DETERMINATION OF DRUG-CYCLODEXTRIN BINDING CONSTANTS BY CAPILLARY ZONE ELECTROPHORESIS

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ABSTRACT

Binding constants of the optical isomers of Deprenyl® (selegiline) and its potential metabolites with (2,6-di-O-methyl)-β-cyclodextrin were determined using electrophoretic mobility data gained from separations performed by capillary electrophoresis, and absorbancies obtained from spectrophotometric experiments. To calculate equilibrium constants 1:1 complex formation have been assumed. The comparison of the equilibrium constants calculated from different methods shows similar values in their order of magnitude. Their difference may probably be explained by the different media of the measurements. The effect of the structure of compounds on chiral discrimination were also elucidated.

1. INTRODUCTION

Cyclodextrins are widely used in capillary electrophoresis (CE) in order to separate drug molecules, especially chiral isomers. The mechanism of the separation is mainly based on the different binding constants of the isomers with cyclodextrins.

To determine such binding constants have various interests:
-the optimum concentration of the chiral selector for enantiomer resolution in CE depends on the magnitude of the complex formation constants and the different mobilities of the free and complexed analytes [1,2].
-knowing these binding constants, experimental conditions to achieve chiral separation can well be predicted.

In this work a simple equilibrium model was used to calculate the formation constants. Enantiomer pairs of Deprenyl® and its metabolites were separated in running buffers containing various concentrations of the chiral selector. The corrected mobilities were used for the estimation of the binding constants.

The formation constants of some compounds were also determined by spectrophotometric method in order to compare data derived from CE and spectrophotometry, as well as from the literature.

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2. MATERIALS AND METHODS

2.1 Materials

The enantiomers of the following standard compounds were used in the sample mixtures: amphetamine (A), propargyl-amphetamine (PA), methamphetamine (MA), p-fluoro-methamphetamine (pFMA), deprenyl (D), p-fluoro-deprenyl (pFD), which compounds were kindly provided by Chinoin Pharmaceutical and Chemical Works (Budapest, Hungary). Pseudoephedrine (PSE), norephedrine (NE) and ephedrine (E) obtained from Sigma (Budapest, Hungary). All other materials were of analytical grade.

2.2 CE apparatus and separation conditions

A CRYSTAL 300 (ATI, UNICAM, Cambridge, UK) capillary electrophoresis system equipped with a variable-wavelength UV absorbance detector set at 190 nm was used. The separations were performed in a 70 cm x 75 μm ID uncoated fused silica capillary, the length to the detection window was 55 cm. Samples were introduced by electrokinetic injection (3KV, 12s). The applied field strength was 300 V/cm, the separation temperature 21 °C. Axxiom 727 software was used for data collection.

20 mM Tris-phosphate pH 2.7 containing 0.5% hydroxypropylmethyl-cellulose (HPMC), and various concentration of heptakis (2,6-di-O-methyl)-β-cyclodextrin (DIMEB) and methanol was used as running buffer [3].

2.3 Spectrophotometry

Conditions: pH=2.7 was adjusted with formic acid, ionic strength =20 mM adjusted with NaCl. Temperature:25±1°C.
The absorbances was measured with Spectromom 195D at λ=520 nm.
The binding constants were determined based on a competing reaction [4] between cyclodextrin and two ligands (methyl orange and drug molecules studied). Upon addition DIMEB to a red-coloured acidic solution of methyl orange, the absorbance decreases on account of the formation of a yellow deprotonated methyl orange-cyclodextrin complex. In the presence of another potential ligand molecule, only a fraction of the cyclodextrin cavities can interact with the methyl orange, resulting in a weaker discoloration effect.

2.4 Viscosity

Viscosity was measured using a Ubbelode suspended level capillary viscometer held in a water bath at 25.0°C.

3. RESULTS AND DISCUSSION

The electrophoretic mobilities of the enantiomer pairs were measured in the 0-48 mM concentration range of the chiral selector, (2,6-di-O-methyl)-β-cyclodextrin.

Application of DIMEB in the buffer decreased the electrophoretic mobility of each test compound. This indicated the inclusion complex formation of separands with the chiral additive, as complexes formed have lower electrophoretic mobilities, than the free analyte. Besides the complex formation, the increasing viscosity of the running buffer containing higher concentration of DIMEB also influences the mobility, and this effect has to be taken into correction.
The sructure of the investigated drugs and the calculated binding constants on the basis of CE and spectrophotometry are shown in Table1.
Comparing the structure of the test compounds and the calculated binding constants the following were found:
-drugs having hydroxyl group at the α position showed weaker interaction;
-the fluoro substitution of the aromatic ring decreased the values of binding constants;
-the binding constants of compounds substituted at the nitrogen were higher in all cases.
The chiral discrimination was found to be the best for compounds substituted by OH group at α position. These compounds have two chiral centres.

The binding constants obtained by CE and spectrophotometry are the same order of magnitude and show chiral discrimination. The observed differences in the values of binding constants obtained from different methods are probably due to the different buffer composition of the measurements. Although the pH and ionic strength were kept constants, the buffer chemicals and additives were unlike. On the bases of these results, the binding constants obtained from CE data are close to the thermodynamic equilibrium constants in a given media. This means that chiral capillary electrophoresis separations can be used to determine binding constants of enantiomers with cyclodextrin derivatives. CE separations provide several advantages compared to other methods used to determine binding constants. These comprise the simple and fast measurements, which can be performed even in fairly complex media.
The constants derived from other measurements can be used as an approximation to optimize the conditions of CE separation.

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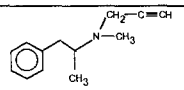
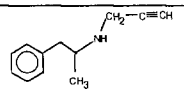
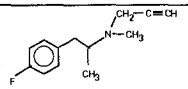
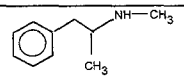
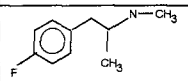
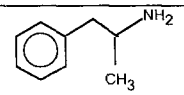
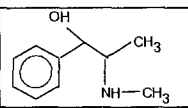
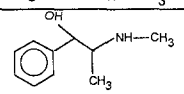
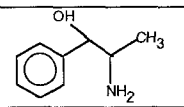
Table 1. The chemical structure of the analytes and the calculated bindindg constants

Name	Structure	Binding constants from CE data K_1 K_2	Binding constants. from photometry K_1 K_2	Literature[a] K_1 K_2
Deprenyl		42.3±4.3 153.3±6.9	156.8 269.5	
Propargyl-amphetamine		K_1=K_2= 151.5±6.6		
p-Fluoro-deprenyl		123.4±4.5 133.2±5.6		
Metham-phetanine		107.0±3.9 111.9±3.2		
p-Fluoro-metham-phetanine		98.9±4.1 100.2±3.9		
Amphetamine		89.1±1.0 94.5±3.3		
Ephedrine		57.6±2.5 63.4±3.1	98.4 130	79.2 71.3
Ψ-Ephedrine		53.8±4.0 77.5±4.5	60.4 114.3	68.9 96.7
Norephedrine		43.8±4.0 52.6±4.1		

Notes: K1 and K2 are the binding constants of (-) and (+) enantiomers, respectively. K = [EC]/ [E] [C] where E is one of the enantiomer C is the cyclodetxtrin and EC is the complex equbilibrium concentration.

a) from ref 6.;The binding constants with β- cyclodextrin were determined by calorimetric titration in phosphate buffer at pH 6.9 .