FORMULATION AND CHARACTERIZATION OF NANOFORMULATION OF APIGENIN

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ABSTRACT

Apigenin, a trihydroxyflavone, recognized as a cancer chemo preventive agent suffers from erratic absorption and low oral bioavailability problems. Hence there is a need to develop a novel formulation for such compounds which can combat the aforementioned limitations.

Traditional strategies, such as micronization, solubilization using co-solvents, the use of permeation enhancers, oily solutions and surfactant dispersions which evolved earlier to tackle the formulation challenges, have limited use. Although reasonable success has been achieved in formulating water-insoluble drugs using liposomes,emulsions, microemulsions, solid dispersion technology and inclusion complexes employing cyclodextrins there is no universal approach applicable to all drugs. Hence, there is a growing need for a unique strategy that can tackle the formulation-related problems associated with the delivery of hydrophobic drugs in order to improve their clinical efficacy and optimize their therapy with respect to pharmacoeconomics.

The aim of the present work was to prepare nanosuspension of apigenin in order to improve solubility and dissolution profile, so as to enhance the oral bioavailability. Apigenin nanosuspension was prepared using high pressure homogenization technique by employing various surfactant stabilizer systems. The size of theoptimized formulation was found to be in the range of 200–210 nm. Morphological studies exhibited regular smooth spheres of apigenin particles in the final formulation. The DSC indicated an unaltered crystalline state thus, exhibiting stability after thehomogenization process.Thus, it can be concluded that apigenin nanosuspension results improved solubility and dissolution profile.

KEYWORDS: Apigenin. Stabilizers, dissolution.

INTRODUCTION

Nanotechnology is defined as the science and engineering carried out in the nanoscale that is 10–9m.The chief goal of nanotechnology is to assist in lowering drug toxicity by specific targeting. This will enable to improve the efficacy as well as safety, as a low dose is sufficient to provide a desired pharmacologic effect. Nanoparticles are preferable to administer water insoluble drugs and to improve the bioavailability. Converting poorly water-soluble drug compounds into nanoparticulate dosage forms can significantly increase dissolution rates, tackling the problem of poor water solubility of hydrophobic drugs. The various nanoparticle drug delivery systems designed and developed to improve the solubility and bioavailability of such poorly water soluble drugs include nanocrystals and nanosuspensions, solid lipid nanoparticles and nanostructured lipid carriers, microemulsions, nanoemulsions and dendrimers (2).

Nanosuspensions have revealed their potential to tackle the problems associated with the delivery of poorly water-soluble and poorly water- and lipid-soluble drugs, and are unique because of their simplicity and the advantages they confer over other strategies. The particle size distribution of the solid particles in nanosuspensions is usually less than 1μm. As the particle size of a crystal is decreased to about 100 nm there is a drastic change in the properties of the material. The decreased size increases the surface area and solubility of drug manifolds and there is proportionate increase in the bioavailability of poorly soluble drugs. However, when the particle size approaches to less than 1-2 μm, the saturation solubility is also a function of particle size. The dissolution pressure increases due to the strong curvature of the particles leading to an increase in saturation solubility. Nanosuspensions thus, not only increase the surface area or saturation solubility but also offer a quick action onset due to faster dissolution and rapid absorption. This is advantageous particularly for drugs where a quick action is desired e.g. naproxen for relief of headache (4).

Nanosuspensions thus, not only increase the surface area or saturation solubility but also offer a quick action onset due to faster dissolution and rapid absorption. This is advantageous particularly for drugs where a quick action is desired e.g. naproxen for relief of headache (4). The bio-availability of various drugs has been found to increase significantly when administered in the form of nanosuspensions. Nanosuspensions can show a strong adhesion because of the increased contact area for van der Waals attraction. The adhesiveness of the

nanoparticles to the gut wall after oral administration enhances absorption and thereby increases the bioavailability. Nanosuspensions may be able to reduce the dose to be administered, provide a sustained drug release and increase patient compliance. The increased bio-availability leads to reduction in dosing frequency which may improve patient compliance. Nanocrystals can be incorporated in various dosage forms which make administration by various routes feasible. Due to better solubility and bioavailability, nanocrystals can be supplied in patient friendly oral solid dosage forms such as tablets and capsules. Nanocrystals of poorly soluble drugs can also be incorporated in cosmetic products where they provide high penetration power through dermal application. A very small size of nanoparticles (200-400 nm) even smaller than the size of the smallest blood capillaries allows the nanosuspensions to be injected intravenously. This provides 100% bio-availability and simultaneously avoids the use of toxic surfactants or co solvents to dissolve the drug. Pulmonary and ophthalmic drug delivery of nanocrystals has also been achieved with better efficiency (5). Nanosuspensions can be used for targeted delivery because their surface properties and changing of the stabilizer can easily alter in-vivo behavior.

The drug nanocrystals / nanosuspensions are a smart delivery system, a universal principle, which can be applied to any drug because any drug can be diminuted to nanocrystals. Furthermore, both lipophilic and hydrophilic drugs can be incorporated as nanocrystals. Another essential prerequisite for entry to the pharmaceutical market is the availability of large scale production methods at sufficiently low cost and

DRUG PROFILE

APIGENIN

Apigenin is a widely occurring flavonoid present in various fruits and vegetables including, parsley, chamomile, oranges, onions, wheat sprouts. It is isolated and extracted from the dried flowers of *Matricariachamomilla*, Asteraceae. Apigenin has potential against carcinogenesis. It had been reported to inhibit the progression of a large number of cancers including, breast, lung, colon, thyroid, small cell lung cancer, leukemia and prostate (11,12,13). Furthermore, it exhibits purgative, anti-inflammatory and anti-viral activities. Apigenin has been reported to exert anticarcinogenic effects by various mechanisms including downregulation of NF-kB activation in cultured human endothelial cells (14,15). Shukla and Gupta, 2007 revealed the modulation of MAPK, cyclin D1 and PI3-Akt thus, leading to cell cycle arrest in human prostate cancer cell lines (16). Kim et al., 2014 has reported inhibition of cell growth by overexpressing p27 and downregulating cyclin dependent kinase 4 and cyclin D1 expression thus, leading to cell cycle arrest at G0/G1

phase. In another study conducted by Zhu *et al.*,apigenin has been found to arrest cell cycle at G2/M phase in T24 human bladder cell lines. It had also been found to induce apoptosis by upregulating caspase-3 activity and PARP cleavage. Also, the apoptotic activity includes the upregulation of Bax, Bak, etc belonging to Bcl family. (13)

Category – Anti-oxidant, Anti-inflammatory, Chemopreventive agent

IUPAC Name – 5, 7-Dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one

Molecular Formula – C15H10O5

Molecular Mass – 270.24 g/mol

MATERIALS AND METHODS

The drug sample of apigenin was obtained from Otto Chemie Pvt. Ltd., Mumbai. The obtained drug sample was identified according to standard procedures.

PREPARATION OF APIGENIN NANOSUSPENSION

The apigenin NS was prepared by high-pressure homogenization method using UltraTurrax® homogenizer. Surfactants were dissolved in 50 mL of water to obtain the aqueous surfactant solution. Apigenin powder (50 mg) was dissolved in 0.3 mL of DMSO solution to form the oil phase solution, which was poured into the aqueous surfactant solution, with continuous magnetic stirring to form pre suspension. The presuspension was subjected to homogenization at 10,000 rpm for 20 minutes with Ultra turrax T25 homogenizer at room temperature to form the final nanosuspension. The samples were withdrawn after the homogenization size reduction steps for size distribution analysis. The suspension was centrifuged immediately to obtain a semi solid cake. The centrifuged product was redispersed with water for oral administration. Re-dispersion was done by manual shaking for 1 minute and the re-dispersion volume being equivalent to the original volume of the NS. Fig 3.1 depicts the schematic representation of the preparation method.

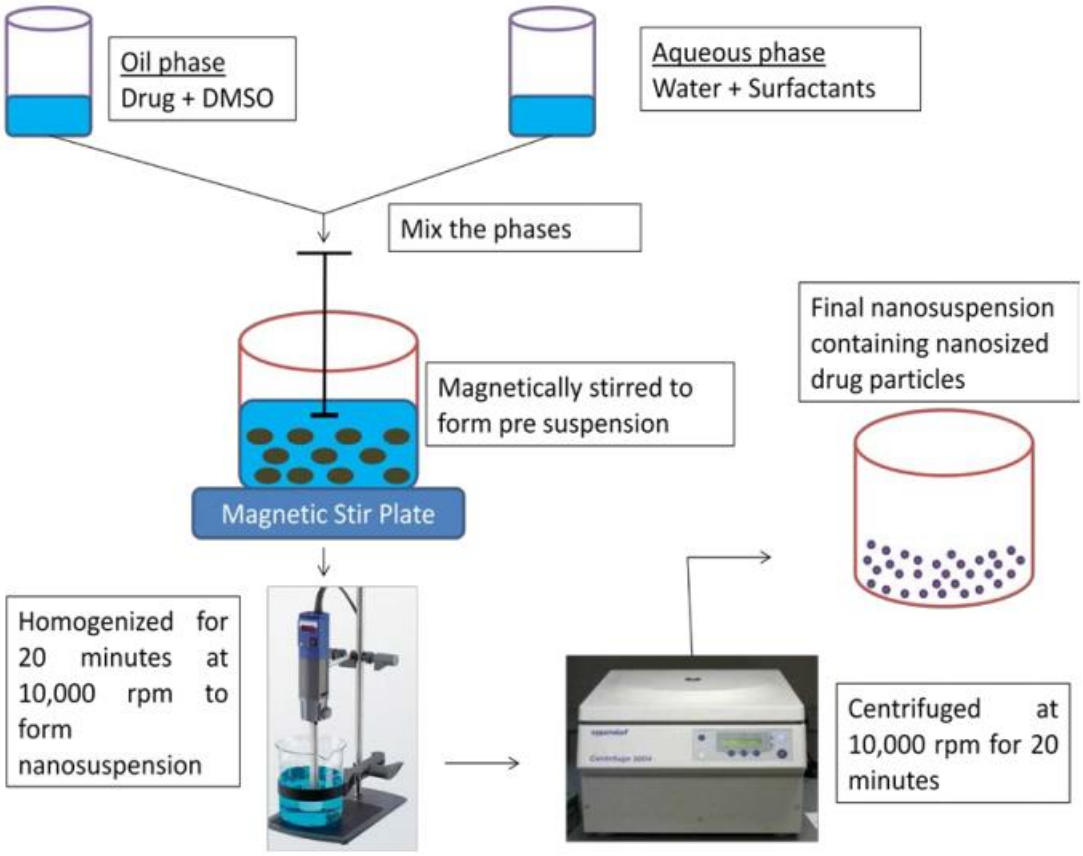

Figure: 1 Schematic representation for preparation of apigenin nanosuspension

EVALUATION PARAMETERES

Impact of different surfactants on the particle size, PDI, and zeta potential of apigenin nanosuspension

Table 1: Impact of different surfactants on the particle size, PDI, and zeta potential of apigenin nanosuspension

Surfactants	Particle size, (nm)	PDI
PEG 400	280.1 ± 1.39	0.222 ± 2.30
Polaxomer 188	343.7 ± 8.63	0.223 ± 1.68
CMC Na	829.2 ± 5.89	0.335 ± 2.72
Tween 80	429.8 ± 2.77	0.254 ± 1.55
Carbopol 934	347.7 ± 0.53	0.244 ± 1.22
Lecithin	1295.5 ± 10.82	0.345 ± 4.62

Mean ± SD, n=3

Impact of different surfactants on the stability of apigenin nanosuspension

Table: 2 Impact of different surfactants on the stability of apigenin nanosuspension

Surfactants	**0 week**	**2 weeks**	**4 weeks**
PEG 400	280.1 ± 1.42	292.6 ± 3. 87	301.1 ± 1.07
Polaxomer 188	343.7 ± 0.88	353.8 ± 4.73	371.9 ± 0.38
CMC Na	829.2 ± 2.08	831.4 ± 0.79	830.8 ± 0.86
Tween 80	429.8 ± 2.13	433.2 ± 1.85	435.5 ± 3.94
Carbopol 934	347.7 ± 6.82	350.8 ± 2.26	353.1 ± 1.61
Lecithin	1295.5 ± 0.53	2241.7 ± 1.29	2805.9 ± 2.31

Mean ± SD, n=3

The impact of combinatorial surfactants on apigenin nanosuspension

Table: 3 the impact of combinatorial surfactants on apigenin nanosuspension

Surfactant Systems	**Particle size, (nm)**	**PDI**	**Zeta Potential, mV**
Carbopol 934 + Polaxomer 188	356.3 ± 2.35	0.173 ± 1.62	-20.69 ±0.55
Carbopol 934 + PEG 400	276.0 ± 0.91	0.217 ± 1.88	-16.12 ± 1.28
PEG 400 + Polaxomer 188	204.3 ± 0.22	0.235 ± 2.35	-31.13 ± 3.04
Tween 80 + Polaxomer 188	436.7 ± 1.59	0.202 ± 3.86	-10.17 ± 3.42
Carbopol 934 + Tween 80	336.9 ± 2.47	0.217 ± 1.71	12.28 ± 0.72

Mean ± SD, n=3

Zeta Potential Determination

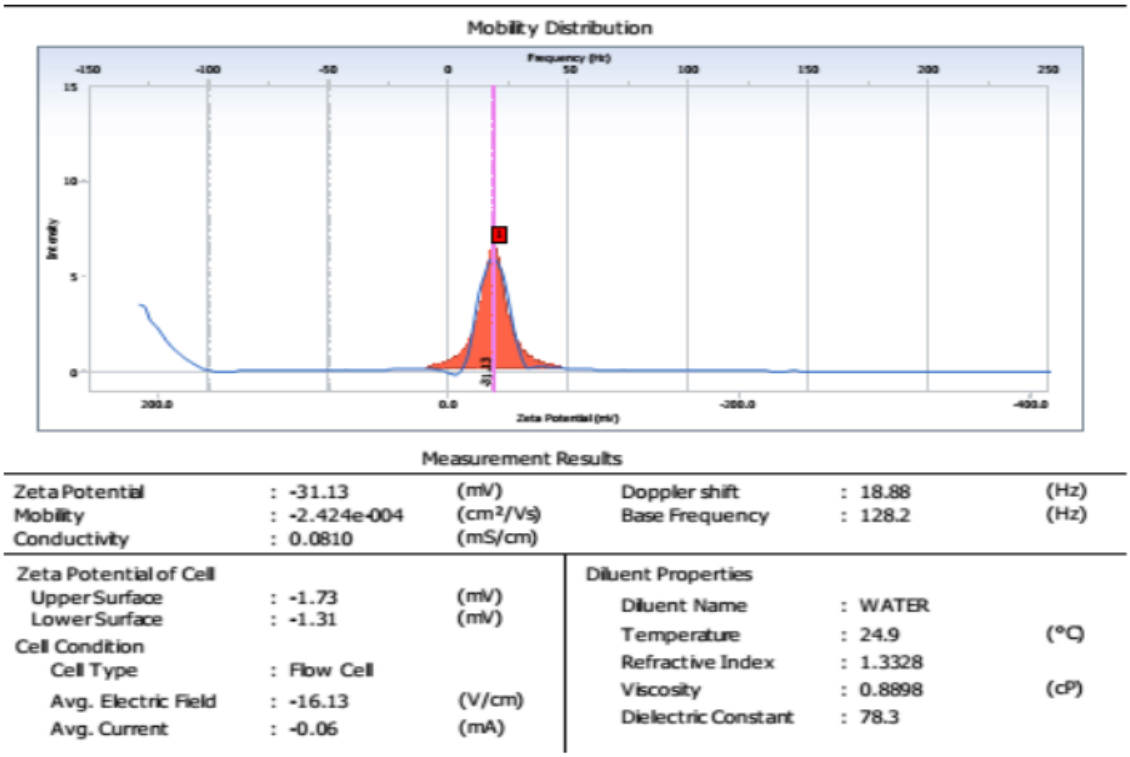

Fig:1 Zeta Potential of optimized formulation

***In-vitro* dissolution study**

The data and release behavior of apigenin CP and apigenin NS in 0.1 M HCl and methanolic PBS (pH 6.8) using dialysis bag method has been shown in Fig. Only about 40% of the drug dissolved from the dialysis bag containing apigenin coarse powder during the 120 min study, which indicated that the dissolution rate might be the limiting step for apigenin to be absorbed in vivo. However, the prepared apigenin NS demonstrated more than 90% of cumulative release within only 20 minutes. According to Cavallari et al; 2013, (24) PEG 400 is a good plasticizer having property of easier distension of its polymer chains thus, leading to faster diffusion of drug from the formulation and in turn providing improved drug release.

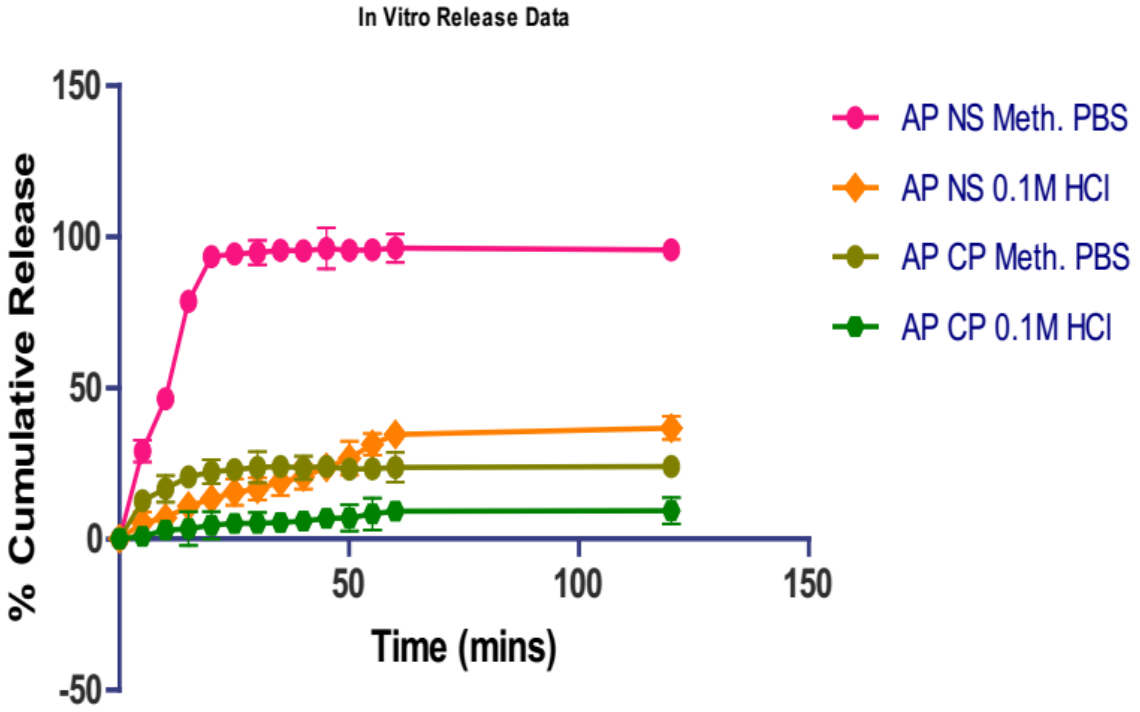

Fig :2 % Cumulative release of drug vs. time curve

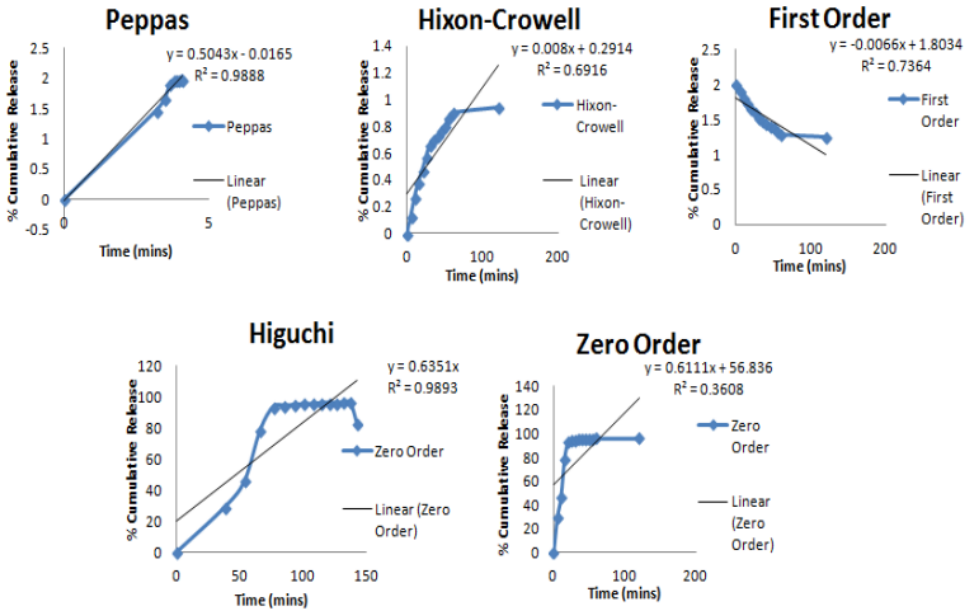

Fig: 3 Various release kinetic models for apigenin nanosuspension

RESULT AND CONCLUSION

Flavonoids has been reported to possess various numbers of effects, like ant carcinogenic, anti-inflammatory, antioxidant, anti-thrombogenic and antiatherogenic as well as antiviral. All these activities are attributed due to the following effects as they may cause decreased oxidative stress and an increased NO bioavailability; modulating signal transduction in cancer cells, interacting with there active species, free radical scavenging and also cell cycle arrest, as well as inducing apoptosis. Apart from this, the flavonoids suffer from the limitation of low solubility and poor oral bioavailability. Apigenin, a trihydroxyflavone, recognized as a cancer chemopreventive agent suffers from erratic absorption and low oral bioavailability problems. Hence there is a need to develop a novel formulation for such compounds which can combat the aforementioned limitations.

Traditional strategies, such as micronization, solubilization using co-solvents, the use of permeation enhancers, oily solutions and surfactant dispersions which evolved earlier to tackle the formulation challenges, have limited use. Although reasonable success has been achieved in formulating water-insoluble drugs using liposomes, emulsions, microemulsions, solid dispersion technology and inclusion complexes employing cyclodextrins there is no universal approach applicable to all drugs. Hence, there is a growing need for a unique strategy that can tackle the formulation-related problems associated with the delivery of hydrophobic drugs in order to improve their clinical efficacy and optimize their therapy with respect to pharmacoeconomics.Nanosuspensions have revealed their potential to tackle the problems associated with the delivery of poorly water-soluble and poorly water- and lipid-soluble drugs, and are unique because of their simplicity and the advantages they confer over other strategies. Nanosuspensions can be defined as colloidal dispersions of nano-sized drug particles that are produced by a suitable method and stabilized by a suitable stabilizer.The aim of the present work was to prepare nanosuspension of apigenin in order to improve solubility and dissolution profile, so as to enhance the oral bioavailability. Apigenin nanosuspension was prepared using high pressure homogenization technique by employing various surfactant stabilizer systems. The size of theoptimized formulation was found to be in the range of 200–210. Morphological studies exhibited regular smooth spheres of apigenin particles in the final formulation.

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