Mechanochemical activation with cyclodextrins followed by compaction as an effective approach to improving dissolution of rutin

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Abstract

Rutin is one of the most important flavonoids with poor bioavailability. This work aimed at addressing the issue of poor biopharmaceutical performance of rutin by applying a combination of complexation with secondary processing into tablets. Mechanical activation was the most suitable method of rutin complex formation with (2-hydroxypropyl)-β-cyclodextrin (HP-β-CD), while the β-cyclodextrin (β-CD) complex successfully formed by kneading with an ethanol/water mixture. Complexation was confirmed by thermal analysis, powder X-ray diffraction and vibrational spectroscopy. Dynamic vapour sorption showed that stability of powders at high humidity conditions was satisfactory, however, the β-CD complex retained around 8% of moisture. The complexes were compacted with or without tricalcium phosphate (TRI-CAFOS) filler at a range of compression pressures (19-113 MPa). The best tabletability was determined for rutin/HP-β-CD, compressibility for the TRI-CAFOS blends with complexes and compactibility for the rutin/HP-β-CD+TRI-CAFOS mix. Dissolution studies showed quicker and more complete dissolution (pH 1.2) of rutin/HP-β-CD tablets, however the compacts comprising the filler were superior than pure complexes. The tablets manufactured in this study appear to be promising delivery systems of rutin and it is recommended to combine rutin/HP-β-CD with TRI-CAFOS and compact at 38-76 MPa.

Keywords

compaction; cyclodextrin; dissolution; mechanochemistry; rutin; solid state; tricalcium phosphate.

1. Introduction

Bioflavonoids are the most frequently characterised components of many plant species, their secondary metabolites, and belong to a class of polyphenol derivatives (Sri et al., 2007). This group of chemical compounds, flavonoids, have been widely investigated as they show favourable properties of therapeutic relevance, such as anti-inflammatory, anti-allergic, anti-viral, anticancer and antioxidant (Cook and Samman, 1996; Yang et al., 2008). Due to the antioxidant properties, bioflavonoids have been employed in clinics for the purpose of prevention of ailments such as circulatory diseases, neurodegenerative diseases, diabetes or osteoporosis (Ferrándiz and Alcaraz, 1991; Gullón et al., 2017). One of the most pharmaceutically important flavonoids is rutin (Fabjan et al., 2003). Chemically, it is a glycoside comprising a flavonoic aglycone residue, quercetin, and a disaccharide, rutinose, (Ganeshpurkar and Saluja, 2017). Studies have demonstrated that rutin possesses some biological activity and its current uses include prevention of the above-mentioned diseases and an improvement in genome stability (Sharma et al., 2013).

Despite the good therapeutic potential of flavonoids, they are poorly soluble in water. Their low aqueous solubility, limited membrane permeability and poor stability result in poor systemic bioavailability (Gullón et al., 2017; Thilakarathna and Vasantha Rupasinghe, 2013). Manipulation of intestinal absorption, such as a modification of the uptake site, and augmentation of metabolic stability have been reported as potentially beneficial approaches aimed at improving bioavailability of flavonoids (Nielsen et al., 2006; Shen et al., 2011; Walle, 2007). However, a simple but efficient method of improving absorption and bioavailability of rutin is to enhance its dissolution rate and to increase its solubility by inclusion complexation (Kwon et al., 2010).

Cyclodextrins (CDs) have been known in pharmaceutical technology as a very important class of pharmaceutical excipients utilised to improve solubility (and/or dissolution

rates) of molecules with inadequate aqueous solubility, both in solution by forming inclusion complexes and in the solid state as a hydrophilic matrix/carrier (Challa et al., 2005). Chemically, CDs are a class of cyclic oligosaccharides comprising α-D-glucopyranose moieties with six, seven, and eight glucopyranose residues forming α-, β- and γ-CD, respectively (Del Valle, 2004). This cyclic structure of CDs results in a unique structural feature, which is a hydrophilic external surface, encompassing primary and secondary hydroxyl rims, and a relatively lipophilic central cavity (Del Valle, 2004). The latter characteristic makes it relatively easy for a range of molecules (solid, liquid and gaseous) to be entrapped in the cavity, thus forming inclusion complexes (Brewster and Loftsson, 2007; Loftsson and Brewster, 2010). Also, to improve the aqueous solubility of natural CDs, chemical modification by substitution of hydroxyl group(s) can be employed (Loftsson et al., 2005).

Even though the chief pharmaceutical purpose of CDs is to improve solubility and/or dissolution rates of poorly soluble drugs, i.e. molecules of the classes II and IV of Biopharmaceutics Classification System (Salústio et al., 2011), this group of excipients also have found a role in tabletting. Several research groups have tested CDs as release-modifying additives, to augment physical and chemical stability of drug molecules, for taste-masking purposes, to reduce or eliminate adverse drug reactions and as single- or multi-functional tablet excipients acting as fillers, disintegrant, binders or showing a combination of these functions (Conceição et al., 2018a, 2018b; Pande and Shangraw, 1995).

Considering the formulation and technological complexity, one can distinguish three main tablet manufacturing processes: direct compression, dry granulation and wet granulation (Leane et al., 2015). The direct compaction process, due to its simplicity and economic advantage, has gained an obvious popularity in the pharmaceutical industry however, the selection of a suitable directly compressible base, compatible with the drug, is critical to the success of this approach. Such a base must not only have good dilution attributes, but also

excellent flowability, compressibility and compaction properties (Drašković et al., 2018). The first decision in an early stage development of a tablet formulation containing CDs is to establish if a simple physical mixture of CD and the drug is adequate in terms of tabletting or perhaps the formation of an inclusion complex is necessary. The former approach of a physical mixture may be satisfactory if the key role of the CD is to work as a tabletting aid, nonetheless, if the solubility of the drug needs to be improved, the tactic that should be implemented is to form an inclusion complex and also incorporate the CD as a tablet excipient, if further addition of this additive is required (Miller et al., 2007). One should be concerned that incorporating a large amount of CD into a solid formulation may result in the tablet being too bulky to be comfortably ingested by the patient (Szejtli, 1991).

Based on our previous work, this work continues investigations into rutin associations with β-cyclodextrin (β-CD), using a range of preparative methods, including mechanical activation and a different molar ratio as well as different analytical methods to those already explored (Paczkowska et al., 2015). The current experiments expand the earlier work by studying the influence of (2-hydroxypropyl)-β-cyclodextrin (HP-β-CD) on the pharmaceutical properties of rutin. The inclusion complexes of rutin with β-CD and HP-β-CD aim to improve both, the aqueous solubility of this compound and to increase the dissolution rate. Based on the studies of Shankarrao and co-workers with olanzapine (Shankarrao et al., 2010), HP-β-CD may act as a channel forming agent thus resulting in short disintegration time of the tablets, but it also could increase permeability of a drug. The work therefore aimed at preparing rutin tablets by using β-CD, HP-β-CD and tricalcium phosphate, a directly compressible excipient, by direct compression with subsequent detailed investigations into the optimum compression pressure, the mechanism of compaction and ultimately – dissolution studies. To the best of our knowledge, no studies have been reported on the tabletting process of rutin complexes with β-CD and HP-β-CD.

2. Materials and methods

2.1. Materials

Rutin trihydrate, β-cyclodextrin (β-CD) and (2-hydroxypropyl)-β-cyclodextrin (HP-β-CD), all with purity >98%, were obtained from Sigma-Aldrich (Poland). Tricalcium phosphate MV 5800 (TRI-CAFOS 500) was kindly donated by Chemische Fabrik Budenheim (Germany). Milli-Q deionised water was used in all experiments and ethanol was HPLC grade (Sigma-Aldrich, Ireland). Potassium bromide (KBr) infrared grade was purchased from Sigma-Aldrich (Ireland). All other chemicals and solvents were of analytical grade.

2.2. Preparation of systems

2.2.1. Preparation of inclusion complexes of rutin with cyclodextrins

Solid inclusion complexes of rutin with β-cyclodextrin (β-CD) or (2-hydroxypropyl)-β-cyclodextrin (HP-β-CD) were prepared by four different methods:

- Method 1 (kneading with an ethanol/water mixture) – Rutin and CD starting material powders in a molar ratio of 1:2 were added to an agate mortar and pestle. Ethanol–water (1:2 v/v) mixture was prepared and used as the wetting liquid. The wet mass was kneaded in an agate mortar and pestle for 30 min.
- Method 2 (kneading with ethanol) – The quantities of materials were as in Method 1, however pure ethanol was used as the wetting liquid and kneading was carried out for 60 min until ethanol had evaporated.
- Method 3 (mechanochemical activation) – The quantities of materials were as in Method 1, however no solvent was used. The materials were subjected to dry mechanochemical activation in an agate mortar and pestle for 30 min.

130 – Method 4 (solvent evaporation) – An aqueous solution of CD was added to an ethanolic
131 solution of rutin (the rutin/CD molar ratio was 1:2). The mixture was evaporated to
132 dryness by using a Rotavapor® R-300 (Buchi) at 45 °C.

133 2.2.2. Preparation of physical mixtures of CD complexes with TRI-CAFOS

134 Physical mixtures of rutin/β-CD and rutin/HP-β-CD complexes with TRI-CAFOS 500 were
135 prepared by combining the complex with the excipient in a 1:1 weight ratio. A quantity of 50 g
136 of each of the complex and TRI-CAFOS 500 were added to a glass jar and thoroughly blended
137 for 10 minutes.

138 **2.3. Characterisation of solid samples**

139 2.3.1. Powder X-Ray Diffraction (PXRD)

140 PXRD was conducted by front-loading powder samples into the cavity of a zero-background
141 silicon sample holder and lightly compressing the samples to ensure they were levelled. All
142 analyses were done using a Cu Kα radiation in a desktop X-ray diffractometer, Rigaku Miniflex
143 II (Japan) at room temperature. The 2theta range applied was 5–40° with a step width of 0.05°
144 2theta and signal collection time of 1 s per step (McDonagh and Tajber, 2020).

145 2.3.2. Differential Scanning Calorimetry (DSC)

146 DSC analysis was performed using a DSC 821e Differential Scanning Calorimeter (Mettler
147 Toledo, Switzerland) equipped with an intracooler system. The powder samples were weighed
148 accurately and encapsulated in pinhole aluminium crucibles. A heating rate of 10 ^{o}C min^{-1} from
149 25 to 200 ^{o}C was applied with a nitrogen purge gas flowing at a rate of 10 mL min^{-1}.

150 2.3.3. Fourier Transform Infra-Red (FTIR) spectroscopy

151 FTIR spectra of the solid samples were produced with a Spectrum One FT-IR Spectrometer
152 (Perkin Elmer, USA) using spectroscopy grade potassium bromide mixed with the samples at

153 a ratio of 1:100 (w/w). The spectra were taken in the transmission mode between 500 and 4000
154 cm^{-1} using the KBr/sample compacts compressed at 8 bar pressure for 1 minute in a hydraulic
155 press. The spectra were background corrected and intensity of the signal normalised.

156 2.3.4. Dynamic Vapour Sorption (DVS)

157 DVS analysis was carried out with a Dynamic Vapour Sorption (DVS) Advantage-1 automated
158 gravimetric vapour sorption analyser (Surface Measurement Systems Ltd., London, UK) at 25.0
159 ± 0.1 ^{o}C (McDonagh and Tajber, 2020). The samples were first equilibrated at 0% RH achieving
160 a stable weight, so that the mass change (dm) over time (dt) was below 0.002 mg min^{-1} and
161 maintained for at least 10 min, and then the equilibrated reference mass was noted. The samples
162 were exposed to variable humidity conditions using the following sorption-desorption profile:
163 0%–90%-0% RH in 10% RH steps. The cycle was then repeated to examine if further changes
164 in the samples occurred. At each RH% stage the sample mass was equilibrated (as above, dm/dt
165 ≤ 0.002 mg min^{-1} for a minimum of 10 min) before changing the humidity. The amount of water
166 sorbed was stated as a percentage of the reference, dry mass of the sample.

167 2.3.5. Laser diffraction particle size analysis

168 A laser diffraction particle sizer Mastersizer 3000 (Malvern Panalytical, UK) with a dry powder
169 accessory was used to measure the particle size and particle size distributions of powders
170 (McDonagh and Tajber, 2020). The results are presented as d(0.1), d(0.5) and d(0.9), defined
171 as follows: d(0.1) [μm] – indicated that 10% of the particle distribution (by volume) is below
172 this value, d(0.5) [μm] – is the median of particle distribution and d(0.9) [μm] – which
173 represents the size where 90% of the particle distribution is below the value. Sauter Mean
174 diameter (SMD), D[3,2], representing an average of particle size and D[4,3], the volume mean
175 diameter, were also estimated.

176 2.3.6. High-performance liquid chromatography (HPLC) method

177 The rutin concentrations in the samples collected from dissolution studies were determined by
178 using the HPLC Diode Array Detection method described previously with modifications
179 (Paczkowska et al., 2017). The separation of rutin in the presence of its impurities, isoquercetin
180 and quercetin, was carried out using a liquid chromatography system (Dionex Thermoline
181 Fisher Scientific) equipped with Chromeleon software version 7.0. Analyses, at 25 ^{o}C, were
182 performed using a Kinetex-C18 column (100 mm length × 2.1 mm with and 5.0 μm particle
183 size). The detection of rutin was performed with a diode array detector at a maximum (λ_{max})
184 wavelength of 353 nm. A mixture of acetonitrile and 0.1% formic acid (20:80 v/v) was used as
185 the mobile phase applying a flow rate of 1 mL min^{-1}.

186 2.3.7. Dissolution studies

187 Dissolution studies were performed using an Agilent 708-DS dissolution apparatus (USA)
188 configured as a type 2 (paddle) dissolution apparatus at 37 ± 0.5 ^{o}C and using a paddle stirring
189 speed of 50 rpm. Gelatin capsules were loaded with either rutin, physical mixtures or rutin/CD
190 inclusion complexes and carefully positioned in a sinker to maintain the capsule at the bottom
191 of the dissolution vessel. Dissolution studies of the capsules were carried out in 900 mL of
192 simulated gastric fluids (pH 1.2). The liquid samples were collected at predetermined time
193 points and replaced with an equal volume of medium equilibrated at 37 ± 0.5 ^{o}C. The collected
194 samples were filtered through 0.45 μm nylon membrane filters and the concentrations of rutin
195 in the filtered solutions were measured by the HPLC method described above.

196 Release profiles were compared using the model proposed by Moore and Flanner, which
197 proposed to use two factor values, f_1 and f_2 (Moore and Flanner, 1996). The difference factor
198 (f_1) determines the error (percent) between two dissolution curves over all time points. The f_2
199 value results from a logarithmic transformation of the sum-squared error of differences between

the test *T*j and reference *R*j system over all time points according to the equations below (Moore and Flanner, 1996):

$$f_1 = \frac{\sum_{j=1}^{n}|R_j - T_j|}{\sum_{j=1}^{n} R_j} \times 100$$

$$f_2 = 50 \times \log\left(\left(1 + (\tfrac{1}{n})\sum_{j=1}^{n}|R_j - T_j|^2\right)^{-\frac{1}{2}} \times 100\right)$$

where *n* is the sampling number, *R*j and *T*j are the percent dissolved from the reference (rutin) and test products at each time point *j*. Dissolution profiles are regarded as similar when the f_1 value is close to 0 and f_2 is close to 100. According to the FDA guidelines, two dissolution profiles are comparable if the f_2 value is between 50 and 100.

2.3.8. *In vitro* permeability studies

Permeability of rutin, rutin/CD systems as well as their mixtures with TRI-CAFOS 500, was investigated through the artificial biological membrane using a PAMPA model simulating the gastrointestinal walls (PAMPA GIT). The system consisted of a 96-well microfilter plate and a 96-well filter plate and was divided into two chambers: a donor at the bottom and an acceptor at the top, separated by a 120-μm-thick microfilter disc coated with a 20% (w/v) dodecane solution of a lecithin mixture (Pion, Inc.). The donor solution was adjusted to pH 2.0. The rutin/CD inclusion systems as well as their mixtures with TRI-CAFOS 500 were dissolved in DMSO (rutin concentration: 5 mg/mL) and 10 μL of this stock solution was transferred to the donor solution. The plates were put together and incubated at 37 °C for 3 hours in a humidity-saturated atmosphere. Rutin concentrations were determined by UV spectrophotometry (λ_{max}=353 nm). The apparent permeability coefficients (P_{app}) were calculated from the following equation:

$$P_{app} = \frac{-\ln\left(1 - \frac{C_A}{C_{equilibrium}}\right)}{S \times \left(\frac{1}{V_D} + \frac{1}{V_A}\right) \times t}$$

where V_D – donor volume, V_A – acceptor volume, $C_{equilibrium}$ – equilibrium concentration $C_{equilibrium} = \frac{C_D \times V_D + C_A \times V_A}{V_D + V_A}$, C_D – donor concentration, C_A – acceptor concentration, S – membrane area, t – incubation time (in seconds). ANOVA was used to compare the results.

2.4. Tabletting studies

2.4.1 Tabletting process

A Natoli NP-RD10 (USA) laboratory scale single punch tablet press equipped with an Enerpac (USA) P-392 manual pump and a RC-104 hydraulic cylinder was used to compress 13 mm in diameter, flat-faced tablets (McComiskey et al., 2019). Compaction pressures between 19 to 113 MPa were employed to fully characterise the compaction properties of the powders studied. The selected pressure was maintained for 60 s. Composition of the tablets is presented in Table 1.

Table 1. Composition of the tablet formulations (in mg).

	Rutin/β-CD	Rutin/HP-β-CD	Rutin/β-CD – TRI-CAFOS 500	Rutin/HP-β-CD – TRI-CAFOS 500
	Content (mg) of compounds in one tablet			
Rutin	50.00	50.00	25.00	25.00
B-CD	186.06	-	93.03	-
HP-β-CD	-	239.34	-	119.67
TRI-CAFOS 500	-	-	118.03	144.67
Total	236.06	289.34	236.06	289.34

2.4.2. Tablet characterisation

2.4.2.1. Tensile strength, solid fraction and porosity of tablets

Tensile strength (σ) values were determined from the breaking force (F) values [N], where d is the diameter of the tablet [mm] and h is the thickness of the tablets [mm]:

$$\sigma = \frac{2F}{\pi d h}$$

Solid fractions (SF) were calculated from tablet weights (W_t, mg), volume of tablets (v, cm^3) and true density of the powder (ρ_{true}, g/cm^3) using the equation below:

$$SF = \frac{W_t}{\rho_{true} v}$$

The tablet porosity (ε) was assessed using SF values using the following equation:

$$\varepsilon = 1 - SF$$

2.4.2.2. Weight, thickness and diameter of tablets

Uniformity of weight was measured based on the method described in Ph. Eur. (Council of Europe, 2017a). A quantity of 20 tablets was randomly selected and thickness and diameter measured using a manual Vernier calliper. Mean values and standard deviations (SD) were calculated.

2.4.2.3. Hardness of tablets

Hardness of tablets was determined with a manual tablet hardness tester (Electrolab, India) and presented as a mean value with SD.

2.4.3. Disintegration of tablets

Disintegration tests were performed according to Ph. Eur. (Council of Europe, 2017b). Tablets were placed in a standard disintegration apparatus (Erweka ZT 44, Germany) using 900 mL of deionised water at 37±0.5 ^{o}C as a disintegrating liquid. The basket rack assembly was allowed to rise and lower at a constant frequency (30 rpm) until the tablets were completely disintegrated and passed through the mesh.

2.4.4. Dissolution of tablets

The study was carried out according to the method described in Section 2.3.7.

2.4.5. Scanning electron microscopy (SEM)

SE micrographs were generated from gold/palladium sputter coated tablets and using a Zeiss Ultra Scanning electron microscope (Germany). A 6 kV accelerating voltage was applied (McComiskey et al., 2019).

3. Results and discussion

3.1. Production and solid-state characterisation of the rutin samples

It has been known that rutin can form complexes with CDs in a liquid phase. The phase solubility studies of rutin complexation with β-CD and HP-β-CD showed that aqueous solubility of this active increased linearly with an increase in CD concertation, forming an A_L type of phase diagrams. The stability constants determined experimentally were 260 M^{-1} and 341 M^{-1} for rutin/β-CD and rutin/HP-β-CD, respectively (Sri et al., 2007). Paczkowska and co-workers performed detailed molecular modelling of the rutin/β-CD 1:1 molar complex and also found that this complex was more potent against *Pseudomonas* (Paczkowska et al., 2015). Limited information, however, can be found about the solid complexes of rutin with CDs.

Solid inclusion complexes of rutin with β-CD and HP-β-CD were prepared by four different methods, including formation in the semisolid state (Methods 1 and 2), formation in the solid state (Method 3) and formation in solution (Method 4) (Jug and Mura, 2018). All samples were initially analysed by PXRD, DSC and FTIR and, based on the results showing the greatest interactions between the components (by DSC and FTIR) as well as the largest degree of solid-state changes (by PXRD), the most appropriate method of formation was chosen. For rutin/β-CD it was Method 1 (kneading with an ethanol/water mixture), while for rutin/HP-β-CD it was Method 3 (mechanochemical activation). Therefore, the rutin complexes with CD for further studies were only prepared by these two techniques. Interestingly, Loftsson and co-workers have stated that methods in the semisolid state used to manufacture CD inclusion complexes resulted only in partial complexation (Loftsson et al., 2016). On the other

hand, mechanochemical activation (co-grinding) was the best method for transforming rutin and HP-β-CD into a complex, which is preferred as no solvents are used and overall this approach is more economical and environmentally friendly (Jug and Mura, 2018).

3.1.1. Powder X-Ray Diffraction (PXRD)

The solid systems were evaluated using PXRD. It was revealed that rutin was crystalline in nature and that the starting material powder was the trihydrate form, as evidenced by the position of diffraction peaks at 5.3°, 7.3°, 14.6°, 15.0°, 16.9°, 22.2°, 26.4° and 26.9° 2θ (Figure 1) (Horosanskaia et al., 2017). The β-CD starting material powder was also crystalline and in its hydrated form (Braga et al., 2003), in contrast to HP-β-CD, which was X-ray amorphous (Figure 1). The complexes showed the rutin diffraction peaks, however they were of low intensity (Figure 1), suggesting introduction of disorder into the samples.

For the inclusion complex to form, the rutin crystal must first disintegrate releasing water molecules. As HP-β-CD is already in a higher energy, amorphous state, the simple mechanochemical activation process of co-grinding was sufficient to break up the crystal lattice of rutin, causing nearly complete amorphisation and complex formation. This is consistent with the mechanism of inclusion complex formation in the solid state firstly requiring a particle size reduction and the crystal lattice defects development followed by the complex formation at the surface of reactants (Jug and Mura, 2018). However, the crystalline, non-stoichiometric hydrate form of β-CD required an addition of ethanol, which may have been required to compete with water to cause disruption of the crystal lattice. It has been reported that in the presence of ethanol the trihydrate form of rutin is metastable (Jug and Mura, 2018) and that crystallisation of rutin from a water/methanol mixture resulted in the pentamethanolate form (Jin et al., 1990). Overall, PXRD showed an evidence of the complex formation between CDs and rutin.

3.1.2. Differential Scanning Calorimetry (DSC)

To further verify that rutin was successfully complexed in CDs, thermal analysis was conducted. The DSC thermograms of the samples are shown in Figure 2. Since the starting material rutin was identified by PXRD as rutin trihydrate, the first broad endotherm with an onset at 105.2 °C and a peak maximum at 136.0 °C was ascribed to dehydration of crystalline water (Horosanskaia et al., 2017; Mauludin et al., 2009). The studies of Horosanskaia and co-workers on the solid state transitions of rutin by thermogravimetry-DSC and temperature controlled PXRD (TC-PXRD) showed that rutin dehydrates in two stages, with the first weight loss of around 3.3% occurring up to 115 °C and then the second stage of dehydrating, associated with a weight loss of 4.6%, ending at around 180 °C (Horosanskaia et al., 2017). TC-PXRD presented that the sample still showed low intensity Bragg peaks at 150 °C, but it was nearly completely disordered at 180 °C, except for a low intensity, broad peak at around 7° 2θ (Horosanskaia et al., 2017). Therefore, it is unclear if the endothermic peak with an onset at 178.5 °C and a peak maximum at 190.0 °C is melting of the remaining rutin trihydrate (or possibly rutin dehydrate) still remaining after the main dehydration events, as no other Bragg peaks than those of rutin trihydrate were present in diffractograms shown by Horosanskaia and co-workers, or it is of a transition to a plastic form (Da Costa et al., 2002; O'Neil, 2013).

The DSC traces of β-CD and HP-β-CD showed a broad endothermal event with a peak maximum at 89.7 °C and 63.6 °C, respectively, assigned to dehydration (Figure 2). The rutin dehydration peak (maximum of transition) in the complexes shifted to 121.6 °C and 110.5 °C in the samples with β-CD and HP-β-CD, respectively, showing destabilisation of the hydrate, consistent with the mechanism of the complex formation. These observations imply the rutin inclusion into the β-CD and HP-β-CD cavity.

3.1.3. FTIR spectroscopy

FTIR spectra of the samples are displayed in Figure 3. The most pronounced differences in positions, intensity and width of the bands was visible in the fingerprint region 1200-500

335 cm^{-1} recorded for rutin/β-CD and rutin/HP-β-CD complexes in comparison to the spectrum of
336 pure rutin. Small shifts to lower wavenumbers were observed for the band assigned to as the
337 aromatic carbonyl of the ketone group, originally located at 1656 cm^{-1} for pure rutin (Sri et al.,
338 2007). Also, peaks of the aromatic C=C stretching at 1600, 1574 and 1556 cm^{-1} were seen to
339 be red or blue shifting by 1-3 cm^{-1}. FTIR confirmed that the dihydroxyphenyl ring in the rutin
340 molecule is the most involved in the interaction with CDs. Further details of changes to the
341 FTIR spectra can be found in the previous work (Paczkowska et al., 2015).

342 3.1.4. Dynamic Vapour Sorption (DVS)

343 Interactions of water with complexes are also usually studied as moisture is a major
344 factor that can cause a phase separation of components. In this work, DVS was employed to
345 study the effect of moisture on the solid-state stability of the rutin/CD systems. The
346 experimentally measured sorption-desorption kinetic profiles of the samples are shown in
347 Figure 4. The rutin/β-CD and rutin/HP-β-CD systems were seen to sorb around 11% and 12%
348 moisture, respectively, at high %RH, however only the HP-β-CD complex was able to
349 dehydrate to below 1% moisture on desorption, while the β-CD sample retained over 8% of
350 moisture. A similar behaviour of both systems was recorded for the second sorption and
351 desorption cycle as presented in Figure 4. The samples post DVS analysis were examined by
352 PXRD, DSC and FTIR and no significant changes to the properties of the systems were noted,
353 therefore the complexes, despite being able to sorb a considerable quantity of water, retained
354 their solid state identity.

355 3.1.5 Solid state characterisation of complexes with TRI-CAFOS 500

356 Physical mixtures of the complexes with a tabletting excipient, TRI-CAFOS 500, were
357 made keeping in mind the secondary processing of powders utilised in the next step. TRI-
358 CAFOS 500 is tribasic calcium phosphate intended for direct compression processes. In acidic

conditions of the stomach TRI-CAFOS 500 not only disintegrates, but also dissolves completely releasing the active compound (Patel et al., 1987). As shown in Figures 1-3, the directly compressible base did not show any indication of interactions with the complexes as studied by DSC, PXRD and IR, therefore, coupled with the ability to dissolve in stomach conditions, it was selected as an appropriate excipient for further processing of the complexes by tabletting.

3.1.6. Particle size analysis

Particle size analysis of the systems revealed that the rutin starting material powder had a monomodal particle size distribution (Figure 5) and a median particle size of 9 μm (Table 2). The complexes had similar particle size distributions as their parent CDs, with rutin/β-CD showing a monomodal distribution with a tail of smaller particles with sizes below 10 μm and rutin/HP-β-CD displaying a nearly identical distribution of particle sizes as that of pure HP-β-CD. The latter inclusion system had a lower median particle size, 12 μm, while the one based on β-CD had a median particle size of 42 μm. TRI-CAFOS 500 had the median particle size and size distribution similar to β-CD, showing that no particle separation should occur during mixing of this tabletting excipient with the CD complexes. The physical mixtures should display the size distributions comprising distributions of the starting material powders. The differences in the particle sizes may impact on how the powders undergo a tabletting process as well as may influence the dissolution of rutin from the system.

Table 2. Particle size parameters: d(0.1), d(0.5), d(0.5), Sauter Mean Diameter D[3,2] and De Brouckere Mean Diameter D[4,3] of the powders.

	Rutin	β-CD	Rutin/β-CD	HP-β-CD	Rutin/HP-β-CD	TRI-CAFOS 500
d(0.1) (μm)	1.8	10.1	5.31	3.8	3.4	5.4
d(0.5) (μm)	9.1	54.1	42.1	12.3	12.3	61.1
d(0.9) (μm)	37.4	140.0	143.0	38.4	38.9	167.0
D[3,2] (μm)	3.8	20.3	11.3	7.8	6.9	12.0
D[4,3] (μm)	16.4	65.9	60.1	17.5	29.1	74.5

3.1.7. Dissolution studies of powders

Dissolution studies of the uncompressed powders were first performed to compare the changes in dissolution rates of rutin. Figure 6 shows that only around 30% of rutin dissolved from the rutin powder "as supplied" with the rutin/HP-β-CD being superior to the β-CD based sample. A complete dissolution of rutin occurred from the HP-β-CD complex, which was not affected by the presence of TRI-CAFOS 500. On the other hand, solubilisation of rutin from the β-CD complex was incomplete after 90 minutes of the studies, however the addition of TRI-CAFOS 500 improved the dissolution of rutin with over 80% of the active solubilised at 90 minutes of the studies. The calculated f_1 and f_2 values confirmed that the dissolution profiles of the rutin systems are different from pure rutin in the acceptor medium at pH 1.2, except for rutin/HP-β-CD and the mixture of rutin/HP-β-CD and TRI-CAFOS 500 which were similar (in bold in Table 3).

Table 3. f_1 and f_2 values calculated for powder dissolution profiles of rutin from the CD complexes and mixtures of CD complexes with TRI-CAFOS 500 (values in bold font indicate profiles which are similar)

	Rutin	Rutin/β-CD	Rutin/HP-β-CD	Rutin/β-CD + TRI-CAFOS 500	Rutin/HP-β-CD + TRI-CAFOS 500
Rutin		f_1=75.45 f_2=19.10	f_1=84.08 f_2=0.25	f_1=72.92 f_2=7.64	f_1=95.52 f_2=1.57
Rutin/βCD	f_1=75.45 f_2=19.10		f_1=72.86 f_2=11.21	f_1=34.03 f_2=26.98	f_1=76.44 f_2=9.02
Rutin/HPβCD	f_1=84.08 f_2=0.25	f_1=72.86 f_2=11.21		f_1=23.99 f_2=25.55	**f_1=4.06** **f_2=59.63**
Rutin/βCD TRI-CAFOS 500	f_1=72.92 f_2=7.64	f_1=34.03 f_2=26.98	f_1=23.99 f_2=25.55		f_1=32.65 f_2=21.49
Rutin-HPβCD TRI-CAFOS 500	f_1=95.52 f_2=1.57	f_1=76.44 f_2=9.02	**f_1=4.06** **f_2=59.63**	f_1=32.65 f_2=21.49	

3.1.8 *In vitro* permeability

Using the PAMPA GIT model, it was possible to study the *in vitro* permeability of rutin through a membrane simulating gastrointestinal walls by passive diffusion and to calculate the apparent permeability values P_{app} for rutin, rutin/CD inclusion systems as well as their mixtures with TRI-CAFOS 500 (Table 4). Introduction of rutin into the β-CD or HP-β-CD cavity resulted

in an increased solubility in the donor liquid. Thus, the amount of rutin which penetrated through the test membrane was almost a 2-fold higher for the rutin/CD inclusion complexes in comparison to the pure compound (Table 4). Permeability coefficients did not decrease in the presence of TRI-CAFOS 500. The P_{app} values of rutin in all samples were higher than $1x10^{-6}$ cm/s, which indicates high permeability properties considering the model used (Yee, 1997), in line with our previous research (Paczkowska et al., 2015). However, it needs to be kept in mind that PAMPA is based on the assessment of kinetics of the passive diffusion process only, while the Caco-2 cell lines model includes the addition of active transport (Zhang et al., 2013).

Table 4. The apparent permeability coefficients (P_{app}) of rutin, rutin/CD inclusion complexes and mixtures with TRI-CAFOS 500

	P_{app} x 10^{-6} [cm/s] ± SD
Rutin	9.85 ± 0.60
Rutin/β-CD	24.58 ± 1.94
Rutin/HP-β-CD	25.10 ± 1.44
Rutin/β-CD – TRI-CAFOS 500	29.73 ± 3.08
Rutin/HP-β-CD – TRI-CAFOS 500	31.47 ± 1.58

3.2. Tabletting studies

3.2.1. Physicochemical properties of tablets

Tablet tensile strength, solid fraction and porosity at a range of compression pressure are the most important parameters describing the material's compaction properties. Tabletability can be described as the capacity of a powder to be converted into a tablet of particular strength under the effect of compaction pressure (Sun, 2016). It evaluates the effect of increasing the compression force on the tablet tensile strength and this effect is shown as a plot of compression pressure (MPa) versus the tensile strength (MPa). Tablet compaction increases tablet strength (tensile strength) through a range of mechanisms including particle fracture and packing rearrangement (Hiestand, 1997). The tablets with the highest tensile strength were made of rutin/HP-β-CD, however at higher compression pressure the powder overcompacted as seen by a decrease in the tensile strength of the tablets (Figure 7a). Generally,

tablets containing TRI-CAFOS had lower tablet strength than the tablets made of complexes compacted on their own. The tabletability of the samples decreased in the following order: rutin/HP-β-CD > rutin/HP-β-CD+TRI-CAFOS 500 > rutin/β-CD > rutin/β-CD+TRI-CAFOS 500. Rutin/HP-β-CD yielded the best results, demonstrating the ability to produce the hardest tablets at low compaction pressures.

Compressibility of a powder is defined as the powder's capacity, while contained in a rigid space, to reduce in volume when subjected to a load (Sun, 2016). This parameter is represented by a compressibility profile, plotting compaction pressure (MPa) versus porosity. There was no large difference in the compressibility profiles between rutin/β-CD and rutin/HP-β-CD and the tablets generally had low porosity (Figure 7b). The general trend of the compressibility profile is that as the pressure load applied to the powder samples decrease the porosity level or increase the solid fraction value. Porosity and solid fraction represent the structure of the compacted particles. The TRI-CAFOS 500-based tablets had better compressibility as relatively high porosity was retained at higher compression pressure values.

Compactibility of a powder is defined as the capacity of a powder to form a coherent tablet during densification (Sun and Grant, 2001). Compactibility is considered to be the most valuable parameter of tablets as it reflects tensile strength and solid fraction, the most notable effects of applied pressure. The aim of measuring compactibility is to determine if the tablets being produced have suitable tensile strengths and solid fraction values upon the application of pressure. Bot parameters may influence the dissolution properties of a tablet. The compactibility profiles for all samples showed that tablet tensile strength decreases as porosity increases. From the graph it is clear that weaker tablets have higher porosity. This is due to the higher percentage of pores in the tablet resulting in weak interparticulate bonding and therefore a lower force is needed to break the tablet. The order of decreasing compactibility appears to be as follows:

448 rutin/HP-β-CD+TRI-CAFOS 500 > rutin/HP-β-CD > rutin/β-CD+TRI-CAFOS 500 > rutin/β-
449 CD.

450 Based on the above parameters, the best tablet properties were obtained for rutin/HP-β-
451 CD with TRI-CAFOS 500 as thus powder demonstrated good tabletability (produced strong
452 tablets at low compaction pressures), good compressibility and the best compactibility
453 (produced the strongest tablet with the highest solid fraction) compared to the other systems.
454 PXRD analysis of the tablets after compaction confirmed that the range of compression forces
455 applied did no cause changes to the solid state properties of the complexes.

456 3.2.2. Scanning electron microscopy (SEM) of tablets

457 Cross-sections of rutin tablets compressed at different compression pressure were
458 analysed by SEM to gain a better insight into the mechanism of compression of the powders.
459 At the lowest compression pressure (19 MPa) the tablets appeared to retain some features of
460 the uncompressed powders, especially apparent for rutin/HP-β-CD. At the highest compression
461 pressure (especially 113 MPa), the particles were strongly deformed and appeared to be fused
462 together, a likely indication of overcompression. SE micrographs confirmed a sponge-like
463 appearance of TRI-CAFOS 500 particles and the plastic deformation mechanism of
464 compression (Patel et al., 1987) in contrast to the CD complexes that compacted by particle
465 fracture and/or fusion.

466 3.2.3. Tablet disintegration

467 All tablets, regardless of the compression pressure used, disintegrated within 15 minutes
468 (900 seconds) and met the pharmacopoeial requirement for uncoated tablets (Figure 9) (Council
469 of Europe, 2017b). The disintegration time of the different formulations did not correlate with
470 tablet porosity, but it appeared to be related to the tensile strength of tablets and for some
471 systems it was dependent on the compression pressure. As the rutin/HP-β-CD tablets had the

highest tensile strength (Figure 7a), they also had the longest disintegration times. Tablets made of mixtures of rutin complexes with TRI-CAFOS 500 were the softest, but also had the greatest porosities, and their dissolution times were the shortest.

3.3.3. Tablet dissolution

The dissolution rate of rutin was markedly improved by the presence of β-CD in the formulations (Figure 10a). The amount of solubilised rutin able to dissolve from the rutin/β-CD tablets was a two-fold greater than that dissolved from the tablets made of pure rutin. The dissolution profiles of the rutin/β-CD tablets were similar, as indicated by the f_1 and f_2 values, and independent on the compression pressure used (Table 5a). A complete solubilisation of rutin was achieved from rutin/HP-β-CD powder (Figure 10b) with the tablets compressed at 19 and 38 MPs releasing a comparable amount of rutin, around 60% of the loaded dose at 90 minutes of the studies. Therefore, the CD complexes improved dissolution properties of rutin, however the impact of CD is similar. Table 5b summarises the similarity and differences between the dissolution profiles. It was clear that the presence of TRI-CAFOS 500 in the tablets had a profound effect on dissolution of rutin (Figures 10c and 10d). This excipient allowed for greater solubilisation of the active, nearly a three-fold difference, when compared with pure rutin, however the impact of the compression pressure on dissolution was not that pronounced as for the tablets containing TRI-CAFOS 500 and the rutin/HP-β-CD complex (Table 5c). The dissolution rate of rutin from rutin/HP-β-CD+TRI-CAFOS 500 tablets increased with decreasing compression pressure (Table 5d). Concluding this part of the studies, the best tablets were those comprising rutin/HP-β-CD and TRI-CAFOS 500 and, considering the tablet properties as presented in Figures 7-9, the compression pressures that are suitable for the compact preparation range between 39 and 76 MPa.

Table 5a. f_1 and f_2 values calculated for powder dissolution profiles for rutin and the rutin/β-CD systems (values in bold font indicate profiles which are similar).

	Rutin (powder)	Rutin/β-CD (powder)	Rutin/β-CD (tablets, 38 MPa)	Rutin/β-CD (tablets, 76 MPa)	Rutin/β-CD (tablets, 113 MPa)
Rutin (powder)		f_1=75.45 f_2=19.10	f1=64.06 f2=22.65	f_1=68.41 f_2=21.23	f_1=70.43 f_2=20.60
Rutin/β-CD (powder)	f_1=75.45 f_2=19.10		**f_1=6.73** **f_2=59.90**	**f_1=4.18** **f_2=69.92**	**f_1=5.24** **f_2=76.63**
Rutin/β-CD (tablets, 38 MPa)	f_1=64.06 f_2=22.65	**f_1=6.73** **f_2=59.90**		**f_1=3.32** **f_2=79.32**	**f_1=3.88** **f_2=71.94**
Rutin/β-CD (tablets, 76 MPa)	f_1=68.41 f_2=21.23	**f_1=4.18** **f_2=69.92**	**f_1=3.32** **f_2=79.32**		**f_1=3.35** **f_2=91.27**
Rutin/β-CD (tablets, 113 MPa)	f_1=70.43 f_2=20.60	**f_1=5.24** **f_2=76.63**	**f_1=3.88** **f_2=71.94**	**f_1=3.35** **f_2=91.27**	

497

498 Table 5b. f_1 and f_2 values calculated for powder dissolution profiles for rutin and the rutin/HP-β-CD systems
499 (values in bold font indicate profiles which are similar)

	Rutin (powder)	Rutin/HP-β-CD (powder)	Rutin/HP-β-CD (tablets, 19 MPa)	Rutin/HP-β-CD (tablets, 38 MPa)	Rutin/HP-β-CD (tablets, 76 MPa)	Rutin/HP-β-CD (tablets, 113 MPa)
Rutin (powder)		f_1=84.08 f_2=0.25	f_1=90.20 f_2=7.26	f_1=91.26 f_2=7.08	f_1=69.11 f_2=21.01	f_1=71.93 f_2=20.14
Rutin/HP-β-CD (powder)	f_1=84.08 f_2=0.25		f_1=31.76 f_2=26.45	f_1=33.27 f_2=26.88	f_1=42.99 f_2=9.98	f_1=42.88 f_2=10.52
Rutin/HP-β-CD (tablets, 19 MPa)	f_1=90.20 f_2=7.26	f_1=31.76 f_2=26.45		**f_1=2.69** **f_2=96.86**	f_1=26.54 f_2=23.68	f_1=25.31 f_2=24.71
Rutin/HP-β-CD (tablets, 38 MPa)	f_1=91.26 f_2=7.08	f_1=33.27 f_2=26.88	**f_1=2.69** **f_2=96.86**		f_1=26.87 f_2=23.31	f_1=25.65 f_2=24.32
Rutin/HP-β-CD (tablets, 76 MPa)	f_1=69.11 f_2=21.01	f_1=42.99 f_2=9.98	f_1=26.54 f_2=23.68	f_1=26.87 f_2=23.31		**f_1=2.14** **f_2=86.72**
Rutin/HP-β-CD (tablets, 113 MPa)	f_1=71.93 f_2=20.14	f_1=42.88 f_2=10.52	f_1=25.31 f_2=24.71	f_1=25.65 f_2=24.32	**f_1=2.14** **f_2=86.72**	

500

501 Table 5c. f_1 and f_2 values calculated for powder dissolution profiles for rutin and the mixtures of rutin/β-CD systems
502 with TRI-CAFOS 500 (values in bold font indicate profiles which are similar).

	Rutin (powder)	Rutin/β-CD TRI-CAFOS 500 (powder)	Rutin/β-CD TRI-CAFOS 500 (tablets, 19 MPa)	Rutin/β-CD TRI-CAFOS 500 (tablets, 38 MPa)	Rutin/β-CD TRI-CAFOS 500 (tablets, 76 MPa)	Rutin/β-CD TRI-CAFOS 500 (tablets, 113 MPa)
Rutin (powder)		f_1=72.92 f_2=7.64	f_1=83.31 f_2=16.95	f_1=81.18 f_2=17.51	f_1=90.55 f_2=15.14	f_1=78.24 f_2=18.32
Rutin/β-CD TRI-CAFOS 500 (powder)	f_1=72.92 f_2=7.64		f_1=19.87 f_2=30.50	f_1=20.80 f_2=29.49	f_1=17.50 f_2=34.34	f_1=22.07 f_2=28.16
Rutin/β-CD TRI-CAFOS 500 (tablets, 19 MPa)	f_1=83.31 f_2=16.95	f_1=19.87 f_2=30.50		**f_1=11.55** **f_2=90.66**	**f_1=4.19** **f_2=69.34**	**f_1=7.94** **f_2=76.42**
Rutin/β-CD TRI-CAFOS 500 (tablets, 38 MPa)	f_1=81.18 f_2=17.51	f_1=20.80 f_2=29.49	**f_1=11.55** **f_2=90.66**		**f_1=9.89** **f_2=64.02**	**f_1=4.54** **f_2=86.02**

Rutin/β-CD TRI-CAFOS 500 (tablets, 76 MPa)	f_1=90.55 f_2=15.14	f_1=17.50 f_2=34.34	**f_1=4.19** **f_2=69.34**	**f_1=9.89** **f_2=64.02**		**f_1=6.91** **f_2=58.24**
Rutin/β-CD TRI-CAFOS 500 (tablets, 113 MPa)	f_1=78.24 f_2=18.32	f_1=22.07 f_2=28.16	**f_1=7.94** **f_2=76.42**	**f_1=4.54** **f_2=86.02**	**f_1=6.91** **f_2=58.24**	

Table 5d. f_1 and f_2 values calculated for powder dissolution profiles for rutin and the mixtures of rutin/HP-β-CD systems with TRI-CAFOS 500 (values in bold font indicate profiles which are similar).

	Rutin (powder)	Rutin/HP-β-CD TRI-CAFOS 500 (powder)	Rutin/HP-β-CD TRI-CAFOS 500 (tablets, 19 MPa)	Rutin/HP-β-CD TRI-CAFOS 500 (tablets, 38 MPa)	Rutin/HP-β-CD TRI-CAFOS 500 (tablets, 76 MPa)	Rutin/HP-β-CD TRI-CAFOS 500 (tablets, 113 MPa)
Rutin (powder)		f_1=95.52 f_2=1.57	f_1=95.38 f_2=3.42	f_1=93.37 f_2=10.26	f_1=70.94 f_2=20.44	f_1=81.28 f_2=17.49
Rutin/HP-β-CD TRI-CAFOS 500 (powder)	f_1=95.52 f_2=1.57		f_1=23.23 f_2=32.79	f_1=30.31 f_2=17.26	f_1=42.73 f_2=8.22	f_1=39.30 f_2=10.10
Rutin/HP-β-CD TRI-CAFOS 500 (tablets, 19 MPa)	f_1=95.38 f_2=3.42	f_1=23.23 f_2=32.79		f_1=16.45 f_2=31.80	f_1=33.06 f_2=16.66	f_1=29.02 f_2=19.49
Rutin/HP-β-CD TRI-CAFOS 500 (tablets, 38 MPa	f_1=93.37 f_2=10.26	f_1=30.31 f_2=17.26	f_1=16.45 f_2=31.80		f_1=19.89 f_2=31.59	f_1=15.04 f_2=37.63
Rutin/HP-β-CD TRI-CAFOS 500 (tablets, 76 MPa)	f_1=70.94 f_2=20.44	f_1=42.73 f_2=8.22	f_1=33.06 f_2=16.66	f_1=19.89 f_2=31.59		**f_1=6.05** **f_2=61.94**
Rutin/HP-β-CD TRI-CAFOS 500 (tablets, 113 MPa)	f_1=81.28 f_2=17.49	f_1=39.30 f_2=10.10	f_1=29.02 f_2=19.49	f_1=15.04 f_2=37.63	**f_1=6.05** **f_2=61.94**	

4. Conclusions

The studies showed that rutin can form inclusion complexes with cyclodextrins, however the most efficient mechanism of the complex formation depends on the physicochemical properties of the type of cyclodextrin. Mechanical activation was the most suitable method of complex formation with HP-β-CD, while the β-CD complex successfully developed by kneading with an ethanol/water mixture. A range of methods was used in this work to confirm the complex formation and postulate the most likely mechanism. The binary rutin systems were stable when stored in humid conditions, however the β-CD complex retained around 8% of moisture, which may not be optimal for solid formulations. The complexes compacted well with rutin/β-CD showing the best tensile strength values. Systems containing

517 HP-β-CD showed better properties than β-CD samples presenting higher porosity while
518 maintaining higher tensile strength and releasing a greater amount of rutin. However, superior
519 compactibility and tablet dissolution were achieved by mixing the complexes with a directly
520 compressible filler, tricalcium phosphate. Our studies showed that the tablets studied in this
521 work appear to be a promising delivery system of rutin and it is recommended to combine
522 rutin/HP-β-CD with TRI-CAFOS 500 and compact at 38-76 MPa.

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678

679 **Figure captions**

680 Figure 1. Powder X-ray diffractograms of the solid samples. 2θ positions of the principal
681 diffraction peaks are shown for rutin, while the traces of crystalline rutin peaks in the complexes
682 are indicated by "R".

683 Figure 2. DSC thermograms of rutin, rutin/CD complexes and physical mixture of rutin/CD
684 complexes with TRI-CAFOS 500.

685 Figure 3. FTIR spectra of rutin, rutin/CD complexes and physical mixture of rutin/CD
686 complexes with TRI-CAFOS 500.

687 Figure 4. DVS kinetic profiles of rutin/CD complexes.

688 Figure 5. Particle size distributions of the powders.

689 Figure 6. Powder dissolution of rutin from the CD complexes and mixtures of CD complexes
690 with TRI-CAFOS 500.

691 Figure 7. (a) Tabletability, (b) compressibility and (c) compactibility profiles of the rutin/CD
692 complexes and mixtures of rutin/CD complexes with TRI-CAFOS 500 powders.

693 Figure 8. SE micrographs of the tablets compressed at the various compression pressures.

694 Figure 9. Disintegration time of the rutin tablets.

695 Figure 10. Dissolution profiles of tablets: (a) rutin/β-HP, (b) rutin/HP-β-HP, (c) rutin/β-HP with
696 TRI-CAFOS 500 and (d) rutin/HP-β-HP with TRI-CAFOS 500.