Chapter

Drug Delivery Approaches for Apigenin: A Review

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Abstract

Apigenin is a naturally occurring plant flavone with prominent antioxidant and anti-inflammatory properties. Although apigenin has the potential to be a promising molecule also for cancer treatment, its delivery to the body requires suitable dosage form design due to physicochemical characteristics that lead to poor bioavailability. It is classified as a BCS (Biopharmaceutical Classification System) II drug with low water solubility and high lipophilicity.

Improving the solubility of apigenin is crucial, various formulation approaches have recently been employed e.g. nanocrystals have increased

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dissolution rate and antioxidant activity, while polymeric micelles increased the solubility to 148 times higher than that crude apigenin and showed enhanced cytotoxicity on hepatoma cells. PLGA encapsulated apigenin nanoparticles cause mitochondrial apoptosis and even DNA targeting could be achieved in skin cancer.

The chapter reviews current approaches to the formulation of drug delivery systems containing apigenin.

**Natural Sources, Intake and Pharmacology**

Apigenin is a 4',5,7-trihydroxyflavone (Figure 1) belonging to a vast group of polyphenolic compounds called flavonoids. Flavonoids occur ubiquitously in the plant kingdom as secondary metabolites. They have a role in plant physiology, especially in pigmentation and flavor and also provide resistance against pathogens and insects. Six subclasses of flavonoids are distinguished - namely flavones, flavonols, flavanones, catechins, anthocyanidins and isoflavones - that have hydroxyl and phenolic groups variously attached to the common diphenylpropane structure (C6-C3-C6) (Ross and Kasum 2002). Generally one or more sugar components are linked to the aglycon molecule with O- or C-glycosidic bond in plants. Thus flavonoids are presented widely as glucosides in food but they are known to demonstrate incomplete absorption. They are abundantly distributed in fruits, vegetables and beverages with plant origin such as wine and tea. The average daily intake of flavonoids in a normal diet is approximately 1-2 g (Havsteen 2002). In the Hungarian population the total flavonoid intake was estimated to be 18.80 mg with only 0.58 mg apigenin (Lugasi, Hóvári et al 2003). Many mechanisms of action have been identified for flavonoids, including antioxidant activity (Procházkova, Bousova et al. 2011), changes in cellular signaling, apoptosis induction, anti-proliferation and anti-inflammation (Birt, Hendrich et al. 2001), thus suggesting their potential in cancer prevention. The use of pure flavonoids as a treatment in many common diseases such as cardiovascular and gastrointestinal disorders is increasing. Researchers have a growing interest in natural active ingredients to prevent or treat cancer, since most drugs currently available in the market have several disadvantages, being very toxic, highly inefficient or highly expensive. Unfortunately, the apparently effective concentrations of polyphenols in vitro are often higher than the absorbed amount measured in vivo.

Apigenin can be found in a variety of fruits and vegetables (Peterson and Dwyer 1998).
The main sources of apigenin are celery, parsley, onion and chamomile, and it is present mainly in glycosides (Database 2014, May). However it occurs in a very low amount in the human diet (Meyer, Bolarinwa et al. 2006). It is recognized that apigenin has many pharmacological activities like free radical scavenging (Horvathova, Novotny et al. 2003, Škerget, Kotnik et al. 2005) and anti-inflammatory activities (Funakoshi-Tago, Nakamura et al. 2011, Choi, Islam et al. 2014). Furthermore, it has anticancer effects via modifying cell signaling pathways (Patel, Shukla et al. 2007, Shukla and Gupta 2010) in several tumor cell lines including skin (Wei, Tye et al. 1990, Tong, Van Dross et al. 2007, Abu-Yousif, Smith et al. 2008), breast (Yin, Giuliano et al. 2001), colon (Wang, Heideman et al. 2000, Chunhua, Donglan et al. 2013) and pancreas (Lefort and Blay 2013, Wu, Yu et al. 2014). Recent clinical studies suggest that a diet high in apigenin could potentially reduce the incidence of ovarian cancer in women (Gates, Vitonis et al. 2009).

All of these findings indicate that apigenin is a very promising drug candidate against cancer and several other diseases. However, the physicochemical and biopharmaceutical properties reveal limited clinical use highlighting a need to develop new drug delivery systems.

**Physicochemical Properties and Bioavailability**

Apigenin was classified as a drug belonging to BCS II. group (Biopharmaceutical Classification System) in a recent study (Zhang, Liu et al. 2014).
This indicates high permeability but low solubility: the log P value is 2.87 (Li, Robinson et al. 1997), but the aqueous solubility is very poor either in water (1.35 µg/mL) (Li, Robinson et al. 1997) or in buffers. The highest solubility value was at pH=7.5 (2.16 µg/mL) (Zhang, Liu et al. 2012) leading to poor intestinal absorption. Several studies have reported that the solubility of flavonoids depends on temperature, nature of solvents, pH conditions, as well as the thermodynamic properties of the compounds which allow formation of hydrogen bonds with the surrounding solvent (Saidman, Yurquina et al. 2002, Tommasini, Raneri et al. 2004, Xiao, Shao et al. 2011).

The bioavailability of these molecules has been shown to be influenced by their chemical form in foods, the food matrix, and the consumer’s microbial flora (Birt, Hendrich et al. 2001). Apigenin could be detected in human plasma following ingestion of apigenin-rich food, but in a very low amount. The first pass metabolism in the small intestine and the liver in conjunction with enterohepatic recycling may play an important role in the poor systemic bioavailability observed (Chen, Lin et al. 2003). Several other factors can cause low bioavailability, including low solubility and dissolution rate, poor membrane permeation, rapid metabolism and elimination. Very less is known about the fate of apigenin in the body.

Gastrointestinal tract is the main absorption site with both active and passive transport mechanism. This was confirmed with an intestinal transport study where apigenin could be detected in the whole intestine, with the main absorption site is probably being the duodenum. The mechanism might be passive transport in the ileum and colon segments with a concentration-independent permeability, while in both the duodenum and jejunum segments an active carrier-mediated transport is involved with concentration-dependent permeability behavior (Zhang, Liu et al. 2012). It seems likely that apigenin in natural form bound to β-glycosides (apiin, apigetrin) provides better bioavailability, as the absorption of other flavonoid glycosides is generally more efficient than the absorption of aglycones. (Hollman P C 1996). This result is more likely because glycosides are water soluble forms of the aglycon and more stable, (e.g. against heating such as cooking), thus more glycosides can get to the body (Nielsen, Young et al. 1999). However, the sugar group makes the molecule quite polar (log P < 0), and therefore hindering cellular uptake and passage through the cell membrane (Walle and Walle 2002, Walle, Hsieh et al. 2004). It was thought that flavonoids could not absorb through the small intestine because glycosides can only be hydrolyzed by a β-glucosidase enzyme of microbial flora in the colon (Pekić, Zeković et al. 1994). However, recently three types of human β-glucosidase enzymes were discovered; one of
them capable of breaking the glycosidic bond and is located in the cells of the small intestine, liver and kidney (Asim 1993, Robert, Venkatakrishnan et al. 1993, Berrin, Czjzek et al. 2003). In blood circulation, apigenin binds to the main carrier protein – human serum albumin (HSA) – with hydrophobic and electrostatic forces. It is noteworthy that there are several type of binding mode between apigenin and HSA, but most of them are very instable (Yuan, lv et al. 2007).

**DRUG DELIVERY APPROACHES**

In the last few decades the use of nutraceuticals to prevent chronic illnesses like diabetes, cardiovascular diseases and cancer has been rapidly emerging. These naturally occurring molecules possess antioxidant activity therefore can be effective against oxidative damage induced diseases. Various bioactive compounds from nutraceuticals and herbal medicines like flavonoids and other polyphenols have proven to be effective and have been applied with safe outcomes. Nevertheless, the exact dose and the complex mode of action are still unfolding areas (Nijveldt, van Nood et al. 2001). There is a need to investigate the influential factors on e.g. antioxidant activity during formulation development.

In a recent study the antioxidant activity influenced by extraction process was measured. It was concluded that temperature and grinding have negative effect on antioxidant activity and flavonoid content, moreover the glucosides have lower antioxidant activity than aglycone (Pápay and Antal 2014). However, their low bioavailability in vivo limits their usage in medical therapy (Nijveldt, van Nood et al. 2001). Researcher’s attention has been drawn by nanotechnology which can change the pharmacokinetics and biodistribution thus improving the bioavailability and effectiveness (Huang, Yu et al. 2010). Controlled drug delivery systems makes targeted drug delivery possible thus leaving the healthy tissues unharmed and decrease the potential of side effects. This is extremely important in case of systemically administered chemotherapeutic agents that can cause severe side effects (Ferrari 2005, Merisko-Liversidge and Liversidge 2008). On the contrary, phytochemicals with anticancer properties have mild or negligible side effects. Several flavonoids including quercetin, luteolin and rutin were loaded into nanoparticles and have been found to be useful as nanomedicine (Nair, Sung et al. 2010). Solubility improvement of apigenin is crucial in order to achieve therapeutic effect as observed previously, and the delivery of apigenin requires
appropriate pharmaceutical formulation to provide protection of the therapeutically active molecular form until delivery to the target tissue. In the last decades a number of drug delivery approaches for apigenin have been developed, including the use of nanotechnology.

Encapsulation into Inclusion Complexes and Colloidal Carriers

Encapsulation into inclusion complexes or colloidal carriers may serve as technologies for enhancement of solubility and bioavailability.

The molecular inclusion complexation can be achieved by using well known pharmaceutical solubilizers, namely cyclodextrins (CD). Natural CDs are consist of 6 (α), 7 (β), 8 (γ) glucopyranose units. These molecules are cyclic oligosaccharides made from starch by bacterial enzymatic conversion. They have truncated cone shape with hydrophobic cavity and hydrophilic rim due to the chair conformation of the units. They have the ability to form non-covalent dynamic inclusion complexes with poorly water soluble drugs thus increasing their aqueous solubility, stability and bioavailability, especially for BCS II. and IV. drugs (Loftsson and Brewster 1996), (Szent and Szejtli 1999). A number of data have been reported in the last years about the inclusion of plant materials (Pinho, Grootveld et al. 2014), but only one about the complexation of apigenin. Kim et al. investigated the aqueous solubility of apigenin with natural β-CD, its methylated (heptakis-(2,6-di-O-methyl)-β-cyclodextrin, DM-β-CD) and hydroxylated (2-hydroxypropyl-β-cyclodextrin, HP-β-CD) derivates. The possible structure of complexation can be seen on Figure 2. The highest solubilization efficiency could be achieved with HP-β-CD: 11.5 fold increase with 2 mM HP-β-CD compare to water. They also conducted phase solubility studies, where 1:1 stoichiometric flavonoid/CD complexation can be assumed from the plotted phase solubility diagrams. Stability constant (Kc) values were the following: 827.6 with β-CD, 1038.6 with DM-β-CD and 4511.5 with HP-β-CD. The strongest complexation occur with HP-β-CD, possibly due to hydrogen bonds between apigenin and hydroxyl groups (Kim H 2008). The degree of hydroxylation affects the hydrogen bonding capacity, and thus interactions with membrane (Ollila, Halling et al. 2002).

For improving biological activity, apigenin can be encapsulated into liposomes or micelles, too (Figure 3).

Lipid nanocapsules are patented biomimetic carriers which have characteristic hybrid structure of polymer nanocapsules and liposomes.
Figure 2. Scheme for the molecular inclusion complexation of apigenin with cyclodextrin.

Figure 3. Encapsulation of apigenin into colloidal carriers.

Lipid nanocapsules (LNC) were applied to encapsulate apigenin with phase inversion method. Generally medium-chain triglycerides build up the oily core which is surrounded by mixture of lecithin and pegylated surfactant.
as a membrane. One of their advantages compared to liposomes is the solvent-free preparation process and longer stability, up to 18 months (Huynh, Passirani et al. 2009). The experiment was optimized by simplex lattice design experiment, for indexes drug loading and encapsulation efficiency was used. The optimal formulation resulted 1.26 ± 0.05% drug loading and 95.86 ± 0.38% encapsulation efficiency. The total apigenin concentration was 5.88 mg/mL with well dispersed, spherical in shape nanocapsules with average particle size 46.1 nm and -28.18 mV zeta potential. The in vitro release behavior showed two phase dynamics process and in vivo MTT assay showed enhanced antiproliferative activity. The study concluded that lipid nanocapsules are potential carrier for apigenin to improve its solubility and biological activity (Ding, Chen et al. 2013).

**Topical Delivery**

One of the studies was to investigate the influence of vehicle, distant topical delivery and biotransformation on chemopreventive activity of apigenin in mouse skin. Dorsal and abdomen skin of female SENCAR mice were treated and compared with apigenin in different model vehicles: DMSO, acetone/DMSO (4:1) and propylene glycol/DMSO (4:1). Apigenin showed to be effective in the order of DMSO > acetone/DMSO > propylene glycol/DMSO. Although most of these vehicles may not be suitable for human use, one of the goals of the study was to investigate whether apigenin can get into the blood circulation through the skin. The results suggested that apigenin permeate only locally to the skin tissues, with no transdermal permeation observed. The biotransformation experiments concluded that sulfate and glucuronide metabolites are not involved in the chemopreventive activity of apigenin (Li, Pinch et al. 1996).

Incorporation of apigenin into a topical liposomal formulation showed success as substitutes for corticosteroid therapy in a clinical study. The apigenin-enriched, standardized chamomile extract were encapsulated into Natipide®II, a semi solid liposome gel, containing 20% of purified phospholipid fraction. The effectiveness of this formulation was compared to a non-liposomal oil-in-water cream, containing 5% Eumulgin® VL 75 as emulsifier. In vitro dissolution studies showed superior characteristics for liposomal creams. In vivo both were well tolerated and reduced inflammation, however, the liposomal formulation had slightly better therapeutic effect despite the same drug concentration. These results indicate that liposomes can
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enhance the penetration into the skin and the formulation strategy has a great importance in therapeutic efficiency. Based on this clinical study, apigenin-enriched formulations showed a great promise as a substitutes of corticosteroid therapy for e.g. eczema without severe side-effects (Arsic, Tadic et al. 2011).

Drug delivery of apigenin into the skin is not only as an alternative but as a first choice treatment showed promising results against skin cancer. This disease is becoming resistant to conventional chemotherapy therefore improved drug delivery like mitochondria and DNA targeting is needed. In a recent study the efficacy of apigenin-loaded PLGA nanoparticles for mitochondrial targeting was tested (Das, Das et al. 2013). PLGA (poly (lactic-co-glycolic acid) is a biodegradable, biocompatible and non-toxic material, one of the most successfully developed polymer. Its hydrolysis leads to two non-toxic metabolites: lactic acid and glycolic acid thus minimal toxicity is associated with the use of PLGA. It is approved by the FDA and European Medicine Agency for parenteral administration. Several formulations and methods of production for various drugs are well described. It is also able to protect the encapsulated drugs from degradation. Furthermore, sustained release and even specific organ or cell targeting can be achieved (Danhier, Ansorena et al. 2012). PLGA nanocarriers have the advantage of improving the permeability of the drug and showed enhanced delivery through mice skin. Thus, the aim of the study was to determine whether nano-encapsulated apigenin have greater anti-proliferative effect on UV-B and benzo(a)pyrene induced skin cancer in mice. Encapsulation was prepared using a one-step procedure of nanoprecipitation. Atomic force microscopy image showed that the majority of the particles are uniform and have spherical shape. The mean diameter was 101 nm with 87.2% encapsulation efficiency. Controlled release up to 72 h could be observed with a biphasic release profile characteristics, the initial burst release lasted for 16 hours. In order to determine the efficacy of nano-encapsulated apigenin histopathological sections and chromosomal aberrations were studied. Results showed that encapsulation improved the effectiveness due to small size and faster mobility; it reduced tissue damage and frequency of chromosomal aberrations. Moreover mitochondrial-apoptosis in cancerous tissues could be observed therefore nano-encapsulated apigenin appears to be promising in the skin cancer therapy (Das, Das et al. 2013). The same group found that PLGA nano-encapsulated apigenin had the ability to enter the nucleus thus directly target DNA. This suggests that PLGA nanoparticles can generate greater apoptotic effect and faster action and can prospectively be a new anti-cancer strategy in drug delivery to the skin (Das, Das et al. 2013).
Recently, a novel topical delivery system was developed for apigenin using ethosomes. This novel type of liposomes can enhance absorption and solubility due to 20-25 % (v/v) of lower alcohol content such as ethanol or propylene glycol. Their high deformability helps to transport the drug into the deeper layer of the skin more effectively than conventional liposomes. The optimal formulation was identified by uniform design experiment. Binary ethosomes were prepared to increase the stability and reduce aggregation using ethanol and propylene glycol. Lipoid S 75 (phosphatidylcholine containing 68-73% of soybean lecithin) was chosen as lipid phase. To compare the effectiveness of ethosomes, empty ethosomes with external apigenin, conventional liposomes and deformable liposomes (containing Tween 80) were prepared with mechanical dispersion method. All formulations were loaded with 0.02% (w/v) apigenin. The volume ratio of the lower alcohols significantly affected the mean ethosome size. With increasing amount of propylene glycol the size increases or decreases with the total amount of alcohols. The particle size of the formulations ranged between 36.61 ± 1.78 nm and 698.33 ± 124.30 nm, with zeta potentials between 10.14 ± 2.04 mV and 27.67 ± 3.23 mV. The optimum formulation had particle size distribution of 67.09 ± 4.10 nm with 19.30 ± 0.89 mV zeta potential. Entrapment efficiency of the optimum ethosomes was 91.22 ± 6.38 %, significantly higher than the other formulations 89.55 ± 1.57 % for liposomes and 81.93 ± 0.63 % for deformable liposomes. Increased level of Lipoid S 75 increased the entrapment efficiency of apigenin and the stability at room temperature for 30 days. Skin deposition and transdermal efflux experiments were also conducted. Skin deposition measurement revealed that increasing amount of propylene glycol decreased the skin deposition of apigenin, however, this increased with higher amount of Lipoid S 75 and lower alcohol concentration in the formulation. High concentration of lower alcohols can positively influence the flexibility and fluidity of the ethosomes therefore increased deformability can enhance the penetration of the drug through stratum corneum. Considering these findings, the optimum formulation should consists of 5% Lipoid S 75 and a mixture of lower alcohols in the ratio of 1:10 (v/v) propylene glycol to ethanol. Transdermal efflux experiments showed similar result regarding the increased propylene glycol ratio to ethanol. Ethanol is a one of the most used penetration enhancer and co-solvent but it often causes excessive transdermal drug flux. Propylene glycol was therefore added to increase the viscosity, hygroscopicity and stability thus enhancing the accumulation into deep skin layers. Superior skin targeting could be achieved \textit{in vitro} with these optimized ethosomes. This improved permeability of
apigenin into the skin is mostly related to the vesicle deformability and smaller particle size of ethosomes than conventional and deformable liposomes. Conventional liposomes did not penetrate deeply into the skin and in case of deformable liposomes the deposition of apigenin was even slower. However, there were number of differences between in vivo and in vitro skin deposition profiles, with ethosomes having the strongest effect on reducing inflammation induced by ultraviolet B (UVB) light. Ethosomes therefore can be a promising tool for superior topical delivery and targeting (Shen, Zhang et al. 2014).

Apigenin nanocrystals were prepared also as a skin protective formulation against UVB. Nanosuspension or so-called nanocrystals - when the drug particles are in crystalline state - are nanosized and carrier free colloidal dispersions consisting of drug particles with only a small amount of stabilizer e.g. Plantacare 2000 UP® (alkyl polyglycoside). The main advantages of nanocrystals are increased dissolution velocity and saturation solubility which results in increased concentration gradient between the formulation and the skin and therefore prolonged contact with the skin due to their adhesiveness (Müller and Peters 1998). Nanocrystals can be produced with “top-down” (reduce particle size) or “bottom-up” (growth of particles from molecules) technologies. The “top-down” technology e.g. wet milling or high pressure homogenization is widely used in pharmaceutical research because of the easier scale up for the industry. Since 2001, smartCrystals® technology has been used. Nanocrystals prepared with this combination technology (pretreatment step followed by high pressure homogenization step) show better characteristics e.g. physical stability. In this study the combination technology - bead milling and high pressure homogenization - was used to prepare apigenin smartCrystals. Significant reduction in size was observed after each bead milling process until the 5th passage therefore it was terminated after 7 cycles, followed by low pressure (300 bar) homogenization step. Generally low pressure (100-500 bar) yields smaller and more homogenous nanocrystals. The photon correlation spectroscopy (PCS) diameter of the prepared nanocrystals was 396 ± 12 nm with 0.205 PdI ± 0.007. Low PdI indicates better physical stability. Light microscopy measurement showed uniform crystal distribution and no large crystals (>1µm) or aggregates could be detected. 1% of Plantacare 2000 UP® stabilizer provide optimal stability. Zeta potential measurements indicated well charged surface stability (-38 mV). X-ray diffraction (XRD) revealed no amorphous stage which would reduce the stability and shelf life of the product. The developed smartCrystals can be easily utilized for further use as gel or cream where possibly a faster dissolution rate and increased permeation would be achieved. Furthermore, the
in vitro antioxidant capacity of the nanosuspension is doubled compared to the original suspension (Al Shaal, Shegokar et al. 2011).

Oral Delivery

Orally administered apigenin has also a great potential in anticancer therapy but the extensive metabolism and poor aqueous solubility make it challenging. Nanocrystals can be further used to improve the oral bioavailability and also produce reproducible oral absorption. A new preparation method, SAS (supercritical antisolvent process) has been developed recently. This “bottom-up” process (recrystallization technology) requires no surfactant or other excipients and based on one kind of supercritical fluid technology. Briefly, the organic solution of the drug is injected into the supercritical fluid (generally CO₂) and the drug precipitates as fine particles as the solvent get extracted and the drug solution gets supersaturated.

The improved dissolution rate of the nanocrystals might be the key factor to increase the bioavailability and an effective formulation strategy for oral drug delivery (Zhang, Huang et al. 2013). Zhang et al measured the physicochemical and pharmacokinetic properties of the coarse powder and the prepared apigenin nanocrystals by SAS process. Morphological evaluation of the nanocrystals by scanning electron microscopy revealed regular shape and smooth surface with particle size of 400-800 nm. The mean particle size was determined to be 562.5 ± 56 nm with 0.92 ± 0.21 PdI according to PCS measurements. The crystals were mainly unaltered considering XRPD (X-ray powder diffractometry) and DSC (differential scanning calorimetry) analysis which means beneficial physicochemical stability. Moreover, FT-IR analysis showed that apigenin remained chemically stable during the process. Conducting in vitro dissolution studies, nanocrystals have higher dissolution platform (more rapid and higher cumulative amount) which can be attributed to the enhanced saturated solubility due to the reduced particle size. Only 40% of the coarse powder was dissolved in a 120 min study under sink conditions (0.1 M PBS 6.8 with 0.5% Polysorbate). In contrast more than 90% dissolved within 20 min, as apigenin nanocrystals, demonstrating good in vitro dissolution behavior. The plasma concentration of apigenin following intravenous administration in vivo decreased rapidly with time and followed a biphasic pattern due to the initial distribution and metabolism in the tissues. The formulation administered orally resulted much lower serum concentration,
but still higher than the coarse powder. The $C_{\text{max}}$ and $\text{AUC}_{0-12\text{ h}}$ were enhanced by 3.6 and 3.4 fold compared to apigenin coarse powder. The absolute bioavailability was enhanced from 2% up to 6.9%. It was concluded that nanocrystals with much smaller size and larger surface area can significantly improve the dissolution rate which contributes to higher $C_{\text{max}}$ and $\text{AUC}_{0-12\text{ h}}$ after oral administration. Furthermore, increased muco-adhesion and gastrointestinal transit time can be assumed.

In another paper mixed TPGS (D-α-tocopherol acid polyethylene glycol succinate) modified phospholipid micelles were designed to increase the oral bioavailability of apigenin. The vesicular structure of the phospholipid complexes have limited stability therefore TPGS - a water soluble derivate of natural Vitamin E - was added. It is formed by the esterification of Vitamin E succinate with polyethylene glycol (PEG) and therefore contains a hydrophilic head (PEG) and a lipophilic tail (Vitamin E) like other surface active molecules. The relatively low CMC (0.02% w/w) makes it an ideal molecular biomaterial for developing liposomes and nanoparticles. It has been used as a solubilizer, stabilizer and absorption enhancer. It can prolong the half-life of the drug in the plasma and enhance cellular uptake but most importantly inhibits P-glycoprotein increasing oral delivery (Zhang, Tan et al. 2012). The apigenin-phospholipid-TPGS micelles were prepared with thin film hydration method. The 3D molecular modeling showed that apigenin was positioned in the hydrophobic chains of lecithin, directed to the glycerol moiety and similarly in the hydrophilic PEG chains of TPGS where intramolecular hydrogen bonds and electrostatic forces possibly play a role in the interaction. The 3D structures is shown on Figure 4. The computer model prediction - that PEG chains are directed to the surface of the micelle – is confirmed by the negative zeta potential data (-12.94 mV). The complexation with phospholipids was verified by FT-IR and NMR spectroscopy measurements while the interaction with TPGS was studied with surface tensiometry. The encapsulation efficiency was 87.35%, drug loading was 12.6% with 137.1±3.4 nm particle size. In vitro release experiments showed an initial controlled release for 2 hours and the plateau was reached after 15 hours. Increased intestinal absorption up to 2.4 fold and higher cellular uptake with significant cytotoxicity effect on A549 cancer cell lines could be achieved.

In vivo 72.9% inhibition in S180 carcinoma mice were observed. These results suggest that TPGS micelles combined with phospholipid complex technology can be a novel way for oral drug delivery (Munyendo, Zhang et al. 2013).
Oral liquid dosage forms are therefore usually preferred; self-microemulsifying systems (SMEDDS) – a novel and promising technology for enhancing oral absorption has gained attention recently. SMEDDS contains oil, surfactant, co-surfactant and the drug. The drug can be solubilized in the oil phase and/or on the surface of the surfactant and co-surfactant in the microemulsion. Therefore the surfactant and co-surfactant play an important role in the solubilization of the drug. Mixing with water can produce thermodynamically stable oil-in-water microemulsion. The drug is dissolved in small droplets (size <100 nm) therefore the large surface area can enhance drug absorption (Pouton 2000). This system shows high drug entrapment and fast dissolution but the increase in the oral bioavailability is still needs to be estimated (Narang, Delmarre et al. 2007).

Zhao et al investigated a self microemulsifying delivery system for apigenin, optimized by a simplex lattice experiment design. Ternary phase diagrams were also constructed from the excipients with high solubility of apigenin to obtain the most efficient concentration ranges for microemulsions. The apigenin exhibited the highest solubility in Capryol™ 90 oil (1.39 ± 0.03 mg/mL), in Tween 80 surfactant (26.59 ± 1.16 mg/mL) and in Transcutol®HP co-surfactant (18.16 ± 0.43 mg/mL). The drug loading depends largely on drug solubility in the oil phase. The surfactant should be relatively hydrophobic (hydrophilic-lipophilic balance, HLB < 12) to obtain high self-microemulsifying ability (immediate formation of o/w droplets) and forms stable microemulsion when diluted with aqueous media (Kohli, Chopra et al. 2008).
Although Tween 80 exhibited the highest apigenin solubility, it could not form SMEDDS with Transcutol® HP and Capryol™ 90. PEG 400 was selected for the co-surfactant screening along with Transcutol® HP, however, its higher hydrophilic property could destroy the emulsion system. Finally, the optimal formulation consisted of 60% Cremophor® EL, 30% Transcutol® HP and 10% Capryol™ 90. 7500-fold increase in water solubility of apigenin could be achieved with 17.1 nm average particle size and zeta potential -5.18 mV. The concentration of apigenin was selected to maximum 7 mg/g since at levels exceeding this apigenin precipitated during dilution with water. Transmission electron microscopy (TEM) images showed spherical droplets without agglomeration. 30 s was enough for the formation of microemulsion after dilution with distilled water and it did not precipitate after 8 hours (1 g of SMEDDS to 100 mL distilled water). The droplet size can be influenced by many factors and it plays an important role in the absorption. Interestingly, the size of this formulation was not affected by pH and ionic strength (diluted with distilled water, 0.9% NaCl, pH 6.8 PBS, 0.1 M HCl) and various mixing ways (vortex, magnetic stirring) but influenced by amount of apigenin. In vitro dissolution study demonstrated fast dissolution: 95% of apigenin was released within 10 minutes. All of the results confirmed that SMEDDS could enhance the solubility of apigenin as a potential carrier for oral absorption (Zhao, Zhang et al. 2013).

Nanosized polymeric micelles with particle size ranging from 10-100 nm have been recognized as a potential drug delivery systems for anticancer drugs. Their small size allows accumulation in cancerous tissues and prolonged circulation time without being recognized by reticuloendothelial system (RES). Conventionally, biocompatible and self-assembly amphiphilic block copolymers can form core-shell structure in aqueous media. The hydrophobic fraction is able to incorporate poorly water soluble drugs as a core while the hydrophilic chains crosslinking outside and form a shell (Croy and Kwon 2006). In a recent study apigenin was successfully loaded into polymeric micelles composed of Pluronic P123 and Solutol HS15, to improve its water solubility with a thin-film dispersion method (Zhai, Guo et al. 2013). Pluronic P123 is one of the most widely used triblock copolymer with a structure PEO-PPO-PEO. The hydrophobic PPO group comprises 70%, thus providing suitable microenvironment for hydrophobic drug while 30% PEO can form a relatively thin shell with reduced stability, necessitating further modifications (Schillen, Jansson et al. 2008). Solutol HS15 (polyethylene glycol-660 hydroxystearate) is recorded in the European Pharmacopoeia as a non-ionic solubilizing agent containing 30% of free polyethylene glycol.
In general, PEG used in nanoformulations can increase stability and systemic circulation time (Vonarbourg, Passirani et al. 2006). The micelle formulations were optimized by central composite design. The solubility of apigenin was increased 148 times higher (320.8 µg/mL) than of crude apigenin (2.16 µg/mL) with low CMC concentration (4.23 x 10^{-5} mol/L) the following formulation: apigenin 1.68 mg, P123 92 mg and Solutol HS15 29 mg. The encapsulation efficiency and drug loading were 96.36% and 1.32 %. Under transmission electron microscope (TEM) the formulation had homogenous morphology. The size was 16.9 nm, smaller than blank micelles (18.9 nm), probably due to hydrogen bonds between phenolic hydroxyl group of apigenin and the carboxyl of the PEG chains. Moreover, in vitro drug dissolution study showed sustained release behavior and cytotoxicity studies on HepG2 and MCF-7 hepatoma cells demonstrated enhanced tumor inhibition (Zhai, Guo et al. 2013).

The solubility behavior of an active ingredient is the most challenging aspect, therefore there is a constant demand for new type of drug carriers in oral formulation development. Although solid dispersions (SDs) are known since 1961, their application is still increasing with new types of dispersing carriers e.g. silica nanopowder. In these dispersions the drug is incorporated in an inert carrier in solid state. Sugar was used as a carrier in the first generation SD. Second generation SD utilized amorphous polymeric carriers e.g. PEG in the late sixties, and more recently self-emulsifying and high surface active carriers belong to third generation SD. These carriers are able to improve dissolution rate significantly by reducing particle size, amorphous drug state and enhanced wettability and porosity can be achieved avoiding drug crystallization. Optimized manufacturing techniques make easier scale-up possible for industrial research (Chiou and Riegelman 1971, Leuner and Dressman 2000, Vasconcelos, Sarmento et al. 2007). One new type of dispersing carrier is carbon nanopowder (CNP). This nanomaterial is built up from carbon with diameter of less than 100 nm. It has many unique features like large surface area, chemical inertness and high dispersibility which helps to improve drug dispersion, and are thus widely used in targeted drug therapy. CNP-Apigenin was prepared by solvent evaporation. Briefly, apigenin and CNP were dissolved in ethanol and evaporated in rotary evaporator at 40°C until a clear powder mixture was formed. In comparison a homogenous physical mixture was prepared by grinding using a mortar and pestle. In vitro drug release and in vivo performance were also evaluated from the solid dispersions of apigenin prepared with CNP. All of the CNP-Apigenin had better dissolution profiles than apigenin powder alone (only 38% was
dissolved). Approximately 92% of apigenin was released from the formulation with weight ratio 6:1 and the drug release dissolution profile was improved by 275% within 60 minutes compared to crude apigenin powder. DSC thermogram did not show the peak of apigenin suggesting it is molecularly dispersed and may be in amorphous form, which facilitated the dissolution. Scanning electron microscopy confirmed that apigenin is dispersed in CNP while XRD patterns show amorphous state. Oral bioavailability was tested in rats. The pharmacokinetic analysis showed 1.83 times increase in the AUC\textsubscript{0-\texttau} for CNP-Apigenin indicating improved bioavailability. The CNP-Apigenin enhanced the relative oral bioavailability by 183%. The in vivo tests were in accordance with in vitro data suggesting that the poor bioavailability can be attributed to poor dissolution and reduced absorption. Preliminary intestinal toxicity test was carried out on jejunum mucosa of rats and did not show any degeneration, necrosis, edema or inflammation. Therefore, it can be assumed that CNP are safe and effective vehicles to enhance bioavailability for poor water soluble drugs. Moreover, CNP-Apigenin did not agglomerate and flowed freely, making it possible to formulate on a large scale. It was concluded that CNP is a promising SD carrier for clinical application (Ding, Zhang et al. 2014).

**Dendrimers for Apigenin Delivery**

Dendrimers are polymeric macromolecules with hyperbranched three dimensional structures (Figure 5). Controlled, globular structure and a large number of functionalities yet single molecular weight are significantly advantageous over traditional linear polymers. These attracted researcher’s attention from the mid-1980s because of their unique shape and potential applications in drug delivery. Since then, a large number of dendrimers have been synthetized with various architectures. Two well documented synthetic strategies are used to prepare dendrimers, with the main difference being polymer growth (Hawker and Frechet 1990, Tomalia, Naylor et al. 1990). Basically, the polymer branches grow divergently or convergently start from a polyfunctional core (Liu and Fréchet 1999). A number of application are known for dendrimers. In the past decades biocompatible dendrimers were synthetized for drug delivery of e.g.vaccines and immunology. Recently, attempts have been made for cancer treatment and photodynamic therapy. Dendrimers can be useful tools as multifunction nanoparticulate systems for imaging, diagnostic and targeting in the therapy (Gillies and Fréchet 2005).
They are a technological breakthrough and their use in nanotechnology is progressively emerging. Although accurate selection of dendrimers is a key factor in drug delivery they have a great potential as an optimal drug delivery system for numerous drugs including DNA, proteins, gene therapy as well as diagnostic and solubilizing agent (Kesharwani, Jain et al. 2014). Apigenin was chosen as a fluorophore core of benzylic dendrimers in a recent study. Fluorescent dendrimers have an advantage as analytical tools or organic light emitting devices (OLEDs). Apigenin possess weak blue light emission and three phenol groups therefore ideal for synthetizing dendrimer according to Fréchet method (Hawker and Frechet 1990, Liu and Fréchet 1999). Size and branching effects were studied with molecular dynamics simulation. Data suggest that larger asphericities can occur at the third and fourth generations of dendrimers. Aggregation phenomena can be suggested for nonspheric dendrimers from the fluorescent spectras (Vinš, Vermachová et al. 2013).

Further Prospects

Phytosome technology is a promising concept for enhancement of oral bioavailability. In water, phytosomes form liposome-like structure, however, there are significant differences (Kidd 2009, Semalty, Semalty et al. 2010).
The phytosome is a molecular complex consisting of the drug and phosphatidylcoline which later is compatible with biological membranes. The phospholipids are amphiphilic molecules and easily incorporate polar or non-polar compounds, thus enhance the absorption through lipid-rich biological membranes. Since they can improve the bioavailability of both water soluble and insoluble materials, phytosomes may offer oral delivery for apigenin in the near future.

**CONCLUSION**

In the last few decades the use of nutraceuticals to prevent chronic illnesses like diabetes, cardiovascular diseases and cancer has been rapidly emerging. However, their low bioavailability in vivo limits their usage for medical therapy. Researchers are drawn towards nanotechnology which can change the pharmacokinetics and biodistribution thus improving the bioavailability and efficacy. Solubility improvement of apigenin is crucial in order to achieve therapeutic effect as discussed in the above section. The delivery of apigenin requires pharmaceutical formulation to provide protection of the therapeutically active molecular form until it reaches the target tissue. A few drug delivery approaches for apigenin have been developed in the past decades to address this issue, including novel techniques in the field of nanotechnology. The solubility behavior of an active ingredient – like apigenin – is still the most challenging part therefore there is a constant demand for new type of drug carriers in formulation development.

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