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# Chiral recognition and quantification of propranolol enantiomers by surface enhanced Raman scattering through supramolecular interaction with $\beta$-cyclodextrin

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ABSTRACT

A simple, fast and accurate method of chiral recognition and quantification of propranolol enantiomers by surface enhanced Raman scattering (SERS) and multivariate regression analysis through supramolecular interaction with $\beta$-cyclodextrin is reported. Computational chemistry served as a tool of elucidating the underlying mechanism of molecular interactions responsible for chiral discrimination. The influence of several factors (nature and concentration of chiral auxiliary, selector-selectand ratio, pH, interaction time, etc.) over the obtained SERS spectra was assessed, followed by the construction of the chemometric model with the optimized operational conditions. The performance of the obtained semi-empirical model was established using a validation set of pure enantiomers and its intended use was demonstrated by the assessment of the enantiomeric excess of propranolol in pharmaceutical formulations (tablets) without the need of tedious and expensive chiral separation. The obtained results were also confirmed by chiral high-performance liquid chromatography.



## 1. Introduction

Chiral recognition and differentiation in living organisms represents one of the most intriguing natural phenomena, which assures in the organism a high fidelity transfer of the molecular information. This phenomenon has a significant role in the pharmaceutical industry, since chirality plays a key role in the development of target drug candidates, being a structural variable parameter that needs elucidation. In this context, the subject of chiral purity gained a particular importance in the pharmaceutical industry.

Most often the enantiomers of chiral drug molecules have different pharmacodynamic and pharmacokinetic properties and therefore the quantitative enantiomeric composition (chiral purity, enantiomeric excess) of these drugs should be determined. This is also the case with propranolol (1-isopropylamino-3-(1-naphthyloxy)-2-propranolol; Fig. 1) which is an important $\beta$-adrenergic blocking agent which has gained widespread use in the treatment of angina pectoris, cardiac dysrhythmias and hypertension. The pharmacological properties of the enantiomers of propranolol are quite different, and the $\beta$-adrenergic blocking activity resides in the (*S*)-(−) isomer, while the (*R*)-(+)-enantiomer has only a membrane stabilizing effect. Furthermore, propranolol undergoes stereoselective first-pass metabolism [1].

The assessment of enantiomeric excess is most frequently achieved by a chiral separation technique, which is usually considered a costly and tedious process. Spectroscopy seems to be the much waited alternative, where chirooptical methods (i.e. optical rotatory dispersion and circular dichroism) are able of rapid enantiodiscrimination without the need of separation, but these methods suffer from relatively low sensitivity and corresponding low tolerance against impurities, especially chiral ones. However, with the aid of chiral auxiliaries (i.e. cyclodextrin derivatives, chiral surfactants and ionic liquids, etc.) non-chirooptical molecular spectroscopy combined with multivariate data analysis allows the assessment of the qualitative nature of the chiral molecular recognition, as well as enables a cost-effective, high-throughput and sensitive quantitative determination of chiral purity [2].

Even though with the aid of spectroscopic techniques (FT-IR and Raman spectroscopy) the functional groups involved in chiral discrimination can be identified, it has been rarely used for the quantitative determination of enantiomeric excess [3,4]. Usually in case of infrared spectroscopy this reluctance stems from two reasons, one regarding poor precision in sampling (especially in ATR-FT-IR) and on the other hand, more often, the convolution of weak

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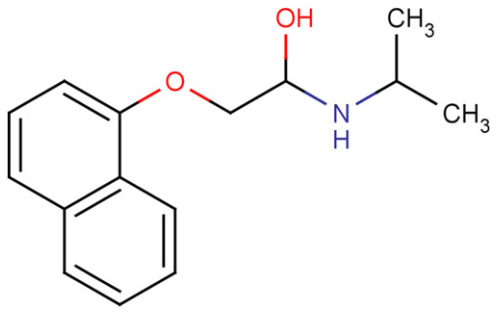


**Fig. 1.** The structure of (+/−)-propranolol.

discriminative spectral features responsible for chiral interaction with other spectral peaks (non-chiral interactions, strong absorbance of solvent and free enantiomers that are not engaged in diastereoisomeric complexes, instrumental noise, etc.). As for Raman spectroscopy, its disadvantages are related to low sensitivity and the frequently occurring fluorescence phenomena. However, these shortcomings are practically overcome by surface enhanced Raman scattering (SERS), upon the formation of localized surface plasmon resonance (LSPR). LSPR excitation results in wavelength-selective absorption with extremely large molar extinction coefficients, resonant Rayleigh scattering, and the enhanced local electromagnetic fields near the surface of a noble metal nanoparticle, which are responsible for the intense signals observed in all surface enhanced spectroscopies [5]. Therefore SERS, upon the interaction of enantiomers with the noble metal hydrosols, allow their trace analysis, concomitantly providing specific, and in some cases, unimolecular information. However, inspite of the advantages offered by SERS, this spectroscopic technique has not yet been exploited to its full potential in chiral probing [6], remaining a promising field for future studies.

Several articles dealing with the enantioseparation of propranolol from different matrices has been published, mostly including liquid chromatographic [7,8] or electrophoretic techniques [9–11]. These chiral separations techniques have proven their value, offering good selectivity and low limits of quantification; however they may also require lengthy and expensive method development and prolonged analysis times. Simultaneous determination of components in a multicomponent drug formulation without separation could be a difficult task, especially when characteristics of these components from the analytical point resemble closely in addition to the presence of other pharmaceutical excipients. Recently, spectroscopic calibration as part of multivariate chemometric methods for the analysis of multicomponent systems, including chiral analysis, have been reported mostly due to the advent of rapid scanning spectrophotometers, fast and affordable computers and user-friendly chemometry software [12–14].

Even though SERS investigations for chiral discrimination has been scarcely described in the literature [6,15], a SERS study on propranolol has been undertaken previously [16], however no chiral discrimination for $\beta$-blockers has been ever reported by this technique to the best of our knowledge. The present study proposes a simple and effective chiral discrimination method for the enantiomers of propranolol allowing also the assessment of enantiomeric excess from pharmaceutical samples with the aid of SERS and multivariate regression analysis in the presence of $\beta$-cyclodextrin.

## 2. Materials and methods

### 2.1. Sample preparation and spectroscopic analysis

(+/−)-propranolol hydrochloride ((+/−)–PRNL) and its enantiomers, (*R*)-(+)-propranolol hydrochloride ((*R*)–PRNL) and (*S*)-(−)-propranolol hydrochloride ((*S*)–PRNL), perchloric acid ($\geq$69%) were all purchased from Sigma-Aldrich, with standard analytical degree. Propranolol containing tablets used in method validation were Propranolol Arena 40 mg from Arena Group SA (Romania). Fine chemical grade $\beta$-cyclodextrin (bCD) was employed as a chiral selector and it was purchased from Cyclolab Ltd, Hungary. Ultrapure water (18.2 MΩ, Barnstead EASYPure RODi) was used for the preparation of buffers and all related aqueous solutions. Hydroxylamine hydrochloride, formic acid (98%) and sodium hydroxide were purchased from Fluka, whereas silver nitrate from Merck and they were used as it is.

For multivariate regression training and validation sets of known propranolol hydrochloride enantiomeric ratios were prepared (83.2 μM total propranolol hydrochloride) with the aid of intermediate working solutions of 0.8 mM (R)- and (S)-propranolol hydrochloride, respectively containing also the chiral selector (1 mM bCD). The intermediate working solutions were prepared from aqueous stock solutions of the pure enantiomers ($\sim$8.62 mM). In all training and validation sets a fixed volume (717 μL) of 1 mM bCD of chiral selector in aqueous colloidal silver hydrosol was added resulting a total final volume of 800 μL which served as samples for SERS analysis. Hydroxylamine-reduced silver colloid (average diameter of 40 nm) was prepared according to the very simple and efficient method of Leopold and Lendl [17] with a measured final pH of 6.90.

As real sample, an accurately weighted portion of the finely powdered tablets corresponding to 20 mg of pure propranolol hydrochloride was extracted by sonication in 10 mL ultrapure water. From the obtained supernatant after centrifugation at 5000 rpm for 15 min the extracted total propranolol hydrochloride's concentration was spectrophotometrically determined at 290 nm using a previously assessed linear regression (range 35–140 μg/mL; Absorbance=0.0074 (μg/mL)+0.0222, $R^2$=0.9979) on a modular AvaSpec-2048 × 14 back-thinned typed CCD fiber optic spectrometer (Avantes, Spain) using 1.0 cm pathway quartz cell. By adding the proper aliquots of supernatant and the aqueous chiral selector's solution (1 mM bCD) the sample was made up of 717 μL aqueous colloidal silver hydrosol to 800 μL resulting 83.2 μM total propranolol hydrochloride, serving as real sample for SERS analysis.

After the calibration of the Raman spectrometer (DeltaNu Advantage 785, excitation laser 785 nm, Intevac, USA) with pure ethanol all samples were analyzed on the range of 200–2000 $cm^{-1}$ with the aid of a liquid vial holder, data collection and processing being made by the provided NuSpec software.

### 2.2. Chiral HPLC

For the confirmation of the obtained results on real samples, chiral separation using high-performance liquid chromatography was employed. Polysaccharide-based, Lux Amylose-2 (5 μm, 250 × 4.6 mm, Phenomenex, USA) chiral stationary phase column was used on Agilent Series 1200 HPLC system equipped with a DAD detector. The mobile phase consisted of 20 mM $NH_4HCO_3$ with 0.1% (v/v) dietylamine:acetonitrile=60:40 (v/v) delivered at 0.7 mL/min at 25 °C. The injected sample volume was 5 μL. The obtained resolution for the two enantiomers of PRNL was $R_s$=1.51 ($t_r$=11.56 min – R-PRNL; $t_r$=12.12 min – S-PRNL).

### 2.3. Study of propranolol interaction with β-cyclodextrin in the presence of colloidal silver hydrosol

The nature and kinetics of interaction between the chiral selector and the studied enantiomers in the presence of hydroxylamine-reduced silver colloid were studied by SERS. Throughout all experiments the concentration of the silver

hydrosol was kept constant, studying the influence over the SERS spectra of selector-selectand ratio, the pH of the hydrosol and the influence of interaction time between the nanoparticle adsorbed enantiomer and chiral auxiliary.

Computational chemistry served as a confirmation tool of the obtained results, also helping in the elaboration of the proposed interaction mechanism responsible for chiral discrimination. Molecular geometry optimization, and vibrational spectra calculations were performed with the Gaussian 09 software package [18] by using density functional theory (DFT) methods with B3LYP hybrid exchange-correlation functional [19,20] and the standard 6–31G(d) basis set. No symmetry restriction was applied during geometry optimization. The vibrational frequencies were computed at the optimized geometry to ensure that no imaginary frequencies were obtained confirming that it corresponds to a local minimum on the potential-energy surface.

### 2.4. Multivariate data analysis

Spectral data were subjected to partial least-squares (PLS) multivariate regression analysis using Simca-$p+v$.12 software (Umetrics, Sweden). PLS regression was performed on the SERS spectral data using full cross-validation of the training set. PLS was used to develop a semi-empirical mathematical model that correlates spectral data over many wavenumbers with the enantiomeric composition, expressed as the (R)-(+)-propranolol hydrochloride mole fraction ($x_R$). The process involves two steps: (a) the model is trained to predict $x_R$ from the calibration set (samples with known enantiomeric composition), and (b) external model validation, in which a second, independent set of samples (validation set, also with known enantiomeric composition) is analyzed over the same spectral range in the same conditions. The predicted enantiomeric composition is then compared with the known reference values.

## 3. Results and discussion

The comparison of the FT-Raman spectra of the pure racemic propranolol with the SERS spectra of its enantiomers in the presence of silver nanoparticles proves the existing SERS effect (Fig. 2). As expected, the recorded SERS spectra for both enantiomers of propranolol (PRNL) are confounded and they show the characteristic vibrational frequencies (Fig. 2) of the functional groups with most intense Raman activity, namely 434 $cm^{-1}$ (naphthalene out of plane bending and ring rocking), 495 $cm^{-1}$ (symmetric longitudinal stretching of the naphthalene ring), 757 $cm^{-1}$ (naphtelene ring breathing), 1019 $cm^{-1}$ ((CC) stretching +(CH) bending) and 1384 $cm^{-1}$ ((CC) naphthalene ring stretching +(CH) bending). The intense band recorded at 227 $cm^{-1}$ is attributed to AgCl and AgO stretching also observed in other similar SERS studies [21] and it is present in every recorded SERS spectra, both in the absence or presence of chiral auxiliaries. The surface selection rules for Raman scattering state that the vibrations of the adsorbed molecules, which have the polarizability tensor component normal to the surface, are preferentially enhanced. Thus, most probably, both enantiomers are adsorbed onto the silver nanoparticle's surface with their naphthalene ring perpendicular to the surface plane, since their SERS spectra are completely identical.

Propranolol has one chiral center located in close proximity of the naphthalene ring and as most of the supramolecular complexes of cyclodextrin derivatives it has been proven to form a 1:1 guest-host inclusion complex with $\beta$-cyclodextrin [22,23]. As the result of DFT simulation (Fig. 3) the distance between the centers of the cyclodextrin ring and the naphtalenic moiety of R-PRNL was found to be 3.156 Å, whereas in case of S-PRNL 6.221 Å was

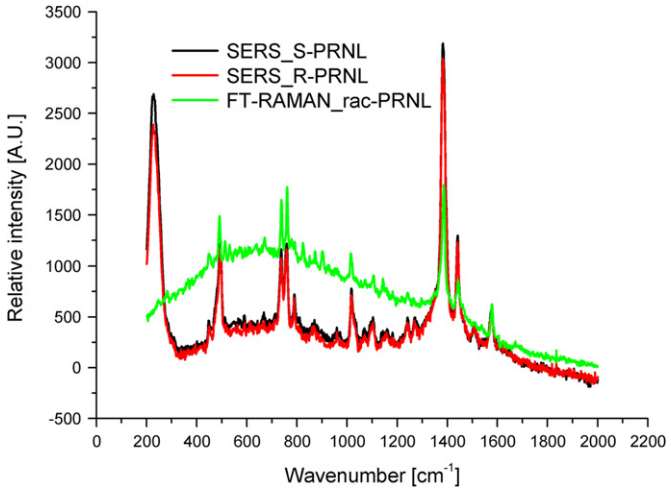


**Fig. 2.** Surface enhanced Raman spectra of *S*-propranolol hydrochloride (black) and *R*-propranolol hydrochloride (red) in aqueous silver nanoparticle hydrosol. FT-Raman spectra of pure racemic propranolol (green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

measured, indicating a selective inclusion of the R antipode into the hydrophobic cavity of the studied chiral selector. Based on the measured bond lengths resulted from the DFT simulation, the interaction between the S-antipod and $\beta$-cyclodextrin seems to be mainly established through two hydrogen bonds between the O atom of the hydroxyl group linked to the chiral center of propranolol and two of the closest O atoms of the hydroxyl groups from the cyclodextrin's wider rim (measured O-H$\cdots$O distance are 2.8 Å and 2.9 Å respectively, within the corresponding limits of a hydrogen bond). The calculated interaction energy for the complex between S-PRNL and $\beta$-cyclodextrin has a value of $-19.57$ kcal/mol, whereas for the corresponding complex with R-PRNL is $-7.12$ kcal/mol. This data supports the stronger interaction between S-PRNL and $\beta$-CD due to hydrogen bonding (the average strength of O-H$\cdots$O bond is around 5 kcal/mol) in comparison with the inclusion complex formed between R-PRNL and $\beta$-CD, stabilized mainly by van der Waals interactions. Despite this stronger interaction, as observed experimentally, the SERS spectrum of the physisorbed S-PRNL in the presence of $\beta$-CD remains unchanged, excepting the intensities of all vibrational peaks which slightly decrease. This is probably due to the interaction of the S-antipode with $\beta$-CD, when the naphtalenic ring gets slightly tilted with respect to the silver nanoparticle's surface, decreasing the normal component of polarizability tensor of the vibrational modes. As also shown by the interaction model resulted from simulation (Fig. 3), upon the addition of $\beta$-cyclodextrin to R-PRNL, its naphthalene ring is included into cyclodextrin's cavity, while the enantiomer remains attached to the silver surface. Upon the inclusion, the naphthalene moiety is pushed closer to the silver surface experiencing better electromagnetic field generated by the excitation of silver surface plasmon oscillations. This theory is also supported by computational chemistry calculations (DFT), where upon the detailed analysis of the enhanced vibrational bands of R-PRNL's SERS spectrum in the presence of the chiral auxiliary (Fig. 4), seems to mainly involve the enhancement of the naphthalene ring's Raman activity (434 $cm^{-1}$ (naphthalene out of plane bending and ring rocking); 680 $cm^{-1}$ (naphthalene ring twisting); 730 $cm^{-1}$ (naphthalene (CH) out of plane bending); 757 $cm^{-1}$ (naphtelene ring breathing); 793 $cm^{-1}$ (naphthalene (CH) and (CC) out of plane bending); 1168 $cm^{-1}$ naphthalene (CH) in plane bending; 1203 $cm^{-1}$ (OH) and (CH) scissoring of the chiral center; 1616 $cm^{-1}$ naphthalene (CC) stretching).

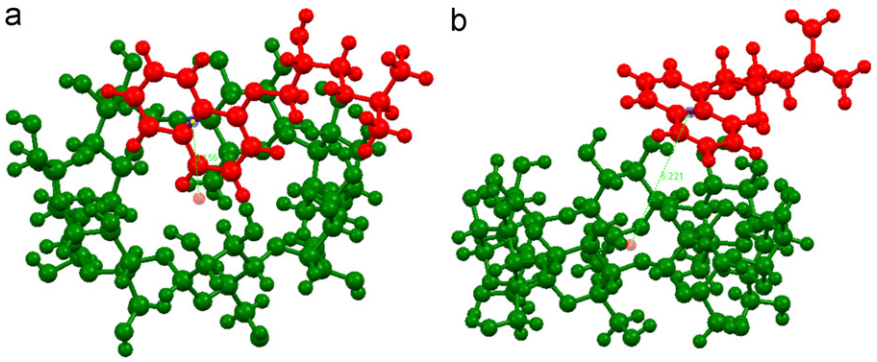


**Fig. 3.** Molecular geometry optimization of the adduct formed between $\beta$-cyclodextrin with R-PRNL (a) and S-PRNL (b).

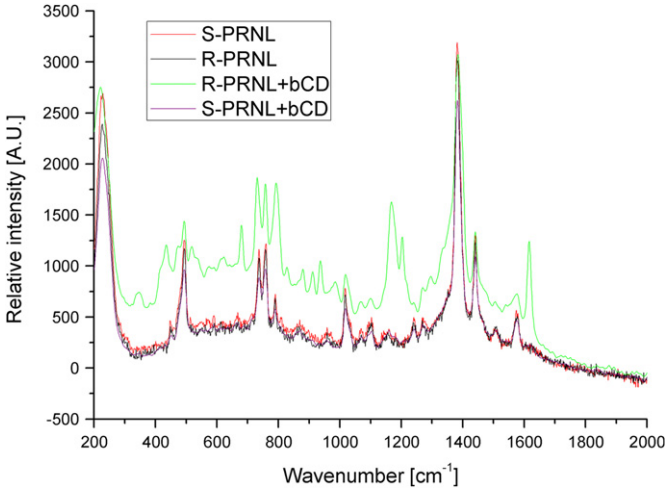


**Fig. 4.** SERS spectra of R- and S-PRNL in the absence and presence of $\beta$-cyclodextrin (fixed ratio of silver colloid).

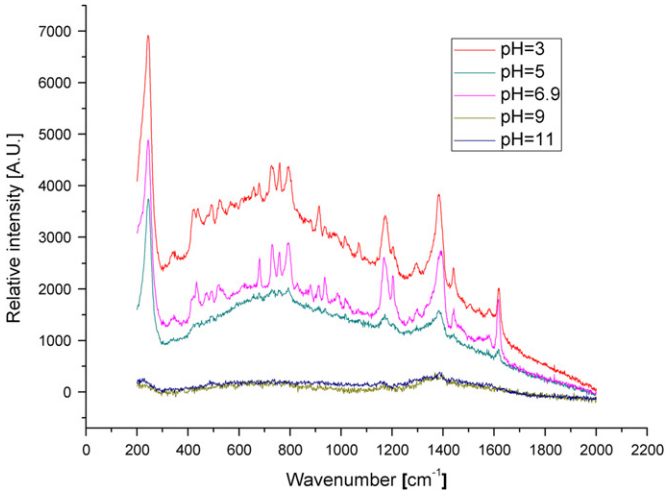


**Fig. 5.** The influence of the silver hydrosol's pH over the SERS spectra of *R*-PRNL (pH adjusted with either 0.1 M HCOOH or NaOH).

It is also worth mentioning that because $\beta$-cyclodextrin has no affinity towards the silver surface independent of its concentration, it does not generate any vibrational band in the SERS spectra.

Therefore during the multivariate regression analysis in all the training, calibration or real samples containing propranolol a fixed concentration of 1 mM $\beta$-cyclodextrin as chiral auxiliary was added.

Moreover, the influence of different experimental factors (the lowest optimum concentration of enantiomers and chiral auxiliary, the nature of buffers and pH, time of interaction, etc.) over the obtained spectra was also assessed. The nature of buffer additives and the pH shows extended influence over the SERS spectra of propranolol's enantiomers, the characteristic spectral features for both antipodes are visible in acidic and neutral (hydrosol's native pH) media and it disappears completely in alkaline media (Fig. 5). In order to avoid the supplementary variables and influences of buffer additives for subsequent studies the pH was kept at the initial value of the obtained hydroxylamine-reduced silver hydrosol (pH=6.9). With the increase of the chiral additive's concentration the signal enhancements experienced for the *R* enantiomer are stronger and they become noticeable in a shorter period of time (under 1 min). However, for short interaction periods the reproducibility of the recorded spectra was poorer, most probably because of the unattained thermodynamic equilibrium of interaction with the added chiral auxiliary.

A compromise had been made between the lowest concentration of $\beta$-cyclodextrin and the necessary interaction time for spectra stabilization capable of inducing measurable changes in the SERS spectra. Thus the addition of 1 mM $\beta$-cyclodextrin was chosen followed by the recording of SERS spectra after 60 min (Fig. 6). This time span was considered useful because it allowed the sequential analysis of a big number of samples, assuring a reproducible interaction time with a minimum consumption of chiral auxiliary.

For multivariate regression the training set consisted of seven samples ($x_R$=0.1, 0.3, 0.4, 0.5, 0.7, 0.8 and 0.9), whereas four were used as the calibration set ($x_R$=0, 0.2, 0.6 and 1.0) all with a total concentration of 83.2 μM propranolol (Tables 1 and 2).

Spectral data analysis began with principal component analysis (PCA) to detect outliers and other anomalies in the data. PCA of the unit variance scaled and mean centered *X*-data gave a three-component model, which explained 99.5% of the variation ($R^2X$=0.995). Examining the loadings, the first component, accounting for more than 93.9%, captures the overall variation in most of the recorded spectra (200–1500 $cm^{-1}$), whereas the loading of the second component describes the remaining unexplained spectral variation in the range of 1500–2000 $cm^{-1}$. Although the third component accounts for merely 1.4% of the spectral variation, it was considered important since it captures the whole fine SERS spectral features of propranolol (data not shown). The PCA-X model, apart from identifying the outliers, can also be useful in giving the first hint of the spectral range loaded with the most useful information considering the chiral analysis, which in this case suggests that the entire spectral range (200–2000 $cm^{-1}$) carries valuable qualitative and eventually quantitative information. Continuing with the PLS modeling of the entire data set, as part of data pre-treatment, all the entire spectral data (Fig. 7) were mean centered, whereas the mole fraction of

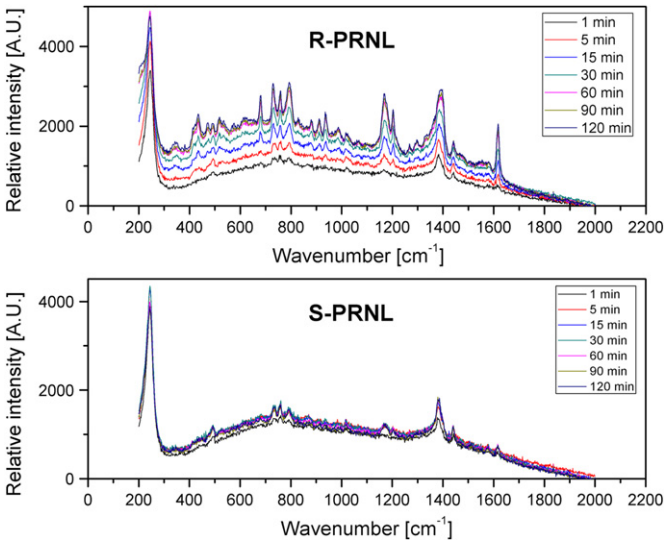


**Fig. 6.** The influence of the interaction time of 1 mM $\beta$-cyclodextrin with 8 μM *R*- and S-PRNL in silver hydrosol.

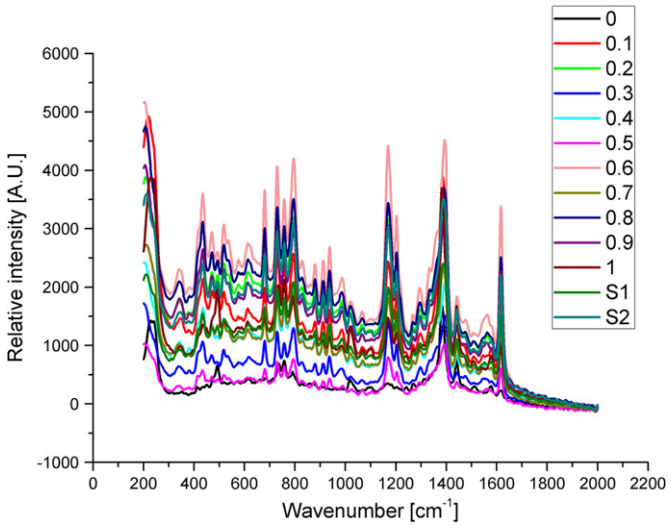


**Fig. 7.** SERS spectra of training and validation set of varying mole fractions of *R*-(+)–PRNL in the presence of 1 mM $\beta$-cyclodextrin; 200–2000 $cm^{-1}$.

**Table 1**
Propranolol hydrochloride training set parameters.

| Observation ID identifier (%) | Mole ratio of R-(+)-PRNL ($x_R$) |
|---|---|
| 10 | 0.09976 |
| 30 | 0.30012 |
| 40 | 0.40024 |
| 50 | 0.50000 |
| 70 | 0.69988 |
| 80 | 0.80048 |
| 90 | 0.90024 |

**Table 2**
Results for the external validation of the PLS model.

| Mole ratio of R-(+)-PRNL ($x_R$) | | Error of prediction (%) |
|---|---|---|
| Real | Predicted | |
| 0.0000 | 0.0039 | 0.39 |
| 0.1995 | 0.2027 | 1.60 |
| 0.5998 | 0.5967 | −0.51 |
| 1.0000 | 0.9955 | −0.45 |

(R)-(+)-PRNL ($x_R$) was unit variance scaled and mean centered. Simple PLS regression of the spectral data did not succeed in building an appropriate model for extracting quantitative information regarding the enantiomeric excess; therefore in order to eliminate the variation in *X* that is unrelated to *Y*, the spectral data was further processed prior to data analysis by the use of different spectral filters. The one that offered the best result turned out to be the orthogonal signal correction (OSC) algorithm built in the Simca-p+ software. OSC is a PLS based solution that removes from the X-data variation that is unrelated to *Y*, the result being a model based on one or more PLS components conveying information about the correction of *X*. Therefore two OSC components were removed, remaining 15.47% of the original sum of squares in the corrected *X*-matrix.

The PLS modeling of the "corrected" training set, where the *X*-block comprised 1801 variables (entire SERS spectral data) and the *Y*-block the mole fraction of *R*-(+)-PRNL ($x_R$), resulted in a two-component model, which according to cross-validation gave an explained variation (goodness of fit) of $R^2Y=0.99997$ and the predicted *Y*-variation according to internal cross-validation (goodness of prediction) of $Q^2_{int}Y=0.99992$. In order to obtain an estimate of the significance of the $Q^2Y$-value a response permutation was carried out, which consist in developing a number of parallel models based on fit to randomly re-ordered *Y*-data, and evaluate the real $Q^2Y$ in the light of a distribution of $Q^2Y$-values of the re-ordered response data, giving a statement of the statistical significance of the estimated predictive power, ruling out any overfitted model ($R^2Y=0.223$, $Q^2Y=-0.407$; Criteria: $R^2Y<0.3$–0.4, $Q^2Y<0.05$). Predictive validation by means of cross-validation and response permutation testing in many ways provides a reasonable first approximation of the predictive ability of the PLS model. However, a more demanding and rigorous way of testing predictive performance consists of computing predictions for an independent set of test observations (validation set). The results of the external validation of the obtained PLS model is presented in Table 2. The very high value of goodness of prediction ($Q^2_{ext}Y=1.000$), the low Root Mean Square Error of Prediction ($RMSEP=0.003182$) and the good correlation ($y=1.009x-0.004169$, $r^2=1.000$) between real and predicted mole fraction values of *R*-(+)-PRNL from the validation set all prove the high analytical value of the obtained model.

The chemometric analysis of the real sample of propranolol (40 mg tablets) based on the recorded SERS spectra of the aqueous extract gave the expected results since the tablets contain the racemate of the active pharmaceutical ingredient, fact also confirmed by the chiral HPLC analysis (Table 3).

## 4. Conclusions

Upon the specific inclusion of the naphthalenic moiety of *R*-(+)-propranolol into the cavity of $\beta$-cyclodextrin, as the aromatic ring gets closer to the colloid's surface, the obtained SERS spectra differs substantially from the one obtained for the *S*-antipode. By computational chemistry calculations it has been confirmed that the nature of atomic groups involved in enantiodiscrimination, and the observed phenomena was employed for the quantitative assessment of propranolol's chiral excess in pharmaceutical formulations. The most important factors influencing the above mentioned phenomena were investigated in order to establish the optimum working parameters. The obtained chemometric model and its performances were characterized by specific validation procedures, proving a high predictive power ($RMSEP=0.003182$) and a very good accuracy

**Table 3**
Mole fraction of R-propranolol enantiomer in real sample.

| Propranolol Arena 40 mg tablets | Mole ratio of R-(+)-PRNL ($x_R$) | | Error (%) |
|---|---|---|---|
| | Real | Obtained | |
| Proposed method | 0.5000 | 0.4986[a] | −0.14 |
| Chiral HPLC | 0.5000 | 0.4798[b] | −4.04 |

[a] $n=2$, $RSD=0.18\%$.
[b] $n=2$, $RSD=0.46\%$.

(under 1% error of prediction in case of real samples) with a minimum consumption of chiral auxiliary.

The present work demonstrate once again the high analytical value of molecular spectroscopy, namely surface enhanced Raman scattering, combined with multivariate regression in chiral recognition and quantification of propranolol in the absence of any time consuming and expensive separation technique. Once the chemometric model is built, by the addition of 1 mM $\beta$-cyclodextrin and a fixed ratio of silver nanoparticle hydrosol into samples containing unknown enantiomeric ratio of propranolol hydrochloride, a high number of chiral analysis can be carried out by merely recording their SERS spectra (total analysis time under 80 min).

The obtained information rich SERS spectra, assisted by computational chemistry allow the elucidation of the underlying molecular interactions responsible for chiral discrimination. Moreover, it opens up new perspectives in the future design of highly enantioselective analytical platforms for fast and sensitive chiral discrimination and quantization of various bioactive compounds.

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