ORIGINAL ARTICLE

Calculation of cyclodextrin-mediated enantiomer ratio shifting of racem norgestrel in aqueous solutions

Dávid Szegvári · Romána Zelkó · Péter Horváth · András Gergely

Abstract Enantioselective solubility of rac-norgestrel was found in the presence of γ-cyclodextrin or hydroxypropyl-γ-cyclodextrin. In both cases the efficacious enantiomer was dissolved in greater extent. Calculating the molar absorptivity and molar ellipticity spectra of the γ- and hydroxypropyl-γ-cyclodextrin aqueous complexes, a simple and rapid direct circular dichroism (CD) spectrometric method was obtained for the determination of the enantiomer ratio in aqueous solutions.

Keywords Enantioselective solubility · Circular dichroism · γ-cyclodextrin · HP-γ-cyclodextrin · Inclusion complexes · Molar absorptivity · Molar ellipticity · Norgestrel

Introduction

Three-fourth of the marketed drugs are chiral and most of them are prepared synthetically. In most cases racemates are obtained, although most frequently only one of the enantiomers (eutomer) is efficacious, while the other (distomer) is of less or no effectiveness and sometimes it causes side effects and toxicity. Application of chiral excipients with racemic drugs could result in different bioavailability of the enantiomers due to the excipient-drug interaction causing diastereomer molecule complexes of basically different characteristics.

Different polysaccharides and cellulose polymers, such as hydroxypropylmethylcellulose (HPMC), were examined mostly for this purpose, and modest enantioselective dissolution were reported in some cases [1–6].

Cyclodextrins (CyDs), as chiral excipients, perfectly comply with the above mentioned requirements because enantiomers of a given racemic molecule could form inclusion complexes of different bond strengths with CyDs. The chiral high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) separation techniques are based on this phenomenon where the CyD is covalently bonded to the solid phase [7, 8], or it is dissolved in the mobile phase [8, 9]. Although contrary to expectations there are only a few articles in the literature concerning the enantioselective solubilizing effect of CyDs. In addition, these works generally failed to prove enantioselective dissolution [5, 6, 10, 11].

Aigner et al. published earlier that water solubility of the practically insoluble norgestrel can be increased using CyDs as solubilizing agent, but there was no attempt to determine its possible enantioselective solubility [12].

It was presented, however, in our previous work [13] that (–)-norgestrel (Fig. 1), the eutomer molecule, was dissolved in greater extent using γ-cyclodextrin (γ-CyD) solution, since the obtained (–)-norgestrel/(+)-norgestrel ratio was 1.27. The observed enantioselectivity was more considerable when hydroxypropyl-γ-cyclodextrin (HP-γ-CyD) solution was applied,

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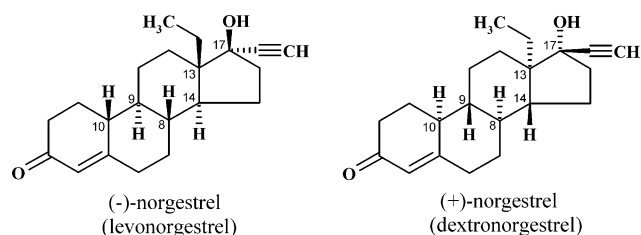
Fig. 1 Structures of levonorgestrel (8R; 9S; 10R; 13S; 14S; 17R) and dextronorgestrel (8S; 9R; 10S; 13R; 14R; 17S)

where the enantiomer ratio reached a value of 1.34 [13]. A circular dichroism (CD) spectroscopic method, based on the measurement of anisotropy factor, previously developed in one of our former works [14], was applied for the determination of the enantiomer ratios. However, the calculation was not possible with the measured data of aqueous solutions, because of the unknown molar absorptivity and molar ellipticity values of the diastereomer complexes. The norgestrel molecules can be extracted quantitatively into octanol from the aqueous complex [13], therefore the enantiomer ratios were determined in octanol phase. Since the CyD molecules remained in the aqueous phase, the complex itself was absent during the measurements [13].

The present work is about to elaborate a principle, which makes the measurements of aqueous solutions sufficient for the determination of the enantiomer concentrations without complex disintegration.

Experimental

Materials

Rac-norgestrel (rac-13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one) and (–)-norgestrel were products of the Chemical Works of Gedeon Richter Ltd, Budapest, Hungary. γ-CyD and HP-γ-CyD were purchased from Cyclolab Ltd., Budapest, Hungary. All other reagents were of the highest analytical quality.

Apparatus

The ellipticities and the absorbances were measured by a Jasco-720 type spectropolarimeter, using the single cell technique. The temperature of the measuring cell was set to $25 \pm 0.1°C$. The samples were shaking by a GFL 1086 Shaking Water Bath. The bath temperature was set to $25 \pm 0.1°C$ and the shaking frequency was $230 \ min^{-1}$. Filtration was carried out through an MSI, MAGNA nylon membrane filter of 0.45 μm pore size.

Sample preparation

Excess amounts of rac-norgestrel or (–)-norgestrel (0.005 g) were weighted into 50 ml volumetric flasks to which 15.00 ml of aqueous γ-CyD or HP-γ-CyD solutions of various concentrations ranging from 5.0×10^{-4} M to 4.3×10^{-3} M were added. The samples were shaken till the solubility equilibrium, which was achieved in 24 h (further studies up to 48 h did not show any difference). The samples were filtered and the CD and UV spectra were recorded. The norgestrel content was extracted into 4×10 ml octanol. The ellipticities were measured at 321 nm, and the absorbances at 250 nm in the combined octanol phases. The concentrations were calculated by the value of molar absorptivity (11567.6 ± 70.1), which was previously determined in water saturated octanol.

Results and discussions

Neither the γ-CyD nor the HP-γ-CyD has measurable ellipticity or absorbance in the applied wavelength range (230–400 nm), although the molar values of the two enantiomer/CyD complexes can be different taking into consideration that the two supermolecules are diastereomers of each other.

The enantiomer concentrations of the unknown sample could be calculated directly from aqueous solutions with the knowledge of all the four molar absorptivity and molar ellipticity values of the complexes.

Equation (1) describes the relationship between the concentration of (–)-norgestrel/cyclodextrin complexes and the ellipticity in the case of sample containing only (–)-norgestrel. Equation (2) is valid for samples containing both enantiomers.

$$[\Theta]_{(-)} = \frac{\Theta}{[(-)Norg] \cdot 10 \cdot l_\Theta} \tag{1}$$

$$[\Theta]_{(+)} = \frac{\Theta - [(-)Norg] \cdot [\Theta]_{(-)} 10 \cdot l_\Theta}{[(+)Norg] \cdot 10 \cdot l_\Theta} \tag{2}$$

where [(–)Norg] and [(+)Norg] are the molar concentrations of (–)- and (+)-norgestrel/cyclodextrin complexes in water (M), Θ is the measured ellipticity in water (mdeg), $[\Theta]_{(-)}$ and $[\Theta]_{(+)}$ are the molar ellipticities of (–)-norgestrel and (+)-norgestrel/cyclodextrin complexes in water for the measuring wavelength (mdeg·cm^2·decimole^{-1}), l_Θ is the path-length of the cell for the measurement of ellipticity (cm).

Fig. 2 Calculated molar ellipticity spectra of the complexes in the wavelength range of 270–370 nm.
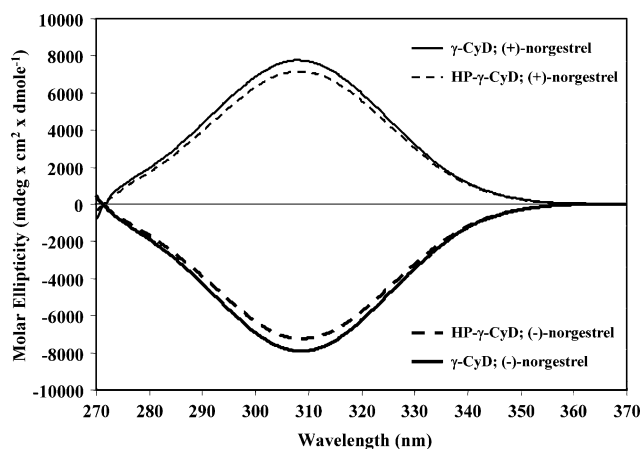

Fig. 3 Calculated molar absorptivity spectra of (–)-norgestrel complexes in the wavelength range of 230–270 nm

Equation (3) describes the relationship between the concentration and absorbance of (–)-norgestrel/cyclodextrin complexes in the case of sample containing only (–)-norgestrel. Equation (4) is valid for samples containing both enantiomers.

$$\varepsilon_{(-)} = \frac{A}{[(-)Norg] \cdot l_A} \tag{3}$$

$$\varepsilon_{(+)} = \frac{A - [(-)Norg] \cdot \varepsilon_{(-)} \cdot l_A}{[(+)Norg] \cdot l_A} \tag{4}$$

where A is the measured absorbance in water, $\varepsilon_{(-)}$ and $\varepsilon_{(+)}$ are the molar absorptivities of (–)- and (+)-norgestrel/cyclodextrin complexes in water for the measuring wavelength, l_A is the path-length of the cell for the measurement of absorbance (cm). The mentioned molar values can be calculated, because [(–)Norg] and [(+)Norg] are known from the previously applied indirect CD spectroscopic method [13].

The wavelengths of the ellipticity and absorbance measurements could be different if the molar values for the measuring wavelengths are available. Figure 2 shows the molar ellipticity spectra of γ- and HP-γ-CyD complexes of (–)- and (+)-norgestrel in water, which were calculated by equations (1) and (2). Figure 3
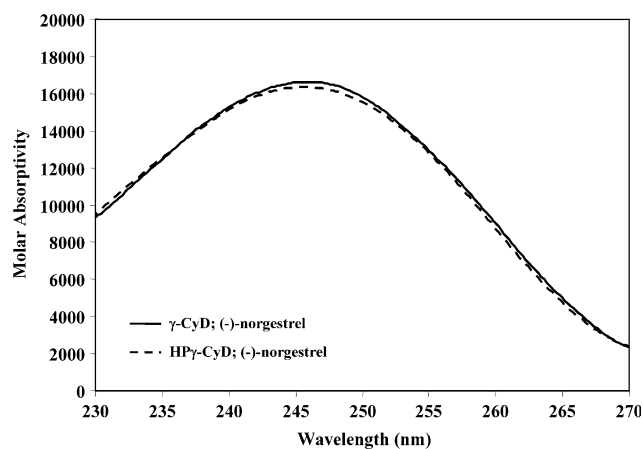
shows the molar absorptivity spectra of γ- and HP-γ-CyD complexes of (–)-norgestrel in water, which were calculated by equations (3). It can be stated, that the wavelengths of the maximum values with the two CyDs are equal in the case of CD and UV peaks, respectively.

Table 1 summarizes the calculated exact molar values corresponding to the peak maxima. It can be stated, that there are significant differences between the molar values of γ- and HP-γ-CyD/norgestrel complexes in the case of the CD curves, while the differences between the molar absorptivities are less considerable. There are no notable differences between the corresponding values belonging to the two enantiomers in all the four cases.

On the basis of the molar ellipticity and molar absorptivity values of the complexes and by the solution of simultaneous Eqs. (2) and (4), the concentration of the enantiomers could be calculated in the samples of unknown enantiomer ratios.

From Eq. (4) the [(-)Norg] can be derived:

$$[(-)Norg] = \frac{A - [(+)Norg] \cdot \varepsilon_{(+)} \cdot l_A}{\varepsilon_{(-)} \cdot l_A} \tag{5}$$

Substituting into the Eq. (2), the [(+)Norg] can be obtained:

Table 1 Molar ellipticity and molar absorptivity values of aqueous CyD inclusion complexes of norgestrel enantiomers corresponding to the peak maxima

	$[\Theta]_{309\ nm} \pm$ SD ($n = 7$)		$\varepsilon_{246\ nm} \pm$ SD ($n = 7$)	
	(–)-norgestrel	(+)-norgestrel	(–)-norgestrel	(+)-norgestrel
γ-CyD	–7904.5 ± 66.8	7753.6 ± 92.9	16539.3 ± 140.3	16637.1 ± 158.5
HP-γ-CyD	–7234.5 ± 87.5	7153.9 ± 85.9	16476.0 ± 127.8	16497.8 ± 135.3

$$[(+)Norg] = \frac{\Theta \cdot \varepsilon_{(-)} \cdot l_A - A}{[\Theta]_{(+)} \cdot \varepsilon_{(-)} \cdot 10 \cdot l_\Theta \cdot l_A - [\Theta]_{(-)} \cdot \varepsilon_{(+)} \cdot 10 \cdot l_\Theta \cdot l_A}$$

$$(6)$$

Based on Eq. (5), $[(-)Norg]$ can also be calculated with the knowledge of $[(+)Norg]$.

Conclusions

The molar ellipticity and molar absorptivity spectra of γ- and HP-γ-CyD complexes of the norgestrel enantiomers for aqueous solutions were calculated. Using the given values, the CyD-mediated enantiomer concentrations could be calculated directly from aqueous solutions where the drug is in complex form. By dropping the time consuming and unpleasant extraction procedure, a quicker and more comfortable method was developed for further examinations, like phase-solubility studies and the determination of complex stabilities of the enantiomers.

The principle can be applied for determining the concentration or enantiomer ratio of similar inclusion complexes in water with other guests of poor solubility.

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