Formulation and characterization of fisetin nanosuspension

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Abstract. Fisetin (3,3,4,7-tetrahydroxyflavone) is a natural antioxidant that has shown to posses anticancer, antioxidant and anti-inflammatory properties. However, the poor solubility leads to poor bioavailability and limits its development. The aim of the study is to investigate the effect of fisetin nanosuspension using a precipitation-sonication method and additional stabilizers polysorbat 80, SLS, F68, PVP, PVA and HPMC on particle size average and the polydispersity index. The suspensions of microcrystalline FIS were prepared by a precipitation method with different proportion of stabilizers fixed. The nanosuspension produced was then characterized using Photon Correlation Spectroscopy (PCS) in term of particle size distribution, polydispersity index and morphology particle (SEM). Result showed fisetin nanosuspension were successfully prepared by anti-solvent precipitation with additional stabilizer SLS and PVA. The nanosuspension containing PVA showed smaller average particle size of 406 nm, a polydispersity index of 0.22±0.1 was obtained. The drug particles precipitated with the PVA as stabilizer were spherical in shape.

Keywords: nanosuspension, fisetin, bottom up, precipitation, ultrasonication

1. Introduction

Flavonoids are a large class of plant secondary metabolites belonging to the wider family of natural polyphenols and generally found in plants, food, fruits and vegetables [1, 2]. They are believed to have beneficial effects against multiple diseases, including cancers, cardiovascular disease, inflammatory, antioxidant and neurodegenerative disorders [3]. Fisetin (FIS, 3,7,3',4'-tetrahydroxyflavone, Figure 1 belongs to the flavonol subgroup of flavonoids found in 65 many vegetables and fruit and is especially rich in apples, strawberries, onions and mangoes [4, 5]. It exhibits antioxidant, antiinflammatory and anticarcinogenic activities. Recently, it was also considered to possess neuroprotective effects against the aging process, cerebral damage and neurodegenerative disorders [5, 6].
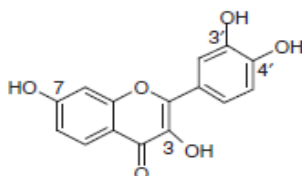
Figure 1. Chemical structure of fisetin [6]

Fisetin has low oral bioavailability (44.1%) probably due to its high lipophilicity (log P 3.2) and low (10.45 µg/mL) aqueous solubility [7]. Moreover, the fisetin molecule bearing 4 hydroxylsubstituents, including a catechol on the phenyl B ring, is extensively metabolised *in vivo* [5]. Consequently, a search for solubilized forms of fisetin is under way, with some promising results obtained by nanoemulsion formation, development of a liposomal formulation, complexation with a cyclosophoroase dimerand nanochelate formation [4-8].

Recently, the nanosuspension technology has been successfully applied to tackle the formulation issue of the poorly soluble drugs. Nanosuspension is a carrier-free colloid drug delivery system containing only minimum stabilizers and pure drug particles with a mean particle size in the nanometer range, typically between 10 and 1000 nm [9, 10]. Nanosuspension properties such as increased drug solubility, high dispersity and homogenization, intravenously injectable, simple production process, universal adaptivity enable its applications in the formulation of large amounts of poorly soluble compounds. Furthermore, the formation of suspensions is one of effective approaches for the stabilization of chemically labile molecules insoluble in aqueous solution [11, 12].

Generally, the nanosuspension techniques are classified as bottom up processes and top down processes according to the differences of the production principle [10]. In the bottom up processes the poorly water-soluble drug is first dissolved in an organic solvent and then precipitated through a non-solvent addition in the present of stabilizers, as in supercritical fluid (SCF) technology, evaporative precipitation into aqueous solution (EPAS), spray-freezing into liquid process, and emulsion-solvent evaporation [13, 14]. These processes were simple and cost effective without any high energy input. However the following prerequisites should be met: (i) the drug should be soluble at least in one solvent. (ii) The solvent is miscible with a non-solvent [4]. The top down methods means the mechanical comminution processes of larger drug particles, as in media milling, microfluidization and high pressure homogenization (HPH) [16]. These methods require no harsh solvents but involve high energy input and low power efficiency [16, 17]. In addition some measures should be taken to minimize the degradation of heat sensitive drugs resulted from the heat generated in the comminution progress [10].

The aim of the present study was to develop fisetin nanosuspension using a precipitation-sonication method and additional stabilizers polysorbat 80, SLS, F68, PVP, PVA, HPMC and Eudragit. The nanosuspension produced was then characterized using photon correlation spectroscopy (PCS) in term of particle size distribution, zeta potential, polydispersity index and morphology particle using scanning electron microscope (SEM).

2. Materials and Methods
2.1. Materials
FIS (98%) raw material was purchased from Shaanxi Dideu Medichem Co. Ltd (Xi'an, China). Polyvinyl alcohol (PVA) were kindly gifted from BASF. Pluronic F68 was purchased from the Sigma-Aldrich Chemical Co. Ltd (USA). Sodium lauryl sulphate (SLS), polyvinyl pyrolidone (PVP) and Hydroxypropylmethylcellulose (HPMC) was obtained from Cognis Indonesia Ltd. Polysorbate 80 (Tween 80®) was obtained from Shino Japan Chemical. All other reagents were of analytical grade.

2.2. Precipitation process (bottom-up method)

Generally, the suspensions of microcrystalline FIS were prepared by a precipitation method with different proportion of stabilizers fixed. The suspensions were prepared by dissolving 0.01 g of FIS in 2.0 mL of ethanol as the organic phase. Then the organic phase was injected slowly at a stirring rate of 600 rpm using a magnetic stirrer (RCT Basic, IKA, Staufen, Germany) into 20 mL of aqueous phase containing polysorbat 80, SLS, PVA, F68, PVP and HPMC (Table 1). During this process, the temperature was controlled using an ice water bath. Low temperature (below 8°C) was maintained through out the process using an ice water bath which controlled the precipitation rate. The treatment was done with an ultrasonic probe sonicator (Q Sonica Sonicator, USA) at ultrasonic power input of 50% for 8 min length. The probe with a tip diameter of 8 mm was immersed 10 mm in the liquid resulting in the wave traveling downwards and reflecting upwards. The suspensions were kept under vacuum at room temperature for 1 hour to remove ethanol.

2.3. Particle size analyses

The particle size and zeta potential value of FIS nanosuspensions were determined by the DelsaTM Nano C Particle Analyzer (Beckman Coulter GmbH, Germany). The samples were adequately diluted with deionised water and placed in an electrophoretic cell. The measured parameters by PCS are the average particle size diameter (ZAve) and the polydispersity index (PI). The mean particle size of three measurements was taken.

2.4. Scanning Electron Microscopy (SEM)

The morphologies of raw FIS and nano-sized FIS were examined using a scanning electron microscope (JEOL JSM-7001F, Japan) operated at an accelerating voltage of 15 kV and a secondary detector. Freshly prepared FIS nanosuspensions and dispersions of raw FIS were deposited on a glass slides following the evaporation of solvent.

Table 1. Composition of FIS nanosuspension

	Formulation (%)				
	A	B	C	D	E
FIS	1.0	1.0	1.0	1.0	1.0
PVA	1.5	-	-	-	-
SLS	-	0.5	-	-	-
Tween 80	-	-	0.5	-	-
HPMC	-	-	-	1.0	-
Eudragit	-	-	-	-	1.0
Water	97.5	98.5	98.5	98.0	98.0

3. Results and Discussion

3.1. Preparation of nanosuspensions

FIS nanosuspensions were produced by precipitation–ultrasonication technique. The aqueous phase containing suitable stabilizer has been used as antisolvent and the use ethanol and acetone as water miscible solvent that having good solubility FIS. Acetone and ethanol have been tried to serve as solvent for the preparations. But acetone-based formulations showed aggregation of particles and settling on storage. The viscosity of the preparation increased indicated flocculation behaviour. Hence acetone has not been used for further studies. Ethanol as solvent produces very uniform-sized nanoparticles on precipitation. The effect of various variables like type of stabilizer, concentration of stabilizer, stirring time and ultrasonication has been observed.

From the preliminary studies no specific effect on particle sizes has been observed at different effect of stirring time (5, 10, 30, 60 min) on the particle size studied. No sign of aggregation due to stirring has been observed, and the particle size did not depend on stirring time. SLS, polysorbat 80, PVA, HPMC and eudragit, at various concentrations (0.5, 1.0 and 1.0%), had been used as stabilizer for the preparations. From the preliminary studies, 0.5% and 1.5% concentration was found to be optimum for both SLS and PVA, respectively (Figure 2). The PVA-based formulations at

concentration 1.5% gave for smaller and more uniform nanoparticles and more stable at storage under room temperature as long four weeks than SLS as stabilizer were a bit bigger.
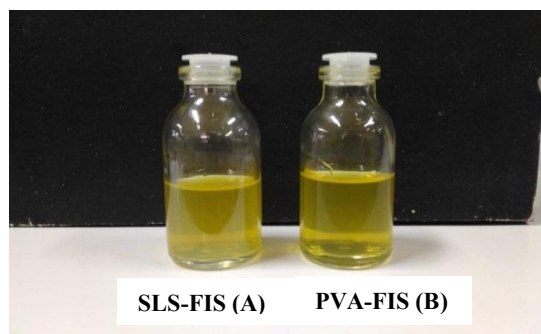

Figure 2. Appearance of fisetin nanosuspensions (A) SLS-Fisetin and (B) PVA-Fisetin

The effect of ultrasonication has been studied since the preparation obtained by precipitation alone showed less homogeneity and particles were obtained in range with average particles size at 406–997 nm. The particle size was considerably decreased and more uniform in case of precipitation followed by ultrasonication for 8 min under cold condition. Hence, precipitations with ultrasonication have been used for further preparations of nanoparticles of FIS.

3.2. *Size measurement and polydispersity index*
The particle size and the polydispersity Index (PI) of the formed drug particles was measured immediately after precipitation and sonication by dynamic laser light scattering (DelsaTM Nano C Particle Analyzer Beckman Coulter GmbH, Germany). The average particle size of FIS nanoparticles was found to be in the range of 406–997 nm (Table 2). The formulations containing 1.5% PVA as stabilizer showed particle size in the range 406 nm smaller than others stabilizer. The formulations were homogeneous as indicated by polydispersity index of 0.220±0.1. The particle size distributions were found to be more uniform as the polydispersity index narrowed down to 0.3–0.5.

Table 2. Input power, mean of PCS particle size and PI FIS nanosuspension

Formulation Code	Parameters		
	Input power (%)	Z-average (nm)	PI
A	50	406	0.220
B	50	574	0.291
C	50	767	0.236
D	50	849	0.201
E	50	997	0.308

3.3. *Scanning Electron Microscopy (SEM)*
Morphology of precipitated drug particles in the suspension after air drying followed by oven-drying. The drug particles precipitated with the PVA as stabilizer were spherical in shape and the size ranges 406 nm was shown in Figure 3. The particles were discrete and uniform in size and there was no sign of agglomerations. The drug particles precipitated with the SLS as stabilizer were slightly bigger and spherical and somewhat cuboidal and the size ranges 575 nm.
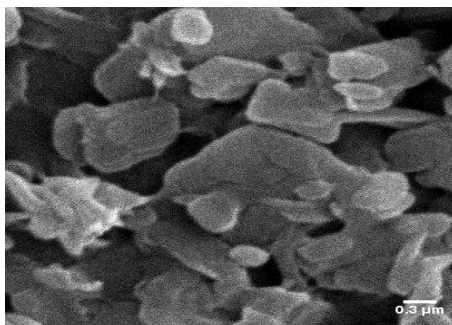
Figure 3. SEM photomicrograph of PVA based Fisetin (X 10.000)

4. Conclusions

It may be concluded that nanoprecipitation with ultrasonication had potential to formulate homogenous nanosuspensions with uniform-sized stable nanoparticles of fisetin. The preparations were found to be physically stable with PVA and SLS as stabilizer.

References
[1]   Leonarduzzi G, Testa G, Sottero B, Gamba P and Poli G 2010 *Current Medicinal Chemistry*. **17** 74-95.
[2]   Chen P Y, Ho Y R, Wu M J, Huang S P, Chen P K, Tai M H, Ho C T and Yen J H 2014 *Food& Function* 1-10.
[3]   Burda S and Oleszek W 2001 *J. Agric. Food Chem.* **49** 2774-9.
[4]   Sowa M, Slepokura K and Jon E M 2014 *Cryst. Eng. Comm.* **16**(46) 10592-10601.
[5]   Wang L, Zhang D Z and Wang Y X 2016 *Pham. Res.* **2** 5-9.
[6]   Ragelle H, Manciet S C, Seguin J, Brossard D, Scherman D, Arnaud P, Guy G and Chabot G G 2012 *Int. J. Pharm.* 452–9.
[7]   Bothiraja C, Yojana, B D, Pawar A P, Shaikh K S and Thorat U H 2014 *Expert Opin. Drug Deliv* **11**(1) 17-29.
[8]   Guzzo M R, Uemi M, Donate P M, Nikolaou S, Antonio Eduardo H, Machado A E H and Okano L T 2006 *J. Phys. Chem. A.* **110**(36) 10545-10551.
[9]   Gao L, Zhang D R and Chen M H 2008 *J. Nanopart. Res.* **10** 845–862.
[10] Keck C M and Muller R H 2006 *Eur. J. Pharm. Biopharm.* **62**(1) 3-16.
[11] Schwitzera J M, Achleitner G, Pomperb H and Müller R H 2004 *Eur. Pharm. Biopharm.* **58** 615–619.
[12] Pu X, Sun, J, Wang Y, Liu X, Zhang P, Tang X, Pan W, Han J and He Z 2009 *Int. J. Pharm.* **379** 167–173.
[13] Li X S, Wang J X , Shen Z G, Zhang P Y, Chen J F and Yun J 2007. *Int. J. Pharm* **342** 26–32.
[14] Rabinow B E 2004 *Nature Rev. Drug. Disc.* **3** 785–796.
[15] Van Eerdenbrugh B, Van den Mooter G and Augustijns P 2008 *Int. J. Pharm.* **364**(1) 64-75.
[16] Patravale V B, Date A A and Kulkarni R M 2004 *J. Pharm. Pharmacol* **56** 827–840.
[17] Liversidge E M, Liversidge G G and Cooper E R 2003 *Eur. J. Pharm. Sci.* **18** 113–120.