Sci.Rep.Kanazawa Univ. **ARTICLES**
Vol. 68, pp. 11-23, 2025

The Study of Structure and Interaction of the Aspirin/Hydroxypropyl-β-Cyclodextrin Complex in Various Solvents Using Molecular Dynamics Simulation

Helmia JAYYINUNNISYA*, Kazutomo KAWAGUCHI, and Hidemi NAGAO

Graduate School of Natural Science and Technology, Kanazawa University
Kanazawa, 920-1192, Japan

(Received December 26, 2024 and accepted in revised form February 6, 2025)

Abstract This study investigates the structural dynamics and interactions of the Aspirin/Hydroxypropyl-β-Cyclodextrin (ASA/HPCD) complex in various solvents using molecular dynamics (MD) simulations. The complex's structural stability, hydrogen bonding, and solvent-accessible surface area (SASA) were analyzed using acetone, ethanol, methanol, and water as solvents. The simulations revealed solvent-dependent behavior, with water displaying the greatest stability due to its strong hydrogen-bonding capability.

Keywords. hydroxypropyl-β-cyclodextrin, aspirin, molecular dynamics simulations, solvent effect

1 Introduction

Acetylsalicylic acid or aspirin is a Nonsteroidal anti-inflammatory drug (NSAID) widely used for its anti-inflammatory, analgesic, antipyretic, and antiplatelet effects. However, aspirin exhibits limited solubility in water, which impacts its bioavailability and presents formulation challenges[1–3].

Beta-cyclodextrin, a cyclic oligosaccharide composed of seven glucose units, can be chemically modified by adding hydroxypropyl groups. This modification results in the formation of hydroxypropyl-β-cyclodextrin, which exhibits enhanced physicochemical properties[4,5]. Adding hydroxypropyl groups into the molecule enhances its water solubility, accordingly assisting as an effective excipient for improving the aqueous solubility of poorly soluble drugs[6]. Hydroxypropyl-β-cyclodextrin exhibits a lower toxicity profile than other cyclodextrins, providing a safer alternative for pharmaceutical formulations[7].

Encapsulating aspirin into hydroxypropyl-β-cyclodextrin leads to an inclusion complex that enhances solubility and stability[8–10]. This complexation can enhance aspirin's therapeutic efficacy while allowing more efficient control of its release profile[11]. Lyophilization is used to form these

* *Corresponding author* E-mail: helmia@wriron1.s.kanazawa-u.ac.jp

complexes; however, aspirin's stability varies by method, with specific methods leading to significant degradation over time[9].

The complex hydroxypropyl-β-cyclodextrin with aspirin is a fascinating research subject in the pharmaceutical industry. This complex has attracted interest due to its potential to enhance aspirin's solubility, stability, and bioavailability, a commonly used medication recognized for its anti-inflammatory and analgesic effects[12–15]. Figure 1 shows the structure of hydroxypropyl-β-cyclodextrin, aspirin, and complex aspirin/ hydroxypropyl-β-cyclodextrin. The complex structure used in this study is the benzene side of aspirin, located on the broad side of the rim of hydroxypropyl-β-cyclodextrin.

Molecular dynamics simulations provide a powerful tool for examining the atomic-level interactions between hydroxypropyl-β-cyclodextrin and aspirin, particularly their potential to form inclusion complexes that enhance the solubility and stability of aspirin. By modeling the structural dynamics of these complexes over time, simulations can identify the most stable configurations and reveal how various formulation techniques influence aspirin's stability within the hydroxypropyl-β-cyclodextrin cavity. This approach offers valuable insights that can streamline the development of pharmaceutical formulations, minimizing the reliance on traditional trial-and-error methods and accelerating the optimization process[9,16,17].

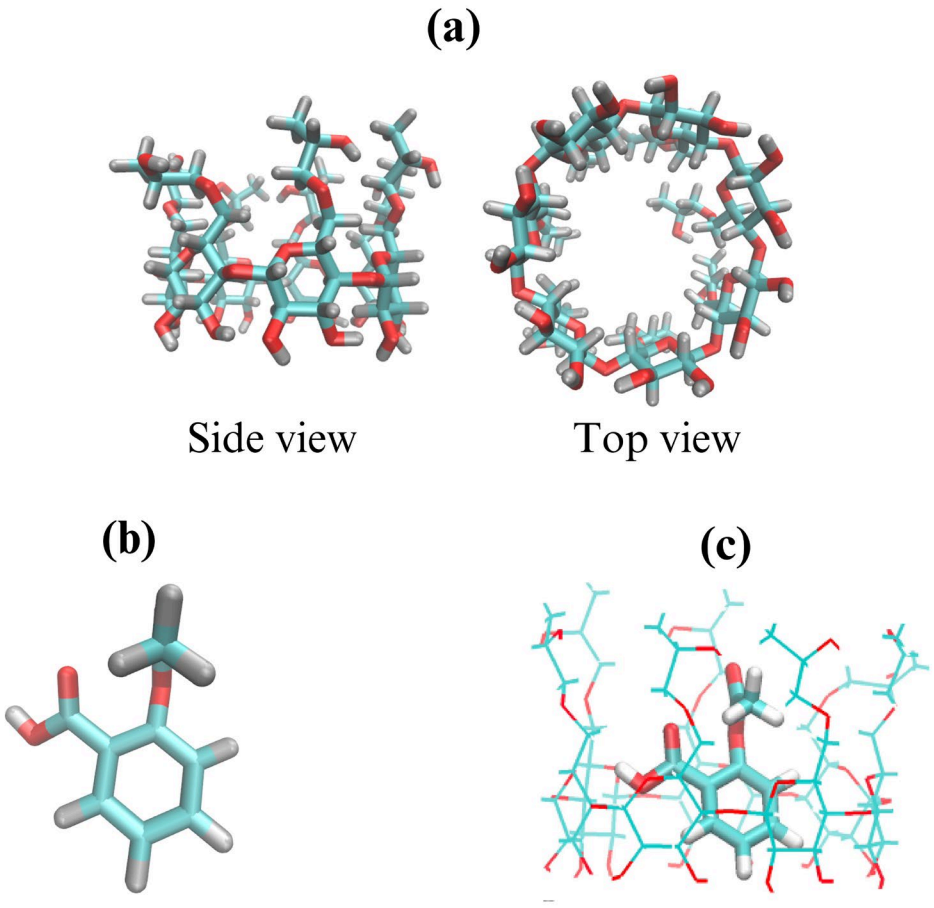

Figure 1. Structure of (a) Hydroxypropyl-β-cyclodextrin (HPCD); (b) Aspirin (ASA); (c) ASA/HPCD complex

This study aims to investigate the structure and stability of the hydroxypropyl-β-cyclodextrin and aspirin complex, emphasizing their molecular interactions and the influence of solvent effects. The investigation includes various solvents, such as water, ethanol, methanol, and acetone, to examine how diverse environments affect the complex's behavior and stability. This study offers insights into the solvent-dependent dynamics and potential applications of the complex in pharmaceutical formulations.

2 Computational Methods

We performed MD simulation using the NAMD program package[18] with the CHARMM36 force field[19–21]. The details of the computational conditions for this simulation are in Table 1. Our prior research derived the initial configuration of the HPCD and ASA complex[22].

The simulations were carried out in a box measuring 40 × 40 × 60 Å^3, defining the space where molecules can move. To ensure a continuous system, three-dimensional periodic boundary conditions were applied, meaning molecules leaving one side of the box to re-enter from the opposite side. Electrostatic interactions between distant atoms were calculated using the particle-mesh Ewald (PME) method, with non-bonded interactions limited to a 10 Å cutoff, following the parameters in Table 1. The temperature was kept constant at 300 K using a Langevin thermostat, and the pressure was maintained at 1 atm. Before starting the primary simulation, the system was equilibrated for 1 ns to allow the molecules to reach a stable arrangement. After equilibration, each system was simulated for 100 ns to collect data.

Hydrogen bond and solvent-accessible surface area (SASA) analyses were performed using VMD[23] to analyze molecular interactions and solvent exposure. Hydrogen bonds were detected based on a donor-acceptor distance of up to 3 Å and an angle of at least 160°, ensuring that only strong and well-defined hydrogen bonds were counted[24]. SASA was calculated using a probe radius of 1.4 Å[25,26], representing a solvent molecule rolling over the surface of the solute to determine its exposure to the solvent. These analyses help understand molecular stability, solvation effects, and interactions within the system.

Table 1. Computational condition

Parameter	Identities
Simulation Program	NAMD
Force field	CHARMM36
Time step	Equilibration; 1ns Production; 100ns
Box size	40 x 40 x 60 Å^3
Cutoffs	10 Å
Temperature Control	Langevin thermostat
Temperature	300 K
Solvent	Acetone (786 molecules) Ethanol (990 molecules) Methanol (1428 molecules) Water (3209 molecules)

3 Results and Discussion

3.1 Root Mean Square Deviation

For stability analysis of ASA and HPCD molecules, the all-atom root-mean-square deviation (RMSD) of ASA and HPCD were plotted using black and red lines, respectively, as shown in Figure 2. RMSD was calculated using the initial equilibrated structure as the reference. Figure 2 shows low RMSD values for all complexes across various solvents, with values consistently remaining below 2 Å. Significant structural changes were observed for ASA in acetone, ethanol, and methanol, whereas HPCD showed limited structural flexibility, with RMSD values consistently ranging from 0.3 to 0.5 Å during the simulations.

In acetone, ASA revealed low RMSD values (0.2 to 0.5 Å) over the first 15 ns, suggesting a conformation comparable to the reference orientation. From 15 to 20 ns, the RMSD showed a slight increase, stabilizing briefly before going through a significant increase after 20 ns. The RMSD increased from 20 ns until the end of the simulation, resulting in an essential structural change in ASA from its reference, with notable steady RMSD fluctuations between 1 and 1.3 Å. In ethanol, ASA maintained low RMSD values (0.2 to 0.8 Å) until 35 ns, then gradually increased to roughly 1 to 1.2 Å, which remained steady for the following 15 ns. The RMSD exhibited minor fluctuations until the end of the simulation.

Methanol showed distinct fluctuations, characterized by a sudden increase to 1 Å within the initial 3 ns, suggesting considerable structural motion. ASA returned to its reference orientation over the next 15 ns, with an RMSD ranging from 0.2 to 0.5 Å. Afterward, from around 20 ns simulation until the end of the simulation, the ASA structure has gripping structural motion, with RMSD fluctuating between 0.2 to 1.4 Å. In contrast to the other solvents, water showed remarkable stability, with ASA remaining highly stable throughout the simulation and RMSD values consistently low (0.3 to 0.6 Å).

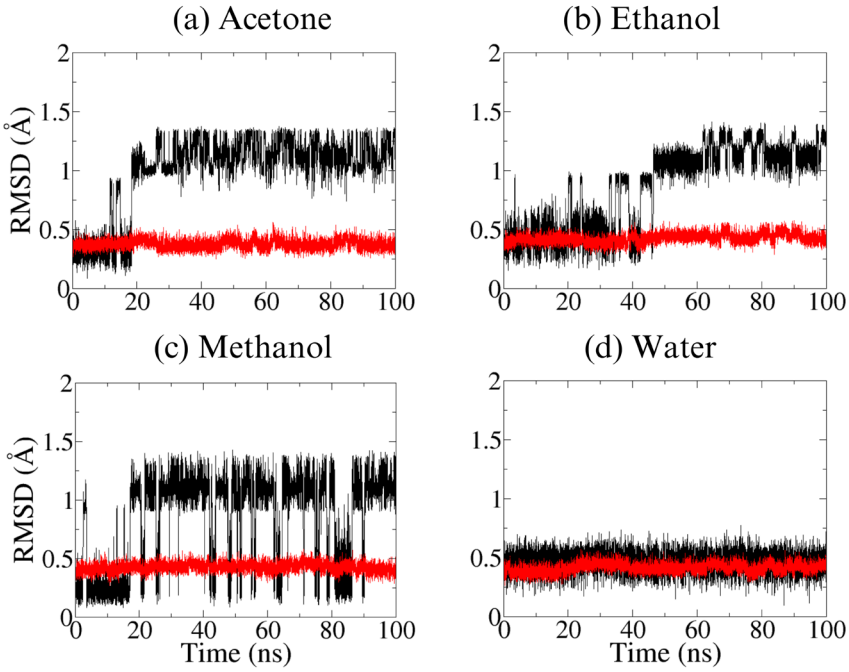

Figure 2. RMSD plots of ASA and HPCD during 100 ns MD simulations. The black and red lines represent the RMSD of ASA and HPCD, respectively.

3.2 Distance between the central of mass of ASA and HPCD

The stability of the inclusion complex has been carefully investigated through the structural deviation of ASA, using all-atom RMSD profiles, as discussed in the previous section. To determine the stability of the inclusion complex, we evaluated the distance between the centers of mass (CoM) of ASA and HPCD during MD simulations, as shown in Figure 3. RMSD indicates structural deviation, whereas CoM distance provides supplementary information regarding the positional behavior of ASA with HPCD.

The CoM distance in water (Figure 3d) remained stable during the simulation, exhibiting minor fluctuations around 5–6 Å. This suggests a strong interaction between ASA and HPCD, maintaining their association within a stable inclusion complex. The blue area indicates the considerable distance the ASA position is inside HPCD. The persistent presence of ASA in the blue zone during the simulation, as illustrated in Figure 3d, verifies that ASA was consistently enclosed within the HPCD cavity.

In contrast, acetone (Figure 3a) revealed that ASA initially remains within HPCD during the first 10-15 ns. Subsequently, ASA showed separation, with the distance fluctuating significantly between 10 and 40 Å, indicating weaker or unstable interactions. This behavior corresponds with the observed increase in ASA's RMSD after 15 ns, as ASA moves outside the inclusion complex.

A similar trend was observed in ethanol (Figure 3b). ASA remained within HPCD for roughly 30 ns, showing low RMSD values ranging from 0.2 to 0.8 Å. Following a time frame of 30 ns, ASA dissociated from HPCD, resulting in fluctuating distances and an increase in RMSD to approximately 1 to 1.2 Å. This suggests that ASA's structural deviation aligns with its positional movement relative to HPCD.

In methanol (Figure 3c), ASA dissociated from HPCD at approximately 19 ns. The distance increased and fluctuated between 10 and 40 Å, aligning with the dynamic motion indicated by the RMSD results. The higher RMSD values for ASA in methanol indicate a less stable position than HPCD, consistent with the more significant fluctuations in the distance.

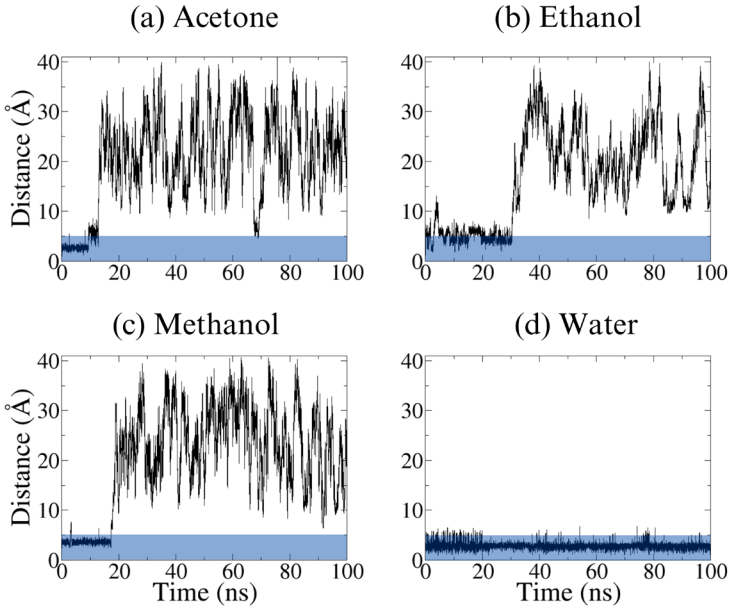

Figure 3. Distance between CoM of HPCD and CoM of ASA in (a) acetone, (b) ethanol, (c) methanol, and (d) water. The blue area indicates the area where ASA is inside the HPCD.

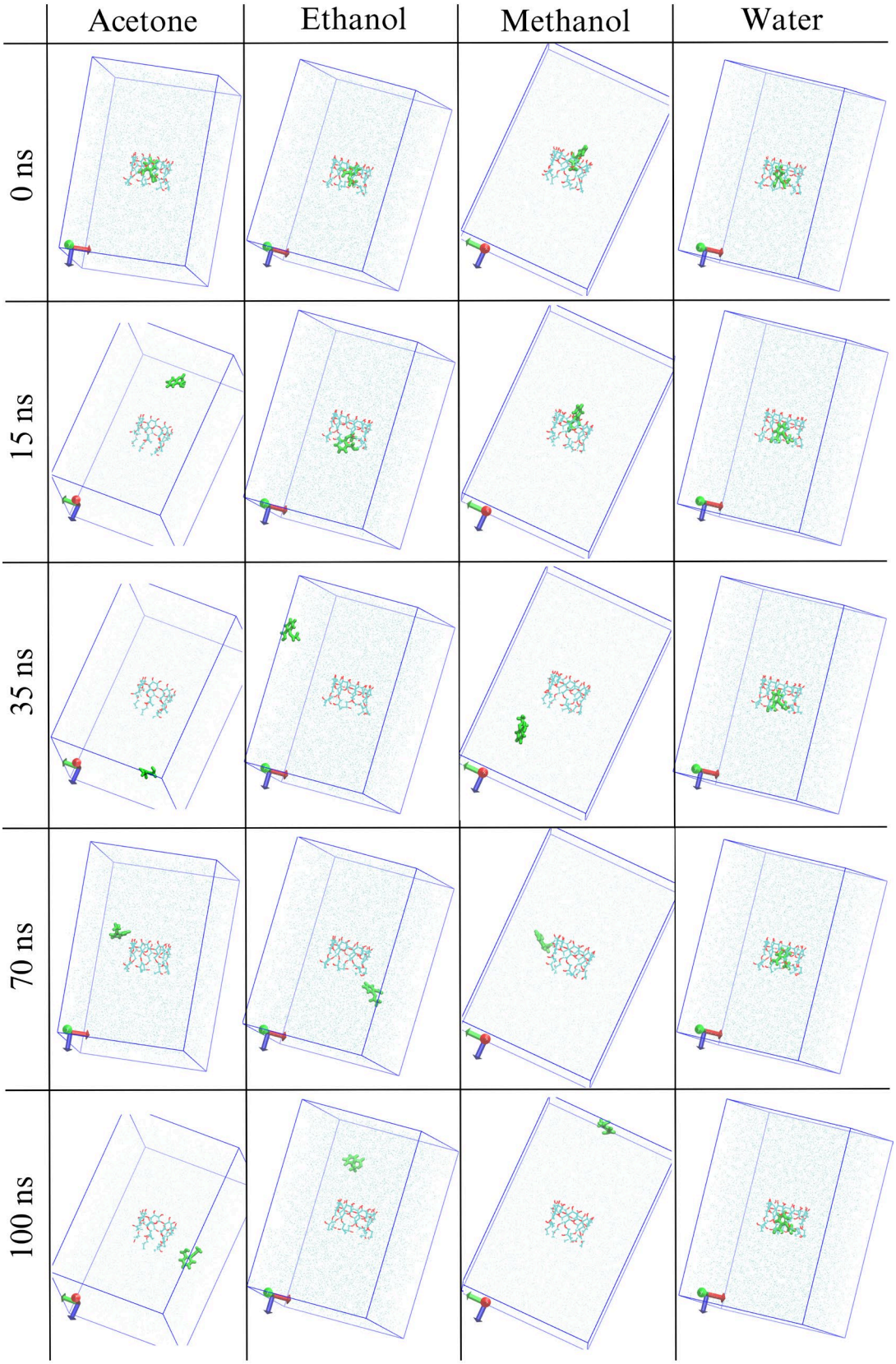

Figure 4. Snapshots of MD simulations in acetone, ethanol, methanol, and water at 0, 15, 35, 70, and 100 ns. The green molecule is ASA, and the molecule in the central box is HPCD. The red, green, and blue arrows in the bottom-left corner represent the *x*, *y*, and *z* coordinates, respectively.

Figure 4 visually supports the findings by showing snapshots from the MD simulations. In acetone, ethanol, and methanol, the ASA molecule moves out of HPCD after 35 ns and stays outside until the simulation ends. This matches the trends in Figure 3, where the CoM distance indicates ASA's separation. In contrast, ASA stays inside HPCD for the entire simulation in water, confirming that the complex remains stable in this solvent. The agreement between Figures 3 and 4 suggests that water is most stable in this study, while organic solvents cause ASA to move out over time.

3.3 Hydrogen Bonding

Figure 5 and Table 2 represent the number of hydrogen bonds established within the ASA/HPCD complex in the analyzed solvents. Most hydrogen bonds were observed around HPCD (red line), with water exhibiting the most significant number due to its natural hydrogen-bonding capacity. HPCD can form hydrogen bonds due to its structure, which has more hydroxyl groups[27].

A limited number of hydrogen bonds were found in acetone, with a maximum of six bonds identified. No hydrogen bonds were formed directly between ASA and acetone. The hydrogen bonds between ASA and HPCD were primarily observed in the first 12 ns, suggesting structural stability during this timeframe. After 12 ns, hydrogen bonds are expected to form between ASA and HPCD due to the interaction of ASA, located outside of HPCD, as indicated by the CoM distance results and the increased RMSD of ASA. This suggests that ASA's move toward HPCD results in decreased interactions and increased structural deviation.

Hydrogen bonds were formed between ethanol and ASA, while interactions within the ASA/HPCD complex were primarily observed between 5 and 30 ns. The decrease in hydrogen bonds after 30 ns corresponds with the separation of ASA from HPCD, as evidenced by the CoM distance and RMSD results. Methanol showed a similar pattern, with occasional hydrogen bonds forming between methanol and ASA. However, bonds within the ASA/HPCD complex were rarely observed.

Water formed the most significant hydrogen bonds between water and ASA and within the ASA/HPCD complex. The hydrogen bonds were consistently observed throughout the simulation, enhancing the stable association observed in water. The consistent distance and low RMSD highlight the water's capacity to maintain the structural stability of the inclusion complex.

Figure 6 illustrates how hydrogen bonds (shown as red dotted lines inside blue circles) connect ASA and HPCD in various solvents. Specifically, oxygen atoms in ASA bond with hydroxyl (OH) groups on HPCD's hydroxypropyl components. This visual supports the data by showing how these bonds help stabilize the ASA/HPCD complex.

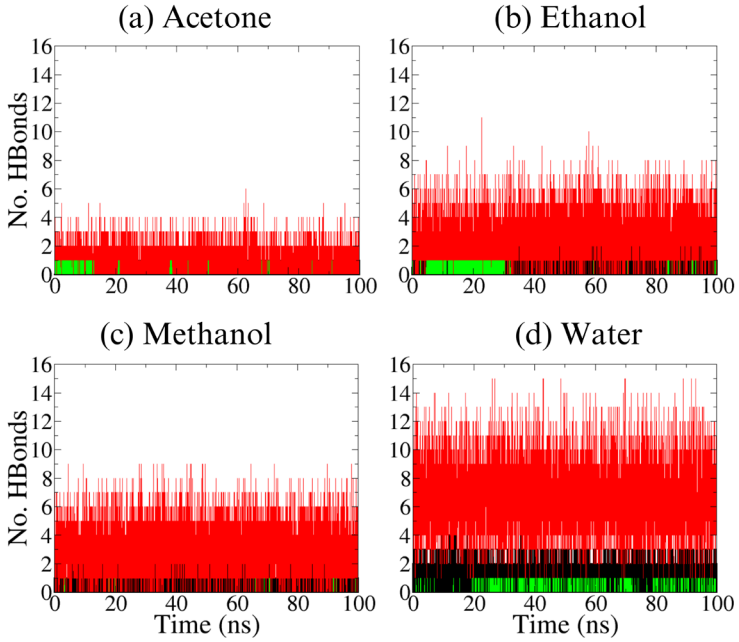

Figure 5. The number of hydrogen bonds of ASA/HPCD complex in the (a) acetone, (b) ethanol, (c) methanol, and (d) water. The black, red, and green lines represent the hydrogen bonds of ASA with solvents, HPCD with solvents, and ASA with HPCD, respectively.

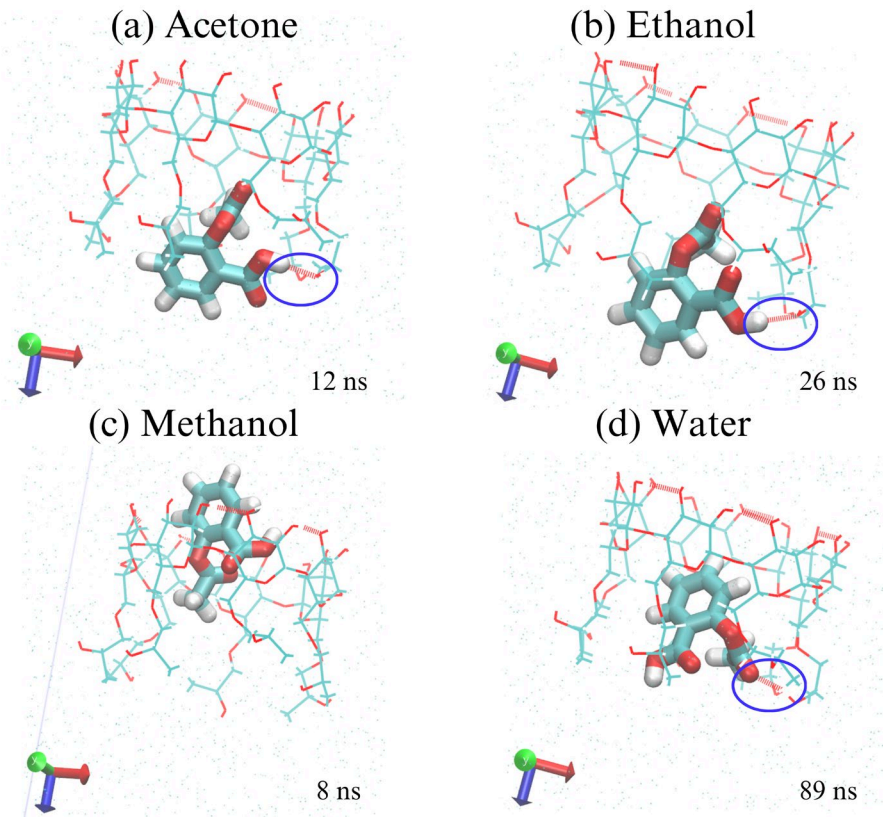

Figure 6. Snapshots showing hydrogen bonds (red dot line in the blue circles) between ASA and HPCD in different solvents: (a) acetone, (b) ethanol, (c) methanol, and (d) water.

Table 2. The number of total hydrogen bonds of complex ASA/HPCD in the different solvents.

Solvent	Average Number of Hydrogen Bonds	Standard Deviation of Hydrogen Bonds	Maximum Number of Hydrogen Bonds
Acetone	1.18	1.02	6
Ethanol	3.32	1.75	11
Methanol	3.70	1.78	11
Water	7.72	2.47	16

3.4 Solvent Accessible Surface Area and Radial Distribution Function

Figure 7 and Table 3 provide the solvent-accessible surface area (SASA) of the ASA/HPCD complex over time across various solvents. SASA offers valuable insights regarding the complex's exposure to the solvent, enhancing the analyses of RMSD and CoM distance. Notably, lower SASA values indicate that ASA remains predominantly encapsulated within the hydrophobic HPCD cavity. At the same time, gradual increases in SASA suggest that ASA becomes increasingly exposed to the solvent, likely due to partial or complete dissociation from HPCD.

The complex showed an average SASA of 1772.62 Å² in acetone, with significant fluctuations. After an initial phase of low SASA during the first 20 ns, the values increased and stabilized at approximately 1800 Å², indicating that the complex remains exposed mainly to acetone. Weak solvent interactions likely contribute to the observed high SASA and fluctuations, which align with the CoM distance and RMSD analyses.

The complex showed an average SASA of 1710.51 Å² in ethanol, which included significant variations. A notable rise in SASA was observed around 20 ns, followed by stabilization near 1800 Å², suggesting enhanced solvent exposure. The increased solvent accessibility indicates that ethanol interacts moderately with the complex, providing a more extended conformation of ASA.

The complex showed an average SASA of 1770.25 Å² in methanol and a standard deviation of 132.72 Å². While the average SASA is comparable to acetone, the higher variability underscores inconsistent solvent exposure. A rapid SASA increase during the first 20 ns was followed by instability, with periodic fluctuations over time. These results imply that methanol provides moderate solvation but less consistent exposure than ethanol or acetone. The elevated SASA values and their fluctuations align with CoM distance trends (ASA dissociates from HPCD after 19 ns) and RMSD data, suggesting structural instability in methanol.

ASA showed the lowest and most stable SASA in waters, averaging 1397.40 Å² with minimal fluctuations. The compact and stabilized structure of ASA in water is associated with low RMSD and stable CoM distance, confirming water's effectiveness in stabilizing ASA.

Figure 8 shows the RDF data for solvent-solvent interactions, specifically C–C interactions for acetone, ethanol, and methanol, and O–O interactions for water. The results highlight the structural characteristics of these solvents. Water peaked at around 2.8 Å, indicating its significant hydrogen-bonding capacity. This observation is consistent with water having the most hydrogen bonds and the lowest SASA. Methanol and ethanol showed peaks of 3.5 to 4.0 Å, suggesting moderate structuring attributed to their hydrogen-bonding properties. These findings correspond to their higher SASA values and the presence of some hydrogen bonds. Acetone showed a broad and low peak in the 4 to 6 Å range, indicating weaker and less well-order interactions assigned to its lack of hydrogen bond formation. The minimal hydrogen bond formation and the high SASA of acetone also support this.

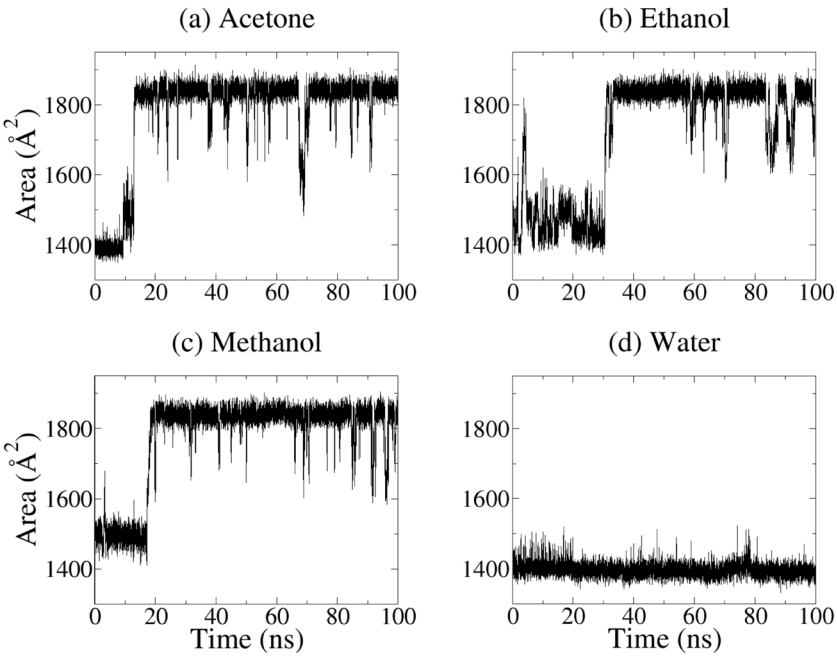

Figure 7. Solvent Accessible Surface area of ASA/HPCD complex in the (a) acetone, (b) ethanol, (c) methanol, and (d) water.

Table 3. Solvent Accessible Surface area value of ASA/HPCD complex

Solvent	Average SASA (Å²)	Standard Deviation of SASA (Å²)	Minimum of SASA (Å²)	Maximum of SASA (Å²)
Acetone	1772.62	145.71	1349.65	1912.99
Ethanol	1710.51	170.05	1371.73	1903.99
Methanol	1770.25	132.72	1411.01	1904.8
Water	1397.40	18.94	1332.41	1523.62

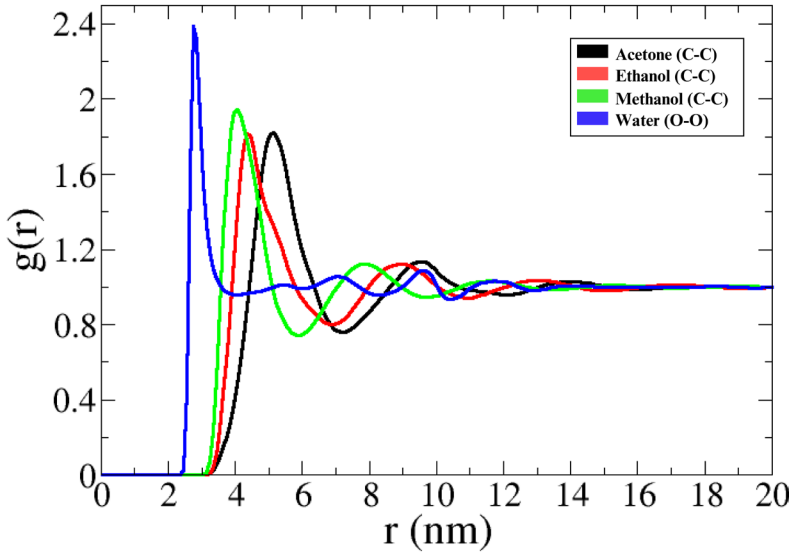

Figure 8. The Radial Distribution Function for solvent-solvent interactions with C-C of acetone (black line), C-C of ethanol (red line), C-C of methanol (green line), and O-O of water (blue line).

4 Concluding Remark

To understand the solvent effect on the stability of the ASA/HPCD complex, we evaluated various solvents, including acetone, ethanol, methanol, and water. This study highlights the critical influence of the solvent environment on the structural stability and dynamic behavior of the ASA/HPCD complex. Water was identified as the most effective medium among the solvents analyzed for promoting the stable inclusion of ASA within the HPCD cavity. A low SASA, minimal structural deviations, and consistent molecular interactions evidenced this effectiveness. In contrast, acetone, ethanol, and methanol exhibited weaker complex stability, marked by pronounced fluctuations and reduced structural coherence.

The findings provide insights into the solvent-dependent stability of the ASA/HPCD complex, underscoring water's suitability as a preferred medium for cyclodextrin-based pharmaceutical formulations. This research aligns with our complementary free energy calculations, which confirmed water's ability to provide the most stable environment for the ASA/HPCD complex. Free energy analyses further revealed that water's strong solvation effects primarily contribute to its stabilizing role.

Acknowledgments

This work was supported by JSPS KAKENHI Grant Numbers 23K03338 (H.N.) and 23K03339 (K.K.), and by JST SPRING, Grant Number JPMJSP2135 (H.J.). A part of the computation was performed using the Research Center for Computational Science, Okazaki, Japan (Project: 22-IMS-C045, 23-IMS-C040, 24-IMS-C040).

References

1. Robertson, D. (2013). Uses, interactions, and side effects of aspirin in older people. *Nursing and Residential Care, 15*(5). https://doi.org/10.12968/nrec.2013.15.5.263
2. Bakar, S. K., & Niazi, S. (1983). Stability of aspirin in different media. *Journal of Pharmaceutical Sciences, 72*(9). https://doi.org/10.1002/jps.2600720914
3. Desborough, M. J. R., & Keeling, D. M. (2017). The aspirin story – from willow to wonder drug. *British Journal of Haematology, 177*(5), 674–683. https://doi.org/10.1111/bjh.14520
4. Jambhekar, S. S., & Breen, P. (2016). Cyclodextrins in pharmaceutical formulations II: Solubilization, binding constant, and complexation efficiency. *Drug Discovery Today, 21*(2). https://doi.org/10.1016/j.drudis.2015.11.016
5. Jansook, P., Ogawa, N., & Loftsson, T. (2018). Cyclodextrins: Structure, physicochemical properties, and pharmaceutical applications. *International Journal of Pharmaceutics, 535*(1–2). https://doi.org/10.1016/j.ijpharm.2017.11.018
6. Pitha, J., et al. (1986). Hydroxypropyl-β-cyclodextrin: Preparation and characterization; effects on solubility of drugs. *International Journal of Pharmaceutics, 29*(1). https://doi.org/10.1016/0378-5173(86)90201-2
7. Gould, S., & Scott, R. C. (2005). 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD): A toxicology review. *Food and Chemical Toxicology, 43*(10). https://doi.org/10.1016/j.fct.2005.03.007

8. Shekh, I., Gupta, V., Jain, A., & Gupta, N. (2011). Preparation and characterization of β-cyclodextrin aspirin inclusion complex. *International Journal of Pharmacy & Life Sciences, 2*(4), 704–710.
9. Williams, R. O., & Liu, J. (1999). Influence of formulation technique for hydroxypropyl-β-cyclodextrin on the stability of aspirin in HFA 134a. *European Journal of Pharmaceutics and Biopharmaceutics, 47*(2). https://doi.org/10.1016/S0939-6411(98)00072-1
10. Nishioka, F., Nakanishi, I., Fujiwara, T., & Tomita, K. I. (1984). The crystal and molecular structure of the β-cyclodextrin inclusion complex with aspirin and salicylic acid. *Journal of Inclusion Phenomena, 2*(3–4), 701–714. https://doi.org/10.1007/BF00662238
11. Zhou, H. Y., Jiang, L. J., Zhang, Y. P., & Li, J. B. (2012). β-Cyclodextrin inclusion complex: Preparation, characterization, and its aspirin release in vitro. *Frontiers of Materials Science, 6*(3), 259–267. https://doi.org/10.1007/s11706-012-0176-2
12. Tee, O. S., & Takasaki, B. K. (1985). The cleavage of aspirin by α- and β-cyclodextrins in basic aqueous solution. *Canadian Journal of Chemistry, 63*(12). https://doi.org/10.1139/v85-581
13. El-Gendy, G. A., Terada, K., Yamamoto, K., & Nakanishi, Y. (1986). Molecular behavior, dissolution characteristics, and chemical stability of aspirin in the ground mixture and in the inclusion complex with di-O-methyl-β-cyclodextrin. *International Journal of Pharmaceutics, 31*(1–2). https://doi.org/10.1016/0378-5173(86)90209-7
14. Brewster, M. E., & Loftsson, T. (2007). Cyclodextrins as pharmaceutical solubilizers. *Advanced Drug Delivery Reviews, 59*(7). https://doi.org/10.1016/j.addr.2007.05.012
15. Rasheed, A., Kumar, C. K. A., & Sravanthi, V. V. N. S. S. (2008). Cyclodextrins as drug carrier molecule: A review. *Scientia Pharmaceutica, 76*(4). https://doi.org/10.3797/scipharm.0808-05
16. Wang, R., et al. (2015). Comparison of three molecular simulation approaches for cyclodextrin-ibuprofen complexation. *Journal of Nanomaterials, 2015*. https://doi.org/10.1155/2015/193049
17. Mazurek, A. H., Szeleszczuk, Ł., & Gubica, T. (2021). Application of molecular dynamics simulations in the analysis of cyclodextrin complexes. *International Journal of Molecular Sciences, 22*(17). https://doi.org/10.3390/ijms22179422
18. Phillips, J. C., et al. (2020). Scalable molecular dynamics on CPU and GPU architectures with NAMD. *Journal of Chemical Physics, 153*(4). https://doi.org/10.1063/5.0014475
19. Brooks, B. R., et al. (2009). CHARMM: The biomolecular simulation program. *Journal of Computational Chemistry, 30*(10). https://doi.org/10.1002/jcc.21287
20. Vanommeslaeghe, K., et al. (2010). CHARMM general force field: A force field for drug-like molecules compatible with the CHARMM all-atom additive biological force fields. *Journal of Computational Chemistry, 31*(4). https://doi.org/10.1002/jcc.21367
21. Guvench, O., et al. (2009). CHARMM additive all-atom force field for glycosidic linkages between hexopyranoses. *Journal of Chemical Theory and Computation, 5*(9). https://doi.org/10.1021/ct900242e
22. Jayyinunnisya, H., et al. (2022). Theoretical study of complex aspirin and hydroxypropyl-β-cyclodextrin in the solvent phase. *Journal of Physics: Conference Series, 2207*. https://doi.org/10.1088/1742-6596/2207/1/012022
23. Humphrey, W., Dalke, A., & Schulten, K. (1996). VMD: Visual molecular dynamics. *Journal of Molecular Graphics, 14*(1). https://doi.org/10.1016/0263-7855(96)00018-5

24. Arunan, E., et al. (2011). Defining the hydrogen bond: An account (IUPAC Technical Report). *Pure and Applied Chemistry, 83*(8). https://doi.org/10.1351/PAC-REP-10-01-01
25. Lee, B., & Richards, F. M. (1971). The interpretation of protein structures: Estimation of static accessibility. *Journal of Molecular Biology, 55*(3). https://doi.org/10.1016/0022-2836(71)90324-X
26. Shrake, A., & Rupley, J. A. (1973). Environment and exposure to solvent of protein atoms. Lysozyme and insulin. *Journal of Molecular Biology, 79*(2). https://doi.org/10.1016/0022-2836(73)90011-9
27. Saenger, W. (1980). Cyclodextrin inclusion compounds in research and industry. *Angewandte Chemie International Edition, 19*(5). https://doi.org/10.1002/anie.198003441