Physico-Chemical and Pharmaco-Technical Characterization of Inclusion Complexes Formed by Rutoside with ?-Cyclodextrin and Hydroxypropyl-?-Cyclodextrin Used to Develop Solid Dosage Forms

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Keywords: antioxidant activity, inclusion complexes, hydroxypropyl-?-cyclodextrin, ?-cyclodextrin, rutoside

Abstract:

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Physico-Chemical and Pharmaco-Technical Characterization of Inclusion Complexes Formed by Rutoside with β-Cyclodextrin and Hydroxypropyl-β-Cyclodextrin Used to Develop Solid Dosage Forms



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Abstract: The aim of our study was to obtain rutoside (RUT) inclusion complexes in β -cyclodextrin (β-CD) and in hydroxypropyl-β-cyclodextrin (HP-β-CD), in a 1:1 molar ratio, using the lyophilization method of complexation in solution. The complexes were confirmed and characterized, in comparison with the raw materials and their simple physical mixtures, by SEM, DSC, and FT-IR analyses. The antioxidant activity of the compounds was assessed by using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and 2'-azino-bis(3-ethylbenzothiazolin-6-sulfonic) acid (ABTS) radicals, determining the radical scavenging activity, and by ferric reducing antioxidant power (FRAP) assay. The results revealed superior antioxidant ability for the inclusion complexes towards rutoside alone. The inclusion complexes were used as active ingredients in formulations of immediate-release tablets. The preformulation studies were performed on the powders for direct compression obtained after mixing the active ingredients with the excipients (Avicel PH 102, Polyplasdone XL-10, magnesium stearate, and talc). The materials were assessed for particle size, flowability, compressibility, and moisture content, establishing they are suitable for a direct compression process. The tablets were characterized regarding their pharmaco-technical properties and the results proved that the formulations lead to high-quality delivery systems, showing a good mechanical resistance with a low friability, excellent disintegration times, and satisfying dissolution rate. The performances were very similar for both formulations and the physico-mechanical properties of the tablets are not influenced by type of the used cyclodextrin, but the RUT- HP-β-CD tablets presented a higher dissolution rate.

Keywords: rutoside; β -cyclodextrin; hydroxypropyl- β -cyclodextrin; inclusion complexes; antioxidant activity

1. Introduction

Rutoside (RUT) (also known as rutin, quercetin-3-O-glucoside, and sophorin) is a natural flavonoid glycoside that has quercetin as the aglycone part, and a disaccharide, rutinose



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Copyright: © 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). $[\alpha$ -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranose], as the glucidic part. Rutoside is found in fruits and vegetables, such as *Sophora japonica*, *Fagopyrum esculentum*, *Rutagraveolens*, *Hypericum perforatum*, *Ginkgo biloba*, *Sambucus nigra*, *Tilia* spp., and *Crataegus* spp. [1–8]. RUT is used in therapeutical practice for its capillary protective (increasing the resistance and decreasing the capillary permeability) and antioxidative properties (direct chelation mechanism) in capillary fragility, venous insufficiency, hemorrhoids, or epistaxis [9,10].

Beyond its scientifically proven effectiveness, rutoside processing in different pharmaceutical forms is still hard to be made, due to its low oral absorption and bioavailability, as a result of poor solubility and chemical instability [11,12]. During the last years, a lot of studies were aimed at enhancing the stability, solubility, absorbability, and bioavailability of rutoside, by using rutoside derivates, particle size reduction, the use of surfactants, or sugar coating [13–15]. A facile and effective method for increasing the solubility, stability, and antioxidant activity of rutoside is its inclusion in the cyclodextrins cavities by forming inclusion complexes [16–18]. Cyclodextrins are cyclic oligosaccharides able to include various molecules in their internal hydrophobic cavity, frequently used to improve the water solubility of encapsulated ingredients due to their hydrophilic external surface [19]. Different studies reported an enhancement of the aqueous solubility, permeability, and absorption of rutoside, and many others proved to improve the chemical stability of rutoside, all by complexing it with β -cyclodextrin [17,20–22]. It appears that the dimension of the rutoside molecule is suitable to fit perfectly in the cavity of β -cyclodextrin, the α -cyclodextrin cavity being too small, and the γ -cyclodextrin too large [21,23,24]. Although, until now, the studies of the preparation and structural characterization of inclusion complexes between rutoside and cyclodextrins by different ways have been advanced, the further development of the inclusion compounds by new methods is required, especially to study the solubility, stability, and antioxidant capacity of rutoside.

In this context, the use of rutoside to form inclusion complexes with CDs has been paid much attention. In the literature, a lot of studies have reported the formulation of rutoside inclusion compounds using various CDs with significant enhancement of its bioavailability. The inclusion complexes between rutoside and β -CD and HP- β CD prepared in a molar ratio of 1:1 by the co-precipitation method show an improved solubility, antioxidative activity, and photostability of rutoside [17]. The RUT- β-CD inclusion complex obtained via a co-grinding technique in a 1:1 molar ratio was found to have a greater stability and solubility compared with free compound but a lower antibacterial activity [25]. The phase-solubility studies of 1:1 stoichiometric inclusion complexes of rutoside with β -CD, HP- β -CD, HP- α -CD, and HP- γ -CD have showed greater stability constants [23]. The authors claimed that the complexes RUT- HP- β -CD and RUT- HP- γ -CD were found to have the greatest stability constants, followed by RUT- β -CD and RUT- α -CD, and in consequence an enhanced antioxidant activity. The inclusion complexes of rutin with β -cyclodextrin and hydroxypropyl- β -cyclodextrin by the kneading method, followed by the evaluation of the antioxidant, antiproliferative, and pro-apoptotic activity against the B164A5 murine melanoma cell line shows an increase of the antioxidant activity compared with free rutoside [26]. Most of these studies have not fully investigated the stability and the antioxidant activity of the inclusion complexes and furthermore the pharmaco-technical of the tablets, including RUT- β -CD and RUT-HP- β -CD inclusion complexes, as active ingredients.

Considering the so-far available studies, the aim of the present study was to prove the enhancement of the stability and the antioxidant properties of RUT by complexation with two cyclodextrins, namely β -cyclodextrin (β -CD) and hydroxypropyl- β -cyclodextrin (HP- β -CD). Therefore, the prepared inclusion complexes of RUT in β -CD and HP- β -CD, using the lyophilization method of complexation in solution, were compared with the simple physical mixtures, obtained in the same molar ratio of 1:1. The complexes were identified and characterized by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and Fourier-transform infrared spectroscopy (FT-IR) analyses. Finally, the inclusion complexes were processed under the form of tablets, showing the preformulation, formulation, and manufacture studies, ending with the quality control performed on the final products.

Moreover, the antioxidant activity of rutoside inclusion complexes with β -CD and HP- β -CD was evaluated by three *in vitro* methods: (i) one using the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH); (ii) the second test was made with 2, 2'-azino-bis(3-ethylbenzothiazolin-6sulfonic) acid (ABTS), determining the radical scavenging activity; and (iii) the third one through ferric reducing antioxidant power (FRAP) assay.

2. Materials and Methods

2.1. Materials

RUT, β -CD and HP- β -CD, DPPH, ABTS, and TPTZ were purchased from Sigma-Aldrich Chemie GmbH, (Steinheim, Germany). The methanol and distilled water were of analytical grade.

For sample weighing, a Mettler Toledo AT261 balance (with 0.01 mg sensitivity) was used.

2.2. Preparation of the Inclusion Complexes

The lyophilization method of complexation in solution was used to prepare inclusion complexes between rutoside and β -CD, and RUT and HP- β -CD, respectively, in a molar ratio of 1:1. The synthesized inclusion complexes were noted as: RUT- β -CD and RUT-HP- β -CD. RUT- β -CD inclusion complex was prepared as follows: 0.25 mmol of β -CD was dissolved in 100 mL distilled water, and, separately, 0.25 mmol of RUT was dissolved in 20 mL of methanol. Using the same procedure as in the case of RUT- β -CD complex, the inclusion complex between RUT and HP- β -CD was synthesized using 0.25 mmol of HP- β -CD dissolved in 100 mL of distilled water and 0.25 mmol RUT dissolved in 20 mL of methanol. The two methanol solution samples were added stepwise to each aqueous solution of β -CD and HP- β -CD, and the two obtained suspensions were stirred at room temperature, for 8 h, at 1000 rpm, in a Heidolph MR 3001K magnetic stirrer. The final two obtained suspensions were lyophilized at -60 °C, for 12 h, in a Christ ALPHA 1–2, Braun Biotech International (Osterode, Germany) lyophilizer.

2.3. Preparation of RUT-β-CD and RUT-HP-β-CD Physical Mixtures

The physical mixtures, in the same molar ratio (1:1) as the inclusion complexes, were prepared by mixing the raw materials accurately weighted, for 5 min, at room temperature, in a mortar. Samples notation: RUT- β -CD physical mixture and RUT-HP- β -CD physical mixture.

2.4. Measurements and Characterization

The obtained inclusion complexes (RUT- β -CD and RUT-HP- β -CD), physical mixtures (RUT- β -CD and RUT-HP- β -CD), and pure compounds (RUT, β -CD, and HT- β -CD) were identified and characterized by scanning electron microscopy (SEM, TECSAN Brno, Czech Republic), differential scanning calorimetry (DSC, Setaram Instrumentation, Caluire, France), and Fourier-transform infrared spectroscopy (FT-IR) analyses. Additionally, the antioxidant properties of rutoside were evaluated.

2.4.1. SEM Analysis

To determine the morphology of the samples, the scanning electron microscope, VEGA II model, produced by TESCAN, (Brno, Czech Republic) was used, working in the following conditions: accelerating voltage of 30 kV, at a working distance of 9.9 mm, with a beam current of 60 μ A, a probe current from 1:8 to 1:12, current absorbed up to \approx 100 pA, and secondary electrons detector. The SEM images were registered at magnifications between 100× and 4000×. The samples were processed by adhesion on a support covered with a graphite double adhesive conducting tape, eliminating the excessing materials by blowing with a compressed nitrogen gun of chromatographic purity.

2.4.2. Differential Scanning Calorimetry

DSC measurements of pure compounds and their physical mixtures (RUT- β -CD and RUT-HP- β -CD) and inclusion complexes (RUT- β -CD and RUT-HP- β -CD) were performed on SETARAM DSC 131EVO (Setaram Instrumentation, Caluire, France), equipped with a cooler system based on liquid nitrogen (95 °C/min), a data detection system PlatinumTM Software, and a DSC Standard Cell RC module. Samples of approximately 5 mg sealed in aluminum pans were heated with a heating rate of 10 °C/min, in the temperature range of 30–210 °C. An inert nitrogen atmosphere with a flow rate of 50.0 mL/min was used. As a reference, an empty sealed aluminum pan was used. The instrument calibration regarding the temperature and the enthalpy was made with the standard material indium (99.98%) with the known melting point $T_{\rm m} = 156.65$ °C. All samples (RUT, β -CD, and HT- β -CD, physical mixtures and inclusion complexes) were analyzed in duplicate.

2.4.3. FT-IR Analysis

The Fourier Transform Infrared spectra were registered using a JASCO FT/IR-4200 spectrometer (Tokyo, Japan) with an ATR PRO450-S accessory, on a spectral range of 4000–400 cm⁻¹ using a resolution of 4 cm⁻¹. The pure compounds (RUT, β -CD and HT- β -CD), the physical mixtures (RUT- β -CD and RUT-HP- β -CD), and the inclusion complexes (RUT- β -CD and RUT-HP- β -CD) were analyzed in duplicate.

2.4.4. Antioxidant Activity of the RUT-β-CD and RUT-HP-β-CD Inclusion Complexes DPPH Radical Scavenging Activity

The DPPH method may be used either in aqueous or nonpolar organic solvents, and is useful to examine both hydrophilic and lipophilic antioxidants. DPPH is a stable free radical, due to the spare electron delocalization over the whole molecule; thus, it does not dimerize, as is the case with most free radicals. Delocalization on the DPPH molecule causes the appearance of a deep purple color, with an absorption band at a maximum of about 520 nm. When DPPH reacts with a hydrogen donor, the reduced molecular form (DPPH-H) occurs, accompanied by the loss of the purple color. Therefore, the decrease in absorption linearly depends on the antioxidant capacity [27–29].

The antioxidant activity of the rutoside-cyclodextrins inclusion complexes was determined using the DPPH free radical scavenging assay described by Margina et al. [30] with some modifications. Briefly, the samples contained 10 μ L of 1 mM ethanolic solution of each tested complex or compound treated with 100 μ L 0.5 mM DPPH solution. The absorbance of the obtained mixture was measured after 5 min and then from 5 to 5 min, for 40 min, at 505 nm. Ascorbic acid was used as the positive control, in this case the reduction of DPPH was evaluated for different concentrations: 2.8 mM–87.5 μ M. The results are presented as the decrease percentage in optical density at 40 min from the initial one.

The DPPH scavenging effect (radical scavenging activity *RSA*%) was calculated using Equation (1):

$$RSA\% = \frac{(Abs_0 - Abs_p)}{Abs_0} \times 100 \tag{1}$$

where Abs_0 is the absorbance of DPPH solution (reference) at t_0 (initial optical density); Abs_p is the absorbance of the samples measured at different period of times t (sample optical density).

ABTS Radical Scavenging Activity

The cationic ABTS radical (ABTS \bullet^+), which shows absorption at 743 nm (giving a bluegreen color), is formed by the loss of an electron through the nitrogen atom of 2, 2'-azino-bis (3-ethylbenzothiazolin-6-sulfonic) acid. In the presence of a hydrogen donor antioxidant compound, the nitrogen atom binds the hydrogen atom, leading to the discoloration of the solution.

The studied compounds were dissolved in ethanol solution 96%, the concentration of the obtained solution being 1 mM. The stock solution containing 7 mM ABTS and 2.45 mM

 $K_2S_2O_8$ was maintained in the dark, at 4 °C, for 16 h, to generate the ABTS•⁺ free radical. The working reagent was obtained by diluting the stock solution with distilled water to $OD_{714 \text{ nm}} = 0.8$. The samples (50 µL) were treated with 100 µL of working reagent and optical density was measured initially and after an incubation of 5 and 10 min, at 714 nm. The results were expressed as %*DOD* (decrease percentage in optical density), this being directly proportional to the antioxidant capacity of the samples, using Equation (2) [31]:

$$\% DOD = 100 \times \frac{\left(OD_{ABTS} + OD_{sample}\right)}{OD_{ABTS}}$$
(2)

FRAP Assay

The mechanism of ferric reducing antioxidant power depends on electron transfer and is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-trys(2-pyridyl)-s-triazine (TPTZ), when an intense blue ferrous ion–TPTZ complex (absorption at 593 nm) is formed [32]. The reaction takes place at acidic medium pH = 3.6, which ensures the iron solubility, increasing the redox potential, which is the main reaction mechanism.

For the FRAP assay, the method described by Nair et al. was used [33]. The reagent was obtained by mixing 10 mL of 300 mM acetate buffer (pH 3.6 adjusted with acetic acid), 1 mL of 20 mM ferric chloride hexahydrate, and 1 mL of 10 mM TPTZ in 40 mM HCl. Then, 100 μ L methanolic solutions of each sample weremixed with 3 mL of the prepared FRAP solution, then incubated for 30 min at 37 °C, protected from light. The absorbance at 593 nm was recorded at 5 and 30 min. The calibration curves were obtained on FeSO₄ methanol sample solutions. The antioxidant activity was reported as μ M Fe²⁺ produced by 300- μ L methanol sample solutions, from a constant Fe³⁺ concentration.

2.4.5. Preformulation and Precompression Studies for Tablets Containing RUT- β -CD and RUT-HP- β -CD Inclusion Complexes

The preformulation studies give useful information for the design of an optimum drug delivery system. For the present study, it was chosen to manufacture the tablets containing RUT- β -CD and RUT-HP- β -CD inclusion complexes by the direct compression method. In order to obtain high-quality tablets, the direct compression material must have good physical properties, including the fundamental ones, flowability and compressibility. As excipients, the following were selected: (i) Avicel PH 102, as filler, which improves the flow of powders, and also shows good binder attributes; and (ii) Polyplasdone XL-10, a non-ionic crosslinked PVP homopolymer, for its superdisintegrant properties due to the porous particle morphology, and magnesium stearate and talc, as lubricants.

Preparation of Powder Formulation

The amounts of inclusion complexes and excipients were selected to obtain tablets containing 40 mg of rutoside per tablet. The complexes were mixed with the direct compression excipients in the following ratios: **F1**—RUT-β-CD: Avicel PH 102: Polyplasdone XL-10: Magnesium stearate: Talc 57:39:2:1:1 and **F2**—RUT-HP-β-CD: Avicel PH 102: Polyplasdone XL-10: Magnesium stearate: Talc 65:31:2:1:1.

The ingredients were passed through a 0.8-mm mesh diameter sieve, then accurately weighed according to the established quantities, and finally, mixed in a ceramic mortar for 20 min, at the room temperature, until a homogeneous powder was obtained.

Assessment of Bulk Powder Physical Properties

Particle size directly influences the flowability of the powder, and also, the content uniformity and the dissolution rate of the tablets. It was determined by the sieving and sorting method, using a CISA Sieve Shaker Mod. RP 10, produced by Cisa Cedaceria Industrial, Spain. The method consists in the passing of the material by mechanical shaking through a set of sieves with well-known mesh sizes, placed under each other, in ascending order of the finesse degree.

Flowability, a critical characteristic, was evaluated by determining the angle of repose, flowing time, and rate, needed for 60 g of powder to flow through an orifice with a standardized diameter. The study was performed with an Automated Powder and Granulate Testing System PTG-S3, fabricated by Pharma Test Apparatebau GmbH, Germany.

Compressibility, also a fundamental property, is established by volumetric characteristics like bulk and tapped density, calculating the Hausner ratio (HR) and Carr Index (CI), which are also used as flowability indicators. It was performed with Vankel Tap Density Tester, produced by Vankel Industries Inc., USA. Bulk density was determined by measuring the volume occupied by 50 g of powder poured into a graduated cylinder, which is, then, subjected to a given number of mechanical shocks, measuring the tapped volume of the material. Hausner ratio (HR) is the ratio between tapped density and bulk density, with a value under 1.25 indicating that the powder is free flowing. Carr index is given by the equation: CI% = (tapped density – bulk density) \times 100/tapped density, and the smaller its value is, the better the flowability and compressibility are.

Moisture content is assessed as the loss on drying, by the Karl Fisher method with a HR 73 Mettler Toledo halogen humidity analyzer.

2.4.6. Formulation of the Tablets Containing RUT- β -CD and RUT-HP- β -CD Inclusion Complexes

After it was concluded that the powder has suitable characteristics to be processed by direct compression, the final formulations of the tablets were established, and they are presented in the Table 1.

Incredients	Quantity (mg/Tablet)		Role in	Producer	
ingreatents	F1	F2	Formulation	i iouucci	
Inclusion complex RUT-β-CD (1:1)	114	-	Active ingredient	Our laboratory	
Inclusion complex RUT-HP-β-CD (1:1)	-	130	Active ingredient	Our laboratory	
Avicel PH 102	78	62	Filler Binder	DuPont™ Nutrition and Health, USA	
Polyplasdone XL-10	4	4	Superdisintegrant	Ashland Specialty Ingredients, USA	
Magnesium stearate	2	2	Lubricant	Peter Greven, Netherlands	
Talc	2	2	Lubricant	Peter Greven, Netherlands	
TOTAL	200	200			

Table 1. The formulations of the tablets with RUT- β -CD and RUT-HP- β -CD inclusion complexes.

2.4.7. Tablets Manufacturing

The immediate release tablets were obtained from the composed powder by direct compression technology, using a medium compression force of 14 kN, in a single-post eccentric machine Korsch EK-O type, equipped with 7-mm flat punches. The machine was adjusted to obtain tablets with an average mass of 200 mg, corresponding to a content of 40 mg rutoside/tablet. After manufacturing, the obtained tablets were subjected to quality control tests.

2.4.8. Quality Parameters of the Tablets Organoleptic Evaluation

The appearance, color, taste, and smell of the tablets were evaluated according to the European Pharmacopoeia specifications [34].

Uniformity of Tablets Dimensions (Diameter and Thickness)

The diameter of the tablets is one of the compendial requirements [35]; meanwhile, the thickness is not included in the pharmacopoeia standards. Both of them are considered to be important for the uniformity of the tablet size, and they can vary due to the different density in the compressed material or to the compression force. The diameter and the thickness were determined on 10 tablets of each formulation with a VK 200 Tablet Hardness Tester, produced by Vanderkamp, USA.

Weight Variation

Mass uniformity is also influenced by the compression parameters. The testing was done by individually weighing 30 tablets of each formulation, then calculating the average mass and finally a comparison among them. The tablets comply with the test if not more than one individual mass is outside the limits of 85–115% of the average mass [35].

Hardness

The tablet crushing strength, even it is not a compendial standard, is very important to be measured, as the disintegration time, the dissolution rate, and the resistance of tablets to breakage under conditions of storage, transportation, or handling before usage all depend on it. Tablet hardness was measured with a VK 200 Tablet Hardness Tester. A tablet was placed in the hardness tester and the load required to crush the tablet was registered.

Friability

This test was performed to evaluate the ability of tablets to withstand abrasion in packing, handling, and transporting. An initial weight of 10 tablets was taken and these were placed in the Wankel Friabilator, rotating at 25 rpm for 4 min, and then weighed again. The difference in the weight was noted and expressed as a percentage. It should be preferably below 1.0%.

2.4.9. In Vitro Disintegration Time

The *in vitro* disintegration time [35] of a tablet was determined using an Erweka DT 3 apparatus, produced by Erweka[®] GmbH, Germany. The test was carried out on 6 tablets of each formulations using distilled water at 37 ± 0.5 °C as a disintegration media and the time taken for complete disintegration of the tablet, with no palpable mass remaining on the screen, was measured in seconds.

2.4.10. In Vitro Dissolution Rate

The dissolution rate was determined using a USP paddle Apparatus II (dissolution tester ERWEKA DT 800), in 900 mL of pH 6.8 phosphate buffer solution [35]. The apparatus was settled at 37 ± 0.5 °C, with a paddle rotating speed of 50 rpm. The amount of dissolved rutoside was determined after 30 min, by the UV-VIS spectrophotometric method (UV-VIS Nicolet Evolution 100 spectrometer) at the absorption maximum of 257 nm.

3. Results

3.1. Organoleptic Evaluation of the RUT- β -CD and RUT-HP- β -CD Inclusion Complexes

The two physical mixtures obtained by mixing RUT with β -CD and RUT with HT- β -CD were yellow crystalline powders, odorless, with a bitter taste. By the lyophilization method, very fine and smooth yellow powders, odorless and bitter tasting, were obtained.

3.2. Identification and Characterization of the RUT- β -CD and RUT-HP- β -CD Inclusion Complexes

3.2.1. Scanning Electron Microscopy (SEM)

The SEM images of RUT, β -CD, HP- β -CD, RUT- β -CD, and RUT-HP- β -CD physical mixtures, and RUT- β -CD and RUT-HP- β -CD complexes are shown in Figures 1 and 2 (between 500× and 4000× magnifications).

The SEM images of RUT, at $500 \times$ and $4000 \times$, from Figure 1A show the presence of non-homogeneous prismatic crystalline particles, with different sizes between 10 and 200 µm. SEM images at different magnifications for β -CD (Figure 1B) showed also crystalline particles, with various shapes and sizes between 5 and 20 µm [36], and HP- β -CD (Figure 1C) showed spherical particles, with an amorphous character [37]. The SEM images for both physical mixtures (Figure 1D,E) showed the crystalline structures of rutoside adhering to the surface of the β -CD and HP- β -CD, confirming the affinity between them, but it is clear that no new substrate occurred. In both mixtures, the individual characteristics of raw material particles remained unchanged, leading to the idea that no inclusion took place yet. The SEM images show only both crystalline structures of the individual components. The same morphologies were found in the literature for similar compounds [36,38,39].



Figure 1. Cont.



Figure 1. SEM images of (**A**) RUT, (**B**) β-CD, (**C**) HT-β-CD, (**D**) RUT-β-CD physical mixture, (**E**) RUT-HT-β-CD physical mixture, at different magnifications.



Figure 2. SEM images of (A) RUT- β -CD inclusion complex, (B) RUT-HT- β -CD inclusion complex, at different magnifications.

Analyzing the SEM images for RUT- β -CD (Figure 2A) and RUT-HP- β -CD (Figure 2B) inclusion complexes, it can be noticed the presence of some homogeneous slightly amorphous powders with fine and regular crystals. It is clear that a new system, with a drastic decrease of particle size and a partial loss of crystallinity towards the initial ingredients, was forming. The important changes in the morphology and shape of the particles reveal the presence of a new solid phase, being a specific characteristic for the formation of the inclusion complexes.

3.2.2. Fourier-Transform Infrared Spectroscopy (FT-IR)

The characteristic FT-IR bands of pure compounds (rutoside, β -cyclodextrin, hydroxypopyl- β -cyclodextrin), and of the two inclusion complexes are shown in Figure 3. The FT-IR spectrum of rutoside (Figure 3 (red line)) shows two broad bands at 3413 and 3334 cm⁻¹, respectively, due to O-H bounds vibrations. The weak peak at 2938 cm⁻¹ can be assigned to alkene C-H stretch. The band at around 1649 cm⁻¹ is characteristic of the double bound C=O from quercetol molecule, and the band at around 1450 cm⁻¹ can be assigned to C=C bound. In the range of 500–1200 cm⁻¹, a series of strong intensity bands are associated with the stretching vibration of the C-C and C-O bounds from the dihydroxiphenyl portion and from the disaccharide structure, and wagging vibration of the C-H bonds.

In the FT-IR spectrum of β -CD (Figure 3 (blue line)), the strong bands at 1021, 1077, and 1151 cm⁻¹ are attributed to the stretching vibration of the C-C, C-O bonds and wagging vibration of the C-H bonds [25]. The large band at 3327 cm⁻¹ is due to the O-H stretching vibrations [36,40]. The band at 2925 cm⁻¹ was assigned to the C-H stretching vibration. The absorption band at 1647 cm⁻¹ was due to the H-O-H bending vibration.

The FT-IR spectrum of HP- β -CD (Figure 3 (black line)) is very similar to the one of β -CD. Several features can be pointed: the large band at 3336 cm⁻¹ is assigned to the O-H stretching vibrations. The strong band at 1022 cm⁻¹ is due to the C-O bound vibration. The band at around 2927 cm⁻¹ is attributed to the alkylic part.

In FT-IR spectra of the two physical mixtures of the compounds (RUT- β -CD and RUT-HP- β -CD), all the above peaks of the compounds appear at the same wavenumbers, indicating no interaction between drug and β -CD or HP- β -CD. The spectra of both physical mixtures showed an approximate superposition of individual spectra of pure compounds.

The FT-IR spectra of RUT- β -CD (Figure 3 (olive line)) inclusion complex is similar with the one of the β -CD, but with a decrease in the intensity and a disappearance of some characteristic bands of RUT, which may be due to the encapsulation of the drug in the β -CD matrix. The differences in the positions and intensity of the ranges 500–1150 cm⁻¹ were recorded for RUT- β -CD inclusion complex in comparison with the pure compounds. In this domain, the bands from the FT-IR spectrum of the inclusion complex were shifted and were broadened.

The FT-IR spectrum of RUT-HP- β -CD (Figure 3 (green line)) inclusion complex shows a high resemblance with the one of the RUT- β -CD inclusion complex due to the structural similarity of the two cyclodextrines. The peak of alcohol is strong; the peaks of C-OH and amine are weak. The lower intensity of these peaks from FT-IR spectrum of the RUT-HP- β -CD inclusion complex is due to the interaction to the hydroxyl group with the secondary amine [41]. These results showed that the inclusion complexes of RUT with β -CD and HT- β -CD were successfully formed.



Figure 3. FT-IR spectra of RUT (red line), β -CD (blue line), HP- β -CD (black line), and the inclusion complexes: RUT- β -CD (olive line) and RUT-HP- β -CD (green line).

3.2.3. Differential Scanning Calorimetry (DSC)

The DSC curves of the studied compounds are given in Appendix A file (Figures A1–A7). Table 2 shows the thermal data (T_m , ΔH_m) obtained from DSC curves for the studied compounds (at a heating rate of 10 K min⁻¹).

Table 2. The characteristic temperatures (*T*) and area of the characteristic peaks (ΔH) obtained from DSC curves.

Commound	$T_1/(^{\circ}C)$	$\Delta H_1/(\mathrm{J~g^{-1}})$	<i>T</i> ₂ /(°C)	$\Delta H_2/(\mathrm{J~g^{-1}})$	
Compound	First Endo	thermal Peak	Second End	Second Endothermal Peak	
RUT	173.0 (melting)	137.2 (melting heat)	-	-	
β-CD	133.9	222.5	-	-	
HP-β-CD	109.3	166.2	-	-	
RUT-β-CD physical mixture	67.7	41.1	146.4	79.8	
RUT-HP-β-CD physical mixture	107.4	70.8	176.4	34.5	
RUT- β -CD complex	121.5/144.8	159.4	178.3	13.1	
RUT-HP-β-CD complex	84.4/98.4	149.3	-	-	

The DSC curve of rutoside (Figure A1) exhibits a sharp endothermic peat at 173 °C, (peak temperature of the DSC curve recorded at a heating rate of 10 K min⁻¹) corresponding to its melting point, with the melting heat of $\Delta H = 137.2 \text{ Jg}^{-1}$ evaluated from the peak area (Figure A1 from Appendix A).

The DSC curve of β -CD (Figure A2) shows an endothermic peak at 133.9 °C with an associated heat of the endothermal process of 222.5 °C, which is due to the release of water from the β -CD cavity [42,43]. No other endothermal effects appear.

The DSC curve of HP- β -CD (Figure A3) showed a large endothermal peak associated with water loss at 109.3 °C. In the 30–210 °C temperature range, no other peaks appear in the DSC curve of HP- β -CD.

The DSC curves of the two physical mixtures were similar to the individual DSC curves of pure compounds. For RUT- β -CD physical mixture (Figure A4), the melting peak of RUT appears at a lower temperature (146.4 °C). The melting endotherm of RUT is attenuated in this case. This is proof that a consistent amount of RUT is included in the β -CD, even in a mixing procedure. From Table 2, it was observed that the peak attributed to the water molecules was at 67.7 °C lower than the parent β -CD, suggesting that the water molecules from the β -CD cavity are replaced by RUT molecules. The small differences in the peak temperatures of RUT-HP- β -CD observed from the DSC curves (Figure A5) is proof that the complexation process did not occur in the physical mixtures.

A difference pattern was observed in the DSC curves of the inclusion complexes. In the RUT- β -CD inclusion complex (Figure A6), the melting point of RUT is still present but is shifted to a higher temperature (178.3 °C). The decrease of the sharpness and the intensity of the characteristic endothermic peak of RUT indicate a conversion of the crystalline RUT into an amorphous form, due to the lyophilization process. The DSC curve of the RUT-HP- β -CD inclusion compound (Figure A7) shows the disappearance of the RUT melting point, suggesting in this case the formation of an inclusion complex. The dehydration process of the inclusion complex appears at a lower temperature than physical mixtures and the parent β -CD. The DSC thermal feature of the two compounds (RUT and HP- β -CD) practically disappears in the DSC curve of the inclusion complex or the disappearance for RUT-HP- β -CD complex of the RUT melting point is proof that the RUT molecule is included in β -CD or HP- β -CD cavities, the inclusion process being complete in the complexes.

3.3. Antioxidant Activity of the Complexes

3.3.1. DPPH Radical Scavenging Activity

Table 3 and Figure 4 show the percentage decrease of the optical density (% radical scavenging activities) of samples and vitamin C used as a positive control.

					% RSA			
Time	5 min	10 min	15 min	20 min	25 min	30 min	35 min	40 min
Ascorbic acid 2.8 mM	11.06	11.48	13.22	15.70	17.69	20.09	22.36	24.27
Ascorbic acid 1.4 mM	9.11	9.05	11.05	13.51	15.57	17.78	20.04	22.36
Ascorbic acid 0.7 mM	3.13	6.27	8.46	10.99	13.33	15.62	17.85	20.62
Ascorbic acid 0.35 mM	3.26	4.64	6.84	9.36	11.78	14.16	16.47	18.80
Ascorbic acid 175 µM	1.56	3.39	6.03	8.38	10.90	13.41	15.72	18.48
Ascorbic acid 87.5 µM	2.12	4.10	6.69	8.86	11.05	13.52	15.65	18.12
RUT	21.76	31.81	34.59	37.24	40.07	42.13	44.17	45.78
RUT-β-CD physical mixture	22.15	30.10	34.86	38.63	41.58	43.94	45.99	47.37
RUT-HP-β-CD physical mixture	25.32	32.31	36.74	40.07	42.64	44.67	46.99	48.34
RUT-β-CD inclusion complex	30.82	39.07	43.45	45.72	46.83	47.48	48.70	50.20
RUT-HP-β-CD inclusion complex	31.45	42.40	47.59	50.18	51.36	52.61	53.98	54.79

Table 3. DPPH radical scavenging activity.



Figure 4. The evolution of DPPH radical inhibition.

All the studied samples exhibit an antioxidant activity, with linear increases in time. It is considered that the ascorbic acid presents strong antioxidative activities, but according to the registered results, rutoside and its inclusion compounds showed a two times better effectiveness than the highest concentration of positive control. After 40 min, RUT exhibited a relatively high DPPH scavenging activity (45.78%) [44]. Both physical mixtures (47.37% for RUT- β -CD physical mixture and 48.24% for RUT-HP- β -CD physical mixture) showed relatively similar reducing power activity as RUT. However, the inclusion complexes presented the highest inhibition of oxidation, having an excellent electron donating ability, revealing a strong antioxidant activity (50.20% for RUT- β -CD inclusion complex and 54.79% for RUT-HP- β -CD inclusion complex), probably due to the increase of rutoside solubility by complexation with cyclodextrins. Still, it can be concluded that the RUT-HP- β -CD inclusion complex had the best behavior, proving the highest DPPH radical scavenging activity.

3.3.2. ABTS Radical Scavenging Activity

Table 4 and Figure 5 present the percentage decrease of the optical density (% radical scavenging activities) displayed by the samples, at time of 5 and 10 min.

% RSA Sample 5 min 10 min RUT 42.41 58.08 β-CD 2.24 -1.91HP-β-CD 2.34-3.21RUT-β-CD physical mixture 53.60 61.67 RUT-HP- β -CD physical mixture 46.82 62.63 54.34 65.60 RUT-β-CD inclusion complex RUT-HP-β-CD inclusion complex 56.36 66.37





Figure 5. The evolution of ABTS radical inhibition.

It has been demonstrated that the ABTS assay is more sensitive in identifying antioxidant activity because of the faster reaction kinetics, and its response to antioxidants is higher. The results proved that both cyclodextrins, β -CD and HP- β -CD, do not possess antioxidant capacities, but they have a significant influence on the ABTS radical scavenging activity of rutoside. The *in vitro* antioxidant assay of RUT revealed its antioxidant potential (42.41% RSA at 5 min and 58.08 at 10 min), which is considerably increased when it is associated with any of the two CDs [45]. Another remark evidenced in Figure 4 is that also the physical mixtures performed better than RUT alone in scavenging the ABTS[•] + radical cation. As can be noticed, the most effective inhibition is provided by RUT inclusion complexes, with β -CD and HP- β -CD being the most active samples after 10 min (65.60% for RUT- β -CD inclusion complex and 66.37% for RUT-HP- β -CD inclusion complex). From the ABTS assay, it can be considered that the inclusion complexes are free radical scavengers of fast action.

3.3.3. FRAP Assay

Table 5. FRAP values of the studied samples.

Table 5 and Figure 6 show the FRAP values determined at a time of 5 and 30 min. Table 5 presents the FRAP values (μ mol equivalent of Fe²⁺ (FeSO₄) /L), calculated using the Fe²⁺ calibration curves in methanol.

 μ mol Fe²⁺ Sample 5 min 30 min RUT 596 1492 RUT-β-CD physical mixture 733 1465 RUT-HP-β-CD physical mixture 701 1555 RUT-β-CD inclusion complex 895 1812 912 RUT-HP-β-CD inclusion complex 1854



Figure 6. The evolution of FRAP values.

The results provided by the FRAP assay strengthen the conclusions obtained by the other two tests that the complexation process produces a significant increase of rutoside antioxidant activity, slightly higher for RUT-HP- β -CD than RUT- β -CD inclusion complex [46]. The complexes also presented a higher reducing ability (around 20% higher) after 5, and even after 30 min, revealing an improved antioxidant efficacy.

3.4. Precompression Studies for Tablets Containing RUT- β -CD and RUT-HP- β -CD Inclusion Complexes

Different studies were performed on the bulk powder for direct compression, in order to establish the behavior of the material during the compression process and to choose the adequate compression parameters (Table 6).

Description	Formulation Code			
Parameter —	F1	F2		
Flow time (s) *	$10.4~{ m SD}\pm 0.73$	$11.1~{ m SD}\pm0.44$		
Angle of repose (θ) *	$28.22~\text{SD}\pm0.86$	$29.17~\text{SD}\pm0.56$		
Flow rate (g/s) *	5.741	5.382		
Bulk density (g/mL)	$0.307~{ m SD}\pm 0.08$	$0.368~\mathrm{SD}\pm0.12$		
Tapped density (g/mL)	$0.373~\mathrm{SD}\pm0.19$	$0.426~\mathrm{SD}\pm0.31$		
Carr Index (CI) (%)	18.2	13.6		
Hausner's ratio (HR)	1.21	1.15		
Moisture content (%)	$3.90~\mathrm{SD}\pm0.97$	$3.66~\mathrm{SD}\pm1.23$		

Table 6. Precompression parameters for the direct compression blend.

* stirring speed: 15 rpm, nozzle: 10 mm.

Concerning the particles dimensions, the histogram exhibited in Figure 7 was obtained by representing the distribution of particle size on granulometric classes for both studied formulations.



% Residue

Granulom etric class (µm)

Figure 7. Granulometric analysis of the material for direct compression.

Figure 7 shows that both compression powders contain a considerable part of the particles with a median particle size between 160 and 600 μ m, with most of them fitting in the smaller fraction on 160–315 μ m.

The values of the flow time, angle of repose, and flow rate, obtained after 5 determinations on 60 g of each powder, with a 15 rpm stirring rate, on a 10-mm nozzle, are very similar for both formulations. The flowability could not be measured using the 10-mm nozzle without stirring or with a 5-rpm and 10-rpm speed rate, because the powders were not flowing steadily and consistently under these conditions.

Flowing time was 10.4 s for **F1** and 11.1 s for **F2**, the angle of repose around 29° for both formulations, and the flowing rate was 5.741 g/s for **F1** and 5.382 g/s for **F2**, values that indicate an excellent flow, but considering the fact that stirring was needed, the powders have a rather poor to moderate free flow. The results were practically the same for both studied materials, leading to the idea that the type of cyclodextrin does not have such a great influence on the flowability of the compression blend.

F1 had lower bulk and tap densities as compared to **F2**. **F2** presented a lower value for CI (13.6) and HR (1.15) than **F1** (18.2 and 1.21), therefore it is considered to have a very good compressibility and a good flow. Meanwhile, **F1** is rated as having a 'fair' flow and a good compressibility [47]. In terms of HR and CI, both formulations have a required free flow and good compressive properties.

Both formulations displayed similar moister contents of 3.90% (F1) and 3.66 (F2), low values which are considered ideal for preprocessed powders for tablets.

Making the correlations between all the physical parameters determined for the tableting mixtures, it can affirm that smaller values of CI and HR for F2 are due to the broader particle-size distribution for a smaller particle-size fraction (160–315 μ m), to an increased bulk and tapped densities, and, also, to a lower moisture content [48]. Considering these remarks, it can be predicted that F2 will have a better compression behavior compared to F1, but the differences between the two formulations are not too significant. It is obvious that in both bulk and tapped, densities increased with decreasing particle size independent of the used CD type. A linear relationship between the mean particle size, moisture content, volumetric properties, flowability, and compressibility of the powders can be observed, with all being characteristic for smooth fluid powders. The results registered for the pharmaco-technical properties of the direct compression mixtures suggest that the two lubricants (magnesium stearate and talc) were well selected, having a good influence on the powder's flowability.

3.5. Quality Parameters of the Tablets

The obtained tablets quality performances were characterized by different physical and mechanical properties and the results of the tests are presented in Table 7 and the digital images of the tablets (**F1** and **F2**) are shown in Figure 8.

T (1 D (Formulation Code			
lested Parameters	F1	F2		
Organoleptic evaluation	round shape tablets with smooth and uniform surface, colored in light yellow	round shape tablets with smooth and uniform surface, colored in light vellow		
Thickness (mm)	$4.19~{ m SD}\pm0.22$	$4.07~{ m SD}\pm0.09$		
Diameter (mm)	$7\mathrm{SD}\pm0.11$	$7{ m SD}\pm 0.08$		
Weight variation	$197~\mathrm{SD}\pm3.20$	$199~\mathrm{SD}\pm2.88$		
Hardness (N)	$69.07~\text{SD}\pm2.63$	$71.20~\mathrm{SD}\pm2.18$		
Friability (%)	$0.34~{ m SD}\pm 0.17$	$0.41~{ m SD}\pm 0.06$		
Disintegration time (s)	$228~{ m SD}\pm2$	$216 \text{ SD} \pm 3$		
In vitro dissolution rate, after 30 min (%)	$84.29~\text{SD}\pm0.85$	$87.12\text{SD}\pm0.64$		

Table 7. The pharmaco-technical evaluation of the tablets.

Based on the above results, the average thickness of tablets is $4.19 \text{ SD} \pm 0.22$ for F1 and $4.07 \text{ SD} \pm 0.09$ for F2, indicating a uniformity between the two formulations and, also, between the tablets from the same lot. In the case of diameter, the differences are even smaller, showing the tablets of both formulations comply with the pharmacopoeial standard. The uniformity in dimensional properties of the tablets proves that the compression parameters were correctly chosen and no undesirable phenomenon occurred during the process.

Regarding the mass uniformity, the average weight of both formulations (197 SD \pm 3.20 for F1 and 199 SD \pm 2.88 for F2) is in accordance with the pharmacopoeial criteria, proving that the materials had a good flowability, which generated a uniform filling of the mold, ensuring the consistency of dosage units during compression.



F1



Figure 8. The digital images of the obtained tablets.

Both formulations present a good mechanical resistance (69.07 SD \pm 2.63 for F1 and 71.20 SD \pm 2.18 for F2) and an excellent friability (0.34 SD \pm 0.17 for F1 and 0.41 SD \pm 0.06), leading to the conclusion that the tablets withstand mechanical shocks during the manufacturing and handling. With friability being closely related to tablet hardness, it is obvious that the studied tablets have the ability to avoid fracture and breaking. This demonstrates that the ingredients and the compression force were properly chosen [48,49].

The disintegration time was below 4 min for both formulations (228 SD \pm 2 for F1 and 216 SD \pm 3 for F2), indicating a short period of time is needed for the disintegration process of the tablets. The fast disintegration was ensured by the use of Polyplasdone XL-10, the chosen superdisintegrant in both formulations and also by the two cyclodextrins, which increased rutoside's solubility in water. Both formulations meet the imposed pharmacopoeia limits for immediate-release dosage forms [50,51].

The results obtained for the tested characteristics prove that high-quality tablets including the RUT- β -CD and RUT-HP- β -CD inclusion complexes as active ingredients were developed. Both formulations' pharmaco-technical attributes were satisfactory and within the limits imposed by rules inforce. They show a good mechanical resistance with a low friability, qualities that ensure the tablets' integrity, and excellent disintegration times, being able to consider them rapidly disintegrating tablets. As the results are very similar for both formulations, it can be admitted that the physical and mechanical properties of the tablets are not influenced by the type of used cyclodextrin.

The dissolved quantity of rutoside rapidly increased within 30 min. The registered dissolution rates after 30 min are characteristic for highly soluble ingredients, and based on the fact that rutoside is a poorly water-soluble drug, the conclusion is that by encapsulating it in the cavity of both CDs, its solubility is significantly enhanced [51]. The RUT-HP- β -CD inclusion complex tablets showed a higher dissolution rate than RUT- β -CD tablets. Beside the influence of CDs on the dissolution profiles of the tablets, probably, a very important role isalso the used method of direct compression, the direct compression excipient Avicel PH 102, and the superdisintegrant Polyplasdone XL-10.

4. Conclusions

In summary, two inclusion complexes in a 1:1 molar ratio, (i) one of rutoside with β -cyclodextrin (RUT- β -CD) and (ii) another one of rutoside with hydroxypropyl- β -cyclodextrin (RUT-HT- β -CD), using the lyophilization method, were successfully prepared. FT-IR spectra showed obvious differences in the positions and intensities in the ranges of 500–1150 cm⁻¹ in both inclusion complexes, but their spectra were similar with the ones recorded for pure cyclodextrines, strengthening the idea that rutoside was successfully

included in their cavity. The morphologies, evaluated by SEM analysis, revealed important changes in the structure and shape of the binary lyophilized systems, proving that new compounds were formed. The recorded DSC curves showed that the complexation was complete in case of RUT-HP- β -CD, and only partial in RUT- β -CD complex. From the antioxidant activities, it was noticed that the two inclusion complexes had a remarkably improved antioxidant activity than rutoside alone or its simple physical mixtures with each CD. Thus, the encapsulation of rutoside in the CDs cavity leads to important increases of its antioxidant properties, probably due to its improved solubility. The physico-chemical and mechanical properties of the solid dosage pharmaceutical forms (F1 and F2) were evaluated in the preformulation studies, the results proving that the new two obtained materials are suitable for the direct compression process. Both drug delivery systems presented a good mechanical resistance with a low friability, and a weight variation within the compendial limits. A rapid disintegration was registered for both series of tables and it can be considered that the main purpose of this study was completely achieved, as the disintegration is the critical quality attribute for tablets. The dissolution rates were satisfying and within the imposed pharmacopeial limits for both formulations. As the performances were very similar for both formulations, the conclusion is that the pharmaco-technical properties of the tablets are not influenced by the type of the used cyclodextrin, but the RUT-HP- β -CD tablets present a higher dissolution rate.

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Appendix A



Figure A1. The DSC thermogram of RUT, dynamic nitrogen atmosphere, $\beta = 10$ K min⁻¹.



Figure A2. The DSC thermogram of β -CD, dynamic nitrogen atmosphere, $\beta = 10$ K min⁻¹.



Figure A3. The DSC thermogram of HP- β -CD, dynamic nitrogen atmosphere, $\beta = 10$ K min⁻¹.



Figure A4. The DSC thermogram of RUT- β -CD physical mixture, dynamic nitrogen atmosphere, $\beta = 10 \text{ K min}^{-1}$.



Figure A5. The DSC thermogram of RUT-HP- β -CD physical mixture, dynamic nitrogen atmosphere, $\beta = 10 \text{ K min}^{-1}$.



Figure A6. The DSC thermogram of RUT- β -CD complex, dynamic nitrogen atmosphere, $\beta = 10 \text{ K min}^{-1}$.



Figure A7. The DSC thermogram of RUT-HP- β -CD complex, dynamic nitrogen atmosphere, $\beta = 10 \text{ K min}^{-1}$.

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