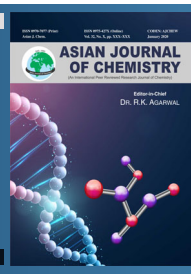
Asian Journal of Chemistry; Vol. 32, No. 4 (2020), 930-934

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2020.22508



Structural Determination of Cephalexin/β-Cyclodextrin Inclusion Complex and its Validation using Molecular Simulation Methods

DINESH SHARMA[1], HARISH SARASWAT[1,*], SYQA BANOO[1] and MAIDUL ISLAM[2]

[1]Department of Chemistry, Mangalayatan University, Aligarh-Mathura Highway, Beswan-202145, India
[2]Department of Chemistry, Aligarh Muslim University, Aligarh-202002, India

*Corresponding author: E-mail: harishsar@gmail.com

Received: 20 October 2019; *Accepted*: 12 December 2019; *Published online*: 25 February 2020; AJC-19814

In this work, molecular simulation methods combining molecular docking and the structure elucidation of inclusion complex of cephalexin/β-cyclodextrin (β-CD) was determined using ^{1}H NMR. Molecular docking studies showed that the complex formation process between β-CD and cephalexin was favourable and spontaneous because of negative values of binding energies. Molecular docking studies also confirmed the entry of aromatic ring into the cavity. Molecular mechanics studies were performed for aromatic ring of cephalexin, in different orientations and from both cavity ends, to measure the parameters of inclusion depth and mode of entry. The resultant structure was studied for HOMO-LUMO gap to validiate the method. The final structure was established using ^{1}H NMR, molecular mechanics, HOMO-LUMO gap and molecular docking.

Keywords: Cephalexin, β-Cyclodextrin, Molecular mechanics, Molecular docking.

INTRODUCTION

The cyclodextrin inclusion complexes are a type of supramolecules that lead to core understanding of basic aspects of binding mechanisms allowing robust evaluations in supramolecular chemistry. Cyclodextrin cavity is wider at one end, narrow at the other and has high potential to form inclusion complexes with different types of hydrophobic guest because of inner hydrophobic cavity [1]. The physico-chemical properties of guest molecules like solubility, stability, bioavailability, *etc*. alter on their inclusion into cyclodextrin cavity [2]. Different types of weak interactions like H-bonds [3], ion-π interaction [4], π-π interactions [5] are responsible to hold these supramolecular complexes by their combined effects. The effects of these interactions can be studied by structural investigations of these complexes. Structural characterization of these complexes is important because of their applications in food and pharmaceutical industries [6,7]. Nowadays structural characterization of complexes to be used in drug delivery and drug protection is being required legally [8]. More investigations into the interesting properties of cyclodextrins in molecular and chiral recognition of molecule make it necessary the structural elucidation of cyclodextrin inclusion complexes [9].

There are different experimental as well as computational techniques used from time to time for structural elucidation of cyclodextrin inclusion complexes. The different types of spectroscopies like UV, FTIR [10], XRD and ^{1}H NMR [11] have been used but ^{1}H NMR is one of the best experimental techniques used widely for structural studies because it gives the direct evidence of inclusion complex formation. Crystal XRD provides highly reliable and accurate structure but it require the crystal formation which is not always possible. The change in chemical shift of cyclodextrin cavity protons towards highfield and guest protons towards the lowfield confirms the complex formation [12]. But this does not provide any information on the mode of entry (wide side or narrow side), depth of inclusion and part of the guest encapsulated into the cavity. Evolution occurred in structural studies of cyclodextrin complexes due to discovery of nuclear overhauser effect (NOE) like rotatory frame overhauser effect spectroscopy (ROESY). In ROESY spectrum, interactions between the guest and cyclodextrin cavity protons help in analyzing the part of the guest

encapsulated into the cyclodextrin cavity [11]. The ROEs can also be studied quantitatively by determining interproton distances and hence determining atom accurate structures [13]. But the mode of entry into cyclodextrin cavity can be predicted by ROESY or not is still a matter of conflict as seen in some cases.

A 3D model studies with experimental support are one of the best techniques for the structural elucidation of cyclodextrin inclusion complexes. These studies provide reliable results, showing mode of entry, inclusion depth as well stable configuration of inclusion complex [11]. Theoretical methods are based on classical mechanics (Newtonian motion) as well as quantum mechanics. Classical methods like molecular mechanics (MM) and molecular docking (MD) are based on classical mechanics provides reliable results when supported experimentally. Quantum mechanical methods like *ab initio*-DFT theory are based on quantum mechanics hence are considered quiet reliable but are highly expensive as compared to classical methods. Classical methods use different force fields like Alinger′s force field, AMBER, *etc*. [14], whereas DFT like methods provides electronic structure [15].

In this article, aim is to elucidate the structural details of interaction between β-cyclodextrin and cephalexin. Cephalexin is a second generation cephalosporin antibiotic is an effective broad spectrum antibiotic that target both Gram-positive and Gram-negative bacteria [16]. Gram-positive bacteria easily absorb the antibiotic drug causing urinary tract infection; on the other hand, Gram-negative bacteria cannot easily absorb antibiotic drug and causes pneumonia. Cephalexin consists of one aromatic ring with side chain containing -NH_2, -COOH groups as the different binding sites which makes it interesting to study its interactions with cyclodextrin. Here, it was considered from previous literature that the complex formed is in 1:1 ratio as the cavity of β-CD is just large enough to accommodate one aromatic ring. The spectroflurometrical investigations for host-guest inclusion complex formation between β-CD and cephalexin showed 1:1 stoichiometry. Using some analytical techniques inclusion constant was calculated to be K = 5.33×10^2 L mol^{-1} [17]. The interaction between β-CD and cephalexin in aqueous form was investigated using ^{1}H NMR and some molecular simulation methods like molecular modeling, molecular docking and HOMO-LUMO orbital interactions.

EXPERIMENTAL

Pure cephalexin and β-cyclodextrin were provided by Shri Ji Pharma International, Vadodara and Sun Pharma India, respectively as gift samples. The sample was prepared in D_2O at room temperature. All ^{1}D and ^{2}D NMR spectra for cephalexin/β-cyclodextrin mixtures were recorded on a Bruker 400MHz NMR instrument, using 5 mm CPDUL ^{13}C spectroscopic probe at 301 K. A 2D 1H-1H COSY for a 1:1 complex was recorded on a 400 MHZ instrument in D_2O at 301 K.

RESULTS AND DISCUSSION

^{1}H NMR analysis: ^{1}D and ^{2}D NMR spectra were used to assign β-CD protons in cephalexin/β-CD mixture. There was chemical shift change in β-CD protons in presence of cephalexin, as seen from the 1H-NMR spectrum of pure β-CD and cephalexin/β-CD mixture (1:1 ratio). The maximum chemical shift change towards highfield was seen in cavity protons of β-CD *i.e*. in H-3′ and H-5′ in comparison to other protons of β-CD. Negligible shift changes were seen in other protons *i.e*. H-1′, H-2′, H-4′ and H-6′ of β-CD on increasing cephalexin concentration. The highfield shift changes in cavity protons of β-CD is because of high shielding due to the presence of aromatic ring which is the π-electron rich group or in other words may be due to anisotropic effect [18]. These changes in chemical shift of H-3′ and H-5′ cavity protons towards highfield suggested the entry of cephalexin molecule into the hydrophobic cavity of β-CD [19]. Fig. 1 shows the expansion of ^{1}H NMR showing the upfield shift of H-3′ and H-5′ protons of β-CD in cephalexin/β-CD (1:1 ratio) complex, compared to pure β-CD. This result confirmed the formation of an inclusion complex between β-CD and cephalexin. Also the large shift in H-3′ as compared to H-5′ cavity proton shows that complexation has occurred from wide side [20]. The drug contains 5 aromatic protons all of which appear as multiplet in one peak near aromatic region.

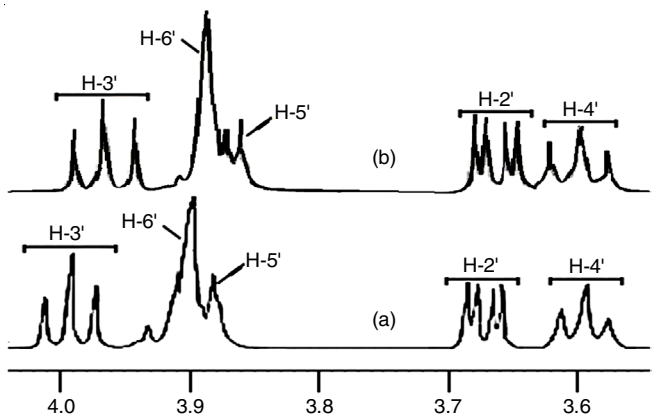


Fig. 1. ^{1}H NMR of pure β-CD (a) and ^{1}H NMR of CPL/β-CD mixture (b)

Molecular mechanics: Molecular mechanics studies were performed using Chem 3D in vapour phase [21] using Allinger′s force field. During the study, it was considered that there is no water present inside the cavity as cavity is hydrophobic. In this study, drug molecule is placed manually in the cavity of cyclodextrin at different depths and different orientations which is then followed by energy minimization. The study was performed using three different modes at three different depths on both narrow as well as wide side of cyclodextrin. The three different depths are shown as W1, W2, W3 on wide side and N1, N2, N3 on narrow side where as three different modes are shown as mode 1, mode 2 and mode 3 which are shown in Figs. 2 and 3. The steric energy of pure cyclodextrin and cepha-

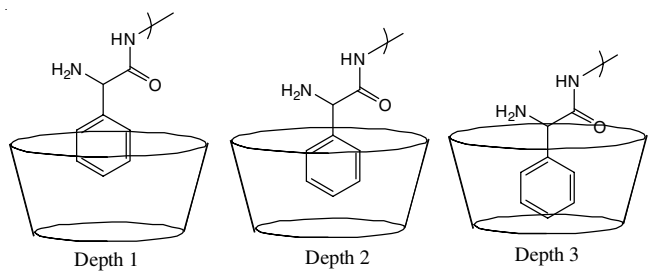


Fig. 2. Different depths at which molecular mechanics studies were done

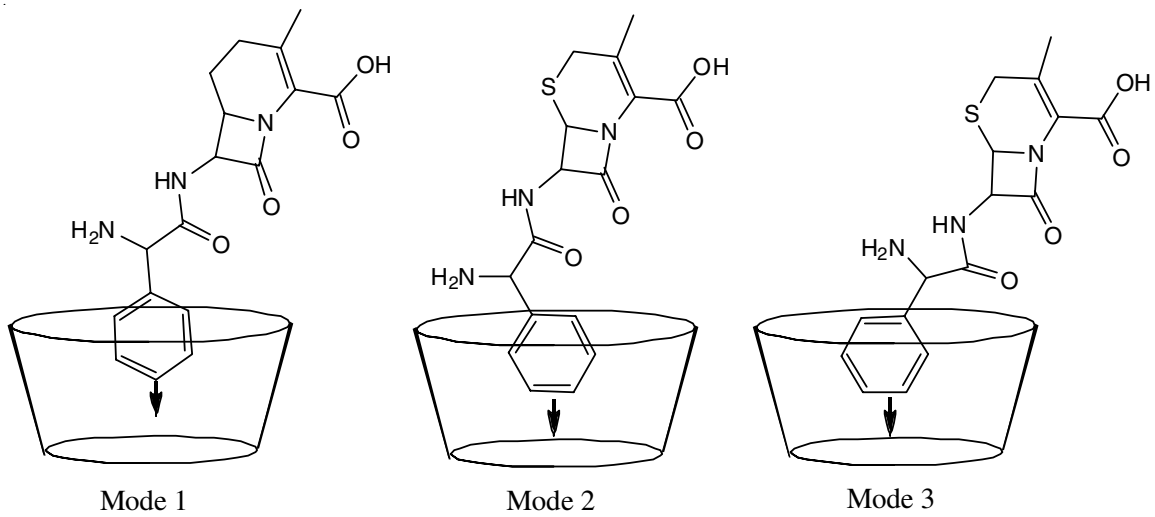


Fig. 3. Different modes of study used in molecular mechanics

lexin was found to be 106.648 and 38.139 kcal/mol, respectively. The binding energy for the inclusion complex formed was calculated using the following formula:

$$E_{\text{binding energy}} = E_{\text{complex}} - E_{\text{pure CD + pure cephalexin}}$$

The binding energy calculations showed that stable complex was formed at the deep bottom of cyclodextrin in mode 3. Higher the negative value of binding energy more stable is the complex [22]. The top, side and bottom view of this stable complex is shown in Fig. 4. The binding energies of inclusion complexes studied in different modes and different depths from both narrow and wide end are summarized in Table-1.

Molecular orbitals studies: The complexes studied in different modes using molecular mechanics were further verified using HOMO-LUMO gap. The molecular orbitals were generated by Chem 3D pro using molecular orbital Huckel calculations. The same mode and orientation as studied by molecular mechanics was found to be stable complex as it is known that higher the HOMO-LUMO gap, more stable is the complex [22]. The HOMO-LUMO gaps for the studied configurations are summarized in Table-2. Hence, W33 was found as the stable complex. The top, side and bottom view of HOMO and LUMO for W33 mode are shown in Fig. 5. The energy for the LUMO is -2.838 eV and that for HOMO is -8.177 eV. The HOMO-LUMO gap for this complex was found to be 5.339 eV. The HOMO and LUMO both were found lying on the guest molecule.

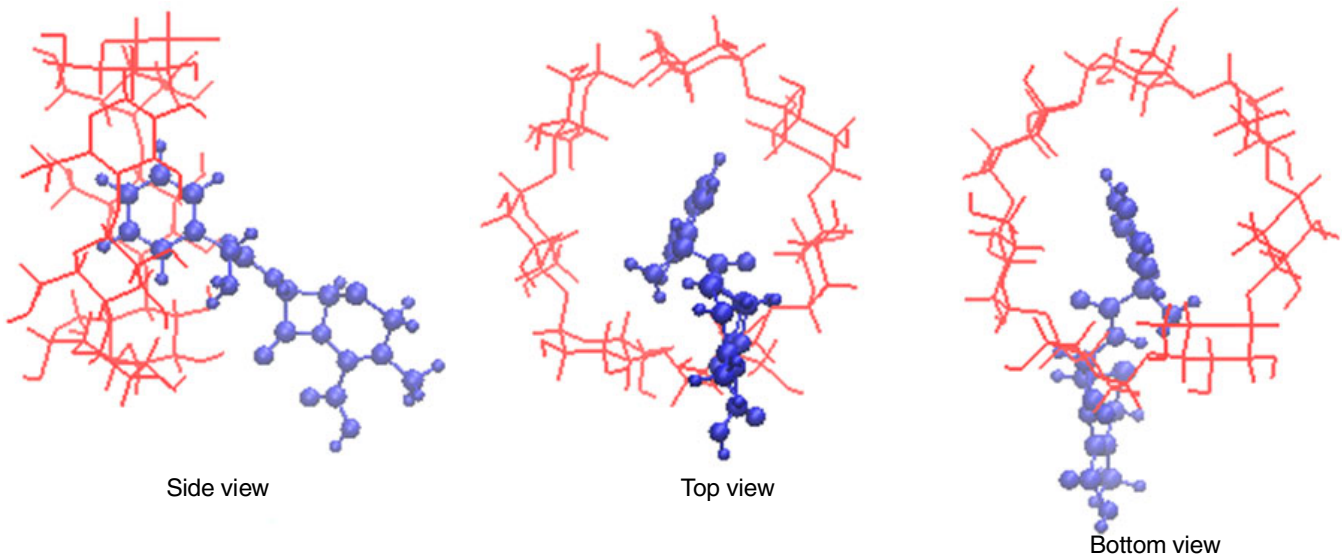


Fig. 4. Side, top and bottom view of the final structure studied by molecular mechanics

TABLE-1
BINDING ENERGIES (kcal/mol) OF INCLUSION COMPLEXES STUDIED AT DIFFERENT DEPTHS IN DIFFERENT MODES BY MOLECULAR MECHANICS

Modes	W1	W2	W3	N1	N2	N3
Mode 1	-7.286	-12.557	-15.646	-11.503	-16.760	-18.278
Mode 2	-7.997	-11.827	-16.127	-12.672	-20.672	-20.936
Mode 3	-8.738	-15.568	-24.377	-12.141	-19.613	-19.527

TABLE-2
HOMO-LUMO GAP (ev) OF INCLUSION COMPLEXES STUDIED AT
DIFFERENT DEPTHS IN DIFFERENT MODES BY MOLECULAR MECHANICS

Modes	W1	W2	W3	N1	N2	N3
Mode 1	5.188	5.202	5.204	5.176	5.150	5.183
Mode 2	5.186	5.191	5.195	5.076	5.046	5.132
Mode 3	5.187	5.207	5.339	5.179	5.156	5.118

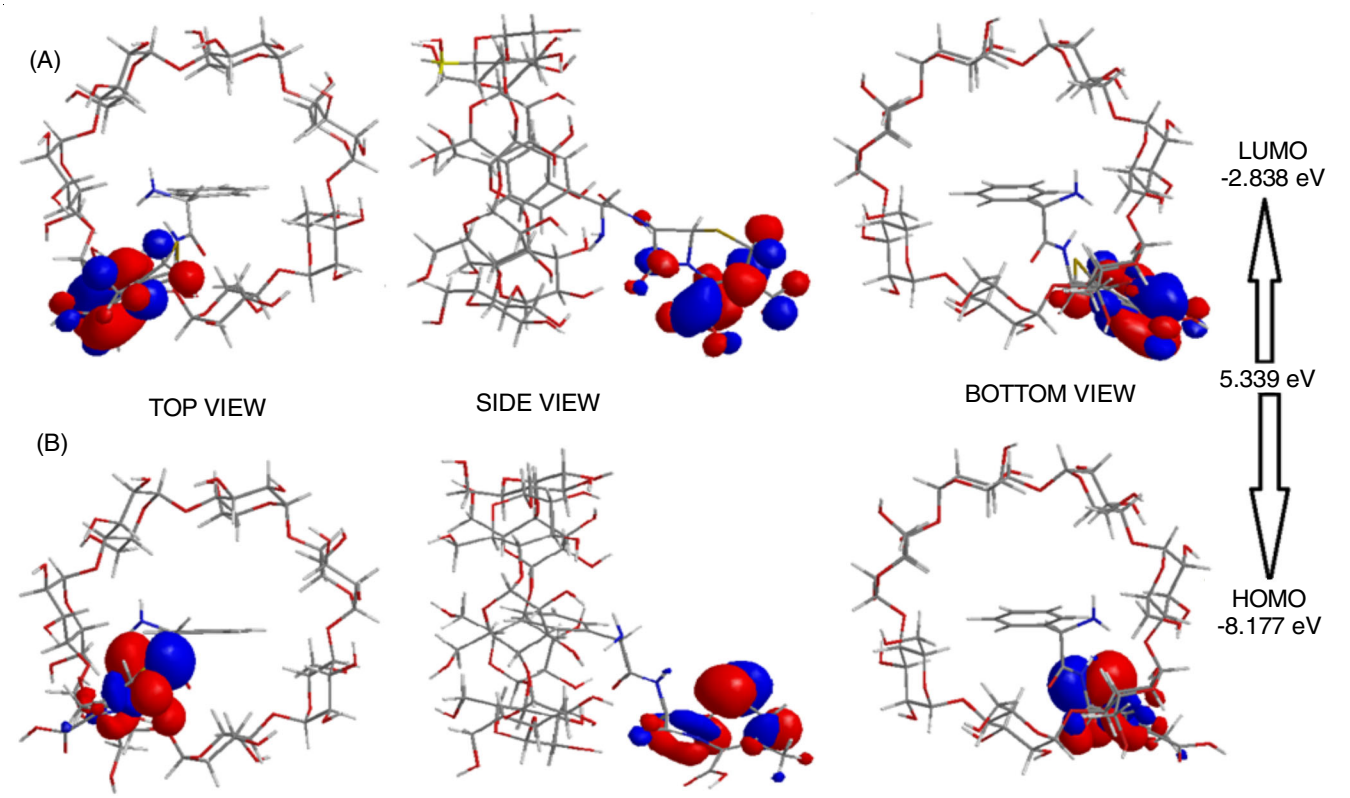


Fig. 5. Top, side and bottom veiw of (A) LUMO (B) HOMO of final structure of inclusion complex

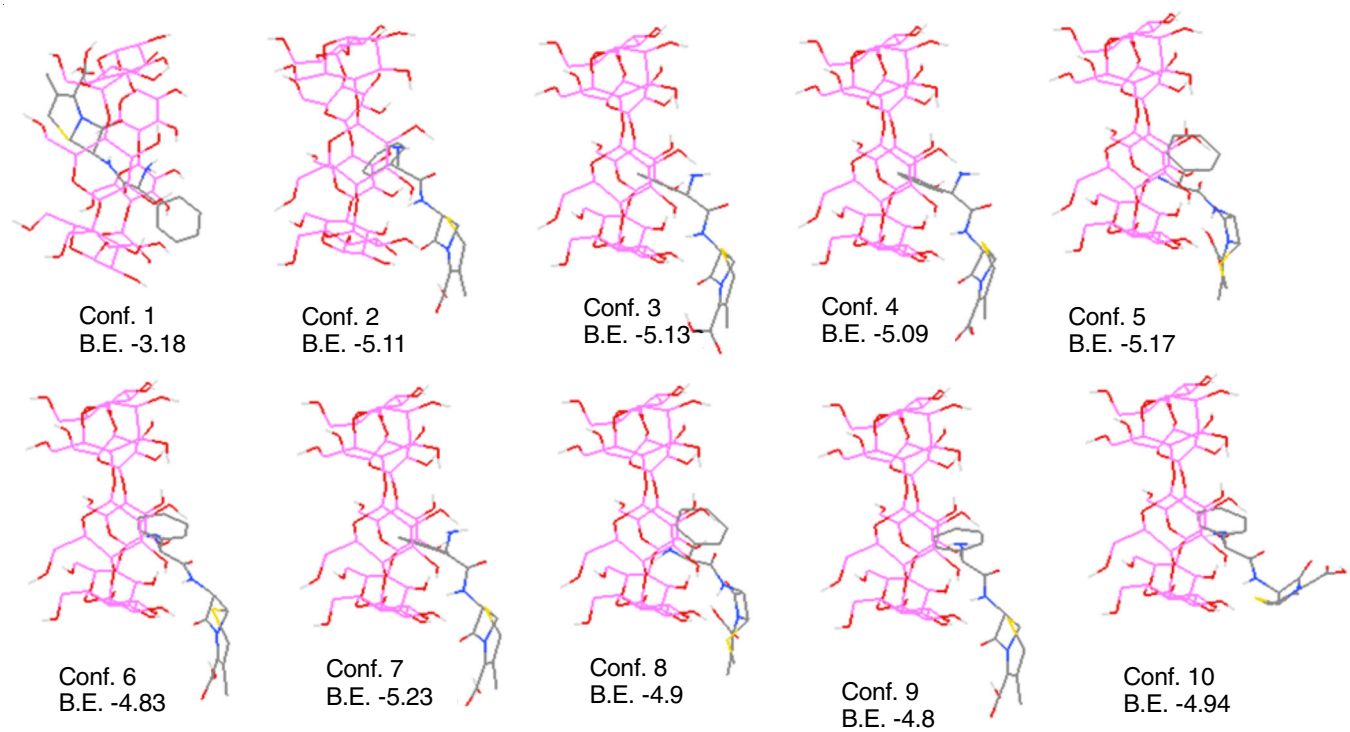


Fig. 6. Conformations obtained from docking simulations

Molecular docking studies: Molecular docking simulations can be used to predict the orientation of guest in β-CD cavity [23]. Docking simulations were performed using Lamarkian Genetic Algorithm in Auto dock 4.2.6 programme keeping the host β-CD as fixed residue and cephalexin guest as the flexible ligand. During docking simulations there was the addition of Gasteiger charges on β-CD however, charges on cephalexin were added automatically. The energetically most stable conformations are found by Autodock in assigned space dimensions of the 3D grid. The docking studies resulted into 10 docked conformations with negative binding energies and the most probable interactions. It was seen that complexation process is favourable because of negative binding energies as shown in Fig. 6. All the docked conformations showed the entry of aromatic ring into β-CD cavity.

Conclusion

A change in ^{1}H NMR chemical shift of β-cyclodextrin cavity protons in presence of cephalexin (guest) has been reported. In this work, the NMR data indicated shielding effects in β-CD and deshielding effects in cephalexin, upon inclusion complex formation. It was confirmed from the results of molecular mechanics, HOMO-LUMO gap and molecular docking studies that cephalexin molecule forms a stable inclusion complex with β-cyclodextrin in the liquid state. Molecular mechanics studied structures were further verified using molecular orbitals studies. Molecular docking studies revealed the insertion of aromatic ring of cephalexin instead of aliphatic portion into β-cyclodextrin cavity during cephalexin/β-cyclodextrin inclusion complexation. All above studies showed that, the mode of penetration of cephalexin into the β-CD cavity was from the wider rim side. These findings may raise the possibility of industrial applications developing novel therapeutic molecules targeting such complexes.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. B. Gidwani and A. Vyas, *Biomed. Res. Int.*, **215**, 198268 (2015); https://doi.org/10.1155/2015/198268
2. C. Szejtli, *Chem. Rev.*, **98**, 1743 (1998); https://doi.org/10.1021/cr970022c
3. D.L. Keeling, N.S. Oxtoby, C. Wilson, M.J. Humphry, N.R. Champness and P.H. Beton, *Nano Lett.*, **3**, 9 (2003); https://doi.org/10.1021/nl025821b
4. M.E. Davis and M.E. Brewster, *Nat. Rev. Drug Discov.*, **3**, 1023 (2004); https://doi.org/10.1038/nrd1576
5. S.E. Wheeler, *Acc. Chem. Res.*, **46**, 1029 (2013); https://doi.org/10.1021/ar300109n
6. Q. Li, H. Pu, P. Tang, B. Tang, Q. Sun and H. Li, *Food Chem.*, **245**, 1062 (2018); https://doi.org/10.1016/j.foodchem.2017.11.065
7. H.R. Ansarian, M. Derakhshan, M. Takafuji and H. Ihara, *Med. Hypotheses*, **71**, 881 (2008); https://doi.org/10.1016/j.mehy.2008.07.033
8. S.M. Ali, S. Khan and G. Crowyn, *Magn. Reson. Chem.*, **50**, 299 (2012); https://doi.org/10.1002/mrc.3807
9. A. Maheshwari, H. Saraswat and S.K. Upadhyay, *Chirality*, **29**, 451 (2017); https://doi.org/10.1002/chir.22712
10. R. Ficarra, S. Tommasini, D. Raneri, M.L. Calabrò, M.R. Di Bella, C. Rustichelli, M.C. Gamberini and P. Ficarra, *J. Pharm. Biomed. Anal.*, **29**, 1005 (2002); https://doi.org/10.1016/S0731-7085(02)00141-3
11. S.M. Ali, S. Muzaffar and S. Imtiaz, *J. Mol. Struct.*, **1197**, 56 (2019); https://doi.org/10.1016/j.molstruc.2019.06.080
12. S.M. Ali and S. Muzaffar, *J. Incl. Phenom. Macrocycl. Chem.*, **94**, 95 (2019); https://doi.org/10.1007/s10847-019-00911-z
13. S.M. Ali and S. Muzaffar, *J. Mol. Struct.*, **1176**, 461 (2019); https://doi.org/10.1016/j.molstruc.2018.08.086
14. N.L. Allinger, *J. Am. Chem. Soc.*, **99**, 8127 (1977); https://doi.org/10.1021/ja00467a001
15. H. Safia, L. Ismahan, G. Abdelkrim, C. Mouna, N. Leila and M. Fatiha, *J. Mol. Liq.*, **280**, 218 (2019); https://doi.org/10.1016/j.molliq.2019.01.019
16. W. Sneader, Cephalosporin Analogues, In: Drug Discovery: A history. Wiley: New York, p. 324 (2009).
17. R.M. Ahmed, M. Dani and A.A. Elbashir, *Int. J. Res. Pharm. Sci.*, **1**, 2278 (2013).
18. S.M. Ali, S.K. Upadhyay and A. Maheshwari, *Spectroscopy*, **21**, 177 (2007); https://doi.org/10.1155/2007/796821
19. S.M. Ali and S. Shamim, *J. Encap. Adsorp. Sci.*, **4**, 63 (2014); https://doi.org/10.4236/jeas.2014.43007
20. S.M. Ali and S. Shamim, *Magn. Reson. Chem.*, **53**, 526 (2015); https://doi.org/10.1002/mrc.4250
21. A. Fifere, N. Marangoci, S. Maier, A. Coroaba, D. Maftei and M. Pinteala, *Beilstein J. Org. Chem.*, **8**, 2191 (2012); https://doi.org/10.3762/bjoc.8.247
22. Z.K. Eddine, M. Fatiha, Z. Amal, N. Leila and M. Rachid, *Comptes Rendus Chim.*, **18**, 193 (2015); https://doi.org/10.1016/j.crci.2014.04.012
23. C. Jullian, S. Miranda, G. Zapata-Torres, F. Mendizábal and C. Olea-Azar, *Bioorg. Med. Chem.*, **15**, 3217 (2007); https://doi.org/10.1016/j.bmc.2007.02.035