ORIGINAL ARTICLE

New insight into the discrimination between omeprazole enantiomers by cyclodextrins in aqueous solution

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Abstract We report results regarding the use of ^{1}H-NMR spectroscopy in the study of the conformational behaviour and optical activity of omeprazole. Changes in the chemical shifts of chosen atoms reveal that the conformational behaviour of omeprazole is temperature and pH sensitive. Separation and identification of omeprazole enantiomers in the presence of natural and derivative cyclodextrins, such as β-cyclodextrin (βCD) and methyl-β-cyclodextrin (MβCD) are achieved using ^{1}H-NMR spectroscopy, with information from molecular dynamics simulation. This work shows that βCD includes preferentially R-(−)-omeprazole, acting as a chiral selector. This discrimination of omeprazole enantiomers by cyclodextrins allows development of pharmaceutical formulations with a better bioavailability profile.

Keywords Cyclodextrin · Enantiomers · Molecular Dynamics · NMR · Omeprazole · Spectroscopy

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Introduction

Omeprazole is a proton pump inhibitor in gastric parietal cells and has been widely used in the treatment of peptic ulcer, reflux oesophagitis and Zollinger–Ellison syndrome [1]. The molecular structure of omeprazole is composed of a substituted pyridine ring linked to a benzimidazole by a sulphoxide group [2]. Normally, this drug is a racemate with a chiral center in the sulphoxide group (Fig. 1).

It was demonstrated that both enantiomers have the same in vitro capacity to decrease gastric acid formation, but stereoselective metabolism by cytochrome P450 2C19 (abbreviated CYP2C19) results in different plasma concentrations. The S-(−)-enantiomer of omeprazole is less susceptible to small intestinal and hepatic metabolism than the (R)-isomer, and therefore, the chiral drug achieves between 70% and 90% higher steady-state serum concentration than the racemic omeprazole [3].

Cyclodextrins are cyclic organic compounds obtained by enzymatic transformation of starch. These compounds have found many applications because of their ability to form inclusion complexes with a large number of molecules [4–6]. Although their main applications are in pharmaceutical, cosmetic, food and agrochemical industries, cyclodextrins have also proven to be of great utility in analytical chemistry, especially in providing separations of positional and optical isomers [7–9]. One example is the use of cyclodextrins as chiral selectors for separation of omeprazole enantiomers by high performance liquid chromatography in plasma samples and by capillary electrophoresis [10].

The present work reports on the importance of cyclodextrins, namely βCD and MβCD, as chiral selectors and the use of ^{1}H-NMR spectroscopy and Molecular Dynamics as tools for isomeric selectivity analysis in order to obtain pharmaceutical formulations with better bioavailability.
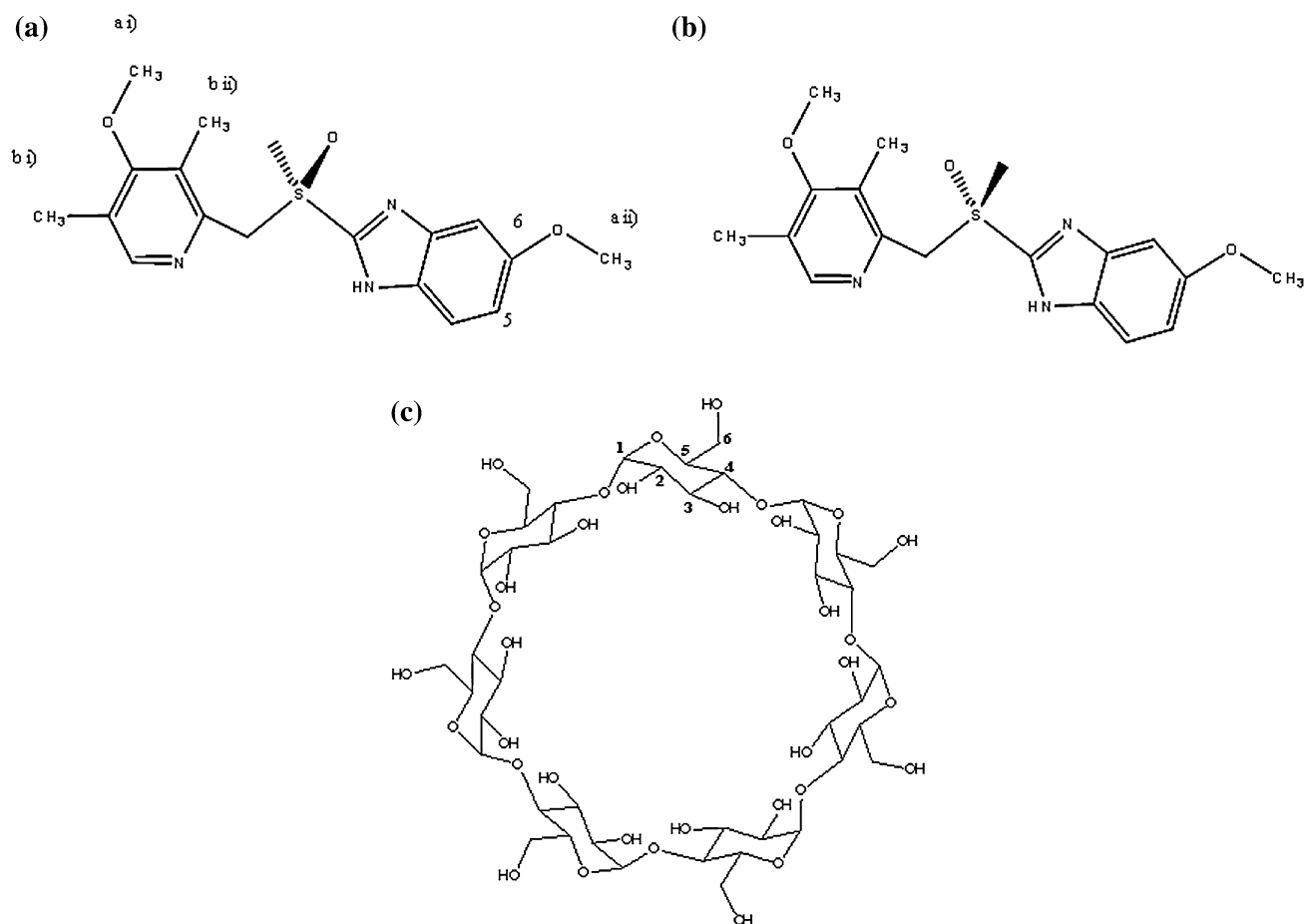
Fig. 1 Chemical structures of omeprazole optical isomers: (**a**) *R* and (**b**) *S* enantiomers and (**c**) *β*-cyclodextrin

Experimental

Materials

Racemic omeprazole (OME) was kindly donated by Belmac Laboratory, S.A. (Madrid, Spain). *β*-Cyclodextrin (*β*CD, $M_W = 1135$) and methyl-*β*-cyclodextrin (M*β*CD, $M_W = 1190$ and an average degree of substitution, DS = 0.5) were kindly donated by Roquette (Lestrem, France). Deuterium oxide (D$_2$O; 99.97%) and NaOD were purchased from Euriso-top (Peypin, France) and Sigma–Aldrich (St. Louis, MO), respectively. All other reagents were of the highest purity available from commercial sources.

Methods

NMR spectroscopy

^{1}H-NMR spectra were recorded at 25 °C, 35 °C and 50 °C on a Varian Unity-500 MHz spectrometer using a 5 mm broadband NMR probe and a simple pulse-acquire sequence with solvent presaturation. Acquisition parameters consisted of 24k points covering a sweep width of 8 kHz, a pulse width of 18 μs, corresponding to a 90 degree rf pulse, and a total repetition time of 15 s. Digital zero filling to 64k and a 0.5 Hz exponential were applied before Fourier transformation. The resonance at 4.681 ppm due to residual solvent (HOD) was used as the internal reference. Samples were prepared by dissolving an amount of free and complexed omeprazole with cyclodextrins, previously prepared by the lyophilized method [11], in D$_2$O to achieve in an omeprazole concentration of 3 mM in all samples.

The final pH was adjusted to 10.30 ± 0.10 so as to minimize the degradation of omeprazole and in some samples the pH was adjusted to 7.55 and 12.04 in order to study the influence of the pH in the omeprazole structural conformation. ^{1}H-NMR variations in the chemical shifts ($\Delta\delta$) were calculated according to $\Delta\delta = \delta$ (complex) $- \delta$ (free).

Simulation details

The Molecular Dynamics simulations were performed using the GROMACS package [12] and employing the GROMACS *ffgmx* force field [13].

The βCD structure was obtained from the HIC-Up on-line database [14]. The initial structure of the omeprazole molecule was also produced as a pdb file. The structures were then converted to GROMACS input files (conformation and topology) using PRODRG [15]. The deprotonated form of OME present in solutions with pH = 10 [16, 17] was obtained by extracting the benzimidazole proton when running PRODRG and setting the partial charges on the molecule according to the charge distributions obtained from GAMESS [18] at the pm3 level, for the protonated and deprotonated forms. The minimized energy structures obtained from GAMESS and GROMACS in the gas phase for the protonated forms presented differences in bond length smaller than 0.05 Å.

In order to maintain the electroneutrality of the systems, one sodium ion was introduced. The long range electrostatic interactions were handled by particle-mesh ewald method [19]. The solvent was considered explicitly using the SPC water model and an algorithm for rigid water molecules [20]. The system was enclosed in a cell with an approximate volume of $33 \times 32 \times 32$ Å^3 and with periodic boundary conditions. Each cell enclosed more than 1020 water molecules. The simulation was conducted in the NPT ensemble at a constant temperature of 300 K with coupling to an external bath [21].

An equilibration run of at least 0.5 ns was done previous to the production trajectory runs, using a timestep of 1 fs, without any constraints. The formation of the inclusion complex is a result of the equilibration process and equilibration was only considered to be completed upon inclusion. The production runs were carried for 30 ns with a timestep of 2.0 ps.

Results and discussion

NMR spectroscopy

One of the problems in obtaining a stable pharmaceutical formulation with omeprazole is the fact that it degrades very rapidly at acidic or neutral pH values. At pH = 10 the molecule is in the deprotonated form, presenting a better stability and larger solubility. The ^{1}H-NMR spectra at 25 °C and pH = 10.30 ± 0.10 of free omeprazole, omeprazole complexed with βCD and with MβCD are shown in Fig. 2a–c, respectively.

The expansion of the ^{1}H-NMR spectrum of the molecule in the region of the methoxy (aii) and methyl (bii) groups at pH = 10.30 °C and 25 °C, revealed that the peaks attributed to these groups were "split", as shown in Fig. 3, with the appearance of a smaller peak at slightly smaller δ values for each resonance. These small peaks were not detectable at pH = 7.55, and at pH = 12.04 appear at the left side of the large peak. At pH = 12.04, these smaller
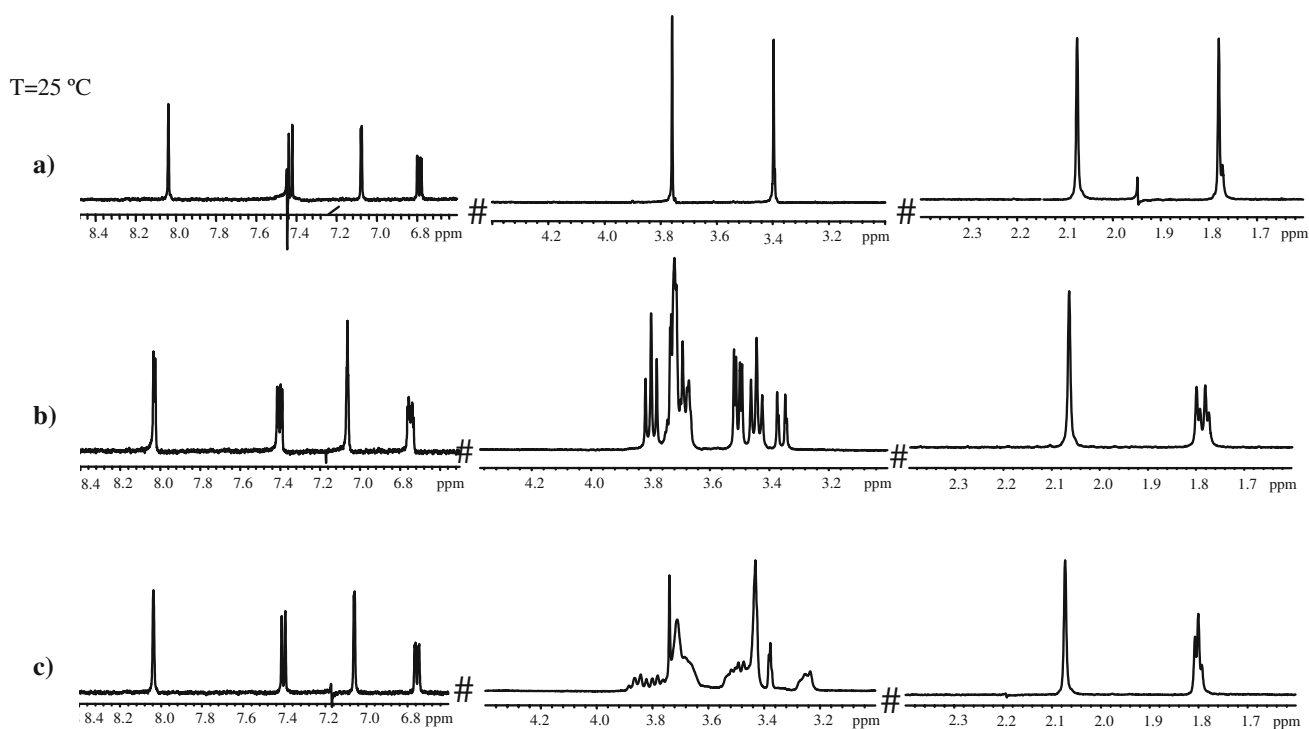

Fig. 2 ^{1}H-NMR spectra of free OME (**a**); OME complexed with βCD (**b**); and with MβCD (**c**) at 25 °C and pH = 10.30 ± 0.10
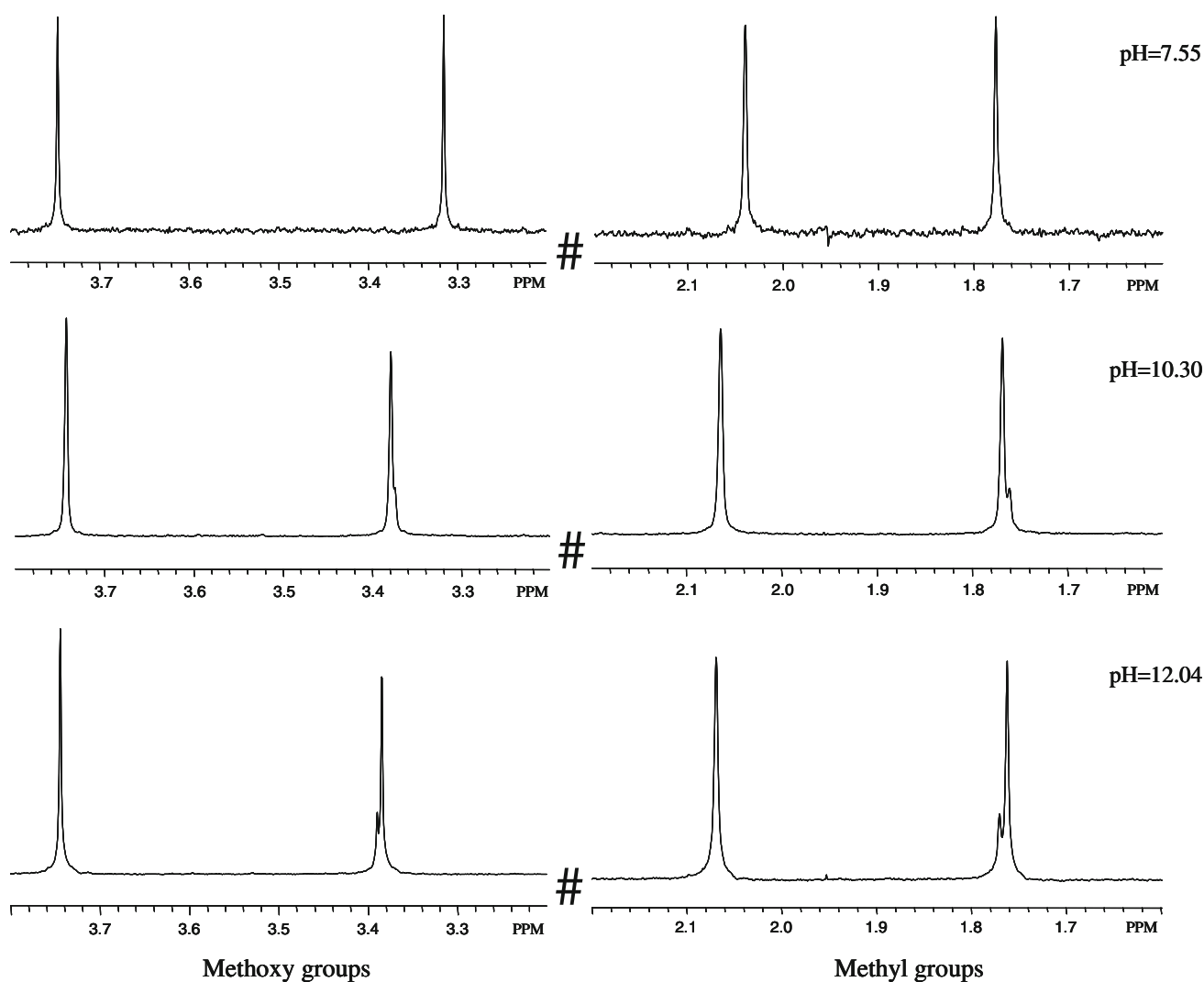
Fig. 3 Expansion of the ^{1}H-NMR spectrum of free OME for the regions of methoxy [(ai) and (aii)] and methyl groups [(bi) and (bii)] at different pHs at 25 °C

peaks present a larger relative area ($\sim 25\%$) compared to the relative area of the same peak visible at pH = 10.30 ($\sim 10\%$). It should also be noted that the effect is identical in both sets of protons, located at different ends of the molecule. This behaviour may be explained by a dependence of the conformational stability on the pH as observed for other molecules [22]. The difference in the values of δ for the large and smaller peaks at pH = 10.30 is probably related to aromatic electronic ring currents caused by opposite ring effects, which at some metastable conformation may deprotect the above mentioned protons. By changing the pH, another metastable conformation is favoured, in this case having a protecting effect. At pH = 10.30, the relative area and δ of the peaks attributed to the protons in the methyl (bii) group vary with temperature, as described in Table 1.

Table 1 Chemical shifts and relative signal areas for peak 1 and peak 2 at pH = 10.30 as function of temperature

Temperature (°C)	Chemical shifts 1 (ppm)	Chemical shifts 2 (ppm)	Signal relative ratio (%)*
25	1.764	1.756	19
35	1.901	1.893	22
50	2.083	2.076	34

* Relative signal area = (peak area 2)/(peak area 2 + peak area 1)

When the temperature is increased from 25 °C to 50 °C, the δ for the largest peak of the methyl group (bii) increases from $\delta = 1.764$–2.083 ppm, with a similar change in the δ of the smaller peak, and the signal relative ratio of the smaller peak increases steadily from 19% at 25 °C to 22% at 35 °C and to 34% at 50 °C. These
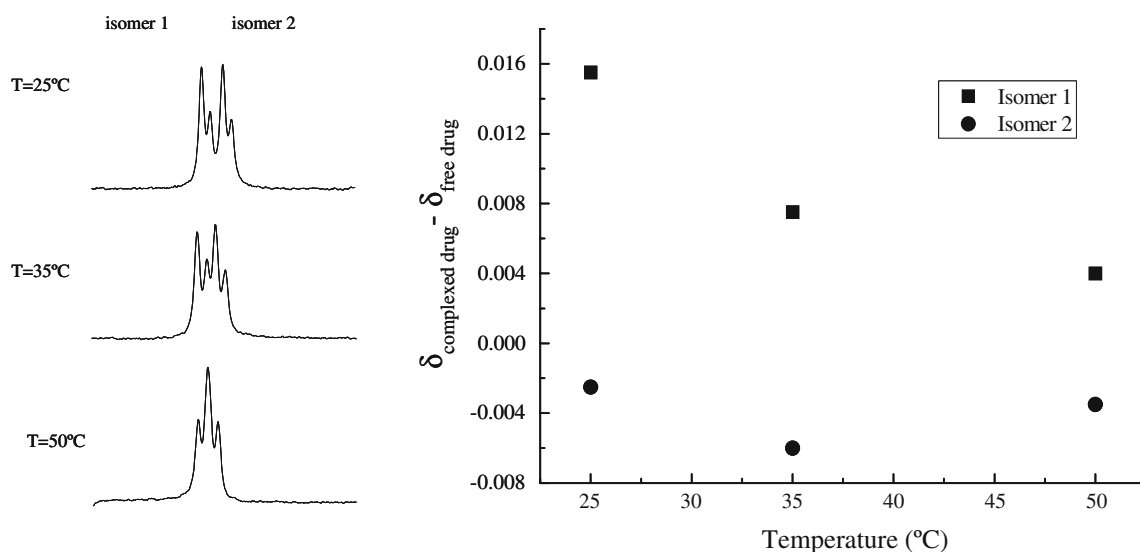
Fig. 4 Expansion of the ^{1}H-NMR spectra and $\Delta\delta$ for the methyl group [(bii)] of OME complexed with βCD as a function of temperature, pH = 10.30 ± 0.10

alterations are consistent with a very dynamic behaviour of the drug, since the alterations in both chemical shifts and ratios of conformers imply a significant alteration in conformation.

Figure 4 shows the expansion of the ^{1}H-NMR spectrum and the δ of complexed omeprazole with βCD in the region of the methyl group (bii). This figure shows, at 25 °C, the appearance of two sets of two peaks, each in the region of the methyl group (bii). Each of these sets is very similar to the respective protons in the absence of cyclodextrin. The relative area of the small peak in each set ($\sim$31%) is, however, quite different from the corresponding areas in the absence of cyclodextrin at 25 °C ($\sim$10%). The separation between the two sets indicates the separation between two different populations, which tend to merge as the temperature is increased. At 50 °C a significant superposition of the resonances occurs.

Molecular Dynamics simulation

Molecular Dynamics studies of the inclusion of the isomers into the cyclodextrin cavity show that there is a slightly deeper inclusion of the R isomer inside the cavity, as visible in Fig. 5.

At the same time, it was observed that the S isomer retains a larger conformational freedom when included in the cavity. This is visible in the larger dispersion of values in the distribution of distances between the oxygens in groups (aii) to the hydrogens on the positions 2 and 3 of the cyclodextrin rings, as presented in Fig. 6.

The different δ values observed for the two sets of peaks may therefore be explained by the different proximity between the methoxy (bii) group and the oxygens in
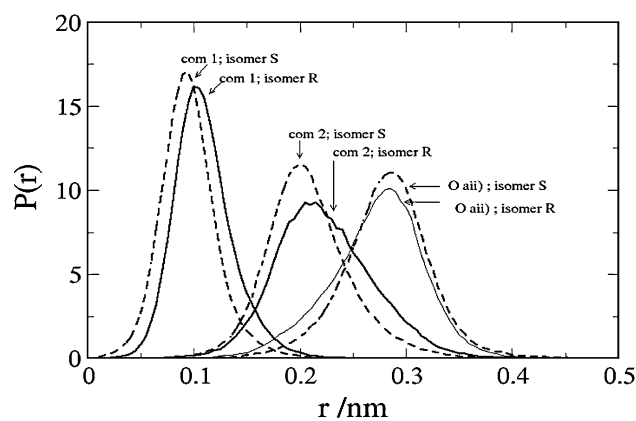

Fig. 5 Distribution of distances for the oxygen in group (aii) and the center of mass of each of the benzimidazole rings (com1 denotes the center of mass of the "imidazole" part and com2 that of the "benzene" ring) to the center of mass of the group formed by O4 of the cyclodextrin. Full line and dashed line correspond to isomers R and S respectively

positions 2 and 3 in the wider side of the cyclodextrin cavity, as shown in Fig. 7. The separation of these sets of peaks in the presence of the cyclodextrin thus results from the action of the latter as a chiral selector. In this view, each set of peaks may be attributed to a specific isomer. Previous work [9] has shown that the S isomer forms less stable complexes with cyclodextrin, in accordance with our observation that this isomer is less deeply included in the cavity. The assignment of the peaks can also be done resorting to the molecular dynamics results. The results presented in Fig. 7 show that there is a significant difference in the proximity between the hydrogens in the OME and the oxygens in the wider entrance of the cyclodextrin, which may have a counterpart in the protection/
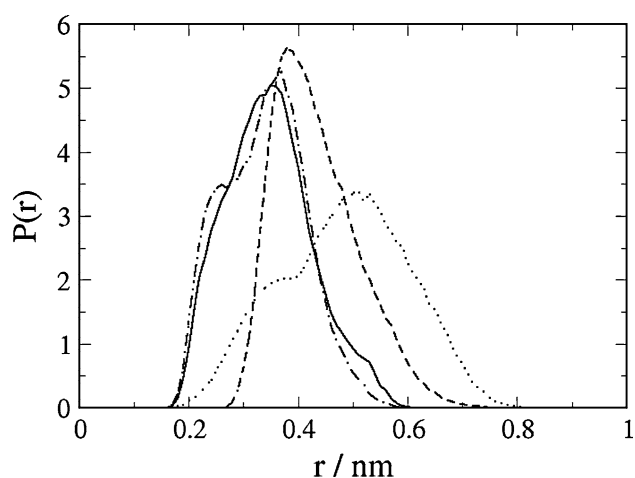
Fig. 6 Distribution of nearest-neighbor distance for the oxygen atom (aii) to the hydrogen atoms in position 6 (full and dotted lines for isomer *R* and *S* respectively) and O (ai) to the hydrogens in positions 2 and 3 (dash-dotted and dotted lines for isomers *R* and *S* respectively) for both isomers when included in the cavity
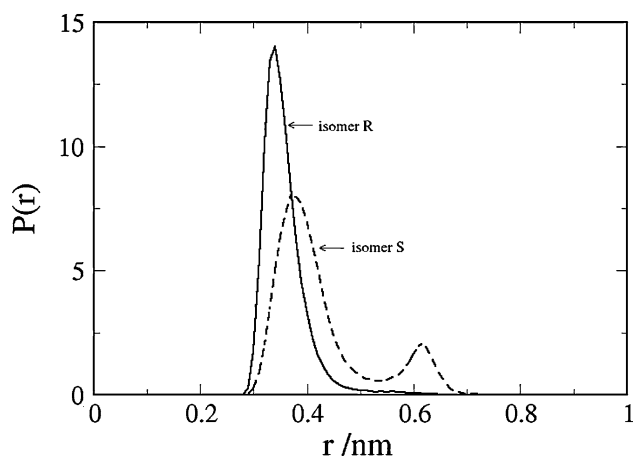

Fig. 7 Nearest-neighbor distances from the carbon in methoxy (bii) to the oxygen atoms in position 2 and 3 of the cyclodextrin. Full line and dashed line correspond to isomers *R* and *S* respectively

deprotection of the former. Following this reasoning, the set with larger δ can be attributed to isomer *R*. The tendency for overlap between the two sets of peaks as temperature increases can be explained by an increased conformational freedom of the two molecules that results in a less specific interaction.

In the case of the drug complexed with MβCD, the methylated derivative, the enantiomeric separation is less marked and with increasing temperature the two enantiomers become almost indistinguishable. We suggest that this observation results from the formation of a more stable inclusion complex between this cyclodextrin and each of the drug enantiomers [23], and that with increasing temperature existing differences in binding constants tend to disappear (smaller $\Delta\delta$).

The results observed indicate that in the presence of cyclodextrin the drug shows enantioselectivity because βCD acts as a chiral selector. However, this fact is not so clearly observed in the presence of MβCD, probably because in this case the inclusion of both enantiomers in the cyclodextrin cavity is deeper [23].

Conclusion

This work allows concluding that the combined use of NMR spectroscopy and molecular dynamics simulation constitutes a clear, simple, and easy methodology to analyse the occurrence of chiral selectivity for drugs, in this case OME, in presence of natural and derivative cyclodextrins, βCD and MβCD. The characteristic features in the spectra have been rationalised resorting to information provided by the Molecular Dynamics simulations, indicating that OME (*R*)-isomer is preferentially included in the βCD cavity, acting this cyclodextrin as a chiral selector for this compound. The same was not observed in the presence of MβCD, because with this cyclodextrin the inclusion of the drug in the cavity is deeper, and this fact hampers the use of this cyclodextrin as a chiral selector in the case of OME.

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