CHARACTERIZATION OF RUTIN-CYCLODEXTRIN INCLUSION COMPOUNDS

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Abstract: The objectives of this study were to examine the potential of beta-cyclodextrin to improve the solubility of rutin and obtain inclusion compounds that were analyzed by different techniques: UV-Vis, IR spectroscopy, thermal analysis. The presence of β-cyclodextrin raises the content of rutin in water. The inclusion compounds were prepared by dry mixing, complexation in semisolid and liquid medium in 1:2 molar ratio rutin - β-cyclodextrin. The UV-Vis and IR analysis demonstrated the obtaining of inclusion compounds and the thermal analysis show that these compounds are more stable than the parent substance.

Keywords: cyclodextrins, inclusion compounds, methods of analysis
INTRODUCTION

Flavonoid is one of the largest groups of natural polyphenols. Rutin (Figure 1), quercetin-3-rutinoside, rutoside, sophorin belongs to flavonoids and is characterized by the presence in molecule of benzopiranic nucleus, substituted with a phenyl in 2 position (quercetin-3-rutinosid). Has important pharmacological properties like increase the strength of the blood vessels, antithrombotic, antioxidant, also is essential for assimilating vitamin C, is benefic in hypertension, builds a protective barrier against infection having an antibacterial effect, anti-inflammatory as a result of diminished formation of proinflammatory mediators, diuretic, lowers the intensity of cholesterol in blood stream, remedy for allergies, prevents cataracts and macular degeneration, disorientation and senility caused by advancing age. Rutin also helps in sustaining collagen - the tissue just below the skin, which sustains the epidermis, the external strata of our skin cells [1, 2]. Is a fine microcrystalline, yellow, tasteless, odorless powder, soluble in 2 mL methanol and 4 mL water at 100°C, hardly soluble in alcohol, practically insoluble in chloroform and ether [3].

The very poor aqueous solubility in water raises the difficulties of formulation, so we try to overcome these drawbacks using cyclodextrins. They have long been used to increase the solubility of lipophilic drugs by forming inclusion complexes with their non polar molecules or functional groups. Also, cyclodextrins and their derivatives play an important role in the formulation development because of their properties: modification of physicochemical properties of drugs, improvement of physical, chemical and thermal stability (against heat, oxidation, light induced decomposition, in aqueous solution), enhancement of bioavailability of poorly soluble and absorbable drugs, ensure of content uniformity in solid dosage forms at extremely low doses, reduction of undesired side effects of drugs, prevention of incompatibility by separating the components in order to prevent drug-drug or drug-additive interaction. The most common naturally occurring cyclodextrins are α, β and γ-cyclodextrins. β-cyclodextrins (Figure 1) are cyclic (α-1,4)-linked oligosaccharides of α-D-glucopyranose containing a relatively hydrophobic central cavity and hydrophilic outer surface. They are toroidal and cone shaped molecules, so the primary hydroxyl groups are located on the narrow side of torus and the secondary hydroxyl groups are located on the wider wedge [4-6].

*Figure 1. Rutin (a) and β-cyclodextrin (b) structures*
In this paper, the possibility of complexation of rutin with β-cyclodextrin for improving the solubility has been investigated, and the molar ratio rutin - β-cyclodextrin was established. Also, some inclusion compounds have been prepared by using different methods: dry mixing, complexation in semisolid and liquid medium. The existence of the inclusion compounds has been demonstrated by UV-Vis and IR spectroscopy and by thermal analysis.

**EXPERIMENTAL**

**Materials**

The materials used were β-cyclodextrin (a.p.) and rutin (a.p.), purchased from Fluka Chemica (Switzerland). All the solvents used were of quality standards of Romanian Pharmacopoeia, Xth edition.

**Phase solubility studies**

The phase solubility studies allow the evaluation of the affinity between β-cyclodextrin and rutin in water and were carried out according to the Higuchi and Connors methods [7]. Excess amounts of rutin that exceeded its solubility were added to aqueous solutions containing different concentrations of β-cyclodextrin. Samples were shaken at room temperature for 12 h. Finally, samples were filtered, diluted and spectrophotometrically analyzed. The concentration of rutin in each solution was determined, by using the external standard method.

**Preparation of inclusion compounds**

As determined from the phase solubility studies, the molar ratio of rutin and β-cyclodextrin required for the inclusion compounds was 1:2, so complexes were prepared using these molar proportion of the constituents by four methods: in solid medium (method 1), semisolid medium (method 2), aqueous medium (method 3), organic solvents medium (method 4) [8]. Samples were coded as follows: R (rutin), R1 (inclusion compound by method 1), R2 (inclusion compound by method 2), R3 (inclusion compound by method 3), R4 (inclusion compound by method 4).

**Analysis of the inclusion compounds**

UV-Vis absorption spectra of rutin and corresponding inclusion compounds were realized with a Jasco V530 spectrophotometer after proper dilution in methanol. The UV-Vis spectra of rutin have been compared with those of the inclusion compounds [9, 10].

IR absorption studies of pure rutin, β-cyclodextrin and the inclusion compounds were carried out using a Jasco FTIR 670 Plus spectrophotometer according to the bromide potassium disk method, recorded wavelength on abscissa and transmittance on ordinate [8-11].
The thermogravimetric curves (TG, DTG, DTA) were recorded on a Paulik-Paulik-Erdey type derivatograph, MOM, Budapest. Each sample constituted by film sample mass 50 mg in platinum crucibles was heated between 25-700°C, with a scanning rate of 10°C/min and a sensitivity of 1/5 [12-15].

RESULTS AND DISCUSSION

Phase solubility studies

The phase solubility diagram for the complex formation between rutin and \( \beta \)-cyclodextrin is shown in Figure 2. Rutin - \( \beta \)-cyclodextrin system exhibited the A\(_p\)-type of solubility curves, suggesting the formation of soluble inclusion compounds, a positively deviating isotherm. The stoechiometry of the complex determined from the initial ascending portion of the phase diagram indicated a rutin : \( \beta \)-cyclodextrin molar ratio of 1:2 in the complex.

**Figure 2. Phase solubility diagram**

**Figure 3. IR spectra of rutin (R) and rutin - \( \beta \)-cyclodextrin inclusion compounds (R1-R4)**

UV-Vis absorption study

Rutin exhibited intense maximums around 273 and 360 nm. The presence of the peak from 360 nm demonstrated the presence in the molecule of the cynamoil radical and -OH phenolic groups; the peak at 273 nm is attributed to benzyl radical.

Spectrum is due to following transitions:

- \( n \rightarrow \sigma^* \), by passing of an electron from a doublet of O atoms in the \( \sigma^* \) level;
- \( n \rightarrow \pi^* \), by passing of an electron from a \( n \) molecular non-bonding orbital in a \( \pi^* \) molecular anti-bonding orbital, characteristic to C=O bond, with lone pairs electrons;
- \( \pi \rightarrow \pi^* \), resulting ethylenic and benzoic bands from aromatic moiety.

The interactions of rutin and \( \beta \)-cyclodextrin in aqueous solution can be examined by comparing the UV-Vis spectra of rutin with those of inclusion compounds (Figure 3).
It can be observed both a modification of the absorption maximum and of the wavelength values. The inclusion compounds exhibited an intense maximum around 271 and 363 nm, the addition of β-cyclodextrin leading to a bathocromic and a hypsochromic effect.

**IR spectral study**

The IR spectra of the inclusion compounds show modifications in the specific peaks of β-cyclodextrin, the most sensitive being OH deformation and CH bending. Table 1 presents the modifications of the characteristic frequencies of β-cyclodextrin, after association with rutin.

*Table 1. Modifications of the characteristic frequencies of β-cyclodextrin, after association with rutin*

<table>
<thead>
<tr>
<th>Sample</th>
<th>νCH</th>
<th>γCH</th>
<th>γOH</th>
<th>νCO</th>
<th>νCO/CC</th>
<th>Pyranosic ring</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>2925</td>
<td>1419</td>
<td>1639</td>
<td>1156</td>
<td>1028</td>
<td>757 ; 862</td>
</tr>
<tr>
<td>R2</td>
<td>2925</td>
<td>1421</td>
<td>1641</td>
<td>1146</td>
<td>1036</td>
<td>760 ; 860</td>
</tr>
<tr>
<td>R3</td>
<td>2951</td>
<td>1432</td>
<td>1696</td>
<td>1117</td>
<td>1008</td>
<td>791 ; 866</td>
</tr>
<tr>
<td>R4</td>
<td>2947</td>
<td>1430</td>
<td>1697</td>
<td>1116</td>
<td>1010</td>
<td>788 ; 866</td>
</tr>
<tr>
<td>β-cyclodextrin</td>
<td>2930</td>
<td>1420</td>
<td>1640</td>
<td>1150</td>
<td>1028</td>
<td>750 ; 850</td>
</tr>
</tbody>
</table>

Frequency changes are more evident in the inclusion compounds obtained in semisolid and liquid medium, which demonstrates that the interaction between rutin and β-cyclodextrin is stronger than in solid medium.

**Thermal analysis**

Comparing the TG, DTG, ATD diagrams of the inclusion compounds with those of rutin, it can be affirmed that both rutin and the inclusion compounds present two steps of decomposition. The initial temperature of the first step, corresponding to dehydration is inferior to rutin. The second step of decomposition is better highlighted on DTG curve, the mass loss varying in the following order: β-cyclodextrin > R4 > R3 > R2 > R1 > R.

All DTA curves indicate an endothermic process at 50 ^°C^ and an exothermic process at 204 ^°C^ for rutin. The inclusion compounds present also an exothermic process at 330 ^°C^ (R1), 400 ^°C^ (R2), 450 ^°C^ (R3), and 260 ^°C^ (R4), respectively, which cannot be found in rutoside curves, probably because of the rather large internal covalent bonds leading to the reticulation.

Both for rutin and the inclusion compounds the activation energy decreases in a first step, then increases. These modifications indicate that rutin interacted with β-cyclodextrin and the strength of the complexes decreases in the following order: liquid medium, semisolid medium, dry mixing.
CONCLUSIONS

The present study showed that the association with β-cyclodextrin can increase the content of rutin in water due to obtaining, most probably, of an inclusion compound 1:2 molar ratio (rutin - β-cyclodextrin).

The modifications observed in UV-Vis and IR spectra indicated inclusion compounds formation between rutin and β-cyclodextrin, the interaction between the two molecules being stronger in semisolid and liquid medium.

The inclusion compounds are thermally more stable than the parent substance, rutin.

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