Available online on 15.04.2019 at at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Nanosized and Enhancement of Solubility Fisetin

Dzakwan Muhammad*[1],Pramukantoro Ganet Eko[2], Mauludin Rachmat[3], Wikarsa Saleh[4]

[1,2]Faculty of Pharmacy, University Setia Budi, Surakarta, Central Java, **Indonesia**
[3,4]Department of Pharmaceutics, School of Pharmacy, Institute Technology Bandung Labtek VII Ganesha 10 Bandung 40132, **Indonesia**

ABSTRACT

Fisetin is a flavonoid compound which has a variety of activities, one of which is an antioxidant. Due to its poor solubility and bioavailability, and limited application in oral and dermal. The main objective of this research is to overcome the low fisetin solubility. Fisetin crystalline nanosuspension was prepared using a precipitation method, organic solvent, stabilizers polysorbate 80, SLS, HPC, HPMC and Eudragit®EPO. Characterization is carried out on particle size distribution, polydispersity index, zeta potential, morphology nanosuspension (TEM) and solubility. Fisetin crystalline nanosuspension can be made by the anti-solvent precipitation method using DMSO as the best solvent, selected stabilizer SLS and polysorbate 80. Crystalline nanosuspension which contains a polysorbate 80 stabilizer has the smallest particle size ($Z_{average}$ 225.7 nm ± 1.31, PI 0.272 ±0.02 and ζ -39.3 ± 0.26) and the most stable for 30 days of storage. The solubility FIS crystalline nanosuspension which was 420µg/ml higher than of the raw material (60, 57 µg/ml), almost seven times higher than coarse fisetin. Conclusion, fisetin crystalline nanosuspension successfully prepared by precipitation method with the polysorbate 80 as stabilizer and DMSO as organic phase. Fisetin's solubility successfully increases significantly after nanosized.

Keywords: Fisetin, precipitation, polysorbate 80, DMSO, solubility

ARTICLE INFO: Received: 26 Dec. 2018; **Review Completed**: 27 Jan.2019; Accepted: 1 March .2019; **Available online**: 15 April 2019

Cite this article as:
Dzakwan Muhammad*,PramukantoroGanet Eko, Mauludin Rachmat, Wikarsa Saleh, Nanosized and Enhancement of Solubility Fisetin ,Asian Journal of Pharmaceutical Research and Development. **2019; 7(2):06-10**
DOI: http://dx.doi.org/10.22270/ajprd.v7i2.465
***Address for Correspondence**
Muhammad Dzakwan, Faculty of Pharmacy, University Setia Budi, Surakarta, Central Java, Indonesia

INTRODUCTION

Flavonoids forms a family of well known natural products present in most of the plant families, considered as active pharmaceutical ingredients (API's), and ubiquitously found in plants, fruit, vegetables and beverage[1]. Flavonoids, e.g., fisetin, luteolin, rutin and kaemferol are widely known to have very diverse activities including antioxidant, anti-inflammatory, antitumor agents, anticarcinogenic and antimicrobialactivity[2-3]. Based on the chemical structure, flavonoids are divided into four groups, flavones, flavonols, flavanones, and isoflavones [4].

The bioavailability of oral delivery of some flavonoid compounds is very low due to the low solubility in water and its limited absorption[5]. Fisetin with chemical name (FIS, 3,7,3',4'-tetrahydroxyflavone, Fig 1) is a flavonoid compound with various activities, as a antioxidant, anti-inflammatory,antiallergic,cardioprotective and anticancer[6], as antiatherosclerosis, antimutagenic, antihydroid, antiaging and has ability to decrease plasma low-density lipoproteins levels[7]. Fisetin has a very low bioavailability of around 10% because of its small water solubility (0.002 mg/ml) and low absorption so that fisetin administration in oral and dermal dosage forms is limited [8-9].

Several methods have been carried out to improve fisetin solubility and bioavailability for the oral and dermal delivery system, including nanoemulsion, co-crystal, loaded nanochelates, liposomal formulation, complexation with β-cyclodextrins and polymeric micell [10-13]. Recently,nanosizing can be interpreted as a process of changing the size of a drug from millimeter or micron size to a sub-micron range in an effort to increase its surface

area and solubility due to its dissolution rate and bioavailability [14].

It means decreasing particle size that have at least one dimension nanometer scale less than 1 µm (1 – 1000 nm), have been used to increase the solubility and bioavailability drugs [15]. The nanosization method can be classified as a bottom-up process, top-down process and a combination of both based on different in production principles[16].

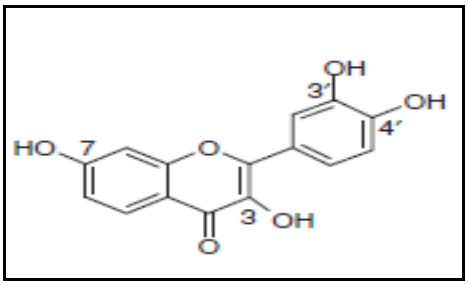

Figure 1: Chemical structure of fisetin

Crystalline nanosuspensions are sub micron colloidal dispersions containing pure drug crystals which are stabilized by adding suitable polymer or surfactant, with particle size below 100 nm[17-18]. Bottom-up methods are 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 [19]. Bottom-up approach is straightforward approach, which is cost-effective, a low energy processes and less expensive and easily be scaled up. This method is compared with other nanosizing methods produce particles with narrow size distribution. In contrast, the disadvantage include the expensive cost, residual solvent, poor drug redispersibility and solid state stability [20], To reduce these losses, a combination method of bottom up and top down has been developed.

Previously, fisetin nanocrystal was successfully developed using a precipitation-sonication combination method with stabilizer SLS and PVA. The nanocrystal containing PVA showed smaller average particle size of 406 nm, a polydispersity index of 0.22 ± 0.1 rather than SLS.

The drug particles precipitated with the SLS as stabilizer were slightly bigger and spherical and somewhat cuboidal and the size ranges 575 nm [21].

The purpose of this research is to further develop fisetin nanocrystal by precipitation method and determine fisetin solubility after nanosized. Crystallin nanosuspension produced was then characterized using photon correlation spectroscopy (PCS) in term of particle size distribution, zeta potential, polydispersity index and morphology nanosuspension using transmission electron microscope (TEM) and solubility before and after nanosizing.

MATERIALS AND METHODS

MATERIALS

Fisetin (FIS) was obtained from Shaanxi DideuMedichem Co. Ltd (Xi'an, China). Eudragit®EPOwas obtained from Evonik (German) as a gift sample.Sodium lauryl sulphate (SLS), Hydroxy propyl cellulose (HPC), Hydroxypropylmethylcellulose (HPMC) were purchased from Cognis Indonesia Ltd.Polysorbate 80was obtained from Shino Japan Chemical. DMSO were purchased from Merck (German). All other reagents used were of analytical grade.

METHODS

Precipitation process (bottom-up method)

One hundred milligram of fisetin was completely dissolvedin 20 mL of DMSO as the organic phase. The organic phase was injected into 20 ml of aqueous phase containing stabilizers at a stirring rate of 600 rpm using a magnetic stirrer (RCT Basic, IKA, Staufen, Germany) (Table 1). Crystallin nanosuspensions were stirred at room temperature for 24 h to remove the organic solvent.

Particle size analyses

The mean particle size of distribution, polydispersity index and zeta potential were determined by a Zetasizer Nano ZS (Malvern Instrument UK). The samples were diluted using ultrapurified water until the appropriate scaterring intensity and placed in an electrophoretic cell

Transmission Electron Microscopy (TEM)

The morphologies of FIS nanosuspensions were examined using a Transmission electron microscope (TECNAL-10, PHILIP) operated at an accelerating voltage of 30 kV and a secondary detector. Freshly prepared FIS nanosuspensions were deposited on a glass slides following the evaporation of solvent.

Table 1. Composition of FIS nanosuspension

	Formulation (%)				
	A	**B**	**C**	**D**	**E**
FIS	10	10	10	10	10
HPC	1,5	-	-	-	-
SLS	-	0,5	-	-	-
Polysobate 80	-	-	0,5	-	-
HPMC	-	-	--	1	-
Eudragit	-	-	-	-	1
Water	88,5	89,5	89,5	89	89

Determination of solubility fisetin

Previously, fisetin crystallin nanosuspensions were dried by lyophilization and it was stored in a desicator. The aqueous solubility of fisetin coarse and nanocrystal were determined by a shaking thermostatic water bath (JulaboGmBH, Eisenbahnstr, German). An excess amount of fisetin (20 mg/ml) was dispersed in 10 ml of water and the dispersions were shaken for 1 week at 37°C. Aliquots were withdrawn and filtered through a 0.22µm Whatmen filter Papers. The filtered solution was suitably diluted and the fisetin concentration in the filtrate was analyzed by UV analysis method at 287 nm to evaluate the a mount of fisetin dissolved.

RESULT AND DISCUSSION

Preparation of Nanosuspensions

FIS crystallin nanosuspensions were stabilized by five different stabilizers and succesfully using precipitation method.The choices of solvent, stabilizer type and concentration are important in nanosuspension preparation. The aqueous phase containing suitable stabilizer has been used as antisolvent and the use DMSO. In this research, DMSO was the best solvent comparing to other solvents, such as ethanol or aceton, as DMSO is able to dissolve fisetin much more. The ethanol - based formulations showed agglomeration of particles, cloudly and settling on storage after 24 hour. In accordance with ICH solvent toxicity, both of them are included class III which presents very low to risk of health. From the result of previous studies, fisetin crystallin nanosuspensions were successfully developed using a combination method of precipitation-sonication with SLS and PVA stabilizers. The nanocrystal containing PVA showed smaller average particle size of 406 nm, a polydispersity index of 0.22 ± 0.1 rather than SLS. Whereas fisetin nanosuspensions using precipitation, only SLS and polysorbate 80 as stabilizers were showed the smallest particle size. The crystallin nanosuspensions containing SLS showed smaller particle size of 225.7nm±1.31 than polysorbate 80 (Table 2). Characteristics and concentration of stabilizers played an important role in stabilizing nanosuspension particles. It must be capable of wetting the surface of the drug crystals and providing a steric or ionic. Less amount of stabilizer induces agglomeration or aggregation and too much stabilizer promotes Oswald's ripening [21].

The type of solvent and antisolvent used in the precipitation process not only influence the particle size of the precipitated drug but also its physical properties [21].

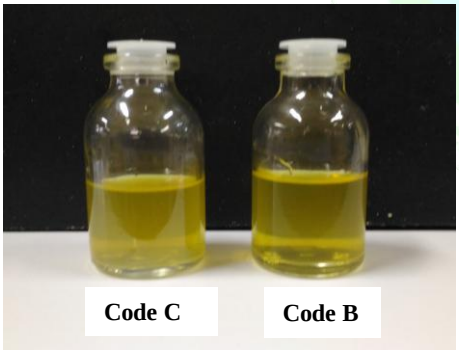

Figure 2: Appearance of fisetin crystallin nanosuspensions (Code C) SLS-fisetin and (Code B) Polysorbate 80-fisetin

Size measurement and polydispersity index

The mean particle size and the Polydispersity Index (PI) of fisetin as function of stabilizer type and concentration after precipitation are shown in Table 2. Crystallin nanosuspensions were measured immediately after precipitation by dynamic light scattering using Zetasizer Nano ZS (Malvern Instrument UK). The FIS nanosuspensions were obtained with the mean particle size of 225.7 –1019.1 nm. The smallest particle size was obtained with formula that contains 0.5 % Polysorbate 80 as stabilizer showed particle size in the range 225.7 nm and stable during 30 days of storage at room temperature. Polysorbate 80 is known to stabilize nanoparticles via steric hindrance to avoid the Ostwald ripening. Unfortunately, Polysorbate 80 in room temperature in the form of liquid is not easy to dry. In this study using crystalline nanosuspension with SLS stabilizer in the room temperature was easily to solidified.

The formulations were homogeneous as indicated by polydispersity index of 0.242 ± 0.02. It is a very important parameter in the nanosuspension system that shows particle size distribution and the long-term stability of nanosuspension storage. The particle size distribution is shown in figure 3.

The particle size distributions were found to be more uniform as the polydispersity index narrowed down to 0.1–0.25 whereas the value is greater than 0,5 indicates a very broad distribution[22]. Fisetin nanosuspensions have a low PI, were found to be 0.242 ± 0.02 indicating a good distribution particle size.

The zeta potential was found to be -39.3 ± 0.26, the zeta potential greater than +30 mV and smaller than -30 mV is normally considered stable. Negative high surface charge produces repulsion between particles and prevent their aggregation and agglomeration. The formulation FIS nanosuspension using polysorbate 80 as stabilizer shows an accepted value good stability. The potential zeta distribution is shown in figure 4.

Table 2: Particle size, PI and zeta potential PI FIS nanocrystal

CODE	Parameters		
	Particle size (nm± SD)	**PI**	**Z-average (nm± SD)**
A	529.3 ± 2.28	0.365 ± 0.02	-29.6 ± 0.37
B	402.8 ± 3.35	0.335 ± 0.05	-31.4 ± 0.53
C	225.7 ± 1.31	0.335 ± 0.05	-39.3 ± 0.26
D	906.3 ± 2.12	0.382 ± 0.07	-14.2 ± 0.87
E	1019.1 ± 2.14	0.455 ± 0.04	-11.5 ± 0.69

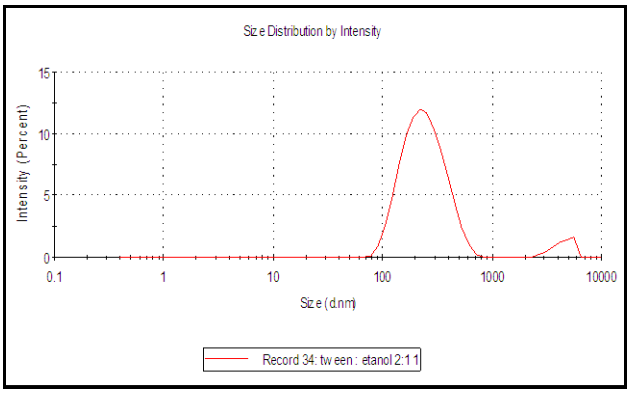

Figure 3: Partizle size distribution and polydispersity index of fisetin nanosuspensions containing stabilizer polysorbate 80 (formula C).

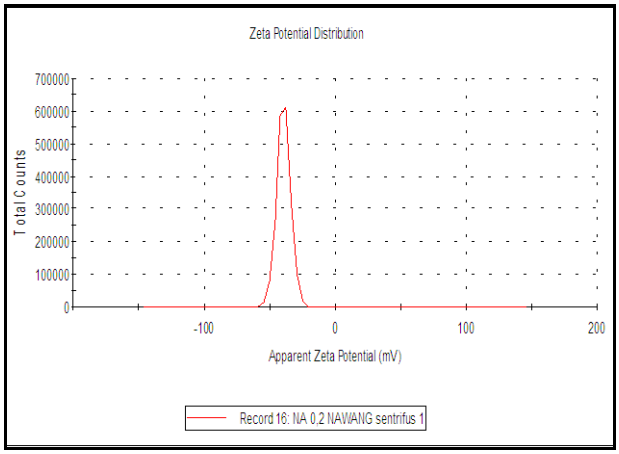

Figure 4: Zeta potential fisetin nanosuspensions containing stabilizer polysorbate 80 (formula C).

Transmission electron microscopy (TEM)

Morphology of FIS nanosuspension after precipitation with Polysorbate 80 as stabiliser was spherical with smooth surface and the size ranges 200 nm was shown in Figure 5. The particles were discreted and uniform in nanosize, and there was no sign of agglomerations.

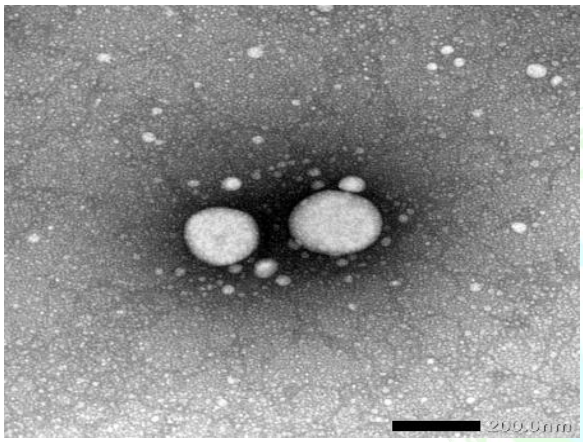

Figure 5: TEM image of fisetin nanosuspension

Solubility of fisetin crystalline nanosuspension

Reducing particle size to nanometer scale will increase surface area and increase saturation solubility. There was an increase in fisetin nanocrystal solubility after particle size reducted into nanometer scale. It increased compared to fisetin microcrystals. The solubility FIS nanocrystal with 420µg/ml higher than the raw material (60,57 µg/ml) shown in Fig 6. The increase of fisetin nanocrystal solubility is in accordance with the Kelvin-Gibbs and Ostwald-Freundlich equations. The equation explains that the reduction in particle size will increase the curvature of the particle surface area followed by an increase in dissolution pressure and an increase in solubility.

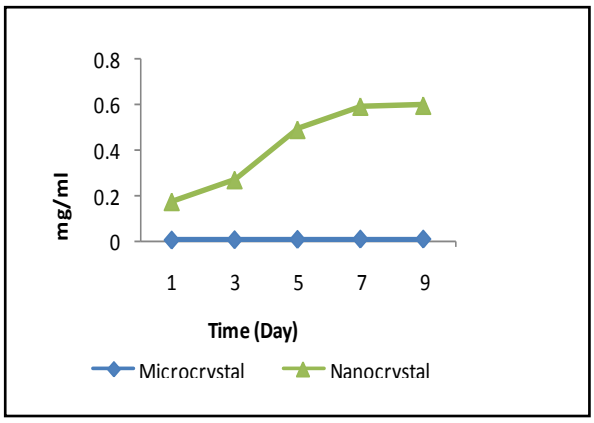

Figure 6: Solubility of the FIS nanocrystal and FIS Microcrystal in water at 37^0 C

CONCLUSION

Precipitation method was employed to produce fisetin nanosuspension, a poorly water-soluble flavonoid compound. The best nanosuspension of fisetin can be obtained by formula C that containing 10% (w/v) fisetin, 0,5 % (w/v) polysorbate 80 and 20 ml DMSO as the organic phase and fisetin nanocrystal has a higher solubility than microcrystal. This research showed precipitation method can thus be a simple and effective to produce nanoparticle of poorly water-soluble flavonoid compound.

ACKNOWLEDGEMENTS

Authors acknowledge the financial support of PKAPT RESEARCH from DRPM, Ministry of Research, Technology and Higher Education Republic of Indonesia.

REFERENCES

1. Elswaifi SF. Palmieri. JR. Hockey. KS and Rzigalinski. BA. Antioxidant nanoparticles for control of infectious disease. Infectious Disorders - Drug Targets. 2009; 9: 445-452.
2. Gacche RN. Shegokar. HD. Gond DS. Yang Z. Jadhav AD. Evaluation of selected flavonoids as antiangiogenic, anticancer, and radical scavenging agents: an experimental and in silico analysis. Cell Biochem Biophys.2011; 61:651–663.
3. Sindhi V. Gupta V. Sharma K. Bhatnagar S. Kumari R. Dhaka N. Potential applications of antioxidants- a review. Journal of Pharmacy Research.2013; 7: 828-835.
4. Leonarduzzi G. Testa G. Sottero B. Gamba P and Poli G. Design and development of nano vehicle-based delivery systems for preventive or therapeutic supplementation with flavonoids. Current Medicinal Chemistry. 2010; 17: 74-95.
5. Odeh F. Al-Jaber H and Khater D. Application of nanotechnology in drug delivery. New York: In Tech; 2014. p. 344-368.
6. Procházková D. BoušováI.Wilhelmová N. Antioxidant and prooxidant properties of flavonoids. Fitoterapia. 2011; 82: 513–523
7. Hu-JunX. Wang-Shu M.Fu-Rong L.Jie-HuiX. Lei. Qun-FangL.Wen-JunF. Radical scavenging activity of fisetin, Acta Phys. -Chim. Sin.2013; 29 (7): 1421-1432.
8. Hong C. Dang Y. Lin G. YaoY. LiG. Ji G.Shen H. Xie Y. Effects of stabilizingagents on the development of myricetinnano suspension and its characterization: an in vitro and in vivo evaluation, International Journal of Pharmaceutics.2014; 477: 251–260.
9. Guzzo MR. Semi M. Donate PM. Nikolaou S. Antonio Eduardo H. Machado AEH.Okano LTA. Study of the complexation of fisetin with cyclodextrins. JournalPhysChem.2006; 110(36):10545-1055.
10. Sowa M. Slepokura K. Matczak -Jon E. Improving solubility of fisetin by cocrystallization. Crys Eng Com. 2014; 16:10592-10601.
11. Seguin J. Brulle L. Boyer R. Lu YM. Ramos Romano M. Touil YS. Liposomal encapsulation of the natural flavonoid fisetin

improves bioavailability and antitumor efficacy. International journal of pharmaceutics. 2013; 444(1-2):146-54.
12. Wang L. Zhang DZ. Wang YX. Bioflavonoid fisetin loaded alpha-tocopherol-poly(lactic acid)-based polymeric micelles for enhanced anticancer efficacy in breast cancers. Pharmaceutical Research. 2017; 34(2):453-61.
13. Bothiraja C. Yojana BD Pawar AP. Shaikh KS and Thorat UH. Fisetin-loadednanocochleates: formulation and Characterization, *in vitro* anticancer testing, bioavailability and biodistribution study. Expert Opin. Drug Deliv. 2014; 11(1):17-29.
14. Al-Kassas R. Bansal M. Shaw J.Nanosizing techniques for improving bioavailability of drugs. Journal Control Release. 2017; 260:202 – 2012.
15. Junghanns AH and Müller RH. Nanocrystal technology, drug delivery and clinical applications. International Journal of Nanomedicines. 2008; 3 (3):295-309.
16. Keck CM. and Muller RH. Drug nanocrystal of poorly soluble drugs produced by high-pressure homogenization. Eur J Pharm Biopharm. 2008; 62(1) 3-16.
17. Sinha B. Muller RH. Moschwitzer JP. Bottom-up approaches for preparing drug nanocrystals: formulations and factors affecting particle size. Int J Pharm. 2013; 453(1):126-141.
18. JunyaprasertVB. and MorakulB. Nanocrystals for enhancement of oral bioavailability of poorly water-soluble drugs. Asian Journal of Pharmaceutical Science. 2015; 10:10-23.
19. De Waard H. Frijlink HW. Hinrichs WLJ. Bottom-up preparation techniques for nanocrystals of lipophilic drugs. Pharmaceutical Research. 2011; 28(5):1220-1223.
20. Möschwitzer JP. Drug nanocrystals in the commercial pharmaceutical development process. International Journal of Pharmaceutics. 2013; 453(1):142-156.
21. Dzakwan M. Ganet E P. Mauludin R Wikarsa S. Formulation and characterization of fisetin nanosuspension. IOP Material Science. 2017; 259 (012016): 1-5.
22. Patravale V B. Date A A. Kulkarni R M. Nanosuspensions: a promising drug delivery strategy. J Pharm Pharmacol. 2004; 56:827-840.