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Co-crystallization of Dihydroquercetin

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Abstract

Dihydroquercetin (DHQ) is a bioflavonoid from the larch wood with the wide range of pharmacological activity. There are certain restrictions in the creation of drugs on basis of DHQ, because the industrial DHQ is characterized by a poor water-solubility at room temperature. Co-crystallization was realized to obtain the new forms of DHQ with improved water solubility. Cooling, lyophilisation, solvent evaporation and sonocrystallization were used.
It has been shown that co-crystals of DHQ with vanillin, nicotinic acid are stable and have a higher solubility than commercial DHQ.

Introduction

Co-crystallization of active ingredients with so called co-formers attracts more and more attention in the pharmaceutical industry. Such approaches are capable of the improvement in dissolution profile, solubility and bioavailability of poorly water-soluble drugs (Kotak, Parajapaty et al. 2015, Apshingekar, Aher et al. 2017). During the process of co-crystallization hydrogen bonds between active pharmaceutical ingredient (API) and co-crystallization partner are formed. Nowadays one focus lies on dihydroquercetin (DHQ) – 2,3-dihydro-3,5,7-trihydroxy-2-(3,4-dihydroxyphenyl)-4H-1-benzopyranone-4. DHQ is the major flavonoid of the larch wood. The antioxidant capacity of this compound and a wide range of proposed pharmacological activities are combined with absence of embriotoxicity, teratogenecity and mutability (Schauss, Tselyico et al. 2015). Some clinically used drugs, such as Diquertin and Ascovertin, were designed on the basis of DHQ (Plotnikov, Tyukavkina et al. 2005, Plotnikov, Aliev et al. 2006, Plotnikov, Logvinov et al. 2007). However, industrially isolated crystal DHQ is poorly water-soluble (Tyukavkina, Selivanova et al. 2015).
The study objective was to obtain a stable water-soluble forms of DHQ by the utilization of co-crystallization.

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Materials and Methods

To date, no crystal structure of DHQ is known but for the hydrate an entry in the Cambridge Structural Database could be found (Selivanova, Tyukavkina et al., 1999). This crystal form of DHQ was used as a starting point for the generation of an API by obtaining of co-crystals with the following co-formers: nicotinic acid (99.5%, Fluka, Germany), vanillin (99%, Sigma-Aldrich GmbH, Germany), cinnamaldehyde (99%, Acros Organics, Belgium) and benzaldehyde (98+%, Carl Roth GmbH, Germany). Starting API-batch and each co-former were dissolved in denatured ethanol (99.8%, Carl Roth GmbH, Germany) in the molecular ratio 1:1. These stock-solutions were used in the co-crystallization process by the following methods.

Solvent evaporation

Solvent from stock-solutions was evaporated slowly by using the rotary evaporator Hei-VAP Value (Heidolph Instruments GmbH, Germany), which worked at 55 °C and 0.15 atm. The residual portion of solvent was removed under vacuum. Co-crystals were obtained from the melt after 3 days.

Sonocrystallization

Stock-solutions were concentrated by using the rotary evaporator, and the melts were obtained. The melts was put into the ultrasound bath Sonorex RK-100 (Bondelin, Germany) to generate crystal forms from the melts. Co-crystals were obtained after 1 hour.

Cooling

Stock-solutions were kept at 4 °C for 7 days. Co-crystals were separated by filtration.

Lyophilisation

Stock-solutions were diluted with distillated water to the 5% concentration of API. Before the lyophilisation all samples were kept at –78 °C for 24 h, then flasks, containing solid samples, were connected with laboratory freeze dryer Alpha 1-2 LD (Martin Christ Gefriertrocknungsanlagen GmbH, Germany), which worked under pressure 0,35 atm and temperature –55 °C for 36 h.

Morphology of crystals, changes in crystal forms, melting points, solubility and stability were used to characterize the new co-crystals. Morphology of crystals was studied by inverted microscope Axiovert S 100 with the camera AxioCam MRc (Carl Zeiss, Germany). Optical microscope with a heating table Leica Galen III (Leica Microsystems, Germany) with the digital temperature meter Testo 720 (Testo Inc., Germany) was used for determination of melting points. Solubility was characterized according to the European Pharmacopoeia. Crystal form was classified as stable on condition that it saved unchanged structure at the air over 24 h.

Results and Discussion

Seven new stable solubility-enhanced forms of DHQ were obtained (*Table*).

Table. Characteristics of co-crystals with DHQ

Method of co-crystallization	Co-formers	Crystal form	Size, μm	Melting point, °C	Stability	Solubility
Solvent evaporation	cinnamaldehyde	needle	87.3	90.5	stable	slightly soluble
	vanillin	needle	24.0	60.1	stable	soluble
	nicotinic acid	island	58.6	67.0	stable	very slightly soluble
Sonocrystallization	cinnamaldehyde	needle	47.2	90.5	stable	slightly soluble
	vanillin	island	11.0	60.1	stable	soluble
Cooling	nicotinic acid	island	137.2	143.2	stable	slightly soluble
Lyophilisation	benzaldehyde	fiber	81.3	64.0	instable	sparingly soluble
	cinnamaldehyde	sheet-like	125.0	58.0	instable	soluble
	vanillin	fiber	46.7	46.0	instable	freely soluble
	nicotinic acid	sheet-like	131.4	102.0	stable	sparingly soluble

Commercial DHQ is very slightly water-soluble substance. It's crystals have small size, approximately 22.04 μm (*Figure, Panel A*). Melting point of this API is 226.0 °C.

Co-crystals of DHQ, obtained by evaporation, sonocrystallization and cooling are stable, but lyophilisats are instable often and convert the following transformations: volume of solid phase is decreased and the color is changed from the white to

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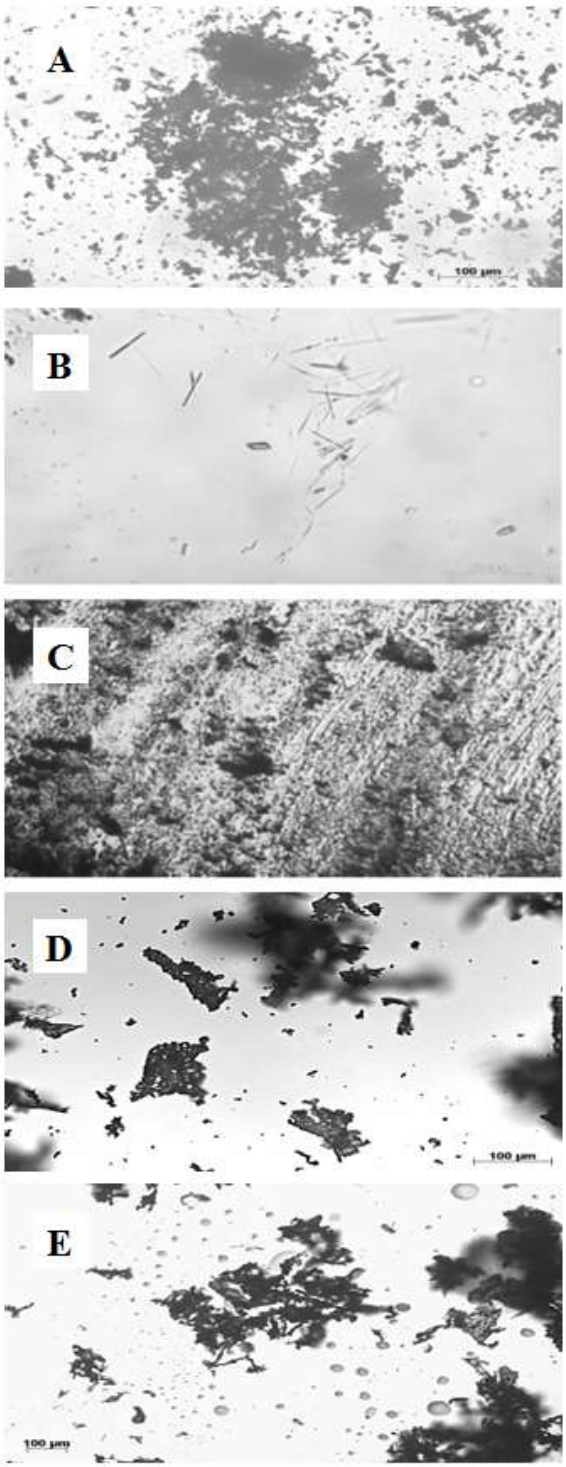

Figure. *Representative photomicrography.* ***Panel A.*** *Commercial DHQ.* ***Panel B.*** *Needle form.* ***Panel C.*** *Island form.* ***Panel D.*** *sheet-like form.* ***Panel E.*** *Fiber form.*

the yellow and even orange. In all probability, hygroscopicity is the cause of these transformations.

The melting point of the obtained co-crystals is lower versus commercial DHQ. This fact gives an opportunity to suggest that there is intermolecular interaction between DHQ and co-formers, because the energy of formation of supramolecular heterosyntons is lower than homosyntons. Co-crystals obtained by cooling with nicotinic acid have the highest melting point and these co-crystals are the most stable among new forms.

During the visual and the microscopic examinations it has been shown that co-crystals have different forms (*Figure, Panel B – E*).The needle form and island form could be obtained by solvent evaporation, sonocrystallization and cooling. It is easy to notice, that the smallest crystals were obtained by sonocrystallization with vanillin and the biggest – by the cooling with nicotinic acid (table). The difference of sizes is attributable to the speed of the co-crystallization. Small co-crystals were produced from the melt under the influence of ultrasound while the big co-crystals – from the solution at the low temperature. It means that the generation of the crystallization points would be much faster in the sonocrystallization method. It is difficult to form large co-crystals at such conditions. Crystal forms of the samples, which were obtained by the lyophilisation, can be classified for the two groups according to the general motifs: sheet-like and fiber. Further investigation of relationship between the form of co-crystals and the method of co-crystallization is required.

The majority of the identified stable co-crystals are comparable or slightly better soluble versus the starting API-batch, resulting for some co-crystals in the enhancement of the solubility by 2 orders of magnitude.

In summary, during this study seven new stable solubility-enhanced DHQ forms were obtained. Co-crystals of DHQ with vanillin, nicotinic acid are stable and they were characterized to have a higher solubility than the starting API-batch. These co-crystals might have the potential to be used as APIs in new pharmaceutical forms.

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