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Surface mediated chiral interactions between cyclodextrins and propranolol enantiomers: a SERS and DFT study

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The nanoparticles mediated enantioselective recognition of propranolol enantiomers through native cyclodextrins complexation has been investigated by using Surface-enhanced Raman Spectroscopy (SERS). The highly efficient chiral recognition mechanism is based on a synergistic interaction between spherical noble metal nanoparticles, propranolol enantiomers and native cyclodextrins (CDs). Amongst the native cyclodextrins, β-CD has the highest chiral recognition ability for propranolol enantiomers, due to its specific shape and cavity size, thus producing the largest difference between the recorded SERS spectra of the two hosted enantiomers. The molecular interaction mechanism responsible for enantioselectivity was furthermore proven by quantum chemical calculations based on density functional theory (DFT). The theoretical calculations and experimental SER spectra allowed the assignment of functional moieties involved in the chiral recognition mechanism. The most important factors governing the highly efficient chiral probing by SERS are the fundamentally different mechanism of interaction between the R- and S-enantiomers and β-CD and the strength of interaction between the nanoparticles surface and the two propranolol-CD complexes. The role of metallic nanoparticles on the enantioselective recognition process detection has been experimentally evaluated by using silver and gold nanoparticles as SERS substrates, given their ability to differently interact with the complexes. The viability of this new method for chiral discrimination has been demonstrated for both substrates and could open new avenues for this kind of applications.

Introduction

Regulatory authorities of pharmaceutical products all over the world provided guidelines for drug manufacturers indicating that preferably only the active enantiomer of a chiral drug should be brought to market. Since currently about 40% of the drugs in use are known to be chiral there is an increased tendency of manufacturers to develop and commercialize chiral drugs as single enantiomers [1]. Propranolol is one of the most known examples in this sense, as the R-propranolol is approximately 40 times more potent than S-propranolol in mediating the antiarrhythmic and antihypertensive activity, whereas only S-propranolol appears to be beneficial in treating angina pectoris [2]. In the drug quality assurance process the assessment of chiral purity requires simple, sensitive, cost-effective, high-throughput and reliable means of enantioselective detection. Most commonly this is achieved with the aid of a chiral separation technique by using gas chromatography (GC), liquid chromatography (HPLC) or capillary electrophoresis (CE). Chiral recognition generally depends on a minimum of three simultaneous interactions between the selector and selectand (three-point interaction rule of Dalgliesh [3]), where at least one of these interactions must be stereoselective to form diastereoisomeric complexes and thereby enable chiral separation [4]. Enantiomers are distinguished and eventually separated on the basis on their interaction with a chiral stationary phase or chiral selector. Finding a suitable chiral selector is still often based on trials, being subject to the occurrence of errors that can make the process tedious and very expensive.

Among the available chiral selectors, native and derivatized cyclodextrins (CD) are among the most widely used ones [5]. β-cyclodextrin (β-CD) has been reported in the literature as a chiral auxiliary in different electro-driven separations [6]. However, when used as background electrolyte modifier, β-CD is not able to promote the baseline separation of propranolol's enantiomers in any conditions [7, 8]. The thermodynamics of the inclusion complexes of racemic propranolol and its enantiomers with native and modified cyclodextrins in aqueous solution revealed some aspects of the nature of the interaction between selectors and selectands [9]. The calorimetric studies

allowed the assessment of the thermodynamic parameters for the formation of 1:1 inclusion complexes, shedding some light on the nature of the interactions between the enantiomers of propranolol and the studied CDs. Even though experiments showed significant changes in thermodynamic association parameters using different native and substituted CDs, for the same chiral selector (β- and γ-CD) the constants for the association (Gibbs energy) of the two enantiomers are almost the same, meaning that there is low enantioselective binding, and explaining the low efficiency as chiral selectors for propranolol in electrophoretic separations [7, 8].

However, considering the low costs of the native CDs and the premises for the proven formation of specific inclusion complexes with enantiomers, their use as chiral auxiliaries in the presence of noble metal colloids was initiated [10, 11]. If the degrees of freedom of the enantiomer molecules are limited by their non-specific immobilization on the surface of the hydrosol nanoparticles (i.e. physi- or chemisorption), upon the addition of a proper native cyclodextrin the three-point interaction rule of Dalgliesh can be more easily fulfilled. The stereospecific interaction distinguishing between the resulting diastereoisomers is generated upon the formation of the inclusion complex with CD, where spectroscopic data carrying enough molecular information (vibration-rotational spectroscopy) might differentiate between the optical antipods.

Raman spectroscopy is gaining more and more popularity in different areas of the pharmaceutical industry based on its ability to provide information on the fundamental vibrational bands (the fingerprint region) of the pharmaceutical compounds [12]. Due to its high sensitivity, information content and non-destructive nature, Raman spectroscopy is now used in many applications across the field of chemistry, biology, forensics, pharmaceuticals and materials science [13]. In addition to providing unique information about the sample, Raman offers several additional benefits, including: minimal or even no sample preparation, sampling directly through glass containers, non-destructive and non-intrusive analysis, minimal or no water and CO_2 interference. With the development of plasmonic nanoparticles synthesis methods and the emergence of Surface Enhanced Raman Spectroscopy (SERS) [14], the increase of the Raman signal have been regularly observed on the order of 10^4-10^6, but it can be as high as 10^{14} for some systems [15]. In this way it became possible to identify molecular species as well as their conformations in ultralow concentrations [16, 17]. Very recently, it was demonstrated that SERS can be also employed for chiral recognition and quantification of hydrobenzoin enantiomers by using a platform composed of polystyrene beads coated with silver nanoparticles functionalized with thyolated β-CDs [18]. The attachment of the analyte to the β-CDs allowed the recording of the hydrobenzoin SER spectra, which cannot be evidenced by using simple metallic SERS substrates. Also, in a previous work, Bodoki et al. [19] have shown that Multivariate Analysis combined with SERS and High Pressure Liquid Chromatography (HPLC) can be employed for quantitative discrimination of the two propranolol enantiomers in the presence of β-CDs but the physical mechanism responsible for this discrimination was not addressed and elucidated.

In this paper, we synergistically employed the SERS technique and DFT calculations in order to get more insights about the molecular mechanism involved in the association of propranolol enantiomers with the three classes of native CDs (figure 1) and to characterize and interpret their specific interactions with the precise aim of elucidating the underlying interaction mechanisms responsible for chiral recognition. The experimental spectroscopic data were compared and interpreted with respect to the theoretical results obtained from quantum chemical calculations. The role of the metallic surface in the chiral probing-process has been addressed by employing spherical silver and gold nanoparticles as SERS substrates. Our results point out the important role of the surface on chiral selectivity and could open new perspectives on the use of SERS for future chiral probing applications.

Figure 1

Experiments

Chemical and Materials

(R)-(+)-propranolol hydrochloride and (S)-(-)-propranolol hydrochloride were purchased from Sigma-Aldrich. Fine chemical grade α-, β- and γ-cyclodextrin were employed as chiral selectors and they were purchased from Alfa Aesar. Hydroxyl-ammonium chloride and sodium hydroxide were purchased from Merck and VWR respectively, whereas tri-natriumcitrate-dihydrate, silver nitrate, and tetrachloroauric(III) acid trihydrate from Roth. Ultrapure water (18.2MΩ, Barnstead EASYPure ROdi) was used for the preparation of aqueous solutions.

Colloids preparation

Silver colloid: 0.017 g of silver nitrate was dissolved in 90 ml double distilled water. In a separate recipient, 0.017 g of hydroxyl-ammonium chloride were dissolved in 10 mL water, followed by the addition of 0.250 mL sodium hydroxide aqueous solution (2M). This solution was then added rapidly to the silver nitrate solution under vigorous stirring. After few seconds a grey-brown colloidal solution resulted and it was further stirred for 10 minutes [20]. The pH value of the silver colloid, measured immediately after preparation, was 6.9. The UV-VIS spectrum of the silver colloidal solution exhibits a single narrow absorption peak at 418 nm, indicating the formation of spherical silver nanoparticles, as can be seen in the TEM image presented in the figure SI-4a.

Gold colloid: 0.0485 g of tetrachloroauric(III) acid trihydrate was dissolved in 100 ml water and boiled under vigorous stirring. 3 ml of 1% sodium citrate aqueouse solution was then added dropwise while boiling and stirring. The solution was left boiling for further 20 minutes with continuous stirring. A significant color shift of the aqueous solution towards a dark red colour was observed [21]. By the end of the synthesis the total volume of the colloidal solution has been adjusted to 100 ml by the addition of water. The pH value of the gold colloid, measured after cooling to room temperature, was 6. The UV-VIS spectrum of the gold colloidal solution presents a single narrow absorption peak at 522 nm, indicating the formation of spherical gold nanoparticles as can be seen in the TEM image presented in the figure SI-4b.

Methods and Instrumentation

All SER spectra have been acquired in back-scattering geometry in the 200-2000 cm^{-1} range using a DeltaNu Advantage spectrometer (DeltaNu, Laramie, WY) equipped with a laser diode emitting at 785 nm. The laser power was 100 mW and the spectral resolution was 5 cm^{-1}. All SER spectra were recorded in 1 ml glass vials filled with 500 µl colloid and 100 µl analyte. Each SER spectrum is the average of five recordings taken with an acquisition time of 30 seconds. The UV-VIS absorption spectra of colloidal solutions were recorded with T92+ UV-VIS Spectrophotometer PG INSTRUMENTS, using standard quartz cells at room temperature, over a spectral range between 190 nm and 900 nm and a spectral resolution of 2 nm. For TEM examination, 5 µl drops of each colloidal solution were deposited on carbon-coated copper grids. After 5 minutes, the excess water was removed by filter paper and the samples were left to dry under the ambient air. TEM images were taken on a Jeol JEM 1010 transmission electron microscope (Jeol Ltd, Tokyo, Japan), equipped with a Mega VIEW III camera (Olympus, Soft Imaging System, Münster, Germany), operating at 80 kV.

Stock solutions of propranolol enantiomers and α, β, γ-CDs having 1 mM concentration have been prepared. For the SERS measurements performed on silver colloid, 10 µl of propranolol enantiomers solution have been added to 500 µl colloid, followed by the addition of 90 µl of α, β, γ-CDs solutions. The resulting final concentration of propranolol enantiomers was 1.6 x 10^{-5} M, while the α, β, γ-CDs concentration was 1.5 x 10^{-4} M, yielding a 1:9 molar ratio between selectand and selector. For reasons described in the Discussion section, in the case of SERS measurements performed on gold colloid, the complexes have been previously formed by mixing 1 ml propranolol enantiomers stock solution with 9 ml α, β, γ-CDs stock solution, followed by incubation at 50°C for 2 h. After that, 100 µl of mixed solution have been added to 500 µl gold colloidal solution. The resulting final concentration of propranolol enantiomers was 1.6 x 10^{-5} M, while the α, β, γ-CDs concentration was 1.5 x 10^{-4} M, yielding to a selectand:selector ratio of 1:9.

The molecular geometry optimizations and vibrational spectra calculations were performed with the Gaussian 09 program [22]. We used density functional theory (DFT) approaches with B3LYP hybrid exchange-correlation functional and the standard 6-31G(d) basis set [23, 24]. To explore the effect of dispersion interactions on the structures and interaction energies of R-propranolol-CD or S-propranolol-CD inclusion complexes we used the methodology proposed by Grimme [25]. The two enantiomers were fully optimized while in case of the inclusion complexes the CDs were kept fixed and the enantiomers were optimized inside the frozen CD cavities obtained from Cambridge Crystallographic Data Centre [26]. All the structures were confirmed as being minima on the potential energy surfaces by an adequate vibrational frequency analysis. The assignment of the experimental bands is based on the observed frequencies and intensity patterns of the experimental spectra and confirmed by establishing a one to one correlation between the observed and theoretical calculated frequencies. The calculated Raman activities were converted to relative Raman intensities according to [27].

It is well-known that London dispersion interactions play an essential role in biological molecular systems [28], being demonstrated that they are the main source of binding in different inclusion complexes [29-35]. Thus, for reliably estimating the stabilization energies in host-guest complexes, such interactions must be properly accounted for. Recent advances in density functional theory have led to specific approaches able to account for dispersion and in this work we have chosen the Grimme's approach (DFT-D, revision 2) [25].

Results and discussion

SERS on silver colloid:

Figure 2 illustrates the SER spectra of R- and S-propranolol adsorbed on the surface of hydroxylamine reduced silver colloid in the absence and in the presence of the three classes of native CDs (α, β, γ-CDs). As previously observed and described [26], the SER spectra of the pure propranolol enantiomers are identical being dominated by some intense bands at 494 cm^{-1} (symmetric longitudinal stretching of the naphthyl ring), 737 cm^{-1} (naphthyl ring breathing), 758 cm^{-1} ((NH) bending + (CNC) bending + (C-CH_3) stretching), 789 cm^{-1} (out of plane deformation of naphthhyl), 1017 cm^{-1} ((CC) stretching + (CH) bending), 1381 cm^{-1} ((CC) naphthyl stretching + (CH) bending), 1440 cm^{-1} ((CH) bending of naphthyl) and 1577 cm^{-1} ((CC) stretching + (CH) bending of naphthyl). The assignment of SERS bands was based on DFT calculations. The great majority of vibrational peaks are attributed to different vibrational modes of naphthalene ring. As it was previously highlighted, the propranolol enantiomers are physisorbed on the silver surface by means of O atoms with the naphthalene ring lying almost perpendicular to nanoparticle surface [36].

The addition of α-CDs to the samples containing R- and S-propranolol and silver colloidal solutions does not produce any modifications of the vibrational bands with respect to the SER spectra of pure enantiomers (figure 2). On the contrary, upon the addition of β-CDs, significant changes occur between the SER spectra of R- and S-propranolol (figure 2). Whereas the SER spectrum recorded for S-propranolol is unchanged (similar with the one recorded in the presence/absence of α-CDs), the one of R-propranolol shows some particularities. New intense peaks appear at 434 cm^{-1}, 680 cm^{-1}, 1168 cm^{-1}, 1203 cm^{-1} and 1616 cm^{-1}. Moreover, the peak intensity at 789 cm^{-1} increases and the SERS signal is significantly improved with many new less intense peaks, arising over the entire spectral region at: 334 cm^{-1}, 345 cm^{-1}, 518 cm^{-1}, 622 cm^{-1}, 828 cm^{-1}, 879 cm^{-1}, 912 cm^{-1}, 937 cm^{-1}, 1296 cm^{-1}. Upon the addition of γ-CDs the SER spectrum of R-propranolol exhibits similar features as the one recorded in the presence of β-CDs (figure 2). In the SER spectrum of S-propranolol recorded in the presence of γ-CD, the vibrational bands located at 434 cm^{-1}, 680 cm^{-1}, 789 cm^{-1}, 1168 cm^{-1}, 1203 cm^{-1} and 1616 cm^{-1} are also present but with a

lower intensity, while the vibrational bands located at 334 cm^{-1}, 345 cm^{-1} 622 cm^{-1} and 1296 cm^{-1} vanish. By comparing the SER spectra obtained for the same enantiomer interacting with α, β, and γ-CDs, it can be observed that the addition of β-CD produces the most significant differences in the SER spectra of the two enantiomers (figure 2).

Figure 2

The modification of SER spectra in the case of R-propranolol after the addition of β- and γ-CDs to the colloidal solution (i.e. the occurrence of new vibrational peaks) raises the question whether or not such modifications could represent vibrational peaks of added CDs. Therefore SER spectra were acquired on colloidal silver solution containing only α, β, or γ-CDs. It turned out that CDs does not present any specific vibrational bands (data not shown here) as it was previously reported in the literature [18]. Moreover, upon the addition of native CDs in the silver colloidal solution, the characteristic surface plasmon resonance (SPR) band of spherical silver nanoparticles slightly broadens and decreases in intensity, but does not shift from the initial value of pure silver colloid (418 nm), suggesting that CDs do not interact with the silver surface. On the contrary, by adding R- or S-propranolol to either pure or CDs containing colloidal silver solution a slight red shift of SPR band from the initial value of 418 nm has been detected, suggesting the attachment of propranolol enantiomers onto nanoparticles surface through a physisorption process [36]. All the new peaks appearing in the SER spectra of complexes can be assigned to vibrational modes of propranolol (figure SI-1 and table SI-1).

Based on the above mentioned observations it can be argued that R- and S-propranolol enantiomers and their corresponding complexes with CDs are attached to the silver nanoparticle surface. As a consequence of selective interaction of β- and γ-CDs with R- and S-propranolol enantiomers physisorbed on silver nanoparticles, differences in the corresponding SER spectra have been recorded. These differences represent a strong experimental evidence of the enantioselective interaction of R- and S- propranolol with β and γ-CDs. It has to be noted that the enantioselective interaction occurs independently of the order in which the selector and selectand are added to silver hydrosol. On the other hand the enantioselective interaction does not occur immediately after sample preparation, a minimum of 30 minutes time lapse being necessary for observing spectral differences.

SERS on gold colloid:

In order to point out the role of the surface in the process of chiral selectivity we have performed the same set of SERS measurements by using citrate capped gold nanoparticles as SERS substrates. The gold nanoparticles have a spherical shape, a narrow size distribution and dimensions comparable to silver ones (~20 nm diameter) as it was demonstrated by UV-VIS and TEM measurements (figure SI-4). The SER spectra of pure R- and S- propranolol and of the complexes they formed with α, β, γ-CDs are presented in figure 3. It can be observed that the SER spectra of pure R- and S-enantiomers (figure 3) present more spectral features than those acquired on either silver colloid (figure 2) or other gold substrates reported in the literature [37, 38]. The peak intensities are 10 times higher than those appearing on silver colloid (figure SI-2). The main vibrational bands of propranolol enantiomers adsorbed on silver colloid are still present at slightly different wavenumbers: 491 cm^{-1}, 736 cm^{-1}, 757 cm^{-1}, 789 cm^{-1}, 1018 cm^{-1}, 1098 cm^{-1}, 1240 cm^{-1}, 1273 cm^{-1}, 1382 cm^{-1}, 1440 cm^{-1}, 1503 cm^{-1}, 1573 cm^{-1}. In addition several new peaks occur over the entire spectral range: 622 cm^{-1}, 667 cm^{-1}, 802 cm^{-1}, 869 cm^{-1}, 959 cm^{-1}, 1068 cm^{-1}, 1159 cm^{-1}, 1178 cm^{-1} and 1620 cm^{-1}. All these new peaks appear in the calculated spectrum of propranolol (figure SI-1) meaning that they represent vibrational bands of propranolol enantiomers. Their assignment can be found in the table SI-1 showed in supplementary information. The addition of propranolol enantiomers on the gold colloid instantaneously produces a change of the colloid color from dark red to blue-violet. The UV-Vis spectrum (not shown), recorded on the same samples used in SERS measurements, indicates that the characteristic SPR band at 522 nm drastically decreases in intensity while a new stronger absorption band at 644 nm occurs. This absorption band is characteristic to the formation of gold nanoparticles aggregates due to a strong interaction between propranolol enantiomers and the gold nanoparticles surface. Since in aqueous solution, at the colloid pH of 6, the propranolol is in the protonated form (pK_a values of propranolol are 9.5 and 13.8), one might speculate that it will strongly attach to the negatively charged citrated capped gold nanoparticles surface, thus promoting nanoparticles aggregation. The propranolol molecules entrapped in the interparticle junctions are responsible for the improved SER spectra obtained on the gold colloid. Given the slight difference between the vibrational bands of propranolol adsorbed on both colloids (less than 5 cm^{-1}), it can be concluded that the adsorption geometry of propranolol enantiomers on both metallic nanoparticles is similar.

Figure 3

The SER spectra acquired upon the addition of CDs on the colloid solutions, already containing propranolol, were identical with those obtained in the case of pure R- and S-propranolol. This finding suggests that the propranolol molecules from the interparticle junctions are not able to subsequently couple with the CDs and to form complexes. Supposing that those propranolol molecules attached at the exterior side of these junctions might form a complex with CDs, the SERS signal given by the complexes is rather weak compared with that of propranolol molecules inside the hot spots. Similar effect is observed if the colloidal solution is immediately added on the propranolol-CDs solutions. Most probably, the very short time elapsed between the preparation of propranolol-CDs solutions and the addition of colloid solution, does not allow the formation of complexes, the propranolol molecules preferring

to attach to nanoparticle surface rather than forming complexes with CDs. For a better understanding of this behaviour, prior to SERS measurements, we have prepared solutions containing the desired amounts of propranolol enantiomers and native CDs. In order to be sure that the complexes are formed, the solutions have been incubated for 2 hours at 50°C.

The SER spectra of R- and S- propranolol incubated with α-CDs fit perfectly band to band and exhibits spectral features of less intensities as compared to pure propranolol (figure 3). The main vibrational bands of propranolol are recorded at the same wavenumbers with a much lower intensity. The intense peak at 1031 cm^{-1} represents vibrational band of the capping agent surrounding the gold nanoparticles. The same observations apply in the case of R- and S-propranolol incubated with γ-CDs, their SER spectra being identical (figure 3). As in the case of silver colloid, the major differences in the SER spectra occur for the R- and S- propranolol incubated with β-CDs (figure 3). It can be observed that the SER spectrum of S-propranolol incubated with β-CDs exhibit similar spectral features with those of S-propranolol incubated with α-CDs and γ-CDs (figure 3). Five additional peaks (423 cm^{-1}, 621 cm^{-1}, 679 cm^{-1}, 1173 cm^{-1} and 1208 cm^{-1}) are present in the SER spectrum of R-propranolol-βCD complex. The vibrational modes associated to these five new peaks are identical with those corresponding to the following peaks: 434 cm^{-1}, 622 cm^{-1}, 680 cm^{-1}, 1168 cm^{-1}, 1203 cm^{-1}, which occur in the SER spectra of R-propranolol-β-CD complex adsorbed on silver nanoparticles (see table SI-1). These experimental finding represent a strong evidence of the capability of β-CD to enantioselectively interact with R- and S-propranolol on the gold colloid as well.

Geometry optimization of complexes

Propranolol has one chiral center located in close proximity of the naphthalene ring and it has been proven to form a 1:1 guest-host inclusion complex with native CDs [9]. For a proper understanding of the experimental differences observed in the SERS measurements, DFT calculations at the B3LYP-D/6-31G(d) level have been carried out for each class of complexes. In order to explain the experimental SERS spectra as a function of surface selection rules, geometry optimization of each class of complexes formed between R and S propranolol and α, β, and γ-CDs was carried out. Expectedly, the full optimization of the CD inclusion complexes requires large computational resources and is very time consuming. Considering the numbers and large sizes of the inclusion complexes that were investigated and also the available computational resources we decided to perform partial optimizations of the inclusion complexes. The enantiomers have been fully optimized inside the frozen CD cavities.

The B3LYP-D/6-31G(d) partially optimized geometries of the six inclusion complexes are given in figure 4. As it can be seen, the inclusion geometries of the two enantiomers in α-CD are very similar and it can be concluded that the interactions of the two propranolol enantiomers with α-CD are identical and take place through the incorporation of propranolol alkyl chain into CD hydrophobic cavity (figure 4). This type of inclusion can be mainly attributed to the small internal diameter of α-CD (4.9Å). The naphtalene ring of both propranolol enantiomers lies outside the CD cavity in a very similar manner (figure 4).

On the other hand, a marked difference is noted between the enantiomers' geometries optimized inside the β-CD cavity. The metrics used to describe these differences consists of three parameters: the distance between the chiral carbon (C*) and the centroid defined by the oxygen atoms linking the glucopyranose units (X_{CD}), the angle between the mean plane defined by the oxygen atoms linking the glucopyranose units (P_{mean}(CD)) and the mean plane defined by the naphthyl group (P_{mean}(naphthyl)) and the distance between the centroid of naphthyl group (P_{mean}(naphthyl)) and P_{mean}(CD). These parameters are summarized in table SI-2 and they clearly show that S-enantiomer is significantly more distant from the center of the β-CD cavity than the R-enantiomer (figure 4).

Moreover, while the plane of the naphthyl group in R-enantiomer is nearly perpendicular to the horizontal mean plane of β-CD and to its wider rim, it becomes more tilted for the S-antipod (figure SI-3) proving a completely different interaction mechanism for the complexes formed between R- and S-enantiomers with β-CD. The naphthalene ring of the R-propranolol fits into the β-CD cavity, while the side chain lays almost perpendicular to the outer rim of β-CD. As a result of this inclusion geometry the distance between the center of the β-CD ring and the chiral carbon of R-propranolol has a value of 5.8 Å. According to DFT calculations, the distance between the oxygen atom of hydroxyl group located near the chiral center and the hydrogen atom in the closest hydroxyl group of the β-CD is 2.067 Å, while the corresponding O...O distance is 3.000 Å. In this case the formation of a hydrogen bond can be envisaged as it was previously suggested [39], the value being within the corresponding limits of a hydrogen bond [40]. These data suggest that the naphthalene ring is incorporated into CD cavity via van der Walls interactions and that the inclusion of naphthalene ring occurs from the wider side of the CD.

Figure 4

For the S-β-CD complex the quantum chemical calculations indicate that the naphthalene ring is not fully incorporated into the β-CD cavity (Figure 4). The distance between the center of the β-CD ring and the chiral carbon of S-propranolol has a higher value (6.5 Å) than in the case of R-propranolol. Based on the measured bond lengths resulted from the DFT simulation, the occurrence of hydrogen bonds cannot be envisaged, all the distances being greater than 3 Å.

At B3LYP-D/6-31G(d) level of theory, the estimated interaction energies of the two enantiomers with β-CD are -67.6 and -64.7 kcal mol^{-1} for R- and S-enantiomers, respectively. Since the basis set superposition error was not accounted for in these calculations, and still trying to avoid the laborious counter-poise method for BSSE correction, we relied on the functional B97-D [25] that has been shown recently to be stable with respect to the basis set change, yet providing an excellent agreement with high-level post-Hartree-Fock calculations [41, 42, 33, 34]. This slightly dependence on the basis sets presumably leads to a much more reliable interaction energies that do not necessarily need further BSSE corrections. Moreover, in a study on weakly interacting molecular complexes, Welsh et al. [34] concluded that using the B97-D and its long range corrected partner ωB97X-D functional they obtained "the most reliable and similar results", and even more,

"with the use of small basis sets and the lack of counter-poise correction" (see Table 1 in ref. [34]). Actually, the interaction energies obtained with B97-D functional are about half of those given by the raw, BSSE uncorrected values, provided by B3LYP/6-31G(d) functional. Such an impact of the BSSE correction through the counter-poise methods on the calculated binding energies is commonly observed in the literature [33, 43, 44]. Thus, the β-CD-R and β-CD-S complexes were partially re-optimized at B97-D/6-31G(d) level of theory, as in the case of B3LYP-D approach, but starting from the geometries delivered by the B3LYP-D/6-31G(d) method. As shown in figure SI-5, the only significant difference between the B3LYP-D/6-31G(d) and B97-D/6-31G(d) methods resides in the relative orientation of the naphthyl group inside the CD cavities. The new obtained interaction energies at B97-D/6-31G(d) level of theory are -36.2 kcal mol^{-1} and -32.8 kcal mol^{-1} for β-CD-R- and β-CD-S complexes, respectively, in line with other similar inclusion complexes [29, 31, 34, 35, 45, 46].

The propranolol enantiomers form inclusion complexes with γ-CD, as well. Given the wider diameter of the rim (~8 Å) both enantiomers adopt very similar inclusion geometries into CD cavity (figure 4). The naphthalene moieties are included into the hydrophobic cavity, while the side chains were found to point out of the CD rim.

Discussions

In a recent report Abalde-Cela et. al. [18] have demonstrated that, by employing as SERS platforms, highly aggregated β-CD functionalized silver nanoparticles supported and stabilized on polystyrene microbeads, chiral discrimination of hydrobenzoin enantiomers can be achieved. However, the very complex synthesis procedure of the SERS substrates and the concept allowing chiral discrimination relying exclusively on the differences in the relative intensities of the characteristic vibrational bands represent two issues that can be considerably improved. In our experiments we have employed silver and gold spherical nanoparticles synthesized using simple, standard methods [20, 21] as SERS substrates, all the measurements being performed in aqueous solutions. Similar to Abalde-Cela et al. [18], for both classes of colloids, differences in the relative intensities of the characteristic vibrational bands of propranolol enantiomers interacting with both β- and γ-CDs, have been detected as well. In addition to this evidence of chiral discrimination, our study introduces a fundamentally new and irrefutable proof of chiral discrimination between enantiomers using SERS technique: the occurrence of new vibrational peaks in the SER spectra of R-propranolol-β-CD complex with respect to the SER spectra of S-propranolol-β-CD complex. Since the occurrence of new vibrational peaks in the SER spectra of only one enantiomer has been detected on both colloids, one can conclude that this feature is characteristic to the complex itself and does not represent a colloidal artefact. The DFT calculations were very useful for the interpretation of the acquired experimental spectra. In the same time they allowed the understanding of the mechanism of interaction between the two enantiomers and the three classes of native CDs. As a general observation we believe that the method could be successfully applied for other classes of chiral molecules.

The nanoparticles surface seems to play also a very important role in chiral discrimination by employing SERS, this assumption being supported by two experimental evidences. Firstly, the SER spectra recorded on gold nanoparticles are better resolved and more intense with respect to those acquired on silver colloid (figure SI-2). Secondly, the stronger interaction between gold nanoparticles and the complexes leads to spectral differences between the two enantiomers only for complexes with β-CDs. By analyzing the interaction geometries of the two enantiomers with the three classes of native CDs it can be observed that only in the case of β-CDs the interaction is fundamentally different (figure 4). As a result, a proper understanding of our SER spectra needs to take into account also the interaction of the nanoparticles surface with the complexes.

The DFT modeling showed that both propranolol enantiomers form complexes with α-CDs in similar manner. This can explain the perfect match between their SER spectra acquired on both colloids. The most intense vibrational bands correspond to vibration modes associated with the naphthalene ring (1573/1577 cm^{-1}, 1440/1440 cm^{-1}, 1382/1381 cm^{-1}, 757/758 cm^{-1}, 736/737 cm^{-1}, 494/491 cm^{-1}), which is located outside the CD cavity. By comparing the SER spectra of the complexes with those of pure R- and S-enantiomers one can conclude that in both cases the naphthalene ring lies in a same geometry with respect to the surface. The SER spectra acquired on gold colloid are more intense as a consequence of a stronger interaction with the gold surface that has the capacity to bring the complexes closer to the surface thus enhancing the Raman signal. However, on the gold colloid, the intensities of the vibrational bands of the complexes are lower than those of pure R- and S-enantiomers. This is mainly due to a weaker interaction of the complexes with the gold surface, which is further proved by the occurrence of the 1031 cm^{-1} peak that belongs to the colloid. This peak is not visible in the SER spectra of pure enantiomers since it is masked by the stronger vibrational bands of propranolol. The faintly visible peak at 1671 cm^{-1} can be assigned to NH_2 bending vibrational mode of propranolol indicating that the enantiomers are in the protonated form.

The SER spectra show the largest difference between the two propranolol enantiomers, in the case of the complexes formed with β-CDs, independently of the type of colloid employed. This major difference can be explained by the different interaction geometries of the propranolol enantiomers with β-CD. The S-enantiomer has the naphthalene ring center situated at 6.5 Å from the center of β-CD and forms an angle of 27^o with the β-CD mean plane (table SI-2 and figure SI-3). The R-propranolol has the naphthalene ring situated deeper in the cavity, at 5.8 Å from the β-CD center and in a plane forming an angle of 77^0 with the β-CD mean plane (table SI-2 and figure SI-3).

Our data are consistent with other results obtained by using NMR [48], X-ray diffraction [39] and molecular docking [49] that have proved the formation of inclusion complexes between the propranolol enantiomers and natural β-CD. Moreover, as it was pointed out earlier, the aliphatic chain of R-propranolol is almost perpendicular to the β-CD largest rim, thus favouring the formation of a hydrogen bond between the oxygen atom of the hydroxyl group bound to the chiral center and the nearest hydroxyl group from the β-CD, with an estimated O-H distance of 2.06 Å. This type of hydrogen bond formation was also proposed by other authors, based on molecular docking data [48], and is considered to be responsible for an improved chiral discrimination between propranolol enantiomers in capillary electrophoresis separations. In a pioneering paper Armstrong et al. [39] pointed out that in order to obtain chiral separation with β-CD, a compound needs at least one aromatic ring (although two would be of greater benefit), a chiral center situated in the

proximity of the ring moieties and that the chiral center or one substituent of the chiral center must be near and interact with the mouth of the CD cavity. As a result, numerous chemically modified CDs were synthetized, and are currently used in separation techniques, by substituting different chemical groups at the CD rim, aiming to increase the interaction difference between the modified CD and the molecules to be separated.

In our case, the R-propranolol inclusion in the natural β-CD cavity, together with the interaction of the molecule with the colloidal metallic surface create a more rigid environment of the molecule, not allowing all the vibration modes as in the case of a free molecule and leading thus to the excitation of new vibration modes which were evidenced in the respective SER spectra. The different inclusion geometries of the two enantiomers inside the β-CD cavity can cause a different orientation of them with respect to the metallic surface and therefore the different effect of the surface selection rules on the enhancement of vibrational modes [47] generates the differences between SER spectra of complexes. The new vibrational peaks can be assigned to vibrational modes belonging to either naphthalene ring or to methylethylamino chain. The spectral differences between complexes exist on both colloids, but are slightly more visible on gold colloid due to a stronger interaction with the surface. It has to be mentioned that for both colloids the new vibrational bands occur at the same wavenumbers (difference less than 5 cm^{-1}) suggesting that the same vibrational modes are responsible for the occurrence of the new SERS peaks.

For complexes formed with γ-CDs, the DFT calculations showed a much similar geometry of interaction for the two enantiomers, the naphthalene ring being included in the CDs cavity in both cases. The SER spectra recorded on gold colloids are similar for the two enantiomers, whereas those acquired on silver nanoparticles exhibit slight differences only in the relative intensities of some vibrational bands. On the other hand, in the case of gold colloids, the new vibration bands, observed for R-β-CD complex, are not evidenced. We consider that the larger inner diameter of the γ-CD cavity creates a more flexible environment for the guest molecule and therefore the new vibration modes are less excited and cannot be evidenced. Because the enhancement produced by the silver colloid is much smaller the ratios between the relative intensities of different vibration bands are also reduced and hence some of the new vibration bands are still noticed when using the silver colloids. This experimental finding demonstrates the important role played by the nanoparticles surface in chiral discrimination using SERS.

Conclusions

We have employed the SERS technique in conjunction with quantum chemical calculations (DFT) for a proper understanding of the physical concept leading to enantioselective recognition of propranolol enantiomers through complexation with native cyclodextrins (α-, β-, γ-CDs). For the ease of interpretation the experiments have been acquired on very common SERS substrates (as-synthesized silver and gold spherical nanoparticles). The experiments and the calculations have been performed for all the complexes of R- and S-propranolol formed with the three classes of native CDs allowing the understanding of the physical concept responsible for chiral discrimination through SERS. Since the interactions of the propranolol enantiomers with α-CDs were identical, taking place through the incorporation of propranolol alkyl chain into CD hydrophobic cavity, no differences in the SER spectra have been recorded. On the contrary, the specific shape and cavity size of β-CD, induces fundamentally different geometries of interaction with propranolol enantiomers. As a result the naphthalene ring of the R-propranolol is incorporated into the β-CD cavity via van der Walls interactions, being situated almost perpendicularly on the horizontal mean plane of β-CD whereas the naphthalene ring of the S-propranolol is not fully incorporated into the β-CD cavity and forms an angle of 27° with the horizontal mean plane of β-CD. The deeper insertion of the R-enantiomer into β-CD cavity allows the formation of at least one hydrogen bond between the oxygen atom situated near the chiral center and the hydrogen atom from the closest hydroxyl group of the CD rim. This result is consistent with the larger interaction energy obtained from DFT data for the R-enantiomer with β-CD as compared to his S counterpart and is probably responsible for the chiral separation of the two enantiomers in CD driven separation techniques. These two types of interaction geometries of R and S-propranolol with β-CD are responsible for the different SER spectra, characterized by the occurrence of new specific vibrational bands of propranolol in the SER spectrum of R-propranolol-β-CD complex. The chiral discrimination of propranolol enantiomers by β-CDs have been recorded on both colloids, paving the way of using the SERS technique for chiral discrimination of other classes of enantiomers in a rapid and sensitive manner. Even though the DFT calculations showed a similar complexation mechanism between propranolol enantiomers and γ-CD, i.e. the naphthalene rings being entrapped in the γ-CDs cavity, the attachment of both complexes on the silver nanoparticles have produced clearly observable differences in the relative intensities of some vibrational bands of propranolol in the SER spectra, which represent a proof of the chiral discrimination of propranolol enantiomers by γ-CD. These differences have not been recorded on the gold nanoparticles, since the interaction of propranolol with the gold surface is stronger. This represents a strong evidence of the important role played by colloids in chiral discrimination using SERS technique.

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Electronic Supplementary Information (ESI) available: the calculated Raman spectra of propranolol together with the assignment of the characteristic vibrational bands occurring in the SER spectra, a description of geometrical parameters characteristic to inclusion complexes with β-CD, the original SER spectra of propranolol enantiomers on both colloids, the optimized geometries of inclusion complexes with β-CD highlighting the planes of naphthyl group of propranolol enantiomers and of the oxygen atoms on the wider rim of β-CD, TEM images with spherical silver and gold nanoparticles employed as SERS substrates and the optimized geometries of R- and S-propranolol inside the β-CD cavity using both B3LYP-D/6-31G(d) and B97-D/6-31G(d) levels of DFT theory are included. See DOI: 10.1039/b000000x/

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