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Determination of the enantiomeric composition of some molecules of pharmaceutical interest by chemometric analysis of the UV spectra of guest–host complexes formed with modified cyclodextrins

Sayo O. Fakayode, Isabel M. Swamidoss, Marianna A. Busch, Kenneth W. Busch*

Department of Chemistry and Biochemistry, Baylor University, One Bear Place 97348, Waco, TX 76798, USA

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Abstract

Multivariate regression modeling techniques (PLS-1 regression modeling) were applied to ordinary UV spectral absorption data obtained on solutions containing inclusion complexes formed between homochiral modified cyclodextrins (methyl-β-cyclodextrin, α-, β-, and γ-carboxymethyl cyclodextrins and α-, β-, and γ-hydroxypropyl cyclodextrins) and four guest molecules of pharmaceutical interest (ephedrine, norephedrine, norepinephrine-L-bitartrate, and tryptophan methyl ester). The PLS-1 regression models were developed by correlating the known enantiomeric composition of laboratory prepared samples with ordinary UV absorption spectral data. The regression models were subsequently validated with laboratory-prepared test sets. The *rms* percent relative error in the predicted mol fraction of (*1S, 2R*)-(+)-ephedrine, (*1S, 2R*)-(+)-norephedrine, (*R*)-(−)-norepinephrine-L-bitartrate, and D-tryptophan methyl ester obtained with the independently prepared test sets was heavily dependent on the host molecule used.

Keywords: Chiral analysis; Multivariate regression modeling; PLS-1; Cyclodextrins; UV–vis spectrophotometry

1. Introduction

Recent studies in our laboratory [1–3] have shown that the enantiomeric composition of samples of chiral molecules can be determined with multivariate regression models developed from ordinary UV–vis absorption spectral data obtained on diastereomeric guest–host complexes formed with native homochiral cyclodextrins. We have observed, for samples that contain a fixed amount of cyclodextrin and a fixed amount of chiral analyte, that the UV absorption spectra of the samples vary slightly in certain wavelength regions as the enantiomeric composition of the chiral analyte is varied. These spectral changes, although small, can be readily correlated with the known enantiomeric composition of a calibration set of samples using standard multivariate regression techniques (partial least squares regression, PLS-1) [4].

Multivariate regression techniques are widely used in chemistry as a means of correlating small changes in spectral data with known compositional changes [5,6]. In our experiments, for example, the absorption spectra contain contributions from both complexed and uncomplexed guest and host molecules, making univariate data analysis problematic. By contrast, multivariate regression modeling is useful because it can focus on those spectral features that correlate with the parameter of interest (in this case, enantiomeric composition). The PLS-1 algorithm is especially powerful in this regard because both the spectral data and the dependent variable (in this case, enantiomeric composition) are actively involved in the construction of the new basis set of variance-scaled eigenvectors that serve as PLS components. As a result, the PLS regression algorithm focuses on those variations in the spectral data that are most important in

* Corresponding author. Tel.: +1 2547103311; fax: +1 2547104272.
E-mail address: kenneth_busch@baylor.edu (K.W. Busch).

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predicting enantiomeric composition. Once developed, the regression model can be used to predict the enantiomeric composition of future samples having the same concentration of chiral analyte and cyclodextrin as used to develop the original model.

Our initial studies of this technique involved the use of native cyclodextrins as chiral auxiliary agents. Native cyclodextrins (CDs) are homochiral cyclic oligosaccharides that are produced from starch [7–9]. The most readily available native CDs are the α-, β- and γ-CDs, which are composed of 6-, 7-, and 8-D-glucopyranose units, respectively [7–10]. The glucose units assume the classical C1 chair conformation and are linked by α-1,4 bonds, producing truncated cone or toroidal structures.

To improve the solubility and guest selectivity of native CDs, particularly with fairly large chiral organic molecules, substituted or modified CDs have been synthesized, where the primary and/or secondary hydroxyl groups on the rim of the cavities of native CDs have been replaced by different chemical moieties [11]. The most common replacement substituents for the hydroxyl groups are amino acids, amines, esters, methyl groups, hydroxypropyl groups, carboxymethyl groups, and carboxylic acids. In most cases, substituted cyclodextrins are produced by random substitution of the particular substituent chemical moiety. The aggregate substitution, that is the number of replacement groups per cyclodextrin, is known as the degree of substitution (DS). Theoretically, a cyclodextrin molecule (α-, β- or γ-) can have up to 3(*n*) substituents, where *n* is the number of glucopyranose units that comprise the cyclodextrin molecule. Therefore, the maximum degrees of substitution for α-, β-, and γ-cyclodextrins are 18, 21 and 24, respectively.

In the present study, the most common commercially available modified cyclodextrins (methyl-β-cyclodextrin, α-, β-, and γ-carboxymethyl cyclodextrins and α-, β-, and γ-hydroxypropyl cyclodextrins) were used in conjunction with four chiral compounds of pharmaceutical interest (ephedrine, norephedrine, norepinephrine-L-bitartrate, and tryptophan methyl ester – see Fig. 1) to produce regression models based on UV absorption spectral data. These regression models were subsequently used to predict the enantiomeric composition of unknown samples of the chiral analytes.

Ephedrine (α-(1-methylaminoethyl)-benzyl alcohol) has two chiral centers, and is popularly used to increase body metabolism and to stimulate the release of fat for fuel. Norephedrine (erythro-α-(1-aminoethyl)-benzyl alcohol) also has two chiral centers, and is a sympathetic nerve stimulating chemical (a so-called "fat burner"). Norepinephrine-L-bitartrate [(−)-α-(aminomethyl)-3,4-dihydroxybenzyl alcohol L-bitartrate] is used for controlling short-term low-blood pressure and cardiac output. Tryptophan methyl ester is a protected tryptophan amino acid commonly used as a food supplement to improve mental health by reducing depression, anxiety or aggression.

2. Experimental section

Enantiomerically pure (*1S*, *2R*)-(+)-norephedrine, (*1R*, *2S*)-(−)-norephedrine, (*1S*, *2R*)-(+)-ephedrine hydrochloride, (*1R*, *2S*)-(−)-ephedrine hydrochloride, (*S*)-(+)-norepinephrine-L-bitartrate, (*R*)-(−)-norepinephrine-L-bitartrate monohydrate, D-tryptophan methyl ester hydrochloride, and L-tryptophan methyl ester hydrochloride were obtained from Aldrich Chemical Company. Methyl-β-cyclodextrin (M-β-CD), carboxymethyl-α-cyclodextrin (CM-α-CD), carboxymethyl-β-cyclodextrin (CM-β-CD), carboxymethyl-γ-cyclodextrin (CM-γ-CD), hydroxypropyl-α-cyclodextrin (HP-α-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD), and hydroxypropyl-γ-cyclodextrin (HP-γ-

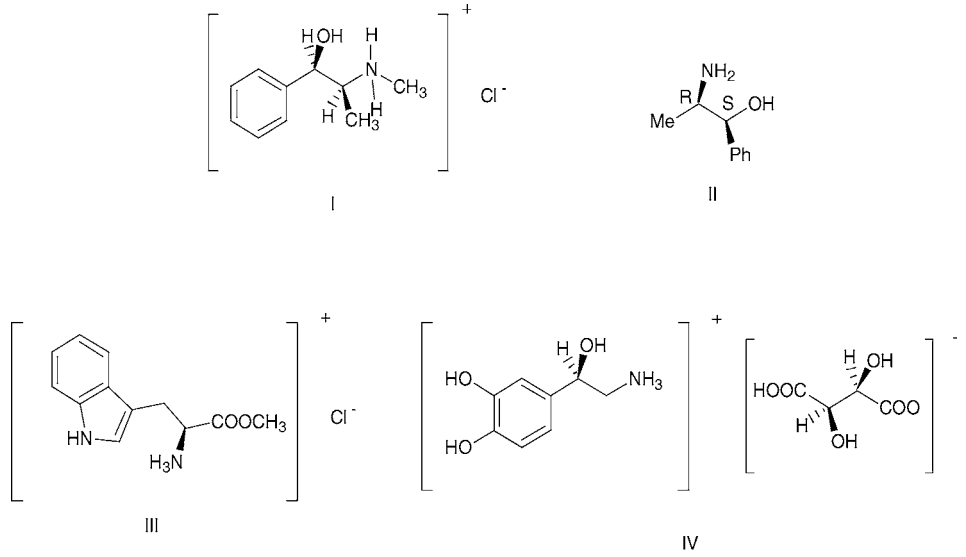

Fig. 1. (I) Ephedrine hydrochloride; (II) norephedrine; (III) tryptophan methyl ester hydrochloride; (IV) norepinephrine-L-bitartrate.

CD) were also obtained from Aldrich Chemical Company.

Aqueous samples of guest molecules with various known enantiomeric compositions were prepared gravimetrically by mixing various proportions of the pure enantiomers of the particular guest molecule as previously reported [1–3,12]. All the samples were made in deionized water.

The spectra of the solutions were recorded with a Hewlett-Packard photodiode array (Model 8455) UV–vis spectrometer using a 1.0 cm path length quartz cell over the wavelength range from 190 to 1100 nm.

The mean-centered spectral data were subjected to multivariate analysis using The Unscrambler 7.6 (CAMO, Inc., Corvallis, OR). Partial least squares regression (PLS-1) was performed on the spectral data using full cross-validation.

3. Results and discussion

3.1. Studies with norepinephrine-L-bitartrate

As an illustration of the technique, the results obtained with methyl-β-cyclodextrin and norepinephrine-L-bitartrate will be discussed in detail. Fig. 2 shows the absorption spectra obtained from 300 to 323 nm for eight sample solutions containing methyl-β-cyclodextrin (15.0 mM) and norepinephrine bitartrate (7.50 mM), where the mol fraction of the (*R*)-isomer of norepinephrine-L-bitartrate was varied from 0.100 to 0.900. It is clear from the figure that the spectra vary with enantiomeric composition.

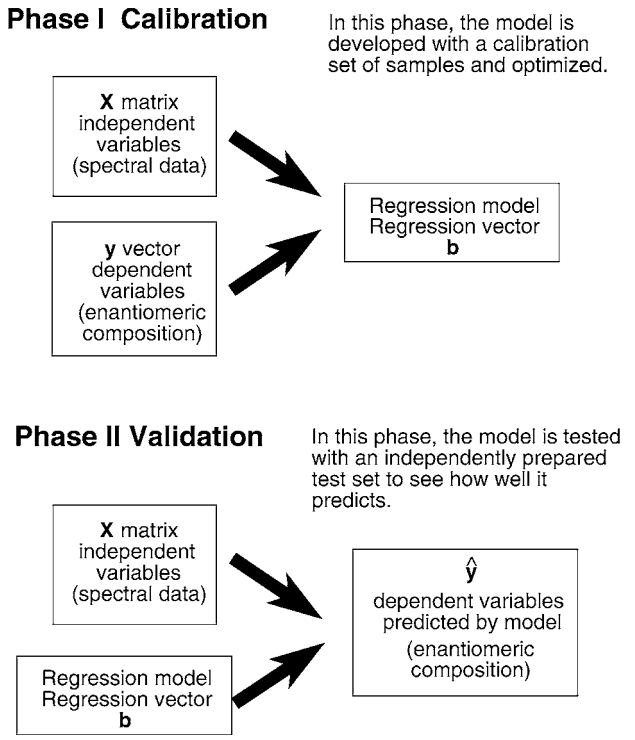

Fig. 3. Steps in multivariate regression modeling.

Multivariate regression modeling is a two-step procedure as illustrated schematically in Fig. 3. In the first step, or calibration phase, a mathematical model is developed with a training set of samples whose enantiomeric compositions

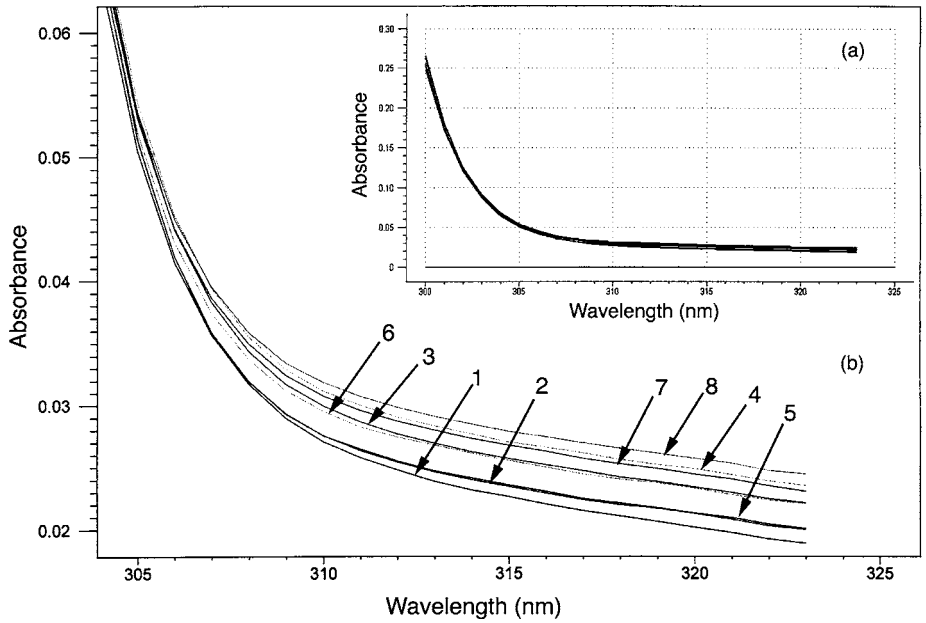

Fig. 2. Absorption spectra of eight solutions containing 15 mM M-β-CD and 7.5 mM norepinephrine-L-bitartrate. (a) Spectrum from 300 to 325 nm; (b) expanded spectrum showing solutions of varying enantiomeric composition: (1) 0.100 mol fraction (*R*)-norepinephrine-L-bitartrate; (2) 0.200 mol fraction (*R*)-norepinephrine-L-bitartrate; (3) 0.300 mol fraction (*R*)-norepinephrine-L-bitartrate; (4) 0.400 mol fraction (*R*)-norepinephrine-L-bitartrate; (5) 0.500 mol fraction (*R*)-norepinephrine-L-bitartrate; (6) 0.600 mol fraction (*R*)-norepinephrine-L-bitartrate; (7) 0.700 mol fraction (*R*)-norepinephrine-L-bitartrate; (8) 0.900 mol fraction (*R*)-norepinephrine-L-bitartrate.

are known independently. In this phase of the procedure, the spectral data shown in Fig. 2, which constitute a matrix of absorbance values versus wavelength (i.e., one spectrum per sample), are correlated by PLS-1 regression modeling with the independently known enantiomeric compositions of the samples in the training set. The result of this regression modeling is a mathematical model, which takes the form of a regression vector **b**, made up of n coefficients where n is the number of wavelengths in the spectral data. The multivariate nature of the model is apparent from the use of a spectral range in the calibration phase rather than an absorbance at a single wavelength.

In the second, or validation, phase of the procedure, a second set of samples is prepared independently, whose enantiomeric compositions are also known. While these validation samples have the same concentrations of chiral analyte and cyclodextrin host as were used in the initial training set of samples, they have different enantiomeric compositions from those used to develop the model. Once again, the spectra of the validation samples are taken over the same spectral range used in the calibration phase of the procedure.

As illustrated in Fig. 3, the new spectral data for the validation samples are then combined with the regression vector from the calibration phase to predict the enantiomeric compositions of the validation samples. In mathematical terms,

$$\hat{y}_i = b_0 + b_1 A_{1i} + b_2 A_{2i} + \cdots + b_n A_{ni} \quad (1)$$

where $\hat{y}_i$ is the enantiomeric composition of the ith sample predicted by the model, b_j are the coefficients of the regression vector determined in the calibration phase of the regression modeling, A_{ji} are the absorbances at the j wavelengths for the ith sample (i.e., the absorption spectrum of the ith sample), and b_0 is given by

$$b_0 = y_{\text{av}} - \mathbf{x}_{\text{av}}^{\text{T}}\mathbf{b} \quad (2)$$

where y_{av} is the average of all the y values in the calibration set, **b** is the regression vector, which consists of the b_j regression coefficients, and $\mathbf{x}_{\text{av}}^{\text{T}}$ is a vector with elements that are the average absorbance values over all the wavelengths in the spectrum for each spectrum in the calibration set.

If the predictions made by the model compare favorably with the known values for the validation set of samples, the mathematical model is considered to be useful to predict the enantiomeric composition of future unknown samples solely from the spectral data of new samples. Once again, the unknown samples must have the same concentrations of chiral analyte and cyclodextrin host as were used in the initial training set of samples.

Using the spectral data in Fig. 2 and the known enantiomeric compositions of the eight calibration samples that were prepared in the laboratory by mixing known amounts of the enantiomers of norepinephrine-L-bitartrate, a PLS-1 regression model with five PLS-components was prepared using commercial multivariate regression software. This model was then used predict the enantiomeric composition of a set of eight independently prepared validation samples.

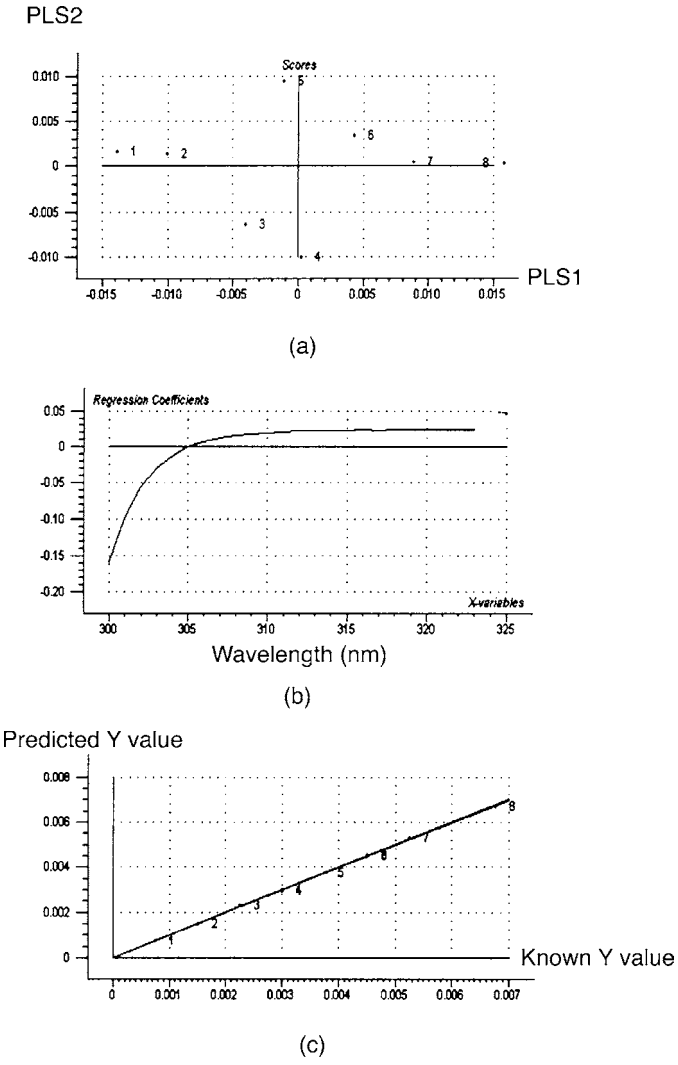

Fig. 4. Summary of regression results obtained with solutions containing (*R*)-norepinephrine-L-bitartrate and M-β-CD: (a) scores plot; (b) regression coefficients as a function of wavelength; (c) plot of predicted *Y*-values vs. known *Y*-values.

Fig. 4 shows a summary of the regression results for norepinephrine-L-bitartrate when M-β-CD was used as the host molecule. Fig. 4(a) shows a scores plot of the first PLS-component versus the second PLS-component. The numbers in the scores plot are the sample numbers of the samples used in the calibration set. Since the mol fraction of the (*R*)-isomer of norepinephrine-L-bitartrate increases with increasing sample number, it is clear from Fig. 4(a) that the first PLS-component of the model is highly correlated with enantiomeric composition because the sample numbers generally increase from left to right in the plot (along the first PLS component).

Fig. 4(b) shows a plot of the regression coefficients obtained for the model as a function of wavelength. The regression coefficient plot resembles a C2 rotation about the

Table 1
Relative errors obtained with a regression model for (*R*)-norepinephredine-L-bitartrate with methyl-β-cyclodextrin as the host molecule

Actual mol fraction	Predicted mol fraction[a]	% Relative error
0.328	0.312	−4.9
0.452	0.443	−2
0.548	0.572	+4.4
0.620	0.629	+1
0.716	0.679	−5.2
0.768	0.763	−0.7
0.844	0.852	+0.9
0.892	0.916	+2.7
RMS%RE		3.2

[a] Five PLS-1 components were used in the model.

wavelength axis of the spectral data shown in Fig. 2. In this model, wavelengths less than 305 nm have negative regression coefficients while wavelengths greater than 305 nm have positive regression coefficients.

Fig. 4(c) shows a plot of the enantiomeric composition of the samples (in terms of concentration rather than mol fraction) predicted by the model against the known values for the laboratory prepared samples. It should be stressed that this plot is not a calibration curve. The correlation coefficient, the slope, and the offset for this plot are 0.99999, 0.99976, and 8.2×10^{-7}, respectively. A perfect model would have a correlation coefficient of 1, a slope of 1, and an offset of zero.

The results of the validation study with (*R*)-norepinephrine-L-bitartrate and methyl-β-cyclodextrin are shown in Table 1. The figure of merit used to evaluate the performance of the model is the root-mean-square value of the percent relative errors for each of the samples in the set, given by

$$\text{RMS \% RE} = \sqrt{\frac{\sum (\%\,\text{RE}_i)^2}{n}} \quad (3)$$

where RMS%RE is the root-mean-square value of the percent relative error, $\%\text{RE}_i$ is the percent relative error for the *i*th sample in the validation set, and *n* is the number of samples in the validation set. Table 1 shows that for this model the root-mean-square value of the percent relative errors for the eight samples was 3.2%. This *rms* relative error is similar to that obtained in previous studies with native cyclodextrins [1–3].

To determine the effect of the host molecule on the results obtained with (*R*)-norepinephrine-L-bitartrate, a series of studies similar to the one described in detail with methyl-β-cyclodextrin was performed. In these studies, the cyclodextrin concentration was maintained at 15 mM and the concentration of (*R*)-norepinephrine-L-bitartrate was maintained at 7.5 mM. Table 2 summarizes the figures of merit for the regression models made with (*R*)-norepinephrine-L-bitartrate and the various cyclodextrins used in this study. Tables 3 and 4 show the validation results obtained with the regression models made when hydroxypropyl- and carboxymethyl-cyclodextrins were used as host molecules. In each case, 5 PLS components were used in the regression model. It is clear from Tables 1, 3 and 4 that the best regression model in terms of predicting the enantiomeric composition of samples of (*R*)-norepinephrine-L-bitartrate was obtained when methyl-β-cyclodextrin was used as the host molecule.

3.2. Studies with other guest–host combinations

To evaluate the technique with other guest–host combinations, the procedure described in detail for norepinephrine-L-bitartrate was repeated for the other guest molecules (ephedrine, norephedrine, and tryptophan methyl ester) with each of the different modified cyclodextrins used in this study. Once again a calibration set of samples with known enantiomeric compositions was prepared in the laboratory and the UV absorption spectra of the resulting solutions were taken.

In each case, the concentration of the host molecule was maintained at 15 mM and the concentration of the guest molecule was fixed at 7.5 mM. After acquisition of the spectral data for a particular system, PLS-1 regression techniques were employed to develop a regression model to predict the enantiomeric composition from the spectral data of future unknown samples. Table 5 gives the figures of merit and the wavelength ranges employed for each model. All models used 5 PLS-components. Following the calibration phase for each model, an independently prepared set of validation samples was prepared to test the predictive ability of each model. The predictive ability of each model was then summarized by calculating the RMS%RE for each validation set as discussed with norepinephrine-L-bitartrate.

Fig. 5 summarizes the results obtained in this study for the four chiral guest molecules and the seven different cyclodextrins. Fig. 5 shows that the predictive ability of the various models was highly dependent on the host molecule used in conjunction with the particular guest molecule as might be expected. This is not surprising since any diastereomeric effects on the absorption spectra should depend on the degree of formation and nature of the diastereomeric complexes that form. Better predictions are expected when effective interac-

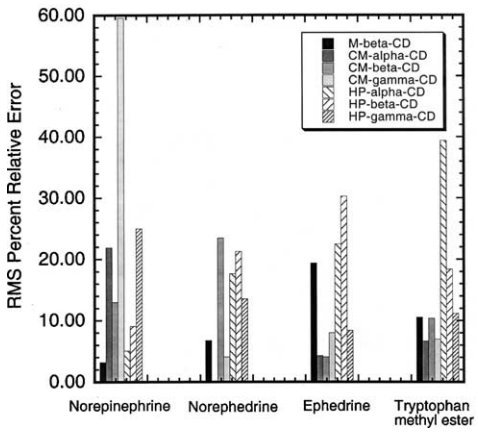

Fig. 5. RMS%RE obtained from regression models for norepinephrine-L-bitartrate, norephedrine, ephedrine, and tryptophan methyl ester with various chiral host molecules.

Table 2
Summary of figures of merit for regression models made with norepinephrine-L-bitartrate and various host molecules

Host molecule	Correlation coefficient	Slope	Offset	Number of PCs used	Wavelength range (nm)
M-β-CD[a]	0.99999	0.99976	8.21×10^{-7}	5	301–324
HP-α-CD[b]	0.99879	0.99759	9.96×10^{-6}	5	299–374
HP-β-CD[c]	0.98915	0.97841	8.10×10^{-5}	5	299–412
HP-γ-CD[d]	0.98097	0.96230	1.41×10^{-4}	5	294–311
CM-α-CD[e]	0.99991	0.99982	6.58×10^{-7}	5	297–410
CM-β-CD[f]	0.99960	0.99921	2.97×10^{-6}	5	294–311
CM-γ-CD[g]	0.99359	0.98722	4.79×10^{-5}	5	296–464

[a] 15 mM M-β-CD and 7.5 mM norepinephrine-L-bitartrate.
[b] 15 mM HP-α-CD and 7.5 mM norepinephrine-L-bitartrate.
[c] 15 mM HP-β-CD and 7.5 mM norephedrine-L-bitartrate.
[d] 15 mM HP-γ-CD and 7.5 mM norepinephrine-L-bitartrate.
[e] 15 mM CM-α-CD and 7.5 mM norepinephrine-L-bitartrate.
[f] 15 mM CM-β-CD and 7.5 mM norepinephrine-L-bitartrate.
[g] 15 mM CM-γ-CD and 7.5 mM norepinephrine-L-bitartrate.

Table 3
Relative errors obtained for (*R*)-norepinephrine-L-bitartrate with hydroxypropyl cyclodextrins (HPCDs)

Actual mol fraction	HP-α-CD		HP-β-CD		HP-γ-CD	
	Predicted mol fraction	% Relative error	Predicted mol fraction	% Relative error	Predicted mol fraction	% Relative error
0.328	0.340	3.7	0.346	5.5	0.234	−29
0.452	0.465	2.9	0.473	4.6	0.668	47.8
0.548	0.567	3.5	0.524	−4.4	0.357	−34.9
0.620	0.596	−3.9	0.610	−1.6	0.472	−23.9
0.716	0.757	5.7	0.619	−14	0.711	−0.7
0.768	0.804	4.7	0.710	−7.6	0.836	8.9
0.844	0.918	8.8	0.721	−14.6	0.842	−0.2
0.892	0.939	5.3	0.794	−11	0.918	2.9
RMS%RE		5.1		9.0		25.0

Table 4
Relative errors obtained for (*R*)-norepinephrine-L-bitartrate with carboxyl methyl-cyclodextrins (CMCDs)

Actual mol fraction	CM-α-CD		CM-β-CD		CM-γ-CD	
	Predicted mol fraction	% Relative error	Predicted mol fraction	% Relative error	Predicted mol fraction	% Relative error
0.328	0.313	4.6	0.268	−18	0.345	5.2
0.452	0.384	−15	0.522	15	0.291	−35.6
0.548	0.383	−30.1	0.451	−18	0.494	−9.9
0.620	0.465	−25.0	0.688	11	0.467	−24.7
0.716	0.559	−21.9	0.692	−3.4	0.604	−15.6
0.768	0.595	−22.5	0.827	7.7	0.703	−8.5
0.844	0.676	−19.9	0.728	−13.7	1.030	22.0
0.892	0.662	−25.8	0.799	−10	2.310	159.0
RMS%RE		21.9		13.0		59.6

tions lead to the formation of stable diasteromeric inclusion complexes between the guest and host molecules.

A major driving force, frequently cited [7,13] in inclusion complex formation, is hydrogen bonding between the OH groups on the rim of the cyclodextrin and the guest molecule. Van der Waals forces, π–π electron interaction, dipole–dipole interaction and electrostatic interactions have also been reported to play a prominent role in complex formation between guest and host molecules. Finally, size is an important consideration in inclusion complex formation. Clearly, the size of the guest molecules and cavity size of the cyclodextrin play significant roles in the interaction between the guest and host molecules. Stable inclusion complex are formed when the guest molecules are of appropriate dimension so that they can fit properly into the cavity of the host molecules.

From Fig. 5, we see that for norepinephrine-L-bitartrate the best model in terms of predictive ability (i.e., smallest RMS%RE) was obtained when methyl-β-CD was used as the host molecule (3.2% RMS%RE). The next best model employed hydroxypropyl-α-CD (5.1% RMS%RE). In the case of norephedrine, the best model was obtained with carboxymethyl-γ-CD (4.1% RMS%RE). For ephedrine, two hosts gave similar predictive abilities (carboxymethyl-α-CD,

Table 5
Summary of figures of merit for regression models made for ephedrine, norephedrine, and tryptophan methyl ester

System	Correlation coefficient	Slope	Offset	Wavelength range (nm)
M-β-CD/ED[a]	0.99795	0.99590	1.52×10^{-5}	242–271
HP-α-CD/ED[b]	0.99857	0.99714	1.07×10^{-5}	245–277
HP-β-CD/ED[c]	0.99198	0.98403	5.99×10^{-5}	243–249
HP-γ-CD/ED[d]	0.96671	0.93453	2.45×10^{-4}	251–285
CM-α-CD/ED[e]	0.99913	0.99826	6.51×10^{-6}	244–260
CM-β-CD/ED[f]	0.99718	0.99437	2.11×10^{-5}	248–263
CM-γ-CD/ED[g]	0.99818	0.99637	1.36×10^{-5}	245–272
M-β-CD/TME[h]	0.99804	0.99608	1.47×10^{-5}	312–332
HP-α-CD/TME[i]	0.99515	0.99032	3.63×10^{-5}	311–324
HP-β-CD/TME[j]	0.99821	0.98643	1.34×10^{-5}	311–339
HP-γ-CD/TME[k]	0.99619	0.99239	2.85×10^{-5}	311–330
CM-α-CD/TME[l]	0.99619	0.99240	2.85×10^{-5}	315–329
CM-β-CD/TME[m]	0.99854	0.99709	1.09×10^{-5}	315–325
CM-γ-CD/TME[n]	0.99944	0.99887	4.23×10^{-5}	314–324
M-β-CD/NEP[o]	0.99880	0.99760	9.01×10^{-6}	247–261
HP-α-CD/NEP[p]	0.99766	0.99533	1.75×10^{-5}	258–284
HP-β-CD/NEP[q]	0.99820	0.99640	1.34×10^{-5}	249–263
HP-γ-CD/NEP[r]	0.98945	0.97900	7.85×10^{-5}	255–263
CM-β-CD/NEP[s]	0.99954	0.99908	3.42×10^{-6}	246–255
CM-γ-CD/NEP[t]	0.99905	0.99812	7.05×10^{-6}	269–310

[a] 15 mM M-β-CD and 7.5 mM ephedrine.
[b] 15 mM HP-α-CD and 7.5 mM ephedrine.
[c] 15 mM HP-β-CD and 7.5 mM ephedrine.
[d] 15 mM HP-γ-CD and 7.5 mM ephedrine.
[e] 15 mM CM-α-CD and 7.5 mM ephedrine.
[f] 15 mM CM- β-CD and 7.5 mM ephedrine.
[g] 15 mM CM-γ-CD and 7.5 mM ephedrine.
[h] 15 mM M-β-CD and 7.5 mM tryptophan methyl ester.
[i] 15 mM HP-α-CD and 7.5 mM tryptophan methyl ester.
[j] 15 mM HP-β-CD and 7.5 mM tryptophan methyl ester.
[k] 15 mM HP-γ-CD and 7.5 mM tryptophan methyl ester.
[l] 15 mM CM-α-CD and 7.5 mM tryptophan methyl ester.
[m] 15 mM CM-β-CD and 7.5 mM tryptophan methyl ester.
[n] 15 mM CM-γ-CD and 7.5 mM tryptophan methyl ester.
[o] 15 mM M-β-CD and 7.5 mM norephedrine.
[p] 15 mM HP-α-CD and 7.5 mM norephedrine.
[q] 15 mM HP-β-CD and 7.5 mM norephedrine.
[r] 15 mM HP-γ-CD and 7.5 mM norephedrine.
[s] 15 mM CM-β-CD and 7.5 mM norephedrine.
[t] 15 mM CM-γ-CD and 7.5 mM norephedrine.

4.3% RMS%RE; carboxymethyl-β-CD, 4.1% RMS%RE). Finally, for tryptophan methyl ester, two hosts again gave similar predictive abilities (carboxymethyl-α-CD, 6.7% RMS%RE; carboxymethyl-γ-CD, 7.0% RMS%RE).

With the exception of norepinephrine-L-bitartrate (where hydroxypropyl-α-CD gave a model with an RMS%RE of 5.1%), the hydroxypropyl series of cyclodextrins did not produce models with satisfactory predictive abilities for norephedrine, ephedrine, and tryptophan methyl ester. Again, with the exception of norepinephrine-L-bitartrate, the carboxymethyl series of cyclodextrins seemed to give good results for norephedrine, ephedrine, and tryptophan methyl ester.

If the results of this study with modified cyclodextrins are compared with previous results obtained with native cyclodextrins [2] for norephedrine, the native cyclodextrins are found to give somewhat better models (3.4% RMS%RE with β-CD and 3.3% RMS%RE with γ-CD). None of the models prepared in this study with modified CDs gave results that were close to those obtained for ibuprofen when native CDs were used (0.8% RMS%RE with β-CD and 0.7% RMS%RE with γ-CD).

In the case of modified cyclodextrins, the degree of substitution of the hydroxyl groups of the cyclodextrin can have a pronounced effect on the binding capability of modified cyclodextrins with respect to a particular guest molecule. At low degrees of substitution, binding is expected to be very similar to that of the native or unmodified cyclodextrin counterpart. At high degrees of substitution, however, binding ability depends upon the particular guest. On the one hand, substituent groups on the rim of the cavity in modified cyclodextrins may actually reduce inclusion-complex formation by steric hindrance, preventing the guest molecule from actually entering the cavity.

On the other hand, substituent groups in modified cyclodextrins may increase the surface area available for guest

molecules to bind. Such complexation, however, may be external to the cavity, and may not result in strong diastereomeric guest–host interactions.

For strong diastereomeric guest–host interactions to occur, it is not unreasonable to suppose that the chiral center of the guest molecule should enter the chiral environment of the cyclodextrin cavity. If substituents on the rim of the cavity prevent the guest molecule from entering the cavity, the use of modified cyclodextrins for chiral discrimination may not be ideal.

The results of this initial study with modified cyclodextrins has shown that the prediction ability of the regression models is, in many cases, worse than that obtained with native cyclodextrins. Clearly more work is necessary to determine whether this preliminary indication is actually true.

4. Conclusions

Small spectral variations in the UV spectra of solutions containing diastereomeric guest–host complexes are observed when the enantiomeric composition of the guest molecule is changed while keeping the concentration of the guest and host fixed. If these spectral variations are correlated by means of PLS-1 regression modeling with the known enantiomeric compositions of a set of calibration samples, regression models can be developed that can predict the enantiomeric composition of future unknown samples directly from the UV spectra of the samples. When modified cyclodextrins are used as host molecules, the predictive ability of the regression models is highly dependent on the particular guest and host involved. Preliminary results with modified cyclodextrins do not suggest that they give better models compared with native cyclodextrins in many cases.

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