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Polysaccharide Coated Nanostructured Lipid Carriers: A Novel Approach of Drug Delivery System

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Abstract: ***Nanostructured Lipid Carriers (NLC) are newly developed drug delivery systems, which have shown a lot of advantages over conventional solid lipid nanoparticles, such as improved drug incorporation and release properties. NLCs are lipids based formulations which have low toxicity. For improvement in biopharmaceutical properties, NLCs are coated with polysaccharides to combine the advantages of lipidic nanoparticles with the biological properties of polysaccharides. Many methods are used for the formulation of polysaccharides coated NLCs such as high pressure homogenization, microemulsion technique, emulsification-solvent diffusion, emulsification-solvent evaporation, solvent injection or solvent displacement, multiple emulsion technique, phase inversion, ultrasonication and membrane contractor technique. Important application areas of nanostructured lipid carriers in pharmaceutics are topical drug delivery, oral and parenteral administration and have found huge applications in cosmetics, food and agricultural products.***

Keywords: Solid lipid nanoparticles, Nanostructured Lipid Carriers, Increased drug load Polysaccharides, Bioavailability enhancer; Colloidal drug carrier

1. Introduction

In the pharmaceutical development today, the latest technologies lead to discover various new drug molecules but only to develop new drugs alone is not enough to assure the advancement in drug therapy [1]. Poor aqueous solubility and intrinsic dissolution rate are common problems that affect oral delivery of many existing drugs which ultimately leads to low bioavailability **[2].** Thus, there is an expanding need to develop a pharmaceutical carrier scheme that overcomes these drawbacks **[3]**. The pharmaceutical carrier system should have aspects such as higher loading capability, no toxicity (acute and chronic) and the viability of drug targeting and control release. The physical and chemical stability for loaded drug should also be provided by the carrier system **[4,5,6**]. The possibility of large scale production with rational overall costs should be available.

Colloidal systems have their own drawbacks. Limitations often experienced with the colloidal system such as liposomes, micro and nanoemulsions, nanocapsules, nanosponges and polymeric nanoparticles have the fast degradation by the stomach pH or by the intestinal enzymes and the bile salts when taken orally, constricted physical and chemical steadiness in every part of storage **[7,8]** ,requirement of large-scale production methods, a quick release of the drug from its carrier system, stability difficulties, the residues of the organic solvents used in the production method, the polymer toxicity etc **[3,9,10]**.

Solid lipid nanoparticles (SLN) are as an alternate carrier scheme to emulsions, liposomes and polymeric nanoparticles **[11,12]**. SLN are formulated from solid matrix only. The advantages of SLNs are the use of physiological lipids, the avoidance of organic solvents and the possibility for large scale production **[13].** As drug delivery carrier system, SLNs can enhance bioavailability, protect the sensitive drugs against environmental conditions, and control drug-release properties **[14]**. However, SLNs show some disadvantages such as drug carriers with an unforeseeable gelation tendency, polymorphic transformation, and low absorption due to the crystalline structure of solid lipids **[15].**

NLC have been developed to resolve the problems raised by SLNs. They are advised to be the second generation of lipid nanoparticles **[16].** Contrasted to SLN, NLC show a higher potential loading capacity by controlling the mixing of solid lipids with liquid oil, leading to special nanostructures in the matrix **[17].** The potential drawbacks of SLNs, such as limited drug-loading capacity and drug expulsion during storage, can be avoided by the new generation of nanoparticles **[18]**.

In the last ten years, different biopolymers, including proteins and nucleic acid, polysaccharides, from animals, plants and microbes origin, have been employed to packaging materials, drug delivery and regenerative medicine **[19, 20]**. As significant types of biopolymers, natural polysaccharides have chemical and structural difference with some magnificent properties **[21, 22].** Due to difference in charge, chain lengths, monosaccharide sequences, and stereochemistry offer the highest extent for the evolution of advanced functionalized substances and biomedicines **[23, 24, 25].** The various types of NLCs and the formulation techniques with different polysaccharide are described in the present review.

Nanostructured lipid carriers (NLCs)

A new generation of nanostructured lipid carrier (NLCs) is a drug delivery system composed of a lipid matrix with a special nanostructure **[18]**. This nanostructure enhance the drug loading capacity and firmly integrates the drug during storage **[26, 27].** The NLC system minimizes some potential problems as so-ciated with SLN **[28**] such as:

- Control or target drug release.
- Pay-load for a number of drugs too low.
- Drug expulsion during storage.
- High water content of SLN dispersions.

Types of NLCs

NLC is a blend of solid lipids along with incompatible liquid lipids. At room temperature, it remains in solid state **[29].** NLCs have miscellaneous advantages like SLN such as use of biocompatible lipids, controlled drug release from the carrier, feasibility of large scale production at economical cost by utilizing the existing equipments, via lymphatic transport it avoids first pass metabolism and protect from biochemical degradation of drug moiety **[28,30]**. NLC have superior drug loading due to the use of liquid lipid, drug expulsion can be avoided during storage for long period of time **[31**]. Different types of NLCs based on the nanostructure (Figure 1), composition and ratios of solid and liquid lipids:

- The imperfect type
- The multiple O/F/W type
- The amorphous type

The imperfect type (imperfectly structured solid matrix): It is produced by mixing different lipids which gives rise to imperfections in the crystal order to accommodate the drug. **[32]**

The multiple O/F/W type (multiple oil in fat in water carrier): It is produced by mixing high amount of oil with solid lipid to form nanocompartments. The drug is loaded in the oil compartments and can be formulated by hot homogenization process. **[33].**

The amorphous type (structureless solid amorphous matrix): It is prepared by mixing solid and liquid lipids in such a way that crystallization can be avoided and are obtained in amorphous state. **[34]**

After Production

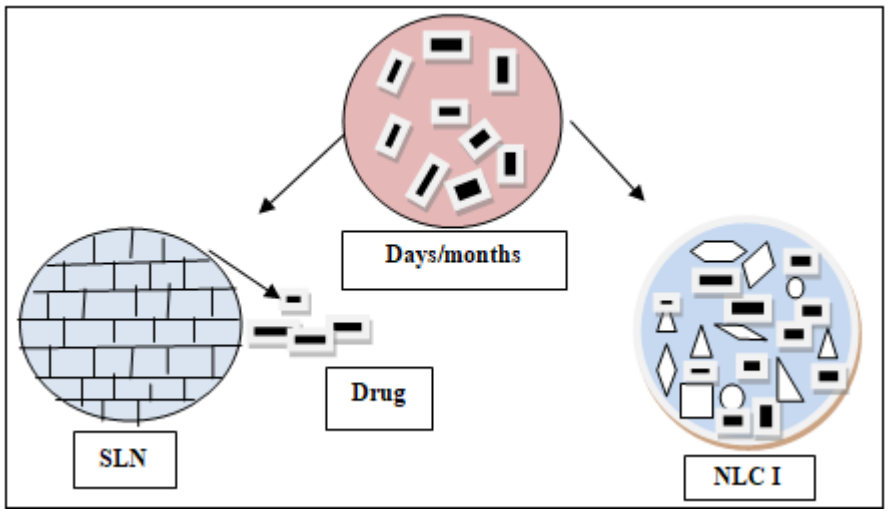

Figure 1: Crystallisation process during storage to perfect crystal in SLN (left) and unchanged remaining NLC 1 structure with imperfections (right)

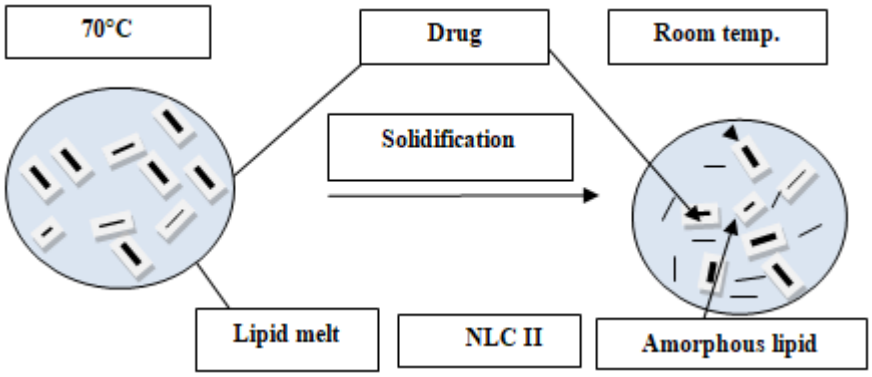

Figure 2: Structureless type II of NLC- the lipid solidifies in the solid but amorphous state.

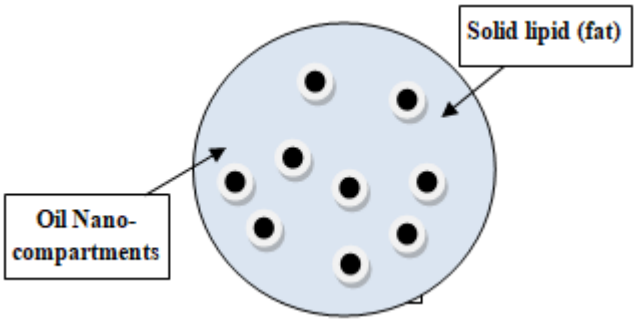

Figure 3: Theoretical proposed structure of multiple types NLCs (oil-in-solid fat-in-water)

Formulation Techniques for Polysaccharide Coated NLCs

Different formulation techniques for the production of polysaccharide coated NLCs, like microemulsion techniques and high-pressure homogenization (HPH) exhibits strong potential for scaling up to industrial production scale **[35, 36].** However, in some instances combination of different methods has been used to produce the nanoparticles. High-Pressure Homogenization (HPH) is a suitable technique for the production of lipid nanoparticles **[37]**. There are two types of HPH, hot HPH and cold HPH **[38]**.

Hot High Pressure Homogenization

In this method, firstly the polysaccharide with lipid(s) are melted at 5–10°C above their melting point(s), then the drug is homogenized and dispersed in the melted lipid(s) **[17]**. Then a surfactant's hot aqueous solution is mixed to the drug–lipid melt and dispersed (pre-emulsion) by a high shear homogenizer. Following, the hot pre-emulsion is put in to a high-pressure homogenizer at the same temperature **[39]**. For the desired average particle size of nanoemulsion, homogenization process is repeated. Then nanoemulsion is cooled to room temperature. During the cooling process, the lipid nanoemulsion droplets re-crystallize and lipid nanoparticles are formed with solid matrix **[38].**

Cold High-Pressure Homogenization Technique

As hot HPH, the lipid with polysaccharide are melted at 5–10°C above their melting points and the drug is dispersed in the melted lipid with homogeneous stirring in the cold HPH technique. Then the drug-lipid melt is cooled down by use of dry ice or liquid nitrogen and then ground to microparticles with the help of a mortar or ball mill. These microparticles are dispersed in a cold aqueous surfactant solution, followed by, homogenization at room temperature to form lipid nanoparticles. This technique is suitable for hydrophilic or thermo-labile drugs as this method can avoid drug degradation due to temperature and aqueous phase drug distribution during homogenization **[40]**. HPH technique is widely used in pharmaceutical and food industry.

Emulsification-Sonification Technique

In this technique, the lipids are melted at a temperature of 5–10°C above their melting points as in HPH and the drug is dispersed in the melted lipids. Then a hot aqueous surfactant solution is added to the drug-lipid melt and dispersed by a high shear mixing instrument. Probe sonicator is used for ultrasonication to obtain coarse hot oil-in-water emulsion for required nanoemulsion size **[41]**. At last, by cooling of hot nanoemulsion at room temperature, lipid nanoparticles are obtained. However, by using probe sonicator metallic contamination may happen with the product during sonication.

Microemulsion Technique

The microemulsion method for the preparation of SLNs was invented by **Gasco *et al.* [42]** and then modified by other researchers **[43,44,45]**. In this technique, firstly, the solid lipids such as fatty acids are melted and the drug is incorporated in the molten lipids. After that, a mixture of aqueous surfactant and co-surfactant solution heated to the same temperature is added to the melted lipid with stirring to obtain transparent, thermodynamically stable microemulsion formulation. Subsequently, the microemulsion is the basis for production of ultrafine nanoemulsion of required size when dispersed in cold water (2–10°C) with mild mechanical stirring which directly crystallize to form nanoparticles **[46].** The ratio of hot microemulsion with cold water should be in the range of 1:25–1:50. Thus, the extra water is reqruied to detach either by ultrafiltration or by lyophilization to get a concentrated dispersion. Requirement of High concentrations of surfactants and co-surfactants are the another drawback of this method, which is not essential. Industrial scale production of lipid nanoparticles is possible by the microemulsion technique.

Solvent Emulsification-Evaporation Technique

In Solvent Emulsification-Evaporation technique, first the polysaccharide hydrophilic drug and lipid(s) are liquified in a water-immiscible organic solvent (e.g., cyclohexane, chloroform, toluene). Then, it is emulsified in an aqueous phase containing surfactants with high speed stirring instrument **[47,48]**. The organic solvent evaporates when the coarse emulsion was passed through the microfluidizer during emulsification process, which results in precipitation of lipid. This technique is the most acceptable for thermo-labile drugs because it is conducted at room temperature. The use of organic solvent is one of drawback, which may remain in the final preparation and can interact with the drug molecules **[49,50].**

Solvent Diffusion Technique

In this technique, moderately water-soluble organic solvents (e.g., benzyl alcohol, ethyl formate) are used **[51,52]**. In this case, both the solvents and water are mutually saturated in order to confirm the starting thermodynamic equilibrium of both liquids. The short term oil-in-water emulsion is passed into water in ratio ranges from 1:5-1:10 with continuous agitation, then the solidification of dispersed phase forms the lipid nanoparticles due to diffusion process of the organic solvent **[53]**. However, dilute nanoparticle dispersion, thus produced needs to be concentrated by ultra-filtration or lyophilization technique. Use of organic solvent is also a concern as some of it may exist in the final preparation.

Solvent Injection Technique

The principle of the solvent injection method is as similar as the solvent diffusion method. In solvent injection technique, lipids and polysaccharides are dissolved in the solvent (e.g., acetone, isopropanol, and methanol) or solvent mixture and then rapidly injected into a surfactant's aqueous solution by the use of an injection needle **[54]**. The solvent migrates

quickly in the water and precipitation of lipid particles takes place in the aqueous solution. Particle size of the nanoparticles depends on the rate of distribution processes **[55]**. The technique have different advantages such as low temperatures, low shear stress, easy handling and fast production process without any high-pressure homogenizer. However, the use of organic solvents is main the disadvantage of this process.

Double Emulsion Technique

The double emulsion (w/o/w) technique is based on solvent emulsification–evaporation method **[43]**. In this technique mainly hydrophilic drugs are loaded in lipid nanoparticles. In this case, the drug and stabilizer are encapsulated in the inner aqueous phase of the w/o/w double emulsion. The primary emulsion is stabilized by adding stabilizer to prevent drug partitioning to the outer aqueous phase containing hydrophilic emulsifier, which is followed by agitation and filtration. Due to the larger particle size in the formulation, it is usually named as 'lipospheres' [56].

Characterization of NLCs

Quality control and stability of NLCs are necessary to confirm by the different methods of physicochemical characterization. Microscopic and Macroscopic techniques are used in development of colloidal system. The structure, mobility and molecular environment of the compounds are determined by various techniques like particle size analysis, zeta-potential, Transmission Electron Microscopy, Differential Scanning Calorimetry (DSC), X-Ray Scattering, Polarized Light Microscopy, Laser Diffraction (LD) and Field-Flow fractionation (FFF).

Particle Size Analysis:

The particle size is major parameter in process control and quality assurance due to physical stability of vesicle dispersion which depends on particle size and as particle size reduces, surface area increases, Photon Correlation Spectroscopy (PCS) based on laser light diffraction imparts a suitable method for examination and can be applied to the particles having range below 200 nm and up to 1µm **[57]**. The types and amount of lipid and emulsifier used in NLCs preparation highly effect particle size. The size can be reduced by the incorporation of additional emulsifiers that consistently facilitates the emulsification and more inflexible structure **[28].**

Electron Microscopy:

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) are used for the measurement of radius and size distribution of NLCs **[58]**. In addition, shape and morphology of NLCs particles can also be observed by using electron microscopy. SEM employs the beam of the electrons passing through the surface of the sample row by row, while TEM utilize electrons transmitted through the specimen. SEM has high resolution and easy preparation of the samples. After freeze-drying or freeze-thawing, TEM permits the visualization of NLCs **[59]**.

X-Ray Spectroscopy:

X-ray spectroscopy is a systematic technique mostly used for the elemental analysis or chemical characterization of a sample product **[60]**. This type of spectroscopy depends on the study of a sample through interactions of the electromagnetic radiation and the matter, analyzing X-rays released by the matter in this particular case. Its characterization ability is mainly due to the basic principle that each element of the periodic table has a different atomic structure **[**61].

Zeta potential (ZP):

Zeta potential is the electric potential, used to evaluate the dispersion and aggregation process which affects the particle stability of the colloidal dispersions. In suspensions the surfaces of particles develop a charge due to electrostatic repulsion **[62]**. This charge depends on the particle's surface chemistry and the media of these particles. A potential is generated around the particle by the surface charge, which is at the greatest near the surface and degrade with distance into the medium. The zeta potential can be determined by the particle's velocity in an electrical field **[63]**.

Differential Scanning Calorimetry (DSC) analysis:

DSC is usually used to get information about melting and re-crystallization behavior of a formulation. DSC measures the heat loss or gain as a result of physical or chemical changes within a sample as a function of the temperature Magdalene **[62].** In the NLCs formulations, DSC analysis is useful to recognize solid dispersions like solid solutions, simple eutectic mixtures or, as, drug and lipid interactions and the mixtures of solid lipids and liquid lipids (oils). Commonly, a melting point reduction is remarked while modifying the bulk lipid to a particulate form in the nanoparticles **[64].**

Polydispersity Index:

Due to polydisperse nature of NLCs, size distribution of the nanoparticles is important to measure by Polydispersity Index (PI) **[65]**. Lower the PI value, the more mono-dispersed nanoparticle dispersion can be obtained. Most of the researchers accept that the optimum value of PI should be less than 0.3 **[55].**

Polysaccharides Used In NLCs

Polysaccharides used in NLCs are natural polymers of mono-saccharides that are different in the type, number, distribution, and bonding of the monomers in the chain. Polysaccharides can be classified on the basis of their charge into neutral, acidic, and cationic **[66**]. Due to Polysaccharide's huge molecular form and their capability to self-assemble, numerous polysaccharides can be utilized to construct delivery systems for bioactive ingredients.

Chitosan

Chitosan, a natural, linear, non-toxic, biocompatible polycationic polysaccharide, composed of glucosamine and N acetyl glucosamine employed as the mucoadhesive polymer coating. According to the method of preparation the two monomers are randomly or block distributed throughout the biopolymer chain. Chitosan have a surface of high-density positive charge. Therefore, it has the property to dissolve in acidic medium. Chitosan prepared from shrimps and crabs has been approved as Generally Recognized As Safe (GRAS) **[67].** Chitosan has different nutritional and health properties, like hypocholesterolemic, immunity-enhancing, anticancer effects, and acceleration of mineral absorption **[68]**. Also, it has antimicrobial properties with

wide spectrum that act against food borne microorganisms **[69].** Chitosan and its maillard conjugates have been widely used to stabilize and upgrade overall emulsions quality. In addition, because of its renewable sources, the compound is inexpensive and further, for encapsulation of food bio-actives, Chitosan has many favorable properties such as nontoxicity, biocompatibility and biodegradability **[70].** Modifications of chitosan are aimed to improve physicochemical properties by chemical and physical treatments. Chitosan can be modified by N-alkylation, hydroxyalkaylation, carboxyalkaylation, and so forth **[71].** Chitosan can also be modified by means of physical treatments such as electromagnetic radiation and sonication processes. After modification chitosan with enhanced properties, such as improved solubility in water at different pH level, regulated surface charges, elevated absorption efficiency, new cross linking sites, find novel applications and can be modified for particular objective.

Pectin

Pectin is natural polysaccharides of complex mixture of different composition which depends upon on the source and the conditions at the time of isolation. Pectin is composed by large amounts of poly-D-galacturonic acid joined in chains by means of α-1, 4-glycosidic linkage. According to pectin's degree of methyl esterification, it can be of two types such as high methoxyl (HM) pectin or low methoxyl (LM) pectin, which produce some differences in its properties **[72]**. Pectin has a few hundred to about 1,000 saccharide units with average molecular weights from about 50,000 to 150,000 Da. These two types of pectin form gels by various mechanisms. The HM pectin forms gel formulation at pH around 3.0 by using a minimum amount of soluble sugars. These gels are thermally reversible. The production of gels from LM pectin can be done at wide range of pH and in the presence of a controlled amount of calcium or other divalent cations **[73]**.

Alginates

Alginates are natural anionic polysaccharides which is isolated from brown marine algae (Phaeophyceae). These are linear copolymers of (1→4)-β-D-mannuronopyranosyl and (1→4)-α-L-guluronopyranosyl units in homopolymeric sequences. The gel formulation can be prepared from Alginates in the presence of divalent cations, such as Ca_2. Thus alginate beads can be formulated by drop wise addition of sodium alginate solution into $CaCl_2$ solution to induce cross linking of the polymer chain to form an egg-box like configuration **[74]**. Particle size of the formulation depends on solution concentration and the initial dimensions of the extruded droplets. Alginate beads are insoluble at low pH values, which make them positive candidates for delivery of acid sensitive bioactive ingredients in the intestine area or to reduce the release of bioactive compounds in acidic foods **[75].**

Gum Arabic Polysaccharide

Gum arabic (GA) is the exudates gum obtained from Acacia trees. Gum Arabic is a complex mixture of arabinogalactan oligosaccharides, polysaccharides, and glycoproteins. It is mainly a branched, neutral, or slightly acidic substance. There are different factors which affect the chemical composition of GA including the source, climate, season, age of trees, rainfall, and time of exudation. GA has been identified to consist of α-(1→3)-linked d-galactopyranosyl units. The side chains are made up of 2–5 β-(1→3) linked with D-galactopyranosyl units, which are joined to the main chain by 1, 6-linkages. Both the main and the side chain have units of α-L-arabinofuranosyl, α-L-rhamnopyranosyl **[76].**

Tamarind Gum Polysaccharide

Tamarind, a branched polysaccharide is obtained from tamarind seeds, another natural polysaccharide belongs to the family *leguminosae* and which has high potential to utilize as hydrophilic polymer in controlled drug delivery system. Tamrind has been used as gelling, thickening, suspending and emulsifying agents in different dosage formulations **[77,78,79].** It has properties like high viscosity, broad pH tolerance, no carcinogenicity, mucoadhesive nature, and biocompatibility. It is used as stabilizer, gelling agent, thickener and binder in food and pharmaceutical industries **[78, 80].**

Fenugreek Polysaccharide

Fenugreek (*Trigonella foenum graecum*) is isolated from the ripe raw fenugreek, used extensively as spice, herb, food and medicine **[81].** It includes active ingredients like galactomannan, saponins, trigonelle, diosgenin and 4-hydroxyisoleucine **[82].** Fenugreek have hypoglycemic property also **[83,84].** Fenugreek galactomannan is present within the seed endosperm. It has moisture retention property which prevents seed from drying **[85].** It is the important source of soluble dietary fiber (17–50%) of dry seed weight **[86].** Due to the presence of high galactose content it shows more water solubility **[87].** Fenugreek is already established as gelling agent, binder, disintegrant, suspending agent, matrixformer and release-retardant **[88**]

2. Conclusion

NLC's are the carrier systems with suitable aspect which provide more flexibility in drug loading, modulation of release and enhance performance in developing final dosage forms such as injectable, creams, tablets, capsules etc. NLC's are the new generation of formulations which incorporates most of the advantages of various novel colloidal systems and avoids some of their disadvantages. Natural polysaccharides have broad application in pharmaceutical field such as in the synthesis of NLCs due to their non-toxic, biocompatible and biodegradable nature. There are different natural polysaccharides available. The different polysaccharides can be isolated by the different methods. They exhibits improved physicochemical properties and can be utilized for development of different pharmaceutical drug delivery systems. Thus, there is considerable chance to develop novel systems with natural polysaccharides.

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