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Determination of enantiomeric compositions of pharmaceutical products by near-infrared spectrometry

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Abstract

A new method for the determination of enantiomeric compositions of a variety of drugs including propranolol, atenolol, and ibuprofen has been developed. The method is based on the use of the near-infrared technique to measure diastereomeric interactions between an added carbohydrate compound and both enantiomeric forms of a drug followed by evaluation of the data by partial least square analysis. The fact that the method works well with all three macrocyclic carbohydrates with different cavity sizes (i.e., α-, β-, and γ-cyclodextrin) and with sucrose, which is a linear carbohydrate, clearly demonstrates that it is not necessary to have inclusion complex formation to produce effective diastereomeric interactions. Rather a simple adsorption of the drug onto a carbohydrate is sufficient. Since inclusion complex formation is not a requisite, this method is not limited to the three drugs evaluated in this study but is rather universal as it can, in principle, be used for the sensitive and accurate determination of enantiomeric compositions of many different types of drugs with only about 1.5 mg/mL concentration and enantiomeric excess as low as 0.80%, in water or in a mixture of water with organic solvent. Furthermore, it does not rely on the use of rather expensive carbohydrates such as cyclodextrins but is equally as effective even with a simple and inexpensive carbohydrate such as sucrose.

Keywords: Near-infrared; Chiral analysis; Partial least square; Cyclodextrin; Sucrose; Ibuprofen; Beta blocker

Differences between the physiological properties and the therapeutic effects of the enantiomeric forms of many compounds have been recognized for some time [1–4]. Very often, an unwanted enantiomer can reverse or otherwise limit the effect of the desired enantiomer [1–4]. For example, propranolol, one of the most widely used beta blockers (i.e., a compound that inhibits the action of the adrenergic agents and reduces the force of the heart muscle contraction and the heart rate), exists in two enantiomeric forms. The enantiomer that is most active in correcting ventricular arrhythmias is much less active as a beta blocker. However, despite this knowledge, only 61 of the 528 chiral synthetic drugs are marketed as single enantiomers while the other 467 are sold as racemates. Recognizing the importance of chiral effects, the FAA in 1992 issued a mandate requiring pharmaceutical companies to evaluate the effects of individual enantiomers and to verify the enantiomeric purity of chiral drugs that are produced [1–4]. Furthermore, even if a drug is to be marketed as a single enantiomer, the pharmaceutical properties and toxicity must be established for both enantiomers [1–4]. It is thus hardly surprising that the pharmaceutical industry needs effective methods to determine the enantiomeric purity of chiral drugs.

Methods currently used to determine enantiomeric purity are based on either separation or spectroscopic techniques [2–11]. Separation-based methods include HPLC, GC, and CE [2–10]. Circular dichroism, NMR, MS, and FTIR are some of the spectroscopic methods that are widely used [11–13]. While these methods have proven to be effective, they all have some drawbacks including being time consuming (separation-based methods), requiring the addition of reagent(s) or isotopic label(s) (NMR and FTIR, respectively), being destructive (MS), and having relatively low sensitivity (circular dichroism) [2–13]. More importantly, none of them are truly universal; namely, they cannot be used

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for all types of chiral compounds [2–13]. A truly spectroscopic method that is capable of quickly and sensitively determining enantiomeric purity of all types of compounds is, therefore, needed. The near-infrared (NIR)[1] technique can offer a solution for this problem.

NIR spectrometry has been used extensively in recent years for chemical analysis and characterization [14]. The popularity stems from the advantages of the technique including its nonintrusive, nondestructive, and wide applicability. Specifically, the NIR region covers the overtone and combination transitions of the C-H, O-H, N-H, and C = O groups, and since all drugs possess at least one or more of these groups, the technique can be used for analysis of all pharmaceutical compounds [14]. Additionally, because NIR light can penetrate a variety of materials, the NIR technique has real-time and on-line capabilities. The chiral analysis method based on the NIR technique is therefore desirable. Unfortunately, despite its potentials, to date, a chiral analysis method based on the NIR technique has not been developed.

To facilitate chiral analysis of a pharmaceutical compound, the addition of a chiral reagent to promote diastereomeric interactions between the reagent and two enantiomeric forms of the drug is generally required. A variety of chiral reagents has been developed and successfully used for chiral analysis [2–13]. However, all reported methods are based on analyzing changes in the either UV or visible spectra of the drug upon the addition of chiral reagents [2–13]. As a consequence, they are not only based on the use of rather expensive chiral reagents but also are limited to reagents and/or pharmaceutical compounds that absorb UV and/or visible spectra. Such a limitation can be ameliorated by replacing the UV–visible spectra methods with the NIR technique. This is because, in principle, the NIR technique can be used to detect any changes induced by diastereomeric interactions between two enantiomeric forms of the drug and an added chiral reagent, regardless of the types of drugs and reagents. In fact, we have recently demonstrated that adding chiral macrocyclic compounds such as cyclodextrins (CDs) to solution of various substances led to changes in NIR absorption spectra of the latter and such changes can be used to determine binding constants between the CDs and the substances [15,16]. More importantly, the NIR results indicate that this method is so sensitive that it is not limited to inclusion complexes formed between CDs and substances but is rather applicable to all types of complexes formed by interactions other than inclusion complex formation including electrostatic interactions [15,16].

[1] *Abbreviations used*: NIR, near-infrared; CD, cyclodextrin; PLS, partial least square; Pro, propranolol; Ate, atenolol; Ibu, ibuproten; ee, enantiomeric excess.

The information presented is indeed provocative and clearly demonstrates that diastereomeric interactions between both enantiomeric forms of a drug with either a macrocyclic compound (e.g., cyclodextrin) or a linear compound (e.g., sucrose) should produce changes in NIR spectra. Chiral composition of the drug can then be determined by analyzing the changes in the NIR spectra. Such consideration prompted us to initiate this study which aims to develop the first universal chiral analysis for pharmaceutical compounds based on synergistic use of the NIR technique to measure diastereomeric interactions of both enantiomeric forms of a drug with added cyclic or linear sugar such as CD or sucrose followed by analysis of the changes in the NIR spectra using a multivariate method for enantiomeric purity. Preliminary results on chiral analysis of beta blockers such as atenolol propranolol and on ibuprofen, a nonsteroidal antiinflammatory drug, are reported.

Experimental

(R)-(+)- and (S)-(−)-4-(2-hydroxy-3-[(1-methylethyl) aminopropoxy] benzenacetamide) (R- and S-atenolol) and (R)-(+)- and (S)-(−)-1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol (R- and S-propranolol) were purchased from Aldrich Chemical (Milwaukee, WI). (R)-(−)- and (S)-(+)-(4-isobutylphenyl) propionic acid (R- and S-ibuprofen) were purchased from BIOMOL Research Laboratories Inc. (Plymouth Meeting, PA). The structures of these three drugs are shown in Fig. 1. The α-, β-, and γ-cyclodextrins were a gift of Cargill Corp. (Hammond, IN).

NIR spectra were taken on the home-built NIR spectrometer based on an acoustooptic tunable filter. This NIR spectrometer has been described in detail in previous papers [15–18]. Normally, each spectrum of a sample (in 5-mm-pathlength cell) was an average of 50 spectra taken at 1-nm interval from 1450 to 2450 nm. Multivariate analyses of data were performed using Unscrambler version 8.0 (Camo ASA), similar to the procedures used in our previous publications [18].

Results and discussion

The NIR spectrum of a solution of 160.0 mM S-propranolol (S-Pro) in water, taken using a 0.1-mm-pathlength cell, is shown as a solid line in Fig. 2. The spectrum shown as a dashed line is that of water taken in the same cell. As illustrated, absorbance of Pro in this spectral region is not only small but also very similar to that of water. For clarity, the spectrum of S-Pro was remeasured in two regions where Pro is known to absorb: 1600–1800 and 2100–2400 nm using cells with relatively longer pathlength (5-mm pathlength for the

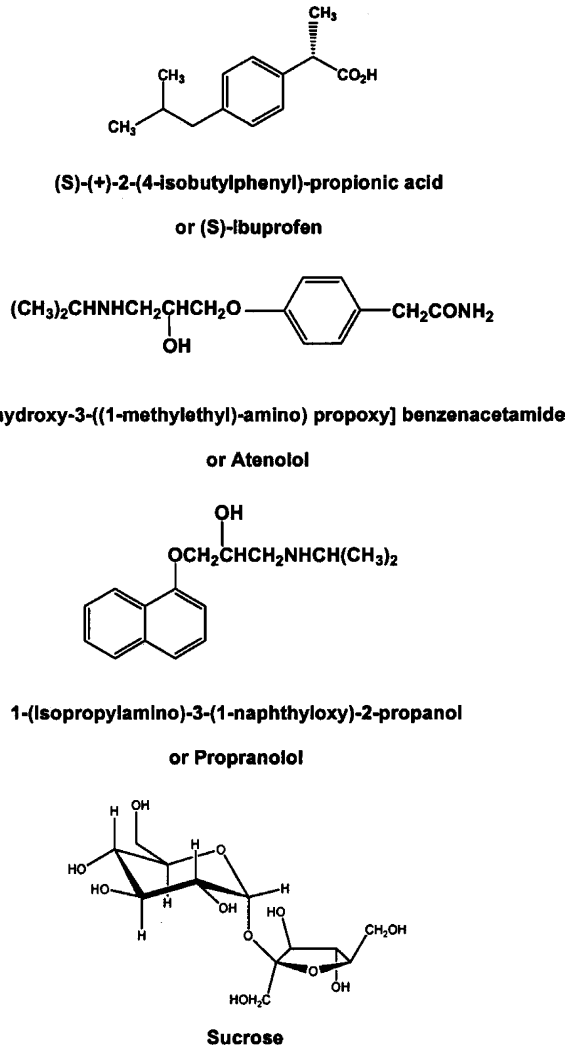

Fig. 1. Structure of ibuprofen, atenolol, propranolol, and sucrose.

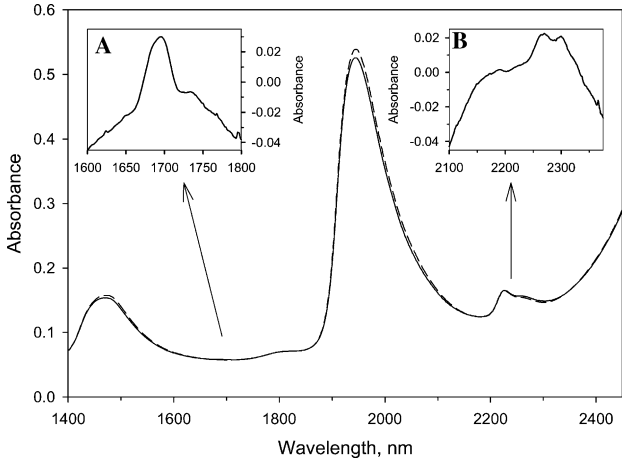

Fig. 2. Absorption spectra of 160.0 mM S-propranolol in water taken in a 0.1-mm-pathlength cell (solid line) and that of water taken in the same cell (dashed line). (Insets A and B) Spectra of the same 150.0 mM propranolol solution taken in a 5- and a 1-mm-pathlength cell, respectively, with background absorption of water subtracted.

1600- to 1800-nm region and 1-mm pathlength for the 2100- to 2400-nm region). The spectra obtained, with background absorption of water subtracted, are shown as insets A and B in Fig. 2. It is relatively difficult to completely and unambiguously assign these bands. Based on our previous studies, the 1696- and 1722-nm bands can be tentatively assigned to the overtones and combinations of the C-H groups [15–18]. Overtones and combination transitions of C-H groups may also be responsible for several broad bands in the 2100- to 2400-nm region [15–18]. Since absorption of C-H groups in the 2100- to 2400-nm region is so high that it necessitates the use of a cell with a very short pathlength, subsequent experiments were performed in the 1600- to 1800-nm region where a cell with 5-mm pathlength can be used.

It was found that adding α-CD to a solution of Pro led to changes in the position and bandwidth of the propranolol absorption. Since α-CD is chiral, it is expected that its interactions with R-Pro will be different from those with S-Pro. Accordingly, a set of 10 solutions of Pro in 80 mM α-CD was prepared. The enantiomeric compositions of these solutions are listed in Table 1. As listed, each of these solutions has the same total Pro concentration (40 mM) but with different enantiomeric composition. Fig. 3A shows the NIR spectra of these solutions (with background absorption of water and α-CD subtracted). It is interesting to observe that changing the enantiomeric composition of Pro led to changes in its absorption spectrum. The magnitude of these changes is, as expected, relatively small. However, they are certainly larger than experimental errors. This conclusion was made by comparing the spectra shown in Fig. 3A with those in Fig. 3B which show absorption of a second set of Pro solutions that are identical to the first set (i.e., same total Pro concentration of 40 mM and different enantiomeric compositions) except that they are in pure water without any α-CD. Since α-CD was absent in the second set, it is not expected that solutions in this set should not have any differences in their absorption spectra. The observed small differences among

Table 1
Compositions of solutions used for calibration

Sample	Mole fraction of R drug	Mole fraction of S drug
1	0.30	0.70
2	0.35	0.65
3	0.40	0.60
4	0.45	0.55
5	0.50	0.50
6	0.55	0.45
7	0.60	0.40
8	0.65	0.35
9	0.70	0.30
10	0.75	0.25
11	0.80	0.20
12	0.90	0.10

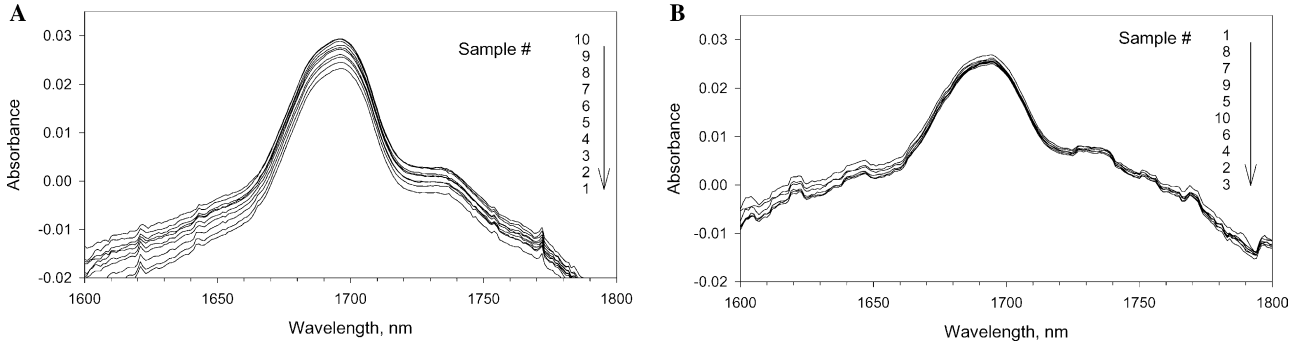

Fig. 3. (A) Spectra of a set of 10 solutions of propranolol in 160 mM of α-CD. Each solution has the same total propranolol concentration (80 mM) but different enantiomeric concentration. Compositions of the solutions are listed in Table 1. Background absorption of α-CD and water was subtracted from the spectra. (B) Spectra of a set of 10 solutions of propranolol in water with the same total propranolol concentration (80.0 mM) but with different enantiomeric compositions. Compositions of the solutions are the same as those corresponding in Fig. 2A. Background absorption by water was subtracted from the spectra.

these spectra are, therefore, due to the experimental errors including drifts and instability of the NIR spectrometer. However, because the differences among spectra of solutions with α-CD (Fig. 3A) are much larger than those without α-CD (Fig. 3B), they are due to the diastereomeric interactions between the α-CD and both enantiomeric forms of propranolol.

It is evident from Fig. 3A that spectra of Pro are dependent on the enantiomeric composition of the solution. There may be a correlation between the spectra and the enantiomeric composition of Pro in solutions. However, it was not possible to use univariate calibration for analysis because not only are the differences small but also there are other effects including instability and drift of the spectrometer. Accordingly, a multivariate method of analysis (i.e., partial least square method; PLS) was used. Calibrations were performed on NIR spectra of the 12 samples of Pro in α-CD (Fig. 3A) using the partial least square analysis and the cross validation method with the Unscrambler Chemometric software package (version 8.0). Results from the PLS cross validation show that calibrations for 12 solutions require a relatively small number of factors for optimal performance (five for R-Pro and five for S-Pro). The root means standard error of prediction (RMSEP) values are 0.124 and 0.127 for R- and S-Pro while the standard error of prediction (SEP) values are 0.125 and 0.128 for R- and S-Pro, respectively.

To evaluate the effectiveness of this method, five samples of Pro (in 80 mM α-CD) with the same Pro concentration (40 mM) but different enantiomeric compositions were prepared, and the concentrations of R- and S-Pro in each sample were calculated using the calibration models. Results obtained are shown in Table 2 and Fig. 4A where the calculated concentrations of R- and S-Pro in five samples were plotted against actual concentrations. As illustrated, for all five samples, the calculated concentrations for both enantiomers agree very well with actual concentrations: the calculated concentrations are linearly related to actual concentrations with the slope of 1.00 and 0.01 intercept, within experimental errors ($y = (0.97 \pm 0.04)x + (0.02 \pm 0.02)$).

Table 2
Actual and predicted enantiomeric compositions of solutions of 40 mM propranolol in 80 mM α-, β-, or γ-CD

Sample	Actual mole fraction		Predicted mole fraction	
	S	R	S	R
α-CD				
1	0.53	0.47	0.56	0.43
2	0.58	0.42	0.54	0.40
3	0.62	0.38	0.65	0.42
4	0.68	0.32	0.71	0.34
5	0.78	0.22	0.80	0.23
6	0.83	0.17	0.79	0.18
β-CD				
1	0.53	0.47	0.50	0.45
2	0.58	0.42	0.57	0.43
3	0.62	0.38	0.65	0.36
4	0.68	0.32	0.66	0.35
5	0.73	0.27	0.74	0.28
γ-CD				
1	0.53	0.47	0.53	0.49
2	0.58	0.42	0.62	0.38
3	0.62	0.38	0.63	0.39
4	0.68	0.32	0.65	0.31
5	0.73	0.27	0.71	0.26

The effectiveness of this method stems from the diastereomeric interactions between the chiral macrocyclic α-CD and both enantiomeric forms of propranolol. However, results presented above do not provide adequate information to elucidate the mechanism for the interactions. One possible type of interaction is the inclusion complex formation. It is, however, also possible that Pro may externally adsorb onto α-CD, and if such external adsorption is strong, it may produce measurable differences between R- and S-Pro with α-CD. To evaluate whether inclusion complex formation is the

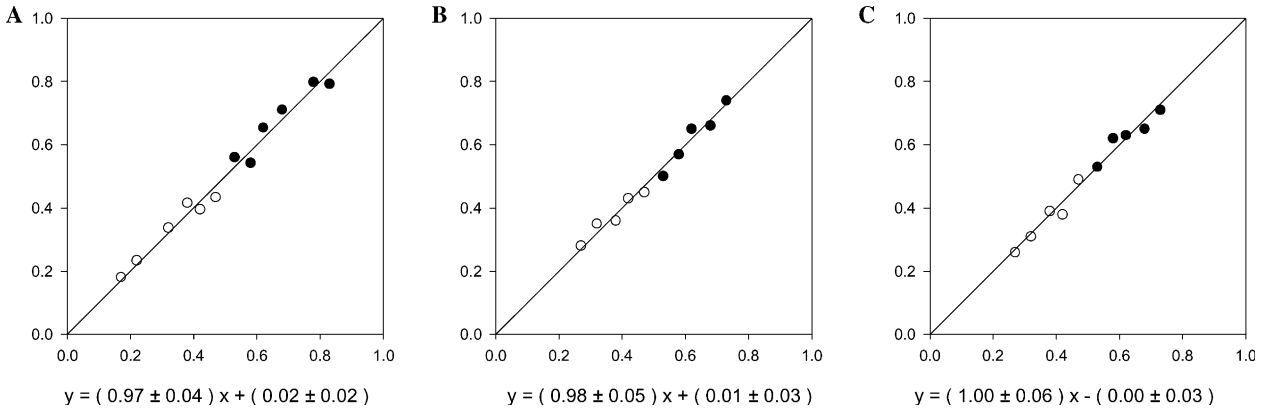

Fig. 4. Plots of predicted versus actual enantiomeric composition for solutions of 40 mM propranolol in 80 mM α-CD (A), β-CD (B), and γ-CD (C). See text for detailed information. Filled circles, S-propranolol; open circles, R-propranolol.

main interaction, similar experiments were performed in which α-CD was replaced with cyclodextrins that have relatively larger cavity, namely β- and γ-CD. Results obtained are shown in Table 2 and Figs. 4B and C where calculated concentrations of a set of five solutions of Pro in β-CD (4B) and in γ-CD (4 C) were plotted against actual concentrations. As illustrated, similar to α-CD, calculated concentrations of R- and S-Pro agree well with actual concentrations for both β-CD and γ-CD. The results seems to suggest that the diastereomeric interaction is not dependent on the type of cyclodextrin used, or more accurately, on the cavity size of the CD. Therefore, it is possible that inclusion complex formation may not be the only one and/or the dominant factor responsible for the interactions between CDs and both enantiomeric forms of Pro.

Additional information on the interaction mechanism can be obtained by replacing Pro with atenolol (Ate) which is also a beta blocker but is relatively smaller than propranolol as the atenolol has a phenyl group while propranolol has a naphthyl group (Fig. 1). Results obtained are listed in Table 3 and plotted in Fig. 5 as calculated concentrations of R- and S-Ate against actual concentrations with all three cyclodextrins: α-CD (Fig. 5A), β-CD (Fig. 5B), and γ-CD (Fig. 5C). As illustrated, enantiomeric compositions for this drug can also be accurately determined by this method, regardless of the size and type of functional group of the drugs and the type of cyclodextrin added. The fact that this method can provide accurate enantiomeric compositions for two drugs with different size and shape and with all three different size CDs again confirms that inclusion complex formation is probably not the only one and/or the most important factor in the diastereomeric interactions responsible for its effectiveness.

Interestingly, it was found that the method is not limited to water-soluble drugs but is also effective for drugs that have relatively low solubility in water. Ibuprofen (Ibu) which is a nonsteroidal, antiinflammatory drug is an example of this type of drug. It is not soluble in water but can be dissolved in a mixture of 30:70 ethanol:water. Similar to propranolol and atenolol, experiments were performed for Ibu and γ-CD in a 30:70 ethanol:water mixture (only γ-CD was used because it is the only cyclodextrin that can be dissolved in the 30:70 ethanol:water mixture at required concentration of 80 mM). Results obtained are shown in Table 4 and Fig. 6 where calculated concentrations of a set of five solutions of Ibu in γ-CD are plotted against actual concentrations. As illustrated, similar to Pro and Ate, calculated concentrations of R- and S-Ibu in all five solutions agree well with actual concentrations.

So far, the method was used to calculate concentrations of R and S enantiomers in a sample. Rather than individually calculating and/or presenting concentrations of each enantiomer, it is often preferred to report

Table 3
Actual and predicted enantiomeric compositions of solutions of 40 mM atenolol in 80 mM α-, β-, or γ-CD

Sample	Actual mole fraction		Predicted mole fraction	
	S	R	S	R
α-CD				
1	0.53	0.47	0.54	0.44
2	0.58	0.42	0.55	0.45
3	0.62	0.38	0.64	0.36
4	0.68	0.32	0.70	0.34
5	0.78	0.22	0.79	0.23
6	0.83	0.17	0.80	0.18
β-CD				
1	0.53	0.47	0.56	0.44
2	0.58	0.42	0.56	0.44
3	0.62	0.38	0.59	0.41
4	0.68	0.32	0.70	0.30
5	0.73	0.27	0.74	0.26
γ-CD				
1	0.53	0.47	0.58	0.44
2	0.58	0.42	0.61	0.42
3	0.62	0.38	0.60	0.40
4	0.68	0.32	0.68	0.29
5	0.73	0.27	0.70	0.27

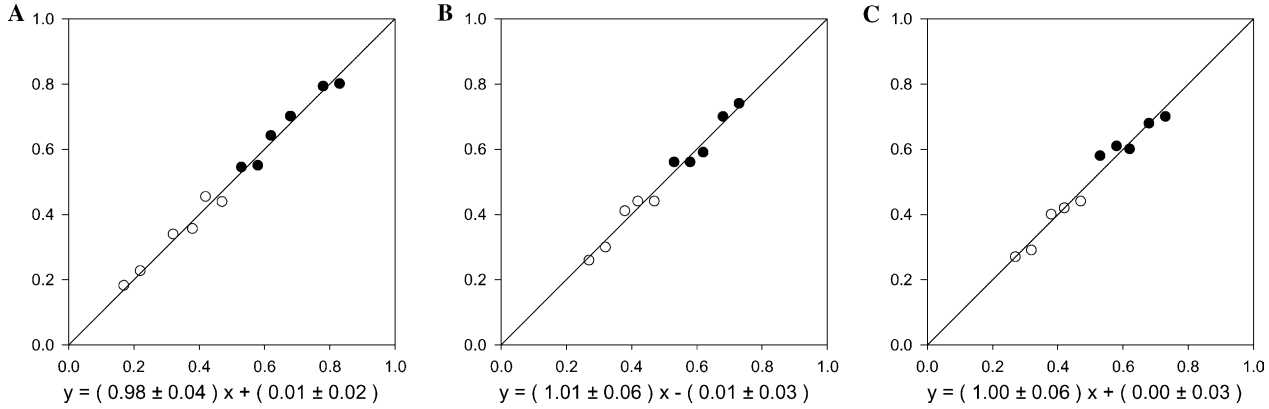

Fig. 5. Plots of predicted versus actual enantiomeric composition for solutions of 40 mM atenolol in 80 mM α-CD (A), β-CD (B) and γ-CD (C). See text for detailed information. Filled circles, S-atenolol; open circles, R-atenolol.

Table 4
Actual and predicted enantiomeric compositions of solutions of 40 mM ibuprofen and 80 mM γ-CD in 30:70 ethanol:water

Sample	Actual mole fraction		Predicted mole fraction	
	S	R	S	R
1	0.53	0.47	0.523	0.463
2	0.58	0.42	0.563	0.427
3	0.62	0.38	0.627	0.384
4	0.68	0.32	0.662	0.321
5	0.73	0.27	0.745	0.302

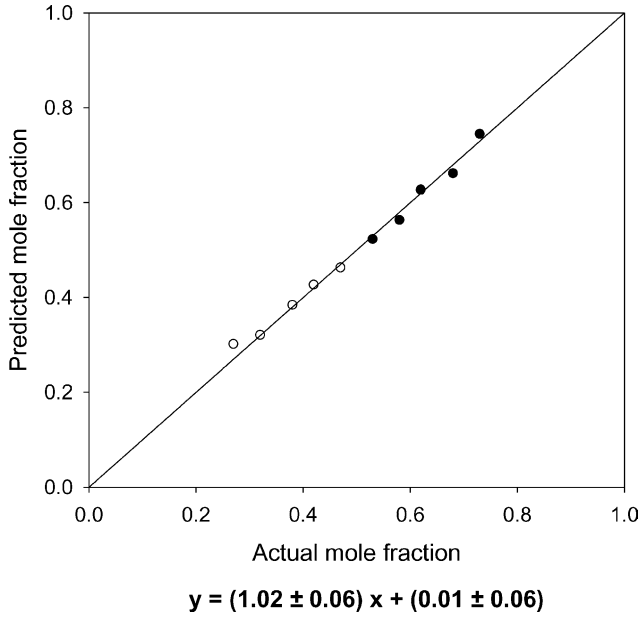

Fig. 6. Plot of predicted versus actual enantiomeric composition for solutions of ibuprofen and γ-CD in 30:70 ethanol:water. See text for detailed information. Filled circles, S-ibuprofen; open circles, R-ibuprofen.

the results as enantiomeric excess % (ee%) which is defined as $ee\% = [(R - S)/(R + S)] \times 100$. Of course, such ee% values can also be readily calculated by this method. Moreover, ee% can be calculated either from R and S concentrations calculated by this method (e.g., calculated values listed in Tables 1–4) or directly by the PLS program. Listed in Table 5 are ee% values calculated using two methods for solutions of 40 mM ibuprofen and 80 mM γ-CD in 30:70 ethanol:water mixture. It is evident that the method can also be used for the accurate calculation of ee% of samples. Interestingly, ee% values calculated directly by the PLS method are more accurate (lower relative errors) than those calculated from calculated concentrations of R and S. This is hardly surprising as it is expected that there are errors associated with calculated R and S concentrations and that the errors will be magnified when the concentrations are used to calculate a ee % value.

It has been known that the hybrophobicity of the cavity of cyclodextrins is highest in water and lower in organic solvents and/or in a mixture of organic solvent and water. As a consequence, the ability of cyclodextrins to form inclusion complexes decreases concomitantly with the concentration of organic solvent. It is, therefore, expected that the possibility for CDs to form inclusion complexes in a 30:70 ethanol:water mixture is much lower than that in pure water. The fact that this NIR method is as effective in this 30:70 ethanol:water mixture as in pure water again seems to suggest that inclusion complex formation is not the main and/or dominant mechanism for the diastereomeric interactions between the CDs and both enantiomeric forms of the drugs.

To obtain more information on the mechanism of diastereomeric interactions, additional experiments in which cyclodextrin was replaced with sucrose were performed. Similar to cyclodextrins, sucrose is also a carbohydrate. However, since sucrose is a linear carbohydrate molecule composed of one glucose and one fructose, it cannot form inclusion complexes. Results obtained for Pro and Ate in 80, 240, and 320 mM sucrose are shown in Tables 6 and 7 and Fig. 7. As evident in the tables and the figure, similar to cyclodextrins,

Table 5
Actual and calculated enantiomeric excess (ee%) of solution of 40 mM ibuprofen and 80 mM γ-CD in 30:70 ethanol:water

Sample	Actual ee%	ee% calculated from calculated R and S values[a]	Relative error[b]	ee% calculated by program[a]	Relative error[b]
1	−6.00	−6.10	1.67	−6.16	2.66
2	−16.00	−13.7	14.38	−16.55	3.44
3	−24.00	−24.04	0.17	−24.12	0.50
4	−36.00	−34.70	3.61	−33.95	5.70
5	−46.00	−42.4	7.83	−44.31	3.67

[a] Defined as ee% = $[(R - S)/(R + S)] \times 100$.
[b] Defined as Relative error = (actual value − calculated value) × 100.

Table 6
Actual and predicted enantiomeric compositions of solutions of 40 mM propranolol in either 80, 240, or 320 mM sucrose

Sample	Actual mole fraction		Predicted mole fraction	
	S	R	S	R
80 mM Sucrose				
1	0.53	0.47	0.52	0.51
2	0.58	0.42	0.69	0.33
3	0.62	0.38	0.67	0.31
4	0.68	0.32	0.66	0.38
5	0.73	0.27	0.76	0.27
240 mM Sucrose				
1	0.53	0.47	0.55	0.45
2	0.58	0.42	0.58	0.39
3	0.62	0.38	0.60	0.35
4	0.68	0.32	0.68	0.33
5	0.73	0.27	0.72	0.30
320 mM Sucrose				
1	0.53	0.47	0.58	0.42
2	0.58	0.42	0.62	0.39
3	0.62	0.38	0.60	0.40
4	0.68	0.32	0.63	0.33
5	0.73	0.27	0.74	0.26

Table 7
Actual and predicted enantiomeric compositions of solutions of 40 mM Atenolol in either 80, 240, or 320 mM sucrose

Sample	Actual mole fraction		Predicted mole fraction	
	S	R	S	R
80 mM Sucrose				
1	0.53	0.47	0.52	0.52
2	0.58	0.42	0.65	0.38
3	0.62	0.38	0.68	0.34
4	0.68	0.32	0.63	0.36
5	0.73	0.27	0.68	0.33
240 mM Sucrose				
1	0.53	0.47	0.58	0.45
2	0.58	0.42	0.59	0.41
3	0.62	0.38	0.60	0.40
4	0.68	0.32	0.70	0.31
5	0.73	0.27	0.71	0.29
320 mM Sucrose				
1	0.53	0.47	0.57	0.44
2	0.58	0.42	0.62	0.41
3	0.62	0.38	0.66	0.40
4	0.68	0.32	0.72	0.33
5	0.73	0.27	0.72	0.31

enantiomeric compositions of both drugs can also be effectively determined with sucrose. However, it seems that, in this case, the method is dependent on the concentration of sucrose. As listed in Tables 6 and 7, for both drugs, relatively larger errors were obtained with 80 mM sucrose; namely, error as large as 22 and 21% were obtained for atenolol and propranolol at this concentration. R^2 values for plot of calculated versus actual concentration for Pro and Ate (Figs. 7A and D) are 0.88 and 0.88, respectively. Interestingly, increasing concentration of sucrose led to better prediction and relatively smaller errors. Specifically, relative errors for both drugs at 240 and 320 mM sucrose concentration are only a few percent. Slopes of the predicted versus actual concentrations for these concentrations are 1, within experimental errors (Figs. 7B, C, E, and F). The results clearly demonstrate that sucrose is as effective as cyclodextrins in providing diastereomeric interactions with both enantiomeric forms of either Pro or Ate. The observation that sucrose is more effective at 240 and 320 mM than at 80 mM may be due to the fact that sucrose has only one glucose and one fructose molecule whereas the smallest CD (α-CD) has six glucose units. As a consequence, to produce a similar effect, sucrose must be used at a concentration at least three times that of CD.

As stated above, the present method has high sensitivity. Its sensitivity and accuracy can be evaluated from two values: the lowest ee% that can be determined at the lowest concentration of a sample. It should be noted that these two terms are interdependent on each other; namely, the limit of detection on ee% can be improved by increasing sample concentration or vice versa. In an attempt to estimate the sensitivity of the method, we performed measurements on 14 samples of 5.0 mM or 1.4 mg/mL of propranolol with different ee%s in 10.0 mM of α-CD in water. Results obtained are listed in Table 8. It is evident from the table that the method is not only sensitive but also very accurate. It can accurately determine samples with concentration as low as micrograms having ee value as high as 97.00% (or −90.00%) and as low as 0.80%. Furthermore, even at ee as low as 0.8%, the relative error was only 4.10%.

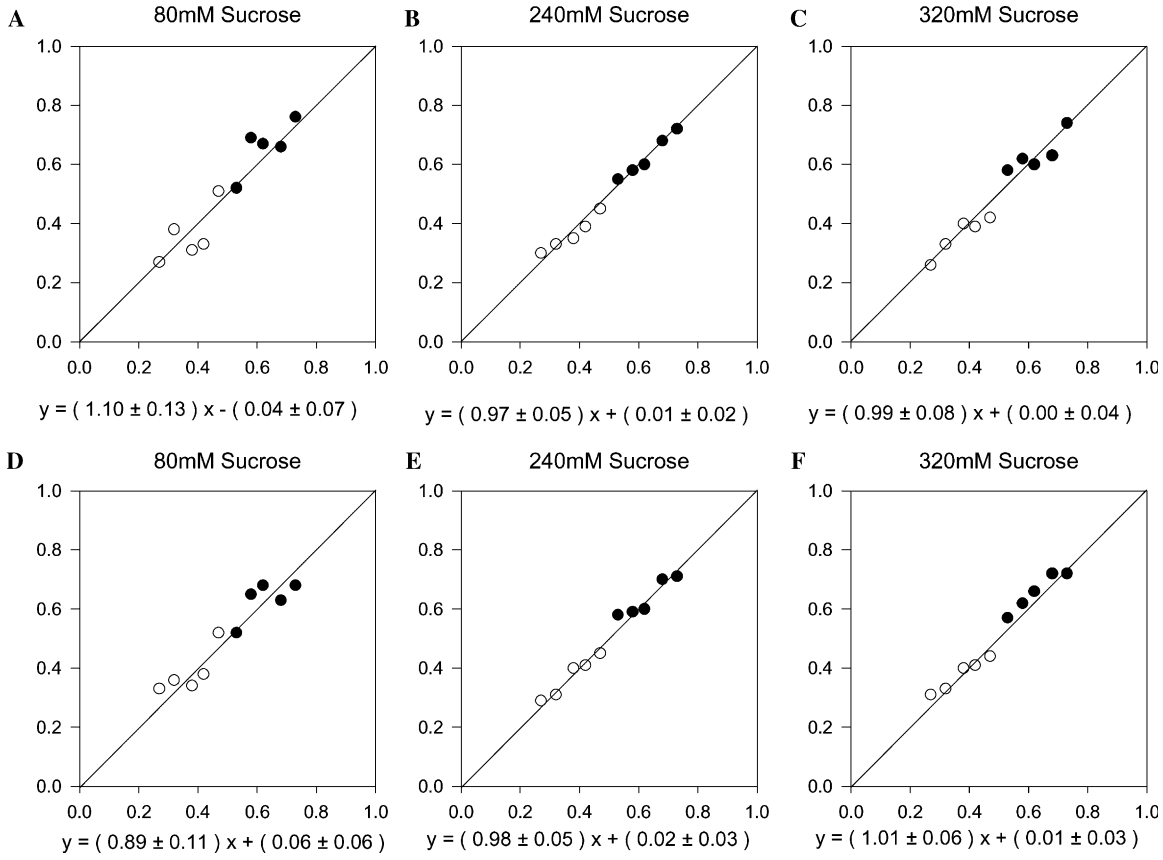

Fig. 7. Plots of predicted versus actual enantiomeric composition for solutions of 40 mM propranolol (A–C) or atenolol (D–F) in 80, 240, and 320 mM sucrose. See text for detailed information. Filled circles, S enantiomers; open circles, R enantiomers.

Table 8
Actual and calculated enantiomeric excess (ee%) of solution of 5.0 mM propranolol in 10 mM α-CD

Sample	R-propranolol (mole fraction)	S-propranolol (mole fraction)	Actual ee%[a]	Calculated ee%[a]	Relative error[b]
1	0.0500	0.9500	−90.00	−92.75	3.06
2	0.1500	0.8500	−70.00	−72.36	3.37
3	0.2500	0.7500	−50.00	−46.27	7.46
4	0.3000	0.7000	−40.00	−41.09	2.73
5	0.3650	0.6350	−27.00	−27.08	0.30
6	0.4800	0.5200	−4.00	−3.76	6.00
7	0.5040	0.4960	0.80	0.83	3.75
8	0.5100	0.4900	2.00	1.97	1.50
9	0.5350.	0.4650	7.00	6.93	1.00
10	0.5850	0.4150	17.00	16.53	2.76
11	0.7250	0.2750	45.00	45.69	1.53
12	0.8000	0.2000	60.00	59.99	0.02
13	0.9000	0.1000	80.00	80.39	0.49
14	0.9850	0.0150	97.00	95.96	1.07

[a] Defined as ee% = [(R-propranolol − S-propranolol)/(S-propranolol + R-propranolol) × 100.
[b] Defined as Relative error = (actual value − calculated value) × 100.

Conclusions

Collectively, the results presented clearly demonstrate that enantiomeric compositions of pharmaceutical products with different shapes sizes, and functional groups can be accurately and sensitively determined by the NIR spectrometric technique. Such analysis is possible because both enantiomeric forms of a drug are differentiated through their diastereomeric interactions with an added carbohydrate compound. The fact that the

method works well with all three CDs and the fact that sucrose is as effective as all three CDs clearly demonstrate that it is not necessary to have inclusion complex formation to produce effective diastereomeric interactions. Rather a simple adsorption of the analyte onto a carbohydrate is sufficient. Since inclusion complex formation is not a requisite, this NIR method is not limited to the three drugs studies here but is rather universal as it can, in principle, be used for the sensitive and accurate determination of enantiomeric compositions for many different types of pharmaceutical products with only milligram concentration and enantiomeric excess as low as 0.80%, in water or in a mixture of water and organic solvent. Furthermore, it does not rely on the use of rather expensive carbohydrates such as cyclodextrins but is equally as effective even with a simple and inexpensive carbohydrate such as sucrose.

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