

KINETIC SOLUBILITY OF Fisetin NANOCRYSTAL

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Abstract

Background: Fisetin (3,3',4',7-tetrahydroxyflavone) is a natural antioxidant with poor solubility leads to poor bioavailability and limits its development. Fisetin nanocrystals were produced by nanoprecipitation technique and transformed into dry powder by lyophilisation. Objective: The aim of the study is to investigate the kinetic solubility of fisetin after produced into nanocrystals. Methods: Fisetin nanocrystals were prepared by nanoprecipitation with different proportion of stabilizers and transformed into dry powder by lyophilization. In a period of 1 week, the kinetic solubility was determined using a shaker water bath at 37°C. Result: Showed fisetin nanocrystals were almost completely dissolved within 15 min in water and buffer pH 1,2. In contrast, only 55% of fisetin raw material at the same condition. Conclusion: Lyophilized fisetin nanocrystals could be re-dispersed completely in water, buffer pH 1,2 and the kinetic solubility in water increased to 420 µg/ml.

Keywords: fisetin, nanocrystal, nanoprecipitation, kinetic solubility

Introduction

Flavonoids, e.g., fisetin, luteolin and kaempferol comprise a vast group of naturally occurring polyphenolic compounds, considered as active pharmaceutical ingredients (APIs), and present in daily consumed vegetables, fruits and products of that origin (Scholz and Keck, 2014., Chen *et al.*, 2014). Fisetin (FIS, 3,3',4',7-tetrahydroxyflavone, Fig 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 (Seguin *et al.*, 2013., Wang *et al.*, 2016). It exhibits antioxidant, anti-inflammatory and anticarcinogenic activities. Recently, it was also considered to possess neuroprotective effects against the aging process, cerebral damage and neurodegenerative disorders (Ragelle *et al.*, 2012., Wang *et al.*, 2016). However, a limiting factor for fisetin is poor solubility, restricting their full antioxidative potential as well as bioavailability (Scholz and Keck, 2014). The drug has dissolution rate limited bioavailability and therefore formulating as nanosuspension might improve its dissolution rate thereby enhance oral bioavailability (Kumar *et al.*, 2008).

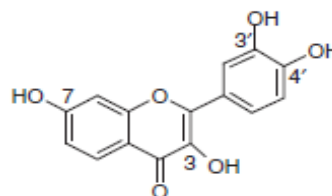


Figure 1 –Chemical structure of fisetin (fisetin (Ragelle *et al.*, 2012))

Over the last decade, there has been an increase in the number of newly developed drug molecules that exhibit poor water solubility as well as poor bioavailability. So, one of the most challenging tasks in drug development is to improve solubility and oral bioavailability of these novel drugs. Various methods to enhance solubility of poorly water-soluble drugs include solubilization (Dulfer *et al.*, 1995), formation of complexes β -cyclodextrin (Calabro *et al.*, 2005), solid dispersions (Kanaze *et al.*, 2006), liposomes (Dupont, 2002), emulsions (Nakano, 2000) and microemulsions (Lawrence and Rees, 2000). However, these methods are successful in some instances and are specific to drug candidates.

The fundamental basis for the observation that size influence the solubility is found in the Kelvin equation (Buckton and Beezer, 1992). The Kelvin equation is equally applicable to the solid–liquid interface, because the vapor pressure in a liquid–gas system corresponds to the dissolution pressure in a solid–liquid phase system. Replacing the pressure terms with solubilities (s), as activity coefficients are effectively set equal to the unity in the dilute solutions considered here, produces the so-called Gibbs–Kelvin relation.

The aim of this study is to assess of kinetic saturation solubility of fisetin nanocrystal. The physicochemical properties of the fisetin nanocrystals and the nanocrystal powder have been investigated regarding crystallinity and kinetic solubility.

Methods

Materials

Fisetin (98%) raw material was purchased from Shaanxi Dideu Medichem Co. Ltd (Xi'an, China). Eudragit was obtained from BASF (German). (Sodium lauryl sulphate (SLS) was obtained from Cognis Indonesia Ltd. Polysorbate 80 (Tween 80®) was obtained from Shino Japan Chemical. All other reagents were of analytical grade.

Methods

Production of fisetin nanosuspensions

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 polysorbate 80 and SLS (Table I).

Particle size analyses

The particle size and zeta potential value of the mebendazole nanosuspensions were determined by a Zetasizer Nano ZS (Malvern Instrument UK). The samples were adequately diluted with deionised water and placed in an electrophoretic cell.

Lyophilization

The fisetin nanosuspensions were dried by lyophilization. Each fisetin nanosuspension (10 mL) was freeze-dried at -80°C in a 20 mL vial using Martin Christ " 1–2/LD plus freeze dry system (Martin Christ Gefriertrocknungsanlagen GmbH, 37507 Osterode am Harz, Germany)

with 50 mbar vacuum for 24 h. The dried powder was stored in a desiccator.

Characterization by DSC

Differential scanning calorimetric (DSC) measurements were carried out using a TA DSC 60 thermal analyzer (TA-60 WS DE 19720, Shimadzu, JAPAN) in a temperature range of 30°C – 300°C at a heating rate of $10^{\circ}\text{C}/\text{min}$ in nitrogen gas.

Powder X-ray diffraction (PXRD)

X-ray diffraction was studied using the Bruker AXS D8 Advance X-ray diffractometer (Bruker AXS, Madison, USA) Cu K α targets at a scanning rate of 0.010 $2\theta/\text{s}$, applying 40 kV at 40 mA, to observe the crystallinity of samples.

Kinetic solubility

Solubility studies were performed with shaking water bath (SW 22 Shaking Water Bath, Julabo GmbH, Eisenbahnstr, Germany) at 37°C . Vials were sealed to avoid changes due to evaporation and placed for 1 week in a thermostated storage at $37\pm 0,5^{\circ}\text{C}$. After the equilibrium was reached, suspensions were filtered through Sartorius® 0.1 mm filters (Sartorius AG, Germany). An aliquot from each vial was withdrawn by a 1 mL glass syringe (Poulsen & Graf GmbH Germany) and assayed by spectrometrically at 287 nm using an ultraviolet spectrometer (Beckman D270, Beckman Coulter GmbH, Germany) to evaluate the amount of FIS dissolved.

Table 1. Composition of FIS nanosuspension

	Formulation (%)		
	A	BC	
FIS	1	1	1
SLS	0.5	-	-
Tween 80	-	0,5	-
Eudragit	-	-	1
Water	98.5	98.5	98

Result And Discussion

Production of aqueous nanosuspensions and Lyophilization

FIS nanosuspensions stabilized by three different stabilizers were successfully produced. The PCS particle size result of all nanosuspensions are shown in Table 2. The nanosuspension stabilized by SLS (formulation A) was chosen for further investigations. Dried

FIS nanocrystals were obtained by lyophilization using the freeze dryer. After 24 h, lyophilized FIS nanocrystals were collected. The physical characteristics of the lyophilized FIS nanocrystals were investigated with regard to crystalline state and kinetic solubility.

Table 2. Mean PCS particle size and PI FIS nanosuspension

	Formulation		
	A	B	C
z-average (nm)	402.8	225.7	1019.1
PI	0,212	0,253	0,277

Crystalline state evaluation by DSC

Thermal analysis of the FIS nanocrystal powder was performed and compared to the raw material and to the data in the literature. The FIS nanocrystal powders investigated were obtained by lyophilization. The results of thermal analysis by DSC can be seen in Fig. 2. According to these results, the melting points of FIS (raw material and both nanocrystal powders) are similar. The only difference observed was a slight shift in melting temperature (from 320,53°C to 323°C). This modification was attributed to the presence of SLS as stabilizer and mannitol as cryoprotectant. The results of the thermal analyses are shown the melting points of the FIS nanocrystals have not changed significantly. The presence of stabilizers has a minor influence on the melting temperature and has not altered the crystallinity of the drug

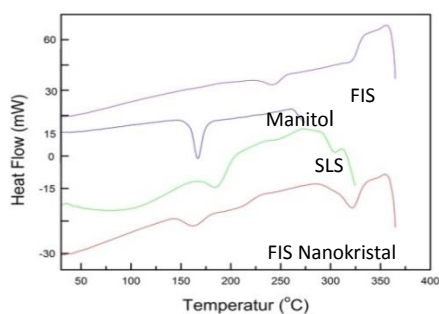


Figure 2 – DSC thermogram of lyophilized, FIS nanocrystals and raw material

Crystalline state evaluation by XRD

To confirm the crystalline state of the dried FIS nanocrystals, X-ray diffraction was performed on the lyophilized FIS nanocrystals. X-ray diffraction patterns were visualized in diffractograms and can be seen in Fig. 3. The crystallinity of lyophilized FIS nanocrystals with SLS as stabilizers. The diffractograms reveal no different peaks for FIS nanocrystals. According to these results, FIS nanocrystals were still in the same crystalline state as the raw material. Applying energy by being homogenized and subjecting them to the drying processes did not transform FIS nanocrystals to be fully or partially amorphous. In general, more crystalline substances are physically more stable compared to amorphous forms.

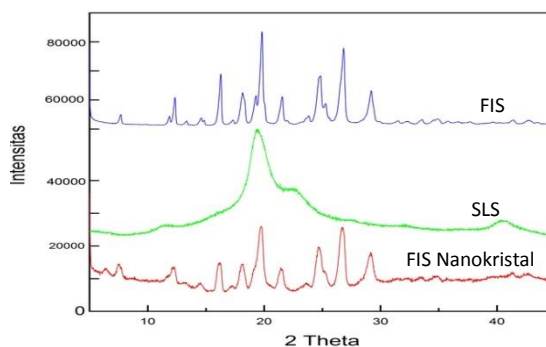


Figure 3 – X-ray diffractogram patterns of lyophilized FIS nanocrystal formulations and the raw material

Kinetic solubility

Thermodynamic solubility is the concentration in the solute in equilibrium with a normal sized powder, this condition being physically stable. Kinetic solubility, e.g. achieved by size reduction or from amorphous powders, is higher than the thermodynamic solubility but physically not long-term stable (Mauludin *et al*, 2009). The kinetic solubility of FIS nanocrystals was investigated over 1 week. The results of this testing showed that the solubility of the FIS nanocrystals in water and pH 1.2 were increased at 37°C. The kinetic solubility was with 420 µg/ml higher than of the raw material (60,57 µg/ml) (Fig. 4).

According to the crystalline state investigation, the solubility enhancement of the FIS nanocrystals is not due to the presence of the FIS amorphous form. It is due to the particle size reduction to the submicron range. This result is in agreement with the Kelvin–Gibbs equation and Ostwald–Freundlich equation

(Buckton and Beezer, 1992; Müller and Akkar, 2004)

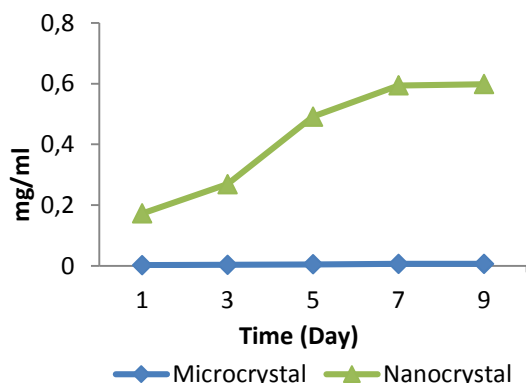


Figure 4 – Kinetic saturation solubility of the FIS nanocrystals and FIS microcrystals in water at 37°C.

Conclusion

FIS nanocrystals could provide superior physicochemical properties. Lyophilized FIS nanocrystals improved the kinetic saturation solubility from 60.57 µg/ml to 420 µg/ml, in comparison to FIS microcrystals.

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