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THE EFFECTS OF INCREASING NaCl CONCENTRATION ON THE STABILITY OF INCLUSION COMPLEXES IN AQUEOUS SOLUTION

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Abstract

Equilibrium constants and standard molar enthalpies of reaction were determined by titration calorimetry for the reaction of 1-butanol with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) in aqueous solution at different concentrations of NaCl (0–1.9 M). The standard molar free energy and entropy changes associated with the complexation were calculated from the corresponding equilibrium constants, *K*, and standard enthalpies determined experimentally. In NaCl solutions the inclusion complexes ButOH/HP-β-CD are more stable than in water and their stability increases at increasing NaCl concentration; otherwise, the standard molar enthalpy associated to the formation of the complexes does not change with the increasing of salt concentration. The dependence of *K* on NaCl concentration were used to evaluate the number of water molecules displaced from the hydration shells of HP-β-CD and ButOH in forming complexes.

Keywords: cyclodextrins, inclusion complexes, isothermal titration calorimetry, NaCl

Introduction

The use of cyclodextrins (CDs) as pharmaceutical excipients in the formulation of poorly water-soluble drugs is particularly intriguing because of the ability of these molecules to improve the pharmacological efficacy of the drugs [1, 2]. In fact, it has widely been observed that the presence of CDs often produces an increase of drug water solubility, bioavailability and stability.

These interesting properties are the result of CDs' structure characterised by a toroidal shape with a hydrophilic outer surface and a hydrophobic central cavity which make them water soluble and able to include molecules having suitable size and polarity.

Although the mechanism of CDs' effects on drugs has not been fully explained, it is known that CDs enhance the permeation of poorly soluble drugs through biological membranes. They act as non-conventional penetration enhancers, by increasing drug availability at the surface of biological membranes without disrupting the lipid

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layers. In particular CDs keep lipophilic drugs in solution and deliver them to the surface of the biological membrane; here the drugs distribute between CDs and membranes [3, 4]. According to this mechanism and to experimental evidence, the lowest CD concentration necessary should be used to solubilize the drug; an excess amount of CDs indeed leads to a decrease of the absorption of the drug [5]. Because the amount of CD necessary to solubilize a given quantity of a drug decreases at increasing of the value of the binding constant, a possible strategy for reducing the amount of CD can be that of introducing in the formulation some substances which are able to improve the efficiency of complexation of CDs. At this aim various water soluble polymers have already been taken into consideration; in fact, they have shown to be able to increase the stability constants of the CD-drug complexes [6–8]. Electrolytes are another important class of substances that can be used for the same purpose. Recently we have undertaken a systematic study on the effect of increasing salt concentration on the complexation efficiency of CDs. In this contribution we present the results of the first system studied. It is composed by 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), one of the most used CDs' derivatives in drug formulations [9, 10], and 1-butanol in aqueous solution of NaCl. The information obtained while studying this system, besides being useful for the above stated applications, can also provide insights into the molecular mechanism involved in the formation of the complexes.

Experimental

Materials

Hydroxypropyl-β-cyclodextrin (HP-β-CD) DS=5.6 and 1-butanol (ButOH) were purchased from Aldrich and FLUKA respectively and used as received. Since HP-β-CD is hygroscopic, the correct concentration value of each stock solution was evaluated by using the procedure reported in literature [11]. NaCl (SigmaUltra) was dried in an oven at about 620 K for 24 h. For each NaCl concentration a stock solution was prepared with doubly distilled water. These solutions were used as solvent in the preparation of HP-β-CD and ButOH solutions.

Calorimetry

Calorimetric measurements were performed using an LKB 2277 (TAM) microcalorimeter equipped with a Thermometric 2250 titration unit. The isothermal titration calorimeter (ITC) was calibrated electrically and its performance was tested as previously described [12]. Each calorimetric titration consisted in three experiments. In the first one the thermal effects (Q_{tot}) associated to 20 injections of 12 μL aliquots of a ButOH solution (0.35 M) into the sample cell containing 1 mL of a HP-β-CD solution (0.02 M), was measured. These heats are the result of three contributes associated to the interaction of ButOH with HP-β-CD (Q), to the dilution of the ButOH aqueous solution ($Q_{dil(ButOH)}$) and to the dilution of the HP-β-CD aqueous solutions ($Q_{dil(HP\text{-}\beta\text{-}CD)}$):

$$Q_{tot}=Q+Q_{dil(ButOH)}+Q_{dil(HP\text{-}\beta\text{-}CD)} \quad (1)$$

The other two experiments were carried out to obtain the values of $Q_{dil(ButOH)}$ and $Q_{dil(HP\text{-}\beta\text{-}CD)}$ respectively in order to isolate from the total effect that associated to the interaction (Q). In particular the values of $Q_{dil(ButOH)}$ were obtained measuring the thermal effects associated to 20 injections of 12 μL aliquots of a ButOH solution (0.35 M) into the sample cell containing 1 mL of water or of a NaCl solution; whereas the values of $Q_{dil(HP\text{-}\beta\text{-}CD)}$ were obtained measuring the thermal effects associated to 20 injections of 12 μL aliquots of water or of a NaCl solution into the sample cell containing 1 mL of a HP-β-CD solution (0.02 M).

All measurements were performed at 298 K.

Treatment of the data

Calorimetric data were analysed using the treatment proposed by Eftink and Biltonen [13].

According to this treatment and assuming the formation of a complex 1:1, Q_i, the sum of the heats Q associated to i injections of a solution of ButOH in a solution of HP-β-CD, is proportional to n_b, the moles of HP-β-CD bound:

$$n_b = Q_i/\Delta H^0 \tag{1}$$

where ΔH^0 is the molar standard enthalpy of complexation. The moles, n_f, of HP-β-CD free in solution are given by the following equation:

$$n_f = [\text{HP-}\beta\text{-CD}]_0 V_0 - Q_i/\Delta H^0 \tag{2}$$

where $[\text{HP-}\beta\text{-CD}]_0$ is the initial concentration of HP-β-CD in the sample cell, before the addition of the ButOH, and V_0 is its volume.

Using the Eqs (1) and (2) to express the concentration of HP-β-CD free and bound in Eq. (3), the association constant becomes:

$$K = \frac{Q_i/\Delta H^0}{[\text{ButOH}]([\text{HP-}\beta\text{-CD}]_0 V_0 - Q_i/\Delta H^0)} \tag{3}$$

The Eq. (3) can be rewritten in the following form which correlates experimental data $Q' = Q_i/([\text{HP-}\beta\text{-CD}]_0 V_0)$ with the concentration of ButOH free in solution ([ButOH]):

$$Q' = \frac{\Delta H^0 K[\text{ButOH}]}{1 + K[\text{ButOH}]} \tag{4}$$

The [ButOH] is calculated by the following equation:

$$[\text{ButOH}] = [\text{ButOH}]_T - [\text{ButOH}]_0 Q'/\Delta H^0 \tag{5}$$

where $[\text{ButOH}]_T$ is the total ButOH molarity.

The values of ΔH^0 and K were obtained from Eqs (4) and (5) by an iterative least square method. The first values of [ButOH] were evaluated assuming that $\Delta H^0 = Q'_{sat}$ that is the value of Q' at saturation at the end of the titration when CD is present in

large excess. The iteration were stopped when two successive values of ΔH^0 differed by less than 2% as reported in literature [14, 15].

Results and discussion

The thermodynamic quantities associated to the formation of inclusion complexes between 1-butanol and HP-β-CD in water and in aqueous solution of NaCl are presented in Table 1. All the equilibrium constants and standard molar enthalpies were evaluated assuming a 1:1 binding model:

$$\text{ButOH+HP-β-CD} \leftrightarrows \text{HP-β-CD/ButOH} \quad (6)$$

Table 1 Thermodynamics parameters for the association between 1-ButOH and HP-β-CD at 298 K

[NaCl]/mol L^{-1}	K/M^{-1}	ΔH^0/kJ mol^{-1}	ΔG^0/kJ mol^{-1}	ΔS^0/J mol^{-1} K^{-1}
0.000	8.7±1.1	10.6±0.3	–5.3±0.3	53.4±0.6
0.184	9.3±1.1	10.5±0.2	–5.5±0.3	53.7±0.5
0.357	10.5±1.2	10.5±0.2	–5.8±0.3	54.7±0.5
0.539	11.8±1.1	10.4±0.3	–6.1±0.2	55.4±0.5
0.718	12.8±1.3	10.4±0.3	–6.3±0.2	56.0±0.5
0.907	14.0±1.3	10.4±0.3	–6.5±0.2	56.7±0.5
1.102	15.3±1.2	10.2±0.3	–6.8±0.2	57.0±0.5
1.289	16.2±1.4	10.0±0.2	–6.9±0.2	56.7±0.5
1.505	18.2±1.4	10.0±0.3	–7.2±0.2	57.7±0.5
1.702	19.2±1.6	10.0±0.2	–7.3±0.2	58.1±0.5
1.900	21.5±1.8	10.0±0.4	–7.6±0.2	59.1±0.6

This assumption was done in agreement with the binding processes in aqueous solutions between CDs and alcohols having a chain composed by less than 7 carbon atoms [16]. This hypothesis was also confirmed by the unfruitfull analysis of experimental data carried out assuming a different stoichiometry. The values of the equilibrium constants reported in Table 1 refer to observed equilibrium constants formulated in terms of concentrations of cyclodextrin and 1-ButOH complexed and uncomplexed as follows:

$$K=[\text{HP-β-CD/ButOH}]/[\text{ButOH}][\text{HP-β-CD}] \quad (7)$$

The standard molar free energy and entropy changes associated to the complexation given in Table 1 were calculated from the corresponding equilibrium constants and standard enthalpies determined experimentally.

To the best of our knowledge, there are no thermodynamic data in literature about the formation of complexes between HP-β-CD and ButOH both in water and in NaCl solutions. From a qualitative point of view, it is possible to compare our K and ΔH^0 values and those relative to the natural β-CD in water. The two inclusion com-

plexes are characterised by different equilibrium constants and standard molar enthalpies. In particular the values of the equilibrium constant and of the standard molar enthalpy of the inclusion complexes formed between 1-ButOH and natural β-CD are (16±2) and (4.3±0.4) kJ mol^{-1} [16] whereas those relative to HP-β-CD are (8.7±1.1) and (10.6±0.3) kJ mol^{-1} respectively. As already reported in literature, the substitution of one or more OH groups with other moieties produces important modifications in the structure and behaviour of CDs [17]. In particular, the lower ability of HP-β-CD to complex compared to the natural β-CD could be due to the steric hindrance of the hydroxypropyl fragments placed close to the cavity entrance [18].

In NaCl solutions the inclusion complexes ButOH/ HP-β-CD are more stable than in water and their stability increases at increasing NaCl concentration, while on the contrary, the standard molar enthalpy associated to the formation of the complexes does not change with the increasing of salt concentration. Therefore the dependence of ΔG^0 on NaCl concentration is the result of an increase of the entropic term.

The standard molar free energy changes evaluated by the K values, defined according to (7), are a measure of the stability of the complexes ButOH/ HP-β-CD relative to that of the uncomplexed ButOH and HP-β-CD in the solution where the complexes are formed. The ΔG^0, in fact, is the result of the differences in the molar free energies of the pure species and in their interactions with other components of the solution; therefore ΔG^0 is highly sensitive to solution composition [19]. In general, the presence of electrolytes in solution can affect the interaction between two molecules and therefore their ability to bind by means of different mechanisms. Neglecting the effects due to a direct participation of ions, which are not important in the case of binding between neutral molecules, the ions in solution produce changes in the dielectric constant and in water activity [20]. The increase of the dielectric constant of the medium, caused by the addition of NaCl, makes the electrostatic contribution, which is one of the noncovalent interactions generally involved in the formation of the inclusion complexes, less important [21]. The effect produced by the change in water activity can be explained rewriting the equilibrium as follows:

$$\text{HP-}\beta\text{-CD}(H_2O)_a + \text{ButOH}(H_2O)_b \leftrightarrows$$

$$\text{But}\leftrightarrows\text{OH}(H_2O)_{b-x}/\text{HP-}\beta\text{-CD}(H_2O)_{a-y} + (x+y)H_2O \quad (8)$$

where a and b are the number of water molecules which constitute the hydration shells of HP-β-CD and ButOH respectively; a includes also the number of water molecules located in the CD's cavity. The coefficients x and y indicate the number of water molecules which are released in the bulk water following the formation of the inclusion complex. The stoichiometry of participation of water can be estimated from the dependence of K on salt concentration by the following simplified form of the equation developed by Record *et al.* for analysing the effects of univalent salt on the binding equilibria of proteins and nucleic acids [17–20, 22]:

$$d\log K/d\log[\text{NaCl}] = 2(x+y)[\text{NaCl}]/[H_2O] \quad (9)$$

In Eq. (9) it was assumed, as already stated, that there is not a direct involvement of ions in the formation of the inclusion complexes. Furthermore, if (x+y), the number of water molecules displaced in forming the complex, can be considered not depending on concentration and nature of electrolyte, the approximate integration of Eq. (9) provides the following equation which explain the dependence of K on the salt concentration:

$$\log K = \log K_{REF,MX} + 0.016(x+y)[NaCl] \quad (10)$$

where $K_{REF,MX}$ is the binding constant in a hypothetical reference state at [NaCl]=1 where (x+y)=0. Equation (10) sets up a linear dependence of log$K$ on NaCl concentration, the slope of which is correlated to the number of water molecules released in the bulk water. The plot of log$K$ *vs*. [NaCl], reported in Fig. 1, shows a linear correlation in agreement with the one expected according to Eq. (10). The slope obtained by the linear regression of data plotted in Fig. 1 was used to calculate the (x+y) value which resulted to be 13. The obtained value is higher than the number of water molecules originally included in the β-CDs' cavity which, not exactly known, has been evaluated to be 6–7 [21]. This result indicates that an important role in the thermodynamics of the formation of the studied complex is played not only by the release of water molecules originally present in the CD's cavity to the bulk water but also by a partial dehydration of the guest molecule. These two processes can be considered responsible for the observed positive change in the entropy of the system. Moreover, the increase of ΔS^0 at increasing NaCl concentration can be explained taking into account the ability of NaCl to change the structure of water. In particular in the water-rich region NaCl having chaotropic properties is a water structure breaker [23]. This means that water molecules in solution of NaCl are less structured than in pure water. Consequently, the displacement of water molecules from an organised structure, such as that present on hydrophobic surfaces, to the bulk water produces an increase of entropy, the amount of which raises with the decreasing of the bulk water structure. Further evidence that the displacement of water molecules has a very important role in the thermodynamics of the process is also given by the almost constant value of ΔH^0 at different NaCl concentrations. In fact, if the change in enthalpy is essentially due to the break of hydrogen bonds between ordered water molecules provoked by the formation of the complex, the number of broken bonds should be almost constant at increasing of salt concentration, producing only little changes in ΔH^0 [24].

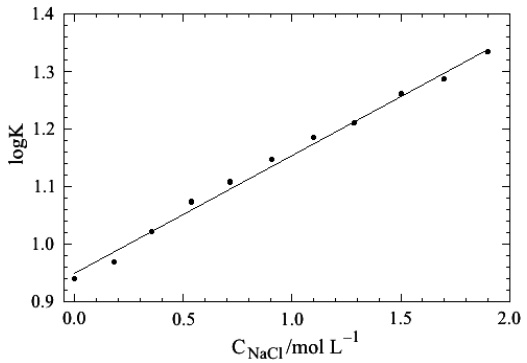

Fig. 1 Plot of the dependence of log$K$ on NaCl concentration (mol L^{-1})

References

1 D. Duchene, D. Wouessidjewe and G. Ponchel, J. Cont. Rel., 62 (1999) 263.
2 T. Loftsson and M. Masson, Int. J. Pharm., 225 (2001) 15.
3 M. Masson, T. Loftsson, G. Masson and E. Stefanson, J. Cont. Rel., 59 (1999) 107.
4 A. M. Siguroardottir and T. Loftsson, Int. J. Pharm., 126 (1995) 73.
5 P. Jarho, A. Urtti, D. W. Pate, P. Suhonen and T. Jarvinen, Int. J. Pharm., 137 (1996) 209.
6 T. Loftsson, H. Fridriksdottir and T. K. Guomundsdottir, Int. J. Pharm., 127 (1996) 293.
7 T. Loftsson and H. Fridriksdottir, Int. J. Pharm., 163 (1998) 115.
8 J. Savolainen, K. Jarvinen, H. Taipale, P. Jarho, T. Loftsson and T. Jarvinen, Pharm. Res., 15 (1998) 1696.
9 M. E. Brown, B. D. Glass and M. S. Worthington, J. Therm. Anal. Cal., 68 (2002) 631
10 A. Agostiano, L. Catucci, M. Castagnolo, D. Colangelo, P. Cosma, P. Fini and M. Della Monica, J. Therm. Anal. Cal., 70 (2002) 115.
11 R. DeLisi, A. Inglese, S. Milioto and P. Pellerito, Langmuir, 14 (1998) 6045.
12 P. Fini and M. Castagnolo, J. Therm. Anal. Cal., 66 (2001) 91.
13 M. Eftink and R. Biltonen, Biological Microcalorimetry, A. E. Beezer (Ed.), Academic Press, London 1980, p. 343.
14 G. Castronuovo, V. Elia, F. Velleca and G. Viscardi, Thermochim. Acta, 292 (1997) 31.
15 G. Castronuovo, V. Elia, D. Fessas, A. Giordano and F. Velleca, Carbohydr. Res., 272 (1995) 31.
16 M. V. Rekharsky, F. P. Scharz, Y. B. Tewari and R. N. Goldberg, J. Phys. Chem., 98 (1994) 10282.
17 U. Uekama, F. Hirayama and T. Irie, Chem. Rev., 98 (1998) 2045.
18 V. Zia, R. A. Rajewski and V. J. Stella, Pharm. Res., 17 (2000) 936.
19 M. T. Record, J. H. Ha and M. A. Fisher, Meth. Enzymol., 208 (1991) 291.
20 M. T. Record, C. F. Handerson and T. M. Lohman, Q. Rev. Biophys., 11 (1978) 103.
21 M. V. Rekharsky and Y. Inoue, Chem. Rev., 98 (1998) 1875.
22 J. H. Ha, M. W. Capp, M. D. Hohenwalter, M. Baskerville and M. T. Record, J. Mol. Biol., 228 (1992) 252.
23 K. D. Collins and M. W. Washbaugh, Q. Rev. Biophys., 18 (1985) 342.
24 R. O'Brien, B. DeDecker, K. G. Fleming, P. B. Singer and J. Ladbury, J. Mol. Biol., 279 (1998) 117.