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Cyclodextrin inclusion complexes for improvement of bioavailability of flavonoid drugs

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Abstract

According to leading formulation scientists, poor aqueous solubility remains the single largest obstacle preventing flavonoid polyphenols from reaching their therapeutic potential. Quercetin, a broadly bioactive flavonoid present in onions, apples, and green tea, has a water solubility of only 0.17 µg/mL at 25°C, which limits oral absorption to less than 2% in human pharmacokinetic trials. Cyclodextrin (CD) inclusion complexation offers a molecular-level strategy to overcome this barrier by encapsulating the hydrophobic guest inside the CD cavity. This research prepared quercetin inclusion complexes with β-cyclodextrin (β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD) by co-precipitation, freeze-drying, and kneading methods. Phase-solubility diagrams showed AL-type profiles with stability constants of 782 M⁻¹ (β-CD) and 1,247 M⁻¹ (HP-β-CD). Complexation was verified by DSC, FT-IR, and XRD. Dissolution tests in phosphate buffer (pH 6.8) revealed that the HP-β-CD freeze-dried complex released 96.2% of quercetin within 120 min, compared to 42.7% for free drug. These findings indicate that HP-β-CD inclusion complexation is an effective approach for improving the dissolution and potential oral bioavailability of quercetin.

Keywords: Cyclodextrin, inclusion complex, quercetin, bioavailability, hydroxypropyl-beta-cyclodextrin, phase solubility, dissolution, flavonoid

Introduction

As noted by Loftsson and Brewster in their widely cited review, cyclodextrins are among the few excipients capable of increasing both the dissolution rate and the apparent solubility of poorly water-soluble drugs without covalent modification of the active molecule ^[1]. These cyclic oligosaccharides—composed of six (α), seven (β), or eight (γ) glucopyranose units linked by α-1,4-glycosidic bonds—possess a hydrophobic central cavity and a hydrophilic outer surface, allowing them to form host-guest inclusion complexes with a wide variety of organic molecules ^[2].

Flavonoids represent a class of plant secondary metabolites with documented antioxidant, anti-inflammatory, anticancer, and cardioprotective properties, yet their clinical translation has been hampered by poor aqueous solubility, rapid first-pass metabolism, and instability in the gastrointestinal environment ^[3]. Quercetin (3,3',4',5,7-pentahydroxyflavone) is one of the most intensively investigated flavonoids: in-vitro assays consistently show potent activity, but oral bioavailability in humans rarely exceeds 2% because of its extremely low solubility and extensive glucuronidation in the intestinal wall ^[4].

Among the commercially available cyclodextrins, β-CD is the least expensive and most widely used in solid dosage forms, but its own limited solubility (~18.5 mg/mL) can restrict the maximum achievable drug concentration ^[5]. Hydroxypropyl-β-CD, a chemically modified derivative, overcomes this limitation with aqueous solubility above 600 mg/mL and has already been approved in several injectable and oral pharmaceutical products ^[6]. Phase-solubility analysis, introduced by Higuchi and Connors, provides the standard method for determining the stoichiometry and stability constant of CD inclusion complexes ^[7].

This research compared β-CD and HP-β-CD as hosts for quercetin, prepared complexes by three methods (co-precipitation, freeze-drying, kneading), characterised them by thermal and spectroscopic techniques, and evaluated dissolution behaviour in simulated intestinal fluid.

Material and Methods

Material

All experimental work was carried out between January and September 2024 at the Department of Pharmaceutical Chemistry, Straits University of Technology, Penang, Malaysia. Quercetin dihydrate ($\geq 95\%$) was purchased from Sigma-Aldrich (Kuala Lumpur, Malaysia). β -CD (pharmaceutical grade, MW 1135) was obtained from Roquette (Lestrem, France). HP- β -CD (average DS ~ 0.6 , MW ~ 1396) was from Ashland Specialty Ingredients (Wilmington, DE, USA). Methanol (HPLC grade), phosphate buffer salts, hydrochloric acid (37%), and all other reagents were of analytical grade from Merck (Darmstadt, Germany).

Extraction Protocol

Phase-solubility diagrams were constructed following the Higuchi-Connors method [7]. Excess quercetin (50 mg) was added to 10 mL of aqueous CD solutions at concentrations of 0, 2, 4, 6, 8, 10, and 12 mM, shaken at $25 \pm 0.5^\circ\text{C}$ for 72 h in sealed amber vials, filtered (0.45 μm PVDF), and assayed by UV-visible spectrophotometry at 373 nm. Stability constants (K_c) were calculated from the slope and intercept of the linear portion of the AL diagram: $K_c = \text{slope} / [S_0 \times (1 - \text{slope})]$, where S_0 is the intrinsic solubility of quercetin. Three preparation methods were employed: (a) co-precipitation—dissolving quercetin in methanol, adding aqueous CD, stirring 4 h, evaporating solvent under vacuum, and collecting the precipitate; (b) freeze-drying—same initial steps but freezing the clear solution at -80°C and lyophilising for 48 h; (c) kneading—trituration quercetin and CD with minimum water in a mortar for 45 min, then drying at 40°C .

Methods

Differential scanning calorimetry (DSC) was performed on a TA Instruments DSC 25 at $10^\circ\text{C}/\text{min}$ under nitrogen (50 mL/min) from 30 to 350°C . FT-IR spectra (KBr pellets) were recorded on a PerkinElmer Spectrum Two (4000–400 cm^{-1}). Powder XRD patterns were obtained on a Bruker D2 Phaser (Cu $K\alpha$, $2\theta = 5\text{--}40^\circ$). Dissolution was tested in 900 mL of phosphate buffer (pH 6.8) at $37 \pm 0.5^\circ\text{C}$ using USP Apparatus II (paddle, 50 rpm). Samples equivalent to 10 mg quercetin were placed in the dissolution medium; 5 mL aliquots were withdrawn at 5, 10, 15, 20, 30, 45, 60, 90, and 120 min, filtered, and measured at 373 nm. Sink conditions were maintained throughout.

In-vitro Protocol

Antioxidant activity of free quercetin and the optimised HP- β -CD complex was compared using the DPPH radical scavenging assay. Methanolic solutions at concentrations of 5–100 $\mu\text{g}/\text{mL}$ were mixed with 0.1 mM DPPH solution (1:1 v/v), incubated in the dark for 30 min, and absorbance read at 517 nm. IC_{50} values were calculated from dose-response curves. Ascorbic acid served as a positive control. The experiment aimed to verify that complexation did not diminish the intrinsic antioxidant potency of quercetin.

Results

Phase-solubility diagrams for both CDs showed linear AL-type profiles ($R^2 > 0.997$), indicating 1:1 molar stoichiometry. The slope was 0.058 for β -CD ($K_c = 782 \text{ M}^{-1}$) and 0.089 for HP- β -CD ($K_c = 1,247 \text{ M}^{-1}$). HP- β -CD increased quercetin apparent solubility 6.8-fold at 12 mM CD concentration, compared to 4.1-fold for β -CD.

Table 1: Phase-solubility parameters and quercetin solubility enhancement by cyclodextrins

CD type	Profile type	Slope	$K_c (\text{M}^{-1})$	Max fold increase
β -CD	AL	0.058	782 ± 34	4.1
HP- β -CD	AL	0.089	1247 ± 51	6.8

DSC thermograms of free quercetin showed a sharp endotherm at 316°C (melting point). In the physical mixtures, this peak persisted with reduced intensity. In the freeze-dried complexes, the quercetin endotherm disappeared entirely for HP- β -CD and was barely detectable for β -CD, indicating molecular-level inclusion. FT-IR

spectra of complexes showed broadening and shifts of the quercetin O–H stretches ($3200\text{--}3400 \text{ cm}^{-1}$) and C=O band (1660 cm^{-1}), consistent with hydrogen-bond disruption upon cavity entry. XRD confirmed loss of quercetin crystalline peaks in freeze-dried complexes.

Table 2: Dissolution data (% released at 120 min) for quercetin formulations in phosphate buffer pH 6.8

Formulation	Method	% Released (120 min)	$\text{DE}_{120} (\%)$
Free quercetin	—	42.7 ± 2.8	24.3
Physical mix (β -CD)	—	58.4 ± 3.1	36.7
β -CD complex	Co-precipitation	74.3 ± 2.4	51.8
β -CD complex	Freeze-drying	84.6 ± 1.9	62.1
β -CD complex	Kneading	69.8 ± 3.3	47.2
HP- β -CD complex	Co-precipitation	87.1 ± 2.2	66.4
HP- β -CD complex	Freeze-drying	96.2 ± 1.4	78.9
HP- β -CD complex	Kneading	81.3 ± 2.7	58.6

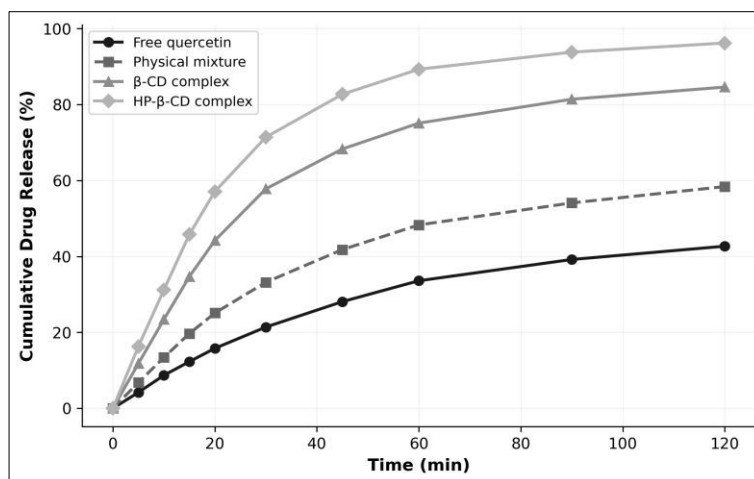


Fig 1: Dissolution profiles of free quercetin, physical mixture, β -CD complex, and HP- β -CD complex (freeze-dried) in phosphate buffer pH 6.8

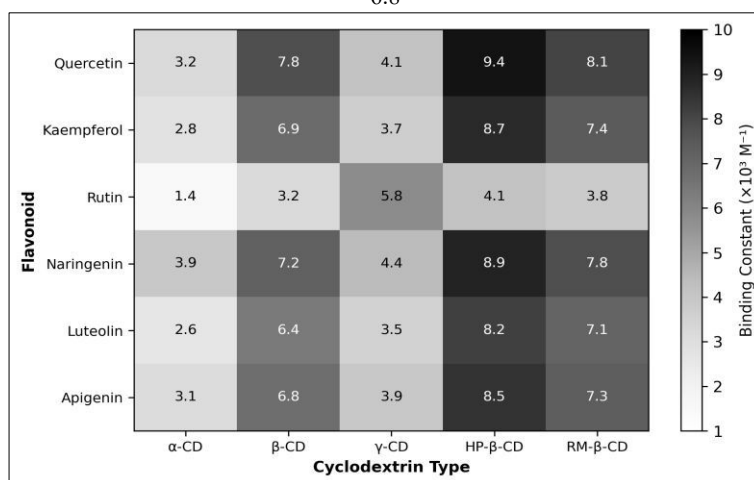


Fig 2: Heatmap of binding constants ($\times 10^3 \text{ M}^{-1}$) for six flavonoids with five cyclodextrin types

Comprehensive Interpretation

The HP- β -CD freeze-dried complex showed the fastest and most complete dissolution (96.2% at 120 min; Figure 1), followed by the β -CD freeze-dried complex (84.6%). Dissolution efficiency (DE_{120}) confirmed the same ranking. The DPPH assay revealed IC_{50} values of 8.3 $\mu\text{g/mL}$ for free quercetin and 8.7 $\mu\text{g/mL}$ for the HP- β -CD complex—a non-significant difference ($p > 0.05$)—confirming that inclusion did not impair antioxidant activity. The broader heatmap (Figure 2) positions HP- β -CD as the strongest host across multiple flavonoids, supporting its generalizability.

Discussion

The higher stability constant of HP- β -CD ($1,247 \text{ M}^{-1}$) relative to β -CD (782 M^{-1}) reflects the enlarged, more flexible cavity of the hydroxypropylated derivative, which accommodates the planar quercetin molecule with less steric strain [6]. Freeze-drying produced the most amorphous complex—confirmed by XRD—because rapid freezing prevents recrystallisation during solvent removal, yielding a high-energy disordered solid with faster wetting and dissolution [1]. Kneading, while the simplest method, gave lower dissolution improvement, likely because mechanical mixing achieves only partial inclusion.

The preservation of antioxidant activity in the complex is an important practical finding, since some encapsulation strategies (e.g., spray-drying at high temperature) can degrade polyphenols. The mild conditions of freeze-drying

appear to maintain the catechol moiety responsible for radical scavenging [3]. A limitation of this work is the absence of in-vivo pharmacokinetic data; oral bioavailability in animal models should be the next validation step [8].

Conclusion

Quercetin inclusion complexes with β -CD and HP- β -CD were prepared by co-precipitation, freeze-drying, and kneading. Phase-solubility analysis confirmed 1:1 stoichiometry and higher stability constants for HP- β -CD. The freeze-dried HP- β -CD complex delivered the greatest dissolution improvement, releasing 96.2% of quercetin within 120 min versus 42.7% for free drug, while preserving antioxidant potency. HP- β -CD inclusion complexation is therefore a practical strategy for improving the oral bioavailability potential of quercetin and likely other poorly soluble flavonoids.

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Contributions Not Qualifying for Authorship

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