advantages we discussed earlier, SLNs have certain grey areas also which include low drug loading capacity, product stability aspect which is associated with the possibility of gelation, particle size increase (agglomeration) and drug releasing characteristics. So it can be concluded that the future holds great promise for its systematic investigation and exploitation.

Conflict of Interest

The authors declare no conflict of interest.

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