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Solid Lipid Nanoparticles Delivery Systems for Colon Cancer Chemotherapy: A Critical Review

[1]Basma Y. Al-Najjar, [2]Saad A. Hussain*,

[1]Department of Pharmaceutics, Faculty of Pharmacy, Al-Rafidain University College, Baghdad, Iraq; Email: alnajjarbasma@yahoo.com
[2]Department of Pharmacology and toxicology, Faculty of Pharmacy, Al-Rafidain University College, Baghdad, Iraq; Email: saad.hussain@ruc.edu.iq
*Address for correspondence: Professor Saad A. Hussain, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Al-Rafidain University College, Baghdad 10052, Iraq; E-mail: saad.hussain@ruc.edu.iq

ABSTRACT

Therapeutic approaches for targeting colon cancers are currently of significant importance important because of possible remission, reduction of cancer metastases, and increased success pf surgery or radiotherapy. Colonic drug delivery is becoming the increasingly preferred route for drug administration; however, it has many limitations that can be avoided by the use of proper carrier systems. Currently, many solid lipid nanoparticle systems (SLNs) were developed to enable the formulation of hydrophobic and poorly water-soluble drugs including those utilized as colonic drug delivery systems. They have many advantages including high bioavailability, high biocompatibility, cost-effectiveness, controlled release, physical stability, and safety, besides, avoidance of using organic solvents and capability of large-scale production and sterilization. Various studies provide important insights into the use of SLN delivery system to treat colon cancer. However, there is a general lack of data from clinical trials and further studies are recommended to evaluate SLNs in animal models.

Keywords: Solid lipid nanoparticles, colorectal cancer, colon drug delivery, chemotherapy

Correspondence:
[2]Saad A. Hussain*
[2]Department of Pharmacology and toxicology, Faculty of Pharmacy, Al-Rafidain University College, Baghdad, Iraq; Email: saad.hussain@ruc.edu.iq
*Address for correspondence: Professor Saad A. Hussain, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Al-Rafidain University College, Baghdad 10052, Iraq; E-mail: saad.hussain@ruc.edu.iq

INTRODUCTION

Cancer is a global concern, accounted for 8.2 million deaths around the world in 2012, and may increase to 10.05 million by 2020.[1] Thousands of people have been diagnosed with colorectal cancer (CRC) and accounted for 693,881 deaths in 2012.[1] It is the third most familiar cancer and accounts for about 9% of the primary cause of cancer-related deaths worldwide.[2,3] The survival rate in CRC patients depends on early diagnosis, and the preferred treatment is surgical resection followed by systemic chemotherapy or chemoradiation.[4] Colonic drug delivery is becoming the increasingly preferred route for drug administration. The conditions of the gastrointestinal tract (GIT) have been used to deliver drugs via modification and manipulation of oral dosage forms.[5] The colon has been investigated widely for the local treatment of many intestinal diseases such as Crohn's disease, ulcerative colitis, irritable bowel syndrome, lymphoma of the colon, and CRC.[6] Also, the colon has been widely investigated for the treatment of immunodeficiency virus (HIV),[7] delivery of proteins and peptides,[8-10] delivery of antihypertensive and anti-asthmatic drugs.[11] Although the colon as a drug target has many advantages such as moderate pH, fewer acids and enzymes, adequate bioavailability of poorly absorbed drugs, longer residence time, reduced dosage frequency and enhanced patient compliance,[12] it has serious limitations including poor water solubility of many chemotherapeutic agents, presence of microflora, low bioavailability of some drugs due to non-specific binding, presence of mucus and food residues and incomplete drug release,[11] tight junctions, lack of villi, and low blood flow.[12,13] The suggested approach to avoid these defects includes the development of suitable alternative carrier systems. The drug carrier system, so-called solid lipid nanoparticle (SLN) was developed to suit the formulation of hydrophobic and poorly water-soluble drugs.[14] Researchers have shown an increased interest in the SLN as a colonic drug delivery system.[13,15-17] SLNs are colloidal drug carriers and have been used widely as drug delivery systems in the treatment of a variety of diseases and as alternative carriers to nanoparticles.[18] SLN can improve the targeting and tissue distribution of many drugs[19] and also can improve the tissue distribution of drugs and enhance their bioavailability.[20]

The formulation of SLNs utilizes the advantages of other drug carrier systems and avoids the disadvantages of many other colloidal carriers. The proposed advantages include many unique properties of SLNs that make them suitable drug delivery systems to treat cancer. This can be attributed to high bioavailability, high biocompatibility, cost-effectiveness, drug targeting, controlled release of the active ingredient, physical stability, biosafety of the carrier, good tolerability, high drug payload, flexible routes of administration (e.g., intravenous, oral, transdermal and pulmonary routes), avoidance of using organic solvents, and capability of large scale production and sterilization.[21,14] However,

one of the problems associated with SLN is its uptake by the reticuloendothelial system (RES). Thus, several studies have been conducted to overcome this problem such as coating the SLNs with hydrophilic polymers. The techniques used to enhance the biodistribution of SLN and reduce the phagocytic uptake are coating with chitosan,[22] PEG,[23,24], and polyvinyl alcohol.[25] Moreover, the coating of the SLNs has been confirmed to enhance their stability and improve transport through the mucous membranes.[26-28] Garcia-Fuentes *et al* have shown that coating oral calcitonin incorporated into SLN with PEG might affect the surface association, and thus the immediate release of the peptide.[29] Ngwuluka *et al* showed that metformin-loaded SLNs were migrated and accumulated in the colon tumor preventing its proliferation.[30] Cholesteryl butyrate SLN (Cholbut SLN) inhibits cancer cell adhesion which is critical for metastasis.[16] The success of such formulations is expected to motivate the pharmaceutical industry to invest further in the development of more SLN formulations to treat cancer. In this review, the studies of SLNs formulation for treating CRC are summarized.

SLN AS DRUG DELIVER SYSTEM

Despite the advances in anticancer drugs, chemotherapy presents poor side effects and safety profiles. These problems include susceptibility to induce drug resistance, high toxicity, poor specificity in terms of both drug biodistribution and pharmacology at the cellular level. Hence, only a small fraction of these drugs reach the tumor site.[31] In this regard, it has been suggested that the SLN drug delivery system may offer promise to enhance the effectiveness and safety of the conventional forms of cancer chemotherapy. SLN is a properly designed nanoparticle system that can offer numerous advantages; thus, it is emerging in the field of chemotherapy drug delivery to achieve passive tumor targeting and minimize the associated problems.

SLNs or the "lipospheres" are delivery systems for lipophilic drugs to ensure progress in drug therapy (Figure 1). They are FDA approved particles of submicron size in the range of 10-1000 nm[14] and made from natural or artificial solid lipids that remain solid at body and room temperature.[32,33] The new generations of SLNs (e.g., lipid-drug conjugate nanoparticles, polymer-lipid hybrid nanoparticles) can incorporate ionic and hydrophilic compounds[34] that can be administered via oral,[35] ocular,[36,37] pulmonary,[38] nasal,[39] dermal,[40-42] intravenous,[43] intramuscular,[44] and subcutaneous[45] routes of administration. These delivery systems permit localized and controlled release of the drug to specific sites.

Structurally, SLNs are composed of physiological materials that make them easy to modify and to be delivered to the targeted site and reduced toxicity.[46,47] Various lipids are used to prepare the SLN system such as mono-, di-, or triglycerides, glyceride mixtures or waxes, and stabilized by the ionic or non-ionic surfactant(s).[34] Also, many emulsifiers such as polysorbate 80, lecithin, sodium glycolate, and poloxamer 188 are compatible with the SLN formulations.[48] The surface physicochemical properties of SLNs can be easily manipulated to enhance the biodistribution and targeting of the drug to the tumor sites.[21] The methods of preparation and characterization of SLN preparations are detailed in many review articles.[21,49] Some approaches aimed to overcome the most significant drawbacks of the cytotoxic drug delivery including low drug release, avoidance of RES clearance, and the incorporation of hydrophilic anticancer agents.[34] Coating SLNs with polyethylene glycol (PEG) reduces the rapid uptake of SLNs by the spleen or liver and increases their circulation time.[50,51] Additionally, it has been observed that coating with PEG enhances SLNs' stability in simulated body fluids and increases their permeation ability across the epithelium.[52] The proposed advantages of SLNs are shown in Table 1.[21,14,50]

TYPES OF SLN SYSTEMS

Based on the pattern of drug incorporation in the lipid matrix, SLNs are classified into solid solution type, drug-enriched core, and drug-enriched shell (Figure 2). In the solid solution type, the drug is molecularly distributed within the lipid matrix and strong interaction with the lipid moiety.[54,55] They are traditionally formulated by a cold homogenization method without using a surfactant or solubilizing agent. In the case of drug-enriched core type, the drug concentrates within the core and precipitates in the melted lipid after cooling the nanoemulsion. Moreover, further reduction of the dispersion temperature results in the recrystallization of the lipid and enveloping the drug as a coating layer.[49] In drug enriched shell type, when the recrystallization temperature of the lipid is achieved, a solid lipid core forms in the center. After further cooling of the melt, the drug disperses in the liquid external layer of the SLN.[56,24]

Additionally, a new generation of SLN has been developed for better drug incorporation. Nanostructured lipid carriers (NLC) are modified SLN carriers characterized by the inclusion of liquid lipids into a solid lipid phase. The NLC type was developed to overcome some drawbacks of SLNs such as enhanced drug payload and the inhibition of drug discharge during storage. However, they combine all the benefits of SLNs.[57] There are three models of NLC: imperfect, amorphous, and multiple models.[56-58] Imperfect NLCs consist of chemically different oils mixed with solid lipid matrix. Such combination results in imperfections in the crystal shape of the lipid structure. Therefore, the distances between fatty acid molecules increased with enhanced drug incorporation within the lipid matrix and inhibition of drug expulsion by the crystallization process throughout storage.[58] Finally, multiple NCLs consist of numerous oils in fat in water (O/F/W). The high amount of oil prevents drug expulsion because lipophilic drugs are less soluble in solid lipids.[59] Moreover, novel strategies have been developed to incorporate hydrophilic drugs such as "lipid-drug

conjugate nanoparticles" (LDC).[60] In such a formula, an insoluble drug-lipid conjugate is formulated either by salt formation or by covalent linking. For nanoparticle formulation, an aqueous surfactant is blended with the LDC bulk by the homogenization method. However, LDC nanoparticle has not been yet investigated as a delivery system for chemotherapeutic agents.[34] In this regard, the 'polymer-lipid hybrid nanoparticles' (PLN) is another novel technology[61,62] that employs complexation of ionic polymers with drugs to deliver chemosensitizers and ionic anticancer drugs.[63-65] Li *et al* have formulated PLN-verapamil HCl complex using a couple of compatible polymers and incorporated them into a lipid.[63] The entrapment efficiency was increased to 90% and partition of verapamil HCl in the lipid matrix was 33%. It has also been found that the entrapment efficiency of ionic drugs (e.g., verapamil HCl and doxorubicin HCl) was more than 80%.[66] Stealth SLN is a polymer-coated drug delivery system, where hydrophilic polymers such as poloxamines, PEG, and poloxamers are used to coat the SLN. In this type, the drug carrier is known as a long-circulating drug delivery system because of its ability to resist RES clearance.[67,68] Few reports described the role of stealth SLN. In 2002, Zara *et al* prepared stealth SLN of paclitaxel and doxorubicin by coating nanoparticles with PEG 2000. It has been shown that the coating agent influences the clearance rates by the RES and the physicochemical properties of SLN.[69] Hence this may affect the safety, stability, and performance of the SLN system.[69] Previous studies have reported the effect of the stealth coating agent on the biodistribution of SLN *in vivo*.[69-71]

DRUG INCORPORATION AND RELEASE

The incorporation of drugs in the carrier system requires the localization of drug in the solid lipid matrix. Drug loading might result in strong changes in the SLN characteristics (lipid structural modification, particle size distribution, zeta potential, entrapment efficiency, etc.). Based on X-ray, DSC, ESR, and NMR techniques, few data are available on the localization and the physical state of the drug molecule during the design and characterization of SLN formulas [21]. In one study, Bunjes used NMR to monitor the physical state of diazepam, where the NMR spectra indicate high mobility of the drug.[72] Meanwhile, Ahlin *et al* reported that a high percentage of lipophilic nitroxides is localized in the polar environment and the distribution process occurs quickly.[73] In another study, it was observed that acyclovir is not molecularly dissolved in the lipid matrix.[74]

Regarding the rate of release from this carrier system, burst release is observed from the SLN, where cold and hot homogenization produced an SLN system that releases etomidate and tetracaine immediately.[75] In contrast, the release profile of prednisolone was retarded by an appropriate selection of the homogenization temperature.[76] Hence, the rate of release of a drug from the SLN, the system could be affected by several factors such as the nature and composition of the lipid matrix,[77,78] surfactants,[47] and technical factors.[43,50] Also, the release kinetics depend on the release conditions such as release medium, sink, or non-sink conditions, etc.[21] Matrix degradation by lipase depends on the emulsifier and the lipid. Olbrich and Müller reported that the release and particle degradation can be modified by the balance between the surfactant and steric stabilizers because lipases need a lipid interface for enzyme activation.[79] Thus, these enzymes did not easily recognize PEG-coated SLN.

STABILITY OF THE FORMULATIONS DURING STORAGE

The SLN formulations should be stored at 4˚C and their stability is better than formulations stored at room temperature.[80,81] Hence, it is recommended to store SLN formulations in refrigerators.[35,39] Factors such as temperature and light should be taken into consideration in SLN stability during storage.[47] It has been reported that SLNs made from miscellaneous lipids enable higher drug loading capacity and stop drug discharging from the SLN matrix and prevent its crystallization during storage.[82,83,50,43,84] SLN cannot be regarded as colloidal dispersions with solidified droplets but it does have colloidal structures such as liposomes and micelles, which contribute to the stability problems of the SLN systems.[21] The major problem of storage stability is the gelation phenomena represented as an increase in particle size and drug expulsion from the lipid carrier. The conversion of the lipid melt to lipid crystals leads to an increase in surface area of the particles and decrease the loading capacity of the lipid, and hence decrease stability. There is a strong relationship between modification of the lipid structure, gelation, particle aggregation, and drug expulsion.[21]

SAFETY OF THE SLN FORMULATIONS

SLNs consist of physiological materials; therefore, they are highly tolerated by humans. Müller *et al* reported that SLNs were the least cytotoxic formulations in comparison to other polymeric nanoparticles.[85] Also, the experimental results of Müller *et al* (1996) showed that SLNs were less toxic than butyl cyanoacrylate particles and polylactide nanoparticles.[86] Furthermore, the finding of Madureira *et al* confirmed the *in vivo* and *in vitro* safety of the SLNs.[87] Other excipients such as surfactants and emulsifying agents that influence the safety profile of SLN formulations should be considered. Two cationic SLNs were prepared using two different cationic surfactants (CTAB (cetyltrimethylammonium bromide) and DDAB (dimethyl-dioctadecyl ammonium bromide)). It has been found using five different human cell lines that DDAB SLNs produced much lower toxicity than CTAB-SLNs.[88] No data were found about the safety of anticancer agents loaded on the SLNs.

SLNs DRUG DELIVERY SYSTEMS FOR TREATMENT OF COLON CANCERS

Several studies have investigated the SLNs as a drug delivery carrier to treat CRC. In this regard, Patel *et al* formulated SLNs loaded 5-fluorouracil (5-FU) by using a temperature-modulated solidification method.[89] It has

been found that *in vitro* drug release was 80% of the encapsulated drug. Also, in Caco-2 cell cultures, 5-FU-containing SLNs showed a concentration-dependent reduction in cell viability. Kamel *et al* confirmed the success of their combined formulation.[90] Minelli *et al* investigated cholesteryl butyrate solid lipid nanoparticles (Cholbut SLN) as a colon drug delivery system of an anti-cancer agent.[16] The results of the study confirmed that Cholbut SLN could be an efficient anti-metastatic agent. Moreover, initial *in vivo* toxicity studies using the intravenous route did not reveal any toxicity on normal cells of mice model. Rajpoot and Jain developed oxaliplatin containing SLNs (OPSLNs) and oxaliplatin SLNs conjugated with folic acid (OPSLNFs) to target CRC.[91] The drug encapsulated in OPSLNFs showed higher cytotoxic activity in HT-29 cells than in OPSLNs. Thus, this novel system can be a potential strategy for the treatment of CRC. Similarly, Serpe *et al* evaluated the cytotoxicity of SLN loaded doxorubicin, paclitaxel, and cholesteryl butyrate (Cholbut) on colorectal cancer cells model (HT-29 cell line).[17] It has been confirmed that SLN formulations loaded doxorubicin and Cholbut had better chemotherapeutic influence than conventional formulations. Ngwuluka *et al* suggested metformin as the anticancer agent and SLN as a delivery system for CRC.[30] The results proposed that SLNs carrying metformin will accumulate within the tumor, inhibit its spread, and hence limit tumor growth. A broader perspective has been adopted by Kulbacka *et al* who applied the electroporation technique to increase the permeability of cell membranes and improve drug delivery.[92] Many SLNs loaded with cytotoxic agents are prepared using the solvent diffusion method and evaluated in hamster ovarian fibroblastoid (CHO-K1) and human colon adenocarcinoma (LoVo). The results suggested that these formulations, which improved by electropermeabilization, can be a potential chemotherapeutic option. Shen *et al* reported efficient cytotoxicity of an orally administered delivery system consisted of SLNs loaded with doxorubicin and superparamagnetic iron oxide nanoparticles (SPIONs).[93]

In 2016, Escalona *et al* developed a formulation of iron oxide loaded magnetic SLNs; in vitro evaluation using magnetic responsiveness, hemocompatibility, and hyperthermia showed a reduction in cell viability.[94] Additionally, Gumireddy *et al* encapsulated curcumin and resveratrol within SLNs with/without 2-Hydroxypropyl β-cyclodextrin (HPβCD) embedded in Gelucire 50/13;[95] the results of the *in vitro* studies showed that curcumin and resveratrol formulations were physically stable with improved the drug release. Moreover, formulations consisting of omega-3 polyunsaturated fatty acids loaded resveratrol-based SLNs; Serini *et al* improve the uptake and inhibit cancer growth.[96] Yassin *et al* utilized a double emulsion method (w/o/w) to formulate SLNs encapsulated 5-FU using triglyceride esters such as Dynasan™118 or Dynasan™114 with soya lecithin;[15] the results illustrate that SLNs can spread the drug in the colon for a long period and cover all the cancer area. In 2019, Campos *et al* reported a convenient procedure to formulate non-steroidal anti-inflammatory drugs (NSAIDs), such as nimesulide in SLNs.[97] Similarly, Spada *et al* (2012) formulate diclofenac sodium- loaded SLNs with a size range of 300-600 nm using the oil/water hot homogenization method.[13] They have identified the influence of hydroxypropyl-β-cyclodextrin, Compritol ATO888, and cryoprotectant on drug permeation rate and drug release from the delivery system to the colon. Fan *et al* modified SLN loaded salmon calcitonin with two types of peptide ligand: IRQRRRR (IRQ) and CSKSSDYQC (CSK) to enhance penetration of salmon calcitonin into the Caco-2/HT29-MTX cell line.[98] The bioavailability of IRQ-SLNs and CSK-SLNs increased to 1.98-fold and 2.45-fold respectively, revealing the usefulness of peptide ligands to improve the bioavailability of protein drugs through the intestinal mucosa. In addition to the available publications, the registered patents that concerned with this topic are thoroughly reviewed by Battaglia and Ugazia in 2019.[99] There is a general lack of outcomes from the clinical trials and further studies are recommended to evaluate SLNs in animal models of CRC.

CONCLUSION

The SLN delivery systems as carriers of cytotoxic agents to treat CRC represent a promising strategy for effective targeting of colon malignancies. However, further experimental and clinical studies are needed to make data available for effective clinical use.

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Conflicts of interest

There are no conflicts of interest.

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Table 1. Advantages of solid lipid nanoparticles delivery systems

Advantages of SLNs
Incorporation of hydrophilic and hydrophobic drugs
High bioavailability
High biocompatibility
High drug payload
Controlled release
Physical stability of SLN
Protection of the labile drug from degradation
Drug targeting and controlled release
Excellent tolerability
Not toxic
Avoidance of using organic solvents
Easy preparation
Easy scaling up
No problems concerning sterilization
Fewer drug leakage and storage problems compared to liposomes.[49]
No reported significant acidity and toxicity.[53]

Table 2. In vitro and in vivo studies of SLNs formulations for treating colon cancer

Anti-cancer agent	**SLN used**	**Subject**	**Reference**
5-fluorouracil (5-FU)	Glyceryl monostearate (GMS) nanoparticles	In vitro Human colorectal adenocarcinoma (Caco-2) cell culture	Patel et al., 2014 [89]
5-FU	Chitosan-Coated Cinnamon/Oregano-loaded solid lipid nanoparticles	Cell Culture Human colon carcinoma (HCT 116) cells	Kamel et al., 2017 [90]
butyrate	Cholesteryl butyrate solid lipid nanoparticles	Cancer cell lines derived from human colon–rectum, melanoma, prostate and breast cancers	Minelli et al., 2012 [16]
OP	OPSLNFs	HT-29 cell line	Rajpoot and Jain, 2018 [91]
chol-but, doxorubicin, and paclitaxel	SLNs	HT-29 cell line	Serpe et al., 2004 [17]
Metformin	SLNs	---	Ngwuluka et al., 2017 [30]
cyanine-type IR-780	SLNs co-loaded with BAI or FIS	LoVo and CHO-K1 cell lines	Kulbacka et al., 2016 [92]
Doxorubicin and SPIONs	DFSLNs	CT26 colon cancer cells Mice	Shen et al., 2019 [93]
Fe_3O_4	MSLNs	the human HT29 colon adenocarcinoma cell line	Escalona et al., 2016 [94]
Curcumin and resveratrol	HPβCD (CRG-CD) and (CRG) SLNs	colorectal cancer cell line (HCT-116)	Gumireddy et al., 2019 [95]
omega-3 PUFA	SLNs	HT-29 CRC cells	Serini et al., 2018 [96]
5-FU	SLNs	---	Yassin et al., 2010 [15]
nimesulide	SLNs	Caco-2 cell line	Campos et al., 2019 [97]
diclofenac sodium	SLNs	Caco2 cells	Spada et al., 2012 [13]
salmon calcitonin	SLNs	Caco-2/HT29-MTX cell line	Fan et al., 2014 [98]

Figure Legend:

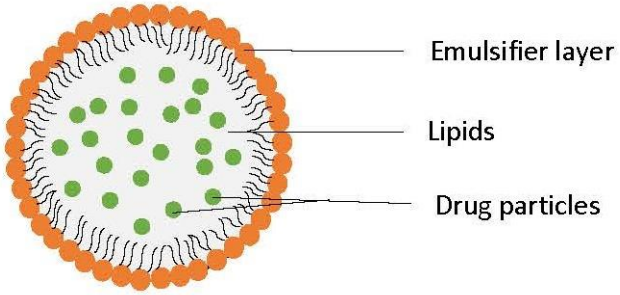

Figure 1: Structure of the Solid-lipid Nanoparticle (SLN).

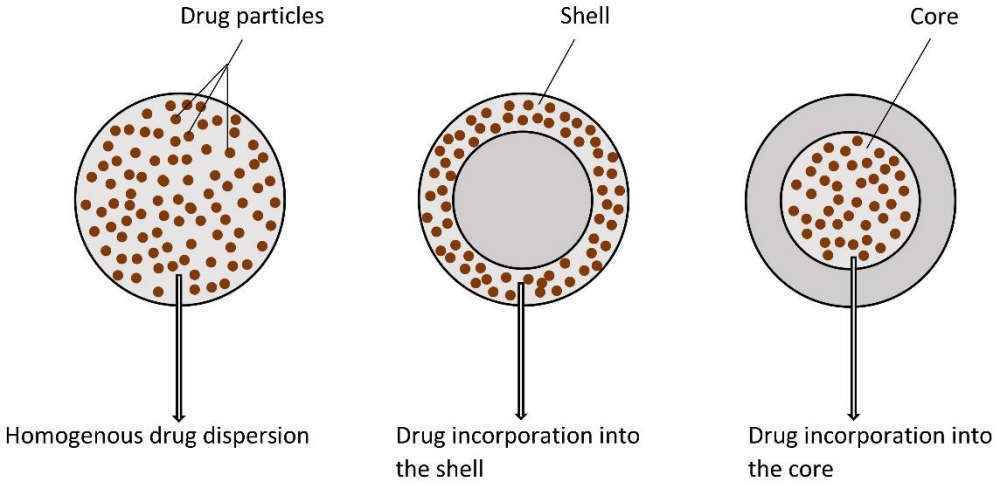

Figure 2: Schematic Presentation of Drug Incorporation Types in SLN Systems.