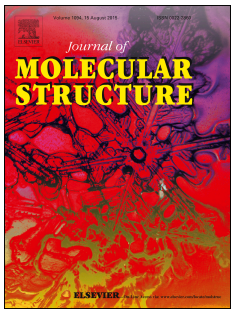
Accepted Manuscript



Solubility profiles, hydration and desolvation of curcumin complexed with γ-cyclodextrin and hydroxypropyl-γ-cyclodextrin

Sergey Shityakov, Ramin Ekhteiari Salmas, Serdar Durdagi, Norbert Roewer, Carola Förster, Jens Broscheit

PII: S0022-2860(16)31318-7

DOI: 10.1016/j.molstruc.2016.12.028

Reference: MOLSTR 23226

To appear in: *Journal of Molecular Structure*

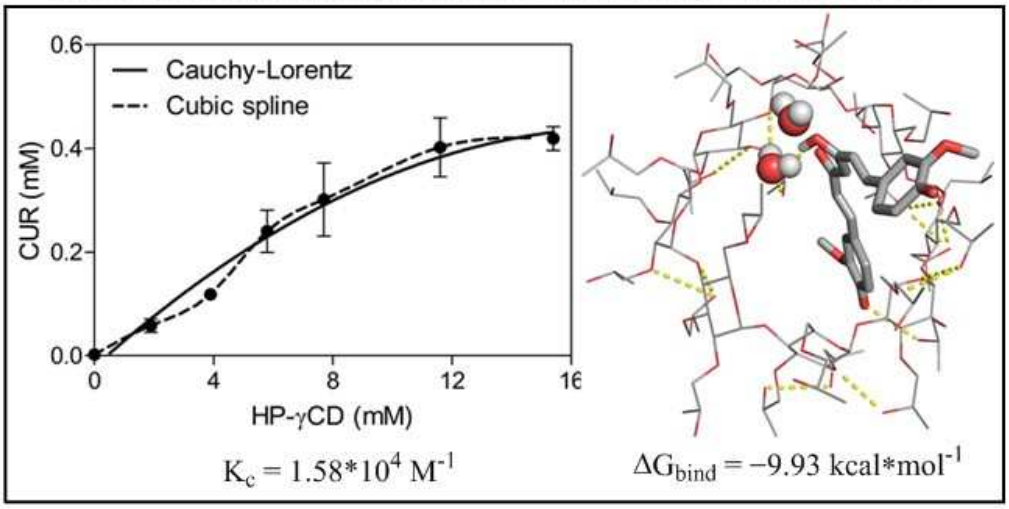
Received Date: 22 July 2016

Revised Date: 6 December 2016

Accepted Date: 9 December 2016

Please cite this article as: S. Shityakov, R.E. Salmas, S. Durdagi, N. Roewer, C. Förster, J. Broscheit, Solubility profiles, hydration and desolvation of curcumin complexed with γ-cyclodextrin and hydroxypropyl-γ-cyclodextrin, *Journal of Molecular Structure* (2017), doi: 10.1016/j.molstruc.2016.12.028.

solubility isotherms and hydrated molecular docking of CUR/HP-γ-CD



Solubility profiles, hydration and desolvation of curcumin complexed with γ-cyclodextrin and hydroxypropyl-γ-cyclodextrin

Sergey Shityakov[1,*], Ramin Ekhteiari Salmas[3], Serdar Durdagi[3], Norbert Roewer[1,2], Carola Förster[1], and Jens Broscheit[1,2]

[1]Department of Anesthesia and Critical Care, University of Würzburg, 97080 Würzburg, Germany

[2]Sapiotec Ltd., 97078 Würzburg, Germany

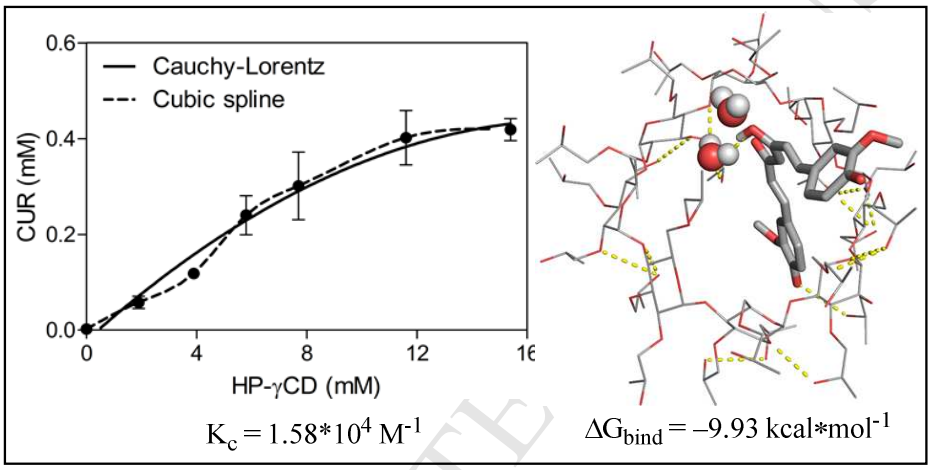
[3]Department of Biophysics, School of Medicine, Bahcesehir University, Istanbul, Turkey

Author to whom correspondence should be addressed; E-Mail: Shityakov_s@ukw.de

Tel: +49-931-2013-0016; Fax: +49-931-2013-0019.

Graphical Abstract:

solubility isotherms and hydrated molecular docking of CUR/HP-γ-CD



Abstract: In this study, we investigated curcumin (CUR) solubility profiles and hydration/desolvation effects of this substance formulated with γ-cyclodextrin (γ-CD) and hydroxypropyl-γ-cyclodextrin (HP-γ-CD) excipients. The CUR/HP-γ-CD complex was found to be more stable in solution with the highest apparent stability constant for CUR/HP-γ-CD (K_c = 1.58*10^4 M^{-1}) as the more soluble form in distilled water. The *in silico* calculations, including molecular docking, Monte Carlo (MC), and molecular dynamics (MD) simulations, indicated that water molecules play an important role in host-guest complexation mediating the CUR binding to cyclodextrins via hydrogen bond formations. The CUR hydration/desolvation effects contributed to the complex formation by elevating the CUR binding affinity to both CDs. The CUR/HP-γ-CD complex after the CUR hydration was determined with a minimal Gibbs free energy of binding (ΔG_{bind} = –9.93 kcal*mol^{-1}) due to the major hydrophobic (vdW) forces. Overall, the results of this study can aid a development of cyclodextrin-based drug

delivery vectors, signifying the importance of water molecules during the formulation processes.

Keywords: curcumin, cyclodextrins, complex stability, hydrated molecular docking, hydrogen bonds, Monte Carlo, molecular dynamics simulations, free energy calculations

1. Introduction

Curcumin (CUR) is a natural chemical produced by some plants of the ginger family, and it is used as a dietary herbal supplement or a food coloring agent (Volate et al., 2005; Baumann et al., 2009). This substance has also been found to possess antiviral and anticancer properties, which were intensively characterized over the last few decades (Nagabhushan and Bhide, 1992; Prasad and Tyagi, 2015). The CUR compound is essentially insoluble at physiological pH and, since it is very unstable, it undergoes a rapid hydrolytic degradation (Tonnesen et al., 2002). Various attempts have been made to produce water-soluble and more stable CUR by formulating it with different excipients, including hydrophilic cyclodextrins (CDs) to minimize its hydrolysis and high decomposition rate (Yadav et al., 2009; Marcolino et al., 2011).

CDs have a wide range of biomedical applications in fields such as pharmacy, chemistry, biotechnology, and medicine. They are oligosaccharides produced by bacteria from starch via its enzymatic degradation and typically exist as pristine hexameric α-, heptameric β- and octameric γ-CD forms and their derivatives (Zidovetzki and Levitan, 2007). CDs have a hydrophilic outer surface, and a less polar but more lipophilic (amphiphilic) central cavity leading to a CD inclusion complexation with different drug-like substances through the electrostatic, van der Waals (vdW), hydrophobic, charge-transfer, and H-bonding interactions. The CD-drug complex formation usually contributes to a retardation of the degradation rate during solvation process (Tonnesen et al., 2002). This complexation process mainly depends on the interaction of the guest molecule with CDs and the difference in the interactions of bound water and water with the bulk solvent. Therefore, studies on molecular recognition and binding require careful consideration of solvent effects.

The solvated drug molecules interact with waters before they bind to CDs with a subsequent release of water molecules to the bulk waters in aqueous solution. This so-called “desolvation process” is usually unfavorable during the change in Gibbs free energy of binding (ΔG_{bind}) and described as a “desolvation” penalty (Baldwin, 2010). However, some water molecules

are not displaced, and they might mediate the CD-drug complexation via altering binding site topography followed by the increase in overall binding affinity.

To tackle this issue, different quantitative and qualitative approaches have been established and applied to assess the energy contribution implied by the presence or displacement of water molecules, such as the hydrated ligand molecular docking (Forli and Olson, 2012). In particular, the AutoDock method was recently revised to evaluate the solvation and desolvation phenomena via including explicit displaceable waters during molecular docking procedure to improve overall docking precision and scoring function without excessive computational needs (Forli and Olson, 2012). On the other hand, to the best of our knowledge, there is no report on the hydration effect of CUR complexed with cyclodextrins so far. Therefore, in the present study, we have investigated the solubility profiles and the CUR hydration/desolvation process contribution to the CUR complexation with γ-CD and hydroxypropyl-γ-CD (HP-γ-CD) using combined experimental and computational techniques.

2. Materials and Methods

2.1. Materials

CUR was purchased from Sigma-Aldrich GmbH (Steinheim am Albuch, Germany). The CUR/γ-CD and CUR/HP-γ-CD complexes as pure forms (98% purity) were prepared as per the following protocols: 45 g of γ-CD and 5 g of CUR were weighed and suspended in 25 ml distilled water with a mortar and a pestle. Similarly, 1 g of HP-γ-CD and 44 mg of CUR were weighed and suspended in 1.5 ml of 96% ethanol and were homogenized as above. After complete homogenization, the suspension was dried *in vacuo* under ambient conditions for three days. Finally, the dried complexes were pulverized in the mortar.

2.2. Quantification of curcumin

The CUR concentrations were determined by UV/Vis spectrophotometry using an Agilent 8453 spectroscopy system (Agilent Technologies, Budapest, Hungary). The samples were diluted with a 50 vol% ethanol-water mixture to yield an absorbance recordable at the 430 nm range. All CUR measurements were performed without any interference from CDs presented in the complex.

2.3. Solubility studies and determination of stability constants

The solubility method was carried out according to the Higuchi & Connors method (Higuchi and Connors, 1965). Solubilities were measured by adding an excess amount of curcumin to distilled water containing different amounts of various kinds of cyclodextrins. The suspension formed was equilibrated under continuous agitation for 24 h at 25 ± 3.0 °C and then filtered through a 0.45 μm nominal pore size PVDF filter to yield a clear curcumin solution. The apparent stability constant (K_c) for a CUR-CD complexes was obtained from the slope of the phase-solubility diagram according to the following equation:

$$K_c = \frac{slope}{S_0(1 - slope)} \quad (1)$$

where S_0 is the saturation concentration of CUR in the solvent without cyclodextrin. The data are represented as means ± S.D. of three independent experiments.

2.4. Molecular docking and semi-empirical calculations

The 3D coordinates of the curcumin structure were retrieved from the PubChem database (Figure 1[A]). Since no 3D structure for HP-γ-CD was available, the molecule was constructed from the γ-CD (PDB ID: 1P2G) crystal structure (Pinotsis et al., 2003) with the PyMol v.1.2 software (Figure 1 [B]). Prior to molecular docking and semi-empirical calculations, the all-simulated structures were minimized with the GTKDynamo v.1.8.1 software (Bachega et al., 2013) using the conjugate gradient method with 200 maximum iterations and a threshold tolerance gradient of 0.1 Å. The host and guest structure preparations for molecular docking included the Gasteiger partial charge assignment (Gasteiger et al., 1980) and rotatable bonds definition. Rigid-flexible standard and hydrated molecular docking were applied to the center of the cyclodextrin structure. AutoDock v.4.2.5.1 (Goodsell et al., 1996; Forli et al., 2015) integrated into the PyMol AutoDock/Vina plugin (Seeliger and Groot, 2010) was used in the study. The grid spacing of 0.375 Å, with a dimension size of 60 Å from x, y, and z, was used to create the grid maps. In order to increase a conformational sampling of the drug, a number of genetic algorithm dockings (ga_run) were set to 100. Docking output results were represented by the approximation function as the estimated Gibbs free energy of binding (ΔG_{bind}). The calculated octanol/water partition (ClogP) coefficient for curcumin using the weighted algorithm with electrolyte concentration of 0.1 mol/dm^3 and its aqueous solubility (ClogS) were determined by the Marwin Sketch v.14.7.14.0 tool. The single-point calculations for all simulated structures using the Austin

Model 1 (AM1) method (Dewar et al., 1985; Rocha et al., 2006) were performed using GTKDynamo v.1.8.1 software (Bachega et al., 2013).

2.5. Atomistic molecular dynamics (MD) simulations

The docking poses of CUR produced by AutoDock v.4.2.5.1 (Goodsell et al., 1996; Forli et al., 2015) were used as an initial structure suitable for MD simulations. All classical MD simulations were performed using a GPU-accelerated version of Desmond 2015.4 code (Bowers et al., 2006). The OPLS 2005 force field (Banks et al., 2005) was used to calculate the interactions between atoms. The long-range electrostatic interaction calculations were set up by using the particle-mesh Ewald (PME) method (Essmann et al., 1995). The short-range van der Waals (vdW) and Coulomb interactions were defined by a cut-off radius of 9.0 Å. A Nose-Hoover thermostat (Hoover et al., 1985) and Martyna-Tobias-Klein method (Martyna et al., 1994) were used to maintain the system at the body temperature of 310 K and the pressure of 1.01325 bars. A time step of 2.0 fs was used during the MD simulations. The systems were minimized using 2000 iterations, and a convergence threshold of 1 $kcal*mol^{-1}*Å^{-1}$. To perform the structural relaxation, all the systems were equilibrated using default algorithms implemented in the Desmond software. Finally, 100 ns atomistic MD trajectories were produced for each of the analyzed systems.

2.6. Monte Carlo simulations

All Monte Carlo (MC) simulations implemented in ROSETTA code (Meiler et al., 2006) were employed in order to generate different systematic conformers for the standard and hydrated variants of CUR bound to γ-CD and HP-γ-CD. The trajectory frames as obtained by the MC protocol were subjected to conformational clustering to be used further for the Molecular Mechanics with Generalized Born and Surface Area Solvation (MM-GBSA) energy analysis (Gohlke et al., 2004).

2.7. MM-GBSA calculations

The MM-GBSA method (Gohlke et al., 2004) was applied to estimate the free energies of binding ($\Delta G_{MM\text{-}GBSA}$) for the CUR-CD complexes. The Prime module in the Schrödinger package (Schrödinger Release 2014-2: Prime, version 3.6) was used to calculate the $\Delta G_{MM\text{-}GBSA}$ values. The VSGB 2.0 solvation model (Li et al., 2011) was used to simulate the implicit solvation for the analyzed systems. The $\Delta G_{MM\text{-}GBSA}$ values were determined by subtracting the

total individual free energies of cyclodextrin (ΔG_{CD}) and CUR (ΔG_{CUR}) from the complex free energy ($\Delta G_{complex}$) as depicted according to the following equation:

$$\Delta G_{MM-GBSA} = \Delta G_{\text{complex}} - (\Delta G_{CD} + \Delta G_{CUR}) \quad (2)$$

or re-written as a sum of the energetic terms:

$$\Delta G_{MM-GBSA} = \Delta G_{\text{Coul}} + \Delta G_{\text{cov}} + \Delta G_{H-bond} + \Delta G_{lipo} + \Delta G_{solv} + \Delta G_{vdW} \quad (3)$$

where $\Delta G_{Coul}, \Delta G_{\text{cov}}, G_{H-bond}, \Delta G_{lipo}, \Delta G_{solv}$ and ΔG_{vdW} are Columb, covalent, hydrogen-bond, lipophilic, GB electrostatic solvation and vdW energies, respectively.

3. Results and Discussion

Molecular modelling techniques, such as molecular docking, have been proven useful in predicting the binding modes and interaction profiles of drug-like molecules complexed with different target structures including CDs (Ansari et al., 2012; Kar et al., 2013; Ahsan et al., 2015; Shityakov et al., 2016). In the standard and hydrated molecular docking experiments, the enol form of CUR, that is more energetically stable in the solid phase (Manolova et al., 2014) because of intramolecular hydrogen bonding, was chosen as the ligand. While γ-CD and HP-γ-CD were selected to be the excipient macromolecules. Taking into account the flexibility of CUR and CD, different conformational variants for the complexes are possible. By adding the ligand molecule to the CDs, 100 docking conformation instances for each CUR/CD pair were generated.

The conformation of a guest compound can depend strongly on the presence of solvent molecules participating in the binding and mediation of the interactions between different ligand substitutes and the receptor (Forli and Olson, 2012). For this reason, studies of molecular complexation involving cyclodextrins require careful consideration of the solvent effects. Nonetheless, the formation of the CUR/CD complex takes place primarily in the aqueous phase, and is followed by the drying step under ambient conditions. Even then, the complex still contains approximately 6.9% residual water, established by the Karl Fischer titration method.

In an aqueous environment, the γ-CD cavity with the largest volume (427 $Å^3$) in comparison to other CDs (174 $Å^3$ for α-CD and 262 $Å^3$ for β-CD) is occupied with water molecules; and most or all of these water molecules are excluded from the cavity upon binding with a guest compound. This mildly lipophilic cavity, which contains about 9 weakly-held and easily displaced water molecules with higher enthalpy than the bulk waters (Tabushi et al., 1978;

Tanhuanpää et al., 2001). Also, because of the H-bond bivalency of this binding site, a water molecule can "invert" a receptor's hydrogen bond acceptor region into a donor (Forli and Olson, 2012). The cavity is further stabilized by intramolecular H-bonds between the adjacent primary and secondary hydroxyl groups in γ-CD and hydroxypropyl groups in HP-γ-CD.

Water molecules were attached to CUR before docking by hydrating all polar groups capable of hydrogen bond interactions. This is important information in *de novo* or early stages of drug design, to optimize the ligands to best fit the binding site. As already mentioned in the literature, γ-CD, and its derivative can be topologically described as torus-shaped molecules with the larger and the smaller entries, where the hydroxyl groups are exposed to the solvent (Munro et al., 2004). Previous studies (Szejtly, 1998; Uekama et al., 1998; Ma et al., 2000; Zhao et al., 2002) have verified that the ligand molecule is usually inserted into CDs via the larger outer rim opening (0.85 nm) instead of the smaller (0.75 nm) one. In fact, our docking simulations demonstrate a similar scenario where CUR was inserted into the binding cavity of the amphiphilic CDs from the larger interface in both the standard and hydrated variants. The water molecules remained in the vicinity of the outer rim, and some of them were deeply submerged into the cyclodextrin binding cavity, interacting with the enolic hydroxyl group of CUR via H-bonds (Figure 2 and 3).

Based on the above observation of the CUR docking poses, only the complex structure with predicted the lowest ΔG_{bind} value in the binding region was considered as the top-docking result. The results are listed in Table 1 for standard and hydrated molecular dockings. CUR was found to bind strongly to CDs exceeding the binder/non-binder energy threshold (non-binders < –6.0 kcal*mol^{-1} < binders), where this threshold for various drug-like molecules in other AutoDock experiments has been determined (Shityakov et al., 2012; Shityakov et al., 2014). The CUR hydration improves its binding affinity to both cyclodextrins, this was confirmed by a minimal predicted Gibbs free energy of binding (ΔG_{bind}) values. The CUR hydration/desolvation effects contributed to the complex formation by elevating the CUR binding affinity to both CDs. The CUR/HP-γ-CD complex after the CUR hydration was determined with a minimal Gibbs free energy of binding (ΔG_{bind} = –9.93 kcal*mol^{-1}) and K_d of 0.05 µM. The thermodynamic equilibrium constants (K_d) for all of the docking poses were calculated from the ΔG_{bind} values as follows: $K_d = \exp([\Delta G_{bind}*1000]/[R*T])$, where R (gas constant) is 1.98 cal*(mol*K)$^{-1}$ and T (room temperature) is 298.15 Kelvin (Shityakov *et al*., 2012).

The increase in CUR binding affinity to CDs after hydration might be explained by the higher number of intermolecular H-bonds formed between the host and the guest: 4 for CUR/γ-CD and 2 for CUR/HP-γ-CD without ligand hydration (Figure 2 [A] and 3 [A]); and 8 for CUR/γ-CD and 4 for CUR/HP-γ-CD with ligand hydration (Figure 2 [B] and 3 [B]). Thus, the CUR hydrated docking to CDs illustrates the capabilities of the new hydration method to predict the position of weakly bound water molecules; and we ranked them in accordance with experimental findings, improving the docking accuracy.

The minimal predicted ΔG_{bind} and K_d values indicated that very lipophilic CUR (logP = 3.28) compound (Pawar et al., 2012) with very low calculated water solubility (0.01 – 0.06 mg*ml^{-1}) appeared to be the strongest binder to HP-γ-CD in both variants of molecular docking. As a result of the higher hydrophobicity potential (ClogP = –5.30) defined for this modified cyclodextrin than for its parental pristine form (ClogP = –14.17).

Following this, the top-docked poses of CUR were analyzed for whether the pose was still the likely bound pose and there are 99 other possible host-guest configurations. Since, there are several commonly occurring docked poses formed during the clustering with the root-mean-square-deviation (RMSD) cut-off value of 2.0 Å, the most popular pose was present multiple times for the standard and hydrated docking simulations. Additionally, when the guest interacts with water before it binds to the host, then those water molecules must be displaced to be further released into the bulk of the waters molecules in the solution (Furuki et al., 1993). The change in ΔG_{bind} in this process is often unfavorable and considered to be the "desolvation" penalty (Furuki et al., 1993). The determination of water to be displaced or bridged is the balance between its energetic contribution to ligand-receptor binding and the ability to stabilize a ligand pose through its displacement (Forli and Olson, 2012). Following this rule, during the hydrated complexation of CUR with the γ-CD and HP-γ-CD molecules most of the water molecules were displaced because of high water free energy (ΔG_{wat} = –0.2 kcal*mol^{-1}) and only few were strongly (ΔG_{wat} = –0.62 kcal*mol^{-1}) or weakly (ΔG_{wat} = –0.36 kcal*mol^{-1}) attached via H-bonds to the inclusion complex (Table 2). Clearly, the CUR inclusion in the CD cavity cannot lead to the displacement of all water molecules presented in it. The more closely a guest can be fitted in the CD binding site, the greater will be the number of water molecules released into the bulk. Thus, the combination of these two effects, such as CUR inclusion and water displacement, impacts the equilibrium formation constants for CD formulated drugs in solution.

Quantitation of CUR was also determined from the solubility isotherms of the CUR/γ-CD and CUR/HP-γ-CD complexes in a distilled water. The solubility diagrams for all complexes were non-linear Cauchy–Lorentz distribution for CUR/γ-CD and showed second-order polynomial (quadratic) curve fitting for CUR/HP-γ-CD (Figure 4 [A, B]). The CUR concentration in CUR/HP-γ-CD was about 60-fold greater than that of γ-CD complex due to improved aqueous solubility. The CUR/HP-γ-CD solution was supersaturated when the concentration of HP-γ-CD was higher than 11.6 mM. The CUR/γ-CD precipitate formation was started at 1.9 mM of γ-CD disturbing the CUR release probably by binding to its free fraction. The plateau section of the curve suggested that the precipitation of the complex was finally attained. An estimate of K_c constant was calculated using the analytical detection limit as highest possible S_0 value (S_0 = 2.37 μM) showing the increase in apparent stability for CUR/HP-γ-CD (K_c = $1.58*10^4$ M^{-1}) in contrast with the CUR/γ-CD complex (K_c = $1.02*10^3$ M^{-1}). Observations from previous complexation studies on curcuminoids also suggested that the bulky moieties of the two phenyl groups of CUR fit better to the bigger γ-CD that β-CD cavity, where the K_c values of CUR in HP-β-CD and HP-γ-CD was reported to be more than $5.0*10^4$ M^{-1} and $16*10^4$ M^{-1}, respectively (Tonnesen et al., 2002).

To compare our experimental and theoretical results, we calculated the reference binding ratio (BR_{ref}) to determine the complexation strength, which is based on the relative content of CUR in the γ-CD ($C_{\gamma-CD}$) or in HP-γ-CD ($C_{HP-\gamma-CD}$) complex, molecular weight of the complex constituents and degree of substitution (*n*) for HP-γ-CD using the following equation:

$$BR_{ref} = \frac{C_{HP-\gamma-CD}(MW_{CUR} + MW_{HP-\gamma-CD} + n \cdot MW_{res})}{C_{\gamma-CD}(MW_{HP-\gamma-CD} + MW_{HP-\gamma-CD})} \quad (4)$$

where MW_{CUR}, MW_{CD}, and MW_{res} are the molecular weights of curcumin, γ-CD, HP-γ-CD and hydroxypropyl residues**.** In reality, the HP-γ-CD structure is randomly substituted having hydroxypropyl substituents at all positions of primary (C6 position) and secondary (C2 and C3 position) interface. To simplify the molecular docking procedure, all hydroxyl groups (n = 24) of the γ-CD molecule were substituted for the hydroxypropyl groups in the case of HP-γ-CD model. Alternatively, the theoretical binding ratio (BR) was simply defined as the ΔG_{bind} ratio between the binding affinities of the CUR/γ-CD and CUR/HP-γ-CD complexes as:

$$BR = \frac{\Delta G_{CUR/HP-\gamma-CD}}{\Delta G_{CUR/\gamma-CD}} \quad (5)$$

As shown in Figures 3 and 4 , the CUR position is roughly detected to be docked in the center of the CD structures using 1:1 complex stoichiometry, where the binding cavity is located.

This stoichiometry was confirmed via a good correlation between the experimental data and the 1:1 binding isotherm for the CUR/CD complexes (Hegge et al., 2009). However, the formation of inclusion complexes with 1:2 (guest:host) stoichiometry is possible for CUR/HP-γ-CD as it was observed in the CUR absorption spectra sing UV-Vis spectroscopy (Hegge et al., 2009). The theoretical molecular docking results were in good agreement with the experimentally derived data judging by the theoretical binding ratio for the standard (BR = 1.15) and hydrated (BR = 1.29) molecular docking in comparison with the reference (BR_{ref} = 1.37).

Furthermore, the three monomers (CUR, γ-CD and HP-γ-CD) and four inclusion complexes, including those with the hydrated ligand were minimized using the conjugate gradient approach in order to perform the single-point AM1 calculation on the assessment of their relative chemical stability. The AM1 energies of 7 species and the energy difference (ΔE) between the inclusion complexes and their constituent monomers are reported in Table 3. The relative stabilities of CUR/CDs inclusion complexes were measured by evaluating their ΔE, which can be addressed as the stabilizing energy of complexation. Hence, the low ΔE values, the more stable the inclusion complex. The energies of all the complexes were lower than the energies of their two constituents except for CUR/HP-γ-CD, indicating that the association of CUR and CDs had formed mainly quite stable complexes. Among the four inclusion complexes, the more hydrated CUR/γ-CD complex was found with the lowest energy term ($\Delta E = -191.55$ kcal*mol^{-1}) due to the numerous stabilizing CD intramolecular H-bonds and less excessive water displacement effect.

Additionally, in order to study the effect of the CDs on the CUR conformational change that can be adapted into the cavity, as well as their influence on the binding property, the CUR/CD complexes were subjected to the MC simulations implemented in the ROSETTA code (Meiler et al., 2006), and 100 ns MD simulations with the MM-GBSA approach in evaluating binding affinity. In classical MD simulations, the conformational changes of compounds and proteins are connected in time. These simulations mimic the dynamical behavior of the studied systems, from which time-dependent values of conformational and thermodynamic properties can be estimated. Series of atomic coordinates (trajectories) are output in the result by using Newton's equations of motion. However, in MC simulations, each conformation can be determined only on its predecessor. The MC protocol predicts conformations randomly and employs energetic criteria to detect whether or not to confirm the new conformation.

To approximate the MD simulations in a more or less realistic way, all the hydroxyl groups were substituted with hydroxypropyl residues in the case of modified cyclodextrin models. Since the biophysical and biological behaviors of a compound often depend mainly upon the conformations that it can adopt, the time-dependent configurations (translations and rotations) of the CUR into the γ-CD and HP-γ-CD were studied and clearly monitored (Supplementary material 1 and 2) based on the MD trajectory frames, in which the CD heavy atoms were aligned with respect to the starting structure. These analyses are feasible to illustrate the level of the conformational changes and movements of the CUR into the different environments from the starting point. The efficiency of this fashion can be enhanced by clustering the different configurations of the CUR that group together the similar conformations, from which the representative conformers can be selected. The relatively straightforward clustering method is the RMSD parameter between pairs of conformations (sometimes referred to as distance analysis). The CUR spatial arrangements were set establishing non-bonded forces with the CDs atoms and the water molecules into the cavity. The strong binding of CUR into the HP-γ-CD provides a high level of shape complementary with the hydrophobic pocket of the HP-γ-CD.

The relative structural stability of the CUR in the HP-γ-CD cavity is dependent upon internal and non-covalent forces between the modified CD and CUR atoms. The CUR molecule in the HP-γ-CD complex incurs a higher entropic penalty compared to CUR in the γ-CD complex. The free energy difference is considered to be a highly important factor in the thermodynamic concept and it would appear to be necessary for estimating the average ΔG_{bind} values between the CUR and CDs based on the MC and MD trajectory frames. The energetic analysis reveals that the highest CD binding affinity to CUR belongs to HP-γ-CD after ligand hydration with the $\Delta G_{MM\text{-}GBSA}$ value of −86.38 kcal*mol^{-1} and the ΔG_{bind} value of −71.4 kcal*mol^{-1}. This was due to strong hydrophobic (vdW) forces causing a decrease in the CUR hydration followed by excessive desolvation effects increasing water displacement (Figure 5 and 6 [A-D]). On the other hand, the hydration/desolvation effect of CUR/γ-CD characterized by less water displacement and high ligand hydration was more pronounced. However, the hydrophobic (vdW) forces in CUR/γ-CD were less prominent.

Furthermore, the relative free energies of binding CUR to the hydrated and dehydrated CDs by the MD simulations were estimated to be −69.67 kcal*mol^{-1} and −46.44 kcal*mol^{-1}, respectively. The same systems (CUR bound to hydrated/dehydrated γ-CD and HP-γ-CD) were examined by the MC simulations, providing free energy differences of −51.90 kcal*mol^{-}

^{1} and −49.41 kcal*mol^{-1}, respectively. Both simulation methods were able to accurately determine the effects of the combinatorial explicit and implicit solvent models of the cavity that were being used in the energy calculations. The stability of the CUR was a consequence of the hydrophobic effect in the pocket of the CDs, from which the contribution of the entropic term could be considered. The vdW forces between the CUR, γ-CD and HP-γ-CD systems were calculated to be −27.59 kcal*mol^{-1} and −40.57 kcal*mol^{-1}, respectively. The vdW interactions of CUR/ γ-CD and HP-γ-CD in the presence of explicit water molecules showed the average energies to be −24.37 kcal*mol^{-1} and −37.03 kcal*mol^{-1}. The observed differences in the non-covalent energies for the explicitly hydrated and dehydrated pockets of the CDs were expected due to the water-mediated Coulombic forces. These results obtained through the MD simulations, obviously were in a satisfactory qualitative agreement with those calculated through the MC method. However, the relative free energy value was highly sensitive to the configurations of the CD complexes used for the above calculations. This was because, the conformations of the molecules were obtained by different simulation methods (MC and MD) in each case. This was considered to be a major drawback of the force-field methods. In principle, free energy is a very difficult value to estimate for flexible systems, such as the CDs used in the current study, which share many closely separated minima. Related thermodynamic terms, such as entropy and partial molar free energy, were also difficult to estimate, due to the poor sampling produced by these simulations.

4. Conclusions

Molecular docking, MC, and MD simulations were performed in this study to investigate the CUR hydration/desolvation effects for its complexation with γ-CD and HP-γ-CD. The calculations indicate that water molecules play an important role in host-guest complexation, mediating the binding of ligands with their targets via hydrogen bond formations. The CUR hydration increases its binding affinity to both CDs, which is confirmed by the ΔG_{bind} and $\Delta G_{MM\text{-}GBSA}$ values. Although the CUR/CD complex affinities were significantly improved after the CUR hydration, as newly formed hydrogen bonds are responsible for the stability of the inclusion complexes, hydrophobic (vdW) forces in CUR/HP-γ-CD are dominant over hydration/desolvation effects. In addition, the CUR/HP-γ-CD complex was found to be more stable in aqueous solution with the highest apparent stability constant for CUR/HP-γ-CD (K_c = 1.58*10^{4} M^{-1}) as the more soluble form in distilled water. Overall, the results of this study

can aid drug delivery vector development, underscoring the importance of hydration effect on the formulation of different drug-like molecules.

Acknowledgments

Special thanks are extended to Dr. Biswapriya B. Misra from the Department of Biology, Genetics Institute, University of Florida for his assistance in the paper's writing. The authors are also grateful to the BMBF (Bundesministerium für Bildung und Forschung) for supporting this work through a grant (BMBF13N11801) to Jens Broscheit.

Conflicts of Interest

The authors declare no conflict of interest.

Reference

1. Volate, S. R., D. M. Davenport, et al. (2005). "Modulation of aberrant crypt foci and apoptosis by dietary herbal supplements (quercetin, curcumin, silymarin, ginseng and rutin)." Carcinogenesis **26**(8): 1450-1456.
2. Baumann, L., H. Woolery-Lloyd, et al. (2009). ""Natural" ingredients in cosmetic dermatology." J Drugs Dermatol **8**(6 Suppl): s5-9.
3. Nagabhushan, M. and S. V. Bhide (1992). "Curcumin as an inhibitor of cancer." J Am Coll Nutr **11**(2): 192-198.
4. Prasad, S. and A. K. Tyagi (2015). "Curcumin and its analogues: a potential natural compound against HIV infection and AIDS." Food Funct **6**(11): 3412-3419.
5. Tonnesen, H. H., M. Masson, et al. (2002). "Studies of curcumin and curcuminoids. XXVII. Cyclodextrin complexation: solubility, chemical and photochemical stability." Int J Pharm **244**(1-2): 127-135.
6. Yadav, V. R., S. Suresh, et al. (2009). "Effect of cyclodextrin complexation of curcumin on its solubility and antiangiogenic and anti-inflammatory activity in rat colitis model." AAPS PharmSciTech **10**(3): 752-762.
7. Marcolino, V. A., G. M. Zanin, et al. (2011). "Interaction of curcumin and bixin with beta-cyclodextrin: complexation methods, stability, and applications in food." J Agric Food Chem **59**(7): 3348-3357.

8. Zidovetzki, R. and I. Levitan (2007). "Use of cyclodextrins to manipulate plasma membrane cholesterol content: evidence, misconceptions and control strategies." Biochim Biophys Acta **1768**(6): 1311-1324.
9. Baldwin, R. L. (2010). "Desolvation penalty for burying hydrogen-bonded peptide groups in protein folding." J Phys Chem B **114**(49): 16223-16227.
10. Forli, S. and A. J. Olson (2012). "A force field with discrete displaceable waters and desolvation entropy for hydrated ligand docking." J Med Chem **55**(2): 623-638.
11. Higuchi T, Connors KA. Phase solubility techniques. In: Reilly CN, editor. Advances in Analytical Chemistry Instrumentation. Vol. 4. New York, NY: Interscience; 1965. pp. 117–212.
12. Pinotsis, N., D. D. Leonidas, et al. (2003). "The binding of beta- and gamma-cyclodextrins to glycogen phosphorylase b: kinetic and crystallographic studies." Protein Sci **12**(9): 1914-1924.
13. Bachega, J. F., L. F. Timmers, et al. (2013). "GTKDynamo: a PyMOL plug-in for QC/MM hybrid potential simulations." J Comput Chem **34**(25): 2190-2196.
14. Gasteiger, J. and M. Marsili (1980). "Iterative Partial Equalization of Orbital Electronegativity - a Rapid Access to Atomic Charges." Tetrahedron **36**(22): 3219-3228.
15. Goodsell, D. S., G. M. Morris, et al. (1996). "Automated docking of flexible ligands: applications of AutoDock." J Mol Recognit **9**(1): 1-5.
16. Forli, S. (2015). "Charting a Path to Success in Virtual Screening." Molecules **20**(10): 18732-18758.
17. Seeliger, D. and B. L. de Groot (2010). "Ligand docking and binding site analysis with PyMOL and Autodock/Vina." J Comput Aided Mol Des **24**(5): 417-422.
18. Dewar, M. J. S., E. G. Zoebisch, et al. (1985). "The Development and Use of Quantum-Mechanical Molecular-Models .76. Am1 - a New General-Purpose Quantum-Mechanical Molecular-Model." J Am Chem Soc **107**(13): 3902-3909.
19. Rocha, G. B., R. O. Freire, et al. (2006). "RM1: A reparameterization of AM1 for H, C, N, O, P, S, F, Cl, Br, and I." Journal of Computational Chemistry **27**(10): 1101-1111.
20. Bowers, K. J., R. O. Dror, et al. (2006). "The midpoint method for parallelization of particle simulations." Journal of Chemical Physics **124**(18).
21. Banks, J. L., H. S. Beard, et al. (2005). "Integrated modeling program, applied chemical theory (IMPACT)." J Comput Chem **26**(16): 1752-1780.

22. Essmann, U., L. Perera, et al. (1995). "A Smooth Particle Mesh Ewald Method." Journal of Chemical Physics **103**(19): 8577-8593.
23. Hoover, W. G. (1985). "Canonical Dynamics - Equilibrium Phase-Space Distributions." Physical Review A **31**(3): 1695-1697.
24. Martyna, G. J., D. J. Tobias, et al. (1994). "Constant-Pressure Molecular-Dynamics Algorithms." Journal of Chemical Physics **101**(5): 4177-4189.
25. Meiler, J. and D. Baker (2006). "ROSETTALIGAND: protein-small molecule docking with full side-chain flexibility." Proteins **65**(3): 538-548.
26. Gohlke, H. and D. A. Case (2004). "Converging free energy estimates: MM-PB(GB)SA studies on the protein-protein complex Ras-Raf." J Comput Chem **25**(2): 238-250.
27. Li, J., R. Abel, et al. (2011). "The VSGB 2.0 model: a next generation energy model for high resolution protein structure modeling." Proteins **79**(10): 2794-2812.
28. Ansari, M. Y.; Dikhit, M. R.; Sahoo, G. C.; Das, P., Comparative modeling of HGPRT enzyme of L. donovani and binding affinities of different analogs of GMP. *International journal of biological macromolecules* **2012,** *50* (3), 637-49.
29. Kar, R. K.; Ansari, M. Y.; Suryadevara, P.; Sahoo, B. R.; Sahoo, G. C.; Dikhit, M. R.; Das, P., Computational elucidation of structural basis for ligand binding with Leishmania donovani adenosine kinase. *BioMed research international* **2013,** *2013*, 609289.
30. Ahsan, M. J.; Choudhary, K.; Jadav, S. S.; Yasmin, S.; Ansari, M. Y.; Sreenivasulu, R., Synthesis, antiproliferative activity, and molecular docking studies of curcumin analogues bearing pyrazole ring. *Med Chem Res* **2015,** *24* (12), 4166-4180.
31. Shityakov, S., R.E. Salmas, et al, (2016) “Characterization, In Vivo Evaluation and Molecular Modeling of Different Propofol-Cyclodextrin Complexes to Assess Their Drug Delivery Potential at The Blood-Brain Barrier Level” J Chem Inf Model **56**(10): 1914-1922
32. Manolova, Y., V. Deneva, et al. (2014). "The effect of the water on the curcumin tautomerism: a quantitative approach." Spectrochim Acta A Mol Biomol Spectrosc **132**: 815-820.
33. Tabushi, I., Y. I. Kiyosuke, et al. (1978). "Approach to Aspects of Driving Force of Inclusion by Alpha-Cyclodextrin." J Am Chem Soc **100**(3): 916-919.
34. Tanhuanpaa, K., K. H. Cheng, et al. (2001). "Characteristics of pyrene phospholipid/gamma-cyclodextrin complex." Biophysical Journal **81**(3): 1501-1510.

35. Munro, I. C., P. M. Newberne, et al. (2004). "Safety assessment of gamma-cyclodextrin." Regul Toxicol Pharmacol **39 Suppl 1**: S3-13.
36. Szejtli, J. (1998). "Introduction and General Overview of Cyclodextrin Chemistry." Chemical Reviews **98**(5): 1743-1754.
37. Uekama, K., F. Hirayama, et al. (1998). "Cyclodextrin Drug Carrier Systems." Chemical Reviews **98**(5): 2045-2076.
38. Ma, X. Y., Z. X. Liao, et al. (2000). "Study of the podophyllotoxin/beta-cyclodextrin inclusion complex." Journal of Inclusion Phenomena and Macrocyclic Chemistry **36**(3): 335-342.
39. Zhao, D. Y., K. J. Liao, et al. (2002). "Study of the supramolecular inclusion of beta-cyclodextrin with andrographolide." Journal of Inclusion Phenomena and Macrocyclic Chemistry **43**(3-4): 259-264.
40. Shityakov, S., J. Broscheit, et al. (2012). "alpha-Cyclodextrin dimer complexes of dopamine and levodopa derivatives to assess drug delivery to the central nervous system: ADME and molecular docking studies." Int J Nanomedicine 7: 3211-3219.
41. Shityakov, S. and C. Forster (2014). "In silico structure-based screening of versatile P-glycoprotein inhibitors using polynomial empirical scoring functions." Adv Appl Bioinform Chem **7**: 1-9.
42. Pawar, Y. B., B. Munjal, et al. (2012). "Bioavailability of a lipidic formulation of curcumin in healthy human volunteers." Pharmaceutics **4**(4): 517-530.
43. Furuki, T., F. Hosokawa, et al. (1993). "Microscopic Medium Effects on a Chemical-Reaction - a Theoretical-Study of Decarboxylation Catalyzed by Cyclodextrins as an Enzyme Model." J Am Chem Soc **115**(7): 2903-2911.
44. Hegge, A. B., M. Masson, et al. (2009). "Investigation of curcumin-cyclodextrin inclusion complexation in aqueous solutions containing various alcoholic co-solvents and alginates using an UV-VIS titration method. Studies of curcumin and curcuminoides, XXXV." Pharmazie **64**(6): 382-389.

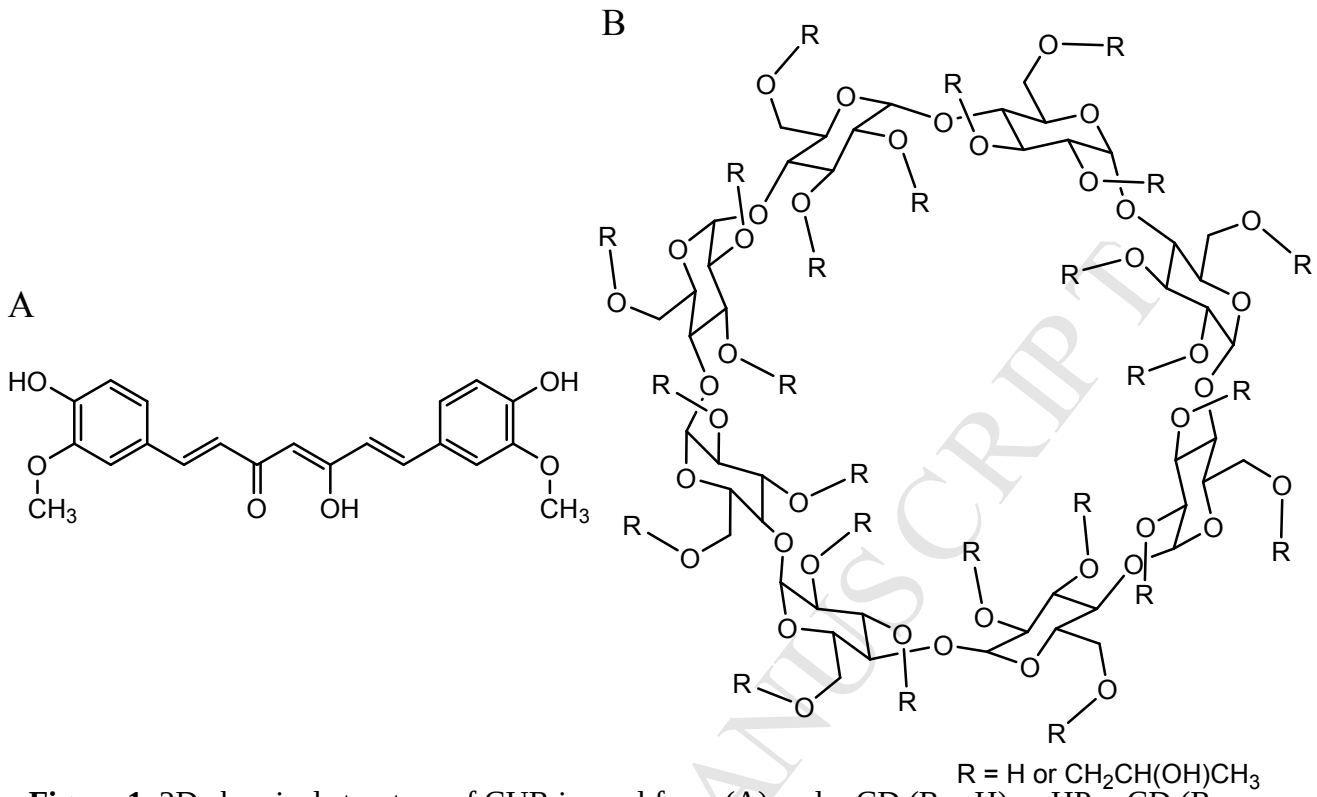


Figure 1. 2D chemical structure of CUR in enol form (A) and γ-CD (R = H) or HP-γ-CD (R = $CH_2CH(OH)CH_3$) molecules (B)

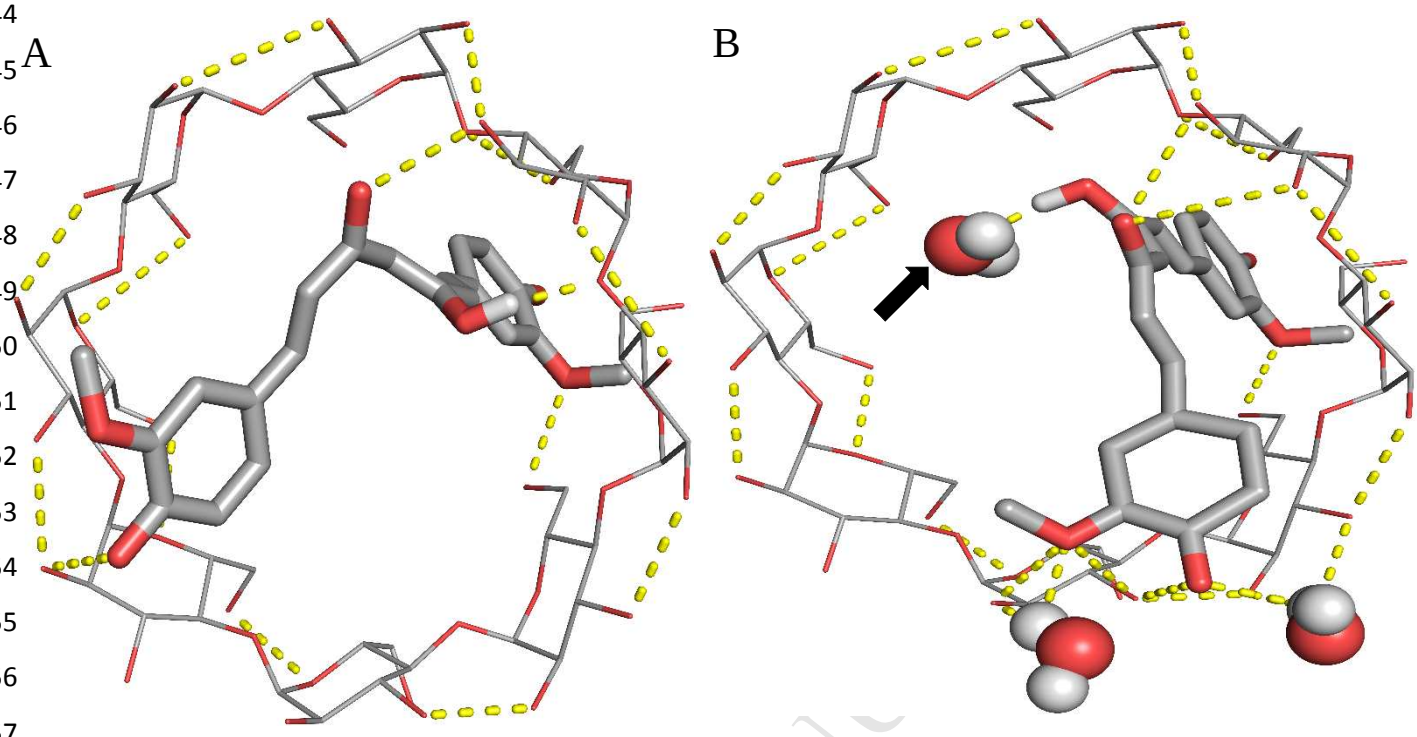


Figure 2: Standard (A) and hydrated (B) AutoDock molecular docking profiles of CUR (enol form) bound to the γ-CD molecule. Cyclodextrin and its ligand are visualized with lines and sticks, respectively. Water molecules are represented in spheres, and the hydrogen bonds are depicted as yellow dashed lines. Water molecule located in the γ-CD binding cavity is pointed by the bold arrow. Molecules are colored according to their atom types. The majority of hydrogens atoms are omitted for clarity.

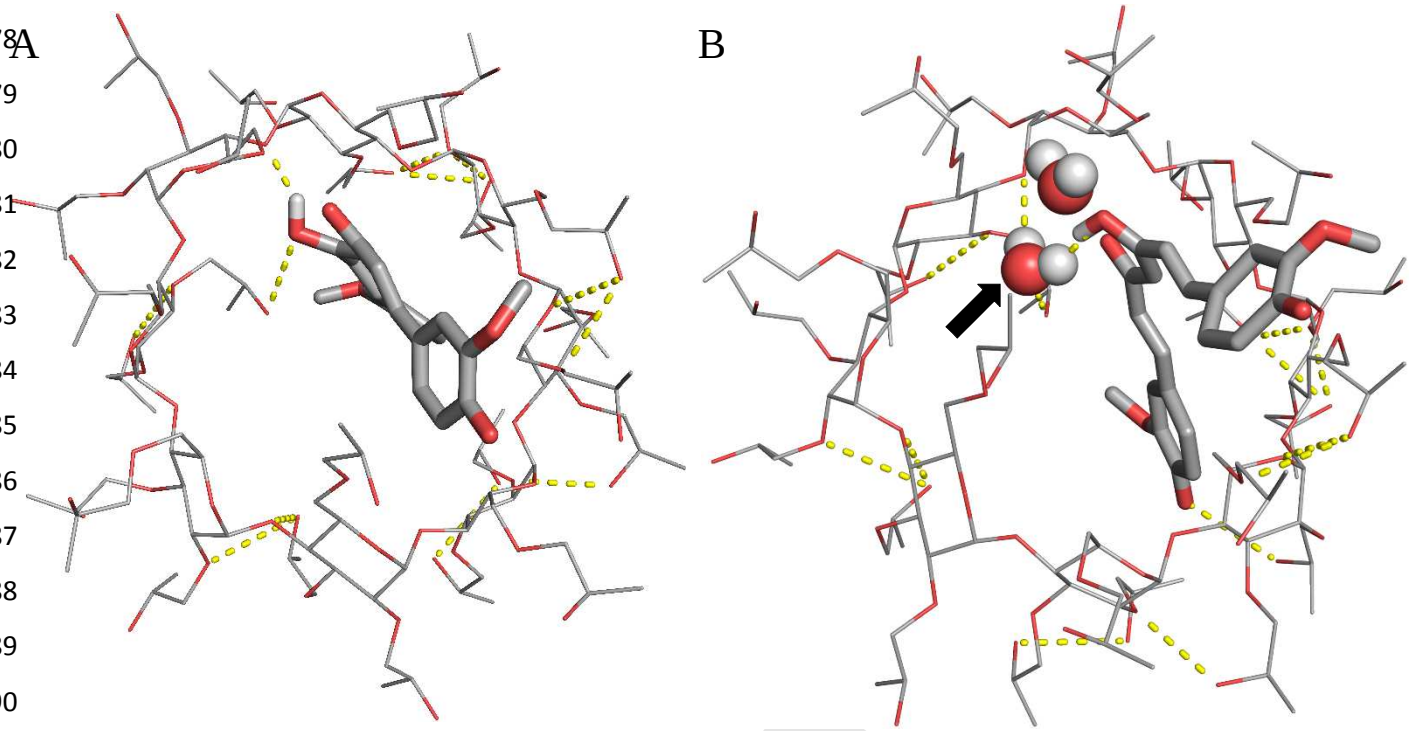


Figure 3: Standard (A) and hydrated (B) AutoDock molecular docking profiles of CUR (enol form) bound to the HP-γ-CD molecule. Cyclodextrin and its ligand are visualized with lines and sticks, respectively. Water molecules are represented in spheres, and the hydrogen bonds are depicted as yellow dashed lines. Water molecule located in the γ-CD binding cavity is pointed by the bold arrow. Molecules are colored according to their atom types. The majority of hydrogens atoms are omitted for clarity.

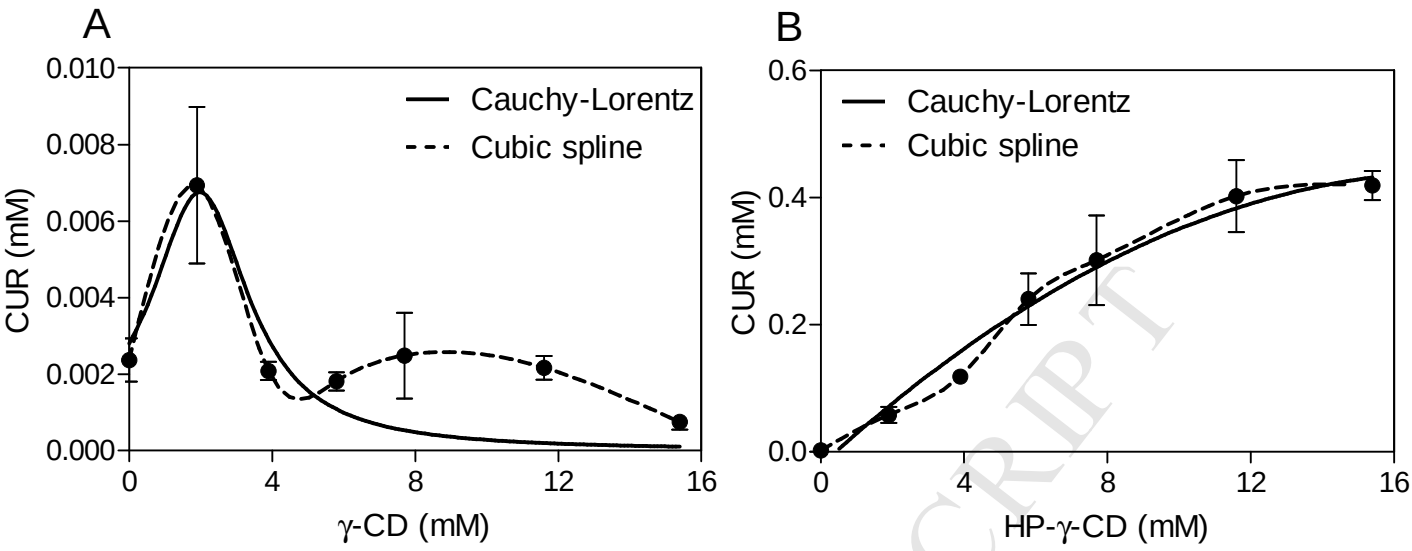


Figure 4. Solubility isotherms of the CUR/γ-CD (A) and CUR/HP-γ-CD (B) complexes determined in H_2O, pH 7.0. The S_0 value (the same for both complexes) states for the CUR saturation concentration in the solvent in absence of cyclodextrin. The solid lines show fitting to the isotherms. The data represent means ± S.D. of three independent experiments.

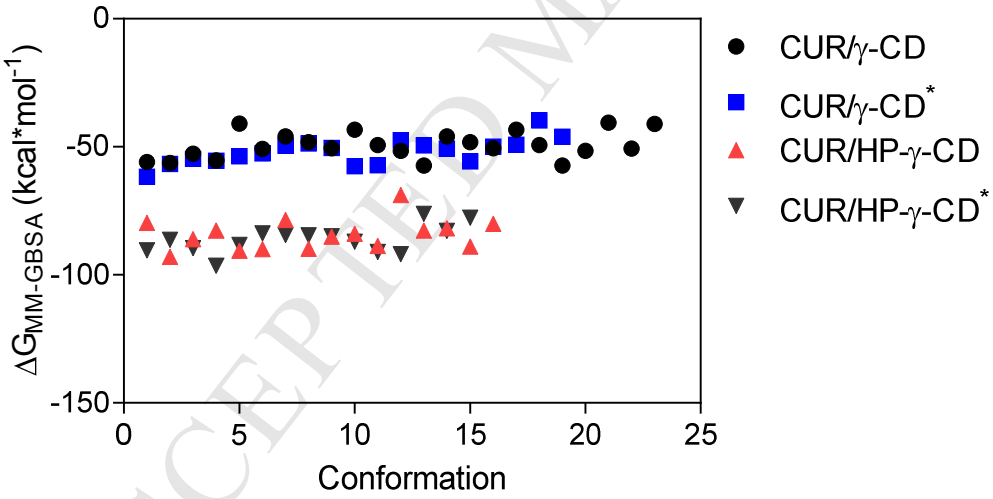


Figure 5: The $\Delta G_{MM\text{-}GBSA}$ values for CUR/γ-CD and CUR/HP-γ-CD with or without CUR hydration (denoted by *) calculated by the MC approach.

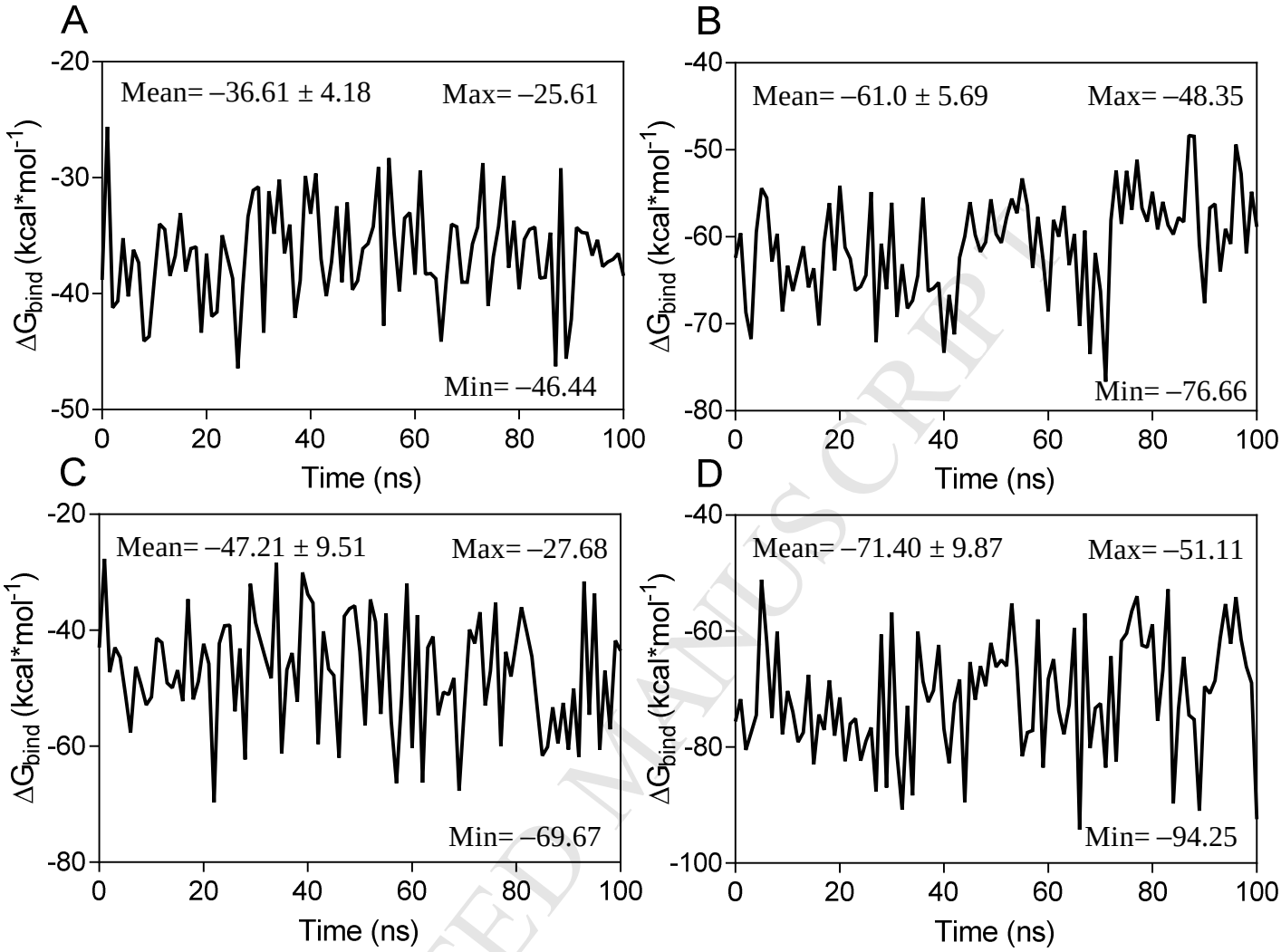


Figure 6: The ΔG_{bind} values (in kcal*mol^{-1}) calculated from 100 ns MD simulations for CUR/γ-CD and CUR/γ-CD* (A, B) together with CUR/HP-γ-CD and CUR/HP-γ-CD* (C, D). CUR hydration is denoted by an asterisk sign. The data represent means ± S.D.

Table 1: Summary of the molecular docking profiles for CUR bound to the γ-CD and HP-γ-CD molecules.

Complex	N_{run}	N_{cl}	ΔG_{bind} (kcal*mol^{-1})	K_d (μM)	LE**	$RMSD_{LC}$ (nm)	N_{ats}***	N_{tors}
CUR/γ-CD	100	66	–6.83	9.45	–0.23	78.02	30	10
CUR/γ-CD*	100	72	–7.69†	2.2	–0.17	78.18	45	10
CUR/HP-γ-CD	100	51	–7.92	1.49	–0.26	77.64	30	10
CUR/HP-γ-CD*	100	65	–9.93†	0.05	–0.22	77.25	45	10

*-hydrated ligand; **- $LE = \frac{\Delta G_{bind}}{N_{ats}}$; ***-waters are described by monatomic pseudoatoms; †- $\Delta G_{bind} = \Delta G_{lig} + \Delta G_{wat}$; N_{runs}-total number of runs; N_{cl}-number of distinct clusters formed in the clustering; $RMSD_{LC}$-the root-mean-square-deviation difference between the lowest energy conformation (LC) in the largest cluster and the reference ligand conformation; N_{ats}-number of heavy atoms; N_{tors}-number of torsions

Table 2: Summary of displaced (DISP), weakly (WK) and strongly (STR) bound water molecules during hydrated complexation of CUR with the γ-CD and HP-γ-CD molecules.

Complex	DISP	WK	STR
CUR/γ-CD	12	2	1
CUR/HP-γ-CD	13	1	1

Table 3: The AM1 energies (in kcal*mol^{-1}) of the 7 species, the energy differences (ΔE) between the inclusion complexes and the molecules (CUR, γ-CD and HP-γ-CD), which form the complexes (CUR/ γ-CD and CUR/HP-γ-CD).

Species	AM1 energy	ΔE**
CUR	–151.19	-
γ-CD	–1891.39	-
HP-γ-CD	–3057.42	-
CUR/γ-CD	–2045.59	–3.01
CUR/γ-CD*	–2234.13	–191.55
CUR/HP-γ-CD	–3199.91	8.7
CUR/HP-γ-CD*	–3324.52	–115.91

*-hydrated ligand; **- $\Delta E = E_{Complex} - E_{CD} - E_{CUR}$

- Curcumin solubility profiles and hydration/desolvation effects of this substance formulated with γ-cyclodextrin and hydroxypropyl-γ-cyclodextrin were investigated.
- Curcumin/hydroxypropyl-γ-cyclodextrin complex was found to be more stable in solution than curcumin/hydroxypropyl-γ-cyclodextrin.
- Water molecules play an important role in host-guest complexation mediating the curcumin binding to cyclodextrins via hydrogen bond formations.
- Curcumin hydration/desolvation effects contributed to the complex formation by elevating the curcumin binding affinity to cyclodextrins.
- Curcumin/hydroxypropyl-γ-cyclodextrin complex was determined with a minimal free energy of binding due to hydrophobic forces.