Solid dispersions of quercetin-PEG matrices: Miscibility prediction, preparation and characterization

Elisabeth van Hecke, Mohammed Benali

Solid dispersions of quercetin-PEG matrices: Miscibility prediction, preparation and characterization

Elisabeth Van Hecke and Mohammed Benali*

Université de Technologie de Compiègne, ESCOM, TIMR (Integrated Transformations of Renewable Matter), Centre de Recherche Royallieu, CEDEX CS 60 319, 60 203 Compiègne, France

*Corresponding author at: m.benali@escom.fr

Solid dispersions of quercetin-PEG matrices: Miscibility prediction, preparation and characterization

ABSTRACT

Quercetin is a bioflavonoid compound with low water solubility in both foods and the gastrointestinal tract, which limits the exploitation of its health-promoting properties. This study aims to formulate solid dispersions (SDs) of quercetin with hydrophilic polyethylene glycol PEG matrices, using the melt-mixing method, to improve the dissolution and antioxidant activity of quercetin. Initially, the total Gibbs free energy for different binary mixtures was modelled to predict the miscibility of the compounds. Then, the physicochemical properties of the formulated SDs were characterised and dissolution tests and antioxidant activity measurements were performed in vitro to evaluate the improved profiles and antioxidant activity of the SDs developed. The group contribution methods of Hoy and Van Krevelen and Hoftyzer, used for PEG solubility parameter calculations, presented a suitable prediction of interaction and miscibility behaviour. The results suggested the presence of amorphous quercetin precipitates in the crystalline matrix of PEG 4000 and PEG 6000, and the formation of an amorphous SD between quercetin and PEG 1000. These formulations achieved good radical scavenging activity and better dissolution, especially the quercetin-PEG 1000 SDs.

Keywords: solid dispersion; quercetin; PEG; solubility parameters; miscibility; radical scavenging activity

1 Introduction

Flavonoids are natural substances with a wide range of uses, including biotechnology, nutraceuticals, and the development of novel bioactive compounds, co-drugs, chemo-protectors, and herbal medicines. Flavonoids are divided into several classes that differ in the degree of saturation of the aglycone heterocycle, its oxidation, and spatial conformation. Among these classes, we find flavanols, anthocyanidins, and flavonols.

Quercetin, *2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one*, is classified as a flavonol and is a major compound of polyphenolic bioflavonoid (El-Saber Batiha et al., 2020). It has been reported that quercetin has several potentially beneficial health effects, including antioxidant, anti-carcinogenic, and anti-hypertensive activities. Recently, quercetin has been reported as exhibiting synergistic antiviral effects when ingested with vitamin C, which may help relieve some of the symptoms associated with COVID-19 (Chow et al., 2020).

In fruits, vegetables or plant extracts, quercetin is found as glycosides with a sugar fraction attached to one of the hydroxyl groups, increasing their solubility in water, such as quercetin glycosides, isoquercetin, and rutin. Thus, quercetin is ingested in the form of glycosides and is then found in the aglycone form in the gastrointestinal tract after hydrolysis. However, quercetin-aglycones have lower bioavailability and solubility in water and the human gut (El-Saber Batiha et al., 2020).

To improve the solubility and bioavailability of quercetin while preserving its activity, various methods and efforts have been deployed, such as encapsulation in nanoparticles (Zou et al., 2021), particle size reduction (Manca et al., 2020), emulsification (Khalid et al., 2016), complexation with cyclodextrins (Yang et al., 2019), and solid dispersions (Gilley et al., 2017). The solid dispersion (SD) method is usually used to enhance low solubility bioactive compounds' release rate and bioavailability. The solid dispersion formulation combines two distinct components: the hydrophobic bioactive compound and the hydrophilic carrier matrix. Solid dispersions were classified according to the state of both bioactive compound and carrier matrix: eutectic mixtures, crystalline or amorphous solid dispersions, and solid solutions (Shah et al., 2014). In the pharmaceutical sector, the first generation of solid dispersions used crystalline carriers and three types of solid dispersions were obtained differently in the bioactive compound or the drug: crystalline compound (eutectic solid dispersion), amorphous compound (amorphous precipitates in the crystalline matrix), or molecularly dissolved (solid solutions) state. The second-generation used natural or synthetic amorphous polymer supports, with drugs in crystalline (glass suspension), amorphous (amorphous solid dispersion), or molecularly dissolved (glass solution) form.

Generally, the solid dispersion of a poorly water-soluble molecule in a hydrophilic carrier (amorphous or crystalline) increases the dissolution behaviour of the hydrophobic compound when this system is exposed to water. This is attributed to several factors, such as improved wettability of the drug by the polymer, reduced particle size, separation of individual drug particles by polymer particles, changing the drug from a crystalline to an amorphous state, and the subsequent prevention of precipitation of the drug in contact with aqueous media (Shah et al., 2014). There are various techniques for preparing the solid dispersion of hydrophobic compounds to improve their aqueous solubility: the solvent evaporation method, hot-melt extrusion, and the melting method (Shah et al., 2014). The main advantages of the latter method of fusion are that it is solvent-free. An important condition for the formation of solid

dispersion using the fusion method is the miscibility of the hydrophobic compound and matrix in molten form. Another important condition is the thermostability of the bioactive compounds and hydrophilic matrix.

To obtain a solid dispersion containing the solute or hydrophobic bioactive compound in a stable amorphous state, the two components must be thermodynamically miscible during the process; ideally, this miscibility must be maintained under storage conditions. In the case of liquid mixtures, the balance between the entropy and enthalpy of the mixture, and therefore the free energy of mixing, dictates miscibility. Based on the concepts of statistical thermodynamics, Flory and Huggins (Ulrich, 1978) proposed modifications to the original regular solution theory to make it applicable to a polymer-solvent binary system. The Flory-Huggins theory of polymer solutions provides an expression for calculating the overall free energy of dissolution per mole of lattice site and is very effective for predicting the behaviour of polymer-solvent systems. This theory was later extended to the drug-polymer system and has demonstrated its usefulness in describing their miscibility by taking into account the large size difference between the two components (Baird & Taylor, 2012; Shah et al., 2014).

The Flory-Huggins equation for calculating the total Gibbs free energy of mixing, $\Delta G_m(J)$, of a drug-polymer system, leads to the following expression:

$$\frac{\Delta \boldsymbol{G_m}}{\boldsymbol{RT}} = \left[\boldsymbol{n_d ln\phi_d + n_p ln\phi_p}\right] + \left[\boldsymbol{n_d \phi_p \chi_{dp}}\right] \quad (\mathbf{1})$$

where R is gas constant (J.mol^{-1}.K^{-1}), T absolute temperature (K), n_s and ϕ_s number of moles and volume fraction

solute, whereas n_p and ϕ_p are the number of moles and volume fraction of polymer respectively; χ_{dp} is the Flory–Huggins interaction parameter, which represents the interaction between polymer segments and solute molecules.

In this expression, the first term on the right-hand side of the equation represents the entropy of mixing, (ΔS_m) and the second term represents the enthalpy of mixing (ΔH_m). A necessary condition for miscibility is that the total Gibbs free energy of mixing should be less than 0. When mixing solutes, especially bioactive molecules or drugs, with a polymer, the entropy contribution will always favour mixing, depending on polymer molecular weight with respect to the drug molar weight. In contrast, it is the enthalpic component of the free energy of mixing that will determine whether $\Delta G_m \leq 0$ or not, and hence whether mixing is going to occur or not (Marsac et al., 2006). Therefore, to predict miscibility between a solute and a polymer, it is necessary to estimate the magnitude of the interaction parameter χ_{dp}. One of the different approaches used for estimating the polymer χ_{dp} is the one based on the solubility parameter of solute δ_s and of polymer matrix δ_m as shown in the following equation:

$$\boldsymbol{\chi_{dp} = V \frac{(\delta_s - \delta_m)^2}{RT}} \quad (\mathbf{2})$$

where V is the volume of the hypothetical lattice.

The equation above shows that two substances exhibiting a similar numerical value of solubility parameter are expected to undergo mutual mixing, whereas a higher difference between the values of δ_s and δ_m indicates decreased tendency to undergo mixing. It has been noted that Eq. (2) would generally be sufficient for systems with van der Waals interactions, but not for systems with specific directional

interactions such as hydrogen bonding (Marsac et al., 2006).

In the present study, polyethylene glycol, with molecular weights from 1000 to 6000 was used, because it has high solubility in water, good stability and a relatively low melting point, around 35-70°C. Polyethylene glycol (PEG) is one of the matrices commonly used in solid dispersion. It has been reported that solid dispersion using PEGs increases the solubility of poorly soluble flavonoids such as naringin, hesperidin, hesperetin (Kanaze et al., 2006) and luteolin (Alshehri et al., 2020).

To our knowledge, no study has been carried out regarding the effect of PEG molecular weights (Mw) between 1000 and 6000 on the enhancement of quercetin dissolution in solid dispersion, using the melt mixing method in a high-speed disperser process. This study aims to investigate the effect of PEG molecular weights on the miscibility and structures of quercetin precipitate, crystalline or amorphous, in solid dispersion. To extract information about the effect of PEG molecular weights on the interaction and compatibility in quercetin-PEG solid dispersions, the total Gibbs free energy of mixing for different binary mixtures was modelled using the solubility parameter. Then, the physicochemical and stability properties of the solid dispersions formulated were characterized and compared using instrumental analysis: Fourier transform infrared (FTIR) spectroscopy, powder X-ray diffraction (XRD), differential scanning calorimetry (DSC), and water vapour sorption isotherms. Finally, dissolution tests and antioxidant activity measurements were performed *in vitro* to evaluate the enhancement of release profiles and antioxidant activity of the quercetin SDs developed.

2 Materials and methods

2.1 Materials

Anhydrous quercetin (purity 99%) was obtained from Verbiese (France), polyethylene glycol with molecular weights 1000 (PEG 1000) and 4000 g/mol (PEG 4000) was purchased from AcrosOrganics (Belgium), and the PEG 6000 was supplied by Merck (Germany). Citric acid and 2.2-Diphenyl-1-picrylhydrazyl (DPPH) was gifted from Sigma Aldrich (USA) and ethanol at 96% was supplied by Carlo Erba Reagents (France). All the other materials and reagents were of analytical grade and purity.

2.2 Equipment

Quercetin-PEG solid dispersions were made by melt mixing in a high-speed disperser Dispermat® LC55, equipped with a double jacket for heating and a temperature control system. A jacketed 50 ml stainless-steel dispersion vessel (height = 50mm, inner diameter = 40mm) was used for batch charging. Dispersion of quercetin was done with help of a stainless-steel disc (diameter = 3mm, thickness = 6mm). The disc speed rotation could be varied from 0 to 20,000 tr/min.

2.3 Preparation of quercetin-PEG physical mixture (PM) and solid dispersion (SD)

A total weight of 10 g of quercetin-PEG SD with an amount of quercetin of 5, 20, 40, and 50% (w/w) was prepared as follows. PEG polymer was placed in a vessel with the calculated amount of quercetin powder, they were mixed at a selected impeller rotation speed of 10,000 rpm and melted at a specific temperature (at 60°C for PEG 1000 and at 80 °C for PEG 4000 and PEG 6000) for 10 minutes. The dispersion obtained was solidified at room temperature. The dispersion was transferred in a desiccator for 24 h and then pulverised using a porcelain mortar and pestle. To obtain particles of uniform size, the solid dispersions were crushed and then sieved (200µm). The resulting powder was stored in an airtight container.

Physical mixtures with different mass amounts of quercetin (5, 20, 40 and 50%) were prepared by mixing quercetin and PEG, using a mortar and pestle. The mixture obtained was sieved (200µm).

2.4 X-ray diffraction analysis

The powder's crystalline state was characterised using a Siemens D5000 X-Ray Powder Diffractometer. This is a theta/theta2 diffraction instrument operating in reflection geometry using Fe Kα radiation (λ = 1.54056 Å), which is focused on a Ge crystal primary monochromatic. The detector is a standard scintillation counter. The scanning range for 2θ was set at 10–45°, the step size was 0.025° with a scanning rate of 0.5 step/s, and using an operating voltage and amperage set to 20.0 kV and 5.0 mA, respectively. The Kα2 radiation of the copper was deleted with the software of the XRD. Experiments were carried out on three replicates.

2.5 Characterisation with FTIR spectroscopy

Infrared spectra were obtained using an infrared spectrophotometer IR-TF Nicolet iS50 (Thermo Scientific^{TM}) in the wavenumber range of 400-4000 cm^{-1} with the method of bromide pellets. 1 mg of the sample was mixed with 8 mg of KBr in a mortar and pestle, the pellet was obtained by pressing the previous melange in a press under 8T. Each experiment is reproduced three times.

2.6 DSC measurement

Heating curves of quercetin, PM, and SD were obtained using a differential scanning calorimeter (Q200 DSC, TA Instruments) equipped with a refrigeration unit. Samples (4–7 mg) were packed in a non-hermetically crimped aluminium pan, and heated under dry nitrogen purge (50.0 ml/min). Samples were heated from 25 °C to 400°C at 10 °C/min.

2.7 Water vapour sorption measurement

The dynamic water vapour sorption analyses, using a gravimetric dynamic sorption analyser SPS device (SPS23, ProUmid GmbH & Co, Germany), were performed to investigate the moisture sorption behaviour of pure compounds, SDs and PMs at 25°C. Samples (100-500mg) were poured into an aluminium pan (50 mm diam. and 10 mm height) after first being dried at 0% RH at 25°C where the equilibrium criterion for the drying step was 0.01% w/w change in 15min and a maximum drying time of 60min. The samples were then exposed to constant relative humidity and temperature for known periods of time. The sample was maintained at each RH until a plateau in the weight grain profile was reached. All samples were maintained in a temperature and humidity-controlled chamber (± 0.6%) and were sequentially weighed using a high precision microbalance (±10 µg). The analysis protocol and the data exploitation were performed according to the literature (Afrassiabian & Saleh, 2020).

2.8 Dissolution tests

The dissolution profiles of pure quercetin, physical mixture (PM), and SDs were obtained in a buffer solution (200 ml) of citric acid/NaOH at pH 5 at 100 rpm speed, at 37°C for 60 minutes, with quercetin content fixed at 0.03 mg/ml. At time intervals (5, 10, 15, 20, 25, 30, 45, and 60 minutes), 1 ml of the solution was collected, filtered (with a Whatman PP 0.45 µm), and replaced with a clean solution. Quercetin concentration was determined by spectrophotometer UV-Vis (Jasco V-530) at 370nm. All measurements were repeated three times.

2.9 In vitro antioxidant activity

The antioxidant activity of quercetin, MP, and SD was measured using a 1,1-diphenyl-2-picrylhydrazyl

(DPPH) radical scavenging assay (Blois, 1958). The hydrogen atom donating ability of the quercetin was determined by the discolouration of the ethanol solution of DPPH. DPPH produces a purple colour in ethanol solution and fades to shades of yellow in the presence of antioxidants.

The quercetin-PEG samples were dissolved in ethanol to obtain a fixed concentration equivalent to IC50 of pure quercetin (0.3 µg/ml). 0.5µL of this solution was mixed with 1.5 mL of DPPH in ethanol solution (0.024mg/mL). The blank sample was prepared with a similar procedure but without Que. The reaction mixture was incubated in the dark at room temperature for 30 min. The activity was determined by spectrophotometer UV-Vis (Jasco V-530) at 517 nm. Percentage of inhibition was calculated using the following equation:

$$\%\ \boldsymbol{Inhibition} = \frac{\left(\boldsymbol{A_{control}} - \boldsymbol{A_{sample}}\right)}{\boldsymbol{A_{control}}} \quad (\mathbf{3})$$

where $A_{control}$ is the absorbance of the control, and A_{sample} is the absorbance of the solid dispersion sample. All the determinations were performed in triplicate.

2.10 Determining solubility parameter δ

The solubility parameter of quercetin was determined using the Hansen solubility parameters (HSPs) approach (Hansen, 2007; S. Abbott, 2010), according to the experimental and numerical procedures detailed in a previous paper (Imatoukene et al., 2020). A total of 32 organic solvents were tested in order to classify them as adapted or non-adapted solvents. A mass of 30 mg of quercetin was placed in a glass vial with 3 mL of the test solvent. The mixture was stirred vigorously and kept to rest for 24 h at room temperature. The behaviour of the compounds was observed by visual inspection: a solvent was considered a "good" or adapted solvent when the solute/solvent mixture formed a homogenous solution (one-phase system), while a solvent was denoted a "bad" or non-adapted solvent when a two-phase system was obtained. The HSPiP software (version 4.0.05 developed by Abbott and Yamamoto) was used to calculate the HSP of quercetin. The solvents are coded in the programme with a number "1" for soluble (one-phase system) or "0" for insoluble (two-phase system). Solvents, their solubility parameters, and experimental scores were tabulated in **Table** 1.

Scores of the solubility tests were computed with HSPiP software providing the Hansen parameters of quercetin and the radius of its solubility sphere by a quality-to-fit function. The coordinates of the solubility volume centre were δ_d= 17.28 $MPa^{0.5}$, δ_p = 16.85 $MPa^{0.5}$ and δ_h = 11.97 $MPa^{0.5}$, while the radius was R0 = 11.7 $MPa^{0.5}$. Therefore, the total solubility parameter δ was 26.9 $MPa^{0.5}$.

The solubility parameters for the polyethylene glycols (PEGs) were determined for the entire molecule by the group contribution according to methods by Small (Small, 1953), Hoy (Hoy, 1985), and Van Krevelen and Hoftyzer (D. W. Van Krevelen, 1976). The solubility parameters of polymers were calculated, *inter alia*, from their molecular structure from the summation of the group molar attraction constants divided by the molar volume of the polymer (Barton, 2017), taking into account the number of monomer units and density. The prediction of quercetin-PEG miscibility was made using the free energy of mixing (Eq. 1).

Table 2 shows the solubility parameters obtained using group contributions methods, density (measured by helium pycnometry), calculated molecular volumes and the Flory-Huggins interaction parameter. This parameter was calculated from the solubility parameter obtained experimentally for quercetin and

234 those of the PEGs obtained by the three group contribution methods (Barton, 2017). The solubility
235 parameters calculated using the group molar attraction constants given by (Small, 1953) are much
236 smaller. Comparison of the values obtained with those obtained experimentally in the literature
237 (Sakellariou et al., 1986) showed that the methods of Hoy and of van Krevelen were acceptably close to
238 the experiment.
239 The values of χ were used for the construction of phase diagrams illustrating the variation of the total
240 free energy for the quercetin-PEG mixture as a function of the polymer volume fraction. The interaction
241 parameter was supposed independent of temperature.

242 # 3 Results and Discussion

243 ## *3.1 Predicting quercetin-PEG's miscibility*

244 In this work, the volume fraction of the quercetin and polymer were calculated by dividing the weight
245 fraction by the true density of the material, and the volume of the hypothetical lattice was defined as
246 being equal to the molecular volume of quercetin. The ratio of the PEG volume to that of the lattice site
247 was considered as the ratio of molar volumes of PEG and quercetin.
248 Using Eq. (1), the entropy of the mixture as a function of the polymer composition was predicted for the
249 quercetin-PEG systems, for three molecular weights of the polymer. The results were presented in **Fig.**
250 **1**. The effect of the different molecular weights M_w (between1000 and 6000) of the polymer on the
251 thermodynamics of the mixture was weak (**Fig. 1**-a). Indeed, the entropy of the mixture with PEG 4000
252 and PEG 6000 is slightly less favourable than with PEG1000. This can be explained by the
253 configurational entropy of the polymer, which was reduced due to the connectivity of the repeat units
254 (Marsac et al., 2006, 2009). The degree of contribution of entropy to the free energy term should
255 therefore have a relative effect. The miscibility would potentially be controlled by the enthalpy
256 interactions in the mixture and therefore by the difference between the solubility parameters of the
257 quercetin-PEG system.
258 **Fig. 1** (b, c and d) shows the evolution of the free energy of the mixture relative to the composition by
259 gradually increasing the volume fraction of the polymer in the quercetin-PEG binary mixture. According
260 to studies reported in the literature (Marsac et al., 2006, 2009; Shah et al., 2014; Thakral & Thakral,
261 2013), three categories of interactions were obtained depending on the group contributions method
262 employed for the PEG solubility parameter:
263 -The first category is characterised by obtaining a downward concave curve, the evolution of free energy
264 as a function of polymer composition, and a positive value of the total free energy of mixture predicting
265 immiscibility for all Quercetin-PEG systems, regardless of the value of the volume fraction of the
266 polymer in binary mixtures. In this case, the quercetin-PEG system tended to form a biphasic mixture
267 (**Fig. 1**-b).
268 - The second is represented by a downward concave curve followed by a concave free energy ascending
269 curve of the mixture with respect to the composition by gradually increasing the volume fraction of the
270 polymer in the binary mixture. The total free energy of the mixture for compositions containing a small
271 proportion of polymer was positive, its value became negative when the polymer fraction increased
272 beyond 60%. The findings indicated that the quercetin-PEG mixture tends to form a biphasic system
273 with a low concentration of the polymer, but the binary system should exhibit a single phase when

increasing the fraction of polymer (**Fig. 1**-b, c).

-The third was only observed in the case of the quercetin-PEG1000 mixture using the contribution groups method of van Krevelen, which gives negative values of the total free energy of mixing and an overall concave upward shape of the free energy curve (**Fig. 1**-c). The low positive value of the enthalpic contribution, in this case, appears to be counterbalanced by the total increase in system entropy and, therefore, the system exhibits negative free energy mixing for all proportions of quercetin and PEG1000. In this particular case, quercetin can be considered miscible with PEG 1000 in all proportions. It is believed that the interacting adhesive forces between quercetin and PEG1000 are thought to be stronger than the cohesive forces and therefore facilitate mixing, leading to the probable formation of solid solution or the crystallization of quercetin at the amorphous state. Moreover, for the PEG 4000 and PEG 6000, the entropy of the system could not counterbalance the low positive value of the enthalpy, but it did contribute to reducing the total free energy of the system to values relatively close to zero.

Taking into account the study that had shown that the methods of Hoy, and of van Krevelen and Hoy, provided PEG solubility parameter values closest to the experimental values (Sakellariou et al., 1986), estimating the free energy of mixing predicts that the miscibility of the quercetin-PEG system would decrease with the increase in PEG molecular weight and in volume fraction polymer. It should be noted that the contribution of the enthalpy of the mixture was estimated from the solubility parameters of quercetin and PEGs at a temperature of 25°C. Therefore, it can be expected that a system that is immiscible at low temperatures can achieve miscibility at a higher temperature; without forgetting the considerable contribution of the mixing process, which generates a significant shear force in the media and consequently improves miscibility.

3.2 X-Ray diffraction

The diffractogram of the pure compounds (**Fig. 2**) showed that the different grades of polymers used, PEG1000, PEG 4000 and PEG 6000, had the same semi-crystalline structure demonstrated by two significant peaks at 19.68° and 23.85° in 2θ (Baird et al., 2010), while quercetin had the peaks at 14.47°, 26.89°, 27.67° and 28.65° in 2θ (Gilley et al., 2017). Thus, given the fact that the crystal structures of quercetin differed from PEGs, it was possible to determine the presence of crystalline quercetin within the solid dispersions (SD). The physical mixtures (PM) with an amount of quercetin of 40% (w/w) were also characterised to compare.

For all physical mixtures, a low presence of crystalline quercetin via its peak at 14.47°, synonymous with the crystalline structure of quercetin in the crystalline matrix, could be observed, while for SD1 (quercetin-PEG 1000), SD4 (quercetin-PEG 4000), and SD6 (quercetin-PEG 6000), containing a quercetin mass composition up to 40% (w/w), the presence of crystalline quercetin was no longer observed. The quercetin peaks intensity in the samples was significantly less than that of pure quercetin. In this case, the state of amorphous quercetin in the precipitated form in the crystalline matrix can be suggested. Moreover, in the case of SD1 40% (w/w), the absence of the characteristic peaks of the crystalline state was observed, not only for quercetin but also for PEG 1000 which becomes relatively amorphous after cooling the formulation, forming an amorphous solid dispersion. This is explained by the favourable molecular interactions, predicted by the free energy of the mixture between quercetin and PEG, and their mutual roles as crystallization inhibitors which become noticeable in the solid

dispersions SD1 40% compared to this class of low molecular weight PEG. However, the diffractogram of all SDs with a quercetin content of 50% (w/w) revealed the crystalline structure of quercetin. On the other hand, in the case of SD1, recrystallization of PEG 1000 was inhibited by quercetin.

In the following steps, for better evaluation and understanding of the effect of molecular weight on the properties of quercetin, the characterisation results of solid dispersions at 40% (w/w) will be developed and discussed.

3.3 Thermal behaviour

Solid dispersions, as well as physical mixtures containing 40% (w/w) of quercetin, were characterised and compared by thermal analysis. The thermograms of binary mixtures containing quercetin and different grades of PEG are shown in **Fig. 3**. Quercetin was characterised by an endothermic melting peak around 330°C and an exothermic peak near 344°C, which refers to its decomposition (De Mello Costa et al., 2011). The endothermic-melting peak for PEGs occurs at 35.60, 61.7, and 67°C for PEG 1000, PEG 4000, and PEG 6000 respectively.

In the case of physical mixtures (PM), **Fig. 3** shows the absence of a fusion endotherm for quercetin. The results correspond to only one peak at the melting of the polymer in the binary mixture. The non-appearance of the endothermic peak of quercetin in PM was observed in the literature (Khan et al., 2011; Thakral & Thakral, 2013) where analysis of samples containing 20%–40% (w/w) of pharmaceutical drugs in the presence of PEG 8000 and PEG 6000 showed only a single peak corresponding to the melting of the polymer. It has been proposed that during the heating process for analysis, especially if the difference in melting point is significant (as the case here between PEGs and quercetin), the molten carrier begins to solubilize the drug by thus dispersing it in its matrix; with the consequence that the drug endotherm completely disappears. The results obtained for the binary physical mixtures also detected that there was sufficient physical interaction between the components leading to the lowering of the melting point of the polymers to 28.5, 60.25, and 62.23 °C for PEG 1000, PEG 4000, and PEG 6000 respectively.

In the case of solid dispersions, for PEG 4000 and PEG 6000, the physical interactions were stronger, resulting in a lowering of the melting point to 58.70 and 60.41°C for PEG 4000 and PEG 6000 respectively. In the case of the quercetin-PEG1000 mixture, we observed the absence of a melting peak for quercetin and the polymer. This can be considered synonymous with the strong miscibility of quercetin with PEG 1000 on the one hand, and with the inhibition of the recrystallization of PEG 1000 in the presence of quercetin in the binary mixture, upon cooling of the solid dispersion in process. These results concur with those obtained by DRX analysis and predicted by the mixing free energy model using the van Krevelen method to calculate the Flory interaction parameter. The latter predicted total miscibility in the binary quercetin-PEG 1000 mixture, resulting in a strong adhesive interaction between the two constituents compared to the cohesive interactions of each component. All these results confirm that quercetin forms an amorphous solid dispersion with crystalline matrixes PEG 4000 and PEG 6000, and a glass solution with amorphous PEG 1000 where the quercetin molecules were dissolved or molecularly dispersed with the amorphous matrix.

3.4 Fourier transform infrared spectroscopy

Further evidence for intermolecular interactions in solid dispersions has been obtained by means of infrared spectroscopic examination. FTIR has been used to explore quercetin-polymer interactions in

SD. IR spectra of quercetin, PEGs, quercetin-PEG (40% w/w) physical mixtures, and solid dispersions are shown in **Fig. 4**. Quercetin and PEG FTIR peaks were found in accordance with those reported in the literature (Dwi et al., 2018; Indra et al., 2020).

The comparison of the infrared spectra of the pure compounds and the mixtures shows the absence of the creation of covalent bonds between the two compounds in the mixture and that they were not degraded during mixing. No clear changes in vibration bands were observed in quercetin with PEGs. In fact, it was the most readable function for seeing if there had been a modification to the hydrogen bonds. To estimate the creation of hydrogen bonds between PEG and quercetin allowing the mixture to be stabilized, the functional group of quercetin studied was C=O (1670 cm^{-1}). The study of the characteristic band C=O made it possible to confirm the creation of intermolecular H bonds. In the solid dispersions, a significant displacement of this band was observed toward 1652, 1653, and 1654 cm^{-1} for PEG 1000, PEG 4000, and PEG 6000 respectively, while this was not observed in the physical mixtures (**Fig. 5**). This shift in the wavenumber of infrared spectra is synonymous with physical interactions caused by weak interactions, such as hydrogen bond interactions between functional groups of quercetin and PEG. A hydrogen bond between the carbonyl group of quercetin and the terminal hydroxyl group of PEGs is quite possible. Moreover, peaks were barely visible around 3300 and 3400 cm^{-1} on the spectra of SD4 and SD6 (**Fig. 4**), indicating that quercetin was only partially amorphous in these solid dispersions (Gilley et al., 2017), in agreement with the DRX analysis (**Fig. 2**).

3.5 Moisture sorption isotherms

A water sorption isotherm at 25°C was used to evaluate the effect of the PEG Mw carrier on quercetin-PEG solid dispersions and their physical stability. PEG is a hygroscopic and semi-crystalline material and can undergo deliquescence. Deliquescence is a process by which highly water-soluble crystalline or semi-crystalline materials undergo a solid-to-solution phase transformation through sorption of large quantities of atmospheric moisture (Dupas-Langlet et al., 2017). The sorption of large amounts of moisture by solid dispersions can potentially have an impact not only on phase behaviour, but also on chemical and physical stability, as well as microbiological properties.

Moisture sorption isotherms for pure quercetin, various PEGs, PMs, and SDs were measured at 25°C between 0 to 85 % RH (**Fig. 6**). Quercetin presented slight moisture sorption of about 2.54 % (w/w) at 85% RH and 25°C, a low uptake level due to surface adsorption, while the PEG isotherms were characterised by a sharp increase in moisture content depending on the contribution of both adsorption and absorption phenomena. In addition to surface sorption, it is widely accepted that semi-crystalline polymers will also sorb moisture into the amorphous region at the lower RH (Schachter et al., 2004). **Fig. 6** a,b and c shows that water content remains negligible (<1%) up to approximately 30, 60, and 65% RH, then a relatively gradual increase in the water content of the polymers, and finally, at higher RH, an increase of water uptake up to 76, 30 and 16% for pure PEG 6000, PEG 4000, and PEG 6000 respectively. Moreover, the inflexion point corresponding to the deliquescence phenomenon of crystalline PEGs, characterised by an intense increase in water content, was observed at a different RH for each grade. The deliquescence relative humidity (RH_0) for PEG 1000, PEG 4000, and PEG 6000, measured by extrapolating the linear parts of the vapour sorption isotherm before and after the deliquescence, was estimated at 54, 77, and 79 % RH, respectively. The values obtained were very close to those reported in the literature (Baird et al., 2010).

PEG can form numerous hydrogen bonds with water through the functional molecular groups of ether oxygen atoms (–O–) in the oxyethylene polymer backbone and hydroxyl (-OH) end groups. The decrease in water uptake, with polymer molecular weight, can be explained by the fact that the relative fraction of the hydroxyl end groups decreases, hence the higher molecular weight grades are less hydrophilic and will have lower water affinity.

The findings have also been explained in the literature by the fact that at low RH, solid PEG crystallizes, obstructing the penetration of water molecules to access oxygen atoms in the PEG chains and thus the water uptake (Thijs et al., 2007). At high RH, it was proposed that when a first hydration shell is formed in the PEG, the PEG chains would adapt the desired conformation or arrangement of the polymer chains to be able to form extensive hydrogen bonds with water, favouring the uptake of more water. The results obtained here obey this proposed mechanism and show that the decrease in the molecular weight of PEG favours those mechanisms for water absorption.

The hydrophobic character and non-deliquescence of pure quercetin were demonstrated by the low sorption of water (2% at 85% RH) in **Fig. 6**. In contrast, the water uptake in solid dispersion SD1 increased to 47% at 85% RH, while those of SD4 and SD6 increased to 30 and 18% respectively. The PMs show a nearly identical sorption isotherm as the solid dispersion.

In other words, the addition of quercetin at 40% (w/w) had a minimal effect on the moisture sorption behaviour of PEG 4000 and PEG 6000, which allocated to the presence of quercetin amorphous precipitates in the crystalline matrix. However, just before the deliquescence relative humidity (HR0 =77%) of PEG 4000, a moderate increase in water uptake could be observed which was attributed to the new molecular arrangement which helps access certain polar functional groups in solid dispersion.

Moreover, compared to PEG 1000 moisture sorption, the SD1 data show a decrease in water sorption at high RH in the presence of hydrophobic molecules of quercetin. These latter obstructed the functional groups in solid dispersion, by means of the steric effect, by making them unavailable to interact with water during sorption. To confirm that, the interaction between quercetin and PEGs was evaluated using the deviation of SD1 water uptake from the ideal behaviour theoretically predicted. The theoretical moisture gain, supposing no interaction between the components, was estimated by summation of the moisture sorption profiles of the two individual components with respect to their mass fractions (Crowley & Zografi, 2002). It is stated in the literature that theoretical moisture gain is valid for a model binary system of two miscible amorphous components. A deviation in the amount of water taken up could be observed for all cases as represented in **Fig. 6**. A decrease in the amount of water taken up in melt mixing SD1 could particularly be noted, which confirmed the relatively strong interaction of quercetin and PEG 1000 and the amorphous structure of SD1, as already indicated.

3.6 Quercetin release profiles

The quercetin dissolution profiles of pure quercetin, PMS and SDs are presented in **Fig. 7**. The dissolution studies were conducted only on SDs and PMs prepared with 40% w/w, to understand the effect of completely amorphous and crystalline systems on dissolution. In this study, the dissolution efficiency of minute 30 (DE30) was selected to compare the quercetin release rate in solid dispersion, under non-sink conditions and without added surfactant. The result was compared to that of pure crystalline quercetin under similar conditions.

The DE30 of quercetin, PM1, PM4, and PM6 was 1.64, 1.78, 1.78, and 0.98% respectively; while for

SD1, SD4 and SD6, the DE30 was 7.71, 3.46, and 6.05 %. The solubility of quercetin in the medium was very low, it was solubilized after only 10 minutes to reach a maximum value (1.6 %). Compared to pure quercetin, all PMs showed relatively faster dissolution during the first 10 minutes, and then it did not result in any change in the dissolution profile and solubility, according to XRD data where no changes in the crystal structure of quercetin were observed in PMs.

All three PEG matrices displayed similarly low quercetin release (<10%), while still much faster and more complete than pure quercetin and the physical mixture. Some results were obtained in the works of Gilley (Gilley et al., 2017), concerning the solid dispersion of quercetin in cellulose derivate matrices (quercetin/cellulose derivate=1/9, pH=6.8), using spray-dried process; it was found within 30 min, quercetin release reached 13–14% then decreased rapidly to 4–5% within 5h. To enhance the dissolution and bioavailability of quercetin, a novel blend of cellulose derivate matrices with hydrophilic PVP was proposed (Gilley et al., 2017).

Fig. 7 shows the increase in the dissolution rates of quercetin in solid dispersion SDs, compared to PMs and pure quercetin, which may be due to the amorphous state of quercetin in a polymeric matrix (Shah et al., 2014). The hydrophilic polymer encapsulated the hydrophobic compound and helped to solubilize the compound readily due to its rapid contact with the dissolution media (Alshehri et al., 2020). Moreover, the SD1 formulation prepared with PEG1000 showed that quercetin release was very rapid. At 5 minutes of dissolution, more than 7.30 % of the quercetin had dissolved from solid dispersion SD1, whereas SD4 and SD6 dissolved 2.17 and 4.30 % respectively.

These results were attributed to the amorphous solid dispersion of SD1, according to XRD and DSC data, where the quercetin was molecularly dispersed with the amorphous matrix. Molecules present in a random arrangement in the amorphous state offered a lower thermodynamic barrier to dissolution and superior molecular motion that made possible a faster dissolution rate. Consequently, the amorphous form showed higher solubility, a higher rate of dissolution, and then better bioavailability than the crystalline structure.

3.7 Antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities of quercetin and solid dispersions (SD1, SD4 and SD6) with a fixed mass ratio (40%) were evaluated. The results are illustrated in the **Fig. 8**. Determining the antioxidant activity of the solid dispersions made it possible to determine the impact of the formulation on this property because all the samples had the same amount of quercetin. The results obtained showed an increase in the anti-radical activity of the different solid dispersions compared to pure quercetin (49.22% ± 3.01). These results obtained show a slight effect of PEG grade on the antioxidant activity.

The significant result of higher antioxidant activity was achieved due to the greater improvement in solubility and dissolution due to the amorphization of quercetin by melt mixing compared to the physical mixtures. Of the three SDs prepared, the highest DPPH radical scavenging activity was achieved by SD6 (76.61% ± 4.06), followed by SD4 (74.60% ± 3.50) and then by SD1 (68.70% ± 3.06). Although characterising SD1 at 40% quercetin revealed the formation of amorphous solid dispersion, the latter did not show superior anti-radical activity compared to other SDs. The results of the study revealed that quercetin-PEG solid dispersions formation showed good radical scavenging activity and antioxidant activities compared to pure quercetin. Statistical analysis using the one-way analysis of

variance confirmed this (p-value < 0.05). The fact that antioxidant activity increased may stem from the better availability of the alcoholic protons of quercetin (or electron e-donation) in the solution that can convert DPPH into DPPH-H. The amorphous state of quercetin was found in several polymeric matrices, which, in the absence of organisation, made possible better availability of antioxidant protons.

4 Conclusion

The quercetin SD formulations were prepared using the melt mixing or fusion method in a high-speed disperser process using PEG 1000, 4000 and 6000 as the hydrophilic matrix to enhance the dissolution rate and antioxidant activity of quercetin.

The theoretical estimation of total Gibbs free energy and Flory-Hugging interaction parameters for quercetin with various molecular weights of PEG was performed using the solubility parameters. This last was experimentally determined using the Hansen solubility parameter method for quercetin while that of PEGs was calculated using the group contribution methods. Thus, it was possible to produce a total Gibbs free energy diagram of the system versus volume fraction polymer in a binary mixture. In this study, Van Krevelen and Hoftyzer's group contribution methods gave a good prediction of interaction and miscibility behaviour, particularly between quercetin and PEG 1000. The theoretical estimation of free energy predicted a decrease in miscibility with the increase in molecular weight of the PEG. The results of X-ray diffraction and differential scanning calorimetry of the dispersion solid, with 40% (w/w) of quercetin, revealed a structure transformation from crystalline to the amorphous state of quercetin in all SDs. Moreover, an amorphous solid dispersion or glassy solution was formed, between quercetin and PEG 1000, with a mutual role as a crystallization inhibitor. The IR study spectra revealed a weak physical interaction involving hydrogen bonds between the quercetin molecules and the polymer chains of PEGs. Moreover, characterising the interaction and stability of quercetin-PEG SDs using DVS made it possible to confirm the presence of quercetin amorphous precipitates in the crystalline matrix of PEG 4000 and PEG 6000, and the amorphous structure of SD1 with low water uptake and relatively strong interaction of quercetin and PEG. The result of this study reveals low enhancement in the dissolution rate of quercetin from all SDs. The amorphous SD1 showed a higher rate of dissolution, about 10%. However, that is still much faster and more complete compared to the release of pure quercetin and physical mixture (<1.6%). All the SD formulations achieved good antioxidant and/or activity radical scavenging generated by the amorphous state of quercetin in the PEG polymer matrix.

Acknowledgements

We thank Region Hauts-de-France for the financial support of the Valantiox project. Valantiox project aims to enhance the antioxidant potential of low-water solubility polyphenols, by formulating solid dispersions to improve their bioavailability.

References

Afrassiabian, Z., & Saleh, K. (2020). Caking of anhydrous lactose powder owing to phase transition and solid-state hydration under humid conditions: From microscopic to bulk behavior. *Powder Technology*, *363*, 488–499. https://doi.org/10.1016/j.powtec.2020.01.033

Alshehri, S., Sarim, S., Altamimi, M. A., Hussain, A., Shakeel, F., Elzayat, E., Mohsin, K., Ibrahim, M., & Alanazi, F. (2020). *Enhanced Dissolution of Luteolin by Solid Dispersion Prepared by Different Methods: Physicochemical Characterization and Antioxidant Activity*. https://doi.org/10.1021/acsomega.9b04075

Baird, J. A., Olayo-Valles, R., Rinaldi, C., & Taylor, L. S. (2010). Effect of Molecular Weight, Temperature, and Additives on the Moisture Sorption Properties of Polyethylene Glycol. *Journal of Pharmaceutical Sciences*, *99*(1), 154–168. https://doi.org/10.1002/jps.21808

Baird, J. A., & Taylor, L. S. (2012). Evaluation of amorphous solid dispersion properties using thermal analysis techniques. *Advanced Drug Delivery Reviews*, *64*(5), 396–421. https://doi.org/10.1016/j.addr.2011.07.009

Barton, A. F. M. (2017). *CRC Handbook of Solubility Parameters and Other Cohesion Parameters*. Routledge. https://doi.org/10.1201/9781315140575

BLOIS, M. S. (1958). Antioxidant Determinations by the Use of a Stable Free Radical. *Nature*, *181*(4617), 1199–1200. https://doi.org/10.1038/1811199a0

Chow, V. T., Manuel, R., Biancatelli, L. C., Colunga Biancatelli, L., Berrill, M., Catravas, J. D., & Marik, P. E. (2020). Quercetin and Vitamin C: An Experimental, Synergistic Therapy for the Prevention and Treatment of SARS-CoV-2 Related Disease (COVID-19). *Frontiers in Immunology | Www.Frontiersin.Org*, *1*, 1451. https://doi.org/10.3389/fimmu.2020.01451

Crowley, K. J., & Zografi, G. (2002). Water Vapor Absorption into Amorphous Hydrophobic Drug/Poly(vinylpyrrolidone) Dispersions. *Journal of Pharmaceutical Sciences*, *91*(10), 2150–2165. https://doi.org/10.1002/jps.10205

de Mello Costa, A. R., Marquiafável, F. S., de Oliveira Lima Leite Vaz, M. M., Rocha, B. A., Pires Bueno, P. C., Amaral, P. L. M., da Silva Barud, H., & Berreta-Silva, A. A. (2011). Quercetin-PVP K25 solid dispersions : Preparation, thermal characterization and antioxidant activity. *Journal of Thermal Analysis and Calorimetry*, *104*(1), 273–278. https://doi.org/10.1007/s10973-010-1083-3

Dupas-Langlet, M., Benali, M., Pezron, I., & Saleh, K. (2017). Characterization of saturated solutions and establishment of “aw-phase diagram” of ternary aqueous inorganic-organic and organic-organic systems. *Journal of Food Engineering*, *201*, 42–48. https://doi.org/10.1016/j.jfoodeng.2017.01.009

Dwi, S., Febrianti, S., Zainul, A., & Retno, S. (2018). PEG 8000 increases solubility and dissolution rate of quercetin in solid dispersion system. *Marmara Pharmaceutical Journal*, *22*(2), 259–266. https://doi.org/10.12991/mpj.2018.63

D. W. Van Krevelen. (1976). *Properties of polymers, their estimation and correlation with chemical structure – (2nd rev. ed.), D. W. Van Krevelen, Elsevier, Amsterdam – Oxford – New York, 1976, 620 pp.* (E. M. Pearce, Ed.; 2nd ed.). https://doi.org/10.1002/pol.1977.130150109

El-Saber Batiha, G., Magdy Beshbishy, A., Ikram, M., Mulla, Z. S., Abd El-Hack, M. E., Taha, A. E., Algammal, A. M., Hosny, Y., & Elewa, A. (2020). *The Pharmacological Activity, Biochemical Properties, and Pharmacokinetics of the Major Natural Polyphenolic Flavonoid: Quercetin*. *9*, 374. https://doi.org/10.3390/foods9030374

Gilley, A. D., Arca, H. C., Nichols, B. L. B., Mosquera-Giraldo, L. I., Taylor, L. S., Edgar, K. J., & Neilson, A. P. (2017). Novel cellulose-based amorphous solid dispersions enhance quercetin solution concentrations in vitro. *Carbohydrate Polymers*, *157*, 86–93. https://doi.org/10.1016/j.carbpol.2016.09.067

Hansen, C. M. (2007). *Hansen Solubility Parameters*. CRC Press. https://doi.org/10.1201/9781420006834

HOY, K. L. (1985). The Hoy tables of solubility parameters. *Union Carbide Corporation, Solvents & Coatings Materials, Research & Development Department.*

Imatoukene, N., Koubaa, M., Perdrix, E., Benali, M., & Vorobiev, E. (2020). Combination of cell disruption technologies for lipid recovery from dry and wet biomass of Yarrowia lipolytica and using green solvents. *Process Biochemistry*, *90*, 139–147. https://doi.org/10.1016/J.PROCBIO.2019.11.011

Indra, P., Zaini, E., Ismed, F., & Lucida, H. (2020). Preparation and characterization of quercetin-polyvinylpyrrolidone K-30 spray dried solid dispersion [Preparación y caracterización de dispersión sólida de quercetina-polivinilpirrolidona K-30 secada por rociado]. *Journal of Pharmacy & Pharmacognosy Research*, *8*(2), 127–134. http://jppres.com/jppres

Kanaze, F. I., Kokkalou, E., Niopas, I., Georgarakis, M., Stergiou, A., & Bikiaris, D. (2006). Dissolution enhancement of flavonoids by solid dispersion in PVP and PEG matrixes: A comparative study. *Journal of Applied Polymer Science*, *102*(1), 460–471. https://doi.org/10.1002/app.24200

Khalid, N., Kobayashi, I., Neves, M. A., Uemura, K., Nakajima, M., & Nabetani, H. (2016). Microchannel emulsification study on formulation and stability characterization of monodisperse oil-in-water emulsions encapsulating quercetin. *Food Chemistry*, *212*, 27–34. https://doi.org/10.1016/j.foodchem.2016.05.154

Li, B., Konecke, S., Harich, K., Wegiel, L., Taylor, L. S., & Edgar, K. J. (2013). Solid dispersion of quercetin in cellulose derivative matrices influences both solubility and stability. *Carbohydrate Polymers*, *92*(2), 2033–2040. https://doi.org/10.1016/j.carbpol.2012.11.073

Manca, M. L., Lai, F., Pireddu, R., Valenti, D., Schlich, M., Pini, E., Ailuno, G., Fadda, A. M., & Sinico, C. (2020). Impact of nanosizing on dermal delivery and antioxidant activity of quercetin nanocrystals. In *Journal of Drug Delivery Science and Technology* (Vol. 55). Editions de Sante. https://doi.org/10.1016/j.jddst.2019.101482

Marsac, P. J., Li, T., & Taylor, L. S. (2009). Estimation of drug-polymer miscibility and solubility in amorphous solid dispersions using experimentally determined interaction parameters. *Pharmaceutical Research*, *26*(1), 139–151. https://doi.org/10.1007/s11095-008-9721-1

Marsac, P. J., Shamblin, S. L., & Taylor, L. S. (2006). Theoretical and practical approaches for prediction of drug-polymer miscibility and solubility. *Pharmaceutical Research*, *23*(10), 2417–

2426. https://doi.org/10.1007/s11095-006-9063-9

S. Abbott, C. M. H. H. Y. (2010). *Hansen Solubility Parameters in Practice* (Hansen-Solubility.com, Ed.; 5th ed.).

Sakellariou, P., Rowe, R. C., & White, E. F. T. (1986). The solubility parameters of some cellulose derivatives and polyethylene glycols used in tablet film coating. *International Journal of Pharmaceutics*, *31*(1–2), 175–177. https://doi.org/10.1016/0378-5173(86)90229-2

Schachter, D. M., Xiong, J., & Tirol, G. C. (2004). Solid state NMR perspective of drug–polymer solid solutions: a model system based on poly(ethylene oxide). *International Journal of Pharmaceutics*, *281*(1–2), 89–101. https://doi.org/10.1016/j.ijpharm.2004.05.024

Shah, N., Sandhu, H., Choi, D. S., Chokshi, H., & Malick, A. W. (Eds.). (2014). *Amorphous Solid Dispersions*. Springer New York. https://doi.org/10.1007/978-1-4939-1598-9

Small, P. A. (1953). Some factors affecting the solubility of polymers. *Journal of Applied Chemistry*, *3*(2), 71–80. https://doi.org/10.1002/jctb.5010030205

Thakral, S., & Thakral, N. K. (2013). Prediction of drug-polymer miscibility through the use of solubility parameter based flory-huggins interaction parameter and the experimental validation: PEG as model polymer. *Journal of Pharmaceutical Sciences*, *102*(7), 2254–2263. https://doi.org/10.1002/jps.23583

Thijs, H. M. L., Becer, C. R., Guerrero-Sanchez, C., Fournier, D., Hoogenboom, R., & Schubert, U. S. (2007). Water uptake of hydrophilic polymers determined by a thermal gravimetric analyzer with a controlled humidity chamber. *Journal of Materials Chemistry*, *17*(46), 4864. https://doi.org/10.1039/b711990a

Ulrich, R. D. (1978). P. J. Flory. In *Macromolecular Science* (pp. 69–98). Springer US. https://doi.org/10.1007/978-1-4684-2853-7_5

Yang, S. L., Zhao, L. J., Chi, S. M., Du, J. J., Ruan, Q., Xiao, P. L., & Zhao, Y. (2019). Inclusion complexes of flavonoids with propylenediamine modified β-cyclodextrin:Preparation, characterization and antioxidant. *Journal of Molecular Structure*, *1183*, 118–125. https://doi.org/10.1016/J.MOLSTRUC.2019.01.046

Zou, Y., Qian, Y., Rong, X., Cao, K., McClements, D. J., & Hu, K. (2021). Encapsulation of quercetin in biopolymer-coated zein nanoparticles: Formation, stability, antioxidant capacity, and bioaccessibility. *Food Hydrocolloids*, *120*, 106980. https://doi.org/10.1016/J.FOODHYD.2021.106980

Table 1

Solvents classification used to solubilize quercetin and the determination of the Hansen solubility parameters (Fit= 1.000, Wrong In= 0, Wrong Out= 0). Solubility parameters were taken from the literature (Hansen, 2007) or predicted using the Yamamoto Molecular Break (Y-MB) group-contribution method (S. Abbott, 2010).

Solvent	δ_D	δ_P	δ_H	Score
Hexane	14.9	0	0	0
Pentane	14.5	0	0	0
Decane	15.7	0	0	0
Heptane	15.3	0	0	0
Ethyl Benzene	17.8	0.6	1.4	0
Toluene	18	1.4	2	0
Chloroform	17.8	3.1	5.7	0
1-Decanol	16	4.7	10.5	0
1-Octanol	16	5	11.2	0
2-Methyl-1-Butanol	16	5.1	14.3	0
Ethyl Acetate	15.8	5.3	7.2	0
2-Butanol	15.8	5.7	14.5	0
Aniline	20.1	5.8	11.2	0
3-Methyl Allyl Alcohol	16	6	15.5	1
1-Propanol	16	6.8	17.4	0
Ethanol	15.8	8.8	19.4	1
Formic Acid	14.6	10	14	1
Acetone	15.5	10.4	7	1
Allyl Alcohol	16.2	10.8	16.8	1
Ethylene Glycol	17	11	26	0
Methanol	14.7	12.3	22.3	0
Triethylene Glycol	16	12.5	18.6	1
Diethyl Sulfate	15.7	12.7	5.1	1
Dimethyl Formamide (DMF)	17.4	13.7	11.3	1
Formaldehyde	12.8	14.4	15.4	1
Ethanolamine	17	15.5	21	1
Nitroethane	16	15.5	4.5	1
Water	15.5	16	42.3	0
Dimethyl Sulfoxide (DMSO)	18.4	16.4	10.2	1
Propylene Carbonate	20	18	4.1	1
Nitromethane	15.8	18.8	6.1	1
Formamide	17.2	26.2	19	1

637 **Table 2**

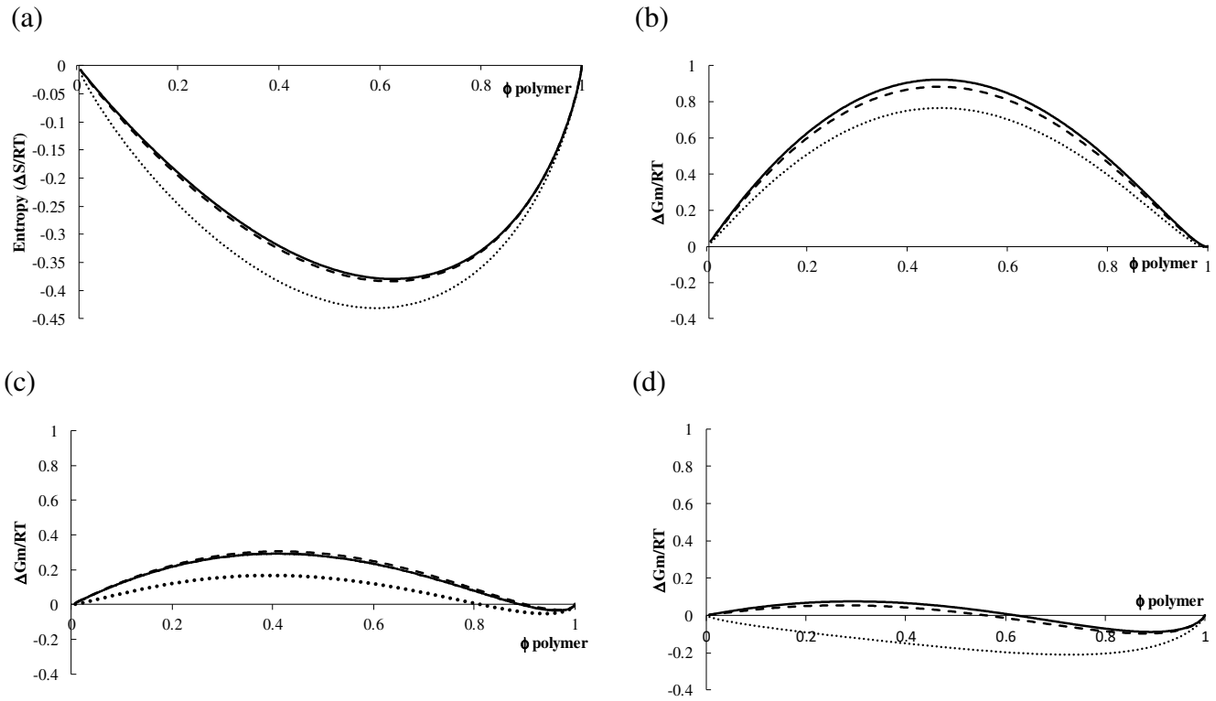
638 PEGs and quercetin properties used to calculate the free energy of mixing

Product	Group contribution methods						Density (kg/m^3)	V_m (cm^3/mol)
	Small		Hoy		Van Krevelen			
	δ ($MPa^{0.5}$)	χ	δ ($MPa^{0.5}$)	χ	δ ($MPa^{0.5}$)	χ		
PEG 1000	19.1	4.6	21.5	2.2	23.4	0.9	1222	869
PEG 4000	18.9	4.9	21.1	2.6	22.5	1.5	1224	3268
PEG 6000	18.8	5.0	21.2	2.5	22.4	1.6	1223	4906

639

640

641



642 **Fig. 1**

643 Entropy (a) and free energy of mixing Quercetin with PEG1000 (dotted lines), PEG4000 (long hashed
644 lines), and PEG6000 (solid lines), as a function of volume fraction polymer, using solubility parameters
645 from the method of Small (b), Hoy (C) and van Krevelen (d).

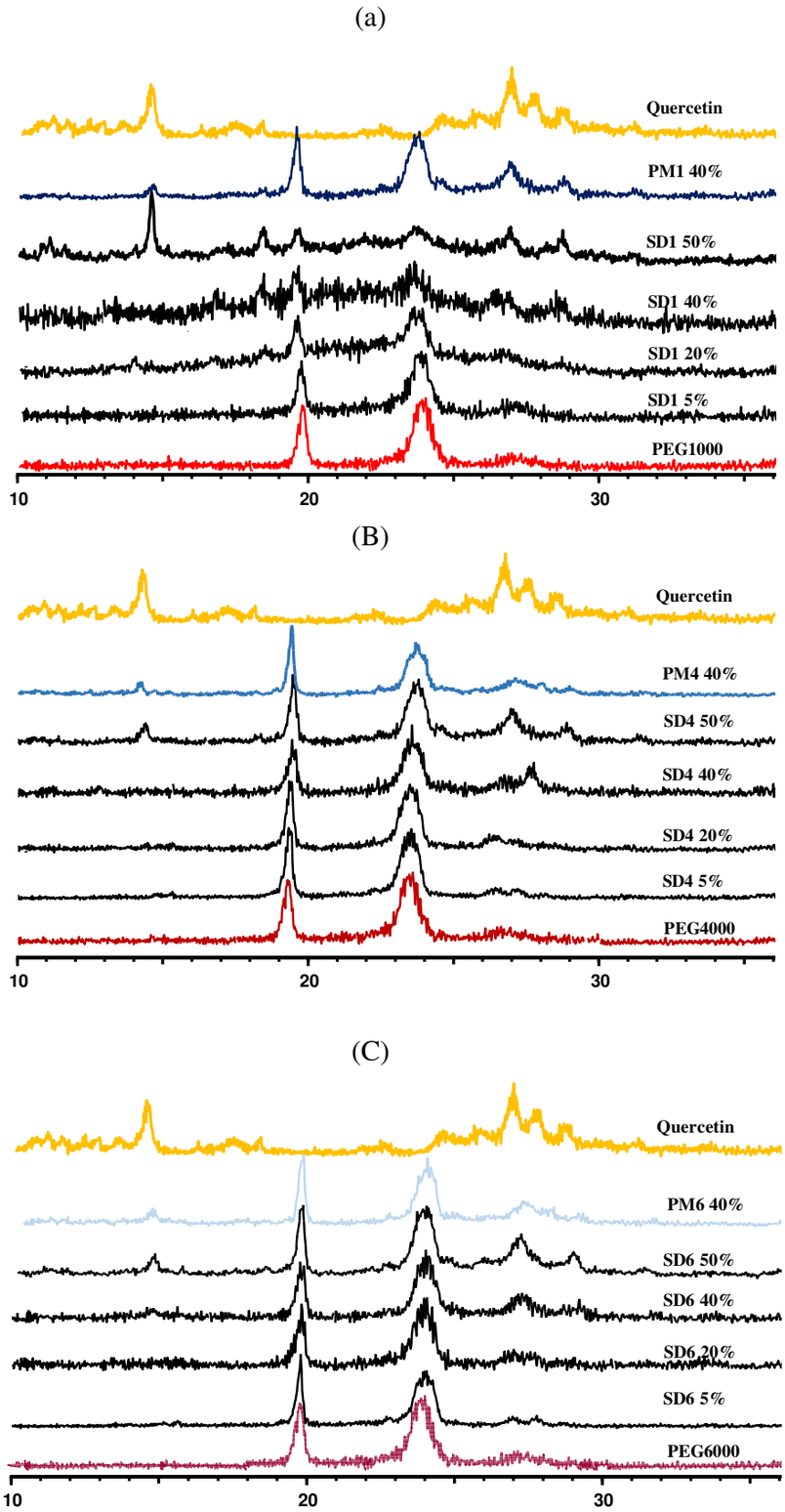
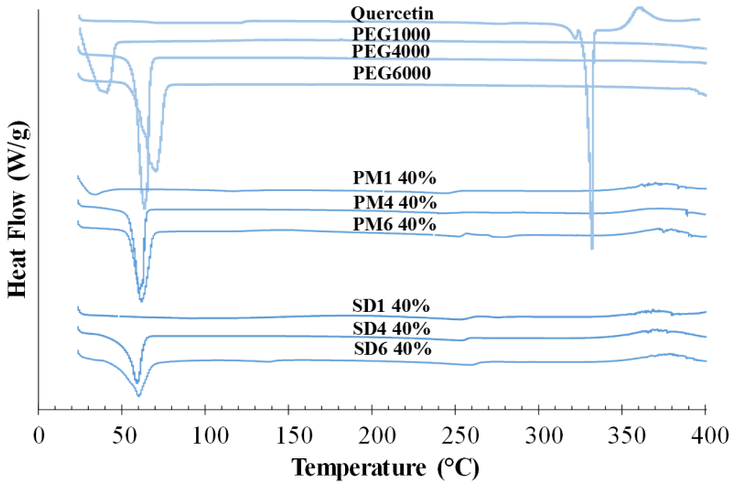


Fig. 2

X-ray diffraction analysis of quercetin, PEGs, Physical Mixtures (PM) and solid dispersions (SD) of varying composition; PM1, PM4 and PM6 correspond to quercetin physical mixtures with PEG1000, PEG4000 and PEG6000 respectively; SD1, SD4 and SD6 correspond to quercetin solid dispersions with PEG1000, PEG4000 and PEG6000 respectively



655

656 **Fig. 3**

657 DSC of quercetin, physical mixtures quercetin-PEG1000 (PM1), quercetin-PEG4000 (PM4) and
658 quercetin-PEG6000 (PM6); and solid dispersions quercetin-PEG1000 (SD1), quercetin-PEG4000 (SD4)
659 and quercetin-PEG6000 (SD6) at composition of 40%

660

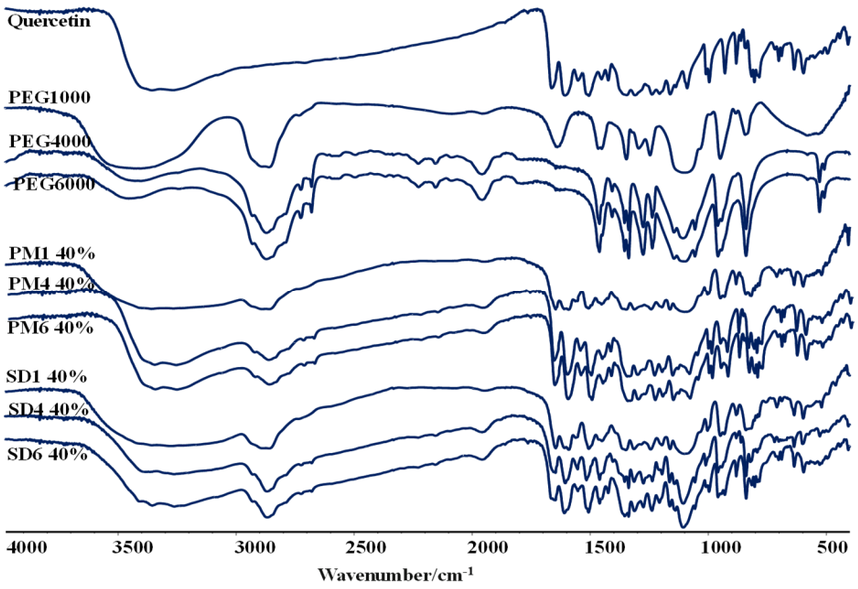


Fig. 4

FTIR spectra of quercetin, physical mixtures quercetin-PEG1000 (PM1), quercetin-PEG4000 (PM4) and quercetin-PEG6000 (PM6); and solid dispersions quercetin-PEG1000 (SD1), quercetin-PEG4000 (SD4) and quercetin-PEG6000 (SD6) at composition of 40%

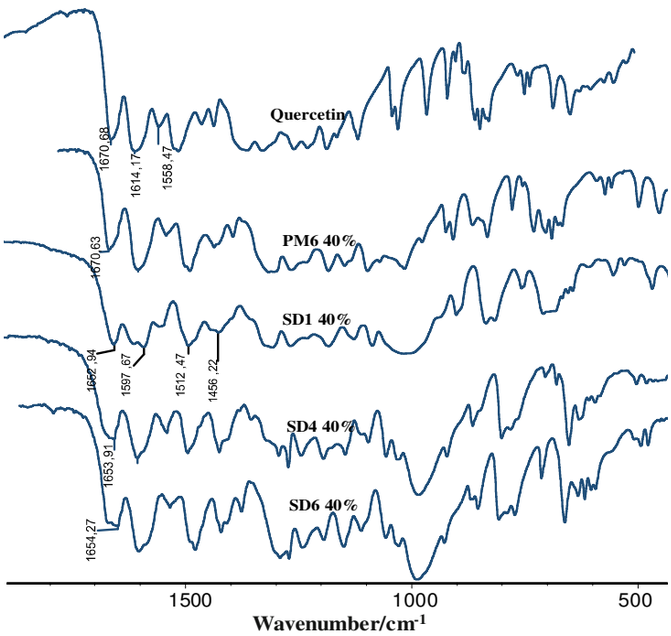
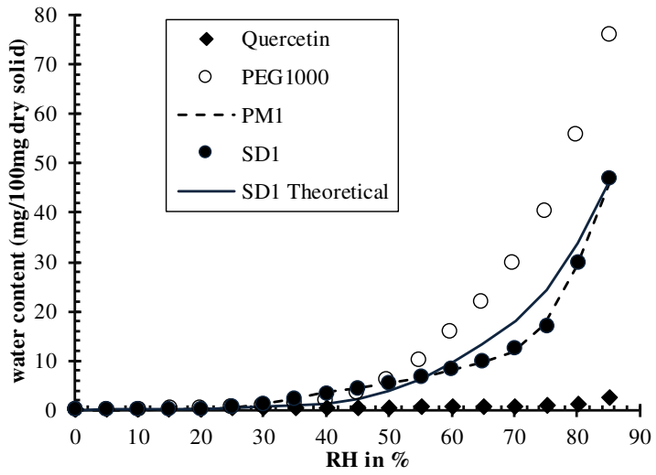


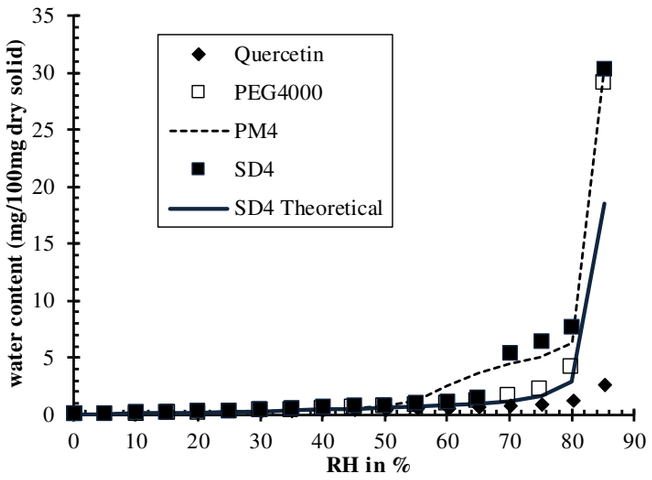
Fig. 5

FTIR spectra, for wavenumber between 450 and 1900 cm^{-1}, of quercetin, physical mixture quercetin-PEG6000 (PM6); and solid dispersions quercetin-PEG1000 (SD1), quercetin-PEG4000 (SD4) and quercetin-PEG6000 (SD6), at composition of 40%

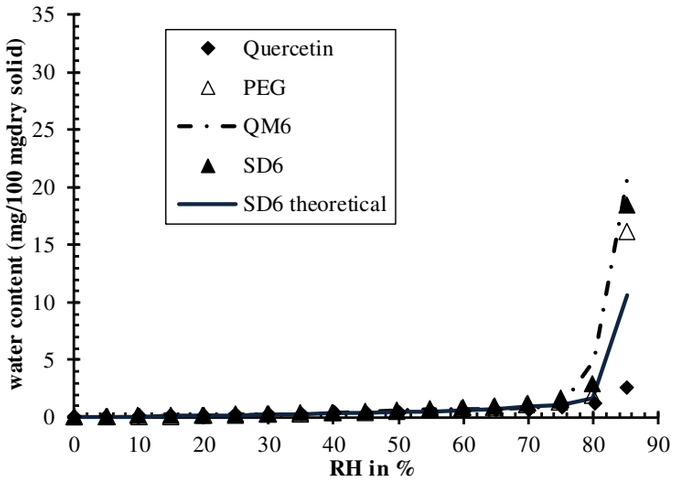
(a)



(b)

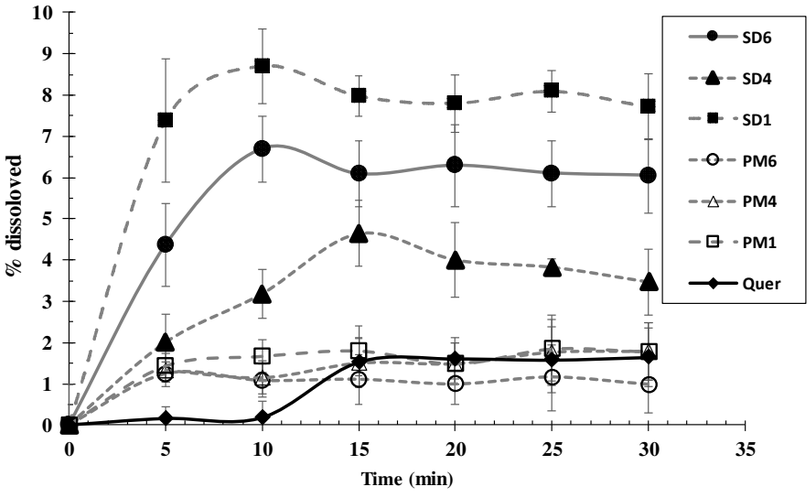


(c)



673 **Fig. 6**

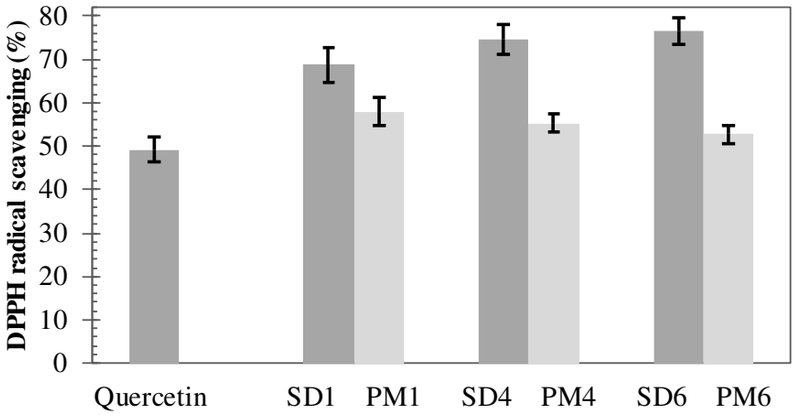
674 Vapor sorption isotherms at 25 °C of SDs (40%), quercetin and various MW PEGs measured by
675 automated gravimetric moisture analysis SPS. (a) quercetin-PEG1000 (SD1), (b) quercetin-PEG4000
676 (SD4), (c) quercetin-PEG6000 (SD6). Solid lines and dashed lines are isotherms of quercetin-PEG
677 theoretical and of physical mixtures PMs respectively.



678

679 **Fig. 7**

680 Dissolution profile of quercetin in physical mixture with PEG1000 (PM1), PEG4000 (PM4) and
681 PEG6000 (PM6); and in solid dispersion with PEG1000 (SD1), PEG4000 (SD4) and PEG6000 (SD6), at
682 composition of 40%.



683

684 **Fig. 8**

685 In vitro antioxidant activity of quercetin, physical mixtures of quercetine with PEG1000 (PM1),
686 PEG4000 (PM4) and PEG6000 (PM6) and solid dispersions of quercetine-PEG1000 (SD1), quercetin-
687 PEG4000 (SD4) and quercetine-PEG6000 (SD6), at 40 % (w/w).

688

689

690