Nanocarriers: More than tour de force for thymoquinone

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Nanocarriers: More than tour de force for thymoquinone

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Abstract

Introduction: Thymoquinone (TQ), 2-isopropyl-5-methylbenzo-1, 4-quinone, the main active constituent of Nigella sativa (NS) plant, has been proved to be of great therapeutic aid in various in vitro and in vivo conditions. Despite the promising therapeutic activities of TQ, this molecule is not yet in the clinical trials, restricted by its poor biopharmaceutical properties including photo-instability.

Area covered: This review compiles the different types of polymeric and lipidic nanocarriers (NCs), encapsulating TQ for their improved oral bioavailability, and augmented in vitro and in vivo efficacy, evidenced on various pathologies. Furthermore, we provide a comprehensive overview of TQ in relation to its encapsulation approaches advancing the delivery and improving the efficacy of TQ.

Expert opinion: TQ was first identified in the essential oil of Nigella sativa L. black seed. TQ has not been used in formulations because it is a highly hydrophobic drug having poor aqueous solubility. To deal with the poor physico-chemical problems associated with TQ, various NCs encapsulating TQ have been tried in the past. Nevertheless, these NCs could be impending in bringing forth this potential molecule to clinical reality. This will also be beneficial for a large research community including pharmaceutical & biological sciences and translational researchers.

Keywords: *Nigella sativa*; Thymoquinone; nano formulations; bioavailability; polymeric nanoparticles; lipid-based formulations

Article highlights

• Thymoquinone, a strong antioxidant derived from black seed, is a promising therapeutic molecule

• Poor biopharmaceutical properties hinder its delivery through conventional methods

• Past research evidenced the usefulness of nano-carrier based strategies to enhance its bioavailability and therapeutic efficacy

• This review is focused on the compilation of various researches carried out on polymeric and lipidic nanocarriers for the improved delivery of thymoquinone

• Design of nano-carrier based strategies could be beneficial for a large research community including pharmaceutical, biological sciences and clinical translational researchers.

1. Introduction

Medicinal plants and their phytoconstituents hold a great therapeutic promise for various ailments, and thus in the recent times, a noteworthy upsurge in the scientific research has been noticed in the area of herbal medicines [1]. *Nigella sativa* (NS) (also known as black cumin seed; family Ranunculaceae) is one of the most promising medicinal plants, generally grown in the Mediterranean region and western Asia (India, Pakistan, and Afghanistan). The biological activity of NS is attributed to its potential chemical constituent i.e., thymoquinone (TQ) (Figure 1). TQ (2-isopropyl-5-methylbenzo-1, 4-quinone), imparts 30–48% of whole constituents of NS seed oil [2]. It was first isolated by El-Dakhakhny in 1963 from NS black seeds using thin-layer chromatography [3]. However, it is also found in other plants like *Eupatorium ayapana,* the leaves of several *Origanum* species, the heartwood essential oils of *Calocedrus decurrens*, oil of different *Satureja* species, aerial flowering parts of *Thymus vulgaris* L. and *Nepeta distans* [4]. TQ belongs to the monoterpenoid class of benzoquinone having the molecular formula $C_{10}H_{12}O_{2,}$ and corresponding to a molecular weight of 164.20g/mol. It contains a basic

quinone ring conjugated to a methyl, and an isopropyl side chain in positions 2 and 5 respectively [5].

The various therapeutic properties of TQ in *in vitro* and *in vivo* models have been reported extensively in the literature [6]. Besides, its anti-oxidant [7], anti-inflammatory [8], chemo-protective and chemo-curative [9] ability is remarkably good. It can also interact with a range of proteins and is competent in inhibiting protein-protein interactions [10]. In normal tissues, TQ acts as a robust anti-oxidant, and inhibits the production of superoxide radicals and lipid peroxidation, or enhance the activities of the antioxidant enzymes like superoxide dismutase (SOD), catalase, reduced glutathione (GSH), glutathione S-transferase, and quinone reductase [11]. However, in tumors cells, TQ induces reactive oxygen species (ROS) generation, and decreases GSH levels in a dose-dependent manner [12]. To understand the molecular mechanisms of TQ, recognition of its binding targets and identification of distribution profiles inside a biologic system can tremendously help [13]. However, in the past, a limited number of studies have been reported pertaining to absorption and disposition of TQ. This could be attributed to its high hydrophobicity, poor solubility and stability in biological fluids [14].

A nanotechnology-based strategy for the effective delivery of thymoquinone could be the most promising area for overcoming the aforementioned limitations related to its poor biopharmaceutical properties. Encapsulation of TQ in the various nanocarriers could improve its *in vivo* solubility, stability, bioavailability, targeted delivery as well as protect it from the unspecific binding [15]. Thus, TQ-Nanoparticles (NPs) are fetching more clinically attractive options than pure TQ owing to their enhanced activity in modulating disease targets *in vitro* and *in vivo* [16,17].

Various researchers have extensively reviewed the therapeutic potential of TQ, mainly for its use as an anti-oxidant, anti-inflammatory, and as an anti-cancer agent. However, the present review is focused onto give an account on different polymeric and lipidic NCs for modulating its biopharmaceutical properties and therapeutic activities.

2. Challenges in the delivery of TQ

Hydrophobicity and poor aqueous solubility of TQ pose problems in the appropriate formulation, and subsequently result in poor systemic bioavailability, and thus might require a higher dosing as shown in figure 2. [18]. Besides, TQ is reported to have low chemical stability due to its degradation in physiological environments and have a high first pass metabolism [19]. Its sensitivity for pH, temperature, and light also poses hurdles in the successful formulation development [20,21]. Therefore, the clinical transition of this molecule had remained far from reality. In the past, various NCs have attempted to overcome the challenges in the delivery associated with this molecule. These NCs based on the biocompatible and biodegradable materials are mainly polymers and lipids. The lipidic NCs include liposomes, niosomes, proniosomes, SLNs, NLCs, lipospheres, nanoemulsions, and SNEDDS, while, polymeric DDS include mainly PLGA, PEG, Chitosan, and cyclodextrin NPs as presented in figure 3.

3. Nanocarriers

3.1. Polymeric NCs

Polymeric nanoparticles have several attributes that make them favorable for drug delivery. These include biodegradability, controlled or sustained release, biocompatibility with tissues and cells and tunable particle size. They are comparatively nontoxic and are stable in blood and lack immunogenicity and thrombogenicity [13]. Polymeric NCs can be useful in targeting the drugs to sensitive regions like brain and central nervous system (CNS) as it can cross the blood brain barrier (BBB). These NCs are mainly evaluated in cancer and brain targeting. NCs can enhance the bioavailability and bioactivity of TQ due to particle size in the nano size range, surface modification, and protecting it from the harsh biological environments. The polymeric NCs could also modulate the pharmacokinetics and diffusion of TQ into various organs by crossing the barriers. A tabular account of various polymeric NCs encapsulating TQ is entailed in the Table 1

Polymeric nanoparticles can be formulated by several approaches depending on the drugs to be encapsulated. Polymeric nanomaterials are drug transporters made of natural, synthetic, or semisynthetic polymers in the nanoscale range. Natural polymers are the polymers that are produced by living organisms. These highly valuable materials can be modified to meet the desired needs of biomedical applications. The natural materials employed for TQ-loaded NCs preparation include chitosan, gum rosin, alginate etc. Synthetic polymers that are exploited for TQ-loaded NCs construction composed of either biodegradable or non-biodegradable polymer backbone, e.g., polyvinylchloride, polyethylene glycol (PEG), Poly lactic-co glycolic acid (PLGA), polyhydroxyalkanoate (PHA), and cylodextrin (CD). In the current section general preparative technology of TQ-loaded NCs employing these aforementioned natural and synthetic polymers is explained. Besides, the research evidences obtained during past decades, regarding the usefulness of these NCs for the biopharmaceutical improvement of TQ, and targeting to different organs, are also enumerated.

3.1.1 Preparation of NCs

TQ-loaded polymeric NCs were mainly prepared by three methods i.e. nanoprecipitation, solvent-evaporation, and ionic gelation method. In the first method, the polymer is dissolved in acetone and then dropwise addition into the aqueous phase, maintained on a continuous rotation. The organic solvent is then evaporated under vacuum. In contrast, the emulsification solvent evaporation method comprises of polymers dissolved in volatile organic solvent, which then added into the continuously rotating aqueous phase, with or without emulsifier and sonicated. Both methods have simple procedures and are appropriate for the encapsulation of lipophilic drugs [22]. The key differences between both methods lie in the scale-up and entrapment efficiency. In the emulsification solvent-evaporation method, the chances of scaling up is comparatively less because it requires high energy in homogenizing and the entrapment efficiency is reasonable too. In contrast, the nanoprecipitation method is easily scaled up and has high entrapment efficiency, which makes it the most commonly used method for the preparation of PLGA nanoparticles [23]. TQ-loaded chitosan NCs are mainly prepared by the ionic gelation method, which is a natural linear

biopolyaminosaccharide. In this method, nanosized particles are prepared by mixing two aqueous phases, one containing chitosan, and the second containing poly-anion sodium tripolyphosphate. The positively charged amino group of chitosan interacts with negatively charged groups of tripolyphosphate to form coacervates, which subsequently leads to the formation of nanosized particles using emulsion cross-linking technique [24].

3.1.2 Natural polymer based TQ-loaded NCs

3.1.2.1 TQ-loaded chitosan NCs

Chitosan (CS), a linear copolymer of β-(1,4)-2-acetamido-D-glucose and β-(1,4)-2-amino-D-glucose, derived from chitin is the most abundant natural polysaccharide after cellulose with good biocompatible properties [25]. TQ-loaded CS–NCs have studied in the past to target various organs like liver and brain. These CS-NCs are proved to enhance the targeting of TQ to the various tissues owing to the cationic nature of the CS, and small size, which can extravagate through biological barriers such as the blood-brain barrier, and thus enhance the therapeutic effectiveness of the encapsulated drug [26].

Alam et al. determined the biodistribution and pharmacokinetics of TQ-loaded CS NCs *via*. nose-to-brain targeting [19]. The NCs formulated by ionic gelation method, and characterized for particle size by dynamic light scattering, morphology by TEM and SEM, *in vitro* kinetics and *ex vivo* release, and X-ray diffractometry (XRD) studies, for investigation of the physical form of the drug inside the NC. Findings suggested the smaller particle size (200 nm) with good entrapment efficiency (63.3%). TQ-NC were spherical in shape, amorphous nature, revealed sustained release pattern and enhanced drug permeation. Furthermore, pharmacokinetics studies revealed approximately 15-fold enhancement in brain targeting efficiency of TQ-CS-NCs in comparison to the TQ solution. This might be due to the cationic TQ-CS systems, which showed higher targeting efficiency due to the interaction of a positively charged amino groups present on the carbon two position of CS, with negatively charged groups on the cell membranes. Similarly, other possible mechanisms could be its ability to cross the

tight junctions of the mucosal epithelial cells. Moreover, smaller particle size of NCs and lipophilic nature of TQ might have resulted in the enhanced partitioning across the BBB.

In another study, Zafar et al. prepared CS-grafted lipid nanocapsules for the co-delivery of docetaxel (DTX) and TQ in the drug-resistant breast cancer cells, i.e., MCF-7 and triple-negative MDA-MB-231 cells [27]. These nanocapsules prepared employing high-speed homogenization and ultrasonication methods and optimized employing 3^3-Box-Behnken design to get the desired quality attributes. Endosome escape study was also performed for the selective and efficient delivery of drug in the tumor site. Results revealed that the optimized nanocapsules exhibited high drug loading of both the encapsulated drugs DTX and TQ, uniform particle size (< 200 nm), and controlled drug release. The CS being a cationic polymers showed pH buffering properties and thus facilitated the delivery of TQ into the cytosol, improved cellular uptake aided in the endosomal escape effect, and led to a significantly higher cytotoxicity against MCF-7 and triple-negative (MDA-MB-231) breast cancer cells. The enhanced effect of these CS nanocapsules might be due to the protonation, which leads to the extensive influx of ions and water into the endosomal sections, initiating the disruption of the endosomal membrane, thus delivering the entrapped TQ.

Recently, Othman et al. were able to demonstrate that CS NCs could encapsulate one hydrophobic (TQ) and other hydrophilic (L-ascorbic acid) drug together, employing ionic gelation method [28]. Results showed that NPs were found to be in the nanosized range, spherical and had good encapsulation efficiency for both the encapsulated drugs. In one study, Aljoufi et al. prepared and evaluated TQ-loaded CS lipidic NCs for the effective treatment of the liver disorder. TQ-loaded CS vesicle were prepared employing solvent evaporation and probe sonication method [29]. The developed NCs were then characterized for vesicle size, entrapment efficiency, morphology, *in vitro* drug release, *ex vivo* drug permeability, mucoadhesive properties and anti-hyperlipidemic activity. Results showed that the prepared NCs showed nano size range (372.8 nm), low PDI (0.175), high encapsulation efficiency (82.23%), optimum drug release profile, significantly higher flux (1.9 fold) and mucoadhesive property (4.4 fold)

vis-à-vis free TQ. Findings from anti-hyperlipidemic activity showed the significant changes in biochemical parameters (SGOT, SGPT, and ALP) and lipid profile (TC, LDL, HDL), which was further confirmed by histopathological evaluation. Histopathology of liver treated with toxic control revealed the inflammatory cells, swelling of hepatocytes and occurrence of hepatitis whereas TQ-loaded NCs treated groups showed minimal central vein inflammation. These lipidic NCs coated with chitosan prolong the circulation time, modify the release behavior, drug targeting, improve the drug permeability and enhance drug stability. These NCs can also prevent the absorption of plasma protein by providing a hydrophilic steric stabilization to the surface of lipid vesicle and thus, improve the absorption of TQ by efficiently crossing the barriers.

3.1.2.2 Gum-rosin-loaded polymeric nanocapsules

Nanocapsules are the typical class of polymeric NCs, composed of one or more active drug substance (core) and a protective matrix (shell) made up of polymeric or lipidic membrane, in which the active constituents may be encapsulated. Nanocapsules have attracted tremendous interest as they can be utilized for the controlled and targeted release of drugs contrary to the protection of enzymes, proteins, and foreign cells [30,31].

Rani et al. formulated two different nanoformulation (NFs), one is glycyrrhizin (GL) loaded nanocapsule prepared via ionic gelation method and other is TQ-loaded gum rosin nanocapsule prepared employing nanoprecipitation method. Both NFs in combination or separately were studied for anti-hyperglycemic potential in streptozotocin-nicotinamide induced type-2 diabetes rat model [32]. The prepared NFs were characterized for particle size, stability, morphology, and *in vivo* behavior. TQ-loaded NCs were found to be stable, spherical in shape with nanometric size range (100 nm) and sustained release behavior as compared to their pure forms. Results from *in vivo* studied endorsed that combined NFs were significantly able to decrease blood glucose level, glycate haemoglobin and improve the lipid profile of diabetic rats as compared to metformin in a dose-dependent manner. This enhanced anti-diabetic effect of NFs might be due to improvement in its bioavailability and increase drug

concentration in the blood, owing to the advantage of nanoscale therapeutics. Additionally, improved anti-diabetic action is the synergistic effect of two NFs, which ultimately improved pharmacological activity.

3.1.3 Synthetic polymer based TQ-loaded NCs

3.1.3.1 TQ-loaded PLGA NCs

PLGA is one of the most abundantly used polymers for the preparation of nanomedicine, which has also been approved by the US-FDA. It has minimal systemic toxicity as it gets hydrolyzed in the body to biodegradable lactic acid and glycolic acids, which are metabolized in the body *via* the Krebs cycle and removed as carbon dioxide and water [33]. Past studies report that PLGA is a non-toxic polymer grounded on cell culture and animal experiments [34,35]. TQ-loaded PLGA NCs were used to target different types of cancer and for their anti-microbial potential.

Nallamuthu et al. determined the anti-oxidant and anti-bacterial potential of TQ-loaded PLGA NCs [36]. TQ-loaded PLGA NCs were prepared by solvent evaporation method and characterised for particle size, morphology, entrapment efficiency, *in vitro* release, antioxidant and anti-microbial activity. The *in vitro* anti-oxidant ability of encapsulated TQ was assessed employing DPPH radical scavenging assay. Whereas, anti-bacterial property were tested by modified agar-well diffusion method against *E. coli, Staphylococcus aureus*, and *Salmonella typhi* strains. Results from particle size and SEM studies revealed the mean particle size of < 200 nm and %EE of about 62%. However, *in vitro* drug release study showed sustain release of TQ at 75% and 54 % respectively for artificial intestinal and gastric juices over the period of 7 days and DPPH radical scavenging activity of the TQ-NCs was found to be 71% at 1 mg/mL concentration. The results showed that PLGA encapsulated TQ–NPs were able to offer sustained release property and enhanced antioxidant as well as anti-microbial activity *vis-a-vis* pure TQ [37].

In other study, Ganea et al. evaluated the anti-cancer potential of TQ using molecular micelle modified PLGA NCs for breast cancer [38]. The NCs were synthesized

employing emulsification solvent evaporation method, using the molecular micelle poly (sodium N-undecenyl-glycinate) (poly-SUG) as an emulsifier and optimized employing Box-Behnken experimental design. TQ-loaded NCs were evaluated for particle size and %EE. The cytotoxic effect of TQ and TQ-loaded PLGA NCs were assessed using MDA-MB231 breast carcinoma cells. Findings showed that molecular micelles provided maximum optimized TQ entrapment efficiency, and uniform particle sizes (200 nm). TQ-loaded PLGA NCs showed approximately five-fold enhancement in cell viability than blank NCs and non-treated cells, and effectively able to inhibit the growth of breast carcinoma cells vis-à-vis free TQ [39]. In breast cancer cells, TQ interfered with PI3K/Akt signaling, and stimulated G(1) arrest, and thus induced cell apoptosis. Moreover, TQ inhibited p53-mutated acute lymphoblastic leukemia cells by the activation of a p73-dependent mitochondrial cell [40,41]. However, enhanced anti-cancer effect of NCs might be due to the interactions between cells and NCs, which leads to the accumulation of NCs in the cells, and thus release the TQ in the extracellular and intracellular spaces.

Similarly, Abdel-Mottaleb et al. evaluated anti-cancer potential of TQ-NCs employing different polymers *viz*. PLGA, ethylcellulose (EC) and polycaprolactone (PCL) for colorectal cancer employing murine mouse model [42]. Solvent evaporation technique was employed for the fabrication of NCs. Prepared NCs were then characterized for particle size, PDI, entrapment efficiency, surface morphology, *in vitro* release and *in vivo* studies. Results showed that the particle sizes were in the nano range with all the polymers used, but TQ-PCL-NCs showed maximum uniformity in size and stability. Further, particles were spherical in shape with smooth surface texture. *In vitro* drug release showed burst release of 50% TQ in the first hour followed by sustain drug release. Findings obtained from *in vivo* studies were similar to Ganea and co-authors, suggesting the superiority of TQ-NCs as compared to the pure TQ, in terms of tumor growth retardation and animal survival. TQ acts as a potent inhibitor of the NF- κB pathway and reduces tumor angiogenesis [43-46]. Besides, TQ bindings to oncogene PAK1, thus changes its conformation and scaffold function, which further interferes with RAF/MEK/ ERK1/2 pathway and controls cancer cell growth [47]. Furthermore, authors

suggested that TQ encapsulation into polymeric NCs could enhance its uptake by the cancer cells especially with the leaky vasculature along with poor lymphatic drainage into tumors, which is also known as the enhanced permeation and retention effect (EPR).

Similarly, Verma et al. also determined the anti-cancer potential of Topotecan-TQ-loaded PLGA NCs employing MTT assay in HEK293 cell lines [48]. Topotecan-TQ-loaded PLGA NCs were formulated employing modified double emulsion solvent evaporation method and optimized using central composite design (CCD). These NCs were then characterized in terms of zeta potential, surface morphology, DSC, XRD and drug release. Optimized formulation showed nano-range particle size (240.7±8.3 nm) with uniform size distribution, good percent entrapment, and loading efficiency (62.6±2.6 and 6.52±0.25) for thymoquinone and for topotecan (42.3±1.2% and 3.6±0.26) respectively. DSC and XRD studies revealed the conversion of drug from its crystalline to amorphous form when entrapped inside the PLGA NCs. Findings were in agreement with previously reported studies suggesting the maximal activity of TQ co-encapsulated PLGA NCs, while offering exposure to tumor cells for a prolonged period of time vis-à-vis pure TQ. Authors proposed that co-encapsulation of topotecan and TQ could be an effective therapy for the treatment of solid tumors. While topotecan is a well acknowledged and broadly used drug for the treatment of various cancers, approved by the FDA, TQ has been extensively explored over fifty years in various carcinomas [49].

3.1.3.2 TQ-loaded PEG NCs

Polyethylene glycol (PEG) is a water-soluble, non-toxic, non-immunogenic and FDA-approved polymer [50]. PEG restricts the passage through the blood-brain barrier thus abolishing neurotoxicity linked with free drug or phytochemical and prolongs the circulating half-life of the free drugs or phytochemicals. PEG has been used as a polymer for the encapsulation of several drugs *viz*. doxorubicin [51] and paclitaxel [52] for treatment of cancer. Anti-cancer potential of TQ-loaded PEG NCs were also studied in the past. This polymer is self-assembled into amphiphilic NCs having a hydrophobic core and hydrophilic shell.

Shah et al. determined the neuroprotective effect of amphiphilic polyhydroxyalkanoate (PHA) monomethoxy polyethylene glycol (mPEG) co-polymeric nano-containers [16]. Co-polymer were synthesized *via.* chemical coupling of poly (3-hydroxybutyrate-co-3-hydroxyvalerate), P(3HB-co-3HV) or poly(3-hydroxybutyrate-co-4-hydroxybutyrate), P(3HB-co-4HB) to mono-methoxy poly(ethylene glycol) (mPEG) through trans-esterification reaction. Findings suggested that the encapsulation of TQ into NPs showed the extended release for TQ compared to pure TQ.

Anti-cancer potential of TQ-loaded PEG4000-NCs was studied by Bhattacharya et al. *via* deregulation of cytoskeletal actin polymerization through miR-34a [17]. TQ-loaded PEG4000-NCs were formulated employing nanoprecipitation method and characterized for particle size and surface morphology. Particle sizes of TQ-PEG4000-NCs were found to be less than 50 nm and nearly spherical morphology with smooth surface texture. Results validated that PEG4000-TQ-NCs significantly augmented the expression of miR-34a through p53. NCs also mediated miR-34a up-regulation, directly downregulated Rac1 expression, monitored by actin depolymerization, thereby disrupting the actin cytoskeleton, which significantly retards the cell migration. In addition, authors evidenced that PEG4000-TQ-NCs exhibited strong specificity to cancer cell migration, showing less toxicity towards the normal cells in comparison to that of TQ alone at a significantly lesser dose than pure TQ.

3.1.3.3 TQ-loaded CD NCs

CD are the family of cyclic oligosaccharides generally including 6–8 d-glucose units, forming inclusion complexes with altered molecules in aqueous solution and in the solid state [53]. CD have been used as complexing agents in many pharmaceutical preparations to improve the solubility, bioavailability, safety and stability of different drugs including anti-cancer drugs [54]. TQ-loaded CD-NCs were prepared to evaluate the anti-cancer potential of TQ.

Abu-Dahad et al. evaluated the anti-proliferative potential of TQ-β-CD self-assembling NCs [55]. These NCs were characterized for particle size, zeta potential, morphology, DSC and FT-IR. Findings suggested that average particle size was found to be 445±100nm with a charge 21.8mV and nearly spherical morphology. Further, the safety

was estimated *via*. cell viability studies employing normal periodontal fibroblasts and anti-proliferative activity by means of the adenocarcinoma cell lines (MCF-7). A very less IC50 value (4.70±0.60 microM) for TQ-CD NCs *vis-à-vis* free TQ solution (24.09 ±2.35 microM) was observed after 72 h of incubation, which means TQ-CD NCs had higher anti-proliferative effects in comparison to free TQ. This enhanced anti-proliferative potential of TQ-CD-NCs, is mainly due to improved cellular permeation. In addition, TQ-CD NCs were found to be less toxic against human periodontal fibroblasts vis-à-vis free TQ**.** Previous reports suggested that TQ could induce apoptosis in MCF-7 breast cancer cells via the up-regulation of p53 expression. Besides, TQ significantly increased the expression of miR-34a via p53, and down-regulated Rac1, led to actin depolymerization, and interruption of the actin cytoskeleton. This damage in the actin cytoskeleton hampered the cell migration [47].

3.1.3.4 Silica NCs

In recent study Khattabi et al. studied the *in vitro* cell toxicity of prepared thymoquinone-melatonin (TQ-MLT) silica NP's towards HeLa cells [56]. Findings suggested that longer polymers showed a more sustained release in a pulsatile manner. The *in vitro* cell viability assay also exhibited that the percentage of cell toxicity to HeLa cells increased with increasing the polymer length. In HeLa cell lines, TQ were found to down-regulate the androgen receptor (AR) and regulate E2F-1 cell proliferation.

3.1.4 Hybrid NCs

Hybrid NCs comprise organic–inorganic, lipidic, polymeric, and natural macromolecule/synthetic polymer based NCs. Hybrid NCs can be prepared via encapsulation and grafting of inorganic components or natural macromolecules by (co)polymerization, precipitation of polymers in the presence of inorganic constituents by solvent displacement techniques [57]. These hybrid NCs exploit the benefits of both systems (lipid and polymer/organic and inorganic materials) in terms of different characterization parameters [58].

3.1.4.1 TQ-loaded PLGA-CS NCs

Xiaxo et al. prepared TQ-loaded PLGA-CS NCs by emulsion solvent evaporation method and characterized for particle size and zeta potential [59]. Findings suggested that TQ-loaded PLGA-CS NCs showed particle size in nano range (183.5±8.2 nm) with positive zeta potential, (33.63±2.25mV). These NCs were evaluated for neuroprotective efficacy and delivered via nose to brain route in the rodent cerebral ischemia-reperfusion model. The pharmacokinetics of TQ-loaded PLGA-CS NCs were also studied in the brain and blood plasma along with localization studies of florescent labelled PLGA-chitosan NCs in brain tissues. Authors showed that the pharmacokinetic and localization studies facilitated the delivery of TQ to brain by intranasal nose to brain transport pathways. The authors found approximately 28-fold enhancement in systemic bioavailability. Authors mentioned that this enhanced intranasal effect was due to improved paracellular transport through epithelial tight junctions via. interaction with the protein kinase C pathway or electrostatic interaction with negative charged sialic acid residues on mucosal epithelial cells.

3.1.4.2 TQ-loaded PEG modified CS Nanocapsules

Kumar et al. evaluated the TQ-loaded PEG modified CS NCs for anti-cancer potential employing MCF-7 cell lines and HEK 293 human embryonic kidney cell lines. TQ-loaded PEG modified CS NCs were prepared *via.* ionic gelation method [60]. Optimization of prepared NCs was done based on particle size, surface morphology, PDI, entrapment efficiency, % yield, *in vitro* release, FT-IR and X-RD studies etc. Findings from *in vitro* drug release studies revealed that TQ-loaded PEG modified CS NCs showed slow and sustained release *vis-a-vis* pure TQ, whereas 100% inhibition of breast cancer cells was observed in 50 mg/mL and 100 mg/mL concentration at 48 h of incubation. Authors suggested that CS readily solubilized in the intracellular and intercellular acidic environment of tumor cells owing to its pKa value (6–6.5), which then release TQ directly in the cancer site and thus prolonged bioavailability in the intracellular environment.

3.1.4.3 TQ-loaded PLGA-PEG-loaded NCs

Admad et al. studied the cytotoxic effects of TQ-PLGA-PEG NCs in Tamoxifen-resistant breast cancer cells [61]. Cytotoxicity studies performed employing MTT assay exhibited enhanced IC50 of TQ-NCs at 20.05 µM and free TQ was 8.25 µM respectively. Findings suggested that the bioavailability of drug found to preserve within NCs compared to free TQ and act gradually on target cells.

3.2. Lipidic NCs

As stated earlier, the major challenge in TQ-loaded formulations is their low aqueous solubility. Lipid-based delivery systems, therefore, provides a noble approach, to improve the bioavailability and stability of sensitive materials amid numerous advantages in drug delivery. Furthermore, the formulation in a lipid matrix system can sustain the drug release, thus contributing to a decrease in the peak drug concentrations in systemic circulation and probably, to avoid side effects attributed to oral delivery. The drug-loaded lipidic NCs can also use to target the particular disease in a specific organ. Lipidic NCs systems are classified mainly into two categories, i.e., particulate type and non-particulate types. Particulate type systems solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), dendrimers, nanogels, microparticles, microsphere, liposomes, niosomes, proniosomes etc. Whereas, non-particulate type carrier-systems include microemulsions, nanoemulsion, and self-nanoemulsifying drug delivery systems (SNEDDS), etc. A tabular account of various lipidic NCs encapsulating TQ is entailed in the Table 2

3.2.1 Preparation of TQ loaded lipidic NCs

TQ-loaded lipidic NCs were mainly prepared by four methods i.e. solvent-injection, microemulsification method, high-speed homogenization, and nanoprecipitation method. Among these methods, solvent injection method is the simplest one. In this method lipid first dissolved in water miscible organic solvent (ethanol, acetone, isopropanol). This solution then injected through a syringe needle in water under stirring. Lipid is precipitated as nanoparticles when encounters water. In microemulsification method, drug dissolved in organic solvent (ethanol, acetone, isopropanol). The lipids are heated

above their melting point (lipid phase), whereas surfactants and co-surfactants are dissolved/dispersed in a portion of water (aqueous phase). Then all the phases i.e., lipid phase, aqueous phase and the drug solution mixed isothermally to form a clear microemulsion. The clear microemulsion so formed then added into ice-cold water at 4°C and continuously stir at 3000 rpm for about 20 min to fetch the nanoparticles. In high-speed homogenization, the polymer and drug heated, melted, and then dispersed, in the aqueous phase containing lipid by continuous mixing, to obtain an emulsion. Emulsion then subjected to high pressure, high-speed impact, and decompression expansion, after which high shear forces steadily break down the fluid droplets to the desired nanoparticle size range. In nanoprecipitation method, polymers and drugs first dispersed in water miscible solvents (e.g., acetone and acetonitrile). Then, the resulting solution added drop wise into a lipid-containing aqueous phase, and the mixed by spinning and homogenization to obtain nanoparticles [62].

3.2.2 Particulate type NCs

3.2.2.1 TQ-loaded NLCs

NLCs are novel colloidal lipid-based systems that create a hybrid blend of incompatible solid and liquid lipids. TQ-loaded NLCs resulted in enhanced pharmacokinetic, bioavailability, controlled drug release and ultimately improved drug absorption by protecting the drug from extensive first-pass metabolism, P-gp efflux of the drug transporters, and intra-enterocyte metabolism. These characteristics of NLCs are mainly attributed to their unique composition, which is constituted of a blend of incompatible solid and liquid lipids [63]. TQ-loaded NLCs were used to target liver and cancer cells. Abdelwahab et al. reported the gastro-protective activity of TQ-NLCs against ethanol-induced ulcers in rats and pharmacokinetic profile was evaluated in the rabbits [64]. TQ-loaded NLCs were evaluated for particle size, zeta potential and *in vitro* toxicity. Findings suggested that the particles size was in nano-range (75 ± 2.4 nm) with negative zeta potential values of −31 ± 0.1 mV. Findings from the *in vivo* study suggested that TQ-NLCs suppressed the formation of ethanol-induced ulcers *via* the modulation of heat shock protein-70 (Hsp70). Moreover, extravascular administration of TQ-NLCs showed enhanced pharmacokinetic profile *viz.* increased bioavailability and

sustained concentrations in blood. Similarly, Elmowafy et al. evaluated the TQ-loaded NLCs for oral bioavailability and hepato-protective activity compared to pure TQ [65]. TQ-loaded NLCs were prepared by high-speed homogenization followed by ultrasonication and evaluated for particle size, polydispersity index, zeta potential and *in vitro* studies. Hepato-protective potential of TQ-NLCs were evaluated employing biochemical parameters and histopathological evaluation. Optimized TQ-NLC formulation showed smaller particle size (141.9±5.1), smaller PDI (0.2), and negative zeta potential values (−58.6±0.5 mV) with high encapsulation efficiency (96.2±1.6%). Pharmacokinetic results revealed that there was approximately 3-fold enhancement in relative bioavailability. Enhanced hepato-protective potential of TQ-NLCs was observed vis-à-vis TQ suspension based on biochemical parameters. This enhanced effect could be due to stabilization of the membranes, which then prevents the leakage of intracellular enzymes. Further, owing to the anti-oxidant potential, TQ exhibited a decline in malondialdehyde and elevation of reduced glutathione levels.

Keat et al. studied the anti-cancer potential of TQ-NLCs on breast cancer cell lines (MCF-7 and MDA-MB-231) and cervical cancer cell lines (HeLa and SiHa) [66]. TQ-NLCs prepared employing high-pressure homogenization and then characterized for various physicochemical parameters and stability. Findings suggested that the mean particle size of TQ-NLC was in nano range (35.66 ± 0.1235 nm) with a narrow polydispersity index (PDI) (0.25) and negative zeta potential (−30 mV). Pharmacodynamic studies showed that TQ-NLCs exhibited enhanced anti-proliferative activity against all the cell lines in a dose-dependent manner *vis-à-vis* pure TQ. TQ induced apoptosis and non-phase specific cell cycle arrest in MDA-MB-231 cells, suggesting it to be a potentially effective chemotherapeutic agent against hormonal independent breast cancer.

Recently, Rathore et al., studied the hepato-protective potential of TQ in the form of phospholipidic nanoconstructs (PNCs) employing Paracetamol-induced hepato-toxicity animal model [67]. PNCs were constructed employing microemulsification technique and optimized by three-factor three level Box-Behnken design. Results showed that

optimized PNC composition exhibited nano size (<100 nm), spherical morphology, within acceptable range of polydispersity index (0.55), high drug entrapment efficiency (>90%), controlled drug release pattern, and neutral surface charge (zeta potential of −0.65 mV). TQ-PNC showed approx. 3.9-fold enhancement in the relative bioavailability vis-à-vis TQ-suspension. Pharmacodynamic data showed a significant decrease in the serum biomarker enzymes in PNCs treated group vis-a-vis control and marketed (SILYBON®) formulations against paracetamol (PCM)-induced liver cirrhosis. This enhanced effect might be due to the higher cellular permeability of TQ achieved by PNCs. PNCs, and are anticipated to interact with the membrane lipids of liver cells resulting in physiologically significant effects. Moreover, the presence of lipidic carrier facilitates capture by the liver and other organs

3.2.2.2 TQ-loaded SLNs

SLNs was introduced in the early 90s and are categorized by the presence of a mixture of one or more solid lipids responsible for controlled drug release. Solid lipid nanoparticles (SLNs) are novel colloidal drug delivery system, formulated to protect the drug from chemical degradation to attain controlled drug release ability, good tolerability, biodegradability, physical stability and efficient encapsulation of lipophilic drugs in their lipid structure [68]. TQ-loaded SLNs can be formulated to target various organs like liver and brain and for bioavailability enhancement [69]. SLNs have potential to be used as alternative drug delivery system for many lipophilic molecules.

Pathan et al. developed a rapid, sensitive and selective UPLC method to estimate TQ in pure form and in NCs formulation (TQ-SLNs) [12]. TQ successfully quantified in TQ-SLNs formulation employing *in vitro* as well as oral *in vivo* pharmacokinetic study. Findings suggested the 2-fold enhancement in the relative bioavailability of TQ-SLNs in the rat's plasma, when administered orally *vis-à-vis* pure drug. Similarly, in other study Singh et al., prepared and evaluated TQ-SLN for modulation in the pharmacokinetics and its hepato-protective activity [70]. TQ-SLNs were prepared by solvent injection method and optimized using BBD. Findings showed that optimized TQ-SLNs were of desired characteristics in terms of particle size (166.1±10.96 nm), entrapment efficiency

(71.60±3.85%) and high drug release (70.95±2.47%). In pharmacokinetic study, authors found nearly 5-fold enhancement in the bioavailability of TQ-SLN compared to pure TQ suspension. Findings from pharmacodynamic data exhibited a significant decrease in the serum biomarker enzymes in TQ-SLNs treated group vis-a-vis control and marketed (SILYBON®) formulations against paracetamol (PCM)-induced liver cirrhosis. It could be due to the passage of TQ-SLNs *via* the endothelial fenestrations, where the hepatic stellate cells (HSCs) are present. A direct inhibition of the activated HSCs by TQ might be the reason of improved hepatoprotective activity of TQ-SLN. In other findings, TQ-loaded SLNs studied for their brain targeting ability [71]. The SLNs prepared by microemulsifcation method and evaluated for 3-nitroproponic acid induced Huntington's disease-like symptoms in Wistar rats, pharmacokinetics and bio-distribution analysis. Pharmacokinetic results were similar to Singh et al. as they also found nearly 5-fold enhancement in oral bioavailability of TQ *vis-à-vis* pure TQ. Further, the drug distribution analysis showed that TQ-SLNs was found to accumulate more in the brain than other organs, hence suitable for the brain targeted drug delivery. Authors asserted that TQ-SLNs comprised of lecithin, a choline containing phospholipid might have resulted in the acetylcholine synthesis and helped in the reduction of various behavioral disturbances in 3-NP intoxicated animals. Moreover, TQ being an anti-oxidant moiety, significantly reduced the endogenous protein carbonyls and lipid peroxidation. Similarly, the same group have also assessed the potential of TQ-loaded SLNs in neuroinflammation and motor abnormalities *via* 3-NP induced Huntington's disease [72]. Findings were similar to the previously reported findings suggesting TQ-SLNs treatment significantly eradicates the nuclear translocation of p-p65 NF-kB and levels of pro-inflammatory markers *viz.* TNF-α, IL-1β, IL-6, iNOS, COX-2. The beneficial effect of TQ-SLNs might be endorsed by the anti-inflammatory potency of TQ.

Recently, our lab has reported TQ-loaded phospholipid NCs (PLNs) prepared *via* micro-emulsification technique employing different phospholipid concentrations, and extensively characterized for particle size, surface charge, surface morphology, entrapment efficiency, and drug release kinetics [8]. Furthermore, these NCs were evaluated for oral bioavailability and anti-inflammatory potential employing rat paw edema model. Results showed that particle size was found to be in nanosized range

(100nm), higher drug entrapment efficiency (> 70%), controlled drug release pattern (Higuchi release), and negatively charged surface (zeta potential of −0.57 mV). After oral administration of single dose of TQ-PLNs approximately 2.3-fold enhancement in relative bioavailability was observed vis-à-vis plain TQ suspension. Findings from pharmacodynamic study showed significantly higher reduction in the percent paw inhibition in case of TQ-PLNs (84.27± 2.93%) vis-à-vis pure drug suspension (81.10± 4.79%) and diclofenac sodium (83.01± 2.63%) respectively. The improved oral bioavailability of the drug from lipidic matrix was probably because of the nano-sized lipidic PLNs. Furthermore, absorption of PLNs through intestinal lymphatic uptake directly transport the drug to the systemic circulation, thus bypassing the liver and resulting in enhanced oral bioavailability vis-a-vis pain TQ. Whereas, the enhanced anti-inflammatory potential of PLN formulation attributed to the encapsulation of drug in lipidic matrix, which subsequently resulted in the slow drug release, for prolonged period of time, vis-à-vis plain TQ, and marketed formulation.

3.2.2.3 TQ-loaded Niosomes

Niosomes are non-ionic surfactant vesicles, have a special role in improving poor bioavailability, stability and acts as a solubilizing matrix, and a local depot for sustained release These are spherical lipid bilayers capable of entrapping water-soluble molecules within an aqueous domain or alternatively lipid molecules within lipid bilayers [73]. They may be unilamellar or multilamellar depending upon the method used for their preparation TQ was encapsulated inside niosomes to target the cancer.

Rajput et al. evaluated the anti-cancer potential of TQ-loaded multilamellar Gold niosomes (Nio-Au-TQ) and small interfering RNA (siRNA) [74]. siRNA-based targeted delivery holds potential as a tumor-selective gene silencing approach in cancer therapy. The prepared niosomes were tested *in vitro* against tamoxifen-resistant (MCF-7/Tam and T-47D/TAM) and Akt-overexpressing (MCF-7/ Akt) cells and *in vivo* in a BALB/c (nu+/nu+) mouse xenograft model of MCF-7/TAM. Results revealed the concentration-dependent decrease in cell growth and viability for TQ, Nio-Au-TQ, and siRNA-Nio-Au-TQ. Further, result showed that siRNA-Nio-Au-TQ is much higher cytotoxic than TQ and

Nio-Au-TQ in resistant breast cancer cells, which might be elucidated *via.* sustained siRNA and drug release. In addition, *in vivo* findings suggested that Nio-Au-TQ and siRNA-Nio-Au-TQ showed significantly higher inhibition of tumor proliferation and apoptosis than pure TQ. Authors elucidated that net positive charge present in niosomes enables its binding to negatively charged cell membranes followed by internalization via endocytosis. Endosomal escape caused due to cationic gold in the niosomes, which induces endosomal swelling and hence rupture. During endosomal rupture, the influx of protons creates an acidic environment that stimulates the dissolution of the niosomes inside the cytosol and thus maintain the therapeutic drug concentrations deep inside the tumor tissues.

3.2.2.4 TQ-loaded proniosomes

Proniosomes are the latest approach in the family of vesicular systems. It is the pro-vesicular approach to niosomes, which are converted to niosomes upon hydration [75]. It escapes many problems related to aqueous niosome dispersion *viz.* aggregation, fusion, leaking, and thus, offers a versatile vesicle delivery concept. There are a number of literature reports available, which prove the usefulness of oral proniosomal formulation for enhanced solubility and bioavailability for hydrophobic drug molecules [76].

Sayeed et al. prepared TQ-loaded proniosome based formulation for hepato-protective activity against methotrexate induced hepato-toxicity [77]. TQ-loaded proniosome prepared by thin-film hydration technique and characterized for particle size and entrapment efficiency. The size of vesicle found to be in nano-metric range with higher encapsulation efficiency. Findings from hepato-protective activity revealed that TQ-loaded proniosomes significantly inhibited the elevated levels of liver enzymes, serum marker enzymes and improved histopathological abnormalities. This decrease in the level of serum marker enzymes suggested that TQ might be effective in the prevention of lipid peroxidation.

3.2.2.5 TQ-loaded liposomes

Liposomes are the most ideal drug-carrier system, composed of a phospholipid bilayer and aqueous core offering the encapsulation of both lipophilic and hydrophilic molecules. Their morphology is similar to that of cellular membranes and because of their ability to incorporate numerous constituents [78]. TQ-loaded liposomes mainly formulated to target different types of cancer cells and to treat bacterial infections, offer various advantages *viz*. improve dissolution profile and bioavailability [79].
Odeh et al. formulated TQ-loaded liposomes, evaluated its anticancer potential in T47D, and MCF-7, breast cancer cell lines, and periodontal ligament fibroblasts cells (PLF) [80]. The liposomes prepared by thin-film hydration technique and evaluated for particle size and entrapment efficiency. Results suggested that TQ-loaded liposomes were significantly effective in suppressing the proliferation in breast cancer cell-lines and showed very low toxicity on normal periodontal ligament fibroblast. Authors elucidated that liposomes led to the enhancement of bioavailability, permeability, EPR and sustained drug release of the TQ in the tumor cells as compared to the pure TQ.
Recently, the same group co-encapsulated Docetaxel (DT) and TQ into PEGylated liposomes and valuated for cytotoxic effect against MCF7 breast cancer cell lines. DT/TQ laded PEGylated liposomes prepared by thin film dispersion method and characterized for encapsulation efficacy. Findings suggested that the combination of DT and TQ resulted in significant synergistic cytotoxicity compared to alone drugs. Authors explained that the cytotoxic effect induced by DT and TQ can be *via* blocking of the PI3K/Akt signaling pathway.

In other study, Khan et al. formulated TQ-loaded liposomes for the treatment of *Candida albicans* infection against murine mouse model [81]. The anti-fungal activity of fluconazole, free TQ and TQ-loaded liposomes were measured through fungal load on the kidney tissue of treated mice. Findings confirmed that fluconazole and free TQ were also effective against *Candida albicans* but TQ-loaded liposomal formulation was significantly effective against both, fluconazole susceptible or resistance *Candida albicans* infection. Further, the authors concluded that TQ countered the anti-fungal activity *via the* immune escaping method. TQ was shown to induce ROS generation,

which managed to initiate the immune response of the host against *Candida albicans*. Similarly, Ahmad et al. evaluated TQ-loaded liposomes for radioprotection and enhanced blood circulation time employing the supercritical anti-solvent technique [82]. TQ-liposomal batch was evaluated for *in vitro* drug release, *in vivo* pharmacokinetic studies and *in vivo* radioprotection effect in rats employing γ-irradiation. Results confirmed that prepared TQ-liposomes were able to prolong circulation, whereas, results from *in vivo* study was translated into enhanced radioprotection for longer duration vis-à-vis pure TQ drug.

3.2.3 Non-particulate type systems

3.2.3.1 TQ-loaded SNEDDS

Self-nano emulsifying drug delivery systems (SNEDDS) is very popular owing to their numerous worthy aspects like microscopic globule size, easy to prepare, improved biocompatibility and higher stability [83]. SNEDDS primarily establish the blend of lipids, surfactants, co-surfactants, and/or co-solvents experiencing spontaneous emulsification. Pre-dissolving the drugs in the mixture of lipidic and emulsifying excipients omits the disintegration/dissolution steps, which are possible rate-limiting factors for oral absorption of poorly water-soluble drugs [84].

Kalam et al. determined the hepatoprotective effect of prepared TQ-SNEDDS for augmentation of its hepatoprotective effects and oral bioavailability [85]. TQ-SNEDDS were formulated *via* construction of pseudo-ternary phase diagrams and characterized for thermodynamic stability, particle size and morphology. Results revealed the 3.87-fold enhancement in oral bioavailability of TQ-SNEDDS in comparison with TQ suspension. *In vivo* hepatoprotective investigations exhibited significant hepatoprotective effects for optimized TQ-SNEDDS vis-à-vis TQ suspension.

3.2.3.2 TQ-loaded nanoemulsions

Nanoemulsions are nano-sized emulsions, manufactured for improving the delivery of active pharmaceutical materials. They are the thermodynamically stable and heterogeneous system where two immiscible liquids are mixed to form a single phase

by means of an emulsifying agent, i.e., surfactant and co-surfactant. This nanosized delivery system is proved to enhance the therapeutic efficacy of drug substance. Nanoemulsion delivery system increases the retention time of a drug in the body, so low amount of drug is required for the therapeutic action [86,87].

Tubesha et al. prepared TQ rich fraction NE for potential toxicity studies in Sprague Dawley rats as per the OECD guidelines [88]. At the end of the study, various parameters *viz*. body weight, hematological parameters, liver and kidney functioning test, and histopathology were studied. Findings suggested that TQ rich fraction NE was non-toxic by the oral route in Sprague-Dawley rats at a dose limit of 20 mL/kg. Results of various parameters of TQ rich fraction NE were compared to the control groups, suggested a wide range of safety for its therapeutic doses. Moreover, no hepatic toxicity was seen in histopathology evaluation. In other study, Dehghani et al. evaluated TQ-loaded nanogel on human breast adenocarcinoma cell line (MCF-7) employing MTT and dye exclusion assay [89]. Findings suggested that the proliferation of MCF-7 cells was significantly inhibited by TQ-loaded nanogel formulation in comparison to pure TQ in a dose-dependent manner. Whereas, Ahmad et al. evaluated TQ-loaded mucoadhesive NE for the treatment of cerebral ischemia [90]. The prepared NE was also validated for pharmacokinetics, biodistribution and brain-targeting efficiency. The results suggested that intranasal to brain targeting revealed enhanced bioavailability of TQ in brain *vis-à-vis* intravenous administration. Improved neurobehavioural activity (locomotor and grip strength) was detected in middle cerebral artery occlusion induced cerebral ischemic rats after intranasal administration of NE *vis-à-vis* pure TQ. Similarly, Ismail et al. evaluated TQ rich fraction-loaded NE for treatment of Alzheimer's disease (AD) in response to high fat/cholesterol diet (HFCD) induced rats [91]. Neuroprotective effect of TQ-rich fraction NE, TQ-NE and conventional emulsion were investigated in response to high fat/cholesterol diet (HFCD)-induced rats. Amyloid-β (Aβ) generation; abnormal amyloid-β precursor protein (APP) processing, β-secretase 1 (BACE1), γ-secretases of presenilin 1 (PSEN1) and presenilin 2 (PSEN2), Aβ degradation; insulin-degrading enzyme (IDE), Aβ transportation; low density lipoprotein receptor-related protein 1 (LRP1) and receptor for advanced glycation end products (RAGE) were

measured in brain tissues. Findings suggested that TQ-NE reduced the accumulation of Aβ in brain that further modulate β- and γ-secretase enzyme activity, and the Aβ degradation and clearance from the brain tissues.

El-Ashmawy et al. prepared doxorubicin (DOX) and TQ-loaded F2 gel nanofibers for enhanced antitumor activity and amelioration of doxorubicin-associated nephrotoxicity [92]. Antitumor potential was studied employing MCF-7 and HEPG2 cells lines and evaluated for apoptosis alongside with cellular proliferation. Findings suggested that nanofibre gel formulation showed a significant increase in apoptosis, caspase 3, and antioxidant enzymes; in comparision to, dramatic fall in cell viability, tumor volume, oxidative and nephrotoxicity markers, and NF-ʁB vis-à-vis free drug therapies.

4. Conclusions

An extensive pharmaceutical research on TQ was done in the past aiming to enhance the stability, improve oral bioavailability, permitting high therapeutic plasma drug concentrations, and minimize the toxicity associated with the drug. In this context, in the recent past years, various polymeric NCs, nanocapsules, nanoemulsions, liposomes, SLNs, NLCs, niosomes have witnessed the enhanced bioavailability and bioactivity of TQ. Despite of an enhanced efficacy over free TQ, TQ nano-formulations have not reached the clinical trials. Towards that aim, human trials should be conducted on TQ nano-formulations to establish the toxicological profiles of these formulations, and to approve their efficacy over free TQ. Only thus, it will be possible to evaluate the real contribution that nanotechnology embraces in the delivery of TQ.

5. Expert opinion

Plant based bioactives have been used in the prevention and treatment of diseases throughout history due to their wide acceptability. Natural bioactives with antioxidant and anti-inflammatory properties have been used in the therapy of various chronic diseases. TQ is one such anti-oxidant molecule, which first identified in the essential oil of *Nigella sativa* L. black seed. During the past years, several studies have shown bright potential of TQ as an antioxidant, anti-inflammatory, and anti-cancer molecule in *in vitro*

and *in vivo* models. However, owing to its poor physico-chemical properties, its chances for FDA authorization and development as a potent medicine clinically is still delayed. TQ belongs to monoterpenes class of chemicals, and like other terpenoids is highly hydrophobic, poor aqueous solubility, high first-pass metabolism, and thus poor oral bioavailability. To deal with poor biopharmaceutical properties associated with TQ, various novel drug delivery colloid-carrier systems encapsulating TQ attempted in the past, with enhanced bioavailability, and therapeutic efficacy. Novel carrier systems based on biocompatible polymers and lipids, protect the drug from external environment, and from the first pass metabolism. These drug delivery systems have the capability to improve the solubility and bioavailability of orally administered, poorly water-soluble and/or lipophilic drugs, while the carrier-based concept of drug delivery was employed. Nevertheless, these carrier systems could be impending in bringing forth this potential molecule to clinical reality.

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Figure legends

Figure 1: Molecular structure of TQ

Figure 2: Schematic representation of the various biopharmaceutical, formulation, and patient-related hurdles associated with the delivery of TQ

Figure 3: Schematic illustration of absorption mechanisms employed by polymeric NCs for oral bioavailability enhancement of TQ presented by the GI tract. Drug transport *via.* epithelial cells as well as reversibly open tight junctions to allow for biologic transport through the paracellular pathway. M-cell mediated transport pathway is associated with the Peyer's Patches, which further enhances lymphatic absorption of the drug and finally the drug absorption

Figure 4: Schematic illustration of absorption mechanisms employed by lipidic nanoparticles for oral bioavailability enhancement of drug. These encompass enhanced permeability across the enterocyte, absorption via. M cells of Peyer's patches, increased transcellular and paracellular transport. Transcellular routes further include transport of drug via. enterocytes viz. macro and pinocytosis, which further enhances the intestinal lymphatic transport and thus, the drug absorption

Figure 5: Various NC systems encapsulating TQ for management of various diseases

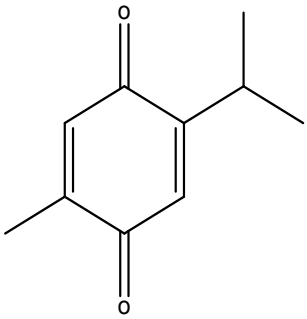


Figure 1

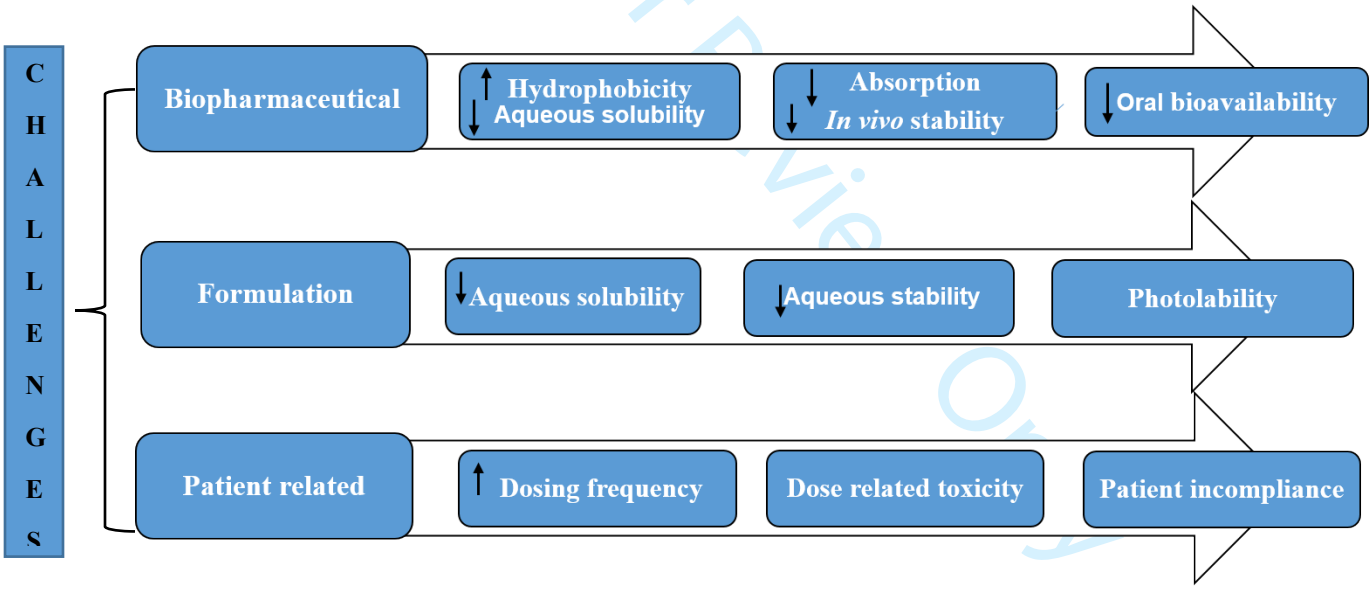


Figure 2

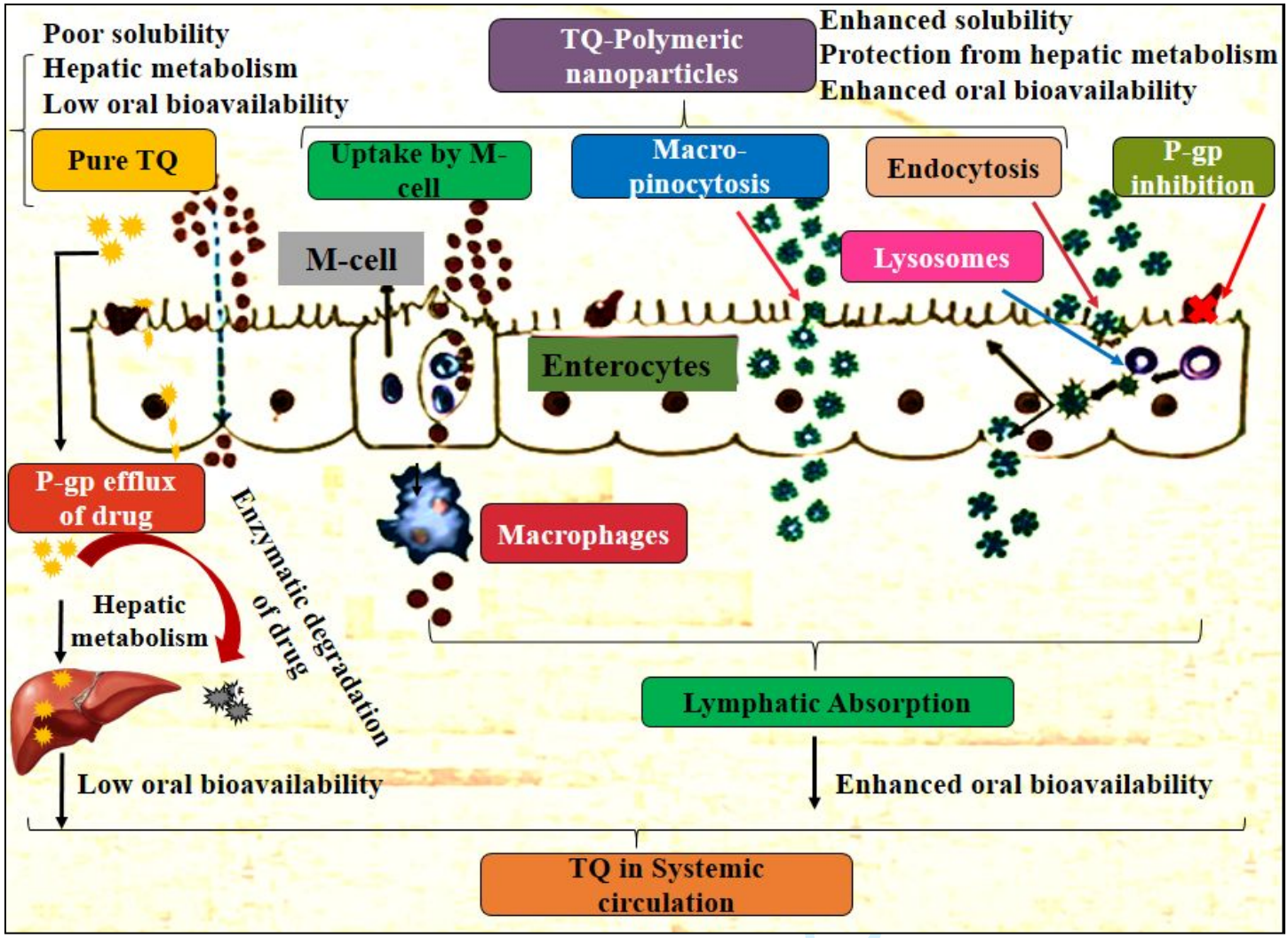


Figure 3

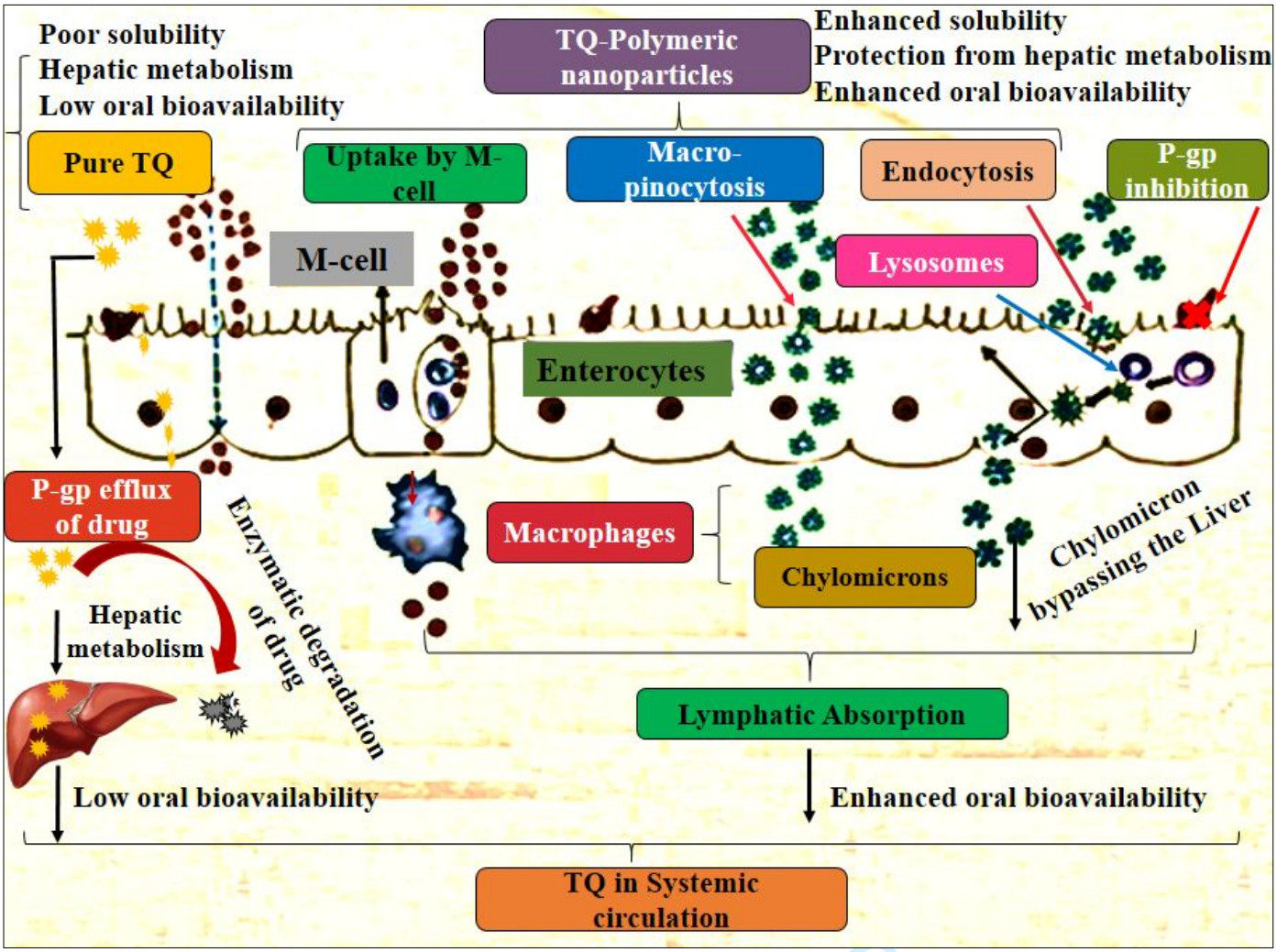


Figure 4

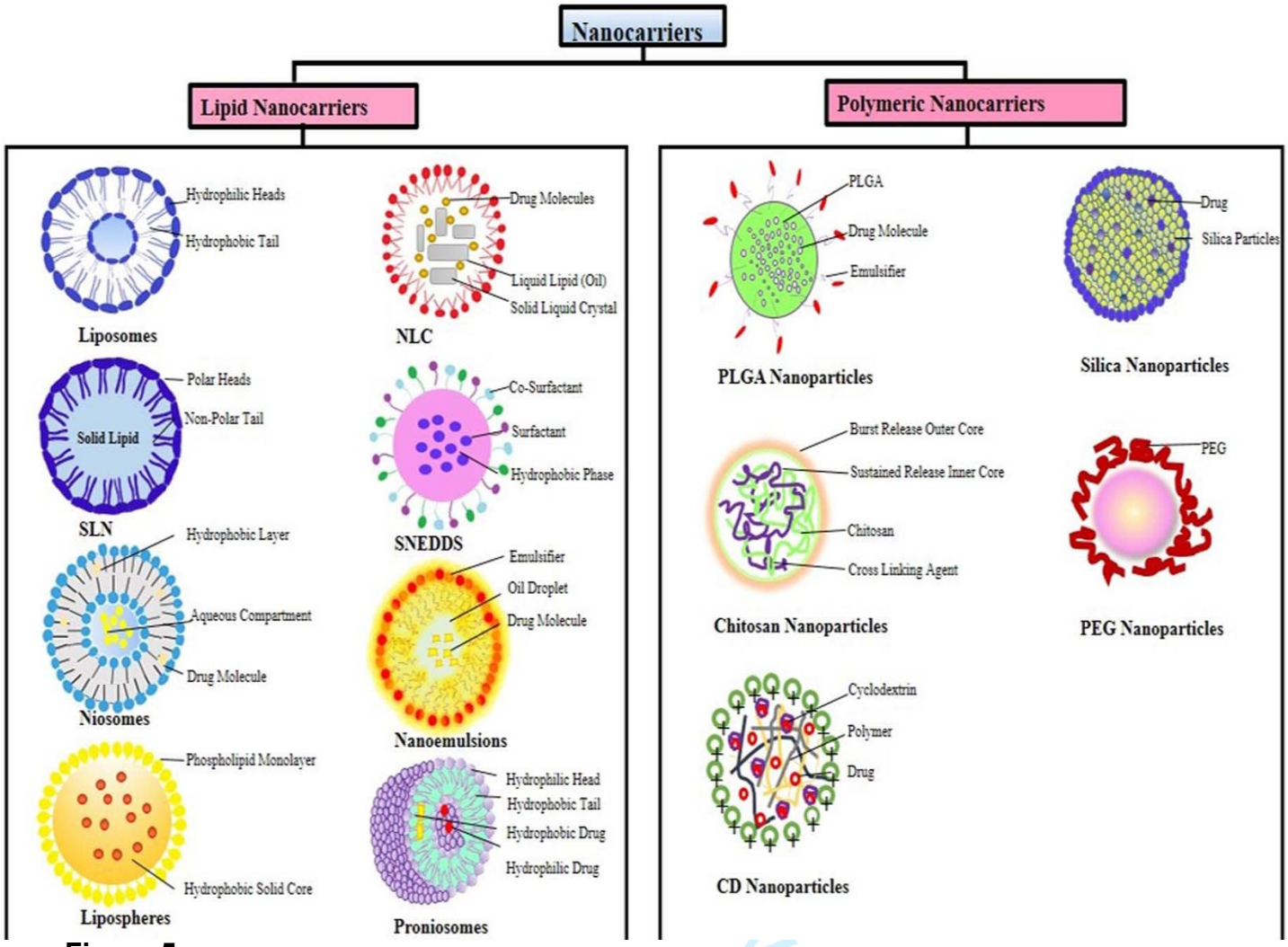


Figure 5

List of tables

Table 1: A tabular account of various TQ-loaded polymeric NC formulations

Table 2: A tabular account of various TQ-loaded lipid NC formulations

Table 1

Formulation	Polymer	Method	Disease/Delivery	Animal model/chemical/strains	Observations	Ref.
Natural polymer-based	Chitosan	Ionic gelation method	Neurodegenerativ/Alzheimer disease	Nose-to-brain drug-targeting	TQ-loaded nanoparticles (TQ-NP1) showed more Effective brain targeting compared to intravenous and intranasal TQ solution	Alam et al., 2012
	Chitosan	Ionic gelation method	Dual drug loaded NCs	-	Enhanced therapeutic effect *via* combining different classes of drugs (hydrophilic	Othman et al., 2019

					and hydrophobic) together	
	Chitosan	Solvent evaporation and probe sonication method	Hepatic diseases	ip injection of the freshly prepared solution of Triton X-100	Significant changes in biochemical parameters (SGOT, SGPT, and ALP) and lipid profile (TC, LDL, HDL)	Aljoufi et al., 2019
	Gum-rosin	Nanoprecipitation method	Diabetes	Streptozotocin-nicotinamide induced type-2 diabetes rat model	Significantly able to decrease blood glucose level and glycate haemoglobin ; and improve the lipid profile	Rani et al., 2018
Synthetic polymer based NPs	PLGA	Solid-in-oil-in-water (s/o/w) solvent evaporation	Anti-oxidant and anti-microbial	Modified agar-well diffusion method against *E. coli, Staphylococcus aureus* and *Salmonella typhi* strains	Exhibited antibacterial property against *E. coli, Staphylococcus aureus* and	Nallamuthu et al., 2013

				in vitro DPPH assay	*Salmonella typhi* strains Whereas, DPPH radical scavenging activity showed that TQ-NP's was found to be 71% at 1 mg/ml conc.	
	PLGA	Emulsification solvent evaporation	Breast cancer	MDA-MB231 breast carcinoma cells	Showed outstanding anticancer properties vis-à-vis free TQ	Ganea et al., 2010
	PLGA-NP's	Solvent evaporation	Colorectal cancer	Murine mouse model	TQ-NPs Showed enhanced therapeutic effects by inhibiting NF- κB pathway and reduced tumor angiogenesis	Mona M.A. Abdel-Mottaleb et al., 2016

					suppressed the expression of androgen receptor and E2F-1giogenesis	
	PLGA	Modified double emulsion solvent evaporation	Anti-tumor	MTT assay *via* HEK293 cell line	NP's Enhanced anti-tumor activity of TQ for a longer duration of time	Verma et al., 2017
	PEG	Emulsification –solvent evaporation	Neurodegenerative	Rat neuronal hippocampal cells and NIH/3T3 fibroblast cell line.	Enhanced stability and amphiphilicity of the particles making them an easy source to assemble into nanosized core-shell structures in aqueous solution.	Shah et al., 2010 and 2011

	PEG	Solvent evaporation technique/ nanoprecipitation technique	Breast cancer	Human mammary carcinoma cell lines (MCF-7, HBL-100)	Showed Significantly increase in miR-34a expression through p53. NPs mediated miR-34a up-regulation directly down-regulated Rac1 expression	Bhattacharya et al., 2015
	CD	Ionic gelation	Anti-cancer	(MCF-7) cell lines	Showed higher anti-proliferative effects and less toxicity in comparison to free TQ	Abu-Dahad et al., 2012
	Silica	Ionic gelation	*in vitro* cell toxicity	HeLa cells lines	Findings suggested that longer polymers showed sustained release in a	Khattabi et al., 2018

					pulsatile manner cell toxicity to HeLa cells showed with increasing the polymer length	
Hybrid NPs	PLGA-Chitosan	emulsion solvent evaporation method	Neurodegenerative	cerebral ischemia-reperfusion model	TQ-loaded PLGA-chitosan NP's facilitated the delivery of TQ to brain Enhanced pharmacokinetic profile in brain tissues	Xiaxo et al., 2016
	PEG modified chitosan	ionic gelation method	Bioavailability enhancement	Pharmacokinetic study	Showed sustained release vis-a-vis pure TQ	Kumar et al., 2019
	PLGA-PEG	Nanoprecipitation technique	Breast cancer	MTT assay	TQ-NP's showed IC50 of at 20.05 µM and free TQ was 8.25	Ahmed et al., 2017

					μM exhibiting its cytotoxic potential	

Table 2

Lipidic formulation	NC	Method	Disease/study	Animal model/chemical/strains	Observations	Ref.
Particulate type	**NLC**	High-pressure homogenization	Gastric ulcers	Ethanol-induced ulcers	Showed inhibition in gastric ulcers *via* modulation of heat shock protein-70 (Hsp70).	Abdelwahab et al., 2013
	NLC	High speed Homogenization	Hepato-toxicity	PCM-induced Hepatic toxicity	Significantly decreased serum alanine amino transferase and aspartate amino transferase enzyme level and showed enhancement in 2, 3 fold in relative bioavailability	Elmowafy et al., 2015

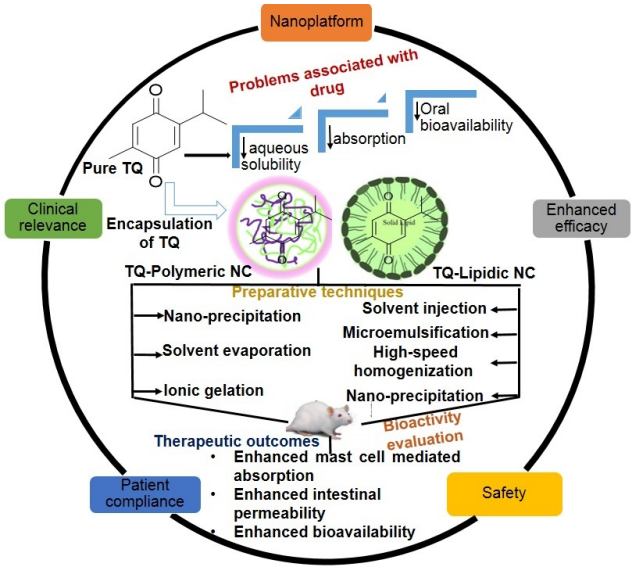
	NLC	hot high-pressure homogenization	Breast cancer	(MCF-7 and MDA-MB-231) cell lines	Exhibited anti-proliferative activity against all the cell lines in dose-dependent manner	Keat Ng et al., 2014
	SLN	Precipitation method	Quantification of TQ and TQ-SLN via UPLC	*In vitro* and *in vivo* pharmacokinetic	Showed two-fold increase in the relative bioavailability of TQ-SLN	Pathan et al., 2010
	SLN	Solvent injection	Hepato-toxicity	PCM-induced hepatic toxicity	Inhibited 5-lipoxygenase and 5-hydroxy-eicosatetraenoic acid production	Singh et al., 2013
	SLN	hot homogenization	In vitro cytotoxicity	In vitro cell viability assay	TQ-SLNs showed concentration-dependent increase in cytotoxic activity	Surekha et al., 2015
	SLN	hot homogenization	Huntington's disease (HD)	3-nitropropionic (3-NP) acid toxin model	Showed changes the mitochondrial succinate dehydrogenase (SDH) inhibition and alter anticholinergic effect upon 3-NP induction	Ramachandran et al., 2016

	SLN	hot homogenization	Huntington's disease (HD)	3-nitropropionic (3-NP) acid toxin model	Showed to impend the glial cell activation and, N-methyl-D-aspartate (NMDA) receptor stimulation, inhibit neuro inflammation and motor deficits	Ramachandran et al., 2017
	SLN	microemulsification	Inflammatory disease	Carrageenan induced paw edema	TQ-SLN showed substantially higher reduction in the percent paw inhibition increase of TQ-SLN vis-à-vis pure drug suspension	Rathore et al., 2019
	Niosomes	film hydration technique	TMX-resistant cancer	(MCF-7/Tam/T-47D/TAM) cell lines and mouse xenograft model	showed significantly higher inhibition of tumor proliferation and apoptosis than pure TQ	Rajput et al., 2015
	Pronioso mes	film hydration technique	hepato-protective	MTX-induced hepato-toxicity	significantly inhibited the elevated levels of liver enzymes, serum marker enzymes and improved	Sayeed et al., 2017

					histopathological abnormalities	
	Liposomes	thin-film hydration	breast cancer	(T47D, MCF-7) breast cancer cell lines and periodontal ligament fibroblasts cells (PLF)	significantly effective in suppressing the proliferation in breast cancer cell lines, and showed very low toxicity on normal periodontal ligament fibroblast	Odeh et al., 2012
	Liposomes	conventional thin-film hydration technique	Anti-fungal	murine mouse model	TQ countered the anti-fungal activity via the immune escaping method	Khan et al., 2014
	Liposomes	supercritical anti-solvent technique	radioprotection	γ-irradiation	TQ-liposomes prolonged circulation, whereas, *in vivo* study was translated into enhanced radioprotection	Ahmad et al., 2017
	Liposomes	ethanol injection method	Analgesic	analgesic animal model	Showed improved analgesic activity in mice in case of NS oil loaded liposomes vis-à-vis pure oil	Rushmi et al., 2017
Non-Particul	**SNEDDS**	Micoemulsification technique	Hepato-protective	CCl_4 induced hepato-toxicity	TQ-SNEDDS showed significant	Kalam et al., 2017

ate type					hepato-protective effect vis-à-vis pure drug suspension	
	Nanoemulsion	high-pressure homogenization	Potential toxicity studies	As per OECD guidelines 425, a test dose of 20 mL for TQ rich faction NE containing (44.5 mg TQ/kg) was given for 2 weeks.	TQ rich fraction NE suggested a wide range of safety for its therapeutic doses vis-à-vis control group	Tubesha et al., 2013
	Nanogel	self-assembly	Breast cancer	MCF-cell lines	TQ-loaded nanogel significantly inhibited the proliferation of MCF-7 cells was	Dehghani et al., 2015
	Nanoemulsion	ionic gelation method	cerebral ischemia	intranasal to brain targeting	nose to brain targeting revealed enhanced bioavailability of TQ in brain vis-à-vis intravenous administration	Ahmad et al., 2016
	Nanoemulsion	high–pressure homogenization	Neuro-protective	fat/cholesterol diet (HFCD) rats	TQ-NE reduced the brain Aβ fragment which further modulate β- and γ-secretase	Ismail et al., 2017

					enzyme activity, and the Aβ degradation and transportation in/out of the brain tissues	
	Nanofibre gel	film hydration	antitumor activity, and doxorubicin-associated nephrotoxicity	Heps liver carcinoma and MCF-7 and HEPG2 cells	Showed an increase in apoptosis, caspase 3, and antioxidant enzymes, inhibited in cell viability, tumor volume, oxidative and nephrotoxicity markers, and NF-ʁB	Zidan et al., 2018



Graphical abstract

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	Liposomes	ethanol injection method	Analgesic	analgesic animal model	Showed improved analgesic activity in mice in case of NS oil loaded liposomes vis-à-vis pure oil	Rushmi et al., 2017
Non-Particulate type	**SNEDDS**	Micoemulsification technique	Hepato-protective	CCl_4 induced hepato-toxicity	TQ-SNEDDS showed significant hepato-protective effect vis-à-vis pure drug suspension	Kalam et al., 2017
	Nanoemulsion	high-pressure homogenization	Potential toxicity studies	As per OECD guidelines 425, a test dose of 20 mL for TQ rich faction NE containing (44.5 mg TQ/kg) was given for 2 weeks.	TQ rich fraction NE suggested a wide range of safety for its therapeutic doses vis-à-vis control group	Tubesha et al., 2013
	Nanogel	self-assembly	Breast cancer	MCF-cell lines	TQ-loaded nanogel significantly inhibited	Dehghani et al., 2015

					the proliferation of MCF-7 cells was	
	Nanoemulsion	ionic gelation method	cerebral ischemia	intranasal to brain targeting	nose to brain targeting revealed enhanced bioavailability of TQ in brain vis-à-vis intravenous administration	Ahmad et al., 2016
	Nanoemulsion	high–pressure homogenization	Neuro-protective	fat/cholesterol diet (HFCD) rats	TQ-NE reduced the brain Aβ fragment which further modulate β- and γ-secretase enzyme activity, and the Aβ degradation and transportation in/out of the brain tissues	Ismail et al., 2017
	Nanofibre gel	film hydration	antitumor activity, and doxorubicin-associated nephrotoxicity	Heps liver carcinoma and MCF-7 and HEPG2 cells	Showed an increase in apoptosis, caspase 3, and antioxidant enzymes, inhibited in cell viability, tumor volume, oxidative and nephrotoxicity markers, and NF-κB	Zidan et al., 2018

Figure 1: Molecular structure of TQ

Figure 2: Schematic representation of the various biopharmaceutical, formulation, and patient-related hurdles associated with the delivery of TQ

Figure 3: Schematic illustration of absorption mechanisms employed by polymeric NCs for oral bioavailability enhancement of TQ presented by the GI tract. Drug transport *via.* epithelial cells as well as reversibly open tight junctions to allow for biologic transport through the paracellular pathway. M-cell mediated transport pathway is associated with the Peyer's Patches, which further enhances lymphatic absorption of the drug and finally the drug absorption

Figure 4: Schematic illustration of absorption mechanisms employed by lipidic NCs for oral bioavailability enhancement of drug. These encompass enhanced permeability across the enterocyte, absorption via. M cells of Peyer's patches, increased transcellular and paracellular transport. Transcellular routes further include transport of drug via. enterocytes viz. macro and pinocytosis, which further enhances the intestinal lymphatic transport and thus, the drug absorption

Figure 5: Various NCs system encapsulating TQ for management of various diseases

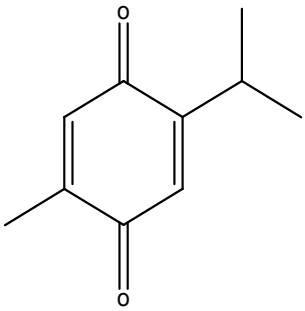

Figure 1

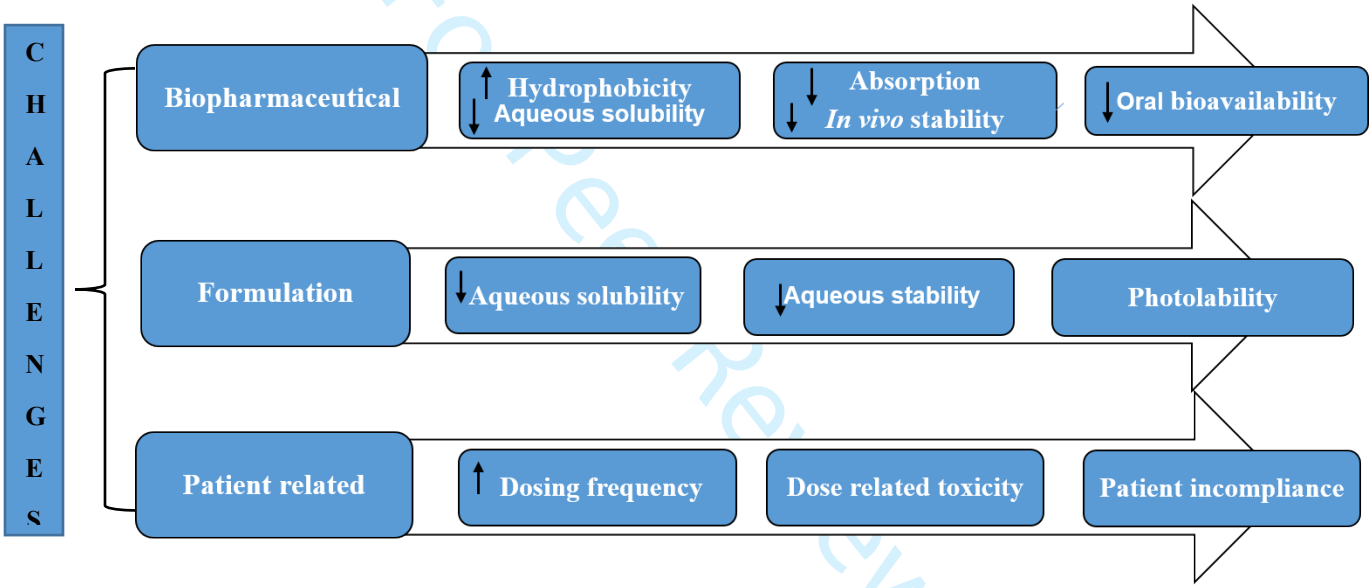

Figure 2

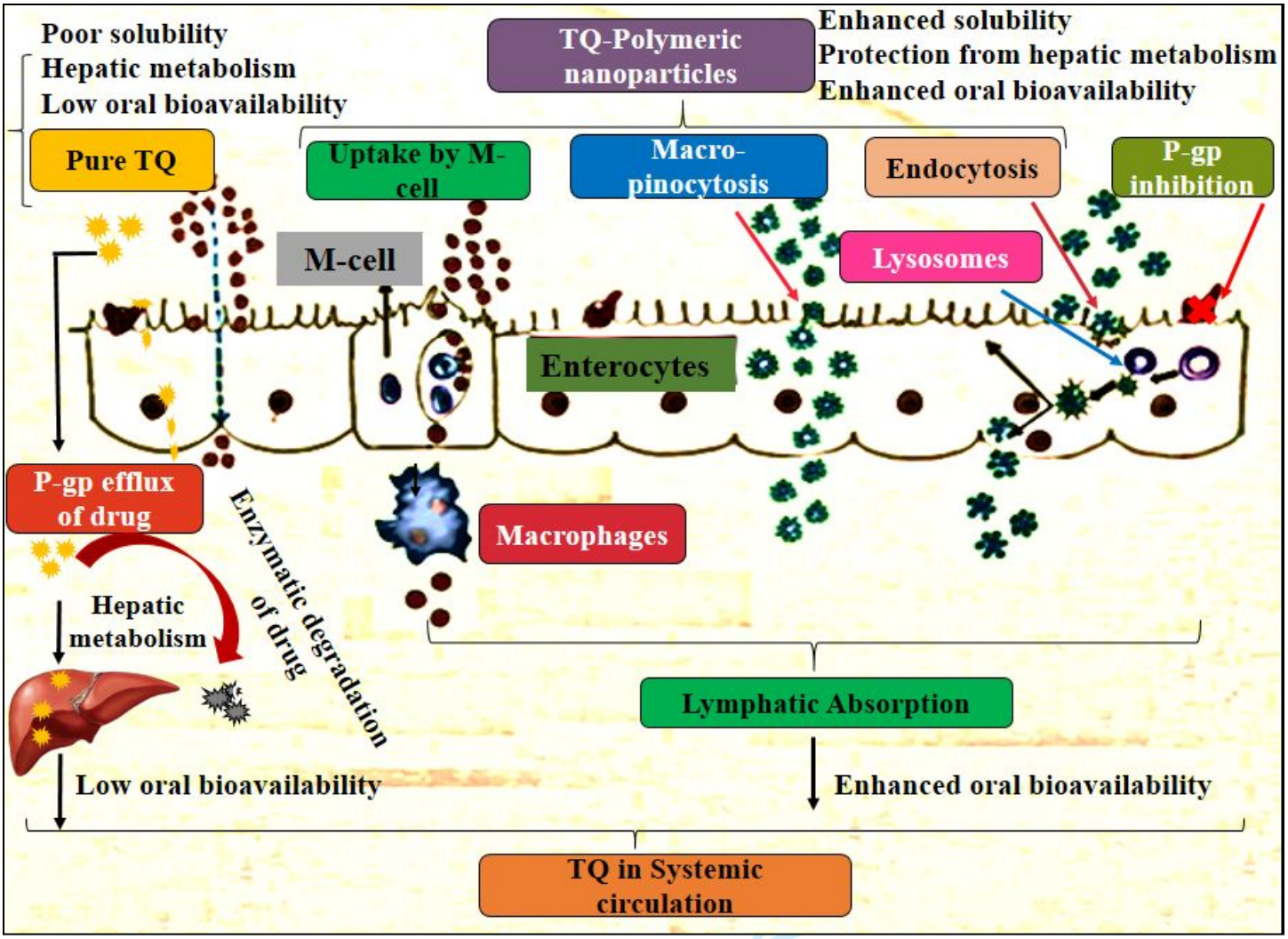
Poor solubility
Hepatic metabolism
Low oral bioavailability
Pure TQ
TQ-Polymeric nanoparticles
Enhanced solubility
Protection from hepatic metabolism
Enhanced oral bioavailability
Uptake by M-cell
Macro-pinocytosis
Endocytosis
P-gp inhibition
M-cell
Lysosomes
Enterocytes
P-gp efflux of drug
Enzymatic degradation of drug
Macrophages
Hepatic metabolism
Lymphatic Absorption
Low oral bioavailability
Enhanced oral bioavailability
TQ in Systemic circulation

Figure 3

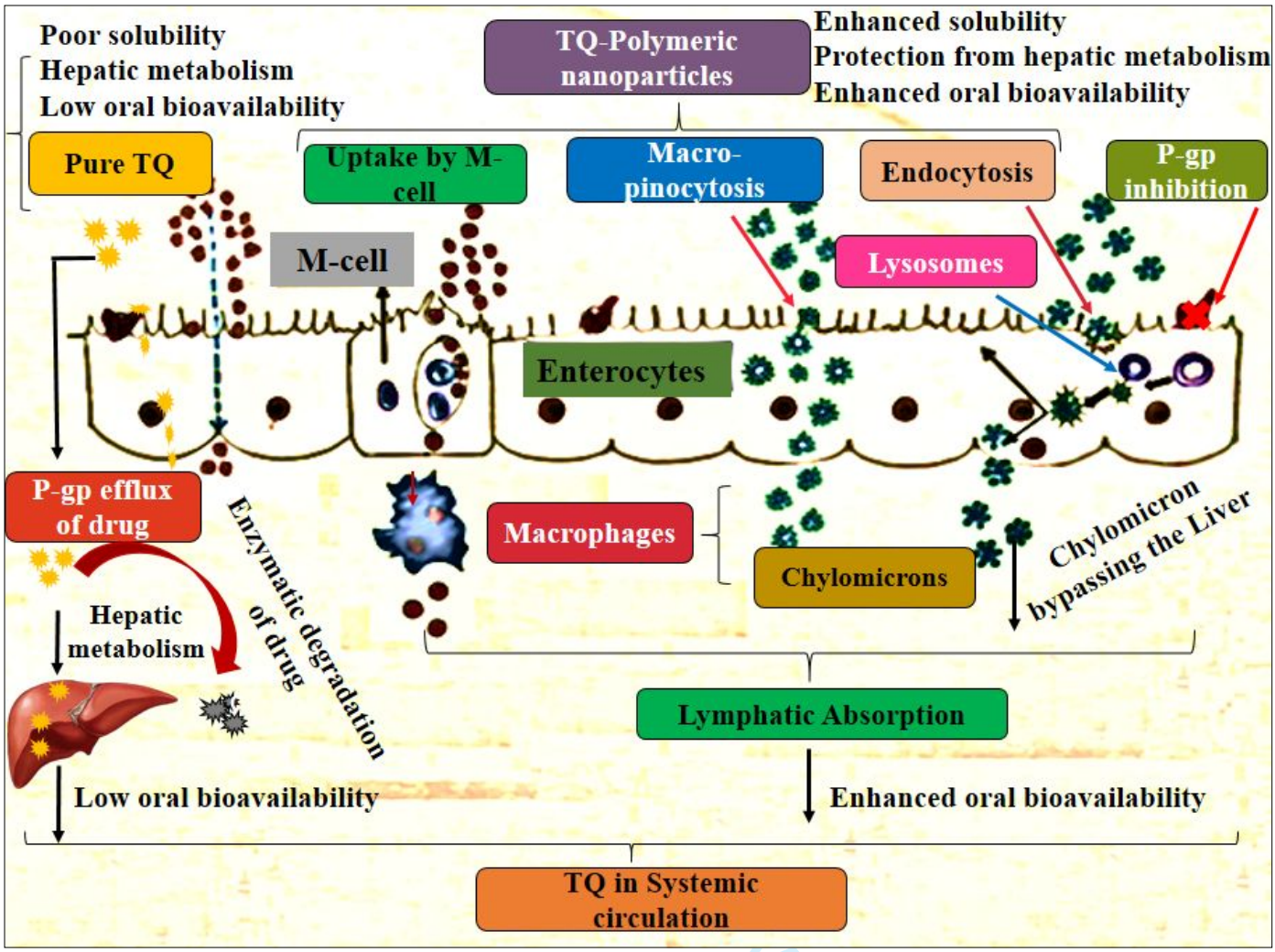

Figure 4

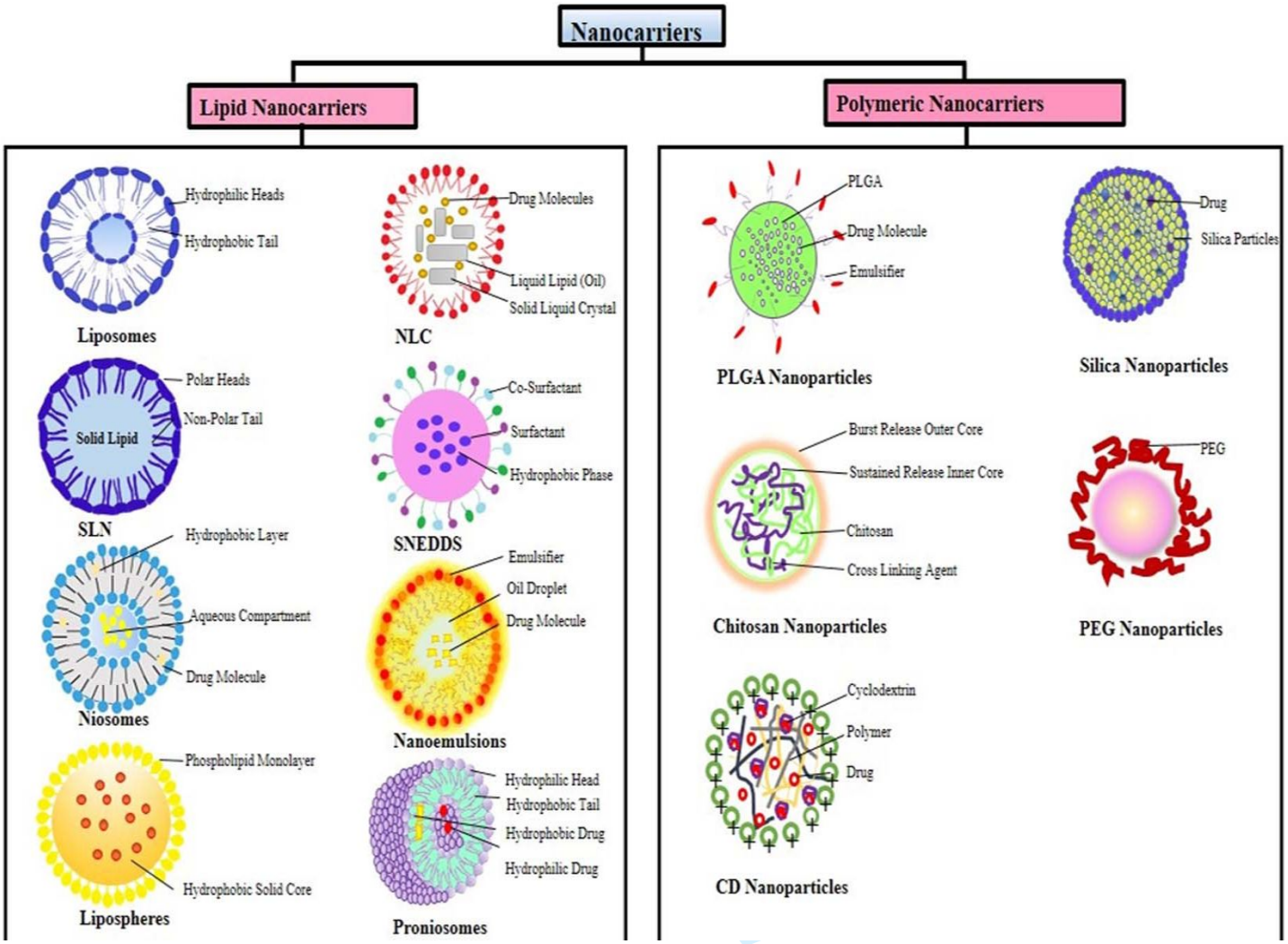
Nanocarriers
Lipid Nanocarriers
Polymeric Nanocarriers
Hydrophilic Heads
Hydrophobic Tail
Liposomes
Drug Molecules
Liquid Lipid (Oil)
Solid Liquid Crystal
NLC
Polar Heads
Non-Polar Tail
Solid Lipid
SLN
Co-Surfactant
Surfactant
Hydrophobic Phase
SNEDDS
Hydrophobic Layer
Aqueous Compartment
Drug Molecule
Niosomes
Emulsifier
Oil Droplet
Drug Molecule
Nanoemulsions
Phospholipid Monolayer
Hydrophobic Solid Core
Lipospheres
Hydrophilic Head
Hydrophobic Tail
Hydrophobic Drug
Hydrophilic Drug
Proniosomes
PLGA
Drug Molecule
Emulsifier
PLGA Nanoparticles
Drug
Silica Particles
Silica Nanoparticles
Burst Release Outer Core
Sustained Release Inner Core
Chitosan
Cross Linking Agent
Chitosan Nanoparticles
PEG
PEG Nanoparticles
Cyclodextrin
Polymer
Drug
CD Nanoparticles

Figure 5