

β -Cyclodextrin Inclusion Complex – *In vitro* Assessment of the Antioxidant Activity

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DOI: 10.46793/ICCBKIG25.457M

Abstract: The formation of β -cyclodextrin (β -CD) inclusion complexes is a well-established strategy to enhance the solubility, stability, and bioavailability of poorly soluble bioactive compounds. In this study, the *in vitro* antioxidant potential of an inclusion complex (**CPD- β -CD**) formed between β -CD and 8,9-dihydroxy-2H-chromeno[2,3-d]pyrimidine-2,4(3H)-dione (CPD) was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The percentage of radical scavenging activity was assessed for the **CPD- β -CD** complex, along with standard antioxidants quercetin and nordihydroguaiaretic acid (NDGA), at concentrations of 25 μ M, 50 μ M, and 100 μ M. Notably, the **CPD- β -CD** complex exhibited strong antioxidant activity, achieving 96.1% inhibition of DPPH radicals even at the lowest tested concentration (25 μ M). Furthermore, the IC_{50} value was determined to be 4.5 μ M, indicating a high degree of antiradical potency. These results suggest that the encapsulation of **CPD** within β -cyclodextrin does not significantly diminish its antioxidant effectiveness. On the contrary, the inclusion complex retains comparable activity to the parent compound, making it a promising candidate for further development in antioxidant applications.

Keywords: Antioxidant Activity, DPPH radical scavenging assay, β -Cyclodextrin Inclusion Complex

1. Introduction

Oxidative stress is a term that increasingly occupies a central place in modern scientific research, as one of the key factors in the development of numerous chronic and degenerative diseases. It is a state of disturbed balance between the creation of reactive oxygen species (ROS) and the ability of the organism to neutralize them through antioxidant mechanisms [1]. The excessive creation of free radicals is significantly

influenced by factors from the external environment, such as pollution, tobacco smoke, UV radiation, unhealthy diet and psychophysical stress. Under physiological conditions, at low concentrations, ROS function as signaling molecules that participate in the regulation of cell proliferation, immune defense, and gene expression. However, when their production exceeds the body's capacity for antioxidant protection, oxidative stress occurs and damage to important biomolecules – lipids, proteins and DNA [2]. Chronic exposure to oxidative stress is associated with an increased risk for the development of cardiovascular diseases, diabetes, neurodegenerative disorders and malignancies.

β -cyclodextrin (β -CD) is a cyclic oligosaccharide that, thanks to its hydrophobic inner cavity and hydrophilic outer surface, can form inclusion complexes with various bioactive molecules, including antioxidants [3]. This ability makes β -CD particularly significant, as it enables the improvement of the solubility of poorly soluble antioxidant compounds, as well as the stabilization of these molecules, thus preventing their degradation under the influence of external factors [4]. In this way, β -cyclodextrin directly contributes to a more efficient delivery of antioxidants to target tissues and cells, thereby indirectly helping to suppress oxidative stress and its negative consequences on the body.

In this study, we investigated the *in vitro* antioxidant potential of an inclusion complex of 8,9-dihydroxy-2H-chromeno[2,3-d]pyrimidine-2,4(3H)-dione (CPD) with β -cyclodextrin (β -CD), referred to as CPD- β -CD, using the DPPH assay.

2. Methodology

2,2-Diphenyl-1-picrylhydrazyl (DPPH), nordihydroguaiaretic acid (NDGA), quercetin, and methanol were obtained from Merck. UV-Vis measurements were performed on a PerkinElmer Lambda 365 spectrophotometer.

2.1 Antioxidant activity of inclusion complex CPD- β -CD

The antioxidant activity of the inclusion complex CPD- β -CD was determined using the DPPH method [5]. The samples were prepared by dissolving 20 μ L of each stock solution (25, 50, or 100 μ M) in 980 μ L of methanol. After that, 1 mL of DPPH solution (0.05 mM in methanol) was added to 1 mL of prepared sample solutions. Samples were then shaken and left to incubate for 30 and 60 minutes at room temperature in the dark. Each sample was tested in triplicate and absorbance was measured at 517 nm in comparison to methanol blank. Quercetin and nordihydroguaiaretic acid (NDGA) were used as reference compounds and the obtained results were shown as mean values \pm standard deviation (SD) of three separate measurements.

3. Results and Discussion

Recently, DPPH radical scavenging was evaluated for the compound 8,9-dihydroxy-2H-chromeno[2,3-d]pyrimidine-2,4(3H)-dione (CPD) [5]. Here is presented antioxidant activity of inclusion complex (CPD- β -CD) obtained in the reaction of this compound

and β -cyclodextrin (Figure 1), as well as the reference antioxidants quercetin and NDGA (Table 1). Across all tested concentrations (25, 50, and 100 μM) and incubation times (30 and 60 min), **CPD- β -CD** showed very high antioxidant activity of 96–99%. **CPD** also exhibited strong dose- and time-dependent antioxidant activity of 91–97%. Quercetin and NDGA maintained consistently high DPPH radical inhibition under all conditions with 94–96% activity. Then, the IC_{50} values were determined to evaluate the antioxidant activity more precisely. Encapsulation in β -CD preserves the excellent antioxidant potential of **CPD**, almost complete inhibition at all tested concentrations and times, but gives a slightly higher IC_{50} value (4.5 μM) than the free compound **CPD** (3.5 μM). Possible explanation for this phenomenon are two effects that are counteracting: improved dispersion and solubility of the **CPD** inside β -CD that maintains a high percentage of inhibition, and the partial shielding of phenolic OH groups inside the β -CD cavity that can slow early hydrogen-atom transfer (HAT) and single-electron transfer (SET) steps in methanol, shifting the concentration–response curve and slightly increasing the IC_{50} value of **CPD- β -CD** [6-9]. Unsurprisingly, quercetin and NDGA remain the most potent antioxidants under these conditions, with IC_{50} values of 1.9 μM and 1.7 μM .

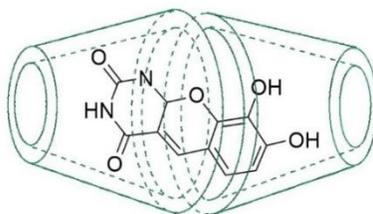


Figure 1. Structure of investigated inclusion complex **CPD- β -CD**

Table 1. *In vitro* interactions of selected compound **CPD** and inclusion complex **CPD- β -CD** with DPPH radical

Compound	DPPH scavenging ability (%)						IC_{50} (μM)
	25 μM		50 μM		100 μM		
	30 min	60 min	30 min	60 min	30 min	60 min	
CPD-β-CD	96.1 \pm 1.9	98.9 \pm 1.6	99.4 \pm 1.7	98.7 \pm 0.5	99.6 \pm 1.5	99.2 \pm 1.1	4.5 \pm 0.1
CPD	91.3 \pm 0.5	91.5 \pm 3.0	93.5 \pm 0.2	93.9 \pm 1.8	96.9 \pm 0.8	97.1 \pm 1.9	3.5 \pm 0.1
NDGA	94.6 \pm 0.7	94.6 \pm 0.6	94.2 \pm 0.7	94.2 \pm 0.7	94.5 \pm 0.2	94.1 \pm 0.7	1.7 \pm 0.1
Quercetin	95.3 \pm 0.8	95.1 \pm 0.9	96.8 \pm 1.0	96.5 \pm 0.9	95.1 \pm 0.9	95.4 \pm 0.8	1.9 \pm 0.1

4. Conclusions

In general, **CPD** is slightly more potent as an antioxidant, while **CPD- β -CD** inclusion complex achieves near the same DPPH radical scavenging activity and may offer formulation advantages typical of β -cyclodextrin complexes. These preliminary results indicate that this compound deserves further experimental research, such as verifying solubility, dispersion and stability improvements, as well as examining some other potential biological activities.

Acknowledgment

This research is funded by the Ministry of Education and Ministry of Science, Technological Development and Innovation, Republic of Serbia, No. 451-03-136/2025-03/200378.

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