Formulation development and process characterization of quercetin nanocrystals by design of experiment[2]

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[2]Presented at the 2020 Controlled Release Society (CRS) Virtual Annual Meeting

INTRODUCTION

The flavonoid quercetin is known for its potent antioxidative and anti-inflammatory activity. Therefore, topically applied quercetin is a highly promising candidate for use against photoaging of the skin and for skin cancer prevention. However, the therapeutic use of quercetin is restricted by its low aqueous solubility and poor permeability. Thus, the skin bioavailability of quercetin is insufficient [1]. A promising and effective formulation approach to boost the penetration into deeper skin layers of poorly soluble actives is the conversion of the untreated bulk material into nanocrystals [2]. Nevertheless, prior to the successful use in therapeutics, a suitable nanocrystal formulation has to be developed, which further allows an economical large-scale manufacturing of the product. It is imperative, that the large-scale production is conducted as a controlled process. Therefore, it is essential to know all process influencing input parameters on the manufacturing process. Hence, design of experiment (DOE) is a great way to evaluate all process influencing factors on the production of nanocrystals. This enables an optimized production, with a consistent quality and efficiency of the quercetin nanocrystals.

AIM

The aim of this study was divided into two steps. The first step was the formulation development of a quercetin nanosuspension via stabilizer screening. The second step was the large-scale production of the nanocrystals and a simultaneous screening for milling process influencing factors with a fractional factorial DOE.

MATERIALS AND METHODS

Materials

Quercetin (Denk Ingredients GmbH, Germany) was used as a model drug. The stabilizer screening included Plantacare® 818UP (PLC 818), Plantacare® 1200UP (PLC 1200), Plantacare® 2000UP (PLC 2000), Poloxamer 188 (PLX 188), Poloxamer 407 (PLX 407) from BASF (Germany), Tween® 80 (VWR, Germany), d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) (Antares Health Products Inc, USA), Brij® L23 (Thermo Fisher GmbH, Germany) and Mirj™ S100 (Croda Personal Care, UK). Hypromellose (Caesar & Lorentz GmbH, Germany) was utilized for viscosity adjustment of the aqueous phase. A PURELAB Flex 2 (ELGA LabWater, Veolia Water technologies Deutschland GmbH, Germany) was used to obtain purified water. All milling trials were conducted with yttria stabilized zirconia beads (SiLibeads Type ZY-E, Sigmud Lindner GmbH, Switzerland).

Production of nanocrystals for stabilizer screening

Bulk suspensions for the stabilizer screening were all prepared with 5% (w/w) quercetin and 1% (w/w) surfactant. The suspensions were then subject to small-scale bead milling according to [3]. Vials were placed on the multiple magnetic stirrer Mixdrive15 with external control unit (2mag AG, Germany) and were milled for 24 h.

Std	Speed [rpm]	Bead size [µm]	Viscosity [mPas]	Bead/Suspension ratio (V/V)	Mass quercetin [%(w/w)]	High speed stirred	D(v)0.5	Span
1	500	100	1.3	60:40	1	no	0.120	18.52
2	1500	100	1.3	60:40	5	no	0.076	2.72
3	500	400	1.3	60:40	5	yes	0.227	10.75
4	1500	400	1.3	60:40	1	yes	0.098	10.82
5	500	100	20	60:40	5	yes	3.884	2.94
6	1500	100	20	60:40	1	yes	0.066	3.35
7	500	400	20	60:40	1	no	1.308	4.18
8	1500	400	20	60:40	5	no	2.386	3.00
9	500	100	1.3	75:25	1	yes	0.202	249.94
10	1500	100	1.3	75:25	5	yes	0.141	62.35
11	500	400	1.3	75:25	5	no	0.121	6.79
12	1500	400	1.3	75:25	1	no	0.075	4.36
13	500	100	20	75:25	5	no	5.348	1.67
14	1500	100	20	75:25	1	no	0.054	2.64
15	500	400	20	75:25	1	yes	0.116	11.66
16	1500	400	20	75:25	5	yes	0.903	153.66

Table 1: Resolution IV fractional factorial design in standard order and results of DLS measurements and span.

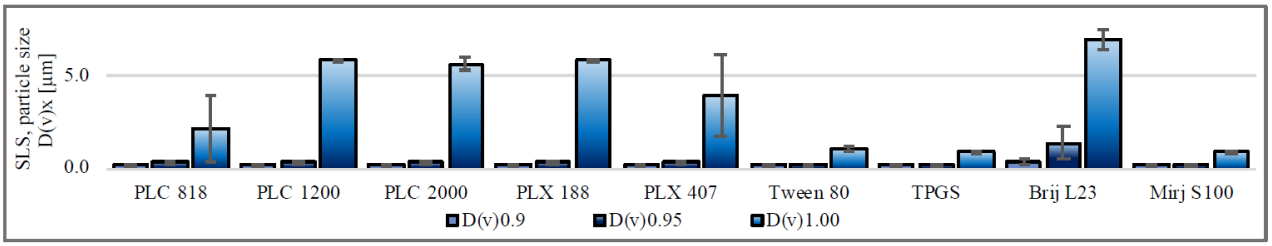

Figure 1: Results of SLS measurements of the stabilizer screening for quercetin. TPGS is the stabilizer of choice.

Large-scale production of NC and factor screening (DOE)

The chosen nanosuspension formulation from the stabilizer screening was produced under large-scale conditions with a state-of-the-art NanoWitt-LAB 100 milling equipment (Frewitt fabrique de machines S.A, Fribourg, Switzerland) in continuous mode with a filling volume of 300 mL. One major advantage of the NanoWitt is that relatively low specific energy is needed for an efficient nanonization of the drug. Therefore, suspension temperature was constant below 18°C, which prevents product degradation. The DOE screening was carried out with a predefined resolution IV fractional factorial design with six different selected input factors (Table 1). All production runs were randomized to avoid unknown effects. Premilling via high speed stirring was conducted at 10,000 rpm for 5 min. Milling time of all suspensions was 2 h.

Characterization of nanocrystals

Static light scattering (SLS) was used to determine the volumetric particle size distribution via a Mastersizer 3000 (Malvern Panalytical GmbH, Germany). Mie-Theory was set for data analysis in combination with a real refractive index of 1.64 and an imaginary refractive index of 0.01. Furthermore, the span ((D(v)0.9-D(v)0.1)/D(v)0.5) was calculated as a parameter to estimate the particle size distribution.

RESULTS AND DISCUSSION

Stabilizer screening and DOE

The stabilizer screening revealed that Tween® 80, TPGS and Mirj™ S100 were suitable stabilizers. Hence, TPGS was selected as the stabilizing surfactant, due to its penetration enhancing properties [4].

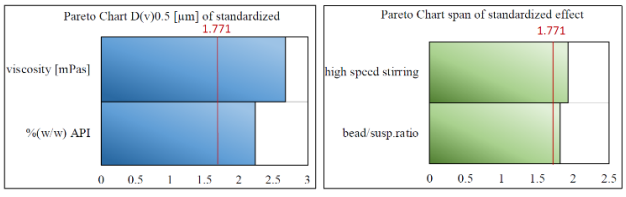

Figure 2: Pareto chart of D(v)0.5 (left) and span (right).

Results from DLS measurements of the DOE factor screening were analyzed with the statistic software Minitab® 18. A 95% confidence interval was set, and insignificant terms were eliminated backwards with $\alpha = 0.1$. The Pareto Charts give an overview of the contribution to the overall variability from each term for the D(v)0.5 and the calculated span (Figure 2). Results for the D(v)0.5 revealed, that the percentage mass of quercetin ($\%_{(w/w)}$) and viscosity were significant. Thus, a higher particle concentration increases the probability of larger particles remaining in the product. An increased viscosity reduced the milling efficacy due decreased kinetic energy of the beads. Significant terms for the particle size distribution expressed as span, were premilling via high speed stirring and the bead/suspension-ratio (bead/susp.-ratio). A higher bead/susp.-ratio and premilling of the suspension via high speed stirring, promotes a homogenous comminution of the particles, leading to a narrow particle size distribution. It should be noticed, that milling speed and bead size were insignificant for the D(v)0.5 and the span, indicating the capability of the NanoWitt to be an effective device for nanomilling.

CONCLUSION

A quercetin nanosuspension was successfully developed and manufactured under large-scale conditions. Process influencing input factors were determined via a fractional factorial DOE. The NanoWitt is capable to achieve an efficient nanonization of quercetin regardless of the used speed and bead size. The possibility to operate with a lower speed is beneficial to avoid abrasion, consequently decreasing the chance of contamination of the product.

ACKNOWLEDGEMENTS

The authors kindly acknowledge the great support of Frewitt fabrique de machines S.A., Switzerland to provide access to the NanoWitt-LAB 100 milling equipment. The study was partly supported by ZIM-Project No. ZF4114902SB7.

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