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Determination of the stability constant for the inclusion complex between β-cyclodextrin and nicotine using capillary electrophoresis

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Abstract

Capillary electrophoresis has been employed in order to determine the stability constant for the inclusion complex between β-cyclodextrin (β-CD) and nicotine. The investigation has been performed in aqueous solution at 25 and 37°C and 100 mM ionic strength in a pH interval ($5.7 \leq pH \leq 9.6$) relevant for systemic delivery of drugs via the oral mucosa. It has been shown that neutral nicotine binds considerably stronger than charged monoprotonated nicotine to β-CD: $K_1 = 22 \pm 12\ M^{-1}$, $K_2 = 242 \pm 11\ M^{-1}$ (25°C) and $K_1 = 22 \pm 12\ M^{-1}$, $K_2 = 194 \pm 10\ M^{-1}$ (37°C) (K_1 and K_2 refer to complexation by monoprotonated and neutral nicotine, respectively). Oral nicotine absorption from a sublingual tablet containing β-CD-bound nicotine is briefly discussed in connection with the use of this tablet in smoking cessation therapy. © 1997 Elsevier Science B.V.

Keywords: Nicotine; β-cyclodextrin; Inclusion complex formation; Stability constant; Sublingual nicotine tablet

α-, β-, and γ-Cyclodextrins (CDs) are cyclic oligosaccharides built up from glucopyranose units; the interior of their torus-shaped structure is predominantly hydrophobic while the outside is hydrophilic (Bender and Komiyama, 1978; Szejtli, 1988; Frömming and Szejtli, 1994). A great variety of molecules form inclusion complexes with CD hosts and the technical applications are manifold (Szejtli, 1988); for example, CDs have been widely used in pharmaceutical formulations to increase the bioavailability of unstable or poorly soluble drugs (Frömming and Szejtli, 1994; Loftsson and Brewster, 1996; Rajewski and Stella, 1996; Irie and Uekama, 1997).

Nicotine-containing pharmaceutical products for oral, nasal and transdermal absorption of nicotine have been successfully used in aiding smoking cessation ever since the first product (Nicorette nicotine chewing gum) appeared on the

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market in 1978. Nicotine is rather volatile and labile towards oxidation and it is normally stabilized in pharmaceutical formulations. For example, nicotine can be protected by formation of an inclusion complex with β-CD and a sublingual tablet containing such a complex has recently been registered as a new smoking cessation product. In general, only the free form of drugs administered as cyclodextrin complexes is absorbed through biomembranes and the rate of absorption may therefore depend on the binding strength of the inclusion complex; thus, a strong complexation may counteract the bioavailability.

Capillary electrophoresis has been shown to be well suited for studies of interactions between different types of ligands and large molecules; for example, studies on binding of ligands to proteins as well as cyclodextrins have been reported (Chu et al., 1992; Honda et al., 1992; Gomez et al., 1994; Kuhn et al., 1994; Kwak and Gomez, 1996). The basis for determination of stability constants using capillary electrophoresis is that the electrophoretic mobility of the ligand changes as it binds to the host molecule. By varying the concentration of the host, it is possible to calculate the stability constant from the magnitude of this change (Gomez et al., 1994). We here report values of the stability constant for the inclusion complex between nicotine and β-cyclodextrin determined using capillary electrophoresis in a pH interval relevant to absorption of drugs in the oral cavity.

The study was carried out at 100 mM ionic strength in the interval $5.7 \leq \text{pH} \leq 9.6$ using phosphate, borate and carbonate buffers. Nicotine from Siegfried AG (99.5%) and β-cyclodextrin from Roquette (12.36% water, determined from loss on drying) were used as received and water was of Millipore Milli-Q quality. Capillary electrophoresis was performed using a Hewlett Packard 3D-CE system with a capillary tubing of uncoated fused silica (Hewlett Packard); total length, 64.5 cm; length from the inlet of the capillary to the detector, 56 cm; internal diameter, 50 μm; and an extended optical path length of 150 μm. Samples (0.35 mM nicotine and 1.4 mM DMSO in buffer) were introduced into the capillary by pressure injection (25 mbar $\times$ 5 s). The experimental conditions were as follows: voltage, 30 kV (yields a current of 80–90 μA); temperature, 25 ± 0.1 and 37 ± 0.1°C; detection, 205 nm. Data were collected and analyzed with the Hewlett Packard Chemstation A.04.01 software package.

Successive electrophoretic runs were performed with increasing concentrations of β-CD in the buffer ([β-CD] = 0, 1.0, 2.0, 3.0, 5.0, 7.0, 10.0 and 12.0 mM). At each concentration, four electropherograms were collected and after each buffer change, the capillary was equilibrated with the new buffer for 12 min. Coelectrophoresis of a neutral EOF $\otimes$ (electro osmotic flow) marker, dimethylsulfoxide (DMSO, Merck, scintillation grade), was necessary to correct for the variable EOF observed when changing buffers or concentration of β-CD (vide infra). To make sure that DMSO does not interact with β-CD, proton NMR spectra of D_2O solutions containing either 1.1 mM β-CD or 1.1 mM β-CD and 8.9 mM DMSO were collected using a Bruker 250 MHz spectrometer working at room temperature. It has been shown that the signals of the H-3 and H-5 protons located in the hydrophobic cavity of β-CD undergo large shifts when a guest molecule binds; NMR therefore efficiently probe formation of inclusion complexes (Djedaini et al., 1990; Djedaini and Perly, 1991). The presence of DMSO did not shift the β-CD signals which, thus, strongly indicate that DMSO does not interact with β-CD.

The observed stability constant, K_{obs}, of the nicotine:β-CD complex was determined by use of Scatchard analysis, Eq. (1) (Gomez et al., 1994). $\Delta\mu_{\text{N,CD}}$ denotes the mobility of nicotine in presence of β-CD ($\mu_{\text{N,CD}}$) relative to the mobility of nicotine in absence of β-CD (μ_{N}) and can be calculated by use of Eq. (2).

$$\Delta\mu_{\text{N,CD}}/[\beta - \text{CD}] = K_{\text{obs}}\Delta\mu^{\max}_{\text{N,CD}} - K_{\text{obs}}\Delta\mu_{\text{N,CD}} \quad (1)$$

$$\begin{aligned}\Delta\mu_{\text{N,CD}} &= \mu_{\text{N,CD}} - \mu_{\text{N}} \\ &= l_c l_d / V\,[(1/t_{\text{N,CD}} - 1/t_{\text{DMSO,CD}}) \\ &\quad - (1/t_{\text{N}} - 1/t_{\text{DMSO}})]\end{aligned} \quad (2)$$

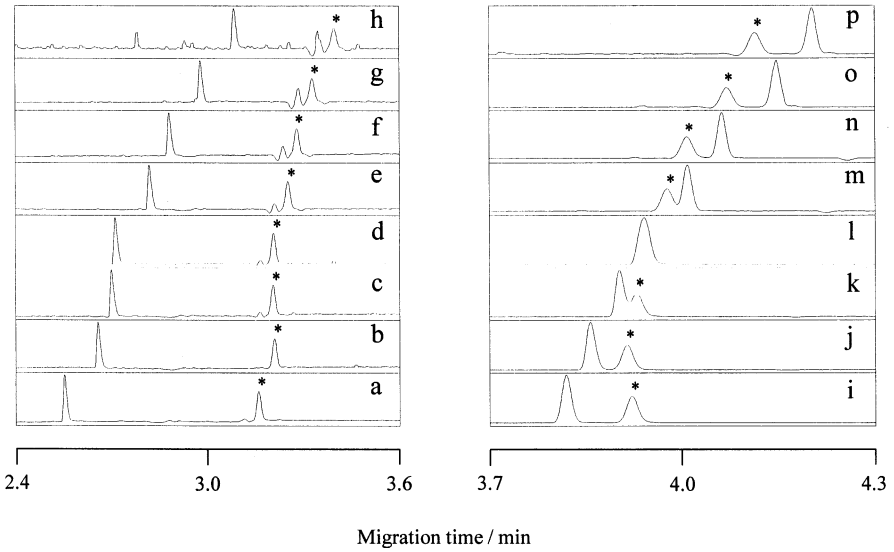

Fig. 1. Representative electropherograms for capillary electrophoresis of nicotine and DMSO, used as a neutral EOF marker, in the absence and presence of β-CD at 25 and 37°C and 100 mM ionic strength. The asterisks (*) mark the position of the DMSO peak while the dominant peak corresponds to nicotine. Electropherograms (a)–(h) were collected at pH 7.80 with [β-CD] = 0 (a), 1.0 (b), 2.0 (c), 3.0 (d), 5.0 (e), 7.0 (f), 10.0 (g) and 12.5 mM (f). Electropherograms (i)–(p) were collected at pH 9.31 with [β-CD] = 0 (i), 1.0 (j), 2.0 (k), 3.0 (l), 5.0 (m), 7.0 (n), 10.0 (o) and 12.5 mM (p). At pH 9.31, the difference in migration times between nicotine and DMSO is less than at pH 7.80 since a smaller fraction of the total nicotine is charged in the more alkaline buffer (pKa (nicotine H^+) = 8.05). Also, at pH 9.31 the migration time of nicotine is less than that for DMSO at [β-CD] < 5 mM while at [β-CD] > 5 mM the order is reversed.

$\Delta\mu^{max}_{N,CD}$ denotes the change in electrophoretic mobility of nicotine when fully bound to β-CD, l_c and l_d are the total length of the capillary and the length from the inlet of the capillary to the detector, respectively, and V is the applied voltage; $t_{N,CD}$, $t_{DMSO,CD}$, t_N, and t_{DMSO} are the migration times of nicotine and DMSO in presence and in absence of β-CD, respectively. The migration times used in the calculations are mean values from three or four consecutive electropherograms.

Fig. 1 shows typical electropherograms of nicotine and DMSO for increasing concentrations of β-CD at two different pH values. The significant changes in mobility of the neutral DMSO marker observed when the concentration of β-CD is increased (Fig. 1) indicate that the EOF is unstable and necessitate the use of an internal standard which has also been the case in many previous capillary electrophoresis studies. (Chu et al., 1992; Gomez et al., 1994; Kuhn et al., 1994; Kwak and Gomez, 1996). The observed stability constant can be determined from Scatchard analysis of the consecutive electropherograms by a linear least-squares fit of Eq. (1) to the data (Fig. 2). Table 1 summarizes the constants determined at 25 and 37°C at different pH. It was not possible to extend the investigation to pH > 9.6 due to serious base line disturbances and very small changes in mobility of nicotine at higher pH.

In the present interval, $5.7 \leq pH \leq 9.6$, neutral nicotine is in equilibrium with its monoprotonated form, Eq. (3).

$$\text{nicotine} + H^+ \rightleftarrows \text{nicotine}H^+;$$

$$K_a = [\text{nicotine}H^+]/[\text{nicotine}]\,[H^+] \quad (3)$$

The observed stability constant is the sum of the constants for the complexes formed by monoprotonated and neutral nicotine, K_1 and K_2, respectively (Eq. (4a), Eq. (4b)), with consideration taken to the relative concentrations of the two species, Eq. (5).

$$\text{nicotine}H^+ + \beta\text{-CD} \rightleftarrows \text{nicotine}H^+{:}\beta\text{-CD};\ K_1 \quad (4a)$$

$$\text{nicotine} + \beta - \text{CD} \rightleftarrows \text{nicotine}{:}\beta - \text{CD};\ K_2 \qquad (4b)$$

$$K_{obsd} = K_1[\text{nicotineH}^+]/C_{nicotine} + K_2[\text{nicotine}]/C_{nicotine} \qquad (5)$$

$C_{nicotine} = [\text{nicotineH}^+] + [\text{nicotine}]$ denotes the total concentration of free nicotine. Combination of Eq. (3) and Eq. (5) gives Eq. (6a) which can be rewritten as Eq. (6b).

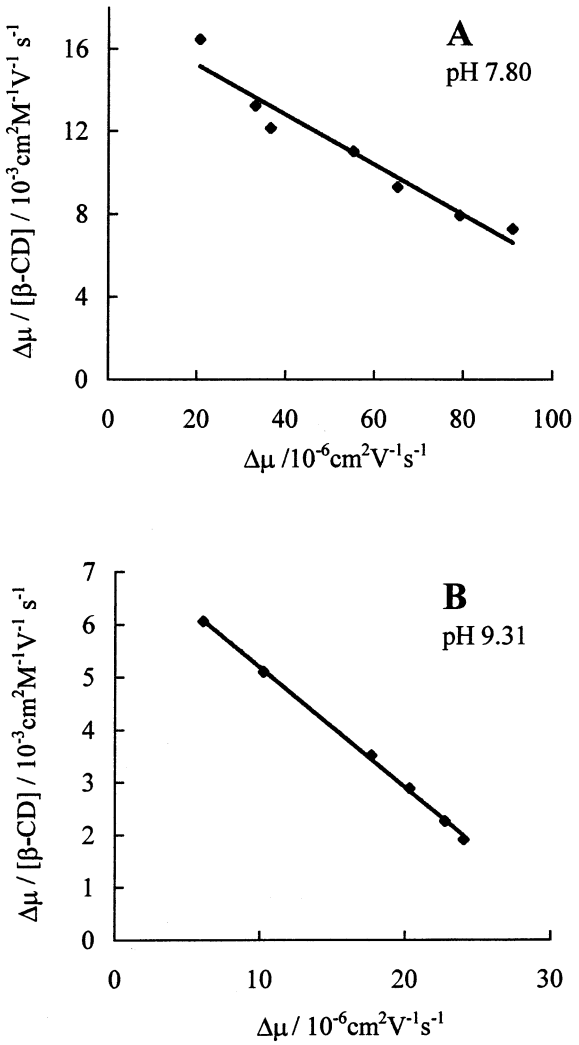

Fig. 2. Scatchard analysis of the electrophoretic data in Fig. 1. The solid line represents a linear least-squares fit of Eq. (1) to the data.

Table 1
The experimentally determined stability constant at 100 mM ionic strength and different pH for the inclusion complex between nicotine and β-cyclodextrin

pH	K_{obs}/M^{-1} (25°C)	K_{obs}/M^{-1} (37°C)
5.70	14 ± 3	34 ± 13
6.36	36 ± 4	22 ± 4
6.91	27 ± 5	32 ± 4
7.00	42 ± 10	
7.47	68 ± 2	80 ± 9
7.80	122 ± 14	126 ± 13
8.00	130 ± 12	
8.60	153 ± 7	148 ± 1
8.95	209 ± 3	172 ± 2
9.20	239 ± 9	
9.31	228 ± 4	197 ± 2
9.58	260 ± 6	209 ± 6

$$K_{obsd} = (K_1[H^+] + K_2K_a)/(K_a + [H^+]) \qquad (6a)$$

$$K_{obsd} = (K_1 10^{-pH} + K_2K_a)/(K_a + 10^{-pH}) \qquad (6b)$$

Thus, the stability constants for the complexes between β-CD and monoprotonated and neutral nicotine, respectively, can be determined by a non-linear least-squares fit of Eq. (6b) to the observed constants at different pH (Fig. 3). With K_a, K_1 and K_2 as adjustable parameters, the following results were obtained: $K_a = (8.9 \pm 4.0) \times 10^{-9}$ M^{-1} ($pK_a = 8.05$), $K_1 = 22 \pm 12$, M^{-1}, $K_2 = 242 \pm 11$ M^{-1} (25°C) and $K_a = (1.7 \pm 0.8) \times 10^{-8}$ M^{-1} ($pK_a = 7.77$), $K_1 = 22 \pm 12$, M^{-1}, $K_2 = 194 \pm 10$ M^{-1} (37°C). It can be concluded that neutral nicotine binds considerably stronger than the charged monoprotonated form to the hydrophobic β-CD cavity, as expected. A change in temperature from 25 to 37°C have a minor but significant effect only on the K_2 value. In a previous study (Han et al., 1984), a value of 358 M^{-1} was reported for the stability constant at pH > 10 (temperature not specified) which is somewhat larger than $K_2 = 242$ M^{-1} at 25°C reported here. In the non-linear fit of Eq. (6b) to the pH data, K_a was treated as an adjustable parameter (values in the range $7.87 < pK_a < 8.02$ for the dissociation of monoprotonated nicotine at 25°C can be found in the literature (Yamamoto, 1966; Dean, 1987; Lide, 1996)). The present value determined at 25°C ($pK_a = 8.05$) agrees well with literature data

for dilute aqueous solutions ($pK_a = 8.02$). It seems clear that nitrogenous bases in general become weaker as the temperature is increased (Albert and Serjeant, 1971), which is in agreement with the present results ($pK_a = 7.77$ at 37°C).

Several factors such as rate of salivation, tablet disintegration rate, and competitive binding to β-CD of other ligands in the oral cavity may influence the bioavailability of nicotine after administration of a sublingual tablet. The pH of whole saliva varies between 6.5 and 7.5 and it can be as low as 5.5 when it leaves the salivary ducts (Mandel and Wotman, 1976; Edgar, 1992). Due to the relatively small values of the stability constant for the nicotine:β-CD complex in this pH region (Fig. 3), a significant amount of nicotine will be uncomplexed and readily available for uptake after disintegration of the tablet; the predominant forms of the free nicotine will be the neutral and monoprotonated species. Recently, the kinetics and mechanisms for nicotine uptake through porcine mucosae have been studied and it has been shown that neutral nicotine permeates much faster than charged mono- and diprotonated nicotine across mucosal membranes (Nair et al., 1997). A more alkaline oral environment than is typically encountered would therefore facilitate the nicotine absorption since a larger fraction of the free nicotine would be in its neutral form (pK_a (nicotineH^+) = 8.05). In this study however, we have shown that neutral nicotine forms a stronger complex than monoprotonated nicotine with β-CD. Thus, since the rate of permeation of nicotine across mucosal membranes and the equilibrium concentration of uncomplexed nicotine have opposite pH dependencies, a pH optimum for nicotine absorption from the sublingual tablet might be expected.

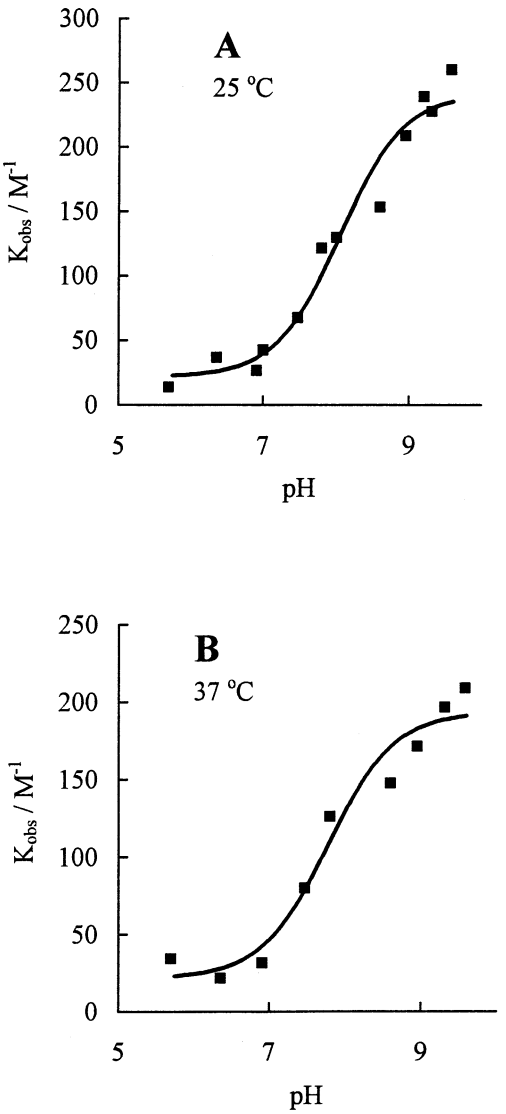

Fig. 3. The observed values of the stability constant for the inclusion complex between nicotine and β-CD as a function of pH at 100 mM ionic strength. The solid line represents a non-linear least-squares fit of Eq. (6b) to the data.

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