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A preliminary study of β-cyclodextrin/metoprolol tartrate inclusion complex for potential enantiomeric separation

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Abstract

The inclusion complex formation between Metoprolol tartarata (MeT) and β-cyclodextrin (β-CD) has been investigated using hyperchromic shift at λ_{max} 274.4 nm of MeT. Different parameters such as stirring time, solvent composition (aqueous and aqueous/methanol solutions with methanol content up to 50%), pH values 4.0 and 8.0 were established for optimal inclusion complex formation and confirmed two stoichiometric compositions 1:1 and 1:2. Preliminary data on usage of MeT/β-CD complex in reversed-phase HPLC indicate the potential application of this complex as a kind of pre-column derivatization for enantiomeric separation of β_1-blockers. © 2001 Published by Elsevier Science B.V.

Keywords: Metoprolol tartrate; β-Cyclodextrin; Inclusion complex; UV-spectrophotometry; Enantiomeric separation

1. Introduction

Most beta-blockers are racemic mixtures and it is known that their enantiomers have different potencies and pharmacological effects. Metoprolol tartrate, (R,S)-3[4-(2-methoxyethyl)phenoxy)-1-(isopropylamino) propan-2-ol] tartrate, used in this study, is a cardioselective β_1-adrenoreceptor antagonist (β_1-blocker) widely used in the treatment of hypertension, angina pectoris and cardiac dysrhythmias [1,2]. Most biological receptors act stereoselectively, reacting with only one enentiomer of a chiral substance, while the other enantiomer is inactive at a specific receptor. One example is the use of β-blockers in reducing blood pressure, where the S-form's therapeutic effects is about 100 times stronger than R-form's, yet the R-form causes various side effects such as anesthesia and cardiodepression at the same dosage [3]. Separation of β-blocker enantiomers included pre-column derivatization by conventional achiral chromatography [4,5]. Using different chiral stationary phases (CSP) or chemically different chiral mobile phase additives, β-blockers have been recently separated [6–9].

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Nowadays, cyclodextrins (CD), which are well-known non-toxic macrocyclic sugars of natural origin, are considered as a whole family of pharmaceutical excipients and drug carriers. Solubility and stability studies of β-CD inclusion complexes with different drugs such as itraconazole, clomiphene and tamoxifen, ranitidine hydrochloride and omeprazole, as well as application of β-CD/salbutamol inclusion complexes to prolong a biological effect and to achieve an efficient dry powder inhalation formulation, have been published recently [10]. Different CD have been used in the pharmaceutical, cosmetics and food industries in order to improve solubility, dissolution rate, stability and the bioavailability of drugs.

Another approach for the direct resolution of racemic mixtures considered the usage of Beta-cyclodextrin-bonded phases [8,11], or application of CD as chiral mobile phase additives in reversed-phase HPLC [12], TLC [13] and capilary electrophoresis (CE) [14].

The objective of this preliminary study was to established the optimal conditions for the reaction between metoprolol tartrate (MeT) and β-CD, applying UV-spectrophotometry. This inclusion complex, β-CD/MeT, could be used as a kind of pre-column derivatization for enantiomeric separation of β_1-blockers in reversed-phase HPLC and some preliminary data are presented.

2. Experimental

2.1. Materials

Metoprolol tartrate (MeT) was purchased from Sigma Chemical Company; β-cyclodextrin (β-CD) was obtained from Laboratories of Pharmaceutical Company 'Zdravlje' a.d. (Leskovac, FR Yugoslavia) and conforms the requirements of Ph Eur'97. Methanol, hydrochloric acid and sodium hydroxide were of analytical purity Merck.

2.2. Instrumentation

UV spectra were recorded on SPECORD M 40 UV–Vis spectrophotometer, (Carl Zeiss Jena, Germany). pH-meter Sentron® Model 2001 pH with standard ISFET probe, Sentron Europe B.V.(AC Roden, The Netherlands) probe was used. Samples were stirred on ultrasonic bath Model USK Ei Nis-RO-VEP (Nis, FR Yugoslavia).

2.3. Solutions

Freshly prepared stock aqueous and methanol/aqueous (25% v/v) solutions of MeT (0.2 mg/ml) and β-CD (3.31 mg/ml) were used. Stock solutions of β-CD and MeT of the same concentrations, but with adjusted pH values (pH = 4.0; pH = 8.0) were also prepared.

Diluted β-CD solutions: aliquots of β-CD stock solutions of 0.5; 1.0; 2.0 and 4.0 ml were refilled with redistilled water or CH_3OH/H_2O (25% v/v) up to 10.0 ml.

For pH adjustment 0.1 M hydrochloric acid and 0.1 M sodium hydroxide solutions were used.

2.4. Procedure for β-CD/MeT inclusion complex formation

Equal volumes of 5.0 ml of stock MeT solution and of diluted β-CD solutions of different concentrations were mixed. UV spectra of these solutions in wavelength range 250–290 nm were recorded: (a) after stirring on ultrasonic bath 40 min for aqueous solutions (50 min for methanol/aqueous solutions); (b) after heating at 80°C (only for aqueous solutions) but without stirring; (c) after rapid cooling under tap water. For the experiments of complex composition, UV spectra of these solutions were recorded only after stirring without heating.

3. Results and discussion

The absorption spectra of MeT [15] in pure methanol solutions display two well defined λ_{max} at 276 and 282 nm (Fig. 1a, solid line). Aqueous MeT solutions in 0.1 M HCl (Fig. 1a, broken line) as well as aqueous solutions without addition of HCl (Fig. 1b, solid line) exhibit only one well defined λ_{max} at 274, 4 nm and an inflexion at 280 nm. The UV spectra of MeT in methanol/aqueous

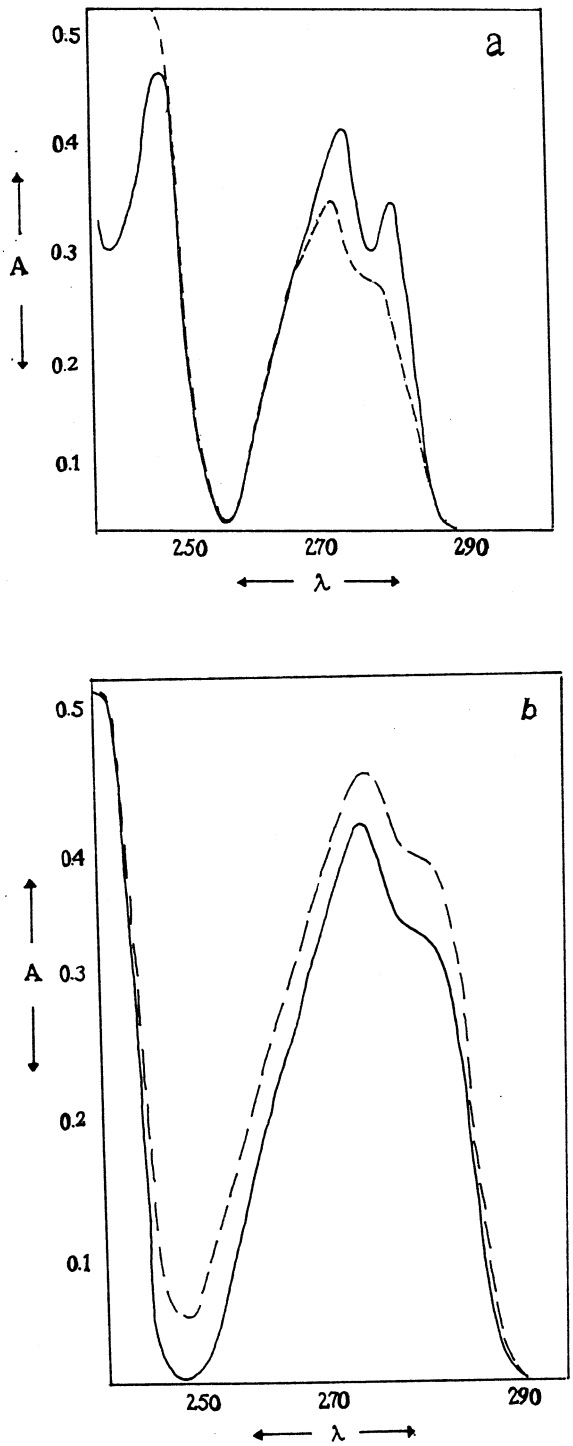

Fig. 1. The absorption spectra of: (a) methanol MeT solution 0.1 mg/ml (solid line); aqueous MeT solution with 0.1 M HCl (broken line); (b) aqueous MeT solution 0.1 mg/ml (solid line) and of MeT/β-CD inclusion complex (broken line) after 40 min stirring for mole ratio 1:1

solutions (up to 50:50) shows the same λ_{max} at 274, 4 nm and shoulder at 280 nm.

The reaction between MeT and β-CD, whereby inclusion complex could be formed, was followed using λ_{max} at 274,4 nm of MeT, since β-CD does not absorb in UV region. Cyclodextrins have doughnut-shaped structure, with hydrophilic external faces and hydrophobic inner surface. Such structure makes them the most important simple organic compounds capable of forming non-covalently bonded inclusion complexes with a wide variety of drug molecules in aqueous solutions [16].

Inclusion complex MeT/β-CD formation was observed, in both solvents, only after stirring on ultrasonic bath at room temperature, and modification of MeT absorption spectra display only hyperchromic shift but without shifts of λ_{max} and inflexion (Fig. 1b, broken line).

The effect of time stirring at different mole ratio of MeT to β-CD up to 1:4 in aqueous solutions (the same volume of solutions for all investigated mole ratios) at $\lambda_{max} = 274.4$ nm is presented in Fig. 2. The solutions of different MeT/β-CD mole ratio without stirring show no significant absorbance changes The most pronounced changes are obtained at MeT/β-CD ratio 1:1, indicating that this ratio corresponds to stoichiometry of inclusion complex. Applying mole ratio method [17], with increasing β-CD concentrations up to 1:10 in respect to MeT concentration 0.2 mg/ml, confirmed formation of 1:1 complex.

The optimum stirring time for aqueous solutions was 40 min and additional 10 min for methanol/aqueous (25% v/v) solutions. Longer optimum stirring for MeT/β-CD complex formation in methanol/aqueous solutions (50 min.) could be explained by lower solubility of β-CD in methanol [16], as well as of inclusion complex. Almost the same absorbance values have been recorded weather MeT and β-CD solutions were mixed or when one of the component was added in solid state to the solution of the other component.

The effects of stirring, temperature, solvent composition and the effect of two pH values on MeT/β-CD inclusion complex formation are summarized in Table 1. Absorbance values, obtained

Table 1
The absorbance values at $\lambda_{max} = 274.4$ nm of MeT/β-CD inclusion complex[a]

Mole ratio MeT/b-cd	Aqeous solutions without pH adjustment				Aqueous solutions with pH adjustment				Methanol/aqueous solutions (25% v/v)			
	Stirring effect		Temperature effect		Solutions pH = 4		Solutions pH = 8		Solutions pH = 4		Solutions pH = 8	
	A_1 without stirring	A_2 stirring (min)	A_1 heated at 80°C	A_2 rapid cooling	A_1 without stirrirng	A_2 stirring (40 min)	A_1 without stirrirng	A_2 stirring (40 min)	A_1 without stirrirng	A_2 stirring (40 min)	A_3 stirring (50 min)	A_4 stirring (70 min)
1:0	0.408	0.414(40)	0.418	0.412	0.400	0.405	0.399	0.406	0.415	0.414	0.420	0.415
1:0.5	0.416	0.417(40)	0.418	0.420	0.401	0.413	0.406	0.406	0.413	0.421	0.424	0.424
1:1	0.416	0.435(40)	0.440	0.431	0.403	0.424	0.408	0.408	0.413	0.425	0.491	0.485
1:2	0.412	0.429(80)	0.415	0.415	0.413	0.447	0.433	0.429	0.413	0.415	0.415	0.420
1:4	0.417	0.415(80)	0.423	0.412	0.413	0.446	0.432	0.428	0.414	0.415	0.415	0.416

[a] The effects of stirring, temperature and solvent composition at different mole ratio for working MeT concentration 0.1 mg/ml.

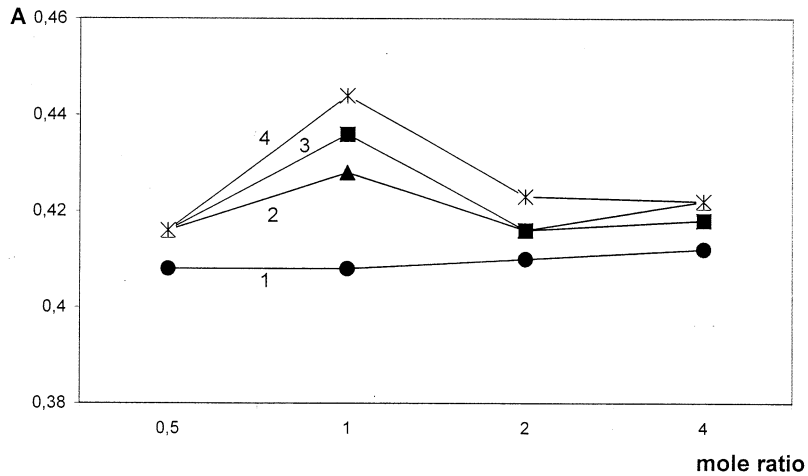

Fig. 2. The effect of time stirring on MeT/β-CD inclusion complex formation for aqueous MeT 0.1 mg/ml at $\lambda_{max} = 274.4$ nm and different mole ratio value; immediately after mixing of solutions but without stirring (curve 1); 10 min (curve 2); 20 min (curve 3); 40 min (curve 4).

for optimum time stirring (40 min) at room temperature and of the solutions heated at 80°C but without stirring on ultrasonic bath, were almost the same and hyperchromic shift was about 6–7% in comparison to starting absorbance value of 0.408. For the solutions with higher MeT to β-CD mole ratio (1:2) longer stirring time was required to obtaine the constant absorbance value (Table 1) but for ratio1:4 lower absorbance value indicates insufficient stirring time. These data in comparison to the results presented in Fig. 2, confirmed that both parameters (mole ratio and stirring time) should be adjusted to follow hyperchromic shift. The pH values of the aqueous solutions of MeT/β-CD complex with different mole ratio values varied over the range 5.8–6.5, and they showed slightly higher values after stirring (pH = 6.1–6.7). The experiments of MeT/β-CD inclusion complex formation at two pH values 4.0 and 8.0, showed some differences (Table 1). In slightly acidic solution two types of MeT salts could exist: hydrochloride and tartrate that caused altered composition of inclusion complex to 1:2, but still stirring was essential. The inclusion complex formation is proportional to the hydrophobic character of the guest molecule. Protonation of MeT amine group decreased the possibility of guest molecule to penetrate into β-CD cavity. Also, hydroxyl groups hinder complex formation, but their hydrophilic effects decrease in the order *ortho* > *meta* > *para*.The secondary alcoholic group of MeT is in the most favorable position (*para*) in respect to hydrophobic part of MeT molecule. Inclusion complex formation at pH = 8.0, whereby MeT is present as the base in sufficient content, also indicate that the complex composition is 1:2, but the hyperchromic shift was obtained without stirring.

The same stoichiometric composition was obtained in methanol/aqueous solution, but higher absorbance values (hyperchromic shift of 18%) as well as longer stirring time indicate the solvent effect (Table 1). MeT/β-CD inclusion complex formation has not been observed in methanol/aqueous solution with increased methanol content (50%) after stirring time for more than 1 h, probably due to lower solubility of β-CD. In this solvent composition, the usage of β-CD derivatives such as 2-hydroxypropil-CD or methylated CDs would be of interest. Methanol/aqueous solutions with higher methanol content (50:50) was considered for sample preparation in the case of other β-blockers, which contain different substituents in different position and particularly if they are not in the form of salts.

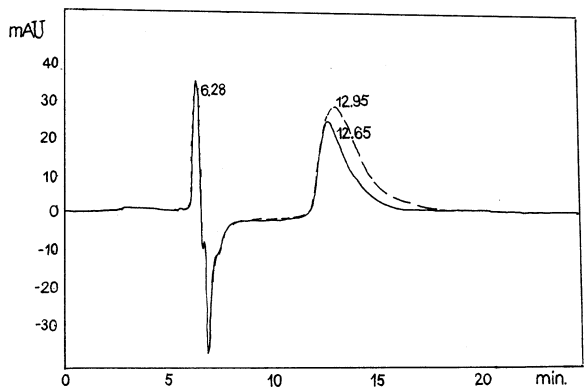

Fig. 3. Chromatograms obtained for samples: 20 μl aqueous solution of MeT 0.6 mg/ml-solid line (12.65 min); 20 μl aqueous solution of MeT /β-CD broken line 12.95 min (MeT 0.6 mg/ml; mole ratioMeT:/β-CD = 1:1 stirring time 40 min) on a 5 μm Lichrosorb RP-18 column (250 × 4.6 mm I.D.); eluent:methanol-aqueous solution of β-CD 6 mM; flow rate 0.5 ml/min; 25°C; detection, UV 230 nm.

The optimal conditions and stoichiometry of MeT/β-CD inclusion complex formation, established in this study using hyperchromic effect in UV spectra, will be confirmed in forthcoming experiments using NMR. This technique is already known as powerful tool for the characterization of inclusion complex, that is recently validated for other β-blockers (atenolol and celiprolol) [18]. The NMR spectra could give the evidence of possible involvement of tartrate particularly through interactions with primary hydroxyl groups of β-CD molecule.

Our preliminary data of potential usage of MeT/β-CD complex, as a kind of pre-column derivatization in reversed-phase HPLC, are presented in Fig. 3. These chromatograms are achieved only with one eluent:methanol-aqueous solution of β-CD. The peak obtained with previously prepared MeT/β-CD inclusion complex, under the optimal conditions established in UV study, confirmed hyperchromic shift in comparison to sample of pure MeT of the same concentration (β-CD does not absorb in UV region) and this peak is significantly broad compared to that one of pure MeT. Both samples showed first peak (6.28 min) of separated tartaric acid. The conditions for enantiomeric separation such as eluent content (buffer addition, organic modifiers addition) as well as flow rate, should be optimized in further study.

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