CHAPTER 2
LITERATURE REVIEWS

2.1 *Butea mon*osperma (Lam.) Taubert (8, 18-20)

Butea monosperma (Lam.) Taubert is the yellow color powdered from flowers (Figure 1)

Figure 1: *Butea monosperma* (Lam.) Taubert flowers (20)

Scientific classification (21)

Kingdom:	Plantae
Division / Phylum:	Magnoliophyta
Class:	Magnoliopsida
Order:	Fabales
Family:	Fabaceae / Leguminosae / Papilionaceae
Genus:	*Butea*
Species:	*Butea monosperma*

Common name: Bastard Teak, Bengal Kino, Flame of the Forest, Parrot tree, Thong-Kwao

Scientific name: *Butea monosperma* (Lam.) Taubert

The morphological characteristics of *B. monosperma* (4, 22): A medium sized deciduous tree of up to 50 feet high (12-18 m) with flower clusters, and the trunk is usually crooked and twisted with irregular branches and rough, grey bark. The *B. monosperma* is native plant of Asia-tropical which distribute in India, Pakistan, Srilanka, Thailand, Myanmar, Loas, Vietnam, Malaysia, Indonesia. The *B. monosperma* is found in northern of Thailand. The *B. monosperma* is a tree of tropical and subtropical climate. Found throughout the drier parts of forests, deciduous forest; open grasslands and wastelands.

Medicinal uses: In the Indian traditional medical system of 'Ayurveda' has used many parts of *B. monosperma* such as roots, gum, seeds, bark, leaves and flowers. In this study interested in the *B. monosperma* flowers.

Chemical composition from *B. monosperma* flowers extract (4): Three chalcones; butein, monospermoside and isoliquiritigenin, one flavone; 7,3',4'-trihydroxyflavone, four flavanones; (-)-butin, (-)-butrin, (+)-isomonospermoside and (-)-liquiritigenin, and three isoflavones; formononetin, afrormosin and formononetin-7-*O*-β-D-glucopyranoside.

In previous researches showed many bioactivities from *B. monosperma* flowers in many of solvents for extraction;

The petroleum ether extract of flowers of *Butea monosperma* exhibited anticonvulsant activity. The fractions protected animals from maximum electro shock, electrical kindling and pentylenetetrazole-induced convulsions in mice. The fractions also inhibited convulsions induced by lithium–pilocarpine and electrical kindling. However, the extract can not protect animals from strychnine-induced convulsions (15). So, the next study of anticonvulsive activity of *B. monosperma* flowers extract was isolated by column chromatography. The active compound of anticonvulsive activity from B. monosperma was found a triterpene (TBM). TBM exhibited anticonvulsant activity against seizures induced by maximum electroshock (MES). TBM also inhibited seizures induced by pentylenetetrazol (PTZ), electrical kindling, and the combination of lithium sulfate and pilocarpine nitrate (Li-Pilo).

However, TBM was not effective against seizures induced by strychnine and picrotoxin. TBM exhibited depressant effect on the central nervous system (16). And the other activity of *B. monosperma* flowers extract, such as an antihyperglycemic activity of the ethanolic extract of *B. monosperma* (BMEE) was studied in glucose-loaded and alloxan-induced diabetic rats. Single dose, oral treatment of BMEE (200 mg/kg) significantly improved glucose tolerance and reduced blood glucose level in alloxan-induced diabetic rats. After that repeated oral treatment with BMEE (200 mg/kg/day) for 2 weeks found the blood glucose, and serum cholesterol were significantly reduced, and improved HDL-cholesterol and albumin as compared to diabetic control group (12). Chemomodulation from *B. monosperma* for protect hepatic cell against thioacetamide-mediated hepatic alterations in Wistar rats. The alcoholic extract of *B. monosperma* used for protect and maintain the function of hepatic cells in dose-dependent. The results showed that the methanolic extract of *B. monosperma* posses hepatoprotective effects and it might suppress the promotion stage from inhibition of oxidative stress and polyamine biosynthetic pathway (11). The anti-inflammatory activity of methanolic extract of *B. monosperma* flowers showed the activity against carrageenin induced paw edema and cotton pellet granuloma in albino rats. When the methanolic extract used into oral route in dose-dependent. The extract was found to inhibit granuloma tissue formation, and reduce in levels of serum, lysosomal enzymes (SGOT, SGPT and ALP), and lipid peroxides as compared to control (13). The NMR spectral data from *B. monosperma* flowers were found into 3',4',7-trihydroxyflavanone 3',7-di-O-β-D-glucopyranoside (butrin) and its hydrolyzed product (butin). The antioxidant activity of *B. monosperma* flowers extract was determined by free radical scavenging test using 2,2-diphenyl-1-picrylhydrazyl (DPPH) spectrophotometric assay. The percent inhibition of butin and butrin were 14.5% and 0%, respectively (2). And the other research of antioxidant activity of *B. monosperma* flowers from ethyl acetate, butanol and aqueous fractions derived from total methanol extract was evaluated by radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, nitric oxide radical, superoxide anion radical, hydroxyl radical and inhibition of erythrocyte hemolysis using 2,2' azo-bis (amidinopropane) dihydrochloride (AAPH). The methanol extract and its ethyl acetate and butanol fractions showed potent free radical scavenging activity (3). The

other activity of *B. monosperma* flowers was antimicrobial activity. The methanol extract of *B. monosperma* was active against the gram-positive bacterium. And phytochemical screening was carried out for phenolics and flavonoids in the extract (10). The study of bioactive flavonoids of the *B. monosperma* flowers were three chalcones, butein, monospermoside and isoliquiritigenin, one flavone, 7,3',4'-trihydroxyflavone, four flavanones, (-)-butin, (-)-butrin, (+)-isomonospermoside and (-)-liquiritigenin, and three isoflavones, formononetin, afrormosin and formononetin-7-*O*-β -D-glucopyranoside. The structure of compounds were elucidated by the spectroscopic techniques. The isolated flavonoids showed antimycobacterial activity with the chalcone, the butein was the most active compound (MIC 12.5 mg/ml) (4). The chemopreventive and anti-cancer properties of the aqueous extract of *B. monosperma* flowers showed the ability of the aqueous extract to impose growth arrest and trigger pro-apoptotic death in cell culture correlated with its chemopreventive effect in vivo when given oral administration (14).

2.2 Antioxidant

Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. The chemical chain reaction has 3 steps; initiation, propagation, and termination step. The reaction caused in cell damage is known as the oxidative stress. Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen which are highly reactive due to the presence of unpaired valence shell electrons. The ROS are produced in biochemical processes in the body and increased when expose to environmental stress (temperature, UV, air) or dietary xenobiotics. The ROS are unstable molecules, which are the main initial reactants for further reaction with the parent molecules in chemical chain reaction (1, 23).

The process of chemical chain reaction has 3 steps (24).

1. Initiation is used to form the low concentration of radical required to start the chain reaction. A special chemical, usually a peroxide, perester or azo compound which undergoes homolytic bond fission on heating is usually used.

   $X^{\bullet} + RH \text{ ----> } R^{\bullet} + XH$

   Note: $R^{\bullet}$ = Free radical

2. Propagation describes the chain reaction itself. There may be thousands of identical propagation steps.

$$R^{\bullet} + O2 \quad \text{---->} \quad RO_2^{\bullet}$$

$$RO_2^{\bullet} + RH \quad \text{---->} \quad ROOH + R^{\bullet}$$

3. Termination is the process by which radical centres are quenched. Termination occurs when two radical centres come into contact and couple together.

$$RO_2^{\bullet} + RO_2^{\bullet} \quad \text{---->} \quad \text{stable product}$$

$$RO_2^{\bullet} + R^{\bullet} \quad \text{---->} \quad \text{stable product}$$

$$R^{\bullet} + R^{\bullet} \quad \text{---->} \quad \text{stable product}$$

The use of antioxidants is one method to minimize rancidity, retard the formation of toxic oxidation product, maintain nutritional quality and increase the shelf life of food product. An antioxidant is any substance which is capable of delaying, retarding or preventing the development of rancidity. Since antioxidants can be classified according to their protective properties at different stages of the oxidation process and since they act by different mechanisms, they are divided into two main types of antioxidants. Primary antioxidants can inhibit or retard oxidation by scavenging free radicals by donation of hydrogen atom or electrons, which converts them to more stable product. Antioxidants in these group are thiols, ascorbic acid or polyphenols. Secondary antioxidants function by many mechanisms, including binding of metal ions, scavenging oxygen, converting hydroperoxides to non-radical species, absorbing UV radiation or deactivating singlet oxygen. Antioxidants in these group are tocopherol, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butyl hydroquinone (TBHQ) or alkyl, propyl, octyl, and dodecyl gallates. The antioxidant agents are synthetic antioxidants and natural antioxidants. Plants may contain simple phenolics, phenolic acids, coumarins, flavonoids, stilbenes, hydrolysable and condensed tannins, lignins and lignans (25). Many plant phenolic compounds exhibiting antioxidant properties have been studied and proposed for protection against oxidation. Several researchers have shown that various tissues from plants contain high concentrations of natural phenolic phytochemicals, including flavonoids. Relatively many Thai plant sources have been studied as sources of phenolic and flavonoid compounds which may be useful for

antioxidant potential (26). Flavonoids and other classes of phenolic compounds are important phytochemicals as effective antioxidants. Flavonoids are a large group of naturally-occurring plant phenolic compounds including flavones, flavonols, isoflavones, flavonones, and chalcones. Flavonoids contain a characteristic C_6-C_3-C_6 structure, with free hydroxyl groups attached to aromatic rings, and they inhibit lipid oxidation by scavenging radicals or by other mechanisms. Many literature reports showed a simple relationship between the content of phenolic compounds and the antioxidant capacity of plant extracts. Other chemical substances in plant including protein, carbohydrate, vitamin and fiber also contribute to the antioxidant capacity (1, 23).

2.3 Nanotechnology

The nanotechnology is an innovation sciences and develops continuously of the knowledge for making particles size in a level of part per billion. The most common definition of nanotechnology is that of manipulation, observation and measurement at a scale as 10 - 1000 nanometres (17). The nanotechnology is widely used in many fields such as medical and pharmaceutical fields especially in drug delivery system (17, 27-28).

2.3.1 Polymeric nanoparticles

The polymeric nanoparticles are nanoparticles which are prepared from polymers. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticles depending on the method of preparation. Nanocapsules are vesicular systems which the drug is retained in a cavity and surrounded by a polymer membrane, while nanospheres are matrix systems which the drug is physically and uniformly dispersed (Figure 2). In recent years, biodegradable polymeric nanoparticles have been interested in a drug delivery system for drug targeting or particular organs/tissues.

Different polymers and methods were used in polymeric nanoparticles which made from various synthetic and semi synthetic polymers, and natural polymers such as gums (Acacia, Guar, etc.), chitosan, gelatin, and sodium alginate. However, the polymers are designed primarily for medical applications and have controlled release

of bioactive agents. Many materials are designed to degrade within the body such as polylactides (PLA), polyglycolides (PGA), poly(lactide-co-glycolides) (PLGA), and polycaprolactone (29). Table 1 exhibited the different polymers and methods for prepared polymeric nanoparticles.

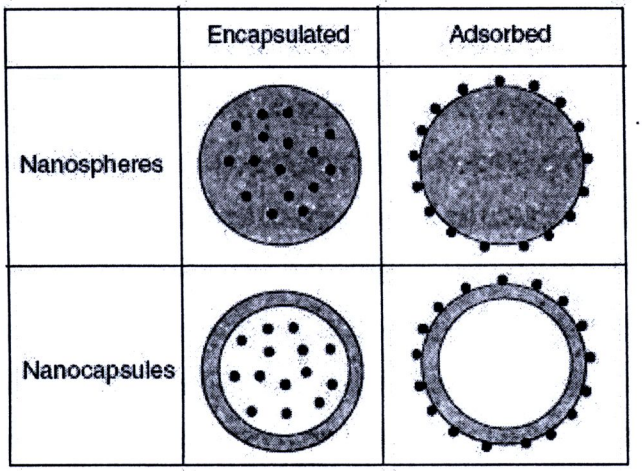


Figure 2: Schematic of drug encapsulated or absorbed at the surface of a polymeric nanoparticle (30).

Table 1 Types of polymers, method of nanoparticles, and nature of the particles

Polymer	Method	Nature of the particles
PLA	Emulsion-evaporation	Spheres
	Emulsion-diffusion	Spheres
	Salting out	Spheres
	Dissolvation	Capsules
	Interfacial precipitation	Capsules
PLGA	Emulsion-evaporation	Spheres
	Interfacial precipitation	Capsules
PACA	Interfacial polymerization	Capsules
	Emulsion polymerization	Spheres
Chitosan	Emulsion-diffusion	Spheres
Eudragit®	Emulsion-diffusion	Spheres
	Interfacial precipitation	Capsules
HPMC-AS	Emulsion-evaporation	Spheres

Key to abbreviations:

PLA	: poly (lactic acid)
PLGA	: poly (lactic-co-glycolide)
PACA	: poly (alkylcyanoacrylate)
Eudragit®	: poly(methyacrylic acid-co-methylmethacrylate)
HPMC-AS	: hydroxypropylmethylcellulose acetate succinate

Chitosan

Chitosan (CS) is a natural polymer which made from animals fiber. Chitin is the precursor of chitosan, has from exoskeletons of crustaceans, molluses and fungi. Chitin is a cationic polysaccharide comprising of N-acetylglucosamine and glucosamine conjugate with beta bonds (1→4). Chitosan (Figure 3) is a linear polysaccharide comprising of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) (31).

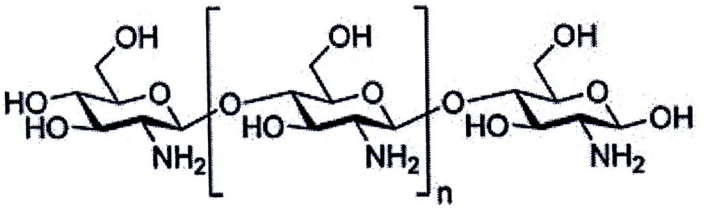


Figure 3: Chitosan (CS) structure (32)

The most common source of the α-CS is crustacean chitin which got from the wastes of crab and shrimp shell. In addition the β-CS was prepared form squid pens (33). CS is obtained by deacetylation of chitin, which is available in variety of commercial products with different grades. The term of CS is involved the different of degree of deacetylation and average molecular weights. The deacetylation of typical commercial CS is usually between 70% and 95%, and the molecular weights are between 10 and 1000 kDa. CS is the polymer which has moderately basic cationic polyelectrolyte, the value of pKa being 6.3 (34). The CS is not soluble in water but can be soluble in weak acid solution. The CS is biocompatible and biodegradable, besides it is low toxicity natural polymer (35). The CS shown mucoadhesive properties as well as an important drug penetration enhancement capacity across

mucosal barriers. Moreover, there are many advantages for many fields such as food industry, agriculture, medicine, cosmetic, and pharmaceutic. There are many techniques for preparing CS nanoparticles including solvent evaporation, and emulsion polymerization method. The other ionotropic gelation is usually a simplier and milder encapsulation.

The CS nanoparticles are obtained by the process of ionotropic gelation on the interaction between the positively charged amino groups of CS and the negative groups of other polymers. This process has been used to prepare CS nanoparticles for the delivery of peptides, proteins, drugs (35), including insulin (36) with tripolyphosphate (37-38), alginate (33), and sodium carboxymethylcellulose (34, 39).

Sodium carboxymethylcellulose (SCMC) [80]

SCMC is also known as Cellulose gum, CMC sodium, E466, Finnfix, Nymcel, Sodium cellulose glycolate, Sodium CMC, or Tylose CB. The typical molecular weight of SCMC ranges from 90000-700000. The appearance of SCMC is white or slightly yellowish, odorless, granular powder. A number of grades of SCMC are commercially available; there are various degrees of substitution (DS) which range from 0.7 to 1.2. The DS is defined as the number of hydroxyl groups substituted per anhydroglucose unit and determined the aqueous solubility of the polymer. The grades of SCMC are classified according to its low, medium, or high viscosity. The SCMC is practically insoluble in acetone, ethanol, and toluene but can be easily dispersed in water. Its pH ranges from 6.0-8.5. The SCMC is incompatible with strong acidic solution, salts solution of iron and some other metals, such as aluminum, mercury, and zinc. Precipitation may occur at pH < 2, and also occur when it is mixed with 95% ethanol (40) (Figure 4).

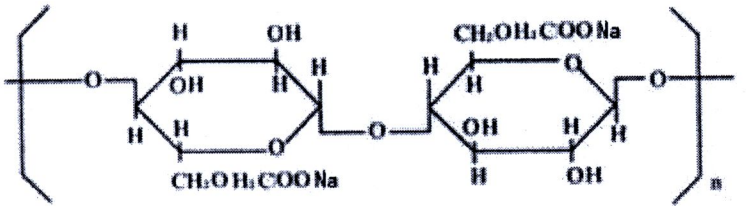
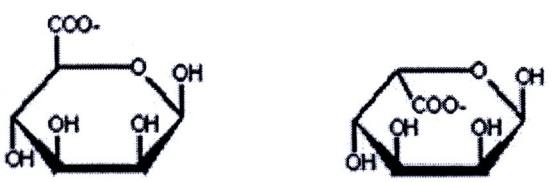


Figure 4: Sodium carboxymethylcellulose (SCMC) structure (41)

The SCMC is used in oral, topical, and some parenteral formulations. It is widely used in petroleum, textile, toiletries, food products, cosmetics, pharmaceutical, and other technologies because of its good solubility in water, non toxicity and non irritation. It may act as a suspending agent, thickener, protective colloid, humectant, and be used for controlling of crystallization of some other components. From these properties of SCMC, which is naturally occurring substance, biocompatible, biodegradable, and non-toxic polymer, make it suitable for drug delivery systems (40, 42).

Alginate

Alginate is an anionic polysaccharide copolymer which is distributed in the cell walls of brown algae. The alginate is a binary copolymer composed of β-D-mannuronic acid and α-L-guluronic acid residues of widely varying composition and sequence (Figure 5-6). A very rapid and irreversible binding reaction of multivalent cations is typical reaction for alginates. Therefore, a direct mix of these two components rarely produces homogeneous gels. The result of mixing is likely to be a dispersion of gel lumps. The cation salts such as calcium gluconate, calcium tartrate, and calcium chloride can be dissolved and gave Ca^{2+} that can be used to form insoluble gel. These gels cannot be dissolved in the acid solution, but can be dissolved in the neutral and alkali solutions. In the other hand, sodium alginate is the biocompatible and biodegradable as same as the chitosan. So the alginate can be applied to drug delivery system with chitosan. (33, 43).



β-D-mannuronate (M) α-L-guluronate (G)

Figure 5: The structure of alginate monomers: β-D-mannuronic acid (M) and α-L-guluronic acid (G) (43)

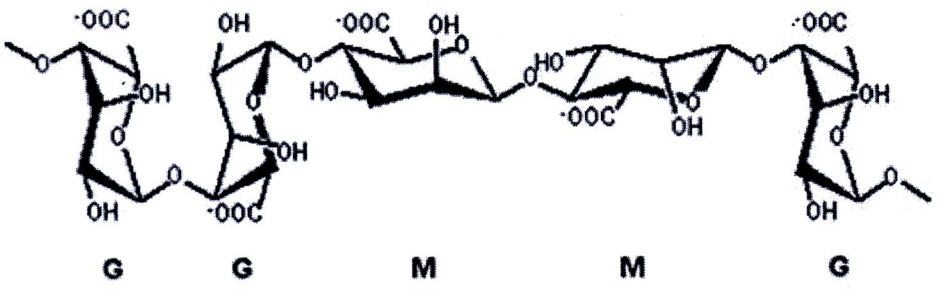


Figure 6: The structure of chain conformation of alginate (43)

Polylactide-co- glycolide (PLGA)

PLGA has interested because of its excellent biocompatibility, biodegradability, and mechanical strength (Figure 7). PLGA was synthesized by a polycondensation reaction or via ring opening polymerization of cyclic diesters. PLGA copolymer was prepared from L-poly lactic acid and polyglycolic acid. The degree of crystallinity and the melting point of the polymers are related to molecular weight. The mechanical strength, swelling behavior, capacity to undergo hydrolysis, and the biodegradation rate are directly influenced by the crystallinity of the PLGA polymer. The crystallinity of the PLGA polymer depends on the type and molar ratio of the monomer components (lactide and glycolide) in the copolymer chain. The glycolic acid is more hydrophilic and is more easily to hydrolysis so it made the polymer degradable quickly. On the other hand, the lactic acid is more hydrophobic than glycolic acid so the PLGA copolymers which have lactide-rich, are less hydrophilic, absorb less water, and degrade slowly. The PLGA copolymers are the biodegradability which were degraded in the body by hydrolytic cleavage of the ester linkage to lactic and glycolic acid (Figure 8). The two monomers of the PLGA copolymer can be metabolized in the body via Krebs cycle and eliminated as carbon dioxide and water. Since the body effectively deals with these two monomers, there is very minimal systemic toxicity associated by using PLGA for drug delivery or biomaterial applications. The commonly methods to formulate biodegradable PLGA nanoparticles, being solvent emulsion-evaporation, emulsification-diffusion and nanopracipitation. The criteria to determining the effectiveness of formulation techniques are particle size and size distribution, toxicity of materials used,

reproducibility, surface morphology, surface chemistry, surface charge, drug encapsulation efficiency, drug release kinetics, and hemodynamic properties of the particle (44-46).

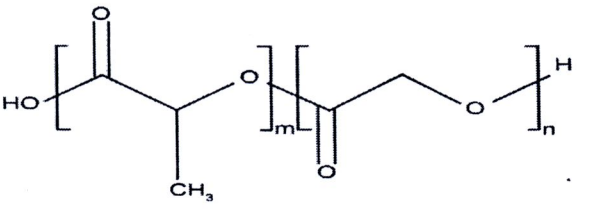


Figure 7: The structure of polylactide-co-glycolide (PLGA) (44)

Abbreviations: m, number of units of lactide acid, n, number of unit of glycolic acid

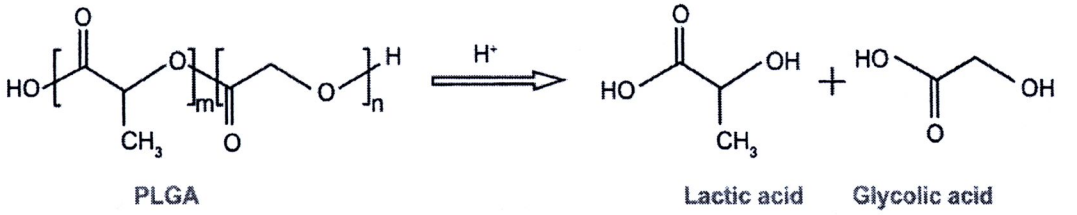


Figure 8: Hydrolysis of PLGA nanoparticles (44)

2.3.2 Lipid nanoparticles

Solid lipid nanoparticles

Solid lipid nanoparticles (SLN) are particles made from solid lipids with a mean diameter between approximately 50 and 1000 nm. SLN have been introduced as carriers for active cosmetic ingredients and pharmaceutical drugs (17, 47-48).

The advantages of SLN are as followed (48).

1. Controlled drug release and drug targeting.
2. Increasing of drug stability.
3. Incorporate of lipophilic drug.
4. No biotoxicity of the carrier.
5. Aviodance of organic solvents.
6. No problem with respect to large scale production and sterilization.

SLN have more advantages for drug delivery system. It was found that SLN can be included a good ability of controlled release, the increase physicochemical stability, could load the both of hydrophilic and lipophilic substances, the non-toxic of lipid particles, the avoided to use the organic solvents in the production and simply to scale up for industries. Furthermore, the extract delivery through the skin with SLNs can increase the chemical stability of loaded extract included the occlusive effect to increase the skin moisture that then support to deliver the extract into the deep skin. But the common disadvantages of SLN were limited drug loading capacity and drug expulsion during storage.

Morphology and structure of solid lipid nanoparticles (SLN) (47)

The type of SLNs depends on the chemical nature of the active ingredient and lipid, the solubility of actives in the melt lipid, nature and concentration of surfactants, type of production, and the production temperature. The three incorporation models have been proposed: (Figure 9)

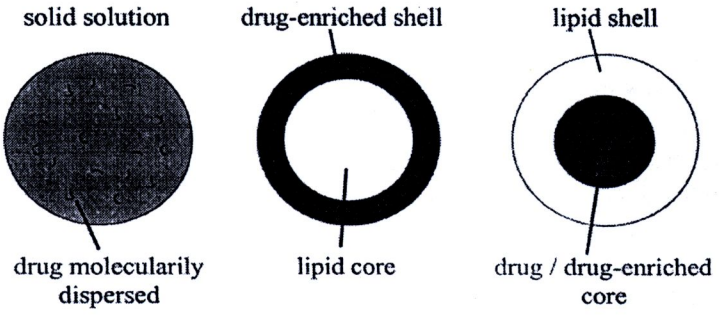


Figure 9: Basic type of solid lipid nanoparticles (47-48). Three drug incorporation models [solid solution model (left), core-shell models with drug-enriched shell (middle) and drug-enriched core (right)]

1. SLN Type I or homogeneous matrix model

   The SLN Type I or homogeneous matrix model is derived from a solid solution of lipid and active ingredient. A solid solution can be obtained when SLNs are produced by the cold homogenization method. A lipid blend can be produced containing the active in a molecularly dispersed form.

2. SLN Type II or drug-enriched shell model

   The SLN Type II or drug-enriched shell model is achieved when SLNs are produced via the hot high pressure homogenizer technique, and the active ingredient concentration in the method lipid is low. During the cooling process of the hot o/w nanoemulsion, the lipid will precipitate first, leading to a steadily increasing concentration of active molecules in the remaining lipid melt with increasing fraction of lipid solidified. An active-free lipid core is form; when the active reaches its saturation solubility in the remaining melt, and outer shell will solidify containing both active and lipid. The enrichment in the outer area of the particles causes burst release.

3. SLN Type III or drug-enriched core model

   The SLN Type III or drug-enriched core model can take place when the active ingredient concentration in the lipid melt is high and at or relatively close to its saturation solubility. Cooling down of the hot oil droplets will in most cases reduce the solubility of the active in the melt; when the saturation solubility is exceeded. Then active molecules precipitated leading to the formation of a drug-enriched core.

Solid lipid nanoparticle for cosmetic

In the cosmetic SLN possess a number of features advantages for the topical route of application. The small particle size ensure close contact to the stratum corneum and should increase the amount of encapsulated agent penetrating into the viable skin. The previous reseach were studied vitamin A loaded SLN in hydrogel and oil/water creams. The SLN dispersion can be successfully incorporated in more convenient topical dosage forms. The characteristic of SLN have been found that increase the UV-blocking capacity and prevents chemical degradation, therefore the possibility of developing a more effective sunscreen system with reduce side effect. The other study was studied tocopherol acetate incorporated into SLN and dispersion incorporated into gel and cream (47, 49).

Nanostructured lipid carriers (NLC)

The Nanostructured lipid carriers (NLC) is a new generation of solid lipid nanoparticles (SLN). It is an alternative carrier of traditional colloidal controlled release drug delivery system. The NLC is consisted of solid lipid matrix and liquid lipid. NLC has many advantages such as biocompatibility, biodegradability, low toxicity, lack of organic solvent during the production process, possibility of production on large scale for industries. Nevertheless, SLN has some limitations such as low drug loading and drug explusion. So the NLC can solved the problems of SLN, NLC has high drug loading, avoidance or minimization of drug explusion and enhancement of chemical stability can be achieved. The limit of drug loading capacity of SLN due to the particle matrix tends to form a crystal lattice leaving limited space to entrap the active compound. The NLC used of lipid mixture with differently structured molecules from the SLN which has perfect crystal. The particles matrix of NLC is an imperfection providing enough space to accommodate the active compounds in molecular form as an amorphous form (Figure 10) (48, 50-52).

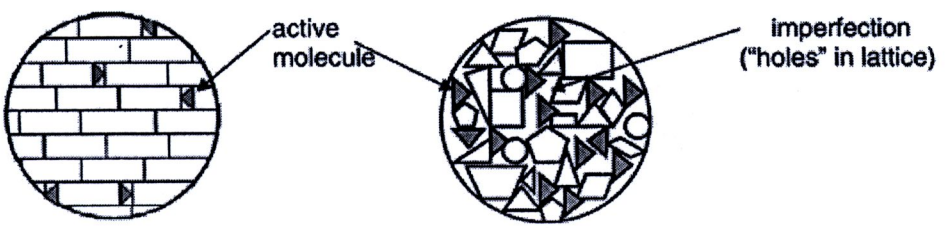


Figure 10: Perfect crystal in SLN comparable with a brick wall and structure with imperfections due to specially very different molecules in NLC type 1 (51)

Morphology and structure of nanostuctured lipid carrier (NLC)

The type of NLC depends on the different of solid lipid and amount of liquid lipid. The chemical nature of the active ingredient, the solubility of actives in the melt lipid matrix. The three incorporation models have been proposed: (Figure 11) (50-52).

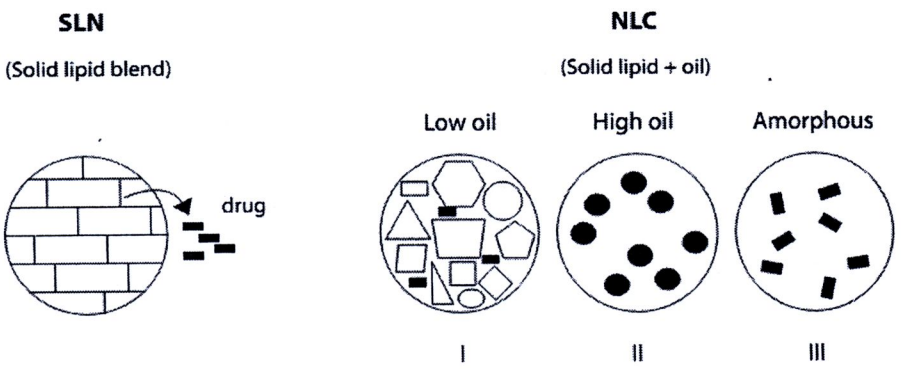


Figure 11: Different types of NLC: I – highly imperfect matrix; II – multiple O/F/W type; III – non-crystalline amorphous NLC (versus SLN with high crystallinity)(50)

1. The imperfect type

   In type I, solid lipids and liquid lipids (oils) are blended. The difference in the structures of the lipids and special requirements in the crystallization process lead to a highly disordered, imperfect lipid matrix structure offering space for drug molecules and amorphous clusters of drugs. In general, drug solubility is higher in liquid lipids than in solid lipids. Based on this, particles were produced with a high content of liquid lipids (oils).

2. The multiple type

   In type II, multiple oil-in-solid lipid-in-water dispersion, the drug can be accommodated in the solid, but at increased solubility in the oily parts of the lipid matrix. At high oil concentrations a miscibility gap of the two lipids (solid lipid plus oil) occurs during the cooling phase, leading to phase separation, that means precipitation of tiny oily nanocompartments.

3. The amorphous type

   In type III, lipids are mixed in a way that prevents them from crystallizing. The lipid matrix is solid, but not crystalline, it is in an amorphous state

Lipid nanoparticles preparation techniques (48, 52-53)

1. High shear homogenization and ultrasonication

High shear homogenization and ultrasonication are the dispersion technique which were initially used for the production of solid lipid nanodispersion and nanostructured lipid carrier. This technique are widespread and easy to handle. However, dispersion quality is often compromised by the presence of microparticles.

2. High pressure homogenization

High pressure homogenization (HPH) is a technology that has been applied for many years in various areas for the production of emulsions and suspensions. A distinct advantage of this technology is its easily for scaling up, even to very large volumes. Typical pressure for production of nanosuspension are 500-1500 bar. Most of the homogenizers used are based on the piston-gap principle, an alternative is the jet-stream technology. In the piston-gap homogenizer the macrosuspension coming from the sample container is forced to pass through a tiny gap (e.g., 10 um), particle diminution is affected by shear force, cativation and impaction. In jet-stream homogenizer the collision of two high-velocity stream leads to particle diminution mainly by impact forces.

Very high shear stress and cativation forces disrupt the particle down to the submicron range. Typical lipid contents are in the range 5-10% and represent no problem to the homogenizer. HPH leads to a product being relatively homogeneous in size, that is processing a higher physical stability of the aqueous dispersion. There are basically two different production methods.

2.1 Hot homogenization

Hot homogenization, the lipid is melted at approximately 5 °C to 10 °C above its melting point. The active-ingredient is dissolved of finely dispersed in the melt and then the active-ingredient containing lipid melt is dispersed by stirring in the hot surfactant solution. The pre-emulsion is homogenized applying a pressure between 500-1000 bar and two to three homogenization cycles. After the homogenization, a hot nanoemulsion is obtained; cooling leads to recrystallization of the lipid and formation of lipid nanoparticles. In general, higher temperatures result in lower particle sized due to the decreased viscosity of the inner phase. However, high temperatures may also increase the degradation rate of the drug and the carrier.

2.2 Cold homogenization

The cold homogenization is contrast the hot homogenization and carried out with the solid lipid. The lipid melt containing the active ingredient is cooled, effective temperature control and regulation is needed in order to ensure the unmolten state of the lipid due to the increase in temperature during homogenization. Cold homogenization has been developed to overcome the following three problems of the hot homogenization technique:

1. Temperature-induced drug degradation.
2. Drug distribution into the aqueous phase during homogenization.
3. Complexity of the crystallization step of the nanoemulsino leading to several modifications and/or supercooled melts.

The first prepared step was the same as in the hot homogenization procedure and included the solubilization or dispersing of the drug in the melt of the bulk lipid. The low temperatures increase the fragility of the lipid and favor. The solid lipid microparticles and dispersed in a cold aqueous surfactant solution. The resulted pre-suspension is homogenized in the solid state at or below room temperature by cooling the high pressure homogenizer. The sheer forces and cativation forces in the homogenizer are strong enough to break the microparticles directly into lipid nanoparticle. The method of cold homogenizatipm minimizes the thermal exposure of the sample, but it does not avoid due to the melting of the lipid/drug-mixture in the initial step. However, when compared with hot homogenization decreased larger particle sizes and broader size distribution.

3. Solvent emulsification/evaporation

Sjostrom and Bergenstahl described the production method to prepared nanoparticle dispersions by precipitation in o/w emulsions. The lipophilic material is dissolved in a water-immiscible organic solvent (e.g.cyclohexane) that is emulsified in an aqueous phase. Upon evaporation of the solvent a nanoparticle dispersion is formed by precipitation of the lipid in the aqueous medium. This solution was emulsified in an aqueous phase by HPH of ultrasonicator. The organic solvent was removed from the emulsion by evaporation under reduced pressure (40-60 mbar). The mean particle size depends on the concentration of the lipid in the organic phase.

The advantage of this procedure is the avoidance of any thermal stress such as HPH. However, disadvantage is use of organic solvents.

4. Microemulsion

Gasco and co-workers developed SLN preparation techniques which are based on the dilution of microemulsions. The microemulsions as two-phse systems compsed of an inner and outer phase (e.g. o/w-microemulsions). Which is typically composed of a low melting fatty acid, an emulsifier, co-emulsifier and water. The hot microemulsion is dispersed into cold water, leading to a breaking of a microemulsion and subsequent formation of an ultrafine nanoemulsion. The temperature gradient and the pH value fix the product quality in addition to the composition of the microemulsion. One disadvantage of this procedure is the dilution of the particle suspension by water, obtaining concentration usually below 1% of particle content. When processing to a final dosage form, a very large amount of water need to be removed.

2.4 Method validation (54)

1. Linearity

The linearity of an analytical procedure is its ability to obtain test result that are directly proportional to the concentration of analyte in the sample within a given range. A response vs. concentration plot is a visual representation of the relationship between the detector response and the analyte concentration for a linear relationship the mathematical equation for a straight line is used: $y = ax + b$. Where y is the concentration, a is the slope of the line, and b is y-intercept. For content assay, linearity should be performed between 80% and 120% of target concentration. A minimum of five concentrations is recommended for linearity studies. The linearity is often report as either the correlation coefficient, r, or the coefficient of determination, r^2 value, an intercept, and a slope. Linearity is generally assessed by calculating a linear regression coefficient (r) and, although on occation it might be acceptable to allow a lower figure, it would usually be expected that $r^2 > 0.999$.

2. Range

The range of an analytical method is the interval between the upper and lower concentration levels of analyte in the sample (including these concentrations) for which the method as written has been shown to be precision, accuracy, and linearity. The range method is validated by verifying that acceptable precision and accuracy is obtained by the analytical method when actual analysis of samples containing analyte is performed throughout the intervals of the range.

3. Accuracy

Accuracy is the measure of how close the method generates is to the "true" value. It is measure as the percent of analyte recovered by assay or by spiking samples in a blind study. To test accuracy for an UV method it is necessary to carry out the method on a sample of know composition. For the determination of method accuracy, the initial step is to generate a response vs. concentration curve using the standard material. The concentration range should ideally cover 50-150% of the target value. Accuracy should be established across the specified range (line of working range) of the analytical method. For assay of the drug substance, accuracy measurements are made by comparison of the results with the analysis of a standard reference material or to compare the results obtained from a second well-characterized independent method.

4. Precision

Precision is a measure of the ability of a method to generate the "same" results for multiple analyses of the same sample. Short-term precision (replicate analyses during the same day) is often refered to as the repeatability of a method. Long-term precision (over a week by the same laboratory or between laboratories) is called reproducibility. The precision of the method is often established over the anticipitated working range of the method and it often determined with be accuracy. The precision of an analysis is often expressed in terms of the relative standard deviation (%RSD) or the coefficient of variation (%CV). These values are calculated from the standard deviation, s, and mean, x, of the data set: %RSD = CV = 100 s/x.

5. Limit of detection

The limit of detection (L.O.D.) is the lowest concentration of the analyte that can be detected, but not necessary quatited under the stated experimental conditions.

For method to determine the signal-to-noise ratio by comparing test results from samples with known concentration of analyte with those of blank samples and establishes the lowest concentration at with analyte can be reliably detected. A signal-to-noise ratio of 2:1 or 3:1 is required. The signal is the peak height and the noise is the amplitude of short term noise in the baseline.

6. Limit of quantitation

The limit of quantitation (L.O.Q.) is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated experiment conditions of the method. This is parameter of the quantitive assays for low concentrations of compounds in samples matrix. The quantitation limit which is similar to the detection limit is expressed as the concentration of analyte in the sample, precision and accuracy of the measurements are also reported. It is equivalent to a higher concentration or weight than the detection limit and accordingly is usually taken as some predetermined multiple of the detection limit. This might typically be in the range 3-5 and almost always be equivalent to a signal-to-noise ratio of at least 10:1 for quantitation.