ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
29 (2002) 425–430

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Chromatographic determination of the association constants between nimesulide and native and modified β-cyclodextrins

Corinne Ravelet [a], Annabelle Geze [b], Annick Villet [a], Catherine Grosset [a], Anne Ravel [a], Denis Wouessidjewe [b], Eric Peyrin [a,*]

[a] *Laboratoire de Chimie Analytique, UFR de Pharmacie, Domaine de la Merci, 38700 La Tronche, France*
[b] *Laboratoire de Pharmacotechnie, Département de Pharmacochimie Moléculaire, UFR de Pharmacie, Domaine de la Merci, 38700 La Tronche, France*

Received 19 December 2001; received in revised form 30 January 2002; accepted 17 February 2002

Abstract

The retention of a non-steroidal anti-inflammatory drug (NSAID), i.e. nimesulide, in high performance liquid chromatography (HPLC) was investigated using a phenyl bond silica column and β-cyclodextrin (β-CD) or hydroxypropyl-β-cyclodextrin (HP-β-CD) as mobile phase additive (0–10 mM). Such a study was carried out in order to determine the most efficient cyclodextrin as a potential drug complexing agent for a future application in pharmaceutical formulation. Assuming a 1:1 stoichiometry, the association constants (K) were calculated from the chromatographic data. At a column temperature of 25 °C and in a highly aqueous medium (98% phosphate buffer-2% methanol (v/v)), K was equal to 523 and 1285 M^{-1} for the nimesulide-β-CD and nimesulide HP-β-CD complexes, respectively. These results were consistent with the data reported previously using phase solubility studies and UV spectrophotometry. As well, the thermodynamic parameters of the inclusion complexes were determined from linear van't Hoff plots for the two inclusion complexes. From the enthalpy and entropy changes, it appeared that nimesulide interact more strongly with HP-β-CD due to a significant hydrophobic effect between the compound and the flexible hydroxypropyl groups.

Keywords: High performance liquid chromatography; β-Cyclodextrin; Hydroxypropyl-β-cyclodextrin; Nimesulide; Association constant

1. Introduction

Several reports have shown the utility of the use of native or modified β-cyclodextrin in pharmaceutical formulation to improve the bioavailibility of drugs [1–6]. Various methods have been used to determine the association constants between cyclodextrin and drugs. UV–visible absorption, NMR, potentiometry fluorescence measurements, capillary electrophoresis and calorimetry have been described [7–11]. Chromatographic experiments have also been previously carried out for the determination of the apparent association constant of various drugs with both native or

* Corresponding author. Tel.: +33-4-7663-7145; fax: +33-4-7651-8667.
E-mail address: eric.peyrin@ujf-grenoble.fr (E. Peyrin).

0731-7085/02/$ - see front matter
PII: S0731-7085(02)00088-2

derivatized cyclodextrins [12–16]. The retention behavior of solute in high performance liquid chromatography (HPLC) is based on the partitioning of the solute between the mobile and the stationary phases. When cyclodextrin is added to the mobile phase, solute retention is split into two main physicochemical processes, i.e. solute complexation by cyclodextrin and transfer of free (i.e. uncomplexed) solute from the mobile to the stationary phase. The association constant K between compound and cyclodextrin can be determined using the well known equation [12–16]:

$$\frac{1}{k} = \frac{1}{k_0} + \frac{K[\mathrm{CD}]^x}{k_0}$$

where k is the solute retention factor, k_0 the solute retention factor without cyclodextrin in the mobile phase, [CD] the cyclodextrin concentration and x the stoichiometry of the complex. For an inclusion complex with a 1:1 stoichiometry ($x = 1$), a linear plot of $1/k$ versus [CD] must be obtained and the K value calculated.

Nimesulide (4-nitro-2-phenoxymethanesulfonanilide, see Fig. 1) is a non-steroidal anti-inflammatory drug (NSAID). It is characterised by its capacity to inhibit selectively the cyclo-oxygenase-2 and block the synthesis of platelet activating factors and tumour necrosis factor. As well, it seems that this drug causes less severe gastrointestinal side effects compared with the other NSAIDs. However, its poor aqueous solubility poses bioavailability problems in vivo [17]. This could be overcome by the formation of inclusion complexes with cyclodextrins. Three previous reports, based on phase solubility studies [18–20] and UV spectrophotometry-based method [20], have been carried out in order to determine the stoichiometry and the apparent association constant of nimesulide-β-cyclodextrin complex.

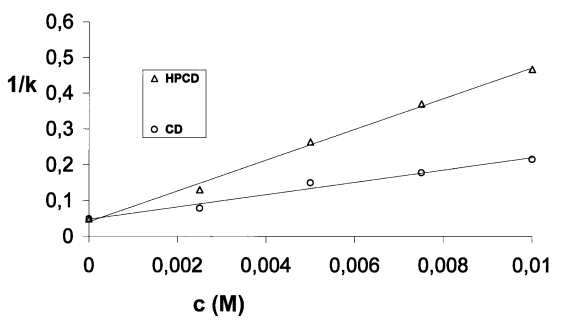

Fig. 1. Plots of $1/k$ vs. β-CD concentration and β-HPCD concentration (assuming 1:1 stoichiometry) for nimesulide at a column temperature equal to 40 °C. Stationary phase: phenyl silica gel; Mobile phase: mixture of phosphate buffer (50 mM) pH 7.7–methanol: 98-2 (v/v).

The aim of this paper was to investigate the stoichiometry and the association constants for the complexes between nimesulide and native (β-CD) or modified (hydroxypropyl: HP-β-CD) β-cyclodextrin using a chromatographic approach. This was done by using a phenyl silica gel as stationary phase and cyclodextrins as mobile phase additives. The association constant data were compared to the values previously determined by phase solubility studies and UV spectrophotometric method. As well, the thermodynamic parameters, extracted from van't Hoff plots, were analysed in order to gain further information about the association mechanism.

2. Experimental

2.1. Apparatus

The HPLC system consisted of a LC Shimadzu pump 10AT (Touzart et Matignon, Courtaboeuf, France), a Rheodyne injection valve model 7125 (Interchim, Montluçon, France) fitted with a 20-μl sample loop, a Shimadzu SPD-10A UV–vis detector. A Nucleosil 250 mm × 4 mm phenyl column (7 μm, particle size) supplied by Macherey–Nagel (Dueren, Germany) was used with controlled temperature in an Interchim Igloocil oven (Montluçon, France). The mobile phase rate was set at 0.8 ml min^{-1} and the wavelength at 240 nm.

2.2. Reagents

Nimesulide was provided from Sigma–Aldrich (Saint Quentin Fallavier, France). Stock standard solutions of nimesulide in methanol (0.85 mg/ml) were prepared and stored at 4 °C. The cyclodextrins β-CD and HP-β-CD were provided from

Roquette (Lestrem, France). Sodium hydrogen phosphate and sodium dihydrogen phosphate were purchased from Carlo Erba Reactifs (Val de Reuil, France). LC-purity methanol was analytical reagent (SDS, Villeurbanne, France). LC grade high quality water was obtained from Stillplus HP system (Oxon, UK). The potassium iodide (RP grade, Merck Germany) was used to determine the void volume of the column.

2.3. Chromatographic conditions

The mobile phase consisted of a mixture of phosphate buffer (50 mM) pH 7.7–methanol: 98-2 (v/v) with various β-CD and HP-β-CD concentrations (0; 0.5; 2.5; 5, 7.5 and 10 mM). Twenty microliters of each solute were injected in triplicate and the retention times measured. All the experiments were repeated three times for each temperature and for each cyclodextrin concentration.

2.4. Temperature studies

The retention factor was determined at the following temperatures: 25, 30, 35 and 40 °C. The chromatographic system was allowed to equilibrate for at least 1 h prior to each experiment. $\Delta H°$ and $\Delta S°$ are, respectively, the standard enthalpy and entropy of transfer of the nimesulide from the mobile phase to the cyclodextrin. These energies can be calculated using the following thermodynamic relationships as previously described [21,22]:

$$\ln K = \frac{-\Delta H°}{RT} + \frac{\Delta S°}{R}$$

where T is the temperature and R the gas constant. For a linear plot of $\ln K$ versus $1/T$, the slope and intercept are respectively $-\Delta H°/R$ and $\Delta S°/R$.

3. Results and discussion

3.1. Chromatographic determination of the apparent association constants for the nimesulide–cyclodextrin complexes and comparison with the literature data

In order to calculate the association constant in a highly aqueous media (98% of aqueous phase), the chromatographic experiments were carried out using a phenyl silica gel as stationary phase. The use of a relatively weak apolar stationary phase allowed the determination of the 'pure' apparent formation constants of nimesulide, i.e. without the use of significant amount of a organic modifier in the mobile phase. A similar approach has been recently reported for the determination of inclusion constants between very apolar compounds (PAHs) and various cyclodextrins [22].

Using the solute retention time and the void time, the k values were determined for all the cyclodextrin concentrations at temperatures of 25, 30, 35 and 40 °C. The coefficients of variation of the k values were $< 0.5\%$, indicating a high reproducibility and a good stability for the chromatographic system. The $1/k$ versus [β-CD] and $1/k$ versus [HP-β-CD] plots were determined and the values of the linear regression coefficients R were calculated. The R values are higher than 0.969 in all cases. For example, Fig. 1 shows the two plots for a column temperature equal to 40 °C. The values of apparent association constants are presented in Table 1 for the various column temperatures. From the R values, it appears clearly that the behavior of nimesulide is well described by the model assuming a 1:1 stoichiometry for the two complex types. This is consistent with the results of Chowdary et al. [18], Vavia et al. [19] and Miro et al [20] who have shown by phase solubility studies that nimesulide forms with β-CD and HP-β-CD a 1:1 stoichiometry complex. Moreover, this 1:1 stoichiometry is classically described for the formation of NSAID-cyclodextrin complexes [23–26]. The K value between nimesulide and β-CD, calculated from the chromatographic experiment, is higher than the association constant determined by Chowdary et al. [18] and Vavia et al. [19] (Table 1). In these phase solubility studies, the operating conditions, i.e. pH, ionic strength, temperature, are not provided by the authors and then, the comparison with our data is not significant. On the other hand, our K value agrees very well with the data from Miro et al. [20] (523 versus 533 M^{-1}/574 M^{-1} at 25 °C) who have performed their experiment in operating condi-

Table 1
Apparent association constants K between nimesulide and β-CD or HP-β-CD determined by chromatographic procedure at various column temperatures and comparison with the literature data

Methods	Chromatographic experiments				Phase solubility studies			UV
References	Present study				[18]	[19]	[20]	[20]
Solution	PB[a] 50 mM–methanol 98/2 (v/v), pH 7.7				na[b]	na	Water	PB 100 mM, pH 7.0
Temperature (°C)	25	30	35	40	na	na	25	25
K (β-CD) (M^{-1})	523	461	385	355	156	94	533	574
K (HP-β-CD) (M^{-1})	1285	1159	1092	1060	–	123	–	–

[a] PB: phosphate buffer.
[b] Not available.

tions similar to our chromatographic conditions (Table 1). Such an observation shows the reliability of the high performance liquid chromatographic method for the evaluation of the association constant and stoichiometry between nimesulide and cyclodextrin. The apparent association constant value obtained for the nimesulide-β-CD complex is in the same order of magnitude than the association constants obtained between cyclodextrins and other NSAIDs. For example, the K values (at 25 °C) determined by capillay electrophoresis [23], UV spectrophotometry [24] or phase solubility analysis [25,26] are equal to 325 M^{-1} for fenoprofen-β-CD at pH 7.1 [23], 340 M^{-1} for sulindac-β-CD association at pH 2 [24], 1280 M^{-1} for ibuprofen-β-CD at pH 7.1 [23], 1702 M^{-1} for naproxen-β-CD at pH ≈ 5 [25] and 4340 M^{-1} for flurbiprofen-β-CD at pH ≈ 5 [26]. As shown in Table 1, the association constant for the nimesulide-HP-β-CD complex is higher than the K value for the nimesulide-β-CD association. This is qualitatively in accordance with a previous study [19] which has shown that HP-β-CD is able to interact more strongly with nimesulide than β-CD (Table 1). Similar behaviors have been described by Bettinetti et al. for the naproxen-HP-β-CD or naproxen-hydroxyethyl-β-CD complexes [25] and Uekama et al. for the flurbiprofen-methyl-β-CD complex [26]. When the native cyclodextrin is replaced by the modified cyclodextrins, the apparent association constant values are enhanced from 1702 to 2581 or 6892 M^{-1} (for the naproxen–cyclodextrin associations) and from 4340 to 10060 M^{-1} (for the flurbiprofen–cyclodextrin associations).

3.2. Thermodynamic parameters for the nimesulide–cyclodextrin complexes

In order to gain information about the mechanistic aspect of the difference in the solute affinity for β-CD and HP-β-CD, the thermodynamic parameters were obtained from van't Hoff plots. The ln K versus $1/T$ plots were obtained for the two cyclodextrins. Linear van't Hoff plots were obtained with correlation coefficient higher than 0.971. Fig. 2 shows the van't Hoff plots corresponding to the two inclusion complexes. $\Delta H°$ and $\Delta S°$ for the two complexes are presented in Table 2 with the corresponding Gibbs free energy $\Delta G°$ at 25 °C. A strong difference is observed between the β-CD and HP-β-CD complexes in

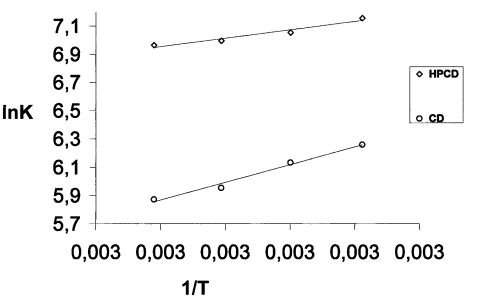

Fig. 2. van't Hoff plots (ln K vs. $1/T$) for nimesulide-β-CD or nimesulide-β-HPCD associations. Stationary phase: phenyl silica gel; Mobile phase: mixture of phosphate buffer (50 mM) pH 7.7–methanol: 98-2 (v-v).

Table 2
Thermodynamic parameters $\Delta H°$, $\Delta S°$ and $\Delta G°$ (at 25 °C) for the associations between nimesulide and β-CD or HP-β-CD[a]

NSAID-CD complexes	$\Delta H°$ (kJ/mol)	$\Delta S°$ (kJ/mol K)	$\Delta G°$ (kJ/mol)
Nimesulide-β-CD	−20.8	−17.9	−15.5
Nimesulide-HP-β-CD	−9.9	+26.1	−17.7
Naproxen-HP-β-CD[b]	−17.2	+7.6	−19.5
Naproxen-ME-β-CD[b]	−11.5	+35.1	−21.9
Naproxen-HE-β-CD[b]	−8.7	+34.5	−19.0
Flurbiprofen-ME-β-CD[c]	−15.0	+24.7	−22.4

[a] Comparison with thermodynamic data for various NSAID-modified cyclodextrin complexes.
[b] Ref. [25]; ME, methyl; HE, hydroxyethyl.
[c] Ref. [26].

terms of enthalpic and entropic parameters. For the nimesulide-β-CD complex, the enthalpic and entropic terms are both negative demonstrating that the association is only enthalpically driven. The formation of an inclusion complex with cyclodextrin is classically caused by interactions such as hydrogen bonding with the OH groups at the periphery of the cavity, van der Waals interactions and hydrophobic effect [27]. In most cases, the solute inclusion in the cyclodextrin cavity is associated to large negative values of $\Delta H°$ and $\Delta S°$ [14,28,29]. The enthalpic term value for nimesulide complexation by β-CD is interpreted as indicating that the binding forces included strong van der Waals–London dispersion interactions. This is associated to a negative value of $\Delta S°$ related to the apparent low degrees of freedom of the solute included in the rigid cyclodextrin cavity. On the other hand, for the nimesulide-HP-β-CD complex, ΔH° exhibits a weak negative value while $\Delta S°$ has a positive value. This demonstrates that the association phenomenon is both enthalpically and entropically driven. At 25 °C, the contributions of the enthalpic and entropic terms to the Gibbs free energy are roughly identical, i.e. 56% for $\Delta H°$ and 44% for $T\Delta S°$. These thermodynamic parameters suggest that the nimesulide-β-HPCD association is dependent on the hydrophobic effect. Water molecules in contact with non-polar species adopt an ordered organisation. The release of water molecules when a solute is transferred from a polar to a nonpolar bulk phase results in an entropy increase ($\Delta S° > 0$) associated to a weak negative value of $\Delta H°$ [30]. It is expected that the increase in the solute affinity for HP-β-CD (relatively to the nimesulide affinity for β-CD) is predominantly governed by a significant hydrophobic effect between nonpolar groups of solute and the hydroxypropyl groups of the modified cyclodextrin. Similar observations have been reported by Bettinetti et al. [25] and Uekama et al. [26] for the NSAID association with both HP-β-CD, hydroxyethyl-β-CD and methyl-β-CD (Table 2). It has been demonstrated that the favorable entropy changes, associated to the enhancement of inclusion ability of HP-β-CD, hydroxyethyl-β-CD and methyl-β-CD toward naproxen and flurbiprofen, is due to the hydrophobic binding contribution of the apolar groups in the host molecule.

4. Conclusion

This paper describes the use of a relatively weak apolar stationary phase such as a phenyl silica gel in order to study the nimesulide–cyclodextrin inclusion phenomenon in a highly aqueous mobile phase. The reliability of this chromatographic procedure is demonstrated by comparing the apparent association constant and stoichiometry to those previously obtained by phase solubility studies and UV-spectrophotometry method in roughly the same operating conditions. It is shown that nimesulide has an affinity approximately two fold more important for the HP-β-CD than for the β-CD. This result is consistent with the data obtained from previous studies on the

association between NSAIDs and native or modified cyclodextrins. From the thermodynamic results, it is expected that nimesulide interacts more strongly with HP-β-CD due to the hydrophobic effect between the compound and the apolar moiety of the flexible hydroxypropyl groups.

References

[1] M. Otagiri, T. Imai, K. Uekama, J. Pharmacobiodyn. 12 (1982) 1027.
[2] X. Deroubaix, A. Stockis, A.M. Allemon, E. Lebacq, A. Acerbi, P. Ventura, Eur. J. Clin. Pharmacol. 47 (1995) 531.
[3] Y.C. Guillaume, E. Peyrin, J. Millet, J. Chromatogr. B 728 (1999) 167.
[4] A.R. Bary, I.G. Tucker, N.M. Davies, Eur. J. Pharm. Biopharm. 50 (2000) 237.
[5] M.S. Nagarsenker, R.N. Meshram, G. Ramprakash, J. Pharm. Pharmacol. 52 (2000) 949.
[6] B. Cappelo, C. Carmignani, M. Iervolino, M. Immacolata La Rotonda, M.F. Saettone, Int. J. Pharm. 213 (2001) 75.
[7] S. Hamai, J. Phys. Chem. 93 (1989) 2074.
[8] K.A. Connors, Binding Constants: a measure of Molecular Complex Stability, John Wiley and Sons, New York, 1987.
[9] P.W. Carr, J.M. Harris, Anal. Chem. 58 (1986) 626.
[10] A. Munoz de la Pena, T.T. Ndou, J.B. Zung, I.M. Warner, J. Phys. Chem. 63 (1991) 1018.
[11] Y.C. Guillaume, E. Peyrin, Anal. Chem. 71 (1999) 2046.
[12] K. Shimada, K. Mitamura, M. Muira, A. Miyamoto, J. Liq. Chromatogr. 18 (1995) 2885.
[13] E. Peyrin, Y.C. Guillaume, Chromatographia 49 (1999) 691.
[14] N. Morin, Y.C. Guillaume, E. Peyrin, J.C. Rouland, J. Chromatogr. A 808 (1998) 51.
[15] K.H. Lamparczyk, P.K. Zarzycki, J. Pharm. Biomed. Anal. 13 (1995) 543.
[16] S. Shuang, M.M. Choi, J. Chromatogr. A 919 (2001) 321.
[17] A.K. Singla, M. Chawla, A. Singh, J. Pharm. Pharmacol. 52 (2000) 467.
[18] K.P. Chowdary, B.N. Nalluri, Drug. Dev. Ind. Pharm. 26 (2000) 1217.
[19] P.R. Vavia, N.A. Adhage, Drug. Dev. Ind. Pharm. 25 (1999) 543.
[20] A. Miro, F. Quaglia, A. Calignano, F. Barbato, B. Cappello, M.I. La Rotonda, STP Pharm. Sci. 10 (2000) 157.
[21] E. Peyrin, Y.C. Guillaume, C. Grosset, A. Villet, A. Ravel, J. Alary, Anal. Chim. Acta 428 (2001) 83.
[22] C. Ravelet, E. Peyrin, A. Villet, C. Grosset, A. Ravel, J. Alary, Chromatographia 53 (2001) 624.
[23] Y.Y. Rawje, D.U. Staerk, G. Vigh, J. Chromatogr. 635 (1993) 291.
[24] M.C. Tros de Ilarduya, C. Martin, M.M. Goni, M.C. Martinez-Oharriz, Drug. Dev. Ind. Pharm. 24 (1998) 301.
[25] G. Bettinetti, F. Melani, P. Mura, R. Monnanni, F. Giordano, J. Pharm. Sci. 80 (1991) 1162.
[26] K. Uekama, T. Imai, T. Maeda, F. Hirayama, M. Otagiri, J. Pharm. Sci. 74 (1985) 841.
[27] A.M. Stalcup, S.S. Chang, D.W. Armstrong, J. Pitha, J. Chromatogr. 513 (1990) 181.
[28] W.Q. Tong, J.L. Lach, T.F. Chin, J.K. Guillory, J. Pharm. Biomed. Anal. 9 (1991) 1139.
[29] K.G. Flood, E.R. Reynolds, N.H. Snow, J. Chromatogr. A 903 (2000) 49.
[30] S.J. Gill, I. Wadso, Proc. Natl. Acad. Sci. USA 73 (1976) 2955.