QUERCETIN LOADED NANOPARTICLES CREATED BY SUPERCRITICAL FLUID EXTRACTION OF EMULSIONS

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ABSTRACT

According to preliminary studies quercetin has strong antioxidant, antiviral, antibacterial, antihistaminic and anti-inflammatory effect. Due to these properties, quercetin is a highly promising material against a wide variety of diseases, including cancer. As quercetin has low solubility in aqueous media, it has low bioavailability in human body. In order to increase the bioavailability of quercetin, we are producing aqueous suspensions, containing quercetin micro-particles using Supercritical Fluid Extraction of Emulsions (SFEE) technology, to extract the organic solvent from the initial oil in water emulsion (o/w) by supercritical carbon-dioxide ($scCO_2$).

Our aim is to optimize the process parameters of the initial o/w emulsion- (concentration of quercetin, surfactant material, organic solvent, duration of emulsification), and of the SFEE as a batch process (numbers and duration of cycles, pressure and temperature) to obtain quercetin nanoparticles as an aqueous suspension, in order to increase the amount of encapsulated quercetin by a surfactant material, and decrease the residual organic concentration in the final product below the restrictions, according by FDA (Food and Drug Administration) criterions.

INTRODUCTION

Quercetin (3,3',4,4',5,7-pentahydroxyflavone) is a bioflavonoid available in various fluids, vegetables and oils. It has a potency to scavenge reactive oxygen species and to down-regulate lipid peroxidation due to its ion chelating and iron stabilizing effect [1].It has also anti-proliferative effects in a wide range of human cancer cell lines. Due to these properties, quercetin is a highly promising active compound against a wide variety of diseases, including cancer [2].

Major limitation of the clinical application of quercetin is the administration of high dose (50 mg/kg) [3] due to its poor solubility in aqueous medium. In order to increase the bioavailability of quercetin, we are producing aqueous suspension containing quercetin micro-particles with high specific surface area using Supercritical Fluid Extraction of Emulsion (SFEE) technology. By SFEE organic solvent is extracted out from the initially prepared emulsion containing the drug by supercritical solvent. Supercritical solvent should

be chose to have high affinity to the organic solvent, while low affinity to the material of interest. Due to solubility differences, $scCO_2$ rapidly dissolving out EtAc from the emulsion, causing the rapid super-saturation of active compound in the solution, and hence the fast precipitation of quercetin in nanometric range inside the surfactant material.

MATERIALS AND METHODS

Quercetin Hydrate (95%) ($C_{15}H_{10}O_7xH_2O$) is obtained from Acros Organicos (CAS: 849061-97-8). Surfactant material is poly-(ethylene glycol)- block –poly-(propylene glycol)- block - poly-(ethylene glycol) (Pluronic L64) is obtained from Sigma Aldrich (CAS: 9003-11-6). Organic solvent to dissolve quercetin is ethylacetate (EtAc) from Pancreac (CAS: 141-78-6).

Before SFEE treatment it is necessary to produce an oil in water emulsion (o/w). Quercetin first is dissolved in an organic solvent producing the oily part-, while surfactant material is dissolved in water, producing water part of the emulsion. Both phase are mixed to produce the emulsion in a rotor-stator device (Ultraturrax) for a predefined emulsifying time. During the extraction of organic solvent, the main part of $scCO_2$ was renewed five times, keeping under constant pressure and temperature the emulsion ~100 bar and ~40°C as SFEE process parameters [4]. Duration of the all extraction process was ~1300 min.

We use TurbiScan Classic laser diffraction device to specify the average droplet size in the initial emulsion, Leica optical microscopy doing visual observations of initial emulsions and SFEE-treated aqueous suspensions, too and Malvern Mastersizer 2000 Light Scattering device to define the particle size distribution in the final aqueous suspension. Shimadzu UV-2550 UV-VIS spectroscopy device is used to determine the encapsulation efficiency of quercetin and for longer term stability measurements of remained quercetin in aqueous samples. Remained organic solvent concentration after SFEE treatment was measured by Head Space Gas Cromatography, Agilent 7890A Gas Chromatography system.

RESULTS

Our main aim is to decrease the final particle size in SFEE produced aqueous suspension, to increase the bioavailability of quercetin. Since there is a significant correlation between initial emulsion droplet size and aqueous suspension's final particle size [5],we made a four factor in two levels experimental plan with three centrum point measurements, according to the average droplet size in the initial emulsion. Factors to be changed in two levels are: quercetin concentration in EtAC, Pluronic L64 concentration, organic/water ratio and emulsifying time. According to our results, one significant factor is the emulsifying time, values were varied between 2 and 6 minutes. According to the centrum point measurements, the effect of emulsifying time on initial emulsion average droplet size (Figure 1) is not linear, therefore, we choose the centrum point value (4 min) for our further experiments. Further significant factors are: the concentration of quercetin in EtAC and the concentration of Pluronic L64 (linearity of the model is accepted). Both of them should be increased in order to decrease the average droplet size in the initial emulsion.

A second experimental plan with three centrum point measurements (*marked by Italic*) was done to study the effect of Pluronic L64 concentration, effect of dissolved amount of quercetin in EtAc and the effect of organic/water ratio, in the remaining organic solvent,

average final particle size in aqueous suspension and quercetin recovery after SFEE treatment (Table 1). Remaining organic solvent concentration in final aqueous suspensions depends on the amount of EtAc used to dissolve quercetin in organic phase. According to this result, concentration of organic solvent should be decreased until the solubility limit of quercetin in EtAc. Best experimental results (repeated twice) **marked by Bold**.

Solvent/water ratio [ml/ml]	**Quercetin concentration in EtAc [g/l]**	**Pluronic concentration [w/w%]**	**Quercetin recovery [%]**	**Droplet size in initial emulsion [µm]**	**50 V/V% of particles are under [µm]**	**Residual EtAc [ppm]**
0.30	*0.10*	*1.0*	*59.3*	*2.3*	*778.6*	*259*
0.30	*0.10*	*1.0*	*64.2*	*2.4*	*150.6*	*354*
0.30	*0.10*	*1.0*	*66.5*	*2.9*	*462.2*	*233*
0.25	0.10	0.8	59.1	3.0	389.1	134
0.25	**0.14**	**0.8**	**60.9**	**2.1**	**13.4**	**170**
0.25	**0.14**	**0.8**	**60.1**	**2.1**	**0.9**	**140**
0.25	0.10	1.2	64.8	2.7	163.9	107
0.25	0.14	1.2	65.0	1.9	108.4	1684
0.35	0.07	0.8	63.4	3.4	126.2	2317
0.35	0.10	0.8	67.0	3.6	93.6	2361
0.35	0.07	1.1	66.9	2.6	87.3	2608
0.35	0.10	1.1	63.9	2.5	7.8	1436
0.35	0.10	1.1	61.6	3.1	3.2	1623

Table 1: Experimental plan for studying effect of factors to emulsion droplet size, final particle size, quercetin recovery, residual organic solvent concentration; (*Centrum point measurements* marked by Italic, **best results** according to final particle size and residual organic content marked by Bold, repetitions underlined.)

Concentration of surfactant material seems not to be significant on the particle size of aqueous suspension, and according to visual observations by microscopy, we obtained needle like quercetin crystals (Figure 2). That result let us conclude that Pluronic L64 is not a suitable surfactant material to encapsulate quercetin, therefore to choose a new surfactant material for our further experiments is requested.

Quercetin recovery after SFEE treatment seems to be independent on all of the studied factors. The recovery was in all experiments between 60-70%. After treatment, samples stored in a glass vial in fridge around 5°C, and according to long term studies, quercetin concentration seems to be constant at least for 3 weeks after treatment.

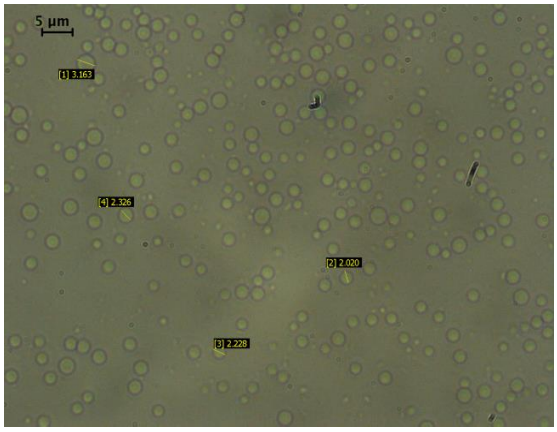

Figure 1: Droplet size in initial emulsion

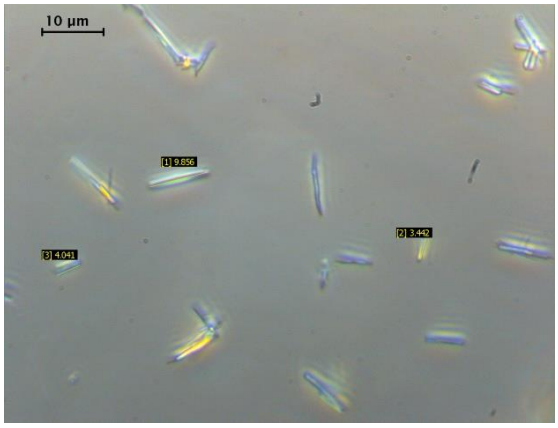

Figure 2: Needle likes quercetin particles

CONCLUSION

To increase bioavailability of quercetin we are using SFEE technology to producing aqueous suspensions containing quercetin micro-particles from o/w emulsions. Experimental plans were done to study influencing factors on initial emulsion average droplet size, final particle size in aqueous suspensions, residual organic solvent and quercetin recovery after SFEE treatment. However Pluronic L64 concentration was found as a significant factor in initial emulsion average droplet size, in final particle size distribution was only significant the concentration of quercetin in EtAc. Surfactant material seems to be not influencing the final particle size, since it could not avoid recrystallization of quercetin after solvent elimination. Quercetin recovery ratio between 60-70% of the originally dissolved quercetin in organic solvent, and suspension is stable at least for three weeks after SFEE treatment.

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