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Complex Formation between Purine Derivatives and Cyclodextrins: A Fluorescence Spectroscopy Study

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Abstract

The molecular encapsulation of two purine derivatives, caffeine and acyclovir by hydroxypropyl-β-cyclodextrin (HPBCD) in water solution has been studied at 25 °C by fluorescence spectroscopy. When the inclusion complex is formed, changes in the fluorescence spectra of the drugs enable the calculation of the association constants of the binding process, by using a nonlinear regression analysis of the experimental data at three different λ^{em}. The inclusion of the drugs into the CD cavity has been supported by preliminary docking studies. The resulting binding constants have been also discussed in terms of the control release of both purine derivatives, evaluating the HPBCD herein studied as a suitable vector for these drugs.

Introduction

Cyclodextrins (CDs), cyclic oligosaccharides with a hydrophobic cavity, have been widely used to enhance solubility, chemical stability, and bioavailability of poorly soluble drugs [1–3]. From a pharmaceutical point of view, CD:Drug inclusion complexes turned out to be very convenient to reduce the local concentration of free drug, thus reducing side effects [1–3]. However, inclusion of the drug within a cyclodextrin will only be effective for these purposes if the association constant of the complex falls within the proper range. Complexes with low association constants readily release the drug, reducing the effect of the complexation on its bioavailability. On the other hand, if the association constant is very high, the release of the drug may be so low that, not only the side effects, but also the therapeutic ones are reduced. Consequently, it is extremely important to choose the proper CD for each drug, in such a manner that the association constant of the CD:Drug inclusion complex will be moderately high [1, 2].

Organized media have been normally used to examine many important photophysical processes [4–7]. Specifically, aqueous solutions of cyclodextrins have also been widely used to enhance the luminescence properties of different compounds [8–11]. The intensification of luminescent processes of lumiphors partial or totally encapsulated by the CD cavity is due to the better protection from quenching and other processes occurring in the bulk solvent. The CD cavity behaves similarly to an organic solvent; it affords an apolar environment and a non-hydrated state for the included probe. The maximum fluorescence intensity is obtained from a molecule, which is totally encapsulated inside the cavity, and the more a molecule is subjected to an aqueous environment, the lower its fluorescence intensity. The observation of steady-state emission can be used as an indication of complexation when the fluorescence spectrum of a fluorophore in an aqueous environment changes markedly on addition of cyclodextrin to the aqueous solution, by increasing [8–16]; or decreasing [17] fluorescence intensity. The reason is that, due to the organizing ability of CD media, cyclodextrin cavities offer a protective, more constrained microenvironment where an electronically excited lumiphor can be isolated from the surrounding environment, the excited states being shielded from extinction processes [10, 15, 16].

The molecular encapsulation of two purine derivative drugs, caffeine and acyclovir (see Scheme 1), by hydroxypropyl-β-cyclodextrin, has been studied through fluorescence emission enhancement measurements in aqueous solutions, at 25 °C. Caffeine is a drug generally used as cardiac and respiratory stimulant and as diuretic, which occurs in tea, coffee, mate leaves, guarana paste and cola nuts. It is believed to act as stimulant of the central nervous system through adenosine receptors and monoamine neurotransmitters, and also to affect cellular calcium levels. Acyclovir, also named acycloguanosine, is an antiviral drug, orally active acyclic nucleoside with inhibitory activity towards several herpes viruses. The aim of this study is to determine the association constant between both CDs and caffeine or acyclovir, $K_{CD:Drug}$, which have been evaluated using a non-linear regression analysis of the experimental fluorimetric data.

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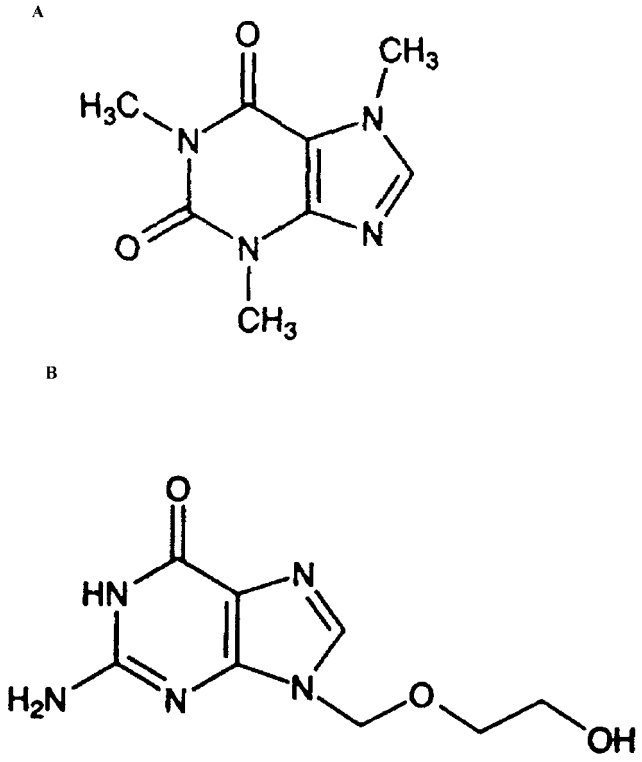

Scheme 1. (A) Caffeine and (B) acyclovir.

Experimental

Materials

3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione, usually named caffeine (CAF) was kindly supplied by Merck (Germany), while 2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-6H-purin-6-one, usually named acyclovir (ACY) was from Sigma. Hydroxypropyl-β-cyclodextrin (HPBCD), containing an average of 0.64 hydroxypropyl groups per glucopyranose unit was from Janssen Biotech (Belgium). All of them, with 99% purity or greater, were used without further purification. HPBCD consists of 3.4% mass of water content, which was considered when calculating solute concentrations. Distilled water was deionized using a Super Q Millipore system before final degassing with a vacuum pump prior to the preparation of the solutions. The homogeneity of the initial solutions was assured by sonicating them for two hours in an ultrasonic bath.

Fluorescence measurements

Steady-state fluorescence experiments were carried out with a Perkin-Elmer LS-50B Luminescence Spectrometer [18]. The equipment is connected to a Pentium PC computer via a RS-232C interface. Data acquisition and analysis of fluorescence spectra were performed with the Fluorescence Data Manager Software supported by the manufacturer. A 10 mm stoppered rectangular silica cell was placed in a stirred cuvette holder whose temperature was kept constant at 25.00 $\pm$ 0.01 °C with a recirculating water circuit. During the experiments, the excitation and emission slits were fixed at 5 and 5 nm, respectively, and the scan rate was selected at 240 nm/min.

In all the titrations, the concentration of the luminescent species was kept constant, while CD concentrations were varied from 0 to a certain value, in such a way that the saturation degree ranges from 0.2 to 0.7–0.8. In fluorescence intensity enhancement studies, care must be taken with the sample preparation procedure [18], which must ensure that the concentration of the fluorophore is equal in the presence and in the absence of cyclodextrin. In order to match this condition, the following protocol of solution preparation was used. Two equal aliquots of a probe stock solution were placed in flasks 1 and 2, and two equal aliquots of a CD stock solution were placed in flasks 2 and 3. The total volume of the probe and CD aliquots in flask 2 was matched in flasks 1 and 3, by adding the necessary volume of water. Working in this way, flask 1 and 2 contain the same concentration of luminescence probe, while flasks 2 and 3 contain the same concentration of CD. An initial volume of 1.5 ml of solution 1 is placed in the fluorescence cell and titrated with different volumes of solution 2, up to a final volume of 2.7 ml. The possible contribution due to the cyclodextrin is corrected by means of a blank titration, where the conditions of the first titration are reproduced but starting with 1.5 ml of water, subsequently titrated with the same volumes of solution 3. In this way, in the first titration the probe concentration is kept constant and its fluorescence is monitored as a function of CD concentration, while in the second titration (blank titration), where no probe is present, the effect of the same CD concentrations in the water is followed. The corresponding difference spectra shows the influence of the inclusion of the probe in the CD cavity. Following this protocol, the experiments reported herein were carried out: (i) at a constant caffeine concentration of 0.524 mM, with [HPBCD] varying from 0 (pure drug) to 4.13 mM, and (ii) at a constant acyclovir concentration of 0.502 mM, with [HPBCD] varying from 0 (pure drug) to 5.23 mM.

Results and discussion

Assuming a 1 : 1 stoichiometry for the inclusion complex between the CD and the drug [1, 19], the encapsulation process is represented by:

$$\mathrm{CD} + \mathrm{Drug} \rightleftarrows \mathrm{CD{:}Drug} \quad K_{\mathrm{CD:Drug}} = a_{\mathrm{CD:DRUG}}/(a_{\mathrm{CD}} a_{\mathrm{Drug}}) \tag{1}$$

where $K_{\mathrm{CD:Drug}}$ is the association constant of the 1:1 complex formed by the CD and the drug as a function of the activities, a_i, of the species present in the solution.

Figures 1 and 2 show, the fluorescence emission spectra of caffeine and acyclovir, respectively, in the absence and in the presence of different HPBCD concentrations. As can be observed, both set of spectra presents maximum peaks, whose intensity increases along with CD concentration.

A quantitative treatment of these fluorescence data starts with the assumption that the intensity is a sum of contributions, as follows [19]:

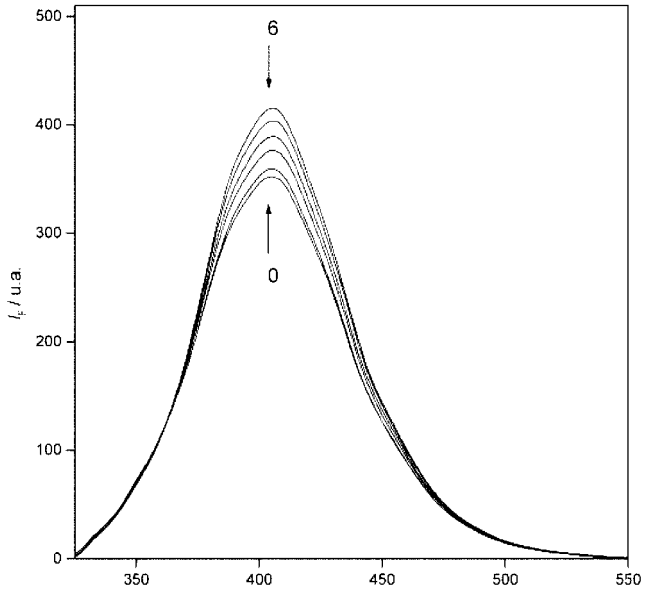

Figure 1. Emission fluorescence spectra (λ_{exc} = 320 nm) of an aqueous solution of caffeine, CAF, at constant concentration (0.524 mM) at 25 °C, in the absence and presence of different concentrations of HPBCD: **0**, 0.000 mM; **1**, 0.384 mM; **2**, 0.952 mM; **3**, 1.690 mM; **4**, 2.760 mM; **5**, 4.130 mM.

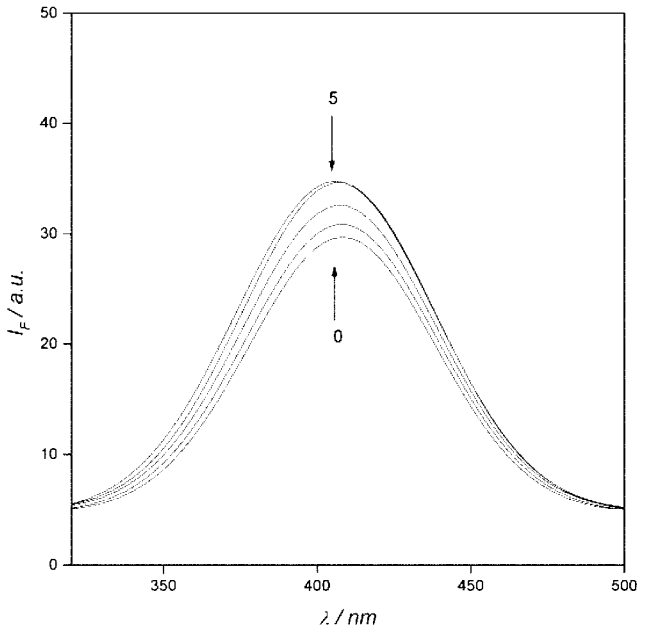

Figure 2. Emission fluorescence spectra (λ_{exc} = 300 nm) of an aqueous solution of acyclovir, ACY, at constant concentration (0.502 mM) at 25 °C, in the absence and presence of different concentrations of HPBCD: **0**, 0.000 mM; **1**, 0.370 mM; **2**, 1.095 mM; **3**, 20828 mM; **4**, 50232 mM.

$$I = k_{\text{Drug}}[\text{Drug}] + k_{\text{CD:Drug}}[\text{CD:Drug}] + K_{\text{CD}}[CD]. \quad (2)$$

Considering Equation (2), the mass balance on substrate, and that cyclodextrins do not fluoresce ($k_{\text{CD}} = 0$), the binding isotherm finally results [1, 19]:

$$\frac{I}{I_o} = \frac{1 + (k_{\text{CD:Drug}}/k_{\text{Drug}})L_{\text{CD:Drug}}[\text{CD}]}{1 + K_{\text{CD:Drug}}[\text{CD}]}, \quad (3)$$

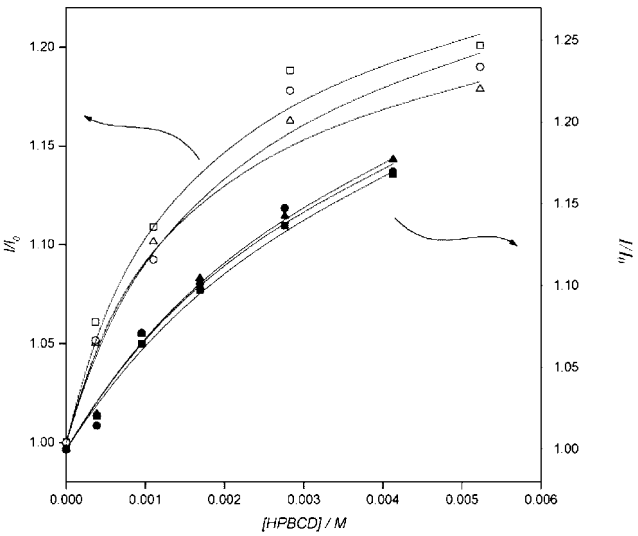

Figure 3. Plot of fluorescence intensity enhancement values, (I/I_o), at different λ^{em}, as a function of HPBCD concentration, for the systems: (a) HPBCD + CAF (■, λ^{em} = 395 nm; ▲, λ^{em} = 404 nm; ●, λ^{em} = 420 nm), and (b) HPBC + ACY (□, λ^{em} = 390 nm; △, λ^{em} = 410 nm; ○, λ^{em} = 420 nm), at 25 °C.

Table 1. Values of the association constants $K_{\text{CD:Drug}}$ and proportionality constant ratio $k_{\text{CD:Drug}}/k_{\text{Drug}}$ of Equation (3) at different λ^{em}, for the systems HPBCD + CAF and HPBCD + ACY, at 25 °C

λ^{em} (nm)	$K_{\text{CD:Drug}}$ (M^{-1})	$k_{\text{CD:Drug}}/k_{\text{Drug}}$	10^5 St. Dev.
HPBCD + CAF			
395	273 ± 38	1.35 ± 0.04	2.1
404	290 ± 43	1.35 ± 0.04	1.9
420	297 ± 60	1.35 ± 0.07	8.1
HPBCD + ACY			
390	764 ± 159	1.26 ± 0.02	8.5
410	770 ± 93	1.23 ± 0.01	2.0
420	605 ± 170	1.26 ± 0.03	9.1

where I_o is the fluorescence intensity in the absence of ligand (CD), but in the presence of the same total substrate concentration $[\text{Drug}]_{\text{tot}}$ ($I_o = k_{\text{Drug}}[\text{Drug}]_{\text{tot}}$). Figure 3 shows the plot of the ratio I/I_o values, as a function of [HPBCD], at the three different λ^{em} for the two systems HPBCD + CAF, and HPBCD + ACY, respectively.

The values of $K_{\text{CD:Drug}}$ and $k_{\text{CD:Drug}}/k_{\text{Drug}}$ are obtained as fitting parameters of a non-linear regression (NLR) analysis of the experimental I/I_o values as a function of [CD]. The NLR procedure is based on a McQuardt algorithm carried out with a TURBO C program developed by us. The results reported in Table 1 show that: (i) the better fits are obtained for the λ^{em} which are closer to the maximum peaks, where the increase on the fluorescence intensity due to the encapsulation is higher; and (ii) the binding constants and proportionality constant ratios are independent of the wavelength at which the intensity enhancement is analyzed, as required [18].

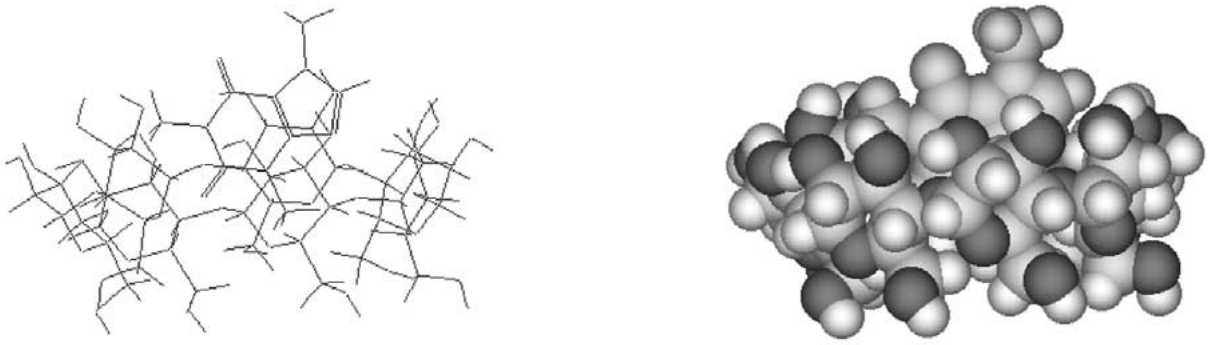

Figure 4. Preliminary geometry of the complex HPBCD:CAF, minimized with the MM+ force field of MM (HyperChem v. 5.1).

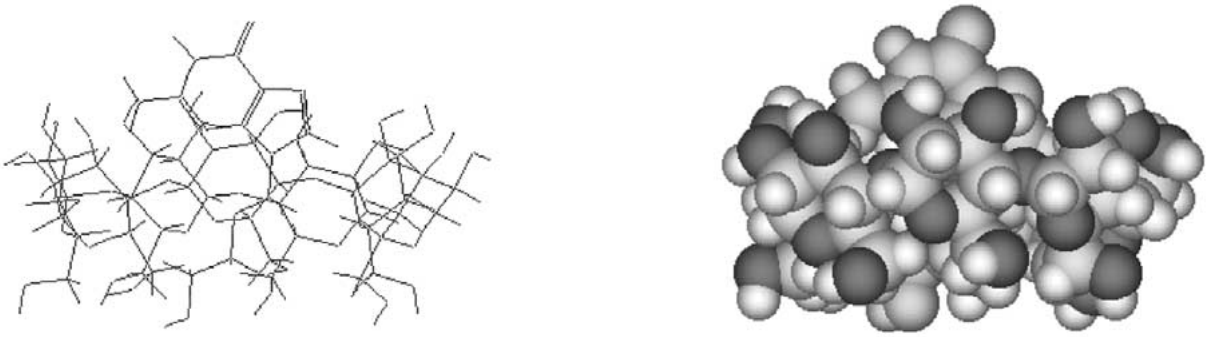

Figure 5. Preliminary geometry of the complex HPBCD:ACY, minimized with the MM+ force field of MM (HyperChem v. 5.1).

As can be seen, the averaged values of $K_{CD:Drug}$ and $k_{CD:Drug}/k_{Drug}$ are $287 \pm 60\ M^{-1}$ and 1.35 ± 0.05, respectively, for the system HPBCD + CAF, and $713 \pm 150\ M^{-1}$ and 1.25 ± 0.02, respectively, for the system HPBCD + ACY. With the aim of clarifying the higher affinity of the HPBCD by acyclovir, with respect to that shown by caffeine, preliminary molecular modeling simulations of the inclusion complexes formed by the parent CD and these drugs were carried out, by using the MM+ force field of molecular mechanics [20], as integrated in Hyperchem v. 5.1. Figures 4 and 5 show a stereoview of the minimized complexes. The atomic coordinates of the crystal structure of the parent CD were used to obtain a conformational minimum for the receptor. The substrates, previously minimized, were docked manually into the cavity by both faces of the receptor and then minimized. It can be noticed that in both cases the purine structural unit, common to both drugs, remains on the wider entrance of the CD; however, the -[(2-hydroxy-ethoxy)methyl] residue of acyclovir is totally inside the apolar cavity, favoring a tighter receptor-substrate interaction, thus justifying the higher value of $K_{CD:ACY}$ with respect to $K_{CD:CAF}$. These possible minimized geometries, calculated for the inclusion complexes studied herein, are thus consistent with the evidence found on the fluorescence emission experiments.

It is well known that as long as the organism "makes use" of the drug, the equilibrium shown by Equation (1) is shifted by mass action toward the release of the active principle, keeping and regulating its presence in the medium. These features rebound the advantages of the use of cyclodextrins as drug carriers [1–3]: (i) the adverse side effects can be substantially reduced since the quantity of the drug which is really free and available in the medium may be much lower than the administered dose; (ii) the time of action of the drug can be prolonged, which in the case of most drugs is particularly important, given its well-known fast onset of action and subsequent short elimination half-life; (iii) as a consequent, the number of doses and its frequency can be reduced with the subsequent decrease of the side effects as well.

It is evident that the value of the association constant of the CD/Drug complex depends on the characteristics of the CD and the drug to be encapsulated. And this overall affinity involves a particular balance between the different non covalent intermolecular forces which take place in the association process, i.e., van der Waals contacts, electrostatic and hydrophobic interactions, hydrogen bonds, solvation processes, etc. It is, then, necessary to determine these association constants and to choose the proper CDs for each specific drug. Association constant values ranging from 200 to 10000 M^{-1} are found to be appropriate for the encapsulating of drugs by cyclodextrins, since they enable the above described controlled release of the drug. For that reason, it can be finally concluded that HPBCD could be an appropriate vector for the drugs caffeine and acyclovir, given that the corresponding binding constants, determined in this work, fall within the optimum range.

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