NANO-BASED DRUG DELIVERY SYSTEM

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3.1. INTRODUCTION

The conventional application of drugs presents challenging problems in the treatment of many diseases for example: therapeutic effectiveness, poor biodistribution, stability, solubility and intestinal absorption, lack of selectivity, side effects and fluctuations in plasma concentration [1-3]. Drug delivery systems (DDS) have been designed to overcome these limitations and drawbacks. DDS provide specific drug targeting and delivery, minimizing undesirable side effects, using lower doses of drug and protecting the drug from degradation. Recent developments in nanotechnology have indicated that nanoparticles (ranging in size from 1–1000 nm) can be successfully used as drug carriers with optimized physicochemical and biological properties (small size, increased drug accumulation and therapeutic effects, ability to cross cell or tissue barriers, controlled drug release, *etc.*). The use of nanoparticles as drug carriers can play an important role in eliminating the challenging problems associated with conventional drugs used for the treatment of many chronic diseases such as cancer, asthma, hypertension, human immunodeficiency virus (HIV), and diabetes [4-10].

Polymeric nanocarriers, dendrimers, polymeric micelles, liposomes, solid lipids nanoparticles (SLNs), metallic nanoparticles (magnetic, gold), carbon nanotubes, nanospheres, nanocapsules and nanogels are examples of nano-based drug delivery systems that are currently under research and development [11-15]. Some of them, especially cancer treatments, have been clinically used and approved by the Food and Drug Administration (FDA).

The use of drug delivery systems in cancer treatment consists of two strategies: passive and active targeting (Figure 1). Each targeting strategy aims to increase the accumulation of the drug within the tumor tissue while reducing the side effects [16,17].

Figure 1. The main characteristics of passive and active targeting

In this chapter, the production techniques, the characterization methods and applications particularly in cancer treatment of certain drug delivery systems including dendrimers, polymeric micelles, liposomes and SLNs will be discussed.

3.2. POLYMERIC MICELLES

Polymeric micelles that consist of amphiphilic co-polymers in the form of core/shell nanostructures can be designed to ensure selective delivery of drugs, especially water insoluble drugs, to their subcellular targets. Polymeric micelles comprise of two structures: an inner core and an outer shell. The hydrophilic shell of the copolymer consists of hydrophilic non-biodegradable polymers (such as poly(ethylene oxide) (PEO), poly(*N*-isopropylacrylamide) (PNIPA), poly(alkylacrylic acid), and poly(ethylene glycol) (PEG)) and sustains stability in the aqueous medium enabling interactions with plasmatic proteins and cell membranes. The hydrophobic core of the micelles encapsulates various non-polar drugs (such as paclitaxel, doxorubicin, tamoxifen, camptothecin, porphyrins, *etc.*) and provides their controlled release. The hydrophobic core may consist of biodegradable, non-biodegradable or poorly biodegradable water-soluble polymers such as poly(propylene oxide) (PPO), poly(β -benzyl-L-aspartate) (PBLA) [17], poly(L-lactic acid) (PLLA), poly(lactic-co-glycolic acid) (PLGA), poly(ϵ -caprolactone) (PCL) or poly(aspartic acid) (PASP), polystyrene (PST), poly(methyl methacrylate) (PMMA), and various polyacrylates [18-28].

Polymeric micelles range in size from 10–100 nm, each type having a narrow size distribution. This narrow size range is the most important property of polymeric micelles, ensuring high stability, sterility and long term circulation in the bloodstream. It is important to point out that polymer chains in the inner core of polymeric micelles affect their stability and it is a key point in drug delivery to avoid interaction of single polymer chains with the loading drug.

The structure of polymeric micelles plays an important role because it consists of different polymer and surfactant according to chosen applications [26-31].

Table 1. The advantages and disadvantages of polymeric micelles [18-31]

Advantages of Polymeric Micelles	Disadvantages of Polymeric Micelles
Small size (10–100 nm)	Difficult polymer synthesis
High structural stability (lipid-core micelles formed by self-assembled amphiphilic polymers)	Not stable (normal self-assembled polymeric micelles)
Large amount of drug loading and sustained drug release	No universal incorporation method
High water solubility when hydrophobic drugs loaded	Possible chronic liver toxicity due to slow metabolic process
Low toxicity	Sometimes toxic side effects exist due to a longer period impacts than free drug
Modification by various chemical species	Not produced in a large industrial scale
Protection of encapsulated drugs from degradation and metabolism	Only few polymeric micelles are approved by FDA and used in clinical applications

3.2.1. Production and drug incorporation into polymeric micelles

The production of polymeric micelles can be separated into two techniques: direct or indirect. What technique will be selected relies on the simple equilibration of the drug and the type of polymers. While the direct method includes the direct solubilization of the amphiphile in aqueous medium followed by encapsulation of the drug, the indirect method depends on the use of water-miscible organic solvents (*e.g.*, acetone, dimethylacetamide) to co-solubilize the co-polymer and the drug and then to separate organic solvents by evaporation or dialysis. However, these methods have been improved in recent studies to increase encapsulation capacity and stability and to control drug release kinetics [32-36].

Drugs can be loaded into micelles by chemical conjugation or by physical entrapment through dialysis or emulsification techniques. The drug loading procedure may influence the entrapment efficiency and the distribution of a drug within the polymeric micelles. Chemical conjugation enables the drug to be incorporated with the hydrophobic polymer by a covalent bond, such as an amide bond. Thus the entrapped drug is protected from enzymatic cleavage due to non-hydrolysis of chemical bonds. The dialysis or oil-in-water emulsion procedure is used in the physical entrapment of drugs. This technique depends

on the removal of the organic solvents used. Whereas soluble (*e.g.*, ethanol, *N-N*-dimethylformamide), non-toxic solvents are used in the dialysis method, water-insoluble solvents (*e.g.*, chloroform, chlorinated) are used in the oil-in-water emulsion method [26,37-42].

3.2.2. Characterization of polymeric micelles

Micelles are characterized by turbidity measurement, critical micelle concentration (CMC) and aggregate size. CMC and CMC ratio along with other components are very important for polymeric micelles. In order to determine CMC, there are various techniques, for example interfacial tension, conductivity, osmotic pressure, *etc.* However, these methods are not convenient for polymeric micelles because of their low CMC values.

In order to measure the size of micelles, dynamic light scattering is a very useful technique, because function of concentration is used as an indicator of the onset of micellization [43,44].

In addition, the hydrodynamic diameter of polymeric micelles can be determined by using dynamic light scattering (DLS). But, since the DLS method is not effective for measuring multimodal size distributions, atomic force microscopy is used for this purpose. Furthermore, DLS instruments often use a detection angle of 90° and this optical configuration may not be sensitive enough for the successful measurement of micelle surfactants [45-47].

The size of micelles is dependent on the encapsulation of the drug. That is, the process of surrounding of the drug in the hydrophobic core causes an increase in the size of the micelles. The size of micelles is generally measured using transmission electron microscopy (TEM) or scanning electron microscopy (SEM) [48,49].

The zeta potential (ZP) of micelles is measured using a zetasizer. ZP values give information about the surface charge of micelles and therefore their aggregation status. The zetasizer machine automatically calculates ZP values in the analyzer using the following Smoluchowski equation:

$$m = \frac{e \cdot z}{h}$$

where *z* is the ZP, *m* the mobility, *e* the dielectric constant and *h* the absolute viscosity of the electrolyte solution [50].

Stability testing of micelles consists of analytical measurements of drug content. Firstly, lyophilized and aqueous solutions of drug-loaded micelles are weighed under fixed and constant conditions and after then in order to determine the drug content, samples are taken at the beginning and at the end of particular time. Content of drug is measured spectrophotometrically and UV spectra are recorded [33,51].

3.2.3. Applications of polymeric micelles in cancer treatment

Different chemotherapeutic agent-loaded polymeric micelles consisting of different polymers have been studied in recent years. The developed formulations are potential drug delivery systems for the loaded agents because they increase the therapeutic efficacy and the accumulation of the drug within the tumor tissue, reduce systematic toxicity and demonstrate excellent multi drug resistant (MDR)-overcoming ability for anticancer drug delivery. These studies are summarized in Table 2 and 3 [52].

Table 2. Clinical trials of doxorubicin, paclitaxel, SN-38, DACH-platin, cisplatin, and epirubicin-loaded polymeric micelles

Product Name	Incorporation drug	Treatment	Trial phase	References
NK-911	Doxorubicin	Solid tumors	II	[53]
SP-1049C	Doxorubicin	Metastatic adenocarcinoma of the upper gastrointestinal tract, breast cancer and NSCLC	II/III	[54]
NK-105	Paclitaxel	Breast cancer	III	[55,56]
		Stomach cancer	II	
Genexol-PM	Paclitaxel	Breast, lung, pancreatic, recurrent breast cancer	Approved	[57-60]
NK-012	SN-38	Breast cancer	II	[61]
NC-4016	DACH-platin	Colorectal cancer (targeted future indication)	I	[62]
NC-6004	Cisplatin	Solid tumors	I/II	[53]
Nanoplatin®	Cisplatin	Solid tumors and NSCLC	I	[62]
		Pancreatic cancer	III	
NC-6300/ k-912	Epirubicin	Breast cancer (targeted future indication)	I	[62]

Table 3. Summary of incorporated drugs, polymer types and treatment of polymeric micelles in published papers

Incorporation drug	Polymer Type(s)	Treatment	References
Doxorubicin (Dox)/wortmannin			
Dox/17-hydroxethylamino-17-demethoxygeldanamycin	PEG- β -p(Asp-Hyd)a	MCF-7 breast cancer	[63]
	Alginate- γ -poly(<i>N</i> -isopropylacrylamide) (PNIPAAm)	<i>in vivo</i>	[64]
	PEG-poly(phosphoester)	MDA-MB-231 tumor model	[65]
	(PCL)2-[poly(2-(diethylamino)ethyl methacrylate- β -PEG methyl ether methacrylate]2 [(PCL)2(PDEA- β -PPEGMA)2]	HepG2 cells	[66]
	PCL β -poly(2-(diethylamino)ethyl methacrylate)- β -PEG methyl ether methacrylate) (4/6AS-PCL- β -PDEAEMA- β -PPEGMA)	HepG2 cells	[67]
Dox	pH-sensitive polyHis- β -PEG micelles	MDA_MB_231 breast cancer tumor model	[68]
	Methoxy poly(ethylene glycol)- β -poly(ϵ -caprolactone) copolymer with citraconic amide	HepG2 and 4T1 cancer cells	[69]
	PCL63- β -PNVP90 block copolymer	K-562, JE6.1 and Raji and mice lymphoma cells (Dalton's lymphoma, DL)	[70]
	PCL/PEG copolymer	Colon cancer mouse model	[71]
	Monomethoxy-PEG-S-S-hexadecyl (mPEG-S-S-C16)	HeLa cells	[72]
Parthenolide (PTL)-Dox	Poly(styrene-alt-maleic anhydride)- β -poly(styrene)	MDR ovarian cancer cells	[73]

	(PSMA- β -PS) and poly(styrene-alt-maleic anhydride)- β -poly(butyl acrylate) (PSMA- β -PBA)		
Dox and gene Msurvivin T34A	MPEG-PCL- γ -PEI labeled with (99)Tc	B16F10 tumor model and lung metastasis model	[74]
Dox/Etoposide	PEG- β -p(γ -benzyl L-glutamate)	CT-26 murine colorectal cancer	[75]
Mitoxantrone (MTX) and Dox	Polyethylene oxide- β -poly(acrylic acid) polymer	A549 NSCLC	[76]
Dox/Paclitaxel (PTX)	PEG- β -PLGA	A549 NSCLC, B16 mouse melanoma, HepG2 liver cancers	[77]
Elacridar- PTX	PEG-PE	PTX resistance in two cancer cell lines	[78]
PTX/17-AAG/rapamycin	PEG- β -PLA	A549 NSCLC, MDA-MB-231	[79]
Cisplatin (CDDP)-PTX	PEG, glutamic acid and phenylalanine (PEG-PGlu-PPh _e)	A2780 ovarian cancer cells	[80]
PTX /17-AAG/Etoposide	Poly(2-methyl-2-oxazoline)	MCF-7 and MDA-MB-231 breast cancer	[81]
PTX /17-AAG/Bortezomib	- β -poly(2-butyl-2-oxazoline)- β -poly(2-methyl-2-oxazoline)	PC3 prostate cancer, HepG2 liver cancer	[81]
PTX /cyclopamine/gossypol	PEG- β -PCL	ES-2-luc and SKOV-3-luc ovarian cancers	[82]
PTX /17-AAG	PEG-DSPE/TPGS	SKOV-3 ovarian cancer	[83]
PTX / CDDP	PEG- β -poly-(glutamic acid)- β -poly(phenylalanine)	A2780 ovarian cancer	[80]
PTX /17-AAG/rapamycin	PLGA- β -PEG- β -PLGA	ES-2-luc ovarian cancer	[84]
PTX -Lapatinib (LPT)	Pluronic F127	T-47D cell line	[85]
PTX- Curcumin (CUR)	PEG2000-PE and vitamin E	NCI-ADR-RES and SK-OV-3TR	[78]

		ovarian cells	
PTX	PEG750-p(CL-co-TMC) copolymer	CT26 colon carcinoma cells and <i>in vivo</i>	[86]
	Tocopherol succinate-chitosan-PEG-folic acid	4T1 mice breast cancer cell line	[87]
	Deoxycholic acid-modified chitooligosaccharide (COS-DOCA) and methoxy PEG - polylactide copolymer (mPEG-PDLLA)	A549 lung xenograft model	[88]
	PEG- β -poly(mono-2,4,6-trimethoxy benzylidene-pentaerythritol carbonate-co-acryloyl carbonate) (PEG- β -P(TMBPEC-co-AC)) and Gal-PEG- β -PCL (Gal-PEG- β -PCL)	HepG2	[89]
	PEG block-poly(2-methyl-acrylic acid 2-methoxy-5-methyl-[1,3]dioxin-5-ylmethyl ester) (PEG- β -PMME)	A549	[90]
	PEG-distearoylphosphatidylethanolamine (PEG-DSPE)	Hep-2, U-937 leukemic cancer cell line	[91]
	Hyaluronic acid-octadecyl (HA-C18) and Folate (FA) conjugated	MCF-7 tumor-bearing mice	[92]
	PEGylated P(CL-co-LLA) (poly(ϵ -caprolactone-co-L-lactide))	S180 tumor-bearing mice	[93]
Platinum	MeO-PEG- β -P(Glu) and Mal-PEG- β -P(Glu)	BxPC3 cells human pancreatic cancer	[94]
Phenformin	PEG-polycarbonate	H460 human lung cancer cell line	[95]
Cyclopamine/Gefitinib	PEG- β -poly(carbonate-co-lactic acid)	MIAPaCa-2 pancreatic cancers	[96]
Camptothecin (CPT)	Poly(cholesteryl acrylate-co-methoxy PEG methacrylate), poly[CHOL(y)-co-	MCF-7	[97]

mPEG(n,x)]			
4-(N)-stearoyl Gem (GemC18)	PEG-poly(d,L-lactide) (PEG-PLA)	Gem high-resistant AsPC-1 cells	[98]
Docetaxel (DTX)	PCL- β -PEG copolymers	LNCaP prostate adenocarcinoma cells	[99]
	Pluronic P105 and F127 copolymers	A549	[100]
	PEGylated poly(amine-co-ester) terpolymers	SK-BR-3 cancer cells	[101]
	Vitamin E TPGS -D- α -tocopheryl PEG 1000 succinate	NIH3T3,SK-BR-3,MCF-7, MDA MB 468, MDA MB 231 and HCC38	[102]
DTX- chloroquine (CQ)	PEG- β -PLGA	MCF-7	[103]
DTX-CDDP	α -tocopherol (Ve) and PEG to poly(L-glutamic acid) (PLG)	B16F1 mouse melanoma cells and <i>in vivo</i>	[104]
CUR	<i>N</i> -Benzyl- <i>N,O</i> -succinyl chitosan (BSCS)	HeLa, SiHa and C33a cervical cell lines	[105]
	Methoxy PEG-poly(lactide)-poly(β -amino ester) (MPEG-PLA-PAE) copolymers	MCF-7 cells and <i>in vivo</i>	[106]
	Poloxamers and D-alpha-Tocopheryl PEG 1000 succinate (TPGS)	Multidrug-resistant ovarian cancer (NCI/ADR-RES) cells	[107]
CUR-PTX	PEG-phosphatidylethanolamine (PEG-PE)/vitamin E	SK-OV-3-paclitaxel-resistant (TR) cells	[108]

3.3. DENDRIMERS

Dendrimers are hyperbranched globular shaped particles having a unique three-dimensional architecture. They can provide perfect control over molecular structure for a nanosized based drug delivery system due to their multiple functional surface groups.

The first poly(amidoamine) (PAMAM) dendrimer was synthesized by Tomalia in 1985 [109]. Recent studies have focused on improving their application and functional design as drug or gene delivery systems, especially in cancer treatment, in order to eliminate their disadvantages.

Dendrimers have three different separate components:

a core, at the center of the dendrimer, determines the size and shape of the dendrimer,

branches, constituted of repeat units lead to a monodisperse, treelike, star-shaped or generational structure,

terminal functional groups, generally located at the exterior surface of the dendrimer. These surface groups enable growth of the dendrimer so they determine the properties of dendritic macromolecules according to their chemical modification.

Dendrimers can be used as a suitable carrier for increasing drug solubilization, enhancing gene or drug delivery and increasing the therapeutic effectiveness of any drug, as well as enabling targeting to specific sites [110-119].

Table 4. The advantages and disadvantages of dendrimers [110-119]

Advantages	Disadvantages
Monodisperse molecular structure unlike linear polymers and providing control over its architecture	Three-dimensional structure and the design of dendrimers especially secondary-structural motifs within the branches are not well understood
Cross biological barriers to circulate in the body with small size and target specific site by modifying	Not suitable for all administration excluding parenteral
Terminal end groups possess positive, negative or neutral charges	Non-specific interaction with a variety of cells and toxic effects

3.3.1. Types of dendrimers

Different types of dendrimer having different functionalities have been developed using recent advances in synthetic chemistry and characterization techniques to overcome limitations and improve applications. The most commonly used types of dendrimer are mentioned in Figure 2 [110,120-133].

PAMAM Dendrimers

Synthesized by the divergent method,
Products up to generation 10 (G 10),
Due to the presence of positive charge on the surface, used in gene transfection.

PPI Dendrimers

Synthesized by divergent method,
Commercially available up to G5,
Applications in material science as well as in biology.

Liquid crystalline (LC) dendrimers

Limited application in the delivery of DNA,
Consist of mesogenic LC monomerase.

Core shell (tecto) dendrimers

Application in the field of nanomedicine including drug delivery,
Different compounds incorporated into dendrimers,
Potential use as diseased cell recognition and diagnosis, drug delivery

Chiral dendrimers

Potential use as chiral hosts for enantiomeric resolutions and as chiral catalysts for asymmetric synthesis

Peptide dendrimers

Produced by divergent and convergent methods,

Used in industry as surfactants, and in biomedical field as multiple antigen peptides (MAP), protein mimics and vehicles for drug and gene delivery.

Glycodendrimers

Encompass sugar moieties such as glucose, mannose, galactose and/or disaccharide,

Potential use as site-specific drug delivery to the lectin-rich organs.

Hybrid dendrimers

Obtained from various polymers,

Generated the compact, rigid, uniformly shaped globular dendritic hybrids,

Potential use as drug delivery.

PAMAM-organosilicon (PAMAMOS) inverted dendrimers

Potential applications for electronics, chemical catalysis, nano-lithography and photonics, *etc.*,

Unique properties; constancy of structure and encapsulate various guest species with nanoscopic topological precision.

Figure 2. Schematic illustration of dendrimer types and their properties [120-133]

3.3.2. Synthesis of Dendrimers

Dendrimers have three traditional macromolecular architectural classes: linear, cross-linked, and branched. Synthesis of dendrimers enables the possibility of generating monodisperse, structure-controlled macromolecular architectures [134].

Dendrimers can be produced by controlled branched chemistry. In the synthesis of dendrimers there are two main strategies: divergent and convergent. The divergent method is both costly and difficult. In this method, synthesis of dendrimers starts from the core. That is, the dendrimer grows outwards from a multifunctional core molecule. There is a disadvantage in that the extension of some branches may not be carried out smoothly causing deformations to occur during the production process. These deformations may impact the functionality of the dendrimer. Impurities are another problem for this method.

In the convergent method, the dendrimers are produced from the functional molecules. The branches then form inwards, eventually attaching to a core. This method is easier because impurities can be easily eliminated, but large quantities of dendrimers cannot be produced by this method [135-138].

The polymers used in dendrimer production are: PAMAM, poly(propylenimine) (PPI), poly(L-lysine), triazine, melamine, PEG and carbohydrate-based citric acid, poly(glycerol-co-succinic acid), polyglycerol, and poly[2,2-bis(hydroxymethyl)propionic acid]. Dendrimers can be based on monodispersed or polydispersed frameworks. Both of these methods influence the size of dendrimers because of the generation number. When dendrimers reach the maximum size, they form a tightly packed ball-like structure. The divergent and convergent methods are most frequently used for dendrimer synthesis. However, hypercores and branched monomer growth, double exponential growth and click chemistry methods have been improved in recent years [110,139,140].

3.3.3. Characterization of Dendrimers

There are many methods for the characterization of dendrimers. For example:

- Ultraviolet-visible spectroscopy (UV-VIS)
- Infrared spectroscopy (IR)
- Nuclear magnetic resonance (NMR)
- Mass spectrometry
- Raman spectroscopy
- Atomic force spectroscopy
- X-ray photoelectron spectroscopy (XPS)
- High pressure liquid chromatography (HPLC)

3.3.3.1. Nuclear Magnetic Resonance (NMR)

NMR is the most widely used technique for characterizing dendrimers. In fact NMR spectra are used during the synthesis of dendrimers but NMR has also been used for determination of dendrimer size and morphology in recent years. Special techniques including Cosy, HMBC, HSQC, ^1H , ^{13}C , ^{31}P , and ^{29}Si have used NMR. According to NMR technique, dendrimers or polymers are dissolved in any solvent, for example chloroform, dimethyl sulfoxide, methanol, *etc.* and then measured in NMR. However, sometimes dendrimers or polymers are not soluble. In that case, solid state NMR should be used [141-144].

3.3.3.2. IR spectroscopy

IR spectroscopy is generally used for the determination of synthesis and for determination of functional groups, conjugation and for the understanding of drug-dendrimer interactions. Moreover, infrared spectroscopy is used to understand the chemical transformations which occur at the surface of dendrimers. The appearance, disappearance, or chemical shift of characteristic peaks gives information about the progress of the synthesis. In general, nitrile groups, amine groups, aldehydes, *etc.* are examined in infrared spectroscopy for the characterization of dendrimers. Disappearance of nitrile groups or amine groups and the appearance or disappearance of hydrogen bonds indicates the synthesis and surface modification of dendrimers [145,146].

3.3.3.3. Ultraviolet-visible spectroscopy (UV-VIS)

UV-VIS spectrophotometry gives information about the synthesis and surface modification on dendrimer molecules by the observation of characteristic absorption maxima. Additionally, UV-VIS spectrometry can be used to determine the functional groups attached to the dendrimers by revealing shifts in the absorption peaks. UV-VIS spectra can be evaluated to measure reaction rates during the synthesis of dendrimers. The UV-VIS spectrum is easily recorded, however solvent selection plays an important role in correct measurement [147,148].

3.3.3.4. X-ray photoelectron spectroscopy (XPS)

XPS is a quantitative method and is used to measure the elemental composition, empirical formula, chemical, and electronic state and thickness of dendrimers. Aromatic rings, carbanile groups, *etc.* can be identified by using XPS [149].

3.3.3.5. Microscopy

Atomic Force Microscopy (AFM) and Transmission Electron Microscopy (TEM) are widely used for imaging dendrimers. AFM is used for surface examination whereas the inner layer and core of dendrimers can be examined by TEM.

Furthermore, the size, shape and structure of the core can be determined by using TEM and AFM [150].

3.3.3.6. Mass spectrometry

Mass spectrometry can only be used to characterize small dendrimers because there is an upper limit on the mass of molecules that can be determined in this way. Generally mass spectrometry is used to check for the presence of impurities in dendrimers. However, dendrimers generally have high molecular mass and so matrix assisted laser desorption ionization time of flight (MALDI-TOF) is used. In particular, PAMAM dendrimers cannot be characterized using mass spectrometry. In addition, it may be possible to determine chemical defects of dendrimers using mass spectrometry [151-154].

3.3.4. Applications of dendrimers in cancer treatment

The applications of dendrimers have been summarized as follows: cancer therapy (site-specific or passive targeting), gene delivery, photodynamic therapy, organ imaging, solubilization, drug delivery (ocular, transdermal, topical, pulmonary administration), vaccine delivery, diagnostic agents, and biomarkers; delivery of bioactives. Here we focus on the applications of dendrimers in cancer therapy.

Dendrimers have been extensively studied for use in cancer therapy owing to their unique properties. Different chemotherapeutic agents have been loaded into dendrimers to increase their therapeutic effects and it has been shown that they preferentially accumulate in cancer cells by passive targeting. On the other hand, dendrimers can be conjugated with a variety of cancer-targeting ligands (biotin, folic acid, amino acids, peptides, aptamers, and monoclonal antibodies) thus they can specifically treat cancer cells by active targeting. Furthermore, dendrimers are suitable as drug delivery systems for gene silencing [155-157]. Kesharwani and Iyer discussed some of the literature about dendrimer-mediated drug and gene delivery in their recent study [110]. The types of dendrimer and the loaded agents or genes which were published in the last year are summarized in Table 5.

Table 5. Summary of the incorporated drugs, and types and treatment of dendrimers in published papers

Incorporation drug	Types of dendimers	Treatment	References
Circulating cancer cells antibody conjugate	Poly(amido amine) (PAMAM) dendrimer	HUVECs	[158]
Diaminocyclohexyl platinum (II)	mPEGylated peptide dendrimer	SKOV-3	[159]

Doxorubicin (Dox)	PAMAM, modified with fluorescein isothiocyanate (FI) and LA (or polyethylene glycol (PEG)-linked LA, PEG-LA)	Liver cancer cells	[160]
	Dendritic poly(L-lysine)	<i>in vivo</i>	[161]
	PEG-PAMAM hybridized gold nanorod	HeLa, <i>in vivo</i>	[162]
	mPEGylated peptide dendrimer	4T1 breast tumor model	[163]
	PEGylated polylysine dendrimer	A syngeneic rat model of lung metastasised breast cancer	[164]
	PAMAM -coated iron oxide nanoparticles	MCF-7/Dox resistant breast cancer	[165]
	Cyclic arginine-glycine-aspartic acid (RGD) peptide-conjugated generation 5 (G5) poly(amidoamine) dendrimers	U87MG cells	[166]
Doxorubicin hydrochloride (Dox)	Poly(propyleneimine) (PPI) dendrimers	MCF-7 cell line	[167]
	Lactose-functionalized poly(amidoamine) dendrimers	A549, DU-145, and HT-1080	[168]
	PAMAM- folate-targeted dendrimer-polymer hybrid nanoparticles	BALB/c athymic nude mice bearing folate receptor (FR)-overexpressing KB xenograft	[169]
	Lysine dendrimers (G1-G3)		[170]
Viologen-based CXCR4 antagonists	-	U2OS human epithelial osteosarcoma and HepG2 cells	[171]
Tat peptide (Tat, T) conjugated	PAMAM dendrimer	S180 cells, Sarcoma 180-	[172]

		bearing mice	
Dicer-substrate siRNA (dsiRNA)	Peptide decorated dendrimer	PC-3 and CR prostatic cancer cells, MDA-MB431, MCF-7	[173]
Head shock protein 27 (Hsp27)- siRNA	PAMAM dendrimer	PC-3, MDA-MB431, <i>in vivo</i>	[174]
	PAMAM dendrimer	PC-3	[175]
Antisense oligonucleotide (ASO)	PAMAM dendrimer	A431, human epidermoid carcinoma cells, <i>in vivo</i>	[176]
siRNA enhanced green fluorescent protein (eGFP) gene	PAMAM dendrimer	A549 lung alveolar epithelial cells	[177]
dsDNA	PAMAM dendrimers conjugated with multiple folate (FA) or riboflavin (RF) ligands for cell receptor	KB cancer cell line	[178]
Akt siRNA-Paclitaxel (PTX)	Triethanolamine-core poly(amidoamine) dendrimer		[179]
Survivin – ASO	PAMAM dendrimer	Hepatic cancer model	[180]
Docetaxel (DTX)-PTX	Dendrimer-D- α -tocopherol PEG succinate (TPGS) mixed micelles	A549, MCF-7 and Chinese hamster ovary (CHO) cells	[181]
DTX	Dendritic copolymer H40-poly(D,L-lactide)	MCF-7	[182]
Methotrexate (MTX)	PAMAM dendrimers	MES-SA cells	[183]
	Folate conjugated-PPI dendrimers	HeLa and SiHa cells	[184]
	Octreotide-conjugated PAMAM	MCF-7 cells	[185]
	PAMAM dendrimer	LNCaP, PC3 cells	[186]
Melphalan	PPI dendrimers	<i>in vitro</i>	[187]

10-hydroxycamptothecin (10-HCPT)	PAMAM-long hydrophobic C ₁₂ alkyl chains, PEG chains and c(RGDfK) ligands	22RV1 cells	[188]
Follicle stimulating hormone receptor (FSHR)	PAMAM conjugated with the binding peptide domain of FSH (FSH33)	OVCAR-3, SKOV-3 cells	[189]

3.4. LIPOSOMES

Liposomes, which were first described by Dr Alec D. Bangham in 1961 (published 1964), are closed spherical vesicles composed of a natural or synthetic phospholipid lipid bilayer in which drugs or genetic material can be encapsulated (Figure 3). This structure presents a unique opportunity, since hydrophilic drugs tend to be encapsulated in the core; while hydrophobic drugs will be entrapped within the lipid bilayers. Liposomes deliver the drugs into cells by fusion or endocytosis mechanisms to provide controlled drug release, which is protected from degradation by plasma enzymes and has reduced side effects. Moreover, liposomes can be easily modified by coating the surface or attaching different specific surface biomarkers PEG, antibodies, and peptides to reach cancer cells by active targeting. The physical and chemical properties of a liposome (size, permeability, charge density, and steric hindrance, *etc.*) are determined by its constituent phospholipids [190-193].

Figure 3. The structure of liposomes [194-196]

Generally, liposomes can be classified as unilamellar or multilamellar (MLV, 100 nm – 20 μ m). Unilamellar liposomes are further divided into two categories: small size [small unilamellar vesicles (SUV), 25–100 nm] or large size [large unilamellar vesicles (LUV), 100–1000 nm]. SUV and LUV are both composed of a single lipid bilayer and a large aqueous core, and thus are suitable for loading hydrophilic drugs, while multilamellar liposomes (MLV) are composed of several lipid bilayers (up to 14 layers) and a limited aqueous space, thus being suitable for loading hydrophobic drugs [197,198].

Table 6. The advantages and disadvantages of liposomes [190-198]

Advantages of Liposome	Disadvantages of Liposome
Liposome are increased efficacy and therapeutic effects of drug by providing changes in the drug biodistribution.	Production cost is high.
Liposome is increased drug stability by control retention of entrapped drugs.	Leakage and fusion of encapsulated drug or molecules.
Liposomes are biocompatible, biodegradable, non-toxic, flexible and nonimmunogenic.	Sometimes phospholipid structure undergoes degradation.
Liposomes are reduction in toxicity of the encapsulated agent.	Short half-life due to chemical and physical instability.
Provides passive targeting or selective active targeting to tumour tissue.	Low solubility and limited encapsulation efficiency.
Liposomes can be generally used in the gene delivery and diagnostic imaging.	Unsterilization.

3.4.1. Liposome preparation methods

Different preparation methods have been used to control the properties of liposomes such as the size, structure, number of bilayers and interaction with drugs, *etc.* as shown in Figure 4. In these methods, drug loading into the liposomes is passive [199-203].

There are several considerations to be taken into account in selecting the loading method:

- 1) The physicochemical properties of the drug or agent to be entrapped must align with the liposome structure;
- 2) The preparation medium in which the liposomes are dispersed is important;
- 3) The effective concentration of the encapsulated drug or agent must be defined;

4) The size, type and delivery method of the liposomes must be determined in accordance with the desired target (active or passive targeting) for the intended application [204-206].

Figure 4. Schematic illustration of the main preparation techniques of liposomes [195,197-217]

Since the classical methods are limited for industrial-scale production of liposomes, a few liposome preparation methods have been improved to provide active targeting. These include the heating method, spray drying,

lyophilization, super critical reverse phase evaporation (SCRPE), the high-pressure extrusion method and the modified ethanol injection method as shown in Table 7 [195,205,218-221].

Table 7. Summary of new liposome preparation techniques [195,205,218-221]

Heating method	Based on the heating of liposome components in presence of glycerol due to providing an increase the stability No degradation lipids Particle size can be controlled Reducing the time and cost of liposome Not need any further sterelisation
Spray drying	Simple and industrially applicable method
Lyophilization	Based on removal of water from products at low pressures, Used to dry products, Provide sterilization, Submicron narrow sized liposomes, Long-term stability
Super Critical Reverse Phase Evaporation (SCRPE)	The SCRPE is based on using supercritical carbon dioxide A high trapping efficiency for both water-soluble and oil-soluble compounds
High pressure exterusion method	Converting MLv to SUV suspension Desirable diameters Homogeneous size distrubitions
Modified Ethanol Injection Method	
[1] The Crossflow Injection Technique	The control of the liposome size and the stability
[2] Microfluidization	Large scale continuous liposome preparation
[3] Membrane Contactor	

3.4.2. Characterization of liposomes

In order to determine the quality of liposomes and to obtain quantitative measures, the main characterization methods include observation of visual appearance and measurement, of size distribution, surface charge phospholipid content, lamellarity, composition, concentration of degradation products, encapsulation efficiency, and stability [195,215,222-224].

3.4.2.1. Determination of liposome size and zeta potential

The size and ZP of liposomes are important characterization parameters especially when the liposomes are used for therapeutic purposes including cancer or gene therapy. ZP gives information about the stability of colloidal systems. When the ZP value of a particle suspension changes within ± 30 mV, the nanoparticles are considered stable.

Some methods used for characterizing the liposomes include dynamic light scattering, static or size-exclusion chromatography (SEC) in conjunction with the aforementioned microscopy techniques, and field-flow fractionation. Additionally, NMR, flow cytometry, and capillary zone electrophoresis have been used in recent studies.

DLS, also known as photon correlation spectroscopy (PCS), is extensively used in drug carrier systems to measure size distribution and ZP. It is a fairly easy technique for obtaining data about low nanometer to low micrometer size particles in native environments with minimal sample volume.

HPLC using SEC is a simple but powerful method which can be used to detect and separate liposomes on the basis of size distribution and stability according to a time-based resolution of hydrodynamic size. Disadvantages of HPLC for liposomes size result from unwanted adsorption of lipids on the HPLC column and therefore, certain data cannot be obtained. However when the shape and chemical composition of liposomes are well matched with the column, liposome size and weight information can be measured. Field-flow fractionation (FFF) is a technique which is used to overcome some of the limitations of HPLC for liposome analysis. While large liposomes separate first in HPLC-SEC, small liposomes elute first in FFF because of its higher diffusion. However the carrier liquid used in FFF should be chosen carefully for the separation of small liposomes [225-228].

3.4.2.2. Encapsulation efficiency and *in vitro* drug release

Encapsulated and un-encapsulated drug fractions are present during liposome preparation. Therefore, it is important to determine the difference between the encapsulated drug within the liposomes and the free drug. Mini-column centrifugation, dialysis, spectrophotometry, fluorescence spectroscopy, HPLC, and FFF can be performed to determine encapsulation efficiency.

In vitro drug release can be measured using the dialysis tube diffusion technique. This technique is based on continuous magnetic stirring at 37 °C with samples of the dialysate taken at specified time intervals and assayed by HPLC or spectrophotometry [229-232].

3.4.2.3. Liposome stability

The liposomes stability is an important consideration for liposome preparation, storage and delivery. The applications of liposomes for drug

delivery are based on high molecular weight drugs loading into liposome suspension which consist of different lipids or polymers. The liposome stability affects the efficient delivery of the contents and successful loading into liposomes. Another important problem is that the average size distribution of liposomes changes upon storage resulting from vesicles affinity for each other leading to larger aggregates. This leads to drug leakage from the vesicles. The chemical stability of the liposomes is also changed by interactions of drugs with the phospholipid layer. Sterilization is a further important parameter to consider in providing microbial stability for therapeutic applications [233,234].

3.4.2.4. Lamellarity

The number of lipid bilayers, the lipid constitution and the preparation technique, impact on the encapsulation efficiency and drug release properties of liposomes as well as their cellular uptake. Liposome lamellarity is often measured by microscopic (TEM, cryo-TEM) or spectroscopic techniques (UV absorbance, NMR, small angle X-ray scattering (SAXS) [235,236].

3.4.2.5. Morphology of liposomes

The visual appearance of liposomes is changed in terms of the composition and particle size. TEM is a technique for size, morphology, and lamellarity characterization of liposomes, especially for small liposomes. However SEM is sufficient to visualize large vesicles. In recent years, cryo-transmission electron microscopy (cryo-TEM) has been used to eliminate structural changes resulting from the use of negative stains such as uranyl acetate or osmium tetroxide in TEM. Additionally, AFM has been utilized to show liposome morphology (especially for small liposomes), size and stability without sample manipulation. AFM analysis is a rapid and powerful technique to obtain information about liposome morphology and aggregation processes during their storage [226,237-239].

3.4.3. Clinical applications of liposomes in cancer treatment

Liposomes encapsulating a wide range of drugs (*e.g.*, doxorubicin, paclitaxel and cisplatin) and even approved by FDA, have been successfully applied for cancer treatment because of their unique properties including their size, their possession of both hydrophobic and hydrophilic structures, their reduced toxicity, increased therapeutic effects and biocompatibility/biodegradability, *etc.* Additionally, liposomes have been used in gene therapy research to encapsulate various genetic materials such as genes, miRNA, siRNA, *etc.*

The first liposomal pharmaceutical product “Doxil” was approved in 1995 and the latest “Marqibo” in 2012 [240]. However, some recent liposomal formulations have been developed to eliminate their disadvantages (degradation of lipids, size problems) and improve efficacy in targeting cancer

cells. The approved liposomal products and ongoing clinical trials are mentioned in Table 8.

In conclusion, new research has continued to achieve better formulations, extend the *in vivo* circulation time, improve tumor delivery and reduce toxicity for the clinical application of liposomes in cancer treatment [241,242].

Table 8. Clinical applications and certain approved liposome-based products currently on the market

Drug	Product Name	Treatment	Trial phase	Approved by FDA	References
Doxorubicin	Myocet	Metastatic breast cancer			[243]
	Lipo-dox	Kaposi's sarcoma, ovarian and breast cancer			[244]
	ThermoDox	Non-resectable hepatocellular carcinoma	III		[245-247]
Daunorubicin	DaunoXome	Blood cancer			[248]
Vincristine sulfate	Marqibo	Acute lymphoblastic leukemia			[249-251]
	LEP-ETU	Ovarian, breast and lung cancers	I		[252]
Paclitaxel	EndoTAG-1	Anti-angiogenesis, breast and pancreatic cancer	II		[253,254]
	SPI-077	Lung, head and neck cancers	I/II		[253,255]
Cisplatin and its analog	Lipoplatin	Pancreatic cancer, head and neck cancer, mesothelioma, breast cancer, gastric cancer and non-small-	III		[253,256-258]

		cell lung cancer.		
		Malignant pleural mesothelioma and advanced colorectal carcinoma	II	[199]
		Advanced or refractory solid tumours	I	[259]
Mitoxantrone	LEM-ETU	Leukemia, breast, stomach, liver and ovarian cancers	I	[252]
Topotecan	INX-0076	Advanced solid tumors	I	[199,253]
Vinorelbine	INX-0125	Breast, colon and lung cancers	I	[199,253,260]
Lurtotecan	OSI-211	Ovarian, head and neck cancers	II	[199]
BLP25 lipopeptide	Stimuvax	Non-small-cell lung carcinoma	III	[199,261]
All-trans retinoic acid	Atragen	Advanced renal cell carcinoma	I/II	[199,262]
Annamycin	Liposome Annamycin	Breast cancer	I/II	[199,263,264]
Cytarabine and daunorubicin	CPX-351	Acute myeloid leukemia	II	[199,265]
Irinotecan HCL and floxuridine	CPX-1	Colorectal cancer	II	[199,266]
	MM-398	Metastatic pancreatic cancer	III	[267]

3.5. SOLID LIPID NANOPARTICLES (SLNs)

SLNs which are in the size range of 10–1000 nm, are attracting major attention as a novel colloidal drug carrier with the potential to overcome limitations in drug delivery including poor drug loading capacity, size problems, unstable properties, uncontrolled drug release associated with polymeric nanoparticles, dendrimers and liposomes. SLNs offer unique properties such as small size, large surface area, high drug loading and entrapment efficiency, low toxicity, excellent physical stability, controlled drug release, protection of drugs from degradation, and avoidance of the use of organic solvents.

The structure of SLNs based on lipids that are solid at room temperature. The lipid structure of SLNs is important to determine whether or not a loaded molecule can be strongly encapsulated within a delivery system. Therefore, the lipids that form the SLNs core structure could be important for high-rate drug loading. Additionally, most lipids that form the structures of SLNs are biodegradable, so SLNs show excellent biocompatibility and have less toxic effects. When the use of SLNs is investigated, it seems that SLNs have often been selected for lipophilic and hydrophilic drug compatibility with lipids that do not have toxic effects as carriers. However, in recent years, increasing attention has also been paid to the coating of SLNs in order to load lipophobic and hydrophilic drugs in the lipid structure and also to provide gene delivery.

Table 9. The advantages and disadvantages of SLNs [268-283]

Advantages of SLN	Disadvantage of SLN
Long-term stability, controlled drug release and targeting.	Poor drug loading capacity.
Decreasing the danger of acute and toxicity of drug or compound.	Degradation of drug in the SLNs during storage.
Enhancing the bioavailability of entrapped drug or compound.	Having high water content.
Chemical protection of labile incorporated compound.	Low capacity to load hydrophilic drugs.
Easily large scale production.	Sometimes burst release of incorporated drugs.

Different methods are being developed to prepare SLNs, such as high pressure homogenization (hot and cold homogenization), microemulsion, solvent emulsion diffusion, solvent evaporation, ultrasonication/high speed homogenization, and spray drying methods. However it is very important to choose the best methods to avoid drug degradation and lipid crystallization according to the properties of the incorporated substances. After production, the second most important step is the characterization of nanoparticles.

Analytical methods can be used to characterize SLNs in terms of particle size, ZP, PCS, surface characteristics (TEM/SEM), crystallinity [differential scanning calorimetry (DSC), XRD], chemical shift, NMR, drug entrapment efficiency, and *in vitro* drug release studies (UV spectroscopy, HPLC). After SLNs are characterized, the third most important step is their application according to the desired properties. Different applications have been studied in the literature, especially in cancer treatment: (i) chemotherapeutic drugs incorporated into SLNs to eliminate their disadvantages and overcome MDR, (ii) gene therapy (delivery of peptides, genes, miRNA, siRNA into cells), (iii) drug targeting, new adjuvants for vaccines, treatment of infectious diseases, cosmetic and dermatological preparations and agricultural applications [268-283].

3.5.1. Production methods of SLNs

Figure 5. Schematic illustration of SLN preparation methods

3.5.1.1. High-pressure homogenization

High-pressure homogenization comprises two basic production techniques for SLNs: hot homogenization and cold homogenization. In both techniques, the drug is dissolved in molten lipid.

3.5.1.1.1. Hot homogenization technique

This technique is the most common method for producing SLNs. In this method the lipid phase is first melted at 80 ± 1 °C with stirring (above the melting point of the solid lipid) and the temperature is set to 80 °C in a thermostated water bath. The drug (in solid state) is added into the molten lipid at the same temperature. Later, a surfactant is added slowly to the mixture and homogenized by ultra-turrax at 20000–25000 rpm. Finally, the obtained mixture is homogenized using a high-pressure homogenizer at a pressure ranging from 100–1500 bar. Three to five homogenization cycles at 500–1500 bar is sufficient. The mixture is then cooled at room temperature and put into glass vials.

Due to the fact that the loss of hydrophilic drugs to the water medium can be considerable, the temperature should be carefully selected. This technique can generally be applied to lipophilic drugs [284-289].

3.5.1.1.2. Cold homogenization

The cold homogenization process is similar to hot homogenization. Initially, the solid lipid phase is melted and the temperature is set to 80 °C in a thermostated water bath with stirring. The drug (in solid state) is added into the molten lipids at the same temperature. Surfactant is then slowly added to the mixture. The mixture is cooled rapidly using liquid nitrogen or dry ice to distribute the drug homogeneously in the lipid matrix. Finally, the obtained mixture is powdered to micro and nanoparticle size using various methods (for example ball milling). It is exposed to high-pressure homogenization at room temperature or below room temperature. At this stage, it is important that the high-pressure homogenization is maintained at an appropriate temperature. In appropriate conditions, the obtained particles have micrometer diameters.

Many heat-sensitive drugs can be incorporated into lipid nanoparticles using this technique, which is more suitable for hydrophilic drugs [270,290].

3.5.1.2. Microemulsion method

A high surfactant/lipid ratio is used in this method. Microemulsions are composed of an inner and outer phase (e.g. oil-in-water). In this method, lipids are melted and surfactant is heated to the same temperature as the molten lipids. The solutions are mixed and a microemulsion is obtained. This mixture is at approximately 60–80 °C and is transparent. Next, the microemulsion is

dispersed in cold water at 2–3 °C with stirring. Liquid droplets are solidified by the cold water because of the high temperature gradient. In this method, the risk of aggregation is very low and the method is easy to perform. However, it has several disadvantages. For example, microemulsions have very low concentrations. The high concentration of surfactants/co-surfactants is another drawback [291-294].

3.5.1.3. Solvent emulsification/evaporation

Another method for the production of nanoparticle dispersions is solvent evaporation. Initially, lipid and drug are dissolved in organic-immiscible solvent such as chloroform, *etc.* The organic solution is emulsified in an aqueous phase using surfactant. Solvent is evaporated from nanoparticulate dispersions and the lipid is precipitated as dispersed nanoparticles. The main advantage of this method is that obtained nanoparticles have diameters between 25–100 nm. However, this method has some disadvantages such as limitation of the lipid concentration [295-297].

3.5.1.4. Supercritical fluid (SCF) technique

This method is performed above the critical temperature and critical pressure of a substance. Under these pressure and temperature conditions, the substance can exist in equilibrium between vapor and liquid form. In order to increase the amount of dissolved compound, the pressure can be increased while viscosity remains constant. Under these temperature and pressure conditions, the fluid can act like an organic solvent and so it simply dissolves lipids and other components. Generally, carbon dioxide is used as a super critical fluid because it is safe, cheap and non-irritating. However, obtained nanoparticles using this method have large diameters (micrometer range). Therefore this method is applied along with other methods *e.g.*, homogenization methods [297-299].

3.5.1.5. Ultrasonication

SLNs can also be produced by sonication. Whereas this method is easily applied in any laboratory, the particle sizes of the obtained SLNs are in the micrometer range. Additionally, physical instability and the potential for metal contamination are cited as other disadvantages of ultrasonication [300].

3.5.1.6. Spray-Drying

The spray-drying method of obtaining an aqueous SLN dispersion can be used as a less expensive alternative to lyophilization. The drawback of this method is that particle aggregation can be caused by the high temperatures, shear forces and partial melting of the particle [273,278,301].

3.5.2. Characterization of SLNs

3.5.2.1. Particle size

There are various techniques for the measurement of the particle size of nanoparticulate systems such as PCS (also known as dynamic light scattering), laser diffraction (LD) and the Coulter counter method. However the easiest and most useful techniques are PCS and LD. As particles move stably in the dispersion, they scatter light passing through the solution. PCS measures fluctuations in the intensity of this scattering light. PCS is an especially good method for nanoparticulate systems because of its working range from 1 nm to 3 μm . The LD method is more effective and useful method than PCS because it is related to the diffraction angle of the particle radius. Thus, small particles give rise to intense scattering whereas big particles cause less scattering. The working range of this method is from 1 nm to 1000 μm . The Coulter counter is hardly used in comparison with other methods because it is quite difficult to measure small particles. However, for electrolytes, which destabilize dispersal systems, it is necessary to use this method. Polydispersity index (PDI) is measured using PCS and is used for measurement of particle size distribution [270,278,297].

3.5.2.2. Zeta potential

ZP has been generally used to characterize colloidal dispersions. ZP, which measures surface charge, gives information about the storage stability of SLNs and their surface morphology. Therefore, ZP is generally used in product stability studies and surface adsorption research. ZP measures the magnitude of the electrostatic repulsion or attraction between particles. If nanoparticle systems show higher surface charge, a higher ZP value can be measured. On the other hand, aggregation of particles occurs when particles have low ZP because there is little electrical repulsion between the particles.

ZP value less than -60 mV is excellent, if it is less than -30 mV there is good electrostatic stabilization, so it can be said that particles show good physical stability. Colloidal systems which contain steric stabilizers are an exception because they can have good long-term stability even though ZP is 0 mV. Various factors such as particle size and shape, molecular weight, pH, surfactants, water impurities and water conductivity affect the ZP value [276,301-303].

3.5.2.3. Differential scanning calorimetry (DSC)

DSC is used to determine the sample structure and interactions between the components such as lipids, surfactants, *etc.* especially in colloidal dispersions. DSC compares the melting enthalpy of the bulk material with the melting enthalpy of the dispersion. That is, firstly, it measures amount of heat required to raise the temperature of a sample, then it determines amount of heat

required to raise temperature of a reference material. Finally, it evaluates differences (positive or negative) between them in heat transfer. During the phase transition, there are some physical changes or variations in the sample compared with known materials, because different lipids have different melting enthalpies and melting points [304].

3.5.2.4. Nuclear magnetic resonance (NMR)

NMR gives information about the physicochemical status of components within the nanoparticle. Other methods give information about mobile or dissolved constituents in colloidal systems, whereas solid state NMR is suitable for characterizing solid particles because of its ability to determine molecular mobility, rotational diffusion of particles, and phase transition of the particle matrix. Spin-lattice in rotating frame, only on proton spin measurement, should be applied to determine particle homogeneity and heterogeneous colloidal systems, especially the different chemical shifts which belong to each specific molecule. For example, methyl protons of lipids give signals at 0.9 ppm whereas protons of PEG chains give signals at 3.7 ppm. As a result NMR experiments provide confidence in the data because of their repeatable results [291,305,306].

3.5.2.5. Drug release

There are three different drug incorporation models (Figure 6). Drug release of solid lipid nanoparticles depends on the lipid matrix, the surfactant concentration and the temperature. In addition, drug release also depends on particle size and particle shape. In general, SLNs have a circular shape and an average 100–500 nm diameter and so drug release is controlled and sustained over a long period. Drug release from solid lipid nanoparticles produced by the hot homogenization method is faster than from those produced by cold homogenization [307,308]. Drug release studies are generally carried out using UV-VIS spectrometry or HPLC.

Figure 6. Three drug incorporation models

3.5.2.6. Entrapment efficiency (EE) and drug loading capacity (DL)

EE is the percentage of active ingredient within the nanoparticle core. EE is determined by measuring the concentration of free active ingredient in the dispersion medium. There are various methods to determine the EE. The most commonly used method is UV-spectrophotometry, because it is applied quickly and easily. The entrapment efficiency is calculated by the following equation [307,309]:

$$EE\% = \frac{M_{\text{initial drug}} - M_{\text{free drug}}}{M_{\text{initial drug}}} \times 100$$

where “ $M_{\text{initial drug}}$ ” is the mass of initial drug and the “ $M_{\text{free drug}}$ ” is the mass of free drug.

Drug loading capacity (DL) is calculated by the following equation:

$$DL\% = \frac{\text{Amount of drug in the particles}}{\text{Amount of drug + excipients added in total to the formulation}}$$

3.5.2.7. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM)

SEM and TEM are used to visualize colloidal dispersions by providing information about the size, shape and morphology of the particles. The surface of nanoparticles can be examined by SEM because SEM detects scattered electrons from the surface of the particles. Inner layers of nanoparticles can be examined by TEM which determines transmitted electrons. There are some disadvantages of both methods such as heating of the sample and difficulty in sample preparation [310-312].

3.5.2.8. X-ray scattering (XRD)

X-ray scattering is a good method for examining the lipid lattice structure of particles. That is, measurement of spacing in the lipid lattice can be performed by X-ray scattering. An advantage of this method is that samples are measured in their native state after production, and there is no process causing changes to the chemical environment. A disadvantage of this method is lack of sensitivity and long measurement time. The method consists of two techniques. The first one is SAXS, the other one is wide angle X-ray scattering (WAXS). Both methods can be used for the characterization of colloidal dispersions [310-314].

3.5.2.9. Powder X-ray diffraction (PXD)

PXD is a commonly used technique whereby the crystal structure of colloidal dispersions can be examined. In this technique, X-rays of fixed wavelength are passed through the sample. Inter-atomic spacing is calculated using the obtained data. However, samples have to be solid form in order to use this method. If a sample is in liquid form, it must be solidified by spray drying or lyophilization [290,310].

3.5.3. Applications of SLNs in cancer treatment

MDR and the disadvantages of chemotherapeutic agents such as adverse side effects, toxicity to normal tissues, poor specificity and stability, *etc.* remain major barriers to the success of anticancer chemotherapy. MDR is often mediated by drug efflux transporters such as P-glycoprotein (P-gp), which are overexpressed in cancer cells and therefore reduce cellular accumulation of the drugs. In addition, cancer cells tend to be more resistant to chemotherapeutic agents because of various permeability barriers, which prevent the accumulation of high drug concentrations. Another problem stems from the different physicochemical properties and highly diverse molecular structures of anti-cancer agents.

Nano based drug delivery systems have been improved to overcome these limitations to the delivery of therapeutic agents. SLNs have attracted attention in the field of cancer biology for the delivery of anti-cancer drugs in targeted organs or tissues. They can provide controlled drug release and reduce the associated toxicity, thus eliminating some disadvantages in the therapy. Additionally, SLNs have improved the stability of drugs, improved the encapsulation of chemotherapeutic agents with different physicochemical properties, enhanced drug effects on tumor cells by enhanced permeability and retention effects (EPR) and improved the pharmacokinetics of drugs. The EPR effect is based on the extra-vascular space which results in accumulating high drug concentration because the EPR effect helps to carry the nanoparticles inside the cancer tissue. The passive targeting of cytotoxic drugs enables their incorporation into SLNs. Furthermore, SLNs can target drugs to specific sites by surface modification.

Different therapeutic agents have been incorporated into solid lipid nanoparticle-based systems and their effects on various cancer cell lines have been evaluated as shown in Tables 10, 11 and 12. When the use of SLNs as drug delivery systems have been investigated, SLNs have been shown to increase the cytotoxic effects of the drugs as well as reducing toxic effects on normal cells. The ability to overcome the resistance of P-gp based MDR has been attributed to increased cellular accumulation of the drug by passive/or active targeting [297,315-319].

Table 10. The incorporation drug, treatment and preparation methods of SLNs associated with MDR

Drug / Agent	Cell Lines	Method of preparation	References
Doxorubicin (Dox)	-EMT6/AR1 (Murine breast cancer cell line)-MDA435/LCC6/ MDR1 (human breast resistant cancer cell line)	Dox loaded polymer lipid hybrid nanoparticles (PLN)	[318-320]
Dox	-MCF-7/ADR cells (Dox resistant cell line)	Solvent emulsification-diffusion methods	[321]
Dox	-MCF7-MCF7/ADR	Solvent emulsification-diffusion method	[310]
Dox and Paclitaxel (PTX)	-NCI/ADR-RES (human ovarian adriamycin resistant carcinoma cell line) -OVCA8-8 (human ovarian carcinoma cell line) -MDA-MB435/ LCC6 /MDR1	Warm o/w microemulsion precursors	[322]
PTX and Dox	-MCF-7 -MCF-7/ADR -SKOV3 (human ovarian cancer cell)-SKOV3-TR30 (human ovarian resistant cancer cell)	Solvent diffusion method	[323]
PTX and Verapamil (VP)	-MCF-7/ADR resistant cells	Hot sonication method	[324]
GG918 (Elacridar) Dox	-MDA-MB 435/LCC6/WT-MDA-MB 435/LCC6/MDR1		[325]
Dox and mitomycin C (MMC)	-MDA-MB 435/LCC6 /WT -MDA-MB 435/LCC6 /MDR1		[326]
Idarubicin and Doxorubicin	-P388 -P388/ADR -HCT-15 cell lines	Co-precipitation method	[327]
Antisense	-NCI/ADR-RES	Microemulsion	[328]

Glucosylceramide Synthase Oligonucleotide (MBO-asGCS)		method combining shear and ultrasonic homogenization techniques
Dox and MBO- asGCS	-NCI/ADR-RES	[329]

Table 11. The incorporated drugs, treatments and preparation methods of SLNs in published papers

Drug / Agent	Cell Lines	Method of preparation	Reference(s)
Cholesteryl Butyrate	-HUVEC (Epithelial cell lines), -HT29, -HCT116 -CaCo-2 Human colon adenocarcinoma -MCF-7, -PC-3 (Human prostate carcinoma), -M14 and LM cells (Human melanoma)	Warm microemulsions	[330]
Hyaluronic Acid (HA) and Vorinostat (VRS)	-A549, -SCC7 (Human alveolar adenocarcinoma cell line), -MCF-7	HA was attached onto the surface of cationic SLNs by electrostatic attraction	[331]
Zanamivir	-Caco-2	A double emulsion (W/O/W) solvent evaporation method	[332]

5-Fluorouracil (5-FU)		Temperature-modulated solidification process	[333]
Docetaxel	-TC-1 cells (murine lung cancer cell line), -M-Wnt cells (murine mammary gland cell lines), -Human breast adenocarcinoma cells (MDA-MB-231)	Emulsion/solvent evaporation method	[334]
	-MCF-7	High-pressure homogenization method	[335]
	-BEL7402 hepatocellular carcinoma	Homogenization method	[336]
	-hCMEC/D3 a primary human brain microvascular endothelial cell line -NO3 glioblastoma cell line	Coacervation technique	[337]
	-A549	Solvent diffusion method	[338]
Paclitaxel (PTX)	-Sarcoma 180 ascited tumor and tumor bearing mice	Solid-liquid lipid nanoparticle (SLLN) modified with folate and PEG	[339]
	-A549	Film dispersion methods modified SA-R8	[340]
	-OVCAR-3 human ovarian cancer cells and -MCF-7	Homogenization	[341]
	-The MXT variant B2 (MXT-B2) was obtained through the isolation of epithelial cells representing different stages of the disease from mice breast adenocarcinoma (murine mammary	Melted homogenization technique	[342]

adenocarcinoma-MXT)			
Paclitaxel (PTX), Methotrexate (MTX), Mitoxantrone (MTO)	-MCF-7		[343]
Butyrate, Dox, PTX	-HT-29	Microemulsion technique	[344]
Podophyllotoxin (PPT)	-HeLa (HL-60) cells	Solvent emulsification–evaporation method	[345]
Mitotane	-Adenocortical carcinoma	Hot high pressure homogenization	[346]
2-methoxyestradiol	-MCF-7, -PC-3, -Glioma (SK-N-SN)	Hot homogenization-ultrasonication method	[347]
Curcumin (CU)	-HCT-116	Coacervation technique	[348]
	-HL-60, -A549, -PC3		[349]
	-MCF-7	Homogenization method	[350]
	MCF-7 cells	High-pressure homogenisation	[351]
	-A549 and xenografts.		[352]
Ferritin	-MDA-MB-468 breast cancer cells		[353]
Methotrexate	-EAC (Ehrlich ascite carcinoma) berain mice	Micro emulsification solidification method	[354]
Tamoxifen	-MCF-7	Microemulsion and precipitation techniques	[355]
		Temperature-modulated solidification methods	[356]

	-MCF-7	Hot high pressure homogenizer technique	[357]
Tamoxifen citrate		Solvent injection method	[358]
Camptothecin(CPT)	-MCF7	Supercritical fluid technology	[359]
Trilaurin (TL) and (PTX)	-Human ovarian cancer cells (SKOV-3)	Hot-melt high-pressure homogenisation	[360]
Carmustine	-The human GBM U87MG cells	Microemulsion catanionic solid lipid nanoparticles (CASLNs) with surface anti-epithelial growth factor receptor	[361]
Etoposide (VP16)	-SGC7901 cells.	Emulsification and low-temperature solidification methods	[362]
Etoposide	-The Dalton's lymphoma tumor cells were maintained in the peritoneum of Balb/c mice	Melt emulsification and homogenization technique	[363]
	-B16F10 melanoma colonization in lung	Hot homogenization	[364]
Doxorubicin (Dox)	-A549, -MCF-7	Solvent injection method	[365]
	-B16F10	Cationic solid lipid nanoparticles	[366]
	-A549	Hot melting homogenization method	[367]
Resveratrol	-Cells from immortalized human keratinocyte cell line NCTC2544	Melt-emulsification process	[368]
Emodin	-MCF-7, -MDA-MB-231, -MCF-10A	High pressure homogenization (HPH).	[369]
Rhodamine 123	-A172, U251, U373 U87	Emulsification	[370]

(R123)	human glioma cell lines -Macrophage cell line (THP1)	process	
Mitoxantrone	-MCF-7 -Model of P388 lymph node metastases in Kunming mice	Dispersion- ultrasonication method	[371]
Shikonin-Act	-A549 cells	Hot homogenization method	[372]
Histone deacetylase inhibitor, vorinostat (VOR),	-MCF-7, -A549, -MDA-MB-231	Hot homogenization using an emulsification- sonication technique	[331]
Retinoic acid	-MCF-7	Hot melting homogenization method using an emulsification- ultrasound	[373]

Table 12. The incorporated drugs, treatments and preparation methods of SLNs associated with gene delivery

Drug/ Agent	Cell Lines	Method of preparation	Reference (s)
c-MeT siRNA	-U-87MG glioblastoma and xenograft model	Cationic solid lipid nanoparticlesbconjugated Pegylated c-Met siRA	[374]
Anti-microRNA for miR-21	-A549	AMO-CLOSS (Cationic lipid binded oligonucleotide (AMO)) loaded SLNs)	[375]
Green fluorescence protein plasmid (pEGFP) and Dox	-A549 and mice beraing A549 tumors		[376]
Pre-miR-107	-SCC15, -SCC25, -CAL27, -UMSCC74A head and neck squamous cell carcinoma	Cationic lipid nanoparticles	[377]
Kinesin spindle protein (KSP) targeting siRNA	Murine endothelial cell line MS1-VEGF - HUVEC	DOPC(DOPC/DOTAP/DOPE- PEG2000) or DOPE (DOPE/DOTAP/DOPE-PEG2000) based preparations the thin film and hydration method.	[378]
miRNA-34a- PTX	-B16F10 -C57BL/6 mice	a film ultrasonic method	[379]
PTX and siRNA	human epithelial carcinoma KB cells	emulsification solidification methods.	[380]

3.6. CONCLUDING REMARKS

The applications of nanotechnology in drug delivery will be a potential priority research area for the pharmaceutical and biotechnology industries in the future due to its unique potential to overcome the limitations and drawbacks of conventional drugs. It may be possible to develop new strategies providing target-specific drug therapy through newly developed drug delivery systems in cancer treatment.

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