Chapter 2
Literature Review

Chapter 2. Literature Review

2.1. Literature Review on Nanocrystals of Anticancer Drugs

Drug nanocrystals (NCs) have garnered significant attention in the field of drug delivery due to their remarkable physicochemical properties. These properties include high drug-loading efficiency, free of organic solvent or solubilizing chemicals, excellent structural stability, consistent dissolution, and prolonged circulation time, making drug NCs an outstanding formulation for effective cancer therapy (Khairnar et al., 2022; Lu et al., 2015; Miao et al., 2018). Nanocrystals also facilitate accumulation in tumor or cancer sites and achieve concentrated intracellular presence in cancer cells due to cellular internalization via endocytosis, resulting in cellular apoptosis and cancer cell death (Chen and Li, 2015; Lu et al., 2017; Mohammad et al., 2019b). The size and shape of nanocrystals can regulate the antitumor action and drug-linked toxicity of anticancer drug (Zhou et al., 2016). Over the past two decades, numerous hydrophobic drugs, such as 2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide (HPPH), camptothecin (CPT), thymectacin, busulfan, cyclosporin A, and paclitaxel, have been successfully formulated into drug NCs for their use in cancer treatment (Miao et al., 2018).

The nanocrystals of 2-devinyl-2-(1-hexyloxyethyl)pyropheophorbide (HPPH), a hydrophobic photosensitizing anticancer drug were prepared by the method of reprecipitation. Prepared nanocrystals were monodispersed, stable, eliminated extra stabilizer (surfactant need), and revealed high and readily cancer cells internalization (Baba et al., 2007). The nanocrystals of Amoitone B (Nur77 agonist) were formulated by microfluidization method to overcome its poor aqueous solubility and short half-life. Amoitone B nanocrystals revealed improved in vitro antitumor activity against stomach, colon, liver and lung cancer cells by inducing G1 cycle arrest and enhancing the cancer cell apoptosis. The tissue distribution of nanocrystals were observed higher

in liver and lung, suggesting their potential in effective treatment of cancer in such organs (Hao et al., 2014). The nanocrystals of 10-Hydroxycamptothecin (10-HCPT) were prepared by acid-base microprecipitation followed high-pressure homogenization technique, to improve the solubility and anticancer activity of drug. The spherical shaped nanocrystals of about 130 nm were obtained with 75 % of drug content. The nanocrystals provided sustained drug release, higher cellular uptake in cancer cells and better antiproliferative activity than 10-HCPT injections. The nanocrystals showed much lower IC50 values than 10-HCPT injections in 4T1, MCF-7, HepG2, A549, and HeLa cells. Nanocrystals can maintain the plasma concentration for a longer period, thereby improving systemic circulation. The nanocrystals observed to increase the mean retention time, half-life and AUC while reducing clearance of drug, as compared to 10-HCPT injection. The nanocrystals also displayed preferential drug accumulation in tumors providing potent anticancer activity in 4T1-bearing mice. (X. Yang et al., 2016).

2.2. Literature Review on Nanocrystals for Intravenous Administration

Nanocrystals for intravenous administration are prepared to prolong the systemic circulation of drug, reduce drug associated toxicity, minimize drug distribution to non-target site and facilitate accumulation at target site. For example; etoposide nanocrystals stabilized using Pluronic F-127 for intravenous injection in mice were observed more effective and safer than Toposar. Nanocrystals offered better half-life and drug tolerance in comparison to Toposar at similar dose (Martin et al., 2020). Nanocrystals of curcumin administered by *i.v.* injections aided the accumulation in lung, spleen, kidney and mainly liver due to RES uptake, leaving only about 33 % NC in blood circulation at 4h (Wang et al., 2018). A high distribution of intravenously administered nanocrystals in MPS-rich organs like liver, spleen, and lungs may result

due to recognition of nanocrystals by mononuclear phagocytic system (MPS) cells as exogenous materials, leading to their passive accumulation (Hollis et al., 2013; Lu et al., 2016b). Liver can act as depot, releasing drug back into circulation for redistribution. The nanocrystals distribution to tumor site and subsequently to cancer cells may also result via enhanced permeation and retention (EPR effect), possibly due to available large inter endothelial pores and disorganized growth of tumors but still can face poor diffusion due to complex structure of tumor (Hollis et al., 2013). The nanocrystals size, shape and surface depending on drug properties and stabilizer used can affect the nanocrystals biodistribution (Lu et al., 2016b). Coating of pure drug nanocrystals with a suitable materials can be done to improve the systemic circulation stability, control drug release, facilitate drug transport to target site and aid interaction with tumor cells (Xia et al., 2023).

2.3. Literature Review on Nanocrystals for Pulmonary Administration

Pulmonary nanomedicine reduces the drug inhomogeneity problem observed with aerosols of drug, alter drug solubility, and decrease mucociliary clearance and macrophage phagocytosis responsible for particle elimination of larger than 1 μm. Despite a lot of research to increase pulmonary nanomedicine deposition to lungs and increase drug bioavailability, a little progress has been achieved. Drug nanocrystals can be formulation of choice for pulmonary drug delivery owing to its sub-micron size, high payload, low excipient addition, high effective surface area, easy scale up feasibility, surface functionalization potential, and high stability towards aerosolization. Nanocrystals are responsible for homogenous particle distribution and deposition to deep lung based on their shape and size, to exert the therapeutic action. Costabile et al., 2020 reported nanocrystals for homogenous distribution in lung on pulmonary administration. The nanocrystals exhibited high aerosol performance with varying

migration rate between large and small-sized crystals. The shape of nanocrystals also varied the diffusion rate showing higher diffusivity of rod-shaped nanocrystals than spherical (Costabile et al., 2020). Nanocrystals have high stability compared to nanosuspension, thus show minimum variation in distribution while administrating as aerosols. Thus, spray or freeze-dried nanocrystals provide good aerosol behavior with a uniform deposition pattern than nanosuspension.

Nanocrystals can either rapidly dissolve in lung fluid to release the free drug taken up by cells or can be taken up by cells in its intact form via clathrin-mediated and caveolae-mediated endocytosis pathways (Lu et al., 2017). Curcumin nanocrystals resulted in selective deposition of nanocrystals in lung (824.27 μg/g) than other body parts. The pulmonary nanocrystals exhibited higher solubility and better penetration, thus resulted in higher C_{max} and 3.2-fold AUC of orally administered drug (Hu et al., 2015). Baicalein nanocrystals also exhibited rapid and extensive absorption on pulmonary delivery (Zhang et al., 2016). In addition, nanocrystals have the capability to interact with biological structures to facilitate the mucoadhesion, drug permeation and cellular internalization (He et al., 2020b). The quality and aerosol behavior of nanocrystals can be determined by assessing fine particle fraction (FPF). FPF is the fraction administered that lies in a size range, necessary for effective penetration and deposition in the airways. The value of FPF for inhaled particles more than 50 % result in minimum retention on capsule and device (Hu et al., 2015). The higher FPF value indicates more extensive penetration to the lung and hence better deposition.

The drug for exerting localized action in lung are required to retain there for a prolonged period. This can be achieved by modifying the nanocrystals surface. Phospholipids mimicking lung membrane (pulmonary surfactant and mucus layer) can be used for modifying nanocrystals surface that slow the drug release, minimize lung

clearance and thus prolong drug retention in lung (He et al., 2023a; Tahara et al., 2016; Yue et al., 2022).

2.4. Literature Review on Surface Modified Nanocrystals

Drug nanocrystal technology can offer an innovative nanomedicine of chemotherapeutic agents for either passive targeting as well as active targeting (Bai et al., 2021). Owing to their nano size and definite shape, nanocrystals (20-500 nm) can achieve passive targeting in leaky vasculature (abundant blood vessels, wide vascular wall spaces, poor structural integrity, and deficient lymphatic drainage) of tumor and thus exhibit superior antitumor activity (Bai et al., 2021; Zhou et al., 2016). However, larger sized nanocrystals are prone to clearance from the blood by reticuloendothelial system (RES) uptake. Therefore, further modification of nanocrystals surface with specific targeting ligands or molecules may require that can bypass the limitations of enhanced permeability and retention (EPR) effect, which is often weak and inconsistent in human tumors. This approach can also minimize non selective drug distribution, avoid drug clearance, thereby reducing the high dose requirement of anticancer drugs, mitigating the unwanted side effects, ultimately enhancing the effectiveness and safety of traditional nanomedicines used in chemotherapy (Lu et al., 2022). The targeting moiety like cetuximab, folic acid, RGD, hyaluronic acid, transferring, peptides and proteins can be used for surface modification of nanocrystals to specify the drug delivery (Kumar et al., 2020a; Lu et al., 2022).

The nanocrystals can be surface modified to improve the pharmacokinetic and pharmacodynamic performance of drug. For example; nanocrystals treated with Pluronic® F68 enhanced the systemic circulation and tumor accumulation potential of drug (Gao et al., 2016). PEGylation of nanocrystals can prolong the systemic circulation, as observed for paclitaxel nanocrystals (PTX-NCs). The size of rod-shaped

nanocrystals increased on PEG coating from 240 nm for PTX-NCs to about 330 nm for PEG-PTX-NCs. PEG-PTX-NCs showed higher stability and better anticancer activity than PTX-NCs and Taxol®, on intravenous administration (Zhang et al., 2015). The surface modification of nanocrystals with Soybean phosphatidylcholine (SPC) and MPEG-DSPE resulted in a modified nanocrystals with no burst effect, higher cellular uptake and improved anticancer activity as compared to unmodified and free drug. The surface modified nanocrystals also exhibited high drug content (~64 %), lower particle size (~50 nm), narrow particle distribution, excellent colloidal stability and sustained drug release. prepared surface modified nanocrystals expected to improve the therapeutic efficacy of paclitaxel and reduced the drug associated side-effects (Guo et al., 2017). Nanocrystals surface modified using DSPE PEG 2000 also improve the systemic circulation and slow the drug release. Resultant nanocrystals displayed 2-fold AUC while reducing the drug clearance also by about 2 fold, as compared to simple PTX-NC (Wang et al., 2019). Surface modified nanocrystals also have potential to improve drug residence at target site, provide superior transmucus penetration, avoid mucosal irritation, and multi-fold the tumor inhibition activity (Duan et al., 2024).

2.5. Literature Review on Paclitaxel and Nanoformulations

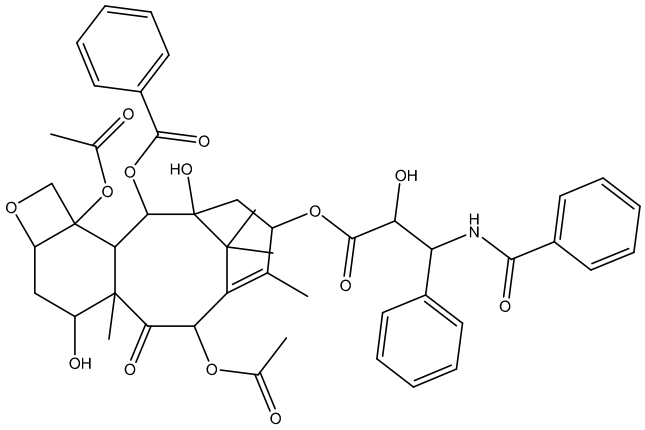

Figure 2.1 Chemical Structure of Paclitaxel

Paclitaxel is a naturally derived tricyclic diterpenoid with potent anticancer activity against all types of cancers, specially, lung, breast, cervical, prostate, colorectal, gastric, ovarian, bone, and brain tumors (Tong et al., 2022; Wen et al., 2016). Paclitaxel is usually given through *i.v.* injection and works by stabilizing the microtubules of cells and inhibiting late G2 or M phases of the cell cycle, causing cells to die. However, the clinical application of its natural form has been limited by its physicochemical characteristics like low aqueous solubility, poor permeability, and p-gp efflux (Haddad et al., 2022). The paclitaxel structure lacks functional groups that can be used for chemical modification to improve its properties. This necessitated the search for a suitable approach for improving the physicochemical properties of paclitaxel and, thus, anticancer efficacy. Although various approaches like the use of co-solvents or surfactants have been reported for improving the physicochemical and pharmacological properties of paclitaxel (Miele et al., 2009). These are associated with side effects or systemic toxicities. More specifically, Cremophor EL: ethanol (50:50 mixture) has been employed as a cosolvent system for formulation (Taxol® or generic equivalents) for intravenous administration(Joshi et al., 2001). Though the idea was suitable for overcoming the limited solubility of paclitaxel, it was associated with toxicities like hyperlipidemia, erythrocyte aggregation, hypersensitivity, sensory neuropathy, and neutropenia. Surfactant like Tween-80 has also been employed for improving drug solubility but are associated with hypersensitivity, peripheral neuropathy, and haemolytic activity (ten Tije et al., 2003). Therefore, there is an urgent requirement of an alternative strategies that not only enhance the physicochemical properties and in-vivo efficacy of drug but also avoid serious and dose-limiting toxicities.

Table 2.1 Physicochemical Properties of Paclitaxel.

Parameters/Properties	**Description**
Brand Name	Abraxane, Taxol
Generic Name	Paclitaxel
Synonyms	benzenepropanoic acid
Chemical name	5β,20-Epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R,3S)-N-benzoyl-3-phenylisoserine
IUPAC Name	[(1S,2S,3R,4S,7R,9S,10S,12R,15S)-4,12-diacetyloxy-15-[(2R,3S)-3-benzamido-2-hydroxy-3-phenylpropanoyl]oxy-1,9-dihydroxy-10,14,17,17-tetramethyl-11-oxo-6-oxatetracyclo heptadec-13-en-2-yl] benzoate
Molecular Formula	$C_{47}H_{51}NO_{14}$
Molecular Weight	853.90
Color	White to off-white crystalline powder
Melting Point	415 to 421 °F or 216-217 °C
Solubility	Insoluble in water, soluble in organic solvent
LogP	3
Optical Rotation	Specific optical rotation: -49 deg at 20 °C/ D (methanol)
UV max absorption	227-229 nm
Route of Administration	Intravenous
Metabolism	Hepatic, metabolized primarily to 6a-hydrox-ypaclitaxel by the cytochrome P450 isozyme CYP2C8; and to two minor

	metabolites, 3'-p-hydroxypaclitaxel and 6a, 3'-p-dihydroxypaclitaxel, by CYP3A4.
Toxicity	Myelosuppression, peripheral neuropathy, neutropenia, leukopenia, anemia, cardiovascular events, hypersensitivity reactions, and mucositis
Applications	Taxoid chemotherapeutic agent, first-line and subsequent therapy for advanced carcinoma; like ovary, breast and lung cancer.

2.6. Literature Review on Bosutinib and Nanoformulations

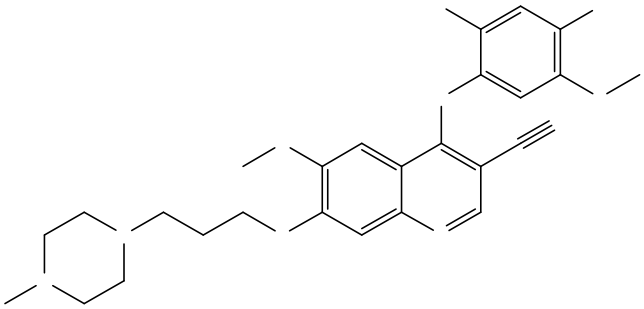

Figure 2.2 Chemical Structure of Bosutinib.

Bosutinib is an TKI that can potentially inhibit mutated oncogenes responsible for advanced-stage cancer (Isakoff et al., 2014). Bosutinib can inhibit ACK1, which attenuates migration and invasion in the context of KRAS mutant NSCLC to fulfil a therapeutic niche through combinatorial treatment approaches (Tan et al., 2014). Bosutinib is also reported to inhibit Src overexpressed in NSCLC for improved patient survival (Karim et al., 2022). Although bosutinib has been reported for cancer therapy, the clinical outcomes are limited due to poor aqueous solubility and permeability. Bosutinib belongs to BCS Class IV drug, exhibiting poor drug bioavailability of only 34 %. This necessitated the development of a dosage form that can effectively improve

the solubility and permeability issue as well as minimize systemic toxicity associated with non-selective drug distribution.

Table 2.2 Physicochemical Properties of Bosutinib

Properties	**Description**
Brand Name	BOSULIF
Synonyms	Bosutinib (SKI-606)
Chemical name	A nitrile, a N-methylpiperazine, an aromatic ether, a tertiary amino compound, an aminoquinoline and a dichlorobenzene, 7-alkoxy-3-quinolinecarbonitrile
IUPAC Name	4-(2,4-dichloro-5-methoxyanilino)-6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinoline-3-carbonitrile
Molecular Formula	$C_{26}H_{29}Cl_2N_5O_3$ or $C_{26}H_{31}Cl_2N_5O_4$ (Hydrate)
Molecular Weight	530.4 g/mol or 548.5 g/mol (Hydrate)
Color	White to off-white powder
Melting Point	131- 134 °C
LogP	3.34
Solubility	Insoluble in water, Soluble in ethanol
Administration Route	Oral (Tablet: 100, 400 and 500 mg; Capsule: 50 and 100 mg)
Bioavailability	34 %
Metabolism	Metabolized by CYP3A4 to major inactive metabolites of oxydechlorinated (M2) bosutinib (19%) and N-desmethylated (M5) bosutinib (25%) with minor inactive metabolite of bosutinib N-oxide (M6).

Toxicity	Hepatotoxicity
Application	An antineoplastic agent and a tyrosine kinase inhibitor

2.7. Literature Review on Fucoidan and Nanoformulations

Figure 2.3 Chemical Structure of Fucoidan.

Marine biopolymers have been widely used to design anticancer drug delivery systems (Jha et al., 2022). Marine biopolymers have extra edges over synthetic polymers owing to their natural abundance, cheap cost, biodegradability, biocompatibility, non-immunogenicity, and non-toxicity (Kumar et al., 2022). Fucoidan is a biopolymer obtained from brown seaweed. Fucoidan (FP) are sulphated polysaccharide with high hydrophilicity, biodegradability, biocompatibility and drug encapsulation potential (Jafari et al., 2020, p.; Venkatesan et al., 2022). Fucoidan has been used to formulate various drug delivery systems to deliver anticancer agents (Venkatesan et al., 2022; Zayed et al., 2022). In addition, fucoidan also exhibit antioxidant and antitumor activity (Lim and Wan Aida, 2017; Lin et al., 2020). Fucoidans are reported to form particles of around 200 nm that can efficiently internalize the cells to exert their action (Chiang et al., 2021). Fucoidan nanoparticles are said to target cancer cells owing to their strong

affinity towards P-selectin (Jafari et al., 2020). Fucoidan being anionic can provide electrostatic stabilization to nanocrystals (Li et al., n.d.; Lu et al., 2014). Therefore, fucoidan was exploited for the fabrication of drug nanocrystals.

Table 2.3 Physicochemical Properties of Fucoidan

Properties	**Description**
Source	*Undaria pinnatifida* found in the waters of China, Japan, Korea, New Zealand, and south-eastern Australia
Synonym	Wakame, brown seaweed, brown alga
Molecular Weight	5 to 1000 kDA
Organoleptic properties	Brown color
Solubility	Water Soluble

2.8. Literature Review on Soluplus and Nanoformulations

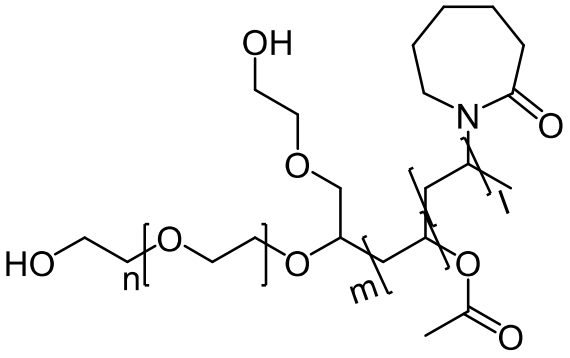

Figure 2.4 Chemical Structure of Soluplus.

Soluplus is a triblock copolymer of Polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol. It is amphiphilic and biodegradable with high dilution stability and outstanding solubilization properties for poorly soluble APIs. It can act as a novel crystallization inhibitor for nanocrystal formulation.

Table 2.4 Physicochemical Properties of Soluplus

Properties	**Description**
Chemical name	Polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft co-polymer
Molecular Weight	Approximately 118,000 g/mol of average weight (90 000 – 140 000 g/mol)
Organoleptic properties	White to slightly yellowish granule with a faint characteristic odor
Critical Micelle Concentration	7.6 mg/L form y 70 to 100 nm in diameter (pH 7 buffer)
HLB	Approximately ~14
Glass transition	~70 °C
Solubility	Soluble in water, acetone (50%), methanol (45%), ethanol (25%), dimethylformamide (50%) and in mixtures of (1:1 m/m) methanol/ acetone (50%) and (1:1 m/m) ethanol/acetone (45%)
Angle of Repose	27.5 °
Density	1.082 g/cm^3