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A Review: Lactoferrin Conjugated Drug Delivery System for Cancer Targeting

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***Abstract*—Delivery of drugs to brain is a subtle task in the therapy of many severe neurological disorders. Solid lipid nanoparticles (SLN) easily diffuse the blood–brain barrier (BBB) due to their lipophilic nature. Furthermore, ligand conjugation on SLN surface enhances the targeting efficiency. Lactoferin (Lf) conjugated SLN system is first time attempted for effective brain targeting in this study. Preparation of Lf-modified anticancer drugs loaded SLN for proficient delivery of them to brain. These lipidic nanoparticles can be evaluate by DLS, AFM, FTIR, XRD techniques and in vitro release studies. Colloidal stability study can performe in biologically simulated environment (normal saline and serum). These lipidic nanoparticles may further evaluate for its targeting mechanism for uptake in brain tumour cells and brain via receptor saturation studies and distribution studies in brain.**

***Index Terms*—lactoferrin, solid lipid nanoparticles, blood brain barriers, targeting**

I. Introduction

The impaired delivery of drug to brain is due to the presence of blood–brain barrier (BBB) that represents the main obstacle in central nervous system. BBB is the specialized system of capillary endothelial cells that protects the brain from harmful substances in the blood stream, while supplying the brain with the required nutrients for proper function [1].

Colloidal drug delivery systems are the effective delivery systems to improve the targeting efficiency [2]. Among all colloidal systems, solid lipid nanoparticles (SLN) have recently been evaluated as potential drug delivery system for brain targeting [3–7]. Colloidal solid lipid carriers such as SLN combine the advantages of polymeric nanoparticles, fat emulsions, liposomes and simultaneously avoid their disadvantages [8–10]. Solid lipid nanoparticles (SLN) delivery can be an ingenious way to administer molecules into the brain by possibly overcoming or alleviating the solubility, permeability and toxicity problems linked with the respective drug molecules. SLN are taken up readily by the brain due to their lipidic nature. Although, SLN deliver comparatively high amount of drug to brain but they show extrapyrimidal side effects because of their non-specificity [11].

SLN being colloidal drug delivery system offer large surface area and the surface can be modified using many charge modifiers such as stearic acid (SA) and stearylamine. SA is an endogenous long-chain fatty acid with low toxicity. It is biocompatible with human tissues and neutral with relevance to physiological fluids. It also provides free carboxylic group and aids in conjugating ligand on SLN surface thus enhances its specificity to the targeting organ [12, 13].

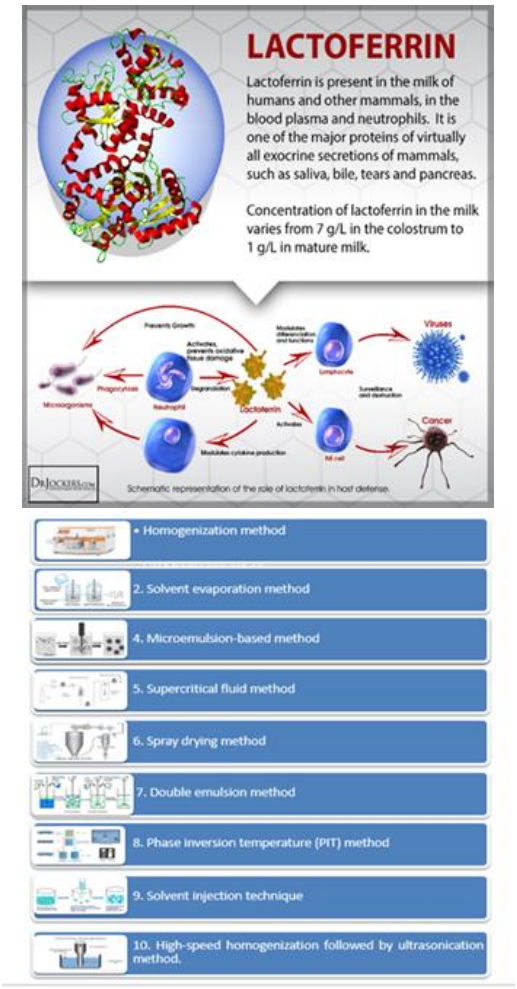

Fig. 1. Methods for preparation of SLN

Lactoferrin (LF), also known as lactotransferrin (LTF), is a multifunctional protein of the transferrin family. It is a globular glycoprotein with a molecular mass of about 80 kDa that is widely represented in various secretory fluids, such as milk,

saliva, tears, and nasal secretions. Lactoferrin is one of the components of the immune system of the body; it has antimicrobial activity (bacteriocide, fungicide) and is part of the innate defense, mainly at mucosa. Lactoferrin interacts with DNA and RNA, polysaccharides and heparin, and shows some of its biological functions in complexes with these ligands. Lf receptor (LfR) has been demonstrated to exist on the BBB in different species and is involved in Lf transport across the BBB in vitro and in vivo receptor mediated transcytosis [19–21]. LfR exist not only on the BBB in different species but also are overexpressed on the cells surface of glioblastomas [22, 23].

II. Methods of Preparation for SLN

A. Emulsion-Solvent Evaporation/Extraction

In this method, the polymer is first dissolved in a water-immiscible, volatile, organic solvent such as chloroform, dichloromethane, or ethyl acetate. The drug is added to this polymer solution and the mixture is emulsified into an outer water phase containing an emulsifier, such as polyvinyl alcohol (PVA), gelatin, polysorbate 80, or polaxamer-188 to yield an o/w emulsion. To harden the nano emulsion droplets into solid nanoparticles, the organic solvent is evaporated or extracted from the system after it diffuses into the external aqueous phase. Emulsification is facilitated by high-speed homogenization or sonication. Forth removal of solvent, the stirring process may be continued for several hours at high-temperature/low-pressure conditions; a quicker option to harden the particles may be to pour the emulsion into water, causing the solvent to phase toward the surfactants in the interface and eventually diffuse out into the aqueous phase.

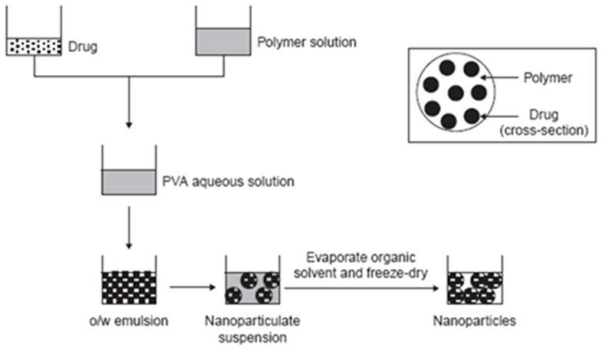

Fig. 2. Preparation of SLN by emulsion-solvent evaporation/extraction

The major existing challenges of this method for the production of nanoparticles are the parameters that control the particle size and the outcome of uniform size distribution for small particles. Moreover, the common solvent used to solubilize thepolymer, dichloromethane, is a class 2 solvent that poses problems in use in pharmaceutical preparations due to its potential toxicity. The common class 3 solvent, acetone, produces highly porous particles that eventually adversely facilitate the drug release, especially for hydrophilic small-molecule drugs. Moreover, processing with acetone must be done very carefully because of its high flammability.

B. Salting Out

The salting-out method and emulsification solvent diffusion techniques for the production of nanoparticles have been developed to meet the US FDA specification on the residual amount of organic solvents in injectable colloidal systems. Polymeric nanoparticles can be prepared by using an emulsion technique that avoids surfactants and chlorinated solvents and involves a salting-out process between two miscible solvents to separate the phases. The preparation method consists of adding an electrolyte saturated (usually magnesium chloride hexahydrate) or a non–electrolyte-saturated aqueous solution containing PVA as a viscosity increasing agent as well as a stabilizer to an oil phase composed of the polymer and the drug dissolved in acetone under continuous mechanical stirring at room temperature. The saturated aqueous solution prevents complete miscibility of both the phases by virtue of the high salt content. After the preparation of the initial water in-oil emulsion (w/o), water is immediately added in sufficient quantity to cause a phase inversion from water-in-oil (w/o) to oil-in-water (o/w) type emulsion; this induces complete diffusion of acetone from the internal non aqueous phase into the continuous external aqueous phase, thus leading to the formation of nanoparticles. The final emulsion is then stirred overnight at room temperature to allow for the complete removal of acetone. Centrifugation may also be used to remove the organic solvent, free PVA, and electrolytes from the raw nanoparticle suspension, after which the nanoparticles can be purified by cross-flow microfiltration and subsequently freeze-dried.

C. Emulsification Solvent Diffusion Method

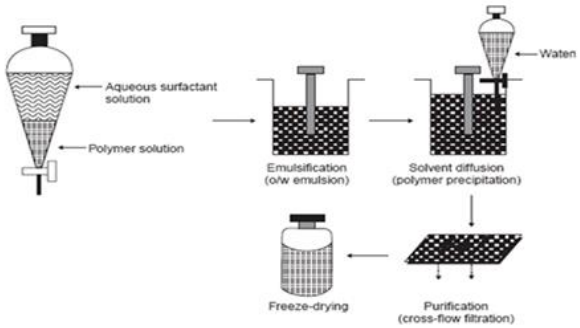

Fig. 3. Preparation of SLN by emulsification of solvent diffusion method

In the technique developed by Quintanar-Guerrero et al., the solvent and water are mutually saturated at room temperature before use to ensure the initial thermodynamic equilibrium of both liquids. Later, the organic solvent containing the dissolved polymer and the drug is emulsified in an aqueous surfactant solution (usually with PVA as a stabilizing agent) by using a high-speed homogenizer. Water is subsequently added under constant stirring to the o/w emulsion system, thus causing phase transformation and outward diffusion of the solvent from the internal phase, leading to the nano precipitation of the polymer

and the formation of colloidal nanoparticles. Finally, the solvent can be eliminated by vacuum steam distillation or evaporation. A schematic diagram of the emulsification-solvent diffusion method is presented in figure.

D. Emulsion Polymerization

This method has been used to prepare poly (alkyl cyanoacrylate) nanoparticles with an approximate diameter of 200 nm. A schematic diagram for preparation of Polyalkyl-cyanoacrylate nanoparticles by anionic polymerization is presented in Fig. The alkyl monomer is dispersed in an aqueous acidic medium containing stabilizers such as dextrans and poloxamers. Surfactants such as polysorbates can be used as well. The low pH favors the formation of stable and high molecular mass nanoparticles. Under vigorous mechanical stirring, polymerization follows the anionic mechanism. The non-polar ends within the interior of the surfactant micelles help solubilize the monomer. In the presence of water-soluble initiators, chain growth commences at the hydrophilic surface of the micelle. When the monomer in the interior of the micelle gets depleted, more monomer droplets from the exterior aqueous phase enter inside; thus, the polymerization reaction proceeds inward and continues until it is terminated by the free radicals. The drug can be solubilized in the polymerization medium either before the monomer is added or later when the reaction has ended. Finally, the nano particulate suspension is purified either by ultracentrifugation or by redispersing the nanoparticles in an isotonic medium. The various factors affecting the formation of particles, their size, and molecular mass include monomer concentration, stirring speed, surfactant/stabilizer type and concentration, and the pH of the polymerization medium.

E. Phase Separation in Non Aqueous System

Unlike the single o/w and double w/o/w emulsion techniques, this process can be used to encapsulate both hydrophilic and lipophilic drugs, offering distinct advantages in terms of the entrapment efficiency over the application of predominantly aqueous systems that wash away highly hydrophilic drugs. In this method, hydrophilic drugs are solubilized in water and added to an organic solution of the polymer (w/o emulsion), whereas lipophilic drugs can be dissolved/dispersed in the polymer solution. Subsequently, an organic non solvent (e.g., silicone oil), which is miscible with the organic solvent (e.g., dichloromethane) but does not dissolve either the drug or the polymer, is added to the emulsion system with stirring; this gradually extracts the organic polymer solvent. With the loss of the solvent, there is a reduction in the polymer solubility, and the coating polymer in the solution undergoes phase separation, with the coacervate phase containing the polymer coacervate droplets. The polymer coacervate adsorbs on to the drug particle surface, resulting in the encapsulation of the drug by the precipitated polymer.

III. Large-Scale Pilot Production of Drug-Loaded Nanoparticles

A. Spray Drying

Some of the challenges faced by this technique include the production of small-sized nanoparticles and the need for innovative methods to increase the drug-entrapment efficiency. However, when compared with other methods, it provides a relatively rapid and convenient production technique that is easy to scale up, involves mild processing conditions, and has relatively less dependence on the solubility characteristics of the drug and the polymer. In this method, a solution or dispersion (w/o) of a drug in an organic solvent containing the polymer is sprayed from the sonicating nozzle of a spray dryer and subsequently dried to yield nanoparticles. A schematic diagram for production of nanoparticles by spray-drying is presented in figure mention below.

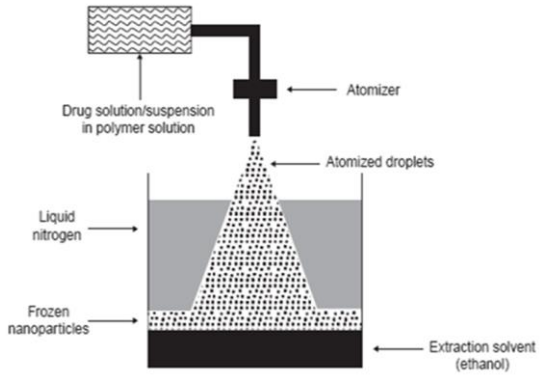

Fig. 4. Production of nanoparticles spray-drying

- *Supercritical Fluid Spraying*

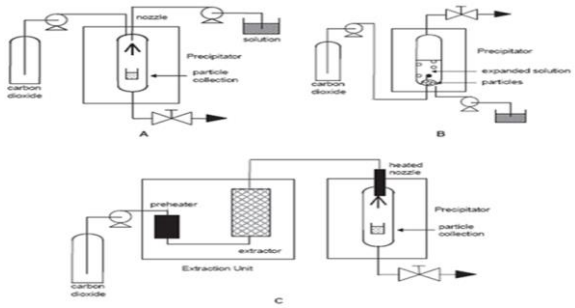

Fig. 5. Schematic diagram of different supercritical fluid spraying

This technology is advantageous in that the use of an organic solvent/surfactant can be avoided or minimized, thus producing nanoparticles that are free from toxic impurities. Carbon dioxide is nontoxic, nonflammable, and environmentally acceptable, and supercritical CO_2 can be easily obtained by pressurizing and heating the CO_2 system to a minimum of 73.8 bars and 31.05ºC, respectively. In the supercritical anti solvent method (76–78), both the drug and the polymer are dissolved in a suitable organic solvent and are atomized through a nozzle into supercritical CO_2. The dispersed organic solvent phase and

the anti-solvent CO_2 phase diffuse into each other and since CO2 is miscible only with the solvent, the solvent gets extracted causing the supercritical fluid–insoluble solid to precipitate as nanoparticles.

B. High pressure Homogenization

Homogenization is a fluid mechanical process that involves the subdivision of droplets or particles into micro- or nano size to create a stable emulsion or dispersion. Homogenization is a very common processing step in the food and dairy industries. It improves product stability, shelf life, digestion and taste. Homogenization can also significantly reduce the amount of additives (e.g. stabilizer) needed in a product. In the cosmetic industry homogenization is essential for the quality and stability of the products and their texture (skin feeling). The bioavailability of the pharmaceutical products can be enhanced by homogenization, also the tolerance of some drugs can be improved. Moreover, high pressure homogenization has some advantages over other size-reducing processes (e.g. ball milling). It is considered to be a superior process from an economical and product quality prospects. The contamination of the products caused by the personnel or coming from the machine (machine parts wearing) is reduced. Also the exposure to thermal stress and microbiological contamination is clearly less due to the shorter production times. There are two types of high pressure homogenizers available on the market, the jet-stream homogenizers e.g. Mirco fluidizer (Microfluidics, Newton, USA), and the piston-gap homogenizers e.g. Micron LAB 40 (APV Deutschland GmbH, Unna, Germany).

C. Characterization of Solid Lipid Nanoparticles

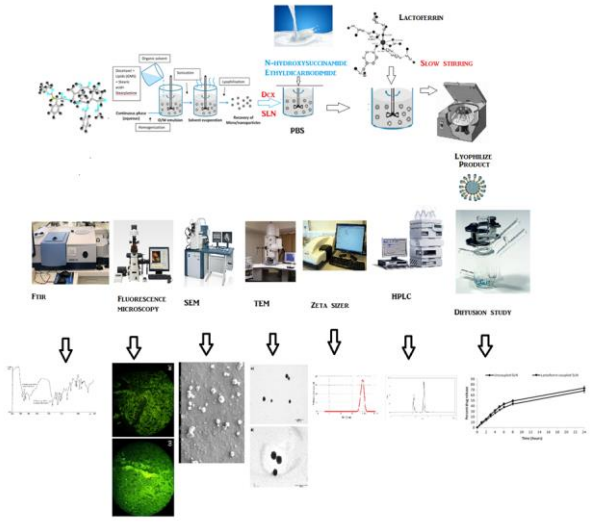

Fig. 6. Characterization of SLN

D. X-RAY Diffraction Method

The phase evolution of calcined powder as well sintered samples can be studied by X-ray diffraction technique using Cu Kα radiation. The generator voltage and current may ranges from 35 KV and 25 mA. Phases present in the sample can be identify with the search match facility available with software.

E. Thermo Gravimetric Analysis (TGA)

Thermal decomposition behavior of the gel can be studied using DSC/TG. The DSC/TG patterns are collected as a function of temperature under N2 atmosphere. Alpha alumina is the type of material used as reference.

F. Laser Diffractometry (LD)

Laser diffractometry (LD) is a technique used for the determination of particle size in the range of 10 nm to 2000 µm. It is based on the phenomenon that particles scatter light in all directions. The laser light is diffracted by the particle surface in a pattern depending on the particle size. Simply, the diffraction angle is small for a large particle, as its surface is less curved, while for a small particle, with a more curved surface, the diffraction angle is larger.

G. Particle size, Polydispersity Index and Zeta Potential

The average particle size and polydispersity index (PDI) of the SLNs can determine by photon correlation spectroscopy using a Zetasizer. The particles size and PDI can be represented by the average diameter of the Gaussian distribution function in the logarithmic axis mode. Surface charge measurement of the SLNs is based on the zeta potential (e) that can be calculated according to Helmholtz–Smoluchowsky from their electrophoretic mobility.

H. Shape and Surface Morphology

In order to examine the SLN surface morphology, the formulations are viewed via scanning electron microscopy (SEM). SEM samples are prepare by lightly sprinkling the lyophilized nanoparticle powder on a double adhesive tape stuck on an aluminum stub. The stubs are then coated with gold to a thickness of about 300 A° using a sputter coater. The photomicrographs can be taken with a scanning electron microscope. Transmission electron microscopy is used to visualize nanoparticles.

I. Entrapment Efficiency

Entrapment efficiency of uncoupled and coupled SLNs can be determined using the method described by Gupta et al. (2007) and Fry (1978). The drug not entrapped is removed from the SLNs by passing the formulation through a Sephadex G-50 minicolumn. The weighed amount of Sephadex G-50 is properly mixed with sufficient amount of distilled water in a beaker and kept for 24 h for complete swelling. After complete swelling, Sephadex dispersion is place in a 1-ml PVC syringe packed with glass wool and a small piece of Whatman filter paper at the bottom end to provide stability for the Sephadex column at 3,000 rpm. The amount of drug not entrapped in the SLNs is determined by passing the formulation from the Sephadex column, centrifuging at 3,000 rpm, and collecting the elution using the equation from Gupta et al. (2007).

After removing the un-entrapped drugs, the SLNs are collected and analyse using drug entrapment is then analysed

spectroscopically.

% Drug entrapment= Theoretical drug content -Practical drug content / Theoretical drug content *100

J. In Vitro Drug Release

The drug release of SLNs and Lf-coupled SLNs can be performed in suitable media using the dialysis bag technique. The dialysis bag retains nanoparticles and allows the free drug into the dissolution media with a molecular weight cut off point 3.5 KD. The bags are soaked in double-distilled water for 12 h before use. One ml of pure SLN formulation containing drug in about 150 mg of SLN free of any unentrapped drug is taken in a dialysis bag and placed in a beaker containing 50 ml of media at 37 ± 1 _C throughout the study. The samples are withdrawn after specified time intervals and replaced with the same volume of media. The withdrawn samples are analyzed for drug content by spectrophotometer.

K. Coupling Efficiency

The Lf concentration in the coupled SLNs can determine by the Bradford method of protein estimation with minor modifications. Briefly, 1 ml of Lfcoupled SLNs containing SLN formulation is placed in a volumetric flask with Coomassie blue G dye solution, and the volume is adjusted with distilled water. To determine the Lf concentration, the absorbance is measured and compare with a blank containing the same amount of dye.

L. In Vivo Organ Distribution Study

Fasted albino rats (average weight 150–200 gm) are divided into three groups each containing 12 animals. Animals of the first group are kept as a control that received an aqueous solution of free drug, while the second and third groups receives uncoupled and coupled formulations, respectively. All studies must be carried out according to the guidelines of the Council for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India and approved by the University Animal Ethical Committee, Sagar (MP), and India. After administration of the formulations, rats from each group are sacrificed after 2, 4, 6 and 24 h. One gram of each organ was homogenized with media using a homogenizer. In the case of organs, the whole organ is use and the amount of drug present in each organ and blood sample can determined by using the high performance liquid chromatography (HPLC) method.

IV. Conclusion

We can concluded that lactoferrin conjugation with drugs are most promising drug delivery systems for those which cant cross blood brain barriers. Solid lipid nanoparticles Solid lipid nanoparticles (SLN) easily diffuse the blood–brain barrier (BBB) due to their lipophilic nature. Furthermore, ligand conjugation on SLN surface enhances the targeting efficiency. And given methods can successfully formulate SLN and are characterize by using listed evaluation parameters.

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