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Inclusion of menadione with cyclodextrins studied by calorimetry and spectroscopic methods

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ABSTRACT: Complex formation of menadione with α-, hydroxypropyl α-, β-, hydroxypropyl-β-, methyl-β- and hydroxypropyl-γ cyclodextrins in aqueous solution at 298.15 K was studied by using isothermal titration calorimetry, ^{1}H NMR, and UV–vis spectrophotometry. The experimental data indicated the partial insertion of menadione into macrocyclic cavity upon formation of two alternative types of 1:1 inclusion complexes, whose thermodynamic parameters (K, $\Delta_c G^0$, $\Delta_c H^0$, and $\Delta_c S^0$) were calculated. The influence of host size on the complex formation process was analyzed. β-Cyclodextrin and its hydroxypropylated and methylated derivatives were found more effective binders towards menadione than α- and γ-cyclodextrins. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: cyclodextrin; menadione; thermodynamics of complex formation; inclusion complexes

INTRODUCTION

Menadione (2-methyl-1,4-naphthoquinone, Fig. 1), also known as vitamin K_3, is a synthetic version of vitamin K. Menadione is often classified as provitamin, which is converted in the animal body into active form of K_1 and K_2 vitamins.[1] Menadione is required for normal blood coagulation and bone calcification.[1,2] Furthermore, menadione exhibits an anticancer activity.[3,4] Besides the numerous positive effects menadione can cause adverse outcomes including anemia, liver damage as well as the mutagenic, irritative, and toxic effects.[5] To improve the physical-chemical and pharmacological properties of menadione, and to reduce its disagreeable side effects the use of the encapsulated forms of menadione can be recommended.

Cyclodextrins (CDs), the natural oligosaccharides possessing the hydrophilic exterior and hydrophobic cavity, are well-known parenterally safe encapsulating materials.[6] Due to their capability to form inclusion complexes with different organic compounds, CDs are used as stabilizing and solubilizing agents as well as suitable carrier materials in drug delivery systems.[6–8] Consequently, the inclusion of menadione with CDs can result in the enhancement of menadione solubility and stability, prolongation of its pharmacological action, and reduction of unwanted side effects. Thus, the investigation on inclusion of menadione with CDs is of practical importance. However, there are only a few publications devoted to study on complex formation of menadione with CDs.[9–11] Stable crystalline inclusion complexes of menadione with β-CD[9] and γ-CD[10] were prepared and analyzed. As it was observed, the complexed menadione showed higher solubility, increased dissolution rate, and improved biological activity.[9,10] The existence of the inclusion complex menadione–β-CD in aqueous solution was also reported by Berzas Nevado *et al.*[11] Up to now, there are no literature data concerning interactions of menadione with α-CD, γ-CD, or substituted CDs. Therefore, the aim of our work was to study on complex formation of menadione with native and modified CDs in aqueous medium by calorimetric and spectroscopic methods. Calorimetry is the useful technique to measure directly the thermodynamics of complex formation, whereas spectral analysis gives information on the binding mode and the stoichiometric ratio. Combination of these experimental methods allows to gain inside (i) the inclusion process; (ii) the driving forces responsible for the complexation process; (iii) the possibility to verify the stability of the complexes by changing of dimensions and hydrophobicity of the CD cavity.

EXPERIMENTAL

Materials

Commercially available menadione (MP Biomedicals), α-CD (Fluka), hydroxypropyl-α-CD (HP-α-CD, Aldrich),

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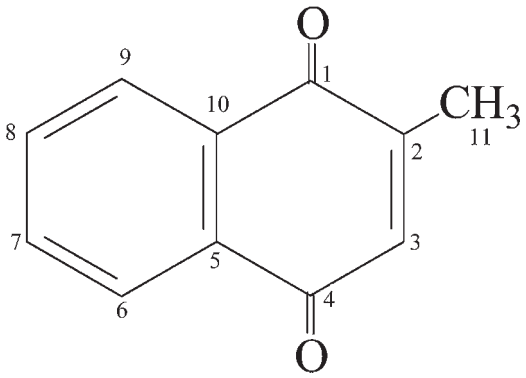

Figure 1. Structural formula of menadione and atom numbering

β-CD (Fluka), hydroxypropyl-β-CD (HP-β-CD, Aldrich), methyl-β-CD (M-β-CD, Aldrich), and hydroxypropyl-γ-CD (HP-γ-CD, Aldrich) of analytical grade were used as supplied. CDs were the stable crystallohydrates and the water content determined by thermogravimetric analysis was consequently taken into account during the sample preparation. Hydroxypropylated and methylated CDs were randomly substituted. The average substitution degree was 1.8 and 0.6 per glucose unit for M-β-CD and all HP-CDs, respectively.

All solutions were prepared by weight using the double distilled, deionized water.

UV–vis spectrophotometry

Absorption spectra were recorded in the range of 200–400 nm at 298.15 K on a UV-2401 PC UV–VIS Recording Spectrometer (Shimadzu, Japan) equipped with TCC-240 A, temperature-controlled cell holder. Quartz cuvettes with a path length of 0.1 cm were employed.

The stoichiometry of the complexes was determined using Job's[12] method. According to this method, 3.9×10^{-5} mol kg^{-1} solutions of menadione (M) and CDs were mixed at different concentration ratios ($R = C_{CD}/(C_{CD} + C_M)$) to constant volume. The stoichiometric ratio was obtained by plotting ΔA against R and finding the R value corresponding to the extreme of this dependence.

For the calculation of the binding constants, the change of absorption of menadione was measured at 250 nm ($\varepsilon = 2.37 \times 10^4$ kg mol^{-1} cm^{-1}) as a function of CD concentrations. The concentration of menadione was fixed at 5.3×10^{-4} mol kg^{-1} and the CD concentrations were changed from 2.0×10^{-3} to 9.8×10^{-3} mol kg^{-1}.

Isothermal titration calorimetry

Calorimetric titrations were performed using isothermal titration calorimeter (Omega MicroCal, Inc.) at 298.15 K. Aqueous CD solutions were injected in 15 μl steps to the menadione aqueous solution placed in the sample cell of the volume of 1.31 ml. A 250 μl Hamiltonian syringe stirred at 400 rpm was used for injection of cyclodextrin solution. The initial concentration of menadione was 5×10^{-4} mol kg^{-1} and the initial CD concentrations were 1.8×10^{-2}, 9.3×10^{-2}, 1.0×10^{-2}, 1.9×10^{-2}, 2.0×10^{-2}, and 2.1×10^{-2} mol kg^{-1} for α-CD, HP-α-CD, β-CD, HP-β-CD, M-β-CD, and HP- γ-CD, respectively. In separate experiments correction values for the heat of dilution of menadione and CD were determined.

The enthalpy of 1:1 complex formation ($\Delta_c H^0$) and the binding constant (K) were calculated simultaneously and fitting the experimental data to a theoretical titration curve using standard instrument software. To obtain more accurate thermodynamic data for low affinity systems the complex stoichiometry determined independently was fixed at known value during the fitting.[13] The other thermodynamic parameters such as the free energy ($\Delta_c G^0$) and the entropy ($\Delta_c S^0$) of complex formation were estimated on the basis of well-known thermodynamic equations:

$$\Delta_c G^0 = -RT \ln K \quad (1)$$

$$\Delta_c G^0 = \Delta_c H^0 - T\Delta_c S^0 \quad (2)$$

^1H NMR: ^{1}H NMR spectra were run on Bruker AC-200 spectrometer operating at 200 MHz and 298.2 ± 0.10 K. Cyclohexane was used as an external reference. All solutions were prepared gravimetrically on the basis of D_2O. The concentration of menadione was constant (5×10^{-4} mol kg^{-1}), while the CD concentration was changed from 0 to 1.7×10^{-2}, 4.5×10^{-2}, 1.2×10^{-2}, 1.8×10^{-2}, 2×10^{-2}, and 8×10^{-2} mol kg^{-1} for α-CD, HP-α-CD, β-CD, HP-β-CD, M-β-CD, and HP-γ-CD, respectively.

RESULTS AND DISCUSSION

UV–vis absorption spectrum of menadione in the absence and presence of β-CD is presented in Fig. 2. As follows from Fig. 2, addition of the excess amounts of β-CD induces the decreasing of absorption. The decrease of absorption intensity upon addition of HP-α-CD, HP-β-CD, M-β-CD, and HP-γ-CD was also noted, whereas the negligible spectral changes was observed when increasing the α-CD concentration.

Decrease in A values can be attributed to complex formation via inclusion of menadione molecule into CD hydrophobic cavities. Stoichiometry of inclusion complexes can be obtained from Job plots, which are presented for several systems in Fig. 3. A symmetric parabolic shape of dependences with an extreme at $R = 0.5$ (Fig. 3) corresponds to the formation of 1:1 complexes. Therefore, the values of stability constants of

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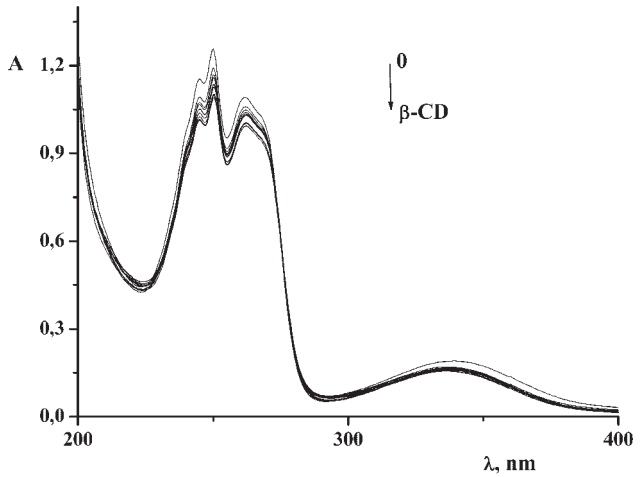

Figure 2. Absorption spectra of menadione (5.3×10^{-4} mol kg^{-1}) in the absence and presence of β-CD (2.0×10^{-3}–9.8×10^{-3} mol kg^{-1}) at 298.15 K

the complexes of menadione with HP-α-CD, β-CD, HP-β-CD, M-β-CD, and HP-γ-CD were calculated from the concentration dependences of A on the basis of well-known Benesi–Hildebrand equation[14] assuming 1:1 stoichiometric ratio:

$$\mathrm{CD} + \mathrm{M} \overset{K}{\longleftrightarrow} \mathrm{CD} \cdot \mathrm{M} \tag{3}$$

$$A = A_0 + \frac{(\Delta\varepsilon K C_{\mathrm{M}} C_{\mathrm{CD}})}{(1 + K C_{\mathrm{CD}})} \tag{4}$$

where A and A_0 are the absorbance of menadione in the presence and absence of CD, respectively; $\Delta\varepsilon = \varepsilon_{\mathrm{CD\cdot M}} - \varepsilon_{\mathrm{M}}$ is the difference in the molar absorptivities between free (ε_{M}) and complexed ($\varepsilon_{\mathrm{CD\cdot M}}$) menadione; C_{M} and C_{CD} are the initial concentrations of menadione and CD, respectively; K is stability constant of the complex. Additionally, K values were calculated with the aid of nonlinear least-squares analysis implemented in Hyperquad 2000 program. The results of calculation are presented in Table 1.

Table 1 also contains the thermodynamic parameters of complex formation of menadione with HP-α-CD, β-CD, HP-β-CD, M-β-CD, and HP-γ-CD determined from the

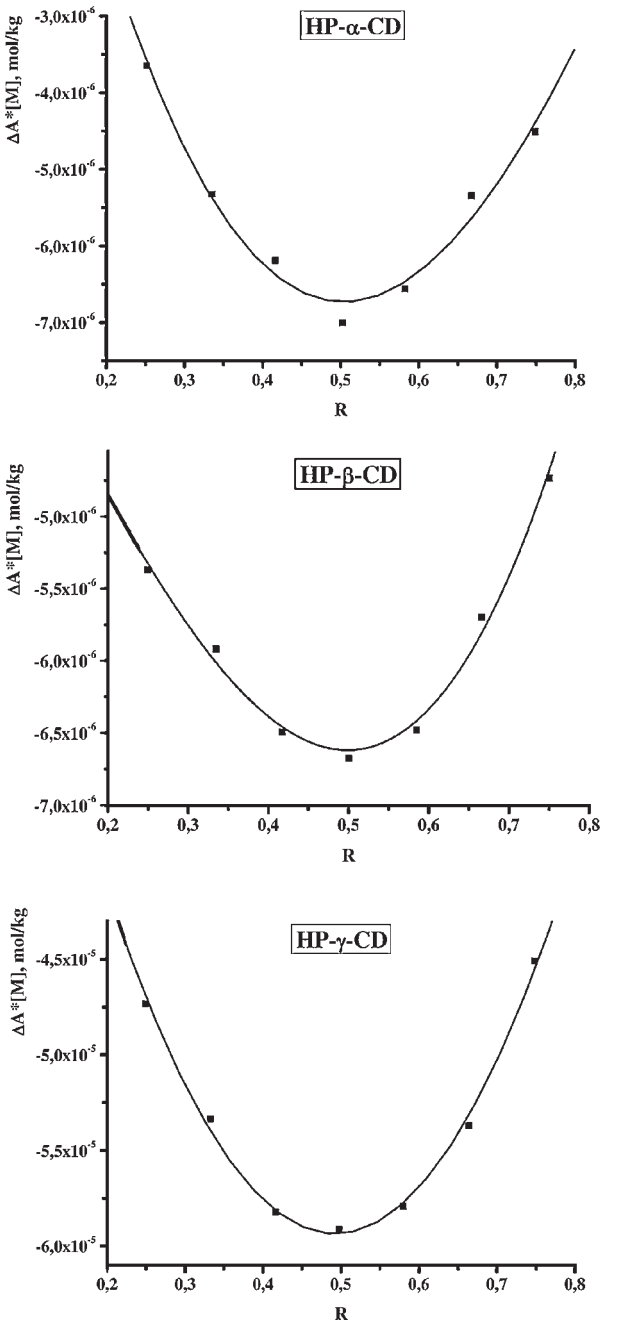

Figure 3. Job plots for complex formation of menadione with CDs in water at 298.15 K

Table 1. Thermodynamic characteristics of complex formation of menadione with cyclodextrins in water at 298.15 K

	UV–vis	Isothermal titration calorimetry			
Complex	K (M^{-1})[a]	K (M^{-1})	$\Delta_c G^0$ (kJ mol^{-1})	$\Delta_c H^0$ (kJ mol^{-1})	$T\Delta_c S^0$ (kJ mol^{-1})
HP-α-CD/M	45 (±25)	14 (±8)	−7 (±4)	−17 (±8)	−10 (±10)
β-CD/M	208 (±37)	240 (±23)	−13.6 (±1.3)	−10.4 (±0.7)	3.2 (±0.5)
HP-β-CD/M	233 (±29)	218 (±31)	−13.3 (±1.9)	−7.9 (±0.7)	5.4 (±1.2)
M-β-CD/M	299 (±20)	281 (±44)	−14.0 (±2.2)	−6.3 (±0.5)	7.7 (±1.8)
HP-γ-CD/M	12 (±10)	16 (±4)	−6.9 (±1.7)	−1.9 (±0.3)	5.0 (±2.0)

[a] K was expressed in M^{-1} under the assumption that solution density was ~1.0 g cm^{-3}.

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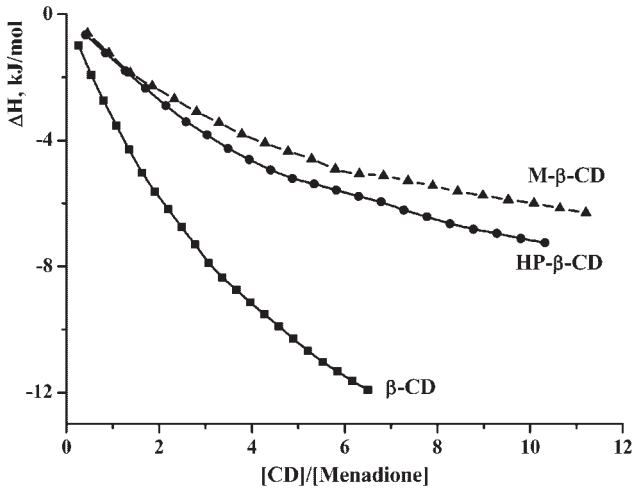

Figure 4. The enthalpy changes for binding of menadione (5×10^{-4} mol kg^{-1}) with β-CD (1.0×10^{-2} mol kg^{-1}), HP-β-CD (1.9×10^{-2} mol kg^{-1}), and M-β-CD (2.0×10^{-2} mol kg^{-1}) in water at 298.15 K *versus* the concentration ratio

calorimetric measurements. Figure 4 shows typical isotherms of binding obtained from the calorimetric titration of menadione with β-CDs in water at 298.15 K. Similar dependences were also plotted for all other systems with HP-α-CD and HP-γ-CD with the exception of α-CD, for which the concentration dependence of ΔH is almost linear (Fig. 5).

Figure 5 illustrates a straight-line concentration dependence of the enthalpy change, which confirms the low α-CD binding affinity to menadione. Unfortunately, calculation of the stability constant and thermodynamic parameters of complex formation was problematic in this case. It can be assumed that favorable enthalpy value is

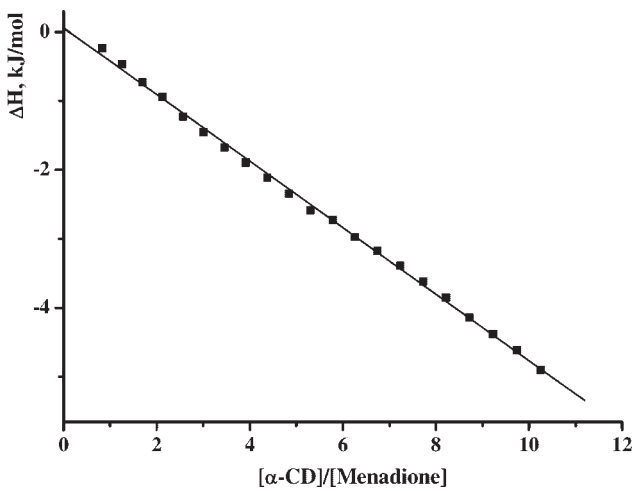

Figure 5. The enthalpy changes for interaction of menadione (5×10^{-4} mol kg^{-1}) with α-CD (1.8×10^{-2} mol kg^{-1}) in water at 298.15 K *versus* the concentration ratio

compensated by the high negative entropy value resulting in a low stability constant ($K < 10\,\mathrm{M}^{-1}$). The negligible changes in the absorption spectrum of menadione induced by the presence of the excess amounts of the α-CD additionally confirm the formation of weak molecular complex in this system. Most likely, attractive interactions and hydrogen bonding that are characterized by exothermal effects and result in the loss of configurational entropy are responsible for the binding of α-CD with menadione.

Substitution of hydroxyl groups surrounding the CD rim by hydroxypropyl groups promotes the complex formation process (Table 1). Perhaps, these bulky substituents increase the α-CD cavity dimensions and the binding of menadione with HP-α-CD becomes more effective. The complex formation of menadione with HP-α-CD is characterized by the negative enthalpy and entropy values (Table 1). Low stability constant of the HP-α-CD–menadione complex (Tables 1 and 2) is a result of enthalpy–entropy compensation. Here, the data quality prevents their detailed interpretation. Probably, van der Waals interactions and possible hydrogen bonding are the main source of the negative enthalpy value. Owing to these interactions severe motional restriction reduces the internal conformational and positional entropy yielding the negative entropy change.[15]

β-CDs consisting of seven glucose units and possessing the larger cavity diameter in comparison with α-CDs form more stable complexes with menadione. As compared to α-CDs, the processes of complex formation of menadione with native and modified β-CDs are characterized by less negative $\Delta_c H^0$ values and more positive $\Delta_c S^0$ values. So, the enthalpy and entropy changes favor the binding of menadione with β-CDs, whereas the binding with α-CDs is only enthalpically favorable. Increase of $\Delta_c H^0$ values in the case of β-CDs can occur due to the prevalence of positive contribution caused by hydrophobic interactions and dehydration of the solutes upon the complex formation. Total entropy change upon binding is the sum of a change in configuration entropy and change in solvation entropy.[15,16] Positive entropy changes can be explained by the prevalence of two processes. First of them is a release of water molecules from the solvation shells to the bulk solvent. The second one may be an existence of various complex configurations.[15] Thus, the increase in the number of microstates forms the basis of entropy enhancement.

The partial substitution of the external hydroxyl groups of β-CD on hydroxypropyl- and methyl-groups changes both the diameter and the depth of CD cavity. Therefore, one can suppose that the insertion of menadione into the cavity of HP-β-CD and M-β-CD is deeper as compared with the native β-CD and is accompanied by a more intensive release of water molecules from the CD cavity and solvation shells of the solutes into the bulk water resulting in enhancement of $\Delta_c H^0$ and $\Delta_c S^0$ values. This assumption will be confirmed below. It is necessary to

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Table 2. Binding constants and asymptotic changes in menadione resonances chemical shifts upon complexation, determined for all the complexes studied in aqueous solution at 298.15 K

		$\Delta\delta_c$ (ppb)[a]			
Complex	K (M^{-1})	H(3)	H(6), H(9)	H(7), H(8)	H(11)
HP-α-CD/M	32 (±2)	138 (±4)	263 (±7)	150 (±4)	79 (±3)
β-CD/M	224 (±10)	101 (±2)	57 (±1)	157 (±3)	53 (±1)
HP-β-CD/M	251 (±7)	122 (±2)	85 (±1)	142 (±2)	63 (±1)
M-β-CD/M	298 (±9)	142 (±1)	72 (±1)	143 (±1)	70 (±1)
HP-γ-CD/M	34 (±6)	~0	~0	83 (±7)	43 (±4)

[a] ppb (part per billion) = 10^{-3} ppm.

note that the nature of these substitutes influences the thermodynamic characteristics of complex formation. In particular, the introduction of more hydrophobic methyl-substituents results in noticeable decrease of the enthalpy contribution and increase of the entropy contribution caused by the hydrophobic interactions, while the hydroxypropyl-groups being more bulky and polar have less pronounced influence on the thermodynamic quantities (see Table 1).

HP-γ-CD comprising of eight glucose units has the largest cavity diameter. However, binding of HP-γ-CD with menadione characterizing by small negative enthalpy and small positive entropy changes results in formation of very weak enthalpy–entropy stabilized complex. Strong binding does not occur because of poor host–guest complementarity. Most likely, the cavity of HP-γ-CD is large enough for the retention of menadione molecule. The similar effects of CD cavity size on the thermodynamic quantities were found in complexation of β- and γ-CD with naphthalene derivatives. As it was obtained from calorimetric titrations, β-CD forms rather stable complexes with 2-naphthalenesulfonate ($\log K = 5.37 \pm 0.07$) and 4-amino-1-naphthalenesulfonate ($\log K = 1.70 \pm 0.03$). The use of γ-CD causes the decrease of stability of complexes with 2-naphthalenesulfonate ($\log K = 1.58 \pm 0.03$) and 4-amino-1-naphthalenesulfonate ($\log K = 1.31 \pm 0.08$).[17]

Undoubtedly, the assumptions concerning the inclusion of menadione inside the CD cavity are based only on the thermodynamic parameters of complex formation and require the additional confirmation. Therefore, the NMR spectroscopy can be the useful technique in this case.

The ^{1}H NMR spectrum of menadione recorded in D_2O consists of the signals of H(3), H(6), H(7), H(8), H(9), and H(11) protons. The numbering of menadione protons is presented in Fig. 1. The signals of H(6) and H(9), as well as H(7) and H(8) are equivalent in the ^{1}H NMR spectrum. Addition of the variable amounts of HP-α-CD, β-CD, HP-β-CD, M-β-CD, and HP-γ-CD induces the downfield shift of the signals of all menadione ^{1}H resonances. Figure 6 illustrates the concentration dependence of proton chemical shift changes ($\Delta\delta$) induced by complex formation of menadione with HP-β-CD. Conversion of this dependence into the double reciprocal Benesi–Hildebrand plot gives a straight lines (Fig. 7) confirming the formation of 1:1 complexes.[14] The plots analogous to that shown in Figs 6 and 7 were obtained for systems with HP-α-CD, β-CD, M-β-CD, and HP-γ-CD.

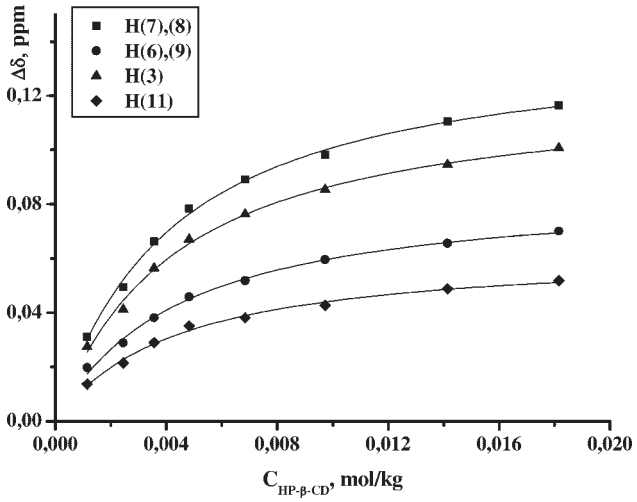

Figure 6. Chemical shift changes for menadione (5×10^{-4} mol kg^{-1}) protons *versus* the HP-β-CD concentration

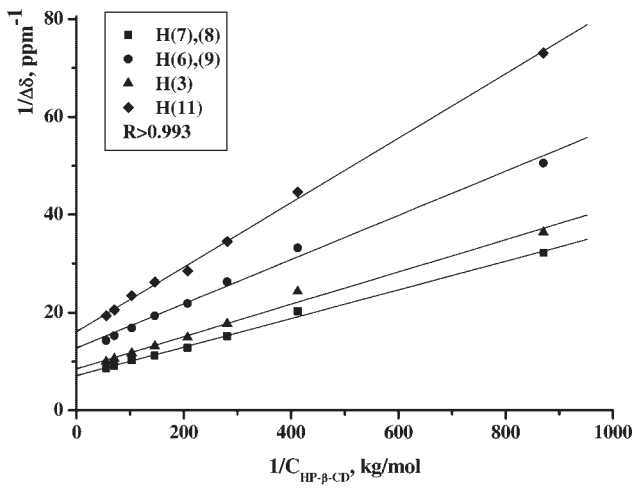

Figure 7. Benesi–Hildebrand plots for complex formation of menadione (5×10^{-4} mol kg^{-1}) with HP-β-CD in water at 298.15 K

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Stability constants (K) of 1:1 complexes were determined using Eqn (5):

$$K = \frac{C_M \cdot \Delta\delta}{\Delta\delta_c \cdot (C_{CD} - C_M \cdot \Delta\delta/\Delta\delta_c) \cdot (C_M - C_M \cdot \Delta\delta/\Delta\delta_c)} \quad (5)$$

where C_{CD} and C_M are the initial concentrations of CD and menadione, respectively; $\Delta\delta$ is difference between the experimentally observed chemical shift and chemical shift of menadione in the free state ($\Delta\delta = \delta_{exp} - \delta_M$), and $\Delta\delta_c$ is the difference between chemical shifts of 100% complexed and free menadione ($\Delta\delta_c = \delta_{CD\cdot MABA} - \delta_M$). The results of nonlinear least-squares fitting are summarized in Table 2. It is necessary to stress a good agreement between K values obtained by titration calorimetry, UV–vis spectrophotometry, and ^{1}H NMR.

Analysis of the chemical shift changes of complex formation ($\Delta\delta_c$) listed in Table 2 together with the thermodynamic parameters of complex formation reported in Table 1 yields the information on the binding mode. As can be seen from Table 2, the signals of almost all menadione protons are shifted upon complex formation with CDs under consideration. Comparison of $\Delta\delta_c$ values allows to emphasize that the signals of menadione protons experience the highest changes for binding with HP-α-CD (Table 2). The noticeable shifting of the signals of all menadione protons induced by the addition of α-CD was also observed. In the case of complex formation with all other CDs the maximal $\Delta\delta_c$ values only for H(7), H(8), and H(3) protons were found (Table 2). This difference can be explained by the different binding mode of menadione with CDs. Probably, menadione fits snugly into α-CD cavity and increasingly loosely in β-CD and γ-CD.

High $\Delta\delta_c$ values for H(7), H(8), and H(3) protons and low $\Delta\delta_c$ values for H(6), H(9) and H(11) protons obtained for complex formation of menadione with β-CDs (Table 2) allow to suggest that two kinds on inclusion complexes are formed in aqueous solution. In one of them the aromatic ring having no methyl-group is incorporated into hydrophobic cavity, and in the second one, on the contrary, the ring carrying CH_3-group is inserted into cavity. The possibility of formation of two kinds of inclusion complexes was found out by Hamai *et al.*[18] for binding of 2-methylnaphthalene with α-CD. It is necessary to note that inclusion of menadione molecule is not deep because of the steric hindrance that can arise from the ketonic groups in the 1 and 4 positions. Conclusion regarding the partial location of menadione inside the β-CD cavity is supported by the hypothetical model of the β-CD–vitamin K_3 inclusion complex proposed by Berzas Nevado *et al.*[11] on the basis of calculation of the geometric dimensions of the reagents. In addition, HP-β-CD and M-β-CD, in comparison with the unmodified β-CD, induce the larger chemical shift changes of menadione protons. This observation can be attributed to the deeper inclusion of menadione inside the cavity of substituted β-CDs and is in agreement with the reported above conclusions based on the thermodynamic parameters of complex formation.

In the case of HP-γ-CD, the $\Delta\delta_c$ values for H(7), H(8), and H(11) protons are highest, while $\Delta\delta_c$ for H(6), H(9), and H(3) protons are about zero. It means, that HP-γ-CD also forms two kinds of complexes with menadione, but the penetration of menadione into cavity is shallow.

CONCLUSIONS

The study on interactions of menadione with CDs clearly demonstrates the formation of 1:1 inclusion complexes in aqueous solution. Inclusion process is very sensitive to the size of CD cavity. Thus, β-CDs are more effective hosts towards the menadione than α-CD and HP-γ-CD. The presence of hydroxypropyl- and methyl-substituents in the CD molecules promotes the complex formation process. Native and modified β-CDs as well as the HP-γ-CD form two kinds of complexes with menadione. In both cases the partial insertion of menadione into macrocyclic cavity was observed.

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REFERENCES

1. Dyke SF. *The Chemistry of the Vitamins*. Interscience Publishers: London, 1965; Ch. 15.
2. Berzas Nevado JJ, Murillo Pulgarín JA, Gómez Laguna MA. *Analyst* 1998; **123**: 287–290.
3. Ham SW, Park HJ, Lim DH. *Bioorg. Chem.* 1997; **25**: 33–36.
4. Hu OY-P, Wu C-Y, Chan W-K, Wu FY-H. *J. Chrom. B* 1995; **666**: 299–305.
5. Chiou T-J, Zhang J, Ferrans VJ, Tzeng W-F. *Toxilcology* 1997; **124**: 193–202.
6. Hedges AR. *Chem. Rev.* 1998; **98**: 2035–2044.
7. Rekharsky MV, Inoue Y. *Chem. Rev.* 1998; **98**: 1875–1917.
8. Uekama K, Hirayama F, Irie T. *Chem. Rev.* 1998; **98**: 2045–2076.
9. Szejtli J, Bolla-Pusztai E, Tardy-Lengyel M, Szabo P, Ferenczy T. *Pharmazie* 1983; **38**: 189–193.
10. Lengyel MT, Szejtli J. *J. Incl. Phenom. Macrocycl. Chem.* 1985; **3**: 1–8.
11. Berzas Nevado JJ, Murillo Pulgarín JA, Gómez Laguna MA. *Talanta* 2000; **53**: 951–959.
12. Job P. *Ann. Chim.* 1928; **9**: 113–203.
13. Turnbull WB, Daranas AH. *J. Amer. Chem. Soc.* 2003; **125**: 14859–14866.
14. Hildebrand JH, Benesi A. *J. Am. Chem. Soc.* 1949; **71**: 2703–2707.
15. Haj-Zaroubi M, Schmidtchen FP. *Chem. Phys. Chem.* 2005; **6**: 1181–1186.
16. Chen W, Chang C-E, Gilson MK. *Biophys. J.* 2004; **87**: 3035–3049.
17. Inoue Y, Hakushi T, Liu Y, Tong L-H, Shen B-J, Jin D-S. *J. Amer. Chem. Soc.* 1993; **115**: 475–481.
18. Hamai S, Ikeda H, Ueno A. *J. Incl. Phenom. Mol. Recogn. Chem.* 1998; **31**: 265–273.

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