Vibrational properties of inclusion complexes: the case of indomethacin-cyclodextrin

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Vibrational properties of inclusion complexes with cyclodextrins are studied by means of Raman spectroscopy and numerical simulation. In particular, Raman spectra of the non-steroidal, anti-inflammatory drug indomethacin undergo notable changes in the energy range between 1600 and 1700 cm\(^{-1}\) when inclusion complexes with cyclodextrins are formed. By using both \textit{ab initio} quantum chemical calculations and molecular dynamics, we studied how to relate such changes to the geometry of the inclusion process, disentangling single-molecule effects, from changes in the solid state structure or dimerization processes.

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I. INTRODUCTION

Inclusion complexes with cyclodextrins have been little studied by means of Raman spectroscopy, although the usefulness of this technique in such systems is now acknowledged. Cyclodextrins (CD) are a family of natural or synthetically modified cyclic molecules, consisting typically of six (\(\alpha\)-CD), seven (\(\beta\)-CD) or eight (\(\gamma\)-CD) glucopyranose units. In water, they take on the peculiar 3-dimensional structure of a truncated cone, with a slightly soluble outer surface and a hydrophobic central cavity, whose size depends on the number of glucose units of the molecule. A remarkable property of CD in aqueous solution, is their ability to form host-guest inclusion complexes with a wide variety of organic and inorganic molecules, provided that the guest molecule is less polar than water. In particular, inclusion complexes of CD with non-polar drugs are a topic of current interest, because these non-covalent complexes increase not only the aqueous solubility of drugs, but also their chemical stability and bioavailability.

Such complexes have been usually investigated in aqueous solution by ultra-violet and visible absorption spectroscopy, fluorescence techniques, and NMR spectroscopy, while X-ray and neutron diffraction are typically utilized in the solid state, but the latter techniques (unlike Raman) require crystalline samples. The effect of the inclusion process on the guest molecules has also been the subject of some Raman studies in particular, Raman and FT-IR investigations of the interactions between CD and some non-steroidal anti-inflammatory agents (like diclofenac sodium, ketoprofen and amorphous piroxicam), have shown significant changes in the vibrational spectrum of the complexed guest molecules with respect to the free ones.

Among the non-steroidal anti-inflammatory drugs, indomethacin (IMC, Fig. 1) is widely used as an analgesic drug in the treatment of rheumatoid arthritis, as well as in other degenerative joint diseases, and recently it has also shown anti-tumor activity, with an important role in the inhibition of human-colon adenocarcinoma cell-proliferation. The molecule of IMC has been characterized by different spectroscopic techniques, and it has been observed that, in the crystalline state, it exhibits three polymorphic forms (called \(\alpha\), \(\beta\) and \(\gamma\), respectively), depending on the sample history. Due to its chemical structure, IMC is poorly soluble in water, resulting in high local drug concentrations responsible for ulcerations in the tissues, and this reduces its therapeutic applications. A strategy to affect the solubility and chemical stability of IMC in water, which is actually employed in commercial drugs, consists in the preparation of inclusion complexes with CD.

Although some models were proposed for the arrangement of the guest molecule into the cavity, unequivocal conclusions were not reached. Moreover, although the vibrational spectra of the various forms of free IMC have been widely studied in the literature by FT-IR and Raman spectroscopy, no Raman data have been reported on its inclusion complex with CD.

In the present work we discuss the results of Raman scattering experiments and numeric simulations on the IMC-CD inclusion complexes in solid state, which provide new insight into the structure of the complexes, and into the effect of the inclusion process on the guest. Additional information is obtained by the comparison of these results with those relative to (i) 5-metoxyindol-3-acetic acid, (ii) the inclusion complexes formed by CD
with 4-clorobenzoic acid and (iii) with the sodium salt of IMC (NaIMC). Additional experiments of electrospray-ionization mass spectrometry and NMR were also performed.

II. EXPERIMENTAL AND COMPUTATIONAL DETAILS

All chemical compounds were purchased from Sigma-Aldrich and used without further purification. Raman spectra were recorded using a Jobin-Yvon HR800 micro-Raman confocal system with a 100x objective, equipped with a liquid-nitrogen-cooled CCD detector. Exciting radiation at 632.8 nm (20 mW) was provided by a He-Ne laser. The Raman spectra were generally recorded using dried samples. In some cases, when the amount of available complex was small, in order to improve the Raman signal, Surface Enhanced Raman Scattering (SERS) was used. In these cases, drops of liquid sample were repeatedly deposited on a suitable metallic substrate (Ti-6Al-4V alloy) and dried by evaporation, thereby increasing the complex concentration.\cite{15,16,17} Mass spectrometry experiments were performed on a Bruker Esquire-LC\textsuperscript{TM} ion trap mass spectrometer equipped with an atmospheric-pressure Electrospray ionization (ESI) source, in the voltage range 99-180 V; the total ion current was acquired with a scan range m/z 100-2000. The sample was directly infused as a 1:1 MeOH/H\textsubscript{2}O solution at a flow rate of 2 \textmu l/min into the ESI source in either positive- or negative-ion mode.\cite{18} \textsuperscript{1}H NMR spectra were taken at room temperature with an Avance 400 Bruker spectrometer; \textsuperscript{1}H was recorded at 400 MHz, with \delta values in ppm in D\textsubscript{2}O relative to the HDO residual signal (\delta\textsubscript{H} 4.70 ppm) and J values in Hz.

All the \textit{ab initio} quantum chemical calculations were performed with the GAUSSIAN 03 program suite\cite{19} utilizing unrestricted DFT\cite{20} and the non-local B3LYP functional hybrid method was employed\cite{21} as well as unrestricted Hartree-Fock (UHF). The standard 6-31G(d) basis set\cite{22} was used for the geometry optimization and frequency analysis of free indomethacin base pairs. Vibrational frequencies were computed at the same level of theory, as well as IR and Raman spectra. In addition, we have also obtained the amplitude of the displacements of the atoms for the different normal modes. When the vibrational frequencies have been computed, the scaling factor of 0.99\cite{23} was taking into account; this has to be introduced to correct for anharmonicity, as well as for the systematic errors inherently present in the calculated harmonic contributions.\cite{24}

Molecular dynamics simulations were performed by utilizing the Amber package.\cite{25} In particular, the molecular dynamics runs have been performed via the module \textit{sander}, while the energy minimization and the search for eigenvalues and eigenvectors of the dynamical matrix have been obtained via the module \textit{ncmode}. The force field employed is \textit{GAFF} (Generalized Amber force field).\cite{26} In some runs, the solvent effects have been incorporated explicitly, by including TIP3 water molecules.\cite{27} The starting structures of IMC and \beta CD have been obtained from the Protein Data Bank.\cite{28} The coordinates for hydrogen atoms were generated by means of the WWW \textit{PRODRG} server.\cite{29} Partial charges were computed by means of the module \textit{antechamber} of the Amber package, utilizing the \textit{AM1-BCC} model.\cite{30}

The IMC-CD complex was prepared according to the procedure adapted from the literature.\cite{31} \beta CD (50.3 mg, 44.4 \mu mol) was dissolved in water (4.2 ml) obtaining a 10.6 \mu M solution, which was added to an equimolar amount of dry IMC (15.9 mg, 44.4 \mu mol). The resulting dispersion was stirred at 60\degree C for 2 hours and cooled at room temperature. After 3 hours, the presence of the complex was confirmed by the precipitation of a white solid, which was collected by drawing the liquid phase with a syringe, dried in a vacuum chamber (over P\textsubscript{2}O\textsubscript{5} as dehydrantant), and finally powdered.

The complexes between hydroxypropyl \beta CD (HP\beta CD) and IMC, and between \beta CD and 4-clorobenzoic acid or sodium salt of IMC, were obtained under the same procedure.

By \textsuperscript{1}H NMR analysis in D\textsubscript{2}O on the complexes of both IMC and NaIMC with \beta CD or HP\beta CD, we have obtained the same \textsuperscript{1}H chemical shifts previously reported by Myles\cite{12} and Fronza.\cite{13}

III. EXPERIMENTAL RESULTS

Before the Raman scattering measurements, preliminary experiments were performed on the complexes using mass spectrometry. In fact, when applied to the analysis of CD complexes, the negative ion ESI-MS technique provides information on the stoichiometry of the complex in gas-phase. In the case of the IMC-\beta CD complex,
as shown in Fig. 2 the cluster signals of the pseudomolecular ion $[\text{M}_{\text{complex}}-\text{H}]^{-}$ of the 1:1 complex is detected at $m/z=1490$, and the signal of the ion $[\text{MC}D-\text{H}]^{-}$ of free βCD at $m/z=1133$. No signals arising from 1:2 complex were observed. Tandem MS/MS experiments, carried out in the ion trap through isolation of the cluster, were observed. Tandem MS/MS experiments, carried out in the ion trap through isolation of the cluster, were observed.

In Fig. 2 we report the Raman spectra of solid γ-IMC (a), 4-chlorobenzoic acid (b), 5-metoxyindol-3-acetic acid (c), and the 1:10 physical mixture of IMC and βCD (d), in the energy range 1500-1750 cm$^{-1}$. The two latter molecules are of interest in the present study because their chemical structures are very similar to subunits of IMC, as shown in Fig. 4.

We focused on the this energy range because it contains no free-βCD peaks, and complexation-induced changes on IMC spectrum can be readily observed. Moreover, this is also the range where C=O stretching energies are expected, so that combining experiment and simulations, useful information on the geometry of the complex (for instance, the position of the involved CO group) may be obtained from the features of Raman spectrum. IMC contains both a C=O amide group (atoms N28-C29-O30 in Fig. 1) and a C=O carboxylic group (atoms C17-O18-O19-H20), which are spatially well separated in the molecule. The comparison among the Raman spectra of Fig. 3 seems to indicate that, actually, the peak of IMC at 1698 cm$^{-1}$ corresponds to the amide C=O stretch. In fact, this peak does not appear in the spectra of compounds (1) and (2) of Fig. 4, which do not contain the amide group; it is also be noted, that this assignment is in agreement with Taylor et al.. Moreover, in the following we shall see that ab initio quantum chemical calculations and molecular dynamics computations confirm this conclusion. Thus we may focus on the peak at 1698 cm$^{-1}$ (from now on the corresponding frequency will be labelled $\omega_{\text{CON}}$, while the frequency of the carboxylic group will be labelled $\omega_{\text{COOH}}$), and on its changes due to the inclusion process, which are shown in Fig. 4. By comparing the spectrum of free IMC (a) to those of the complexes formed by HP/βCD (b) and βCD (c), we note a marked broadening of the peak at $\omega_{\text{CON}}$, as well as a shift from $\approx 1700$ cm$^{-1}$ to $\approx 1670$cm$^{-1}$. Such a large shift for $\omega_{\text{CON}}$ is rather unusual in the study of the complexation processes where, generally, smaller variations are observed. From the above results one might infer that it is the amide C=O group of IMC (and the neighboring atoms) to be mainly affected by the inclusion process. However, in order to understand the precise way in which such atoms are influenced by complexation, other possible effects should be discussed. Generally speaking, changes of vibrational frequencies might be due to the presence of the uncomplexed guest in dimeric form. This is, for example, what happens to the 4-chlorobenzoic acid which, in the crystalline state, may exist also in dimeric form due to intermolecular hydrogen-bonded interactions, and whose Raman spectrum is shown in Fig. 4 (b): here, the broad peak at 1628 cm$^{-1}$ has been assigned to the carboxylic C=O stretch of the dimer and thus corresponds to $\omega_{\text{COOH}}$. In Fig. 4 we report the spectra of free 4-chlorobenzoic acid (a), of the 1:10 physical mix-
tured of 4-chlorobenzoic acid-βCD, respectively (b), and of the βCD-complexed 4-chlorobenzoic acid (c). In case (b), only 4-chlorobenzoic acid monomers are expected: as a matter of fact, the breakdown of hydrogen bonding patterns of 4-chlorobenzoic dimers is observed in the shift of the C=O stretch from 1628 cm$^{-1}$ (Fig. 6(a)) to higher energy ($\approx$ 1684 cm$^{-1}$) (Fig. 6(b)). Moreover, the comparison between Fig. 6 (b) and (c) shows that the complexation process further shifts the peak corresponding to C=O stretch by $\sim$ 10 cm$^{-1}$. Note that this high-energy shift for the 4-chlorobenzoic acid is at odds with the IMC case, where a decrease of $\omega_{CON}$ was observed upon complexation.

The case of the 4-chlorobenzoic acid shows that complexation may affect the Raman peaks in at least two ways: (i) by breaking the intermolecular bonds, (ii) by modifying the strength of the interatomic bonds (C=O in this case). In order to check the possibility that the former effect may affect the complexed form of IMC, we have measured the Raman spectrum of a 1 : 10 physical mixture of IMC-βCD (Fig. 3 (d)). In the energy range between 1500 and 1750 cm$^{-1}$, this spectrum exhibits the same characteristic peaks as in Fig. 3 (a), indicating that the shift of the amide C=O stretch, observed in Fig. 5 (a) and (b), is actually related to complexation and not to the cleavage of hydrogen bonding patterns of IMC dimers.

Similar results have been obtained on the inclusion complex formed by βCD with IMC sodium salt (Fig. 7): also in this case, we clearly observe a broadening and shift to lower energy of the peak at 1675 cm$^{-1}$, which is generally assigned to the amide C=O stretching, when the guest is included in βCD. All these results confirm the trend already observed in the vibrational spectra of IMC complexes, and further support the hypothesis that the amide C=O of IMC is directly involved in the process of complexation with βCD. Finally, the vibrational properties of IMC-βCD inclusion complexes have been also investigated in the low-frequency region (10-100 cm$^{-1}$), using a Jobin Yvon U1000 Raman spectrometer with Ar laser excitation at 514.5 nm; preliminary results seem to evidence, in this spectral region as well, the presence of significant differences, probably due to extended vibrations of the system.

Actually, there is also a third phenomenon that might induce changes in the experimental spectra, namely the possible change of the solid state of IMC due to the complexation. In general, below its glass transition temperature IMC crystallizes in the stable γ-form, whereas at higher temperatures the formation of the metastable α-form is predominant. The polymorphic α and γ forms differ not only in the packing density of the crystals, but also in the unit cell arrangements, leading to a deep
difference in their Raman spectra. Thus, one could argue that the large structural change undergone by the solid state due to complexation, could be related to the observed shift of $\omega_{\text{CON}}$. In order to investigate this possibility, we performed numeric simulations describing the complexation process between a single molecule of IMC and a single molecule of $\beta$CD. We shall see that they reproduce sufficiently well the experimental findings and that structural changes do not need to be taken into account.

IV. NUMERIC SIMULATIONS

A. *Ab initio* quantum chemical computation

Focusing only on the energy range described in the experimental section, the main result from the *ab initio* computation is that, in the region 1500 − 1750 cm$^{-1}$ (a complete assignment of frequencies for free IMC has been attempted in Ref.\(^\text{34}\)), the eigenvector with the largest Raman signal involves the largest displacement over the atoms C29-O30, i.e. it corresponds to the amide C=O stretching. The value for $\omega_{\text{CON}}$ found from the UHF *ab initio* computation (1696 cm$^{-1}$) is rather close to the experimental one, as shown in Fig. 8. Furthermore, in the comparison between experimental and computed Raman intensities, the signals falling in the region between about 1600 and 1630 cm$^{-1}$ can probably be assigned to aromatic C=C stretch bond of IMC. The vibrational analysis of complexes has been performed using classical simulation (see next section), which allows larger systems to be simulated with respect to the *ab initio* calculation.

B. Molecular dynamics computation

The advantage of numeric simulations, with respect to the experiments, is that the positions of the IMC and CD atoms can be located exactly. On the other hand, numeric simulations of the complex are rather CPU-time demanding, especially when the effects of the solvent are taken into account. The length of our runs ranged from 1 ms, for a molecule of IMC in vacuum, to only a few ns when thousands of molecules of water were added. To answer in a reliable way the question about the geometry of the inclusion process, one should perform long enough runs of many IMC and $\beta$CD molecules, in the presence of a solvent, in order to detect many spontaneous inclusion events, and then perform a statistical study. As we shall see, because of the long CPU-time needed, this goal has been only marginally achieved, since only one of such events has been observed. On the other hand, it has been possible to gain further physical insight by supporting this result with somewhat less ambitious simulations. In order to study the inclusion of IMC in $\beta$CD we have simulated the following systems: (1) single IMC molecule in vacuum, (2) single IMC molecule in a box of 1114 molecules of water, (3) single IMC-$\beta$CD inclusion complex in vacuum, (4) single inclusion complex in a box of 633 molecules of water and (5) 9 IMC molecules plus 9 $\beta$CD molecules in a box of 1400 molecules of water. In systems (3) and (4) the inclusion complex was prepared by docking the Cl atom of IMC into the cavity of $\beta$CD, and minimizing the energy of the resulting configuration (see Fig. 9). During the molecular dynamics runs, we recorded the distance $d$ between the Cl atom of IMC and the center of mass of $\beta$CD, in order to determine the formation of an inclusion complex. More precisely, a complex is defined by the fact that $d \sim 0$ (or, at least, much lesser than the typical distance between free IMC and $\beta$CD). Moreover, starting from the thermodynamic equilibrium configuration, we sought for the minima of the energy potential. In the minimum configurations we computed the eigenvalues $\omega$ and the corresponding eigen-
vectors $\vec{e}_i(\omega)$ of the dynamical matrix, in order to determine the vibrational properties of the different systems. From such quantities we have obtained the density of vibrational states $g(\omega)$ and the quantity $Y_i \equiv \vec{e}_i \cdot \vec{e}_i$, which measures the amplitude of the vibration of the $i$-th atom due to the mode of frequency $\omega$. The thermodynamic parameters chosen for the simulations are room temperature and room pressure, with the coupling to the external bath introduced via a weak coupling scheme. In

In Fig. 10 we show the behavior of the distance $d$ during different molecular dynamics runs. The solid line represents the distance between the Cl atom of IMC and the center of mass of $\beta$CD, as a function of time for different simulated systems (see text). After 1 ns, $d$ reaches a value of approximately 2 Å, which we interpret as the signature of an inclusion event, with the Cl located quite close to the center of mass of $\beta$CD. In our runs, we did not observe other inclusion processes, thus we are not able to perform a detailed study of the phenomenon (characteristic times, etc.). It is interesting to note that $d$ is much smaller than the position of the first peak of the pair correlation function between free IMC and CD molecules (horizontal line). Furthermore, the value of $d$ reached in the spontaneous inclusion process found in system (5) is very similar to the stationary value of the distance in the run of system (4) (dashed line), where the complex was initially created and put in a box of water. Both results support our claim that in system (5) (which is the most similar to an experimental sample) we actually observed a single complexation process. Interestingly, the complex is not stable without water: in fact, we see that although system (3) (dotted line) starts from the same initial configuration as system (4), the absence of water leads to an immediate destruction of the complex.

Unlike the ab initio quantum chemical computations discussed previously, from classical molecular dynamics (MD) simulations it is not possible to obtain in a straightforward way the Raman spectra; in fact, the Raman signal $I(\omega) \propto g(\omega) C(\omega)$, where $C(\omega)$ is the so-called coupling function which, in classical computations, is unknown unless the mechanism of polarizability modulation is specified. On the other hand, with MD simulations one can obtain the eigenfrequencies and the eigenvectors of the inclusion complex.

The interpretation of the density of vibrational states $g(\omega)$ proceeds through different steps. In Fig. 11 we identify the frequencies $\omega_{\text{CON}} = 1628 \text{ cm}^{-1}$ (Raman active according to the ab initio calculation, see Section IV A) and $\omega_{\text{COOH}} = 1649 \text{ cm}^{-1}$ (Raman inactive), as the ones corresponding to the modes which are most localized on the atoms of the amide and of the carboxylic C=O groups, respectively. There is a slight difference between the values of these two characteristic frequencies and the ones computed with the ab initio study, and between $\omega_{\text{CON}}$ as computed with the MD approach and the experimental value. These discrepancies might be ascribed to the force
field utilized.

![Graph 1](image1)

**FIG. 12:** Top: projection of the eigenvector corresponding to $\omega_{\text{CON}}$ over the atoms of IMC (labelled as in Fig. 1), in free IMC (solid line, system (1)) and IMC-$\beta$CD complex (dotted line, system (4)). Bottom: projection of the eigenvector corresponding to $\omega_{\text{COOH}}$ in free IMC (solid line, system (1)) and IMC-$\beta$CD complex (dotted line, system (4)).

In Fig. 12 is shown the effect of complexation on the eigenvectors corresponding to $\omega_{\text{CON}}$ and $\omega_{\text{COOH}}$. In fact, in the complexed system (4), we have looked for the frequencies whose corresponding eigenvectors were the most similar to the ones of free IMC shown in Fig. 11. While $\omega_{\text{COOH}}$ is not affected by the inclusion process, one can see that $\omega_{\text{CON}}$, corresponding to the largest experimental Raman signal, shifts to lower frequencies by $\sim 14 \text{ cm}^{-1}$. Thus, from a qualitative point of view, we find results in agreement with the experimental findings described in the above section. Quantitatively, one has to note that this shift is about one half of the experimental one. It seems reasonable to ascribe this difference to the limitations of the GAFF force field in describing a molecule of the complexity of IMC, rather than to the fact that in these simulations the structural properties of the solid state (and their possible modifications due to the complexation) are not taken into account. As a matter of fact, in Fig. 13 we show that for a much simpler molecule like the 4-chlorobenzoic acid (for which the GAFF force field is expected to be more reliable), the MD simulations show a shift of $\omega_{\text{COOH}}$ to higher energy of $\sim 10 \text{ cm}^{-1}$, which is in good agreement with the one found in experiments in the complexation of the monomeric form of 4-chlorobenzoic acid.

**V. CONCLUSIONS**

In the present paper we have reported the results of a multi-technique study of the complexes formed by CD with the molecule of IMC. In particular, we have performed Raman scattering experiments and we have compared the experimental findings with the outcome of *ab initio* quantum chemical calculations and of molecular dynamics simulations. Focusing on the energy range $1500 - 1700 \text{ cm}^{-1}$, where Raman spectra show the largest modifications after the complexation process, we conclude that the geometry of the IMC-$\beta$CD complex is the one shown in Fig. 9, characterized by the 4-chlorobenzoyl unit inserted into the cavity of $\beta$CD through its larger rim, as early proposed by Fronza et al.\textsuperscript{13} We have also computed the density of vibrational states of free IMC, of $\beta$CD, and of their inclusion complex, and compared it to the corresponding experimental Raman spectra. Though it is difficult to reach complete quantitative agreement between experiment and simulations, the latter explain the shift to lower frequencies of the most intense Raman peak, whose eigenvector is localized on the atoms of the amide C=O group; this shift is due only to the inclusion process, whereas dimerization processes or structural changes in the solid state seem not to be involved. This multi-technique, experimental and numerical approach, appears very promising for the study of other inclusion complexes as well.

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1. S. Santesson, J. Johansson, L.S. Taylor, L. Levander, S. Fox, M. Sepaniak and S. Nilsson, *Anal. Chem.* **75**, 2177 (2003).
2 G. Fini, J. Raman Spectrosc. 35, 335 (2004).
3 S. Li and W.C. Purdy, Chem. Rev. 92, 1457 (1992).
4 A.M. Amado, A.M. Moreira da Silva, P.J.A. Ribeiro-Claro and J.J.C. Teixeira-Dias, J. Raman Spectrosc. 25, 599 (1994).
5 P.J.A. Ribeiro-Claro, A.M. Amado, J.J.C. Teixeira-Dias, J. Raman Spectrosc. 27, 155 (1996).
6 R. Spivey, R.L. Swofford, Appl. Spectrosc. 53, 435 (1999).
7 T. Iliescu, M. Baia and V. Miclaus, Euro J. Pharm. Sci. 22, 487 (2004).
8 S.H. Choi, S.Y. Kim, J.J. Ryoo, J.Y. Park and K.P. Lee, Anal. Sci. 17, 1785 (2001).
9 M. Albrecht and J.A. Creighton, J. Am. Chem. Soc. 99, 342 (1977).
10 A. Anderson, The Raman effect, Vol. 1, Marcel Dekker, New York (1971).