



# Inclusion Complex Formation of *p*-Nitrophenol and *p*-Nitrophenolate with Hydroxypropyl- $\beta$ -Cyclodextrins

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**Abstract.** The interaction of the *p*-nitrophenol/*p*-nitrophenolate conjugate has been investigated with different 2-hydroxypropyl- $\beta$ -cyclodextrin hosts. The measured values for stability constants attain a maximum as a function of increasing average degree of substitution. The quotients of constants, characterizing the inclusion of *p*-nitrophenolate and *p*-nitrophenol, respectively, are decreased showing that the effect of London dispersion forces is greatly overshadowed by the hydroxypropylation.

**Key words:** 2-hydroxypropyl- $\beta$ -cyclodextrin, *p*-nitrophenol, *p*-nitrophenolate, average degree of substitution, substitution pattern, stability constant

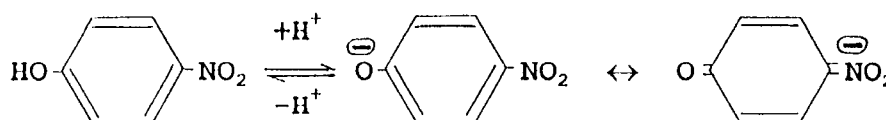
## 1. Introduction

The interest in cyclodextrins (CDs) and among them in 2-hydroxypropyl- $\beta$ -CD (HP-CD) is continuously growing as demonstrated by the excellent monograph published recently [1]. The parent  $\beta$ -CD is hydroxypropylated using propylene oxide in alkaline solution [2, 3] and both the average degree of substitution (DS) and the substitution pattern depend on the experimental conditions, mainly on the alkalinity used during the preparation [2, 3, 4]. These parameters of the product have been shown to greatly influence its inclusion complex forming ability [4].

The study of the interaction between CDs and nitrophenols (NPs) started with the supposition that the acidity of phenols should be generally strengthened by inclusion complex formation [5]. It soon became clear that the finding was mainly valid for *p*-NP [6], as most of the other phenols form complexes of less acidic character (i.e. the complex formation constant of the undissociated phenols with CDs are higher than that of their charged conjugate). E.g. the  $pK_A$  of phenol itself changes from 9.82 to 10.75 when complexed with  $\beta$ -CD, the ratio shows a similar trend in the case of *o*-NP (7.05  $\rightarrow$  7.21) and *m*-NP (8.09  $\rightarrow$  8.33) and only *p*-NP

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Scheme 1.

has a reversed character: its  $pK_A$  is 6.90 while the value changes to 6.40 when complexed by  $\beta$ -CD [7] (for explanation of different  $pK_A$ s consult Figure 2).

The phenomena can be explained considering that the resonance charge delocalization (depending also on pH, see Scheme 1) in the  $p$ -NPate hinders the hydration but promotes the interactions by London dispersion forces [7, 8, and Refs. therein].

The interaction can be easily followed by spectrophotometry, and  $p$ -NP was recommended as a competing reagent for stability constant determination in systems containing  $\alpha$ -CD [9]. The  $p$ -NP- $\alpha$ -CD system seems to be the one studied most intensively and the collected data [10] are used to demonstrate the reproducibility of equilibrium constants measured by different researchers and methods.

As a gradual decrease of stability constants as a function of increasing DS has been measured using phenolphthalein as guest [4], the investigation of the interaction between the  $p$ -NP/ $p$ -NPate conjugate and HP-CDs of different DS seemed to be promising.

## 2. Experimental

All chemicals used were of analytical grade. The HP-CD samples investigated were from Cyclolab Ltd., Hungary. Based on the ratio of substitution on the primary and secondary hydroxy rims [2], the samples can be roughly divided into three categories: (i) the ratio of substitution numbers characterizing the primary and secondary hydroxy groups separately is  $\ll 1.0$ , (ii) it is  $\sim 1.0$ , and (iii) the value is  $\gg 1.0$ .

The spectra were recorded on a Perkin-Elmer Lambda 15 spectrophotometer, and the absorbances measured with a Spectromom 195D spectrophotometer. The final concentration of NP was  $5.0 \times 10^{-5}$  and  $1.0 \times 10^{-4}$  M, that of different HP-CDs was changed in the range of  $1.0 \times 10^{-4}$ – $1.0 \times 10^{-2}$  M. All systems were investigated at three pH values (pH = 6.4; 6.8 and 7.2, using phosphate buffers of appropriate capacity) and at three wavelengths ( $\lambda = 300$ ; 317 and 402 nm). The ionic strength was adjusted to 0.2 M using NaCl, and the temperature was kept constant at  $25 \pm 1$  °C.

Induced circular dichroism spectra were measured using a Jobin-Yvon-Dichrograph Mark VI.

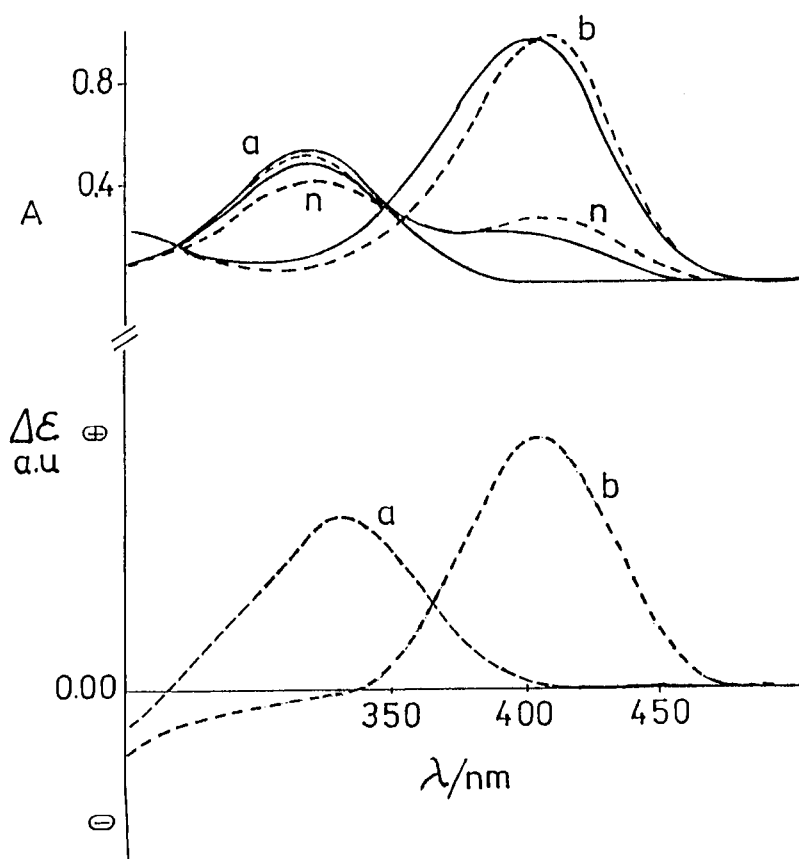


Figure 1. Induced circular dichroism (bottom) and uv-vis absorption (top) spectra of *p*-nitrophenol and its complex with a HP-CD sample (DS = 5.0). Full lines:  $5.36 \times 10^{-5}$  M *p*-NP itself, dashed lines: in the presence of  $5.0 \times 10^{-3}$  M HP-CD; a: pH = 2, b: pH = 12, n: pH = 6.4. (The molar absorptivities calculated at  $\lambda = 300, 317$  and  $402$  nm have the following values:  $\lg \epsilon_{110}^{\lambda} = 3.990, 3.939$  and  $-$ ;  $\lg \epsilon_{010}^{\lambda} = 3.107, 3.413$  and  $4.281$ ;  $\lg \epsilon_{111}^{\lambda} = 3.907, 3.992$  and  $-$ ;  $\lg \epsilon_{011}^{\lambda} = 2.945, 3.167$  and  $4.256$ ; respectively.)

### 3. Results and Discussion

HP-CD has a similar effect on the *p*-NP/*p*-NPate system to that of  $\beta$ -CD [7], as evidenced by uv-vis and induced circular dichroism spectroscopies (Figure 1).

The system can be characterized completely as

$$c_{\text{NP}} = \sum \sum \sum q \beta_{pqr} [\text{H}^+]^p [\text{NPate}^-]^q [\text{HP-CD}]^r \quad (1)$$

and

$$c_{\text{HP-CD}} = \sum \sum \sum r \beta_{pqr} [\text{H}^+]^p [\text{NPate}^-]^q [\text{HP-CD}]^r \quad (2)$$

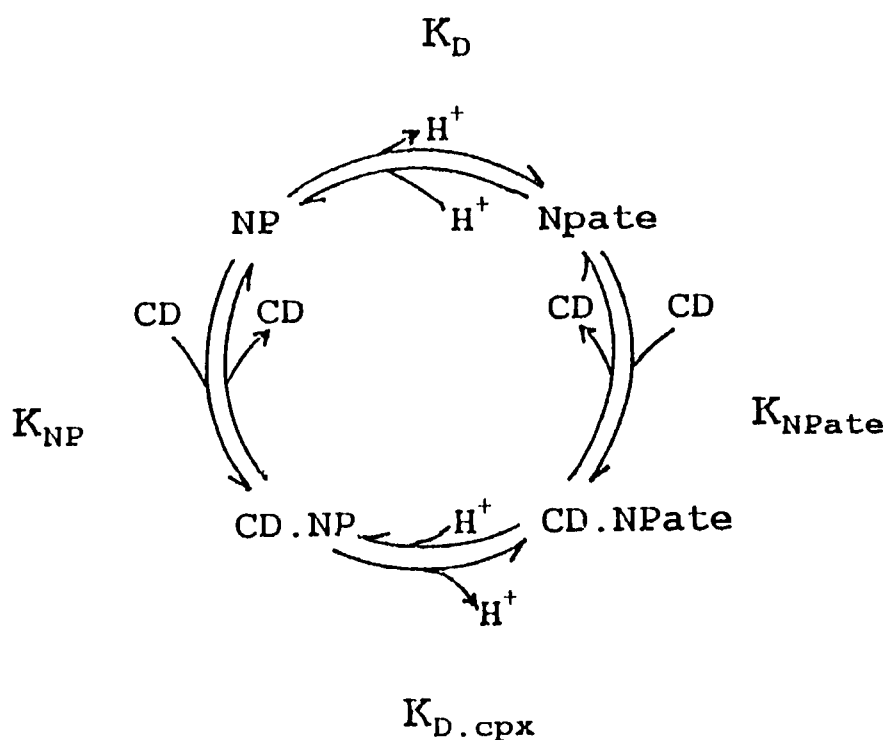


Figure 2. Interrelation of equilibria in the *p*-nitrophenol/*p*-nitrophenolate-HP-CD system.

where  $c_{\text{NP}}$  is the total concentration of *p*-NP,  $c_{\text{HP-CD}}$  is that of HP-CD, square brackets denote equilibrium concentrations, while  $\beta_{pqr}$  is the constant characterising the equilibrium:



The measured absorbance ( $A$ ) can be expressed as:

$$A_\lambda = \Sigma \Sigma \Sigma \epsilon_{pqr}^\lambda \beta_{pqr} [\text{H}^+]^p [\text{NPate}^-]^q [\text{HP-CD}]^r \quad (4)$$

where  $\lambda$  is the wavelength used and  $\epsilon_{pqr}^\lambda$  is the molar absorptivity of the given species (at the given  $\lambda$ ).

$\epsilon_{010}^\lambda$  (the molar absorptivity of NPate) and  $\epsilon_{110}^\lambda$  (the molar absorptivity of NP) can be determined separately in alkaline, and acidic solution (at any wavelength). Measuring these values and a great number of absorbances with solutions of different composition, (of known total concentrations ( $c_{\text{NP}}$  and  $c_{\text{HP-CD}}$ )), using Equations (1–4) and some starting assumptions for the different  $\beta_{pqr}$  and  $\epsilon_{pqr}^\lambda$  constants, the 1 : 1 stoichiometry of the system was proved.

Thus, the system can be characterized as represented in Figure 2. (Later, the  $K_{\text{NP}}$  and  $K_{\text{NPate}}$  complex formation constants will be used instead of  $\beta_{111}$  and  $\beta_{011}$ , as they are defined in Figure 2.)

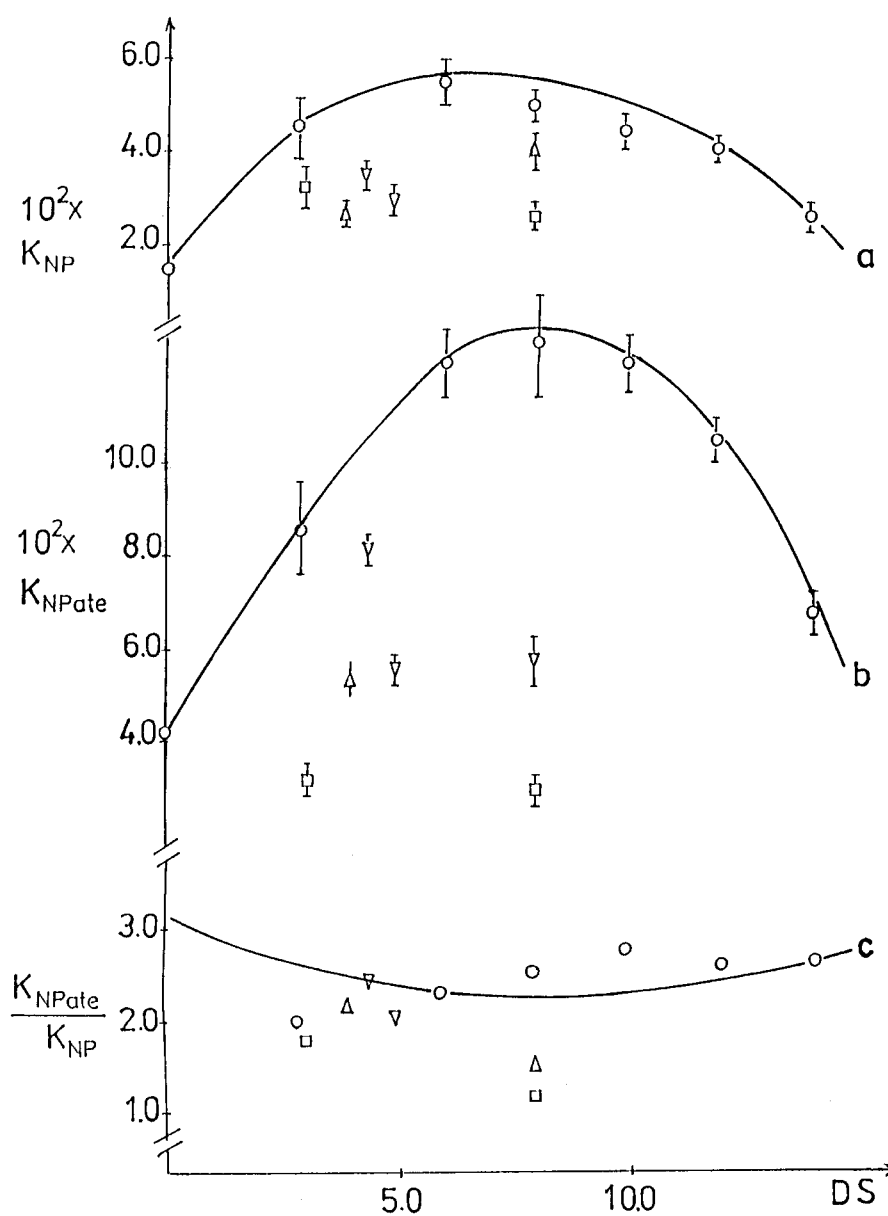


Figure 3. Effect of the change in the average degree of substitution (DS) on the complex formation constants of *p*-nitrophenol (a), *p*-nitrophenolate (b), and on the  $K_{NPate}/K_{NP}$  quotient (c). HP-CD samples:  $\circ$  = the ratio of substitution on primary vs. secondary hydroxy rim is  $< 1.0$ ;  $\Delta$  and  $\nabla$  = this ratio is  $\sim 1.0$ ;  $\square$  = the ratio is  $\gg 1.0$ .

According to our experiences, to determine the equilibrium constants from measurements at different pH values around the  $pK_D$  (6.90) and at the maxima of absorbances (see the experimental procedures and Figure 1) as recommended also by Gelb et al. [9] is better both in sensitivity and reproducibility than the method using the wavelength of the isosbestic point [11] (where the absorbance is lower and the isosbestic point itself is shifted a little as a function of  $c_{CD}$ ). Figure 1 demonstrates this solution, where, for comparison, the molar absorptivities calculated for a HP-CD sample of  $DS = 5$  are also given.

The measured and evaluated data of  $K_{NP}$  values (together with the reference to their reproducibility) are shown in Figure 3a and prove that they increase with increasing  $DS$  up to a maximum ( $DS \sim 8$ ), after which further increase of  $DS$  evokes a fast decrease of  $K_{NP}$ . As Figure 3b demonstrates, the picture is also similar with  $K_{NPate}$  values. In both Figs., the effect of the substitution pattern is explicit, first of all for group (i) mentioned previously, and the inclusion seems to be mainly influenced by the substitution on the primary hydroxy groups as in the case of phenolphthalein [4].

It follows that a relatively small substitution of  $\beta$ -CD by HP groups does not hinder but promotes the inclusion of both  $p$ -NP and  $p$ -NPate. This effect can be explained by (i) extending and (ii) (partly) covering (“protecting”) the CD cavity by HP substituents (iii) the possibility of new H-bondings. (As in addition to the guest and CD’s hydroxy groups, HP hydroxy groups are also able to act as proton donors and/or acceptors. In spite of the fact, that no direct evidence is given in this special case, this effect cannot be excluded, as experienced earlier [12].) When the  $DS$  becomes larger (i.e. the number of HP substituents is high), effect (ii) dominates the others, and the increase of stability of inclusion complexes is stopped, or even decreased because the “protecting” effect turns to steric hindrance.

Comparing Figure 3a and 3b, the stability constants seem to change parallel at first sight but representing the  $K_{NPate}/K_{NP}$  quotients as a function of  $DS$  (Figure 3c), (i) the highest value can be seen at  $DS = 0$  (i.e. in the case of  $\beta$ -CD itself); (ii) it is also influenced greatly by the substitution pattern, and (iii) the quotients show a very slight but recognizable minimum at about  $DS = 8$ . The generally decreased ratio suggests that the role of London dispersion forces is surpassed by the other contributions, i.e. the increase in  $K_{NPate}$  is relatively smaller.

These findings point again to the extremely fine balance among the forces producing the inclusion complexes.

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