RESEARCH ARTICLE

Potential of Native Cyclodextrins and L-Lysine for Enhancing Ellagic Acid Aqueous Solubility

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Received: 30th March, 2024; Revised: 03rd May, 2024; Accepted: 20th May, 2024; Available Online: 25th June, 2024

ABSTRACT

This study aimed to examine the influence of β cyclodextrin (βCD) and γ cyclodextrin (γCD) alone and in combination with L-lysine on ellagic acid (EA) solubility. Indeed, complexation with cyclodextrins can be used to improving the solubility of drugs poorly soluble in water such as EA. EA is a Biopharamceutical Classification System (BCS) IV bioactive polyphenol with numerous therapeutic activities, including antimalarial activities. However, its unfavorable physicochemical properties limit its therapeutic use. Therefore, after a phase solubility study, we successfully prepared EA-βCD and γ CD binary solid complexes using the freeze-drying technique. Methods including proton nuclear magnetic resonance (^{1}H-NMR) analysis and fourier-transform infrared (FTIR) spectroscopy were used to characterize the inclusion behaviors of the complexes of EA with cyclodextrins both in solution and solid forms. The results of the phase solubility study indicated Ap-type diagrams of EA with the two cyclodextrins (CDs). The solubility of EA was multiplied by 9.92 and 2.98 in the presence of γCD and βCD respectively. Moreover, the complexes characterization by FTIR spectroscopy revealed the involvement of the C=O and EA OH groups in the interaction with the CDs. The results of NMR spectroscopic characterization revealed that the formation of EA inclusion complexes with βCD was partial. However, these results did not indicate the appearance of EA inclusion complexes with γCD. These relatively modest results in terms of increased EA solubility, obtained with cyclodextrins, prompted us to use a third compound, l-lysine, to enhance CDs complexation. Thus, the formation of ternary complexes led to a very significant increase EA solubility. Indeed, the incorporation of EA into EA-L-lysine-βCD and γ CD complexes increased its water solubility at pH 7.4 by a factor of 555 and 663, respectively.

Keywords: Antimalarial, L-lysine, Cyclodextrins, Ellagic acid, Solubility.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.2.48

How to cite this article: Nyamba I, Sombie CB, Lechanteur A, Semde R, Evrard B. Potential of Native Cyclodextrins and L-Lysine for Enhancing Ellagic Acid Aqueous Solubility. International Journal of Drug Delivery Technology. 2024;14(2):925-931.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The number of deaths due to malaria is still very high worldwide. Indeed, 627,000 deaths were reported in 2020.[1] Malaria is transmitted to humans by the bite of a mosquito called the female *Anopheles*. It is caused by four species of parasites of the genus *Plasmodium*, namely *Plasmodium vivax, P. knowlesi, P. falciparum, P. ovale,* and *P. malariae*.[2] Africa is by far the most affected continent, accounting for 95% of malaria deaths. The main plasmodial species responsible for these deaths is *P. falciparum*, and children under 5 are the most affected.[3,4] Artemisinin-based combination therapies (ACTs), are the drugs currently recommended by World Health Organization (WHO) for the treatment of malaria. Depending on the severity of the disease, they can be administered orally, intravenously or intramuscularly. However, despite the existence of this effective therapeutic arsenal, the number of malaria patients has been increasing for years, rising from 228 million in 2018 to 247 million in 2020. One of the main explanations is the spontaneous development and spread of *plasmodium* resistance to artemisinin derivatives.[1,5] Indeed, almost all antimalarial drugs available in Southeast Asia are subject to resistance by *P. falciparum*. Moreover, an artemisinin-resistant strain of the same species was recently isolated in Equatorial Guinea.[6,7] Therefore, the search for new antimalarial chemotherapies has become increasingly urgent.[3,8]

Ellagic acid (EA) could be a promising candidate. Indeed, EA is an active pharmaceutical ingredient (API) with antimalarial and other promising therapeutic activities,

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isolated from medicinal plants employed by traditional healers for the treatment of the febrile disease induced by Plasmodium species, including *Terminalia mollis*, as well as *Punica granatum*.[9] EA inhibits *P. falciparum* growth, both in vitro on multiple sensitive and resistant strains (IC_{50} between 105–330 nM), and *in-vivo* after intraperitoneal injection (ED50 ~ 1 mg/kg/d). Furthermore, this molecule is globally non-toxic since no major toxic or side effects were reported even at high doses, more than 5 g/kg/d orally. Unfortunately, its oral efficacy was significantly reduced (ED50 > 100 mg/kg), what seems to mainly originate from its low oral bioavailability, less than 1% in humans. Indeed, the reasons for ellagic acid's low plasma concentrations are its low water solubility, its extensive metabolic transformation and degradation prior to absorption.[10,11] Improving aqueous solubility of EA may lead to a better bioavailability.[12] In this sense, different approaches such as particle size reduction,[13] lipid formulations,[14] and solid dispersions formation[15,16] have been proposed by formulation scientists. Complexation utilizing CDs provides a method to effectively improve the physicochemical characteristics of pharmaceutical compounds.[16] Nevertheless, constraints such as formulation size, manufacturing capabilities, and expenses impose limitations on the quantity of CD that can be integrated into the majority of drug formulations. This suggests that merely one in every three or four CD molecules is engaged in forming a pharmacologically significant complex with the drug.[16,17] Therefore, salt formation using counterions such as L-lysine can be envisaged to improve complexation efficiency.[18] Depending on the physico-chemical properties of the guest molecule namely its size and the size of the cyclodextrin cavity, complexes of different stoichiometry can be formed. The structural formula EA and native cyclodextrins are shown in Figure 1. Different publications have documented a rise in EA solubility due to its combination with βCD and its derivatives.[19,20] The researchers behind these publications did not examine all CDs, particularly the γ-CD which possesses a cavity size more appropriate for EA inclusion. Furthermore, as far as we are aware, the synergistic impact of natural CDs and L-lysine on EA solubility has not been assessed yet.

The objective of the current study was to assess the impacts of β-cyclodextrin and γ-cyclodextrin individually as well as in conjunction with L-lysine on EA solubility and to investigate the characteristics of the relationships between EA and the various CDs.

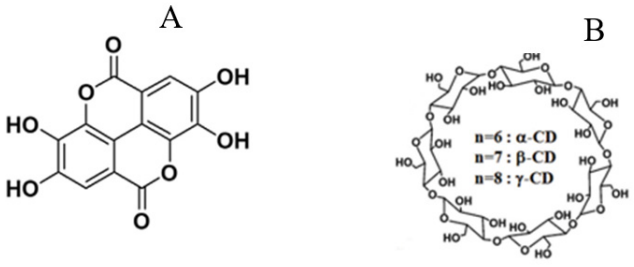

Figure 1: structure of Ellagic acid (A), Native cyclodextrins (B)

METHODS AND MATERIALS

Materials

Ellagic acid (Fluorochem Ltd, United Kingdom), acetonitrile (J.T. Baker, Deventer, Netherlands) and monosodium phosphate (Ph. Eur, Merck, Darmstadt, Germany) were purchased. Deuterium oxide, 99.99% and L-Lysine were obtained from Sigma Aldrich, (Saint-Louis, USA). γ-CD (Cavamax™, Ashland Industries Europe GMBH, Schaffhouse, Suisse) was purchased. β-cyclodextrin was given as a gift by Roquette®. A milliporea system (Milli-Q) was used for purified water production.

Methods

Study of phase solubility

The investigation of phase solubility was conducted in accordance with the methodology established by Higuchi and Connors as replicated by Xiao *et al.*[21] Various solutions containing escalating levels of β-CD (0–14 mM) and γ-CD (0–150 mM) were formulated and supplemented with an excess amount of ellagic acid. The mixture underwent continuous agitation within a precisely regulated temperature chamber set at 37 ± 0.5°C, rotating at a consistent velocity of 144 revolutions per minute (rpm) over a duration of 24 hours. Subsequently, the suspension was subjected to filtration using a syringe equipped with a 0.45 µm PVDF filter for subsequent chromatographic examination. The phase solubility diagram was employed to deduce the stability constants of the complexation (K1:1 and K1:2) in accordance with the equations 1 and 2, wherein [S0] represents the intrinsic solubility of ellagic acid in aqueous media, while [St] and [Lt] denote the concentrations of ellagic acid and cyclodextrin in the solution, respectively.

$$K\,1{:}1 = \frac{Slope}{S0\,(1 - slope)}$$

$$\frac{[St]-[S0]}{[Lt]} = K1{:}1[S0] + K1{:}1K1{:}2[S0][St].$$[22]

Preparation of EA and CDs complexes

Freeze-drying technique was used to prepare the complexes. EA solutions obtained by complexation according to the method previously described (section 2.2.1), using β-CD (10 mM) and γ-CD (50 mM) were produced. After production, 50 mL of each solution was divided equally into open glass containers and freeze-dried according to the method previously described by N. Koch, *et al.*[23]

HPLC analysis

A high-performance liquid chromatographic method previously described by I. Nyamba, *et al.*[15] was used for the quantification of the EA.

Characterization by Fourier transforms infrared spectroscopy

The spectra of EA, native cyclodextrins and CD-EA complexes were conducted in accordance with the methodology previously outlined I. Nyamba, *et al.*[15] The FTIR spectrophotometer employed for the analysis was a Perkin Elmer instrument from

Waltham, USA, which was equipped with a diamond crystal ATR device and a DTGS detector, utilizing a scanning range spanning from 650 to 4000 cm^{-1}. A comparative analysis was performed between the FTIR spectra of the complexes and those of pure EA and cyclodextrins.

^{1}H-NMR spectroscopy

Investigating possible encapsulation modes of EA/CD, we analyzed the ^{1}H-NMR spectra of inclusion complexes of EA with CDs. The spectra were determined by the method previously used by H Zimé-Diawara *et al.*[24]

Preparation of l-lysine salts and EA-l-lysine-CDs ternary

Excess amounts of ellagic acid (1 g) were added to 20-mL solutions of 100 mM L-lysine, 50 mM γCD + 100 mM L-lysine and 10 mM β-CD + 100 mM L-lysine. The mixtures were agitated in a temperature-controlled water bath at 37 ± 0.5°C for a duration of 24 hours, followed by filtration through a 0.45 µm filter and subsequent analysis of ellagic acid content.

RESULTS AND DISCUSSION

Study of Phase Solubility

The studies of phase solubility were conducted to examine the solubility of EA in β-CD and γ-CD and to identify the resultant diagram classification. Despite its relatively limited solubility in water and organic solvents, β-CD is extensively utilized in pharmaceutical formulations owing to its cost-effectiveness, substantial production volume (exceeding 10,000 tons yearly with an average wholesale price of around 5 USD per kg), and its cavity's compatibility with typical guests having molecular weights ranging from 200 to 800 g/mol.[25] In general, γ-cyclodextrin (γ-CD) is utilized with lower frequency. Certainly, following oral administration, γ-CD undergoes rapid and complete digestion within the gastrointestinal tract through the action of α-amylase, while αCD and βCD, on the other hand, are primarily metabolized by colonic bacteria. Nevertheless, γ-CD possesses a larger cavity size that is conducive to the encapsulation of molecules like EA, thus sparking interest in the utilization of these natural CDs in our investigation. A depiction in Figure 2 illustrates the phase solubility patterns of EA with β-CD and γ-CD. In both instances, non-linear solubility graphs were generated, falling under the classification of type Ap. Indeed, Higuchi and Connors established a categorization system for the various types of diagrams that represent substrate-ligand interactions, a framework that can be applied to interactions between cyclodextrin and guest molecules.[26] Thus, they defined two broad categories of solubility diagrams, namely type A and type B profiles. Type A diagrams are representative of the formation of soluble complexes. When the complexes stoichiometry formed is one molecule of cyclodextrin for one molecule of API, the diagram is of A_L type. The phase solubility diagram of Ap type, the inclusion complexes formed are initially of 1:1 stoichiometry for low CDs concentrations before the formation of complexes of 1:2 stoichiometry.[27] The A_N type diagrams are difficult to interpret. They are

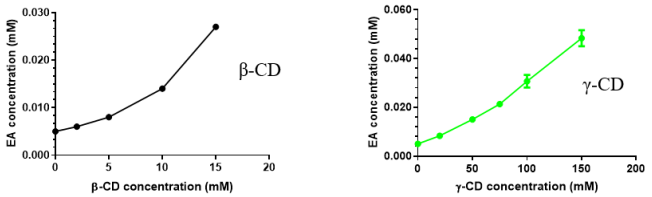

Figure 2: Phase solubility diagram of EA in water at 37°C depending on the cyclodextrin concentration (β-CD and γ-CD)

characterized by a decrease in the solubilization of the active ingredient at higher cyclodextrin concentrations due either to the self-assembly of cyclodextrin molecules or to a modification of the dielectric constant of the medium. Type B diagrams indicate the formation of a complex with limited solubility in the medium. The BS diagram indicates an initial increase in apparent solubility, followed by a plateau evolution and the diagram is B_I if the complex formed is insoluble.[28] The diagram of β-CD appears linear for concentrations below 10 mM. Our result is similar to that of Savic *et al.* who found an AL-type diagrams with β-CD and HP β-CD.[29] Bulani *et al.* on the other hand obtained an A_N type diagram with βCD and HP βCD with concentrations ranging from 0 to 24 Mm.[19,20] These disparities could be explained by different methodological approaches, in particular the complexation temperature. The stability constants of the β-CD/EA complex were 178 and 47 M^{-1} for K 1:1 and K 1:2, respectively. With the γ-CD/EA complex were 136 and 63 M^{-1}, respectively for K 1:1 and K 1:2. Moreover, increasing CD concentrations enhance the EA solubility. The solubility of EA increased from 1.48 ± 0.37 to 4.40 ± 0.27 and 14.68 ± 1.72 µg/mL, respectively with β-CD and γ-CD corresponding to a 2.98 and 9.92-fold increase in solubility. At equal concentrations, β-CD increases EA solubility more than γ-CD. However, β-CD 's low aqueous solubility limits its complexation capacity. This is why most pharmaceutical applications use hydroxypropyl β-CD.

Analysis by Fourier Transforms Infrared Spectroscopy

Fourier transforms infrared spectroscopy (FTIR) spectroscopy has been utilized over an extensive period to directly detect the creation of CDs-guest inclusion complexes. This examination promptly offers insights into the inclusion of a guest within the CD cavity through a comparative analysis of the complex's spectrum with those of the guest and the relevant CD. Typically, the CD bands experience a straightforward alteration due to complexation, while the bands linked to the segment of the guest translocated into the CD cavity are prone to being obscured or modified by the CD spectrum's bands. The Figure 2 exhibit the spectra of EA, β-CD, γ-CD, and the EA/CD complexes. The hydroxyl groups (O-H) stretching vibration is attributed to the bands at 3474 and 3151 cm^{-1} in the pure EA spectrum, with the ketone groups (C=O) stretching vibration at 1717 cm^{-1}, and the C=C stretching vibration at 1615, 1582, and 1507 cm^{-1}. The spectrum of βCD shows characteristic bands of the OH groups at 3266 cm^{-1}, the C—H bonds at 2917 cm^{-1}, and at 1636 cm^{-1}. The spectra of EA, β-CD, and their complex

are depicted in Figure 3 A. The γ-CD spectrum (Figure 3B) displays an O-H stretching band at 3259 cm^{-1}, a C-H stretching band at 2921 cm^{-1}, an H-O-H bending band at 1628 cm^{-1}, and a C-O-C stretching vibration band at 1006 cm^{-1}. The freeze-dried complex spectra closely resemble those of native cyclodextrins, possibly due to the lower EA concentration in the inclusion complex. Nevertheless, noticeable changes in intensity and position are observed in some EA bands. For instance, the distinct peak at 3474 cm^{-1}, related to the valence vibration of the O–H group of EA, is absent in the complex spectrum, indicating potential involvement of EA's O–H groups in CD interaction. Moreover, valence vibration bands of the O-H groups of β-CD and γ-CD in the complex spectra are shifted towards higher wavenumbers. The ketone group's valence vibration band of EA at 1722 cm^{-1} appears in the EA/β CD complex spectrum but at 1695 cm^{-1}. This discrepancy suggests interaction between this group and the CD. Bands at 1618 and 1580 cm^{-1}, associated with the valence vibration of the C=C bond of EA's aromatic ring, are noticeable in the lyophilized β-CD and γ-CD complex spectra. Consequently, the slight peak shifts and disappearance of characteristic EA bands in the complex spectra imply absence of covalent bond formation, indicating interactions solely between the ketone and OH groups of the compounds.

^{1}H-NMR analysis

To investigate the potential inclusion behavior of EA by the native CDs, the ^{1}H-NMR spectra of β-CD, γCD, EA- β-CD, and EA- γCD (Figure 4) were acquired and juxtaposed. The chemical shifts of the γCD and βCD protons in both free and complexed states are detailed in Table 1A and B. NMR spectroscopy stands out as one of the most valuable and comprehensive analytical methods for scrutinizing interactions between CDs and guest compounds.[30] The generation of cyclodextrin-host complexes indeed results in chemical shift alterations, which can be utilized to offer conclusive proof of the presence or absence of inclusion complexes.[31] The segment of the cyclodextrin molecule implicated in the interaction can be discerned by analyzing these modifications.[32] Evidence supporting the formation of inclusion complexes can be observed through alterations in the chemical resonance of protons positioned at 3 (H3) and 5 (H5) within the cyclodextrin cavity.[25] Moreover, one can assess the extent of permeation of the active substance within the cyclodextrin. Specifically, as the H-5 proton is situated at a greater depth compared to the H3 proton within the cyclodextrin cavity, a modification in its chemical displacement signifies a profound integration of the API in the cyclodextrin. In cases where the alteration pertains solely to the chemical displacement of the H3 proton, the integration of the active substance within the cyclodextrin cavity is considered to be incomplete.[21] One of the primary limitations of proton-nuclear magnetic resonance (^{1}H-NMR) spectroscopy is the inadequate solubility of samples in

Table 1: A ^{1}H chemical shifts corresponding to β-CD in free and in complexed state; B ^{1}H chemical shifts corresponding to γ CD in free and in complexed state

A

Proton	*δ (free) ppm*	*δ (complex) ppm*	*Δδ = δ (complex)- δ (free)*
β-CD			
H1	4,9863	4,9857	-0,0006
OH2	3,8816	3,8808	-0,0008
OH3	3,8816	3,8808	-0,0008
OH6	3,8214	3,8209	-0,0005
H2	3,7937	3,7939	0,0002
H3	3,7937	3,7939	0,0002
H5	3,7807	3,7797	-0,001
H6	3,7727	3,7716	-0,0011
H4	3,5756	3,575	-0,0006

B

Proton	*δ (free) ppm*	*δ (complex) ppm*	*Δδ = δ (complex)- δ (free)*
γ-CD			
H1	5,0242	4,9474	-0,0768
OH2	3,8479	3,7711	-0,0768
OH3	3,8479	3,7711	-0,0768
OH6	3,7833	3,7062	-0,0771
H2	3,7644	3,6871	-0,0773
H3	3,7644	3,6871	-0,0773
H5	3,5835	3,5081	-0,0754
H6	3,5636	3,4882	-0,0754
H4	3,5011	3,4239	-0,0772

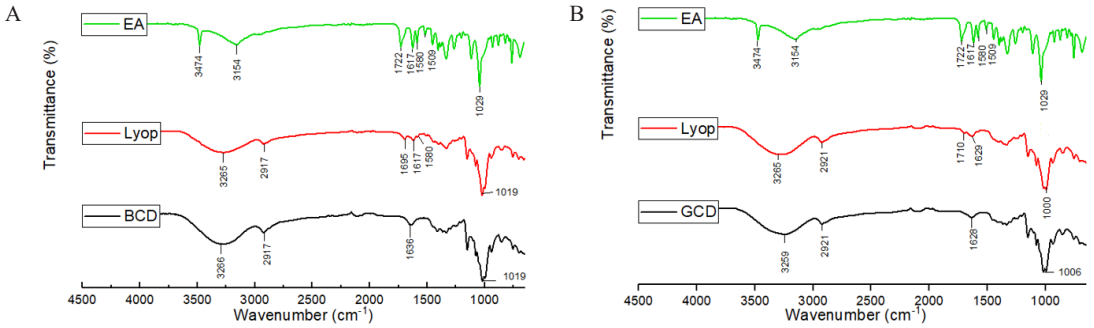

Figure 3 : (A) Spectra of EA (green), and βCD (black), freeze-dried complex (red) ; (B) FTIR spectra of EA (green), γ CD (black) and freeze-dried complex (red)

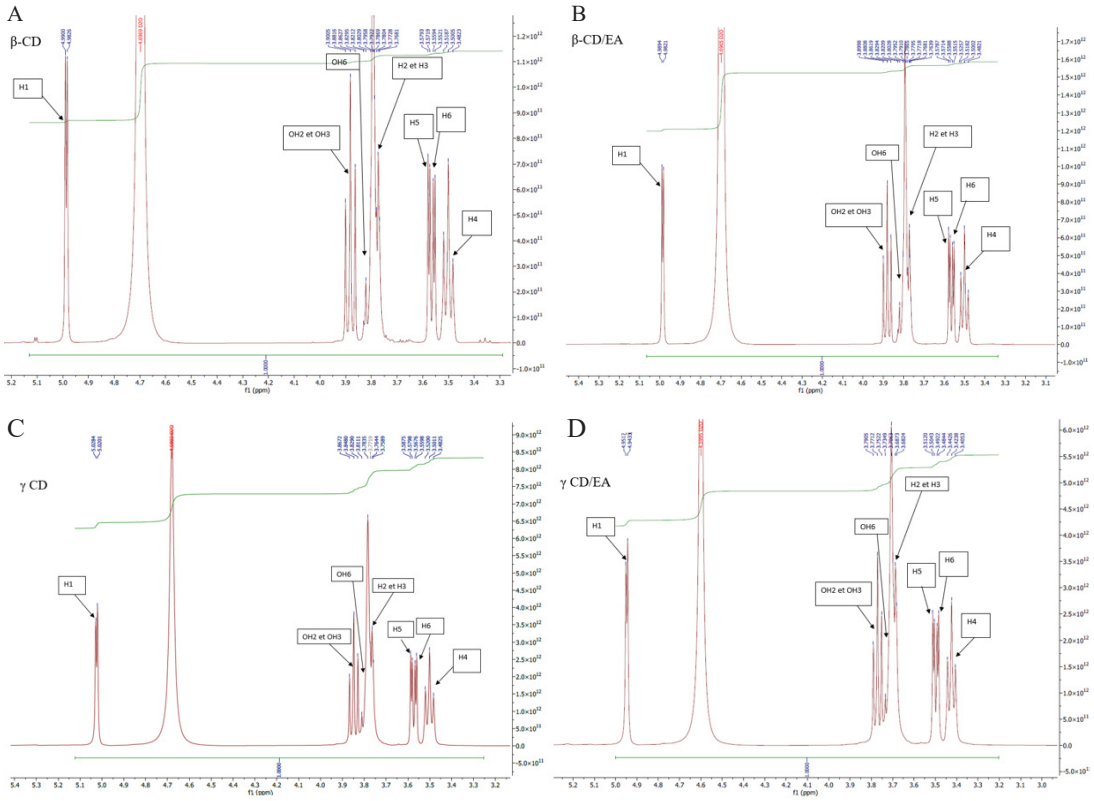

Figure 4 : (A) ^{1}H-NMR spectra of β-CD, (**B)** EA- β-CD complex in D2O at 25°C and (**C)** γCD and (D) EA- γCD complex in D2O at 25°C

Table 2 : Solubility of ellagic acid in L-lysine solution (100 mM) and in combined CD and L-lysine solutions

Solutions	*Dissolved ellagic acid (mg/mL)*
L-Lysine (100 mM)	18.5 ± 0.45
β-CD (10 mM) + L-lysine (100 mM)	18.88 ± 0.24
γ CD (50 mM) + L-lysine (100 mM)	22,54 ± 0.37

deuterated water, thus frequently requiring the utilization of alternative solvents that could potentially influence host-guest interactions in contrast to the basic aqueous environment.[33] The chemical shift values of H-3 and H-5 protons in complexes EA- βCD and EA- γ CD are 0.0002, -0.001, -0.0773, and -0.0754 ppm, respectively. Although these alterations may not be deemed substantial when compared to those of other protons, there is a noticeable upward displacement of the H-3 proton in the EA-βCD complex, suggesting a degree of host inclusion. EA, being a predominantly planar molecule, experiences limitations in its accommodation within the CD cavity due to steric hindrances. This limitation could elucidate the minor variations observed in the NMR spectra. The FTIR and ^{1}H-NMR analyses indicate that the interaction between EA and β-CD primarily involves the H-3 proton of CD, as well as the C=O and O-H groups of EA. These findings closely resemble those reported by Bulani *et al.*[20] Nevertheless, the chemical shifts acquired from the EA- γCD complex did not suggest the creation of an inclusion complex.

Effects of Both CDs and L-Lysine on the Solubility of Ellagic Acid

The solubility of EA in the solutions containing L-lysine, as well as in binary solutions of βCD-L-lysine and γCD-L-lysine, is presented in Table 2. These recorded solubility values signify substantial increases of 544-, 555-, and 663-fold in the solubility of EA in aqueous medium at pH 7.4 when L-lysine, βCD-lysine, and γCD-L-lysine are present, respectively. Notably, the solubility of EA is influenced by the pH of the solution, with its peak value being 33.1 µg/mL, achieved in phosphate buffer at pH 7.4 to 14 L-lysine, an indispensable amino acid, serves as a crucial component in pharmaceutical research by functioning as a counterion for salt formation. The utilization of salt formation represents a favored strategy aimed at improving the aqueous solubility and bioavailability of ionizable drugs such as EA.[34,35] Its pKa is 10.93. Thus, in accordance with the pKa principle, it is plausible that L-lysine may serve as a viable option for forming a salt with EA, leading to a notable enhancement in solubility. Nevertheless, the coexistence of L-lysine and the two indigenous CDs failed to elicit the anticipated cooperative impact on enhancing EA solubility. Notably, the processes of drug ionization and salt formation represent well-established strategies capable of enhancing the efficacy of cyclodextrin complexation in aqueous mediums through augmenting the apparent intrinsic solubility of the drug.[18] The enhancement of cyclodextrins' complexation efficiency in aqueous solutions may be increased through drug

ionization and salt formation. This advancement is observed when the creation of a more water-soluble salt or the ionization of the drug does not markedly diminish its capacity to develop CD complexes.[18]

CONCLUSION

In this work, two natural CDs, β-CD and γCD were tested to study an inclusion complex formation of EA and to evaluate the effect of the presence of these CDs on apparent solubility of EA. Phase solubility studies yielded Ap-type diagrams of EA with both CDs. The solubility of EA was multiplied by 9.92 and 2.98 in the presence of γCD and βCD respectively. Moreover, the characterization of the complexes by FTIR spectroscopy revealed the involvement of the ketone groups (C=O) and OH groups of EA in the interaction with the CDs. The results of NMR spectroscopic characterization revealed the formation of partial inclusion complexes of EA with β-CD. However, these results did not indicate the formation of inclusion complexes with γCD. The size of the β-CD cavity seems to be the most suitable for EA inclusion, but given the low solubility of this CD, results of solubility enhancement are rather limited. More soluble β-CD derivatives should be tested. The relatively modest effect of both CDs in terms of increasing EA solubility led us to use another strategy involving L-lysine, to improve solubility of EA and complexation efficiency. The use of L-lysine alone led to a very significant increase in EA solubility. Indeed, the apparent solubility values obtained, corresponded to 544-fold, increases in EA solubility in water at pH 7.4. On the contrary, the concomitant use of L-lysine and cyclodextrin did not produce synergistic results in terms of increased EA solubility. In view of these results, salt formation using suitable counterions could be an interesting approach to the development of solid pharmaceutical forms based on EA.

REFERENCES

1. WHO. Word Malaria Report 2021. Word Malaria report Geneva: World Health Organization. (2021). Licence: CC. 2021. 2013–2015 p.
2. Haidara M, Haddad M, Denou A, Marti G, Bourgeade-Delmas S, Sanogo R, *et al. In-vivo* validation of anti-malarial activity of crude extracts of Terminalia macroptera, a Malian medicinal plant. Malar J [Internet]. 2018;17(1):1–10. Available from: https://doi.org/10.1186/s12936-018-2223-7
3. Kos J, Degotte G, Pindjakova D, Strharsky T, Jankech T, Gonec T, *et al.* Insights into Antimalarial Activity of N-Phenyl-Substituted Cinnamanilides. Molecules. 2022;27(22):7799.
4. Aditya NP, Vathsala PG, Vieira V, Murthy RSR, Souto EB. Advances in nanomedicines for malaria treatment. Adv Colloid Interface Sci [Internet]. 2013;201–202:1–17. Available from: http://dx.doi.org/10.1016/j.cis.2013.10.014
5. Uwimana A, Legrand E, Stokes BH, Ndikumana JLM, Warsame M, Umulisa N, *et al.* Emergence and clonal expansion of in vitro artemisinin-resistant Plasmodium falciparum kelch13 R561H mutant parasites in Rwanda. Nat Med. 2020;26(10):1602–8.
6. Müller O, Lu GY, Von Seidlein L. Geographic expansion of artemisinin resistance. J Travel Med. 2019;26(4):1–6.
7. Baudou E, Lespine A, Durrieu G, André F, Gandia P, Durand C, *et al.* c or r e sp ondence Deletion of. new engl J Med. 2020;Feb ruary(Coorespondance):2008–9.
8. Soh PN, Witkowski B, Olagnier D, Nicolau ML, Garcia-Alvarez MC, Berry A, *et al.* In vitro and *in-vivo* properties of ellagic acid in malaria treatment. Antimicrob Agents Chemother. 2009;53(3):1100–6.
9. Muganga R, Angenot L, Tits M, Frédérich M. In vitro and *in-vivo* antiplasmodial activity of three rwandan medicinal plants and identification of their active compounds. Planta Med. 2014;80(6):482–9.
10. Seeram NP, Lee R, Heber D. Bioavailability of ellagic acid in human plasma after consumption of ellagitannins from pomegranate (Punica granatum L.) juice. Clin Chim Acta. 2004;348(1–2):63–8.
11. Xiang Q, Li M, Wen J, Ren F, Yang Z, Jiang X, *et al.* The bioactivity and applications of pomegranate peel extract: A review. Vol. 46, Journal of Food Biochemistry. 2022.
12. Li Y, Zhang Y, Dai W, Zhang Q. Enhanced oral absorption and anti-inflammatory activity of ellagic acid via a novel type of case in nanosheets constructed by simple coacervation. Int J Pharm [Internet]. 2021;594(November 2020):120131. Available from: https://doi.org/10.1016/j.ijpharm.2020.120131
13. Zheng D, Lv C, Sun X, Wang J, Zhao Z. Preparation of a supersaturatable self-microemulsion as drug delivery system for ellagic acid and evaluation of its antioxidant activities. J Drug Deliv Sci Technol [Internet]. 2019;53(August):101209. Available from: https://doi.org/10.1016/j.jddst.2019.101209
14. Nyamba I, Lechanteur A, Semdé R, Evrard B. Physical formulation approaches for improving aqueous solubility and bioavailability of ellagic acid: A review. Eur J Pharm Biopharm. 2021;159(November 2020):198–210.
15. Nyamba I, Jennotte O, Sombié CB, Lechanteur A, Sacre PY, Djandé A, *et al.* Preformulation study for the selection of a suitable polymer for the development of ellagic acid-based solid dispersion using hot-melt extrusion. Int J Pharm. 2023;641(May).
16. Bijwar R, Tare H. Solubility and Taste Masked Behaviour of Cyclodextrin Molecular Inclusion Complex of Lumefantrin. 2023;3–7.
17. Price DJ, Nair A, Kuentz M, Dressman J, Saal C. Calculation of drug-polymer mixing enthalpy as a new screening method of precipitation inhibitors for supersaturating pharmaceutical formulations. Eur J Pharm Sci [Internet]. 2019;132(February):142–56. Available from: https://doi.org/10.1016/j.ejps.2019.03.006
18. Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins : 2010;1607–21.
19. Bulani VD, Kothavade PS, Nagmoti DM, Kundaikar HS, Degani MS, Juvekar AR. Characterisation and anti-inflammatory evaluation of the inclusion complex of ellagic acid with hydroxypropyl-β-cyclodextrin. J Incl Phenom Macrocycl Chem [Internet]. 2015;82(3):361–72. Available from: http://dx.doi.org/10.1007/s10847-015-0498-7
20. Bulani VD, Kothavade PS, Kundaikar HS, Gawali NB, Chowdhury AA, Degani MS, *et al.* Inclusion complex of ellagic acid with β-cyclodextrin: Characterization and in vitro anti-inflammatory evaluation. J Mol Struct. 2016;1105:308–15.
21. Xiao CF, Li K, Huang R, He GJ, Zhang JQ, Zhu L, *et al.* Investigation of inclusion complex of Epothilone A with cyclodextrins. Carbohydr Polym. 2014;102(1):297–305.
22. Ribeiro A, Figueiras A, Santos D, Veiga F. Preparation and solid-state characterization of inclusion complexes formed between

miconazole and methyl-β-cyclodextrin. AAPS PharmSciTech. 2008;9(4):1102–9.
23. Koch N, Jennotte O, Gasparrini Y, Vandenbroucke F, Lechanteur A, Evrard B. Cannabidiol aqueous solubility enhancement: Comparison of three amorphous formulations strategies using different type of polymers. Int J Pharm [Internet]. 2020;589(August):119812. Available from: https://doi.org/10.1016/j.ijpharm.2020.119812
24. Zime-diawara H, Dive G, Moudachirou M, Frederich M, Evrard B. Understanding the interactions between artemisinin and cyclodextrins: spectroscopic studies and molecular modeling. 2012;305–15.
25. Li H, Chang SL, Chang TR, You Y, Wang XD, Wang LW, *et al.* Inclusion complexes of cannabidiol with β-cyclodextrin and its derivative: Physicochemical properties, water solubility, and antioxidant activity. J Mol Liq [Internet]. 2021;334:116070. Available from: https://doi.org/10.1016/j.molliq.2021.116070
26. Muankaew C, Jansook P, Stefánsson E, Loftsson T. Effect of γ-cyclodextrin on solubilization and complexation of irbesartan: Influence of pH and excipients. Int J Pharm [Internet]. 2014;474(1–2):80–90. Available from: http://dx.doi.org/10.1016/j.ijpharm.2014.08.013
27. Lv P, Zhang D, Guo M, Liu J, Chen X, Guo R, *et al.* Structural analysis and cytotoxicity of host-guest inclusion complexes of cannabidiol with three native cyclodextrins. J Drug Deliv Sci Technol [Internet]. 2019;51(February):337–44. Available from: https://doi.org/10.1016/j.jddst.2019.03.015
28. Lu Y, Yang L, Zhang W, Xie S, Zhao F, Peng X, *et al.* Enhancement of the oral bioavailability of isopropoxy benzene guanidine though complexation with hydroxypropyl-β-cyclodextrin. Drug Deliv [Internet]. 2022;29(1):2824–30. Available from: https://doi.org/10.1080/10717544.2022.2118400
29. Savic IM, Jocic E, Nikolic VD, Popsavin MM, Srdjan J, Savic-gajic IM, *et al.* The effect of complexation with cyclodextrins on the antioxidant and antimicrobial activity of ellagic acid. Pharm Dev Technol [Internet]. 2019;24(4):410–8. Available from: https://doi.org/10.1080/10837450.2018.1502318
30. Mura P. Analytical techniques for characterization of cyclodextrin complexes in aqueous solution: A review. J Pharm Biomed Anal [Internet]. 2014;101:238–50. Available from: http://dx.doi.org/10.1016/j.jpba.2014.02.022
31. Qiu N, Zhao X, Liu Q, Shen B, Liu J, Li X, *et al.* Inclusion complex of emodin with hydroxypropyl-β-cyclodextrin: Preparation, physicochemical and biological properties. J Mol Liq [Internet]. 2019;289:111151. Available from: https://doi.org/10.1016/j.molliq.2019.111151
32. Li H, Zhao QS, Chang SL, Chang TR, Tan MH, Zhao B. Development of cannabidiol full-spectrum oil/2,6-di-O-methyl-β-cyclodextrin inclusion complex with enhanced water solubility, bioactivity, and thermal stability. J Mol Liq [Internet]. 2022;347:118318. Available from: https://doi.org/10.1016/j.molliq.2021.118318
33. Das S, Subuddhi U. Studies on the complexation of diclofenac sodium with β-cyclodextrin: Influence of method of preparation. J Mol Struct. 2015;1099:482–9.
34. Elder DP, Holm R, De Diego HL. Use of pharmaceutical salts and cocrystals to address the issue of poor solubility. Int J Pharm [Internet]. 2013;453(1):88–100. Available from: http://dx.doi.org/10.1016/j.ijpharm.2012.11.028
35. STEPHENSON GA, ABURUB A, A.WOODS T. Physical Stability of Salts ofWeak Bases in the Solid-State. J Pharm Sci. 2012;101(7):2271–80.