Efficiency of cephalosporin complexation with aromatic compounds

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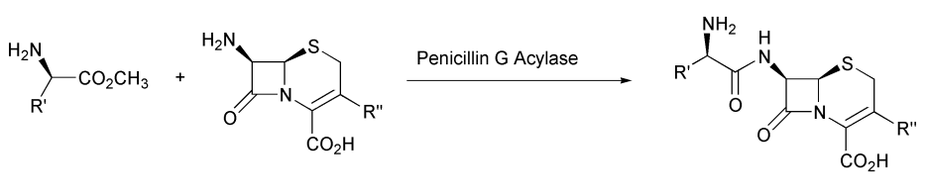
The cephalosporin antibiotics cephradine, cephalexin, cefaclor and cefadroxil form complexes with β-naphthol and several other naphthalene derivatives. In these clathrate-type complexes, the cephalosporins form the host lattice for the naphthalene derivatives. Complexation with β-naphthol analogues can be employed to withdraw cephalosporins selectively from an aqueous solution. In this process, the most important parameter is the complexation efficiency, which expresses the extent to which the cephalosporins can be withdrawn from a solution. The complexation efficiencies for a series of guest molecules are explained in terms of both the thermodynamics of the complexation reaction and the structural features of the cephalosporin complexes. In this manner, insight is gained into the subtle relationship between the molecular structure of naphthalene derivatives and the stability of their complexes with the antibiotics. It is shown which molecular properties of the guest molecules are the most important ones for an optimal complexation efficiency of cephalosporins.

Introduction

The cephalosporin antibiotics cephradine **1**, cephalexin **2**, cefaclor **3** and cefadroxil **4** (Scheme 1) form clathrate-type complexes with β-naphthol.[1,2] In these clathrates the cephalosporin lattice acts as host to the guest molecule β-naphthol, which is also referred to as the complexing agent. On the basis of the crystal structures of the clathrates of cephalosporins **1–4** with β-naphthol a series of novel complexing agents has been identified.[3] Complexation of **1–4** with β-naphthol or some of its analogues provides a convenient method for the isolation of these life-saving antibiotics from aqueous reaction mixtures. Novel production routes to cephalosporin antibiotics make use of enzymatic coupling reactions of D-amino acid side chains and the β-lactam nucleus (Scheme 1).[4] Since these enzymatic coupling reactions take place in water, the products have to be isolated from aqueous solutions. Due to the presence of the β-lactam moiety, cephalosporins are extremely sensitive to degradation. In addition, cephalosporins are susceptible towards secondary hydrolysis by the enzyme, which essentially is a reversal of the coupling reaction producing the amino acid side chains and the β-lactam nucleus. Owing to this undesired degradation and hydrolysis, the yield of cephalosporin product may be seriously reduced. The success of the enzymatic process is, therefore, strongly dependent on how effectively degradation and hydrolysis of the product can be prevented or suppressed. In this respect, selective complexation is a powerful method to tackle such problems.[5] Moreover, complexation provides a method for purification of the cephalosporin products obtained either *via* the conventional chemical routes or *via* the above-mentioned enzymatic synthesis.

Detailed investigation of the underlying concepts of the complexation process is highly relevant from an industrial point of view, since the overall yield of cephalosporin product after isolation or purification strongly depends on the efficiency of the complexation process. Conversely, this efficiency is determined by the molecular properties of the complexing agent used. Highly relevant questions are, therefore: which compound is the most suitable complexing agent for each individual cephalosporin and how can such a compound be identified?

It is known that β-naphthol is a reasonable complexing agent for all four cephalosporins.[5] The aim of the research described



1 Cephradine (R' = cyclohexa-1,4-dienyl, R'' = Me)
2 Cephalexin (R' = phenyl, R'' = Me)
3 Cefaclor (R' = phenyl, R'' = Cl)
4 Cefadroxil (R' = *p*-hydroxyphenyl, R'' = Me)

Scheme 1 The enzymatic coupling reaction of D-amino acid esters with a β-lactam nucleus leading to cephalosporins **1–4**.

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Table 1 The residual concentrations of cephradine **1**, cephalexin **2**, cefaclor **3** and cefadroxil **4** after complexation with naphthalene derivatives

		Residual concentration of cephalosporins **1–4** (mM)												
	t	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**k**	**l**	**m**
1	0	28	29	27	16	3.2	9.8	29	1.4	4.5	5.4	3.6	3.2	29
	30	23	29	19	8.0	2.9	5.3	26	1.1	1.8	3.6	4.5	2.9	26
	90	19	27	1.3	8.0	2.6	6.3	25	1.1	1.8	3.6	4.2	2.9	23
	24h	17	17	1.3	8.0	2.6	9.8	16	1.1	1.8	3.0	3.9	2.9	3.6
	90[a]	*2.7*	*3.7*	*1.3*	*8.0*	*2.6*	*9.8*	*1.7*	*1.1*	*1.8*	*3.0*	*3.9*	*2.9*	*3.6*
2	0	19	29	13	[b]			11	5.5	13	8.7		29	
	30	10	21	2.3				5.5	5.5	4.6	6.6		6.1	
	90	10	17	2.3				5.2	4.0	4.6	6.4		5.8	
	24h	9.2	7.8	2.0				3.2	4.0	3.8	4.3		5.2	
3	0	29		29					6.0					25
	30	13		21					4.9					20
	90	12		5.9					4.9					18
	24h	7.8		3.3					4.9					8.5
4	0	26	—[c]	31	—	—	—	—	13	—	5.8	3.6	—	—
	30	13		27					11		5.5	3.0		
	90	13		22					11		5.2	2.8		
	24h	12		20					11		5.2	2.8		

[a] The complexing agent was added as a solution in methanol. Concentration measured after 90 min. [b] Not measured. [c] No complex formation. **a** β-Naphthol, **b** naphthalene, **c** α-naphthol, **d** 1,2-dihydroxynaphthalene, **e** 1,3-dihydroxynaphthalene, **f** 1,4-dihydroxynaphthalene, **g** 1,5-dihydroxynaphthalene, **h** 1,6-dihydroxynaphthalene, **i** 2,3-dihydroxynaphthalene, **j** 2,6-dihydroxynaphthalene, **k** 2,7-dihydroxynaphthalene, **l** quinoline, **m** 8-hydroxyquinoline.

in this paper is to find the optimal complexing agent for each individual cephalosporin. The present study is a systematic investigation of a series of naphthalene derivatives as potential complexing agents for cephalosporins. The thermodynamics of the complexation reaction have been studied to reveal which parameters of a guest molecule influence the complexation efficiency. In addition, the correlation of the complexation efficiency of a complexing agent with its molecular structure has been investigated using structural information of the respective complexes.

Results

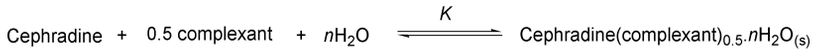
A series of complexing agents has been identified for all four cephalosporins. A relatively large number of these agents form isomorphous clathrates with the cephalosporins **1–3**.[3] Cefadroxil **4** forms clathrates with only a small set of agents, which are not isomorphous with the clathrates formed from **1–3**.[3] The influence of a guest molecule on the complexation efficiency can be investigated by comparing β-naphthol and a newly identified complexing agent. The cavities present in the host framework of **1–3** differ from those in the framework of cefadroxil **4**. Thus it may be concluded that the most efficient complexing agent for **1–3** is not *per se* the optimal one for **4**. On the other hand, cephalosporins **1–3** may have the same optimal complexing agent. As cephalosporins **1–3** form isomorphous clathrates, one of them can, in principle, serve as a model for the other two. In this paper the main focus is on cephradine, cephalexin and cefadroxil, while cefaclor receives less attention due to the limited availability of this antibiotic.

The complexes derived from β-naphthol have been used as a reference for the systematic investigation of the influence of the molecular structure of the guest molecule on the complexation efficiency. The influence of the hydroxy function in β-naphthol was studied by comparing its efficiency with that of naphthalene. Conceivably, a hydroxy function can serve as a hydrogen bond donor and acceptor to the surrounding water molecules[2,3] and hence it can contribute to the overall stability of the crystal, which may result in a more efficient crystallisation. The behaviour of β-naphthol has been compared with that of α-naphthol to investigate the influence of the position of the hydroxy function on the naphthalene skeleton on complexation. The influence of an additional hydroxy function and of its relative position has been studied using a series of dihydroxynaphthalenes as complexing agents. The study was restricted to commercially available dihydroxynaphthalenes. Quinoline and 8-hydroxyquinoline have been examined as guest molecules in order to shed light on the effect of a strong hydrogen bond acceptor in the aromatic system.

Complexation experiments were performed on a 1.5 mmol scale and with a two-fold excess of complexant, *i.e.* using equimolar amounts of cephalosporin and complexant.† The complexation was studied by measuring the decrease of the concentration of cephalosporin with time after addition of the complexing agent. The complexing agent was added in this way to the cephalosporin solution as this procedure most closely resembles the use of the complexation methodology in a production process. Except for quinoline, all of the complexing agents are solids. The concentration data, which reflect the kinetics of the complexation process, are collected in Table 1. These data reveal that some complexing agents form complexes very rapidly; in fact, the remaining concentration of antibiotic does not change further after 90 minutes. Clearly, the solubility of the complexant in water and its rate of dissolution are of importance.‡ If the rate of dissolution of the complexant is too slow, it may not reach its maximum complexation efficiency within 24 h, which was the cut-off time for the monitoring of the antibiotic concentration. Examples are naphthalene and 1,5-dihydroxynaphthalene for cephradine. To facilitate the dissolution of the complexant in water it was added as a solution in a small amount of methanol (2 ml). In this manner a supersaturated solution of complexant in water was obtained, which results in an almost instantaneous clathration with the antibiotic. Moreover, the excess of complexing agent is present as a saturated solution in water and, in part, as a precipitated solid, implying that the concentration of complexant in this complexation procedure remains constant. The residual

† One equivalent of complexing agent (1.5 mmol in 50 ml, 30 mM) is added to the cephradine solution. As the ratio of cephradine : complexant in the complex is 2 : 1 this corresponds to a 100% excess, which implies that even after 100% complexation of cephradine 0.75 mmol of complexing agent remain.

‡ Solubility of the naphthalenes in g l^{-1}: α-naphthol 1.11,[6,7] β-naphthol 0.74,[7–9] naphthalene 0.03,[7,10] 1,3-dihydroxynaphthalene 1.82,[7] 1,5-dihydroxynaphthalene 0.165,[7] 1,4-dihydroxynaphthalene 0.86,[11] 2,3-dihydroxynaphthalene 3.9,[11] 2,6-dihydroxynaphthalene 1.1,[12] 2,7-dihydroxynaphthalene 3.9,[11] 8-hydroxyquinoline 0.39 (at pH = 6),[9] quinoline 6.1.[13]



Scheme 2 The complexation reaction.

Table 2 The equilibrium concentrations of cephradine and complexing agent (CA) and the equilibrium constant (K) and the resulting Gibbs free energies of complexation (ΔG)

Complexing agent	[**1**]/mM [a]	[CA]/mM	K	ΔG/kJ mol^{-1}
β-Naphthol	2.7	5.1	370	−14.7
Naphthalene	3.7	0.2	270	−13.9
α-Naphthol	1.3	7.7	769	−16.5
1,2-Dihydroxynaphthalene	8.0	[c]		
1,3-Dihydroxynaphthalene	2.6	11.4	385	−14.7
1,4-Dihydroxynaphthalene	9.8	5.4	102	−11.5
1,5-Dihydroxynaphthalene	1.7	1.0	588	−15.8
1,6-Dihydroxynaphthalene	1.1	[c]		
2,3-Dihydroxynaphthalene	1.8	15.9 [b]	4406	−20.8
2,6-Dihydroxynaphthalene	3.0	6.9	333	−14.4
2,7-Dihydroxynaphthalene	3.9	17.0 [b]	1967	−18.8
Quinoline	2.9	16.5 [b]	2684	−19.6
8-Hydroxyquinoline	3.6	2.7	278	−14.1

[a] The complexant was dissolved in methanol prior to addition to the cephradine solution. [b] The equilibrium constant was calculated according to eqn. (2). [c] The solubility was not reported in the literature.

concentration of cephradine was measured after 90 minutes of incubation. These data are also included in Table 1, *viz.* bottom row for cephradine **1**. Except for β-naphthol, naphthalene and 1,5-dihydroxynaphthalene the residual concentrations are the same as observed for the addition of complexant after 24 h, implying that in these cases an equilibrium has been reached between the antibiotic, the complexant and their complex. Thus, when the complexing agent is sufficiently soluble in water the efficiency of the complexant is the same as that achieved when methanol is used as a mediating solvent for the complexing agent. It is important to emphasize that the use of methanol to prior dissolution of the complexing agent considerably enhances the rate of complexation in most cases.

The residual concentrations of cephradine obtained by using methanol to dissolve the complexant represent the antibiotic concentrations during the complexation process at equilibrium, for which, in most cases, the complexant is present as a saturated aqueous solution (*vide supra*). Hence, these data can be utilized to calculate some relevant thermodynamic parameters of the complexation process (Scheme 2), which are helpful in understanding the efficiency of the clathrate formation. Under complexation conditions for which the complexant concentration is constant, the equilibrium constant can be simply derived from eqn. (1). When, however,

$$K = 1/[\text{cephradine}] \quad (1)$$

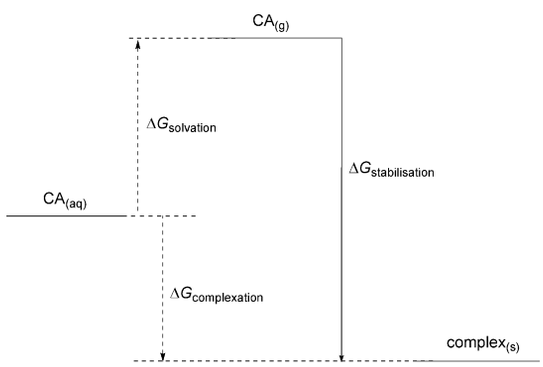
the conditions of saturation are not fulfilled then the K value can be derived by inserting the actual concentration of complexant and antibiotic into eqn. (2). This situation is

$$K = 1/[\text{cephradine}][\text{complexant}]^{0.5} \quad (2)$$

encountered for three cases, *viz.* quinoline, 2,3-dihydroxynaphthalene and 2,7-dihydroxynaphthalene. The K values obtained thus and the Gibbs free energies of complexation derived from them using eqn. (3) are collected in Table 2.

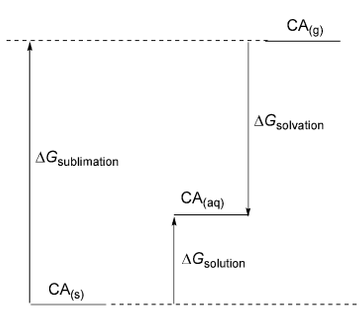
$$\Delta G_{\text{complexation}} = -RT\ln K \quad (3)$$

By comparing the efficiencies of the complexing agents for cephradine shown in Table 2, the intriguing question arises as to whether the overall stability of the complexes can be correlated with the structure of the complexant. It is important to note that the complexation efficiency, which is directly related to the ΔG of the complexation reaction, depends on the stability of the clathrate formed and on the energy of solvation of both the complexing agent and the cephalosporin in water. The data in Table 2 refer to complexation experiments with the same cephalosporin, *viz.* cephradine; thus, only the differences in solvation energy between the individual complexing agents have to be accounted for. Prior to stabilisation by complexation with cephradine, the complexant has to be desolvated from water, which costs energy. Thus, the intermediate state of the complexing agent can be represented by the molecule in the gas phase deprived of all intermolecular interactions. The Gibbs energy of complexation ($\Delta G_{\text{complexation}}$), which is related to the efficiency of the complexation reaction (Table 2), is the difference between the Gibbs energy of stabilisation ($\Delta G_{\text{stabilisation}}$) and the Gibbs energy of solvation ($\Delta G_{\text{solvation}}$). Consequently, the complexation efficiency is determined both by stabilising interactions within the complex ($\Delta G_{\text{stabilisation}}$) and by the Gibbs energy of solvation of the complexing agent ($\Delta G_{\text{solvation}}$), which is sacrificed upon complexation. Therefore, it is only justified to directly attribute variations in the complexation efficiency to structural features of the complexes, when the difference between the energy of solvation of the complexing agents is relatively small compared with the differences between their energy of complexation. The energy diagram of the complexation reaction is depicted in Scheme 3.



Scheme 3 The energy diagram for the complexation reaction. CA stands for complexing agent.

The $\Delta G_{\text{solvation}}$ can be deduced from the energy of sublimation ($\Delta G_{\text{sublimation}}$) and the energy of solution ($\Delta G_{\text{solution}}$) according to Scheme 4. The $\Delta G_{\text{solution}}$ can be derived from the solubility of



Scheme 4 The energy diagram correlating the Gibbs energy of solvation, the Gibbs energy of solution and the Gibbs energy of sublimation of a complexing agent (CA).

the complexing agent, which, in most cases, is reported in the literature.[7] For the five of complexants shown in Table 2 the

Table 3 Comparison of the Gibbs energy (kJ mol^{-1}) of complexation ($\Delta G_{complexation}$), solvation ($\Delta G_{solvation}$) and stabilisation ($\Delta G_{stabilisation}$) for the complexation of cephradine with five different complexing agents

Complexing agent	$0.5 \times \Delta G_{solvation}$	$\Delta G_{complexation}$	$\Delta G_{stabilisation}$ [a]
Naphthalene	−1.4	−13.9	−15.3
α-Naphthol	−11.6	−16.5	−28.1
β-Naphthol	−12.7	−14.7	−27.4
Quinoline [b]	3.5	−19.6	−16.1
8-Hydroxyquinoline	−11.4	−14.1	−25.5

[a] $\Delta G_{stabilisation}$ is calculated from $\Delta G_{stabilisation} = 0.5 \times \Delta G_{solvation} + \Delta G_{complexation}$ (as the ratio complexant : cephradine is 2 : 1). [b] For quinoline $\Delta G_{solvation}$ is calculated from $\Delta G_{vaporisation}$ § and $\Delta G_{solution}$.

$\Delta G_{sublimation}$ is reported.§ Hence, only for these five complexing agents the thermodynamical quantities of complex formation with cephradine can be discussed in more detail. The respective numbers are compiled in Table 3.

Discussion

The data in Table 1 reveal that the residual concentration of the antibiotic in solution is strongly dependent on the complexing agent used. Evidently, the efficiency of the complexation process can be controlled by choosing the most suitable complexing agent. It should be noted that considerable improvement in efficiencies can be achieved with respect to our reference compound β-naphthol. For an efficient isolation of the antibiotics from an aqueous reaction mixture, the residual concentration of antibiotic should be as low as possible. For cephalosporins **1–3**, the highest efficiency is obtained for α-naphthol as the complexant. In the case of cephradine only 1,6-dihydroxynaphthalene is slightly better than α-naphthol, however, this complexant performs much worse in the cases of cephalexin and cefaclor. The difference of 0.2 mM between the residual concentrations of cephradine obtained with α-naphthol and 1,6-dihydroxynaphthalene, respectively, is within the limits of accuracy of approximately 0.3 mM. In practice, the performance of these two complexing agents may be regarded as the same. The finding that cephalosporins **1–3** are most efficiently complexed with α-naphthol can be explained by the isomorphism of their complexes.

The complexation data of cefadroxil shown in Table 1 reveal that β-naphthol is neither the best complexing agent for this cephalosporin. However, α-naphthol behaves the worst in this series, in strong contrast to the results obtained with the cephalosporins **1–3**. The best performing complexing agent for cefadroxil in this study is 2,7-dihydroxynaphthalene. The difference in behaviour between the cephalosporins **1–3** on one hand and cefadroxil **4** on the other, as far as the best complexant is concerned, can be attributed to differences in the host cavities of the respective antibiotics.

The data in Table 2 show some remarkable results, confirming that the complexation efficiency is not fully controlled by the effect of the complexing agent on the complex stability. As the cavity in the crystal lattice formed by cephradine has a two-fold symmetry, it was expected that the dihydroxynaphthalenes arising from applying a two-fold symmetry operation on α- and β-naphthol would be more efficient than their monohydroxy analogues. However, the data in Table 2 show that 1,5-dihydroxynaphthalene performs worse than α-naphthol and 2,6-dihydroxynaphthalene performs worse than β-naphthol, despite that the second hydroxy function could be used for additional stabilisation through hydrogen bonding. The dihydroxynaphthalenes in general, with 1,6-dihydroxynaphthalene as an exception, perform worse than α-naphthol, indicating that the second hydroxy function has no beneficial effect on the complexation efficiency. Similarly, 8-hydroxyquinoline performs worse than quinoline. Whether polar groups do contribute in a positive sense to the stabilisation energy or their beneficial effect fades out due to their increasing $\Delta G_{solvation}$ can only be ascertained when this solvation energy of the complexing agent in water is known. For the subset of five molecules shown in Table 3, $\Delta G_{solvation}$ in water was deduced from literature data.[14–17] For these five complexants, the $\Delta G_{stabilisation}$ can be correlated with the molecular structure of the guest molecules, thereby revealing the essential interactions responsible for complex stabilisation. The observation that $\Delta G_{stabilisation}$ for quinoline is only marginally higher

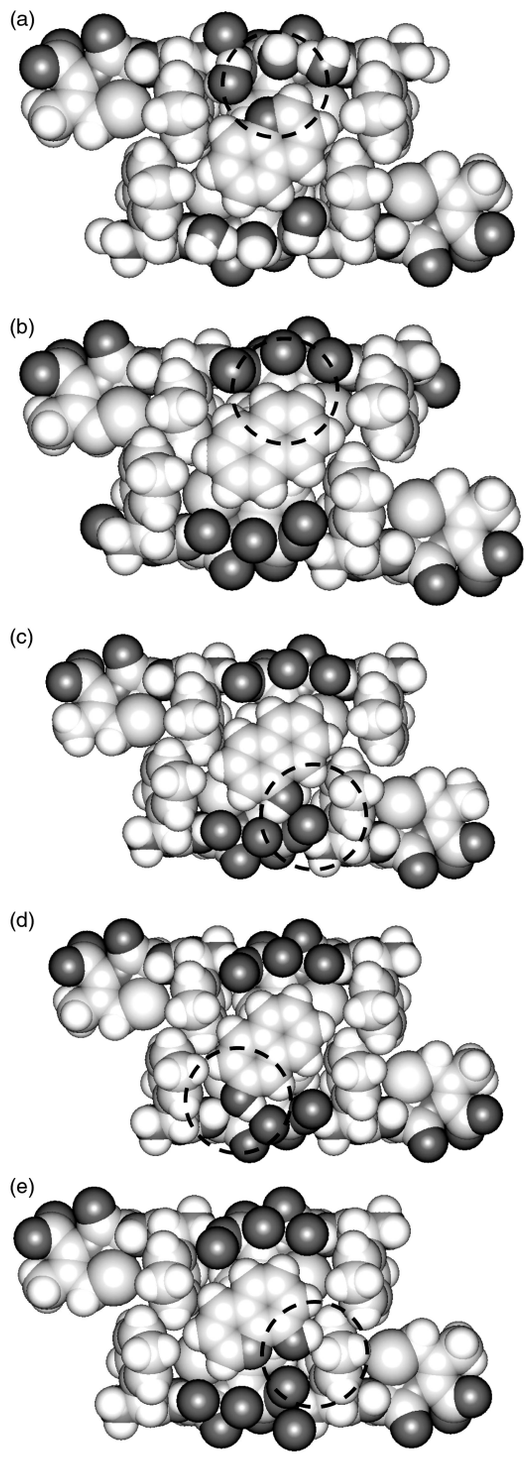


Fig. 1 Complexes of cephradine and (a) quinoline; (b) naphthalene; (c) α-naphthol; (d) β-naphthol; (e) 8-hydroxyquinoline.

§ Gibbs free energies of sublimation (kJ mol^{-1}) from the literature: naphthalene 23.4,[14] α-naphthol 35.1,[15] β-naphthol,[15] quinoline 0.5,[16] 8-hydroxyquinoline 36.4.[17]

than that for complexation with naphthalene, at first sight, seems illogical. It was expected that quinoline would contribute much more to the stabilisation energy than naphthalene by hydrogen bonding with water in the cavity, using nitrogen as a hydrogen bond acceptor. The crystal structure of the complex of cephradine and quinoline was determined very accurately: even the hydrogen atoms of the water molecules could be located. The structure of this complex, which is pictured in Fig. 1a, reveals that the nearest water molecule is positioned with its oxygen toward the nitrogen atom of quinoline, which represents a *repulsive* interaction energy. In contrast, naphthalene can form a weak hydrogen bond ($C_{ar.}H–O_{water}$) with a neighbouring water molecule (Fig. 1b), which contributes favourably to the stabilisation energy. In comparison with naphthalene, solvation phenomena in α- and β-naphthol contribute much more to the stabilisation energy due to hydrogen bonding ($OH–O_{water}$) with these guest molecules, as is evident from the crystal structures shown in Fig. 1c and 1d. In addition to its role as a hydrogen bond donor, the hydroxy function in these complexants can also serve as a hydrogen bond acceptor for a water molecule or, as in the case of the β-naphthol complex, the amide proton of cephradine. The difference in $\Delta G_{stabilisation}$ between the complexes derived from naphthalene and α- or β-naphthol, respectively, of *ca.* 12.5 kJ mol^{-1} must be attributed to the presence of the hydroxy function in the naphthols. The difference in $\Delta G_{stabilisation}$ between the complexes of quinoline and 8-hydroxyquinoline is significantly smaller (9.1 kJ mol^{-1}). From the crystal structure of the cephradine–8-hydroxyquinoline complex shown in Fig. 1e it is evident that the hydroxy group of 8-hydroxyquinoline does not serve as a hydrogen bond donor toward water, but only plays a role as a hydrogen bond acceptor, implying that its contribution to the stabilisation of the complex must be smaller than that of the hydroxy function of α- and β-naphthol in their complexes. In summary, this study of the correlation between the stabilisation energy and the structural features of these five complexes reveals that the complex stability can be enhanced by hydroxy groups present in the guest molecule *via* hydrogen bonding. However, stabilisation of the complex does not necessarily result in more efficient complexation, due to the profound influence of the solvation energy of the complexant in some cases. The term $\Delta G_{solvation}$ may become of comparable importance as the $\Delta G_{complexation}$ in the equation $\Delta G_{stabilisation} = 0.5 \times \Delta G_{solvation} + \Delta G_{complexation}$. Thus, a structural variation in the complexant designed to stabilise the clathrate complex may be accompanied by an uncorrelated contribution to the energy of solvation of the complexant, which may be even larger and thus counterproductive for the complexation efficiency. An anticipated beneficial effect of polar groups in the complexant may be entirely counterbalanced by an increased energy of solvation. Such phenomena are of general importance in host–guest chemistry.

Experimental

Monitoring of the complexation experiments was performed on a Pharmacia LKB.LCC 2252 HPLC using a reversed phase column (Merck 50983 LiChrospher 100RP18, 5 μm, 250 × 4 mm). A UV detector ($\lambda = 254$ nm) from Farmacia LKB.UV-MII was used for detection. An appropriate eluent for the analysis was a mixture of acetonitrile (HPLC grade) and a 50 mM phosphoric acid buffer of pH = 2.7. The complexing agents α-naphthol, β-naphthol, 1,4-dihydroxynaphthalene, 1,5-dihydroxynaphthalene, 2,7-dihydroxynaphthalene, quinoline, 8-hydroxyquinoline and naphthalene were purchased from ACROS; 1,2-dihydroxynaphthalene, 1,3-dihydroxynaphthalene, and 2,6-dihydroxynaphthalene and 1,6-dihydroxynaphthalene were purchased from Aldrich. The cephalosporins **1–4** were a generous gift from DSM (Geleen, The Netherlands).

The crystal data of the complexes of cephradine and naphthalene, α-naphthol, β-naphthol and quinoline have been published previously.[3]

Complexation experiments

Cephalosporin (500 mg) was dissolved in demineralised water (50 ml). The pH was adjusted to 6.3 with 5% ammonia (in several applications of selective complexation a pH of 6.3 is used). The concentration was determined by HPLC analysis using standard solutions of the cephalosporin. To the stirred cephalosporin solution, complexing agent (1.5 mmol) was added in pure form and immediately the $t = 0$ samples were taken. An approximately 200 mg solution was injected through a filter in a small flask and the weight of the filtrate was determined accurately. To the filtrate 7.5 ml of acetonitrile was added and the volume was subsequently increased to 50 ml with 50 mM phosphoric acid buffer of pH = 2.7. After homogenisation, the solution was analysed by HPLC. In the same way samples were taken and analysed after 30 min, 90 min and 24 h.

X-Ray structure analysis of the cephradine–8-hydroxyquinoline complex ¶

Crystals of the cephradine–8-hydroxyquinoline complex, suitable for X-ray diffraction studies, were obtained from a water–methanol mixture by slow cooling. A single crystal was mounted in air on a glass fibre. Intensity data were collected at room temperature. An Enraf-Nonius CAD4 single-crystal diffractometer was used, Mo-Kα radiation, θ–2θ scan mode. Unit cell dimensions were determined from the angular setting of 16 reflections. Intensity data were corrected for Lorentz and polarisation effects. Semi-empirical absorption correction (ψ-scans)[18] was applied. The structure was solved by the program system DIRDIF[19] using the program ORIENT and TRACOR[20] to orient and position a β-lactam fragment in the Patterson map and was then refined anisotropically, by full-matrix, least-squares on F^2 (program SHELXL[21]) using anisotropic parameters for the non-hydrogen atoms. The hydrogens of the ammonium group, the methyl group and the hydroxy group of the 8-hydroxyquinoline molecule were refined as rigid rotors to match maximum electron density in a difference Fourier map. The hydrogens of the water molecules could not be localised and are therefore not included in the model. All other hydrogens were placed at calculated positions and were refined riding on the parent atoms.

$C_{41}H_{58}N_7O_{15.5}S_2$, $M_w = 961.06$, $T = 293(2)$ K, Monoclinic, $C2$, $a = 23.443$ Å, $b = 7.0872$ Å, $c = 14.896$ Å, $\beta = 108.61$, $U = 2345.5$ Å^3, $Z = 2$, $\rho = 1.361$ Mg m^{-3}, ref. col./uni. 2983/2911 $R_{int} = 0.0212$, R (all data) $R1 = 0.0768$, $wR2 = 0.1511$.

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