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Nanostructured Lipid Carriers Employing Polyphenols as Promising Anticancer Agents: Quality by Design (QbD) approach

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Abstract

Cancer is one of the leading causes of death worldwide. There are several hurdles in cancer therapy because of side-effects which limits its usage. Nanoparticulate drug delivery systems have been tested against cancer in a range of scientific studies. In the recent years, advanced research on Nanostructured Lipid Carriers (NLCs) has garnered considerable attention owing to the advantages over their first-generation counterparts, Solid Lipid Nanoparticles (SLN). NLCs facilitate efficient loading of poorly water soluble drugs with simple methods of drug loading. Recently, there is an increased interest in polyphenols because of the evidence of their promising role in prevention of cancer. Polyphenols are produced as secondary metabolites by plants. Their role in prevention of development of tumors through variety of mechanisms and reduction of tumor cell mass has been reported. This article aims to review the science behind development of NLCs and role of polyphenols as promising anticancer agents. Principles of Quality by Design (QbD) have also been explained which are used in formulation-development of many nanoparticles, including NLCs, as reported in literature.

Graphical abstract

Figure showing uptake of NLC through leaky tumor vasculature via EPR effect.

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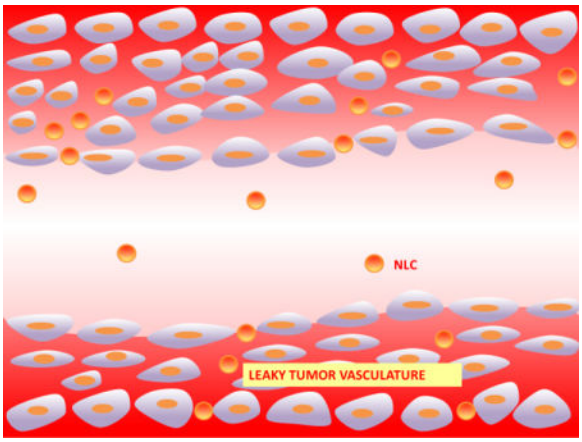

Keywords

Cancer; Nanostructured Lipid Carriers (NLCs); mechanism of action of polyphenols; types of NLC; production methods; design of experiment by Quality by Design (QbD) approach

1. INTRODUCTION

One of the most distressing and life-threatening diseases responsible for several deaths worldwide is cancer. The treatment and control of this disease is a major cause of concern for both developing and developed nations. It was estimated that there were 10.9 million newly detected cases, mortality of 6.7 million, and a whopping 24.6 million humans living with cancer worldwide in 2012 (Ferlay et al., 2015). Typically, cancerous cells may break away from the site of tumor origination and consequently enter the blood-stream, proliferating and metastasizing as the invasion proceeds. There are sensitive lab tests to determine the circulating tumor cells. Such tests cannot fathom the longevity of patients, although they can give a measure of the population of patients with recurrent cancer and can also gauge whether the treatment is working. The major attribute of cancer stem cells is the potential to undergo self-renewal, while alongside giving rise to daughter cells armed with the same potential.

The main forms of treatment for cancer are surgery, radiation and chemotherapy. It is upon the discretion of the doctor which therapy is to be used either alone or in combination, depending upon the severity of the tumor metastasis. The most preferred therapy prevalent clinically is chemotherapy that involves administration of anticancer drugs to the patient. Chemotherapy reacts with the genetic material of the cell (DNA). Chemotherapeutic agents cause cross-linking of bases in DNA strands, leading to blocking of replication of nuclear DNA during mitosis (cell division). Few drugs that are currently used in the chemotherapy of cancer include Tamoxifen, Paclitaxel, Docetaxel, Doxorubicin, Cyclophosphamide, Methotrexate, 5-Fluorouracil, Vinca Alkaloids-Vincristine & Vinblastine, Gemcitabine, Epirubicin, etc. Chemotherapy drugs are usually given in 2–4 week cycles, either orally or parenterally. In adjuvant and neo-adjuvant settings, they are usually given in combinations of two or more drugs. Administering a single drug may be an option in treating cancer that has

already spread to other areas. Although responses to combination chemotherapy are higher, toxicity is also greater as compared to single agent chemotherapy. It can also suppress and damage the immune system to the extent that patients can die from infections rather than the cancer. These treatments often provide only temporary improvement and are associated with several side effects like nausea, hair loss, loss of healthy dividing cells, menopausal symptoms, fertility issues, diarrhea, memory loss, weight changes, anemia, vision problems, heart issues, osteoporosis, etc. The conventional treatments with chemotherapy/radiation are frequently effective in helping cancer patients survive for first four years following surgery. Medicinal herbs have been on the forefront in the cancer treatment.

2. Polyphenols in cancer

Phytoconstituents derived from herbs have been used in various formulations to enhance activity of immune cells of the body, further promoting production of cytokines including interleukin, interferon, tumor necrosis and colony stimulating factor. Such herbal-based formulations help the body to fight cancer more effectively and reduce toxic side effects of chemotherapy and radiation therapy stages of cancer (Sakarkar and Deshmukh, 2011). Thus, owing to the number of problems associated with synthetic anticancer agents, there arises a pertinent need to explore the vast domain of naturally derived anticancer drugs: more specifically those derived from plant sources because of their advantage compared to synthetic anticancer drugs. However, they have issues like poor solubility and high dose requirement. Nevertheless, these problems can be overcome by employing nano-drug delivery systems for plant-derived anticancer drugs. Natural product derived substances, especially polyphenolic compounds with very little toxic effects on normal cells have attracted great attention in the therapeutic arsenal in clinical oncology due to their chemopreventive, antitumoral, radiosensibilizing and chemosensibilizing activities against various types of aggressive and recurrent cancers. The recent advances on the cancer preventative activities of the polyphenolic compounds, including flavonoids have been investigated by many workers (Ferry, 1996; Ishii et al., 2010; Michael et al., 1993; Mimeault and Batra, 2011; Reddy et al., 2003; Singh et al., 2011; Yuan et al., 2012a).

Polyphenols are produced as secondary metabolites by plants. Recently, there is an increased interest in polyphenols because of the evidence of their role in prevention **and amelioration** of cancer, neurodegenerative and cardiovascular diseases. There are many drugs available in the market for treatment of these diseases; however, the emphasis recently is on the exploitation of natural principles derived from plants. Many polyphenols have been investigated for their preventive and curative role in cancer therapy. Most polyphenols show low *in vivo* bioavailability, thus limiting their application for oral drug delivery. This low bioavailability could be associated with low aqueous solubility, first pass effect, metabolism in GIT, or irreversible binding to cellular DNA and proteins (Kaur and Kaur, 2014).

2.1. Classification of Polyphenols

A generic classification of polyphenols is listed in table 1, with representative examples. Each of the classes of polyphenols have been reported to have potent anticancer activity.

2.2. Probable Mechanism of Action

Polyphenolic compounds have been shown to induce apoptosis in various malignant cells including solid tumors and hematologic malignant cells. It is well known that polyphenolic compounds possess both antioxidant and prooxidant activity. Alteration in redox potential has been implicated in antitumor and chemotherapeutic activities, leading to induction of apoptosis. Apoptosis is a mechanism where cells are induced to commit suicide and involves very complex biochemical and molecular process. Many biological enzymes work in harmony to execute the natural death for the cells. It has been accepted that the aim of anticancer therapy is generally focused on apoptosis induction in premalignant and malignant cells, although other multiple molecular mechanisms such as modulation of carcinogen metabolism, anti-angiogenesis and induction of differentiation are also known to be implicated in its anticancer activity. So far, two principal signal pathways of apoptosis have been identified. The intrinsic mechanism of apoptosis involves a mitochondrial pathway. Cytochrome c is the critical protein released from the leaky mitochondrial membrane during apoptosis under the influence of Bcl-2 (B-cell leukemia/lymphoma) which then disintegrate the mitochondria. Once cytochrome *c* is released it activates caspase-9 (initiator caspase) through the interaction with Apaf-1 (apoptotic protease activating factor-1) and dATP, which ultimately leads to caspase-3 and -7 (effector caspases) activation. On the other hand, the extrinsic pathway induced by death receptors, such as tumor necrosis factor receptor (TNFR) and Fas, is responsible for the activation of caspase-8 and caspase-10 (initiator caspase) accompanied by the activation of caspase-3 and -7. Caspase 3 and 7 act at the final link in the intrinsic and extrinsic apoptotic processes that ultimately lead to cell death. Additionally, literature reports that endoplasmic reticulum (ER) stress and caspase-12 constitute the third probable mechanism associated with the cell death. Several markers have been utilized to reveal apoptosis status including cell viability, cytochrome *c* release, caspase-3 activation, poly (ADP-ribose) polymerase (PARP) cleavage, and DNA fragmentation. Cell growth and multiplication of the cancer cells may be attributed to the reactive oxygen species (ROS). Furthermore, oxidative stress, because of alterations of redox homeostasis due to an imbalance between ROS production and elimination, is known to be involved in many diseases such as hypoxic injury. Generation of the reactive oxygen species is important for the normal cell growth and survival. Furthermore, cancer cells exhibit increased ROS production and altered redox status. Past experimental literature suggests that cancer cell biology like overexpression of certain protein and enzymes can be exploited for therapeutic benefits to develop anticancer agents. One of such target may be ROS which is generated in higher quantity in the cancer cells at the advanced stage of this disease. But, this phenomenon can also lead to the development of the resistant cancer cells. It can be one of the potential way to selectively target the cancer cells over normal cells and to kill them further (Indran et al., 2011; Shimizu et al., 1999; William et al., 1999) Yuan et al., 2012b).

Ellagic acid (EA), a phenolic acid is abundantly present in walnuts, pomegranates berries and is known to have anticancer activity against breast cancer, prostate cancer, lung cancer, colon cancer, cardiovascular disease and neurodegenerative disorders due to its antioxidant, anti-inflammatory, anti-tyrosinase and anti-mutagenic activities, anti-proliferative, anti-invasive and pro-apoptotic properties on breast and prostate cancer cells and lung carcinoma

cells (Seeram et al., 2005; Tokton and Ounaroon, 2014). The estrogen and tyrosine kinase receptor pathways are the drivers of cell proliferation and are crucial to the development of both primary and recurrent breast cancers. EA not only interacts with and alters the effects of these pathways but also induces cell death (apoptosis and autophagy) by influencing kinase signaling *in vitro* and *in vivo* (Zhang et al., 2014). EA is found to possess maximum solubility of 9.3 μg/mL in water (Li et al., 2013). Quercetin (QR) is a flavonoid and is present in citrus fruits, apple, onions, parsley, sage, tea and red wine. It exhibits anti-inflammatory, antioxidant, antihistamine, and anti-arthritis properties. Documented literature revealed that flavonoids exhibit potential anticancer activities where quercetin exerted dose dependent killing of cancer cells. It exerts anticancer activity by competing with hormonal receptor sites or by inhibiting some phase of cell cycle. Androgen receptors (AR), epidermal growth factor receptor (EGFR) and death receptor (DR) have been reported in the literature for the antitumoral activities of quercetin.

Irrespective of the anticancer benefits of quercetin, the use of quercetin is restricted owing to its poor water solubility (0.3μg/mL). Thus, solubility enhancement of quercetin remains a challenge to increase solubility (Kaur and Kaur, 2014). Polyphenols may exhibit synergistic, additive or antagonistic anticancer effects. It has been demonstrated that QR and EA interacted synergistically in the induction of apoptosis in the human leukemia cell line, MOLT-4. The results clearly indicate synergistic interaction of quercetin and ellagic acid at the ratios 1:1 and 1:4 (Susanne et al., 2003). Furthermore, the possible cellular mechanisms were investigated and it was found that EA potentiates the effect of QR on $p21^{waf1/cip1}$ protein levels, p53 phosphorylation, and to a lesser extent, on $p21^{waf1/cip1}$ mRNA levels. Possibly, the activation of JNK1,2 and p38 may be involved in the synergistic activation of p53 by both QR and EA, resulting in synergistic expression of $p21^{waf1/cip1}$ and finally, apoptosis through the activation of caspase-3 (Vitro et al., 2005). Apigenin arrests the cell cycle in some cancers including prostate cancer and pancreatic cancer, by decreasing the levels of cyclins and CDKs. It also induces apoptosis in cancer cells through the inhibition of signaling pathways, cellular factors and oncogenes (Helena et al., 2009). Hesperetin (3′, 5,7-trihydroxy-4- methoxyflavanone) inhibits cellular proliferation, the induction of a cell cycle arrest at G1, and apoptosis in human breast MCF-7 cancer cells (Roohbakhsh, 2015; Roohbakhsh et al., 2015). Index of peroxides in the human leukemia cells is increased by Cyanidin-3-rutinoside and this hike may result in the process of apoptosis. This phytochemical activates p38 MAP kinase enzyme and Bcl-2 protein resulting in the leak of apoptogenic factor from the mitochondria leading to cell death. In addition to this the level of ROS in human peripheral blood mononuclear cells (PBMC) is not much affected by this phytochemical, therefore, less toxic to the normal cells (Feng et al., 2007). Genistein inhibits ovarian carcinogenesis by pleiotropic mechanisms. A higher affinity to estrogen receptor β is one probable explanation for its ability to reduce the risk of ovarian cancer. Genistein also targets multiple cellular signal transduction pathways associated with cell cycle regulation and apoptosis (Lee et al., 2011).

3. Nano-scaled Carriers

Formulation of novel drug delivery systems can provide enhanced efficacy and/or reduced toxicity for anticancer agents. Research and development in cancer nanotherapeutics is

rapidly progressing and being implemented to solve several limitations of conventional drug delivery systems such as non-specific biodistribution and targeting, lack of water solubility, poor oral bioavailability and low therapeutic indices. With the aim to enhance the biodistribution and increase the circulation time of cancer drugs, particles in the nano range have been developed, taking into consideration optimum particle/globule size and engineering of surface characteristics. The domain of cancer nanotherapeutics is consistently progressing and being used to remove the various limitations of conventional methods available for the diagnosis and treatment of cancer. Nanoparticles provide an interdisciplinary area for research in imaging, diagnosis and targeting of cancer. With advantages, such as advanced physicochemical properties and better bioavailability, they show prolonged blood circulation with efficient tumor targeting. Nanoparticles can reduce cytotoxic effects of the active anticancer drugs by increasing cancer cell targeting in comparison to conventional formulations. Polymeric drug micelles, liposomes, dendrimer, carbon nanotubes, and nanorods are the most common nanoparticles currently undergoing preclinical and clinical trials stages of formulation (Sharma et al., 2013). Long circulating macromolecular carriers can exploit the ‘Enhanced Permeability and Retention’ (EPR) effect for preferential extravasation from tumor vessels (Park, 2002). The delivery of the drug to the target tissue can be achieved primarily in two ways—passive and active. Passive targeting can be achieved through leaky vasculature by virtue of the EPR effect, whereas, active targeting can be brought about by carbohydrate, receptor and antibody targeting. Active targeting strategies accentuate the ability of the nanoparticle to achieve its exact desired site of action. They have the ability to accumulate in cells without being recognized by P-glycoprotein, one of the main mediators of multidrug resistance, resulting in the increased intracellular concentration of drugs (Cho et al., 2008). There are a number of nanoparticles under research, to name a few, liposomes, emulsions, polymeric nanoparticles, SLN, NLC, nanosuspensions, etc. Polymeric nanoparticles serve various advantages like controlled drug release, avoidance of drug leakage, low toxicity, good biocompatibility and higher bioavailability. Problems associated with the use of these systems include: possible cytotoxic effects after phagocytosis of the polymeric material by macrophages and human granulocytes, possible impairment of the reticulo-endothelial system due to relatively slow degradation of the polymeric material (up to 4 weeks), residual contamination from the production process, limited physical stability and drug leakage during storage, lack of a method for production on a large industrial scale, expensive production methods and products unacceptable for registration by the regulatory authorities due to quality problems, stability problems associated with autoclaving. Sterilization needs to be performed by gamma irradiation possibly leading to the formation of radicals and subsequently toxic reaction product. Compared to liposomes and emulsions, solid particles possess some advantages, e.g. protection of incorporated active compounds against chemical degradation and more flexibility in modulating the release of the compound. Advantages of liposomes and emulsions are that they are composed of well tolerated excipients and they can easily be produced on a large scale, the pre-requisite for a carrier to be introduced to the market. Such advantages are exploited in the making of Solid Lipid Nanoparticles (SLN). SLN are prepared by exchanging the liquid lipid (oil) of the emulsions by a solid lipid which remain so even at body temperature (Radtke and Wissing, 2002). They are an alternative system to emulsions, liposomes, microparticles and nanoparticles based on synthetic polymers for

various application routes because of their numerous advantages. SLN poses the same advantages as those of polymeric nanoparticles. However, drug loading capacity, drug expulsion during storage (β modification) and high water content (70–95%) of aqueous SLN dispersions are the limitations. Drug loading in SLN depends on several factors like the extent of solubility of drug in the solid lipid matrix, physical state, and polymorphism of the matrix. Imperfections in the lipid matrix pose problems for higher drug loading. Thus, inclusion of more complex lipids like mono-, di-, tri- glycerides, different chain lengths, is more sensible for effective drug loading. SLN have a tendency to form a stable, orderly, β polymorphic state during storage. This modification, known as β modification, leads to drug expulsion from SLN. Since NLC employ mixing of spatially different lipids, crystallization upon cooling is avoided and thus the polymorphic transformation to β state does not occur, thus preventing drug expulsion as observed with SLN (Souto et al., 2004).

3.1. Nanostructured Lipid Carriers (NLC) (Chew et al., 2011; Chinsriwongkul et al., 2012; Hu et al., 2005; March and Technology, 2006; Radtke and Wissing, 2002)

Nanostructured Lipid Carriers (NLC), are novel lipid nanoparticles formed as a result of mixing spatially different lipids together in a blend. On blending together solid and liquid lipids, the regular lattice structure is disrupted, thus increasing the irregular crystal structure of the nanoparticles and drug loading capacity of the nanocarrier. NLC can be called as second generation SLN that overcome the limitations posed by the latter. NLCs can more strongly immobilize drugs and prevent the particle from coalescing by virtue of the solid matrix compared to emulsions. NLC are endowed with decreased risk of systemic side effects compared to other nanoparticles. Compared to SLN where there is possibility of drug expulsion because of β-modification, there are very few chances of drug expulsion from NLC. Additionally, NLC can be suitably formulated avoiding the use of organic solvents. NLC offer much flexibility in manufacturing various dosage forms like creams, tablets, capsules, injectables. To produce NLC, specially very different lipid molecules are mixed, i.e. blending solid lipids with liquid lipids (oils). The resulting matrix thus composed of solid-liquid lipid mixture remains solid at room temperature despite a depression in melting point as compared to the solid lipid alone. Different types of NLC are produced which is a function of the method of production and the composition of the lipid blend. The pay-load of the active compound and the rate of expulsion from the matrix depends on the nanostructure of the matrix. Besides, NLCs have the usual particle diameter ranging 10–1000 nm. Liquid lipids with a different chain length of fatty acids compared to solid lipids will produce NLC with a disorganized crystalline structure and enable enhanced drug loading capacity. Liquid lipids are better solubilizers of drugs than solid lipids. Most of the lipids used in NLC are GRAS-approved lipids, or have been manufactured widely on a commercial scale. Because of their small size, the lipid particles are in intimate contact with the stratum corneum, because of which the quantity of drug penetrating into the mucosa or skin is increased (Souto et al., 2004). The solid lipid matrix enables controlled release from the lipid matrix, which ensures supply of the drug over a prolonged period, reduction of systemic absorption whenever necessary and during instances when high concentration of the drug results into irritation.

NLCs offer extended release of the drug because of the solid lipid-liquid lipid matrix. They are easy to prepare and are industrially scalable. The particle size of NLCs can be controlled. Because of the GRAS-approved lipid components, NLCs are preferred for topical preparation (Nasr et al., 2008).

NLCs have smaller particle size which gives them larger surface to volume ratio, thus enabling higher drug release from the lipid matrix. Conversely, sustained action is achieved on uniform dispersion of the drug in the lipid matrix. The partition coefficient of the drug and drug release show an inverse relationship (Ittrick et al., 2016). These principles explain how imperative it is to incorporate optimized production parameters in manufacturing of stable NLC systems.

3.1.1. Types of NLCs—Depending upon the content of the lipid mix, the following types of NLCs have been described (Selvamuthukumar and Velmurugan, 2012b):

- **i.** Imperfect type
- **ii.** Amorphous type
- **iii.** Multiple type

i. Imperfect type NLC (imperfectly structured solid matrix): Mixing of spatially different lipids, amongst variety of oils with solid lipids, introduces imperfections in the crystal order. Glycerides composed of different fatty lipids are examples of spatially different lipids, which enable fairly large distances between fatty acid chains. Such imperfections serve to our benefit to accommodate drug with a high payload.

ii. Multiple type (multiple oil in fat in water (O/F/W) carrier): NLCs are subject to cooling process after homogenization which decreases solubility of the drug in the lipophilic phase. Similarly, the drug solubility in lipid is negatively affected during crystallization process that occurs during storage. Such modifications with respect to drug solubility in lipid can eventually lead to drug expulsion from the lipid matrix at high drug concentration. Generally, a liquid lipid offers better drug solubility compared to the solid lipid. In cases of poor drug solubility in lipid, addition of increased amount of liquid lipid not only enhances the solubility but also thwarts drug leakage.

iii. Amorphous type (structureless solid amorphous matrix): NLC belonging to this category are formulated by using solid lipids combined with special liquid lipids. The NLCs thus produced are in amorphous and not in the crystalline state. Hence, expulsion of drug during storage by the process of β modification is prevented.

3.1.2. Production methods of lipid nanoparticles (Nautyal et al., 2015; Selvamuthukumar and Velmurugan, 2012b)—Several methods for preparation of nanoparticulate drug delivery systems have been reported, the choice of which depends on drug solubility, drug stability in excipients, composition of the lipid matrix, route of administration, degradation temperature of the drug and excipients, etc.

A. High Pressure Homogenization Technique: HPH has been known to have applications on large scale production of lipid nanoparticles with a good yield. This method utilizes forcing the liquid under high pressure of around 100–200 bars through a narrow orifice. High pressure induces shear and cavitation to break-down particles to nano-range. Scale-up is easier in HPH as compared to other methods of production. HPH is divided into two types: hot homogenization and cold homogenization. In both the techniques, the lipid is kept at approximately 5–10° C above the melting point and drug is added to the molten lipid.

B. Hot Homogenization Technique: Herein, the drug-molten lipid solution is uniformly dispersed in aqueous surfactant solution that is maintained at the same temperature as the drug-lipid solution. The assembly is conjugated to a high shear device to allow continuous stirring. The pre-emulsion thus obtained is forced through a piston gap homogenizer and the nanoemulsion can recrystallize by cooling, forming nanoparticles.

C. Cold homogenization technique: Hot homogenization has some drawbacks with respect to drug degradation at high temperature and partitioning of the drug into aqueous surfactant phase, leading to drug loss. These problems are circumvented by using cold homogenization. The first step is like hot homogenization, followed by rapid cooling which distributes the drug uniformly in the lipid matrix. This method is suitable for thermos-labile drugs.

D. Microemulsion technique: The molten lipid mix containing the drug is taken and heated to the same temperature as the aqueous phase that contains water, surfactants and co-surfactants/solubilizers. A provision for mild stirring is made. The heated aqueous phase is added dropwise to molten lipid mix upto a point where a transparent, thermodynamically stable solution is formed. The correct ratio of aqueous phase: lipid phase is noted, which forms the basis of formation of nanoparticles of desired size. The method described uptil this point produces a microemulsion, which is then dispersed in cold water under mild mechanical agitation to allow oil droplets to recrystallize and form nanoparticles. An advantage of this method is avoidance of organic solvents and complex mechanical devices.

E. Solvent emulsification-evaporation technique: This technique utilizes dissolving the hydrophobic drug and lipid in water immiscible organic solvent like cyclohexane, chloroform, etc. and emulsification of this mixture in aqueous phase using high speed homogenizer. The organic solvent is then evaporated under reduced pressure, leaving behind precipitates of the lipid nanoparticle. This method avoids using high temperatures that may degrade thermolabile drugs. However, usage of organic solvents remains a disadvantage.

F. Solvent emulsification-diffusion technique: For this method to work, the solvent used must be partially miscible in water. The solvent is eliminated after the final formation of nanoparticles. This method is compatible to be carried out in either oil or aqueous phase. The thermodynamic equilibrium of both the solvent and water is achieved by saturating mutually, either in presence or absence of heating, depending on the solubility of the drug. Lipid mixture containing the drug is then dissolved in the solvent saturated with water which constitutes the organic phase, i.e. internal phase. The dispersed phase consists of solvent saturated aqueous solution with which the internal phase is emulsified by means of

mechanical stirring. The o/w emulsion thus formed is diluted with water which leads to aggregation of lipid in form of nanoparticles. Care is taken to maintain both the phases at the same temperature.

G. Phase inversion temperature (PIT) method: The PIT method uses the ability of some polyoxyethylated surfactants to modify their affinity towards oil or water phases, depending on the temperature. If the temperature is increased above the PIT, the emulsion type changes from o/w macroemulsion to w/o emulsion. Conversely, if the temperature is lowered, it leads to the formation of o/w nanoemulsion. The oil phase consists of lipid mix and surfactants, whereas the aqueous phase consists of water supplemented with salts like NaCl (Battaglia et al., 2015).

H. Melting dispersion method: In this method, the lipid mix containing the drug, i.e. oil phase is added dropwise to the aqueous phase, maintaining both the phases at the same temperature. The emulsion obtained hereby is stirred vigorously under magnetic stirrer and cooled to form nanoparticles.

I. High Shear Homogenization or Ultrasonication Technique: In this method, the aqueous phase is added dropwise to lipid melt containing the drug; both phases maintained at the same temperature. The system is emulsified by using either mechanical high speed stirrer or magnetic stirrer. The pre-emulsion thus formed is ultrasonicated using probe sonicator. The sonicator assembly must be maintained in ice bath to absorb heat dissipated due to cavitation.

J. Solvent injection (or solvent displacement) technique: Liquid is dissolved in a solvent that diffuses very rapidly in water, example ethanol or DMSO. The mixture is injected into an aqueous solution of surfactant. The solvent, because of rapid diffusion in water, migrates quickly to the aqueous phase, leaving behind lipid particles in the precipitate. Though this method employs low shear, usage of organic solvents remains a disadvantage.

K. Double emulsion technique: This method is suitable for encapsulating hydrophilic drugs and uses w/o/w double emulsion technique. The drug and surfactant are encapsulated in the inner phase of the double emulsion. Suitable stabilizer dispersed in the aqueous phase containing hydrophilic emulsifier is required for stabilizing the primary emulsion.

The concept of drug-loaded NLC with plant-derived polyphenols as the payload has been tested by scientists with promising results. The outcomes of such studies broadly assert the advantages of using NLC as superior drug delivery system for polyphenols over other nanocarriers. In a study conducted by Kumar et al. for studying NLC and SLN formulations of quercetin, smaller particle size (67.46 nm) was observed for NLC versus 74.61 nm for SLN. The drug entrapment and drug loading efficacy for NLC was also higher compared to SLN. Moreover, NLC showed 5.4-fold increase in bioavailability *in vivo* as compared to 3.5-fold for SLN (Kumar et al., 2016).

In yet another study, different nanoformulations viz. SLN, NLC, ethosomes and liposomes were studied with loading of tretinoin. NLCs showed the best particle size (79.5 nm) and

stable zeta potential (−23.5 mV) compared to the rest of the nanocarriers. The entrapment efficiency for NLC was also highest (92.13 %) compared to other nanocarriers. Moreover, NLCs demonstrated better skin permeation of tretinoin over others (Raza et al., 2013a).

In order to improve the biopharmaceutical properties of a compound, studies have been undertaken to load phospholipid complexes in NLCs, taking into consideration advantages of the latter like high drug loading and stability. Such nanocarriers have demonstrated promising results both *in vitro* and *in vivo* (Khurana et al., 2017).

4. Quality by Design (Patil and Pethe, 2013.; Yu, 2008)

ICH Q8 defines quality as “The suitability of either a drug substance or drug product for its intended use. This term includes such attributes as the identity, strength, and purity.” Pharmaceutical QbD is a systematic, scientific, risk-based, holistic and proactive approach to pharmaceutical development that begins with predefined objectives and emphases product and processes understanding and process control (Services, 2006). Pharmaceutical QbD implies designing and development of both formulations and manufacturing processes underlying them in order to meet the quality objectives set aside for the product. The main role of QbD is identification of specific characteristics that define the quality of the product from the point of view of patients, further translating them into critical attributes or features that the final drug product should possess to make it safe for consumption by the end consumer. For achieving the critical attributes, it is essential to understand how the critical process parameters for the final product need to be varied and optimized to consistently produce the final product with the required characteristics. The first and foremost essential part of QbD is to establish a relationship between formulation and process variables and further layout the steps in a typical QbD process.

A QbD development process may begin with defining the target product profile, i.e. TPP, which are the parameters one would desire in the final product. TPP are defined based on clinical safety of the product, prior knowledge about the drug substance and excipients used in the product, patient compliance with the final formulation, and all such attributes that would essentially define the essence of the final product. Based on TPP, critical quality attributes, i.e. CQAs are identified, which are factors selected based on TPP, that are a source of variability and greatly affect the final product quality. CQAs must be controlled by series of experiments to achieve the TPP and design a robust and flexible manufacturing process that gives desired product output consistently. Risk assessment analysis is done to identify which attributes are low, medium and high risk. Studies are then designed to convert high and medium risk factors to low risk factors, reducing the sources of variability. Risk assessment analysis thus helps in prioritizing process parameters. Prior knowledge is combined with experiments to establish design space and control strategy for the entire process. Control strategy may include anything from limiting input quantities to fixing process parameters. Any strategy that will be continuously monitored to pointedly ensure consistent production of the final product will be termed as the control strategy.

The typical tools used in QbD process, wherever applicable, include Design of experiments (DoE), risk assessment and process analytical technology (PAT).

QbD has a number of advantages over classical optimization, the most important being reduction in the number of experiments compared to classical optimization process. In classical optimization, one factor is considered at a time; whereas, in QbD, there is an interplay between several factors that can optimized at the same time. This not only saves time, but also saves the material in consideration for optimization process. Under QbD, emphasis is on patient safety and product efficacy. The critical factors are meticulously analyzed using statistical tools to achieve desired results. Scientific understanding of pharmaceutical process and methods involved therein is done with utmost care. Scientific risk assessment is a major advantage of QbD which helps to focus on minimalizing the high risk and medium risk factors to low risk factors to achieve desirable product design. Thus, QbD ensures a robust yet flexible product development which is highly benefit to any industry from business perspective.

The theoretical principles underlying QbD which are science- and risk-based product development, risk assessment and design of method of analysis are explained in ICH ICH Q8 Pharmaceutical Development, ICH Q9 Quality Risk Management, ICH Q10 Pharmaceutical Quality System and ICH Q11 Development & Manufacture of Drug Substances (Sangshetti et al., 2014).

$$\text{Quality by Design} = \text{ICH Q8} + \text{ICH Q9} + \text{ICH Q10} + \text{ICH Q11}$$

4.1. Design of Experiment (DoE)

DoE is a systematic way of planning and executing experiments such that the data thus gained can be analyzed for conclusive and consistent results. According to Q8 (R2), Pharmaceutical Development, Design of Experiment (DoE) is a structured, organized method for determining the relationship between factors affecting a process and the output of the process.

4.2. Need of DoE in QbD

Employing DoE in QbD comes with a number of advantages. It gives the ability to collect exhaustive information by performing minimum number of experiments, enabling optimization of process variables in shorter span of time as compared to classical optimization, identification of interaction between factors and the degree of interaction, ability to transform variables to fit within data, evaluation of the effect of critical factors and critical material/process attributes so as to achieve TPP and finally the ability to establish the design space in order to reduce variability between interacting variables.

4.3. Screening

The most important goal of QbD is identifying CQAs that play a crucial role in defining the final quality of the product. CQAs greatly influence the critical material attributes (CMAs) as well. While CQAs encompass factors like particle size, entrapment efficacy, % drug loading, etc., CMAs typically will include material or excipient factors that play crucial role in dictating the CQAs. In the study conducted by Garg and colleagues, CMAs like concentrations of solid lipid, liquid lipid, surfactant and co-surfactant heavily influence

CQAs like particle size, permeation flux, release and entrapment. Since the influence of these CMAs is large on determining the outcome of the CQAs, the latter are classified as high risk factors (Garg et al., 2016). Screening is necessary for identifying the key variables that are responsible for the final product quality. Screening reduces all those variables which do not play a dominant role in ensuring the consistency of the product, allowing one to focus for optimization on only the most important process variables. Two-level full and fractional factorial designs and Plackett-Burman designs are usually used for screening purposes.

4.4. Optimization

Screening allows one to identify the most important variables. Post that, it is necessary to identify the best or optimum values for these selected factors for further experimentation. Optimization is necessary to arrive at the best values for critical factors after performing experiments that establish consistent relationship between the critical factors. It can be done by full factorial designs, response surface designs (Central Composite Design, Box Behnken Design), mixture designs (Simplex Centroid, Simplex Lattice) and Taguchi designs. In a study by Raza and group, a face-centred cubic design (FCCD) with 2 factors at 3 levels was employed for formulation design (Raza et al., 2013b).

4.5. Model Diagnostic

The importance of selected parameter is best performed using analysis of variance, i.e. ANOVA method that uses statistical F-test to define significance of the model terms in the study.

4.6. Illustration of Design Space

According to ICH guideline Q8 (R2), “design space is a multidimensional combination of input variables, their interactions and process parameters that have been demonstrated to provide assurance of quality. Working within the design space is not generally considered as a change of the approved ranges for process parameters and formulation attributes.” However, working out of the design space is counted as a ‘change’, necessitating regulatory guidelines for change post-approval.

The design space establishes the range of values within which the optimized process will give desired values. The design space is determined after optimization studies on the data set are performed. Design space is best illustrated by contour plots, three-dimensional plots and overlay plots.

4.7. Contour plots

A contour plot provides two-dimensional graphical representation among three numeric variables. The X- and Y- axes represent the two variables and the remaining third variable is indicative of contour levels.

4.8. Three-dimensional plots

These plots are used to illustrate the effect of two input variables on an output variable at the same time and in three-dimensions.

4.9. Overlay plots

The overlay plots are indicative of the design space, which shows various values of critical factors that provide results within desirable range. Based on the design space after series of optimization steps, the software predicts the best possible outcome for the given set of data with its statistical significance. For a NLC preparation for which the critical factors are lipid/surfactant concentration, the overlay plots will give estimated points within the design space which will produce results consistent to those expected.

5. Conclusion

Studies suggest the anticancer role of plant polyphenols in prevention and amelioration of tumor growth. Based on their promising evidence, it is unfair to overlook the anticancer role of phytochemicals in betterment of patient condition. Though we are not close to suggesting plant-derived polyphenols as potent alternatives to synthetically derived anticancer agents, we have clearly achieved favorable milestones in that direction. One advantage of the polyphenol anticancer compounds is elimination of the severity of toxic side-effects posed by synthetic anticancer compounds. With the advent of novel drug delivery systems like NLCs, scientists are aiming to improve the solubility of such potent polyphenols which incidentally suffer from poor aqueous solubility and poor bioavailability. Lipid-based drug delivery systems like NLCs improve the solubility, bioavailability of the drugs and protect them from degradation by lipid-surfactant matrix system. Using QbD approach, one can improve the large-scale productivity of such delivery systems so that they can be realized to the market.

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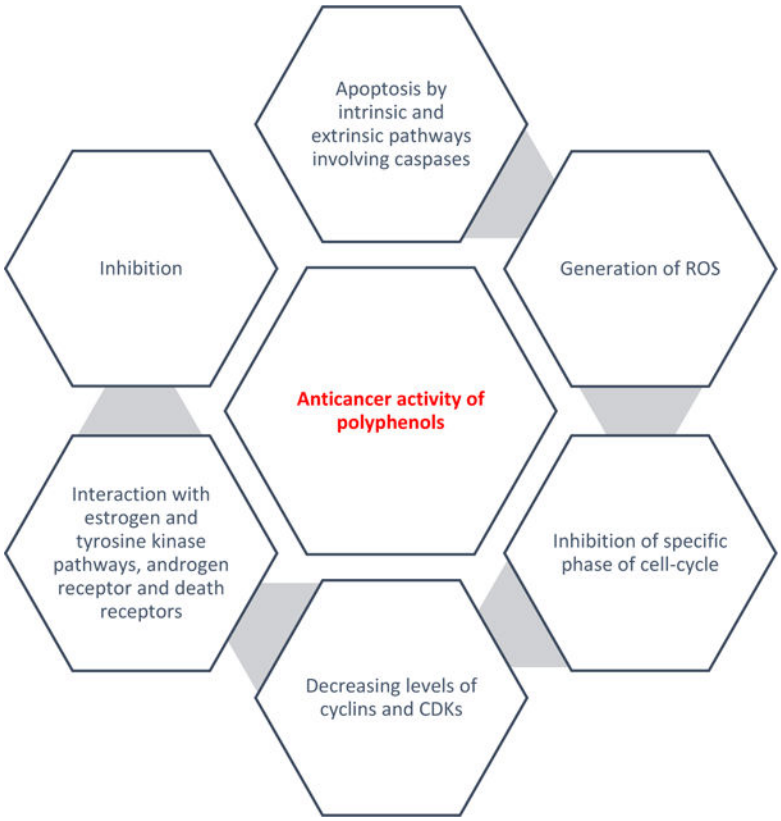

Figure 1.
Probable anticancer mechanisms of action of polyphenols

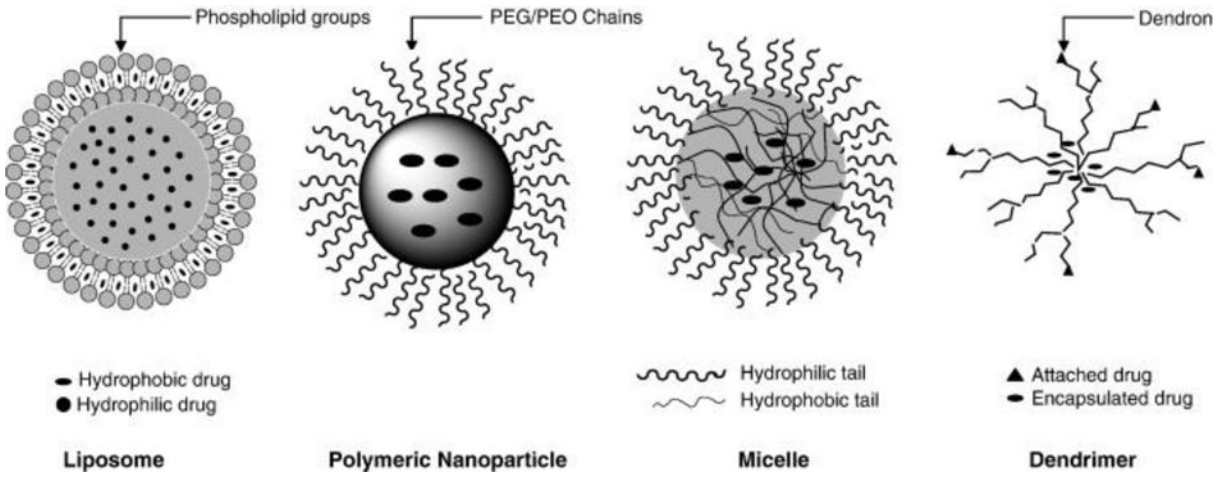

Figure 2.

Different types of nanocarriers. Taken permission and adopted from Journal of Controlled Release (Ganta et al., 2008).

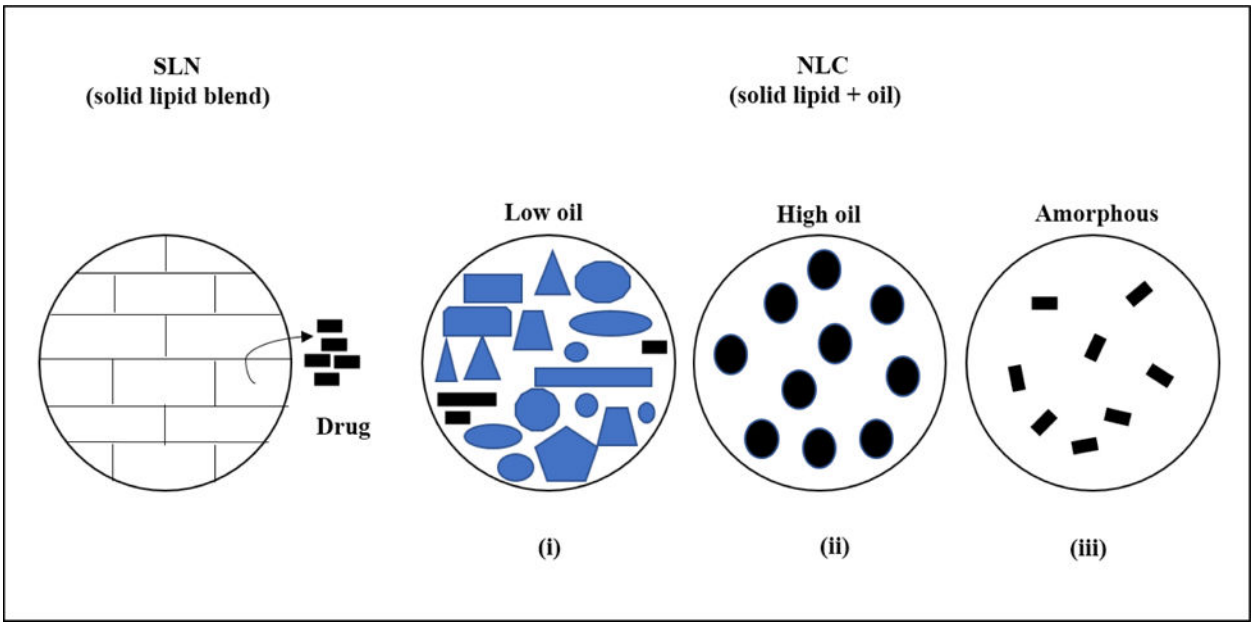

Figure 3.

Illustration of SLN with high crystallinity and 3 different types of NLC, viz., (i) imperfect type, (ii) multiple type and (iii) amorphous type. Modified from Selvamuthukumar and Velmurugan (Selvamuthukumar and Velmurugan, 2012).

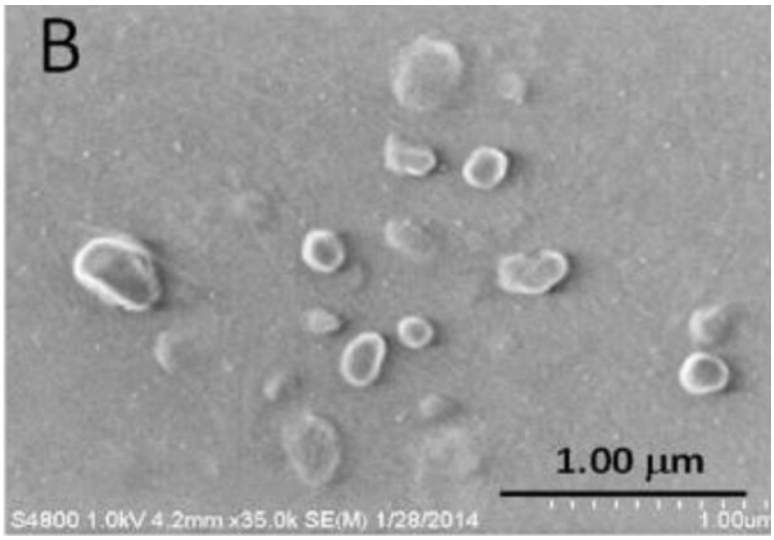

Figure 4.

Scanning Electron Microscope (SEM) images of drug-loaded NLC. Taken permission and adopted from International Journal of Pharmaceutics (Mussi et al., 2015).

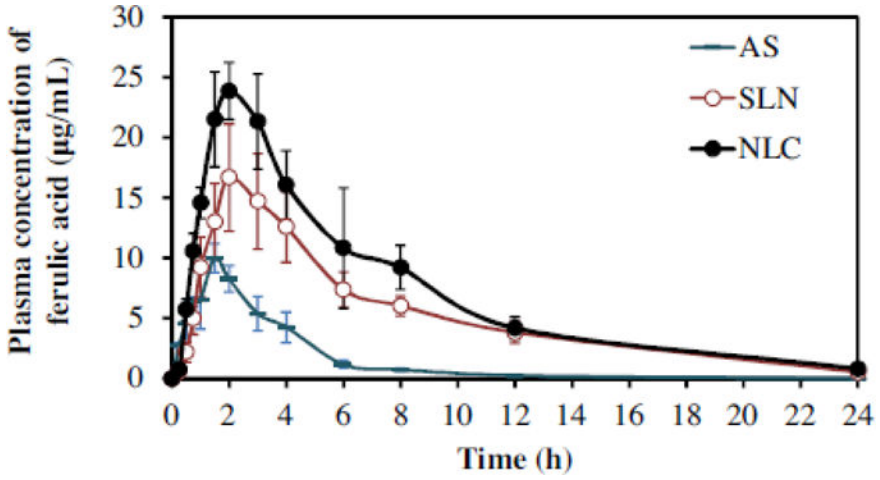

Figure 5.

Mean plasma concentration–time curves for trans-ferulic acid (TFA) in rats after oral administration of TFA aqueous solution (TFA-AS), TFA-loaded NLCs (TFA-NLCs), and TFA-loaded SLNs (TFA-SLNs), all at doses equivalent to 80 mg/kg of TFA (n = 5). Values are mean standard deviation (n = 5 per group per time point). Taken permission and adopted from International Journal of Pharmaceutics (Zhang et al., 2016).

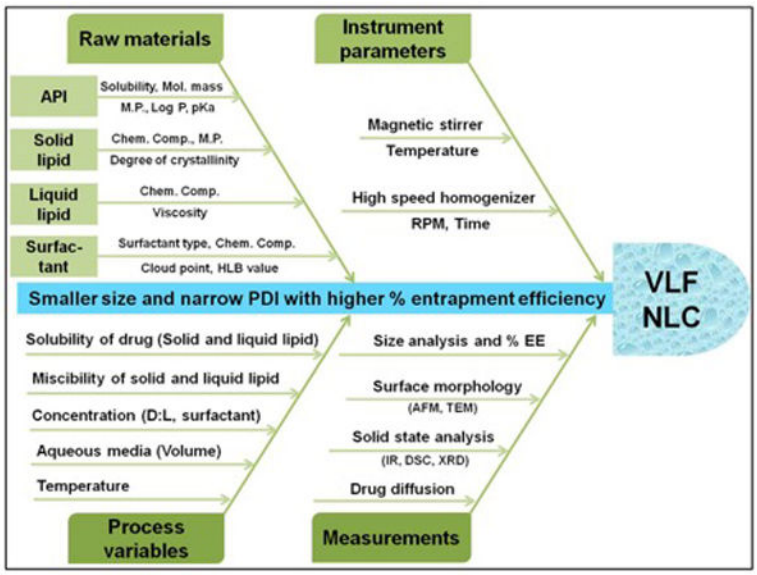

Figure 6.

Ishikawa diagram showing critical parameters affecting development of representative NLC. Taken permission and adopted from Journal of Drug Delivery Science and Technology (Shah et al., 2016).

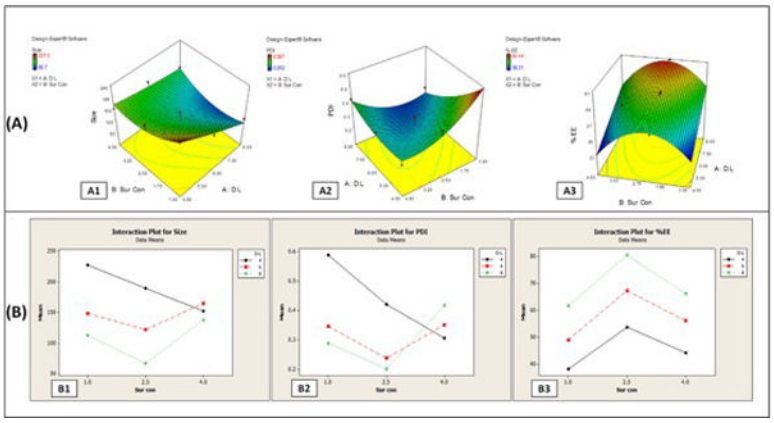

Figure 7.

3D response plots and interaction plots for the response. Taken permission and adopted from Journal of Drug Delivery Science and Technology (Shah et al., 2016).

Table 1

Classification of polyphenols with examples (Kaur and Kaur, 2014):

Polyphenol	Example
Phenolic acid	Ellagic acid, Gallic acid, Protocatechuic acid and Vanillic acid
Stilbenes	Reseveratrol
Flavonols	Quercetin
Flavones	Apigenin
Flavanones	Hesperetin, Naringenin
Anthocyanins	Cyanidin-3-rutinoside, Delphinidin-3 rutinoside
Isoflavones	Genistein, Daidzein