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Cyclodextrins as phototoxicity inhibitors in drug formulations: studies on model systems involving naproxen and β-cyclodextrin

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Abstract

The binding of the phototoxic non-steroidal anti-inflammatory drug naproxen (NX) to β-cyclodextrin (β-CD) in the presence of alcohols or protein (bovine serum albumin, BSA) is described. Changes in the NX fluorescence spectrum shows that it forms a moderately strong 1:1 complex with β-CD in pH 7.4 aqueous buffer at room temperature ($K_{app} = 475 \pm 35\,M^{-1}$). The presence of 1% linear alcohols (ethanol to pentanol) systematically lowers K_{app} for the β-CD:NX complex. The greatest reduction is observed for the longest alkyl chain. Detailed analysis of K_{app} as a function of [pentanol] suggests that three-member complexes involving the CD, NX and pentanol form ($K_3 = 141 \pm 32\,M^{-1}$). Quenching the fluorescence of NX with NaI shows that the β-CD cavity protects NX from this aqueous phase quencher. Addition of pentanol inhibits this protective effect. Ultra-violet irradiation of NX at pH 7.4 leads to formation of photoproducts. An amount of 10 mM β-CD strongly inhibits NX photoconversion. Addition of 1% pentanol essentially eliminates the photosuppressive effect of β-CD. By contrast to the moderate CD/NX binding, NX binds very strongly to BSA ($K_{app} = (3.2 \pm 1.6) \times 10^5\,M^{-1}$). Changes in the shape and energy of the NX fluorescence spectrum in the presence of BSA and separation experiments based on molecular weight support the view that BSA essentially displaces NX entirely from the CD environment. The implications of these results for application of CDs as in vivo phototoxicity inhibitors are discussed.

Keywords: Phototoxicity; Drug formulations; Cyclodextrins; Naproxen

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently prescribed therapeutics for alleviation of minor aches and pains, the treatment of rheumatic fever, rheumatoid arthritis and osteoarthritis [1]. They also have potential as prostaglandin synthesis inhibitors [1]. Naproxen (NX), or 6-methoxy-α-methyl-2-naphthalene acetic acid, is a NSAID based on the aryl acetic acid structure (Scheme 1).

NX, marketed under the trade names Anaprox and Naprosyn, is typically used to treat rheumatoid and gouty arthritis. For such ailments the treatment is chronic which means an individual will be exposed to NX for prolonged time periods. Chronic exposure raises the concern of side effects. Indeed NX is well known to exhibit a number of side effects including dizziness, drowsiness, nausea and gastrointestinal tract irritation [1]. In common with several other aryl acetic acid NSAIDs [2], NX can also induce skin photosensitivity in some patients [3,4]. NX and the related drug ketoprofen, also exhibit photohemolitic activity towards red blood cells [5] and NX is capable of causing photocleavage of DNA [5–7].

The photosensitizing effects of NX and related structures can of course be traced to their photochemistry. The photochemistry of NX has been examined by Moore and Chappuis [8]. Under flash photolysis conditions NX undergoes photoionization and triplet formation with comparable quantum yields. Photolysis of NX in air-saturated aqueous buffer results in decarboxylation leading to a primary carbon-centered radical. This radical then reacts with molecular oxygen probably yielding a peroxy radical which further reacts to yield 1-(6-methoxy-2-naphthyl)ethanol which is subject to oxidation leading to 2-acetyl-6-methoxynaphthalene. Both of these stable products contain the naphthalene chromophore. In addition NX, and presumably its photolysis products as well, is a source of singlet oxygen [8,9]. Thus, the phototoxicity of NX may arise from radicals generated during photolysis or from singlet oxygen generation or both. In fact these species were found to be responsible for NX photoinduced lipid peroxidation of unilamellar liposomes of egg phosphatidylcholine [10].

It is clear that a means to suppress photosensitization by NX is desirable. The suppression can be introduced at any stage in the photochemical processes open to NX. That is,

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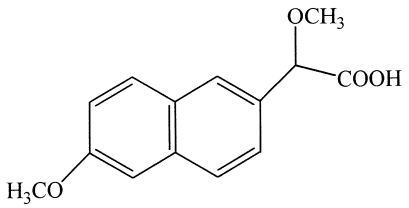

Scheme 1. Naproxen.

methods to suppress the phototoxicity might include addition to NX formulations of compounds which act as UV screens, addition of species which rapidly quench excited states of NX, addition of species which trap radicals or singlet oxygen, etc. For example, de Guidi and co-workers have shown that radical-trapping metal ions such as Cu(II), Mn(II) and Co(II) can protect membranes and other lipid material against NX-photoinduced damage [10,11]. However, to have realistic potential for development as a drug excipient any UV screening agent, radical trap or excited state quencher must be bio-compatible. This places fairly severe limits on the types of species that can be used.

An alternate approach is to include NX in a host/guest complex with β-cyclodextrin (β-CD). β-CD and related structures are essentially non-toxic, being largely excreted unchanged from the body, and biodegradable [12]. β-CD is a naturally occurring oligosaccharide consisting of seven α-1,4-linked D-glucose units. The β-CD molecule itself is water soluble but possesses a hydrophobic cavity ("host" component) which can include organic molecules ("guests") which would normally have little or no aqueous solubility. Recently there has also been a lot of interest in using CDs as drug delivery media [12–16]. CDs are valuable in this context because they increase the aqueous solubility, stability and bio-availability of drugs [12–16].

It is well established that complexation of an organic guest by β-CD can be used to control both thermochemical [17–19] and photochemical [20–23] reactivity. For example, β-CD-complexed reagents can exhibit photochemistry which is quite different from that observed in homogeneous solution [20–24]. It is also well known that complexation by β-CD isolates the guest from other species, such as excited state quenchers (e.g. O_2), present in the aqueous bulk medium [25–28]. This reactivity control and protection effect, coupled with the desirable bio-compatibility characteristics of β-CD, make it potentially a useful tool to control unwanted drug side effects that arise from drug thermo- or photochemistry.

de Guidi et al. have reported that NX mediated photolysis of red blood cells can be inhibited by addition of β-CD. The protective effect was explained in terms of a reduction in the quantum yield of decarboxylation in the CD included NX molecules with a resulting decrease in the amount of radical product [5]. Although not specifically mentioned by these authors, complexation of NX by β-CD might also reduce the amount of singlet oxygen produced in the system by protecting the NX excited state from endogenous oxygen. CD suppression of photodecarboxylation has also been indicated for ketoprofen [24], suprofen [29] and tolmetin [2].

Typically pharmaceutical preparations are complex mixtures of drugs and excipients which may include buffer salts, anti-microbial agents, surfactants, etc. [16,30]. Once introduced into the body they are exposed to a wide range of endogenous species like ions and proteins. In fact the latter have been suggested to be a major source for the displacement of drugs from CD cavities in vivo [15]. For a cyclodextrin to be a useful tool to suppress NX side effects the NX must remain bound to the CD both in its initial formulation and in vivo. Is this likely to be the case? This question can be answered by recognizing that a more realistic in vitro model for the NX/β-CD system can be obtained by simply adding a third component to the system.

Recently the impact of adding a third component on CD host/guest binding has attracted attention. Studies of "third-party effects" have examined inter- and intramolecular excimer formation within CD cavities [31–34], alteration of quenching of guest fluorescence by quenchers that might also be CD complexed [35–38], variation in chemical behavior with surfactants as the third component [39–43] and the influence of amino acids on pyrene binding [44]. Addition of small amounts of water soluble polymers to CD/drug solutions has been shown to improve drug solubility and bio-availability relative to simple CD/drug formulations [45,46].

Alcohols have also been studied as "third parties" for a variety of CD/guest systems [27,47–55]. The behavior of CD/guest systems has been probed by both steady-state [25,27,37,38,49,50,52,53,56,57] and time-resolved fluorescence [25,58,59] techniques. By far the largest portion of these studies have made use of polyaromatic hydrocarbons (PAHs), especially pyrene derivatives, as guests. PAHs are strongly fluorescent and bind to the CD because of their hydrophobicity [60]. Addition of alcohols can increase the extent of binding by formation of ternary CD/PAH/alcohol complexes [47–49,52]. Naphthalene binding to CDs has also been studied fairly extensively [27,39,61–63], as has the influence of certain alcohols on these complexes. Again, ternary complexes can form [27,48]. Similar results have been found for binding of the more water soluble α-(naphthyloxy)-acetic acid with γ-CD [60], although ternary complexes do not play a role in the β-CD/2-naphthol/alcohol systems [25]. From these studies a picture of the binding in ternary CD/PAH/alcohol complexes has been emerging. The size and geometry of the alcohol seem to be important factors [47,48] as is its ability to alter the hydrophobicity of the CD cavity [49]. The presence of the primary and secondary hydroxyl groups of the CD are also essential for formation of ternary alcohol complexes [49], the stoichiometry of which depends on the specific guest, type of CD and type of alcohol [48,49].

Considering the interest in suppressing the phototoxicity of NX and related compounds, coupled with the fact that

real NX/CD formulations will be exposed to "third-party" components, we decided to undertake a detailed fluorescence study of the binding of NX to β-CD in the presence of a number of common alcohols and the plasma protein bovine serum albumin (BSA). While values for the NX binding constant with β-CD have been recently reported [64,65] no detailed study of the nature of the binding in the presence of additives has been carried out. We have also carried out an examination of the impact of additives on the efficiency of the photoconversion of NX in the absence and presence of β-CD. This work is part of an ongoing investigation of the binding of naphthalene derivatives to CDs in the presence of additives.

2. Experimental

NX (98% purity, Fluka) was used as received as was sodium iodide (NaI, Merck). β-CD (Aldrich) was recrystallized twice from water. Bovine serum albumin (BSA, 64 kDa, Fluka) was used without additional purification. All alcohols (Aldrich, Fluka) were of spectroscopic or HPLC grade and used without additional purification. Buffer salts (disodium hydrogen *ortho*-phosphate, potassium dihydrogen phosphate) were of analytical quality and were purchased from BDH. Water was conductivity grade (Millipore "Academic" model).

Fluorescence measurements were made at pH 7.4 (phosphate buffer). The buffer solutions were prepared according to literature methods [66]. Samples for fluorescence analysis were prepared by dissolving an appropriate mass of NX in the appropriate buffer. If an alcohol was to be added, the neat alcohol was injected via a Hamilton microliter syringe directly into 10 ml of the β-CD:NX solution. If BSA was to be added it was introduced at the stage of preparing the NX/buffer stock solution. The CD samples were stirred overnight to obtain equilibrium. Typically the NX concentration in the measurement cuvette was about 10^{-4} M. Alcohol concentrations were typically 1% v/v. BSA concentrations were in the μM range. Fluorescence quencher stock solutions in buffer were prepared just before use by dissolving an appropriate mass of NaI in the appropriate β-CD:NX solution with or without added alcohol. Quenching experiments were performed by injecting small aliquots of quencher stock into 2 ml samples of β-CD:NX solutions immediately prior to measurement. Exposure to light was kept to a minimum during all sample preparation and handling. All samples were air saturated.

Separation of protein from samples containing BSA was carried out using pre-washed Centricon YM-10 centrifugal filter devices (designed to cut-off a MW = 10,000, Millipore). The samples were placed in the sample reservoir and spun in a centrifuge (Brinkmann, JA17 rotor head) at 5500 rpm at 4°C for 1 h. The filtrate was collected, diluted with buffer and its fluorescence spectrum recorded.

Steady-state fluorescence measurements were carried out at 22 ± 1°C with a Perkin-Elmer LS-50B instrument. Instrument control, data collection and preliminary data processing were carried out by a computer interfaced to the fluorimeter. Samples for fluorescence measurements were contained in 1 cm × 1 cm standard quartz cuvettes (Hellma). The spectra were recorded with excitation at 280 nm. Spectra were scanned between 300 and 440 nm. The band pass was typically 2 nm. In the systems containing BSA excitation was at 320 nm to avoid light absorption by the protein. In the BSA systems the spectra were scanned between 340 and 440 nm. All emission spectra were uncorrected. Fluorescence intensities were determined by integrating the emission spectra between 300 and 440 nm or estimated by recording the peak height at 355 nm (BSA experiments). Absorption spectra were recorded with a Perkin-Elmer Lambda 40 UV–VIS spectrophotometer. Calculations of the association constants were performed within the Kaleidagraph (Abelbeck) framework.

UV-photolysis experiments involving NX were carried out in air saturated pH 7.4 aqueous phosphate buffer, with or without added β-CD/alcohol. The NX concentration was about 5×10^{-4} M. The samples were irradiated, for varying lengths of time, in Pyrex tubes. The light source was a water-cooled 200 W Ace-Hanovia high-pressure Hg lamp housed in a "merry-go-round" photoreactor cabinet (Ace Glass). The impact of irradiation under the different experimental conditions was evaluated by monitoring the changes in the NX absorption spectrum as a function of irradiation time.

3. Results and discussion

Fluorescence measurements were made on 10^{-4} M NX in aqueous solutions at pH 7.4. This pH was chosen as it matches physiological pH. The pK_a of NX is 4.2 [67] so at pH 7.4 the NX will be in the anionic deprotonated form.

The fluorescence intensity of NX in aqueous solution at pH 7.4 is sensitive to added β-CD, as shown in Fig. 1. When the integrated intensity values are plotted as a function of [β-CD] a typical binding isotherm [68,69] is observed (Fig. 2). According to the literature on the binding of naphthalene derivatives to β-CD, it is expected [23,25,33,34,39,61–63] that the β-CD:NX complex is a so-called 1:1 complex formed via the equilibrium process

$$\text{NX} + \beta\text{-CD} \rightleftarrows \beta\text{-CD} : \text{NX} \quad (1)$$

which has the binding constant defined by

$$K_1 = \frac{[\beta\text{-CD} : \text{NX}]}{[\text{NX}][\beta\text{-CD}]} \quad (2)$$

In systems containing alcohols the following equilibria must also be considered:

$$\text{ROH} + \beta\text{-CD} \rightleftarrows \beta\text{-CD} : \text{ROH} \quad (3)$$

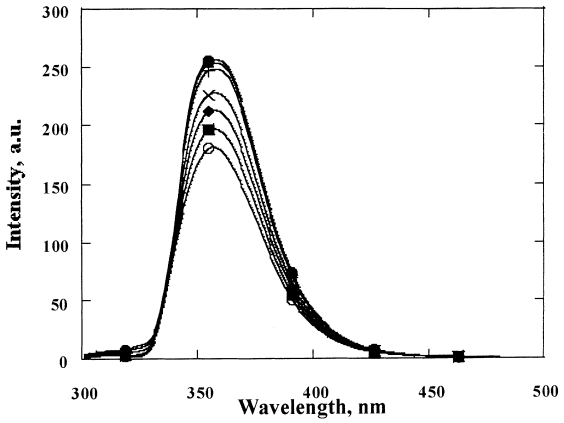

Fig. 1. Changes in 1×10^{-4} M naproxen fluorescence as a function of [β-CD] in pH 7.4 buffer containing 1% ethanol. The β-CD concentrations were 0 mM (○), 0.5 mM (■), 1 mM (◆), 2 mM (×), 5.5 mM (+), 8 mM (▲) and 10 mM (●).

$$\text{ROH} + \beta\text{-CD/NX} \rightleftarrows \beta\text{-CD : NX : ROH} \quad (4)$$

We can combine these two equilibria with that of Eq. (1) into the general process

$$\text{ROH} + \beta\text{-CD} + \text{NX} \rightleftarrows \text{NX}_{\text{bound}} \quad (5)$$

for which we can write an apparent binding constant

$$K_{\text{app}} = \frac{[\text{NX}]_{\text{bound}}}{[\text{NX}][\beta\text{-CD}]} \quad (6)$$

under conditions of excess alcohol. We can use a modified version of Eq. (6) to quantify the binding of NX to BSA in the absence of CD and alcohols. We do not specify the detailed nature of the binding except that there be "bound" and "free" NX. The only modification required to Eq. (6) is, then, that [β-CD] be replaced by [BSA].

The treatment for extracting the value of K_{app} from the binding isotherm has been described previously [25,53,70]. Essentially the binding isotherm data are fit by the model

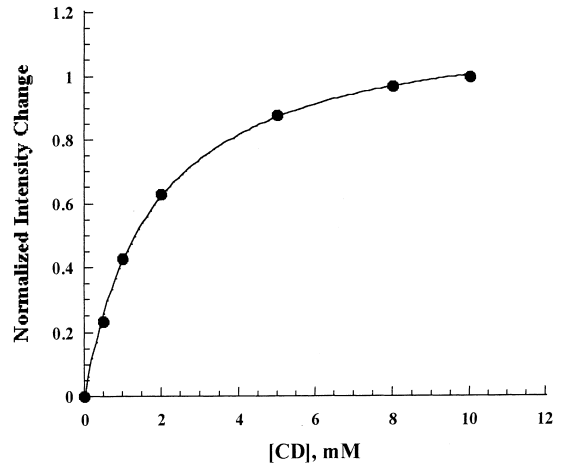

Fig. 2. Binding isotherm for 1×10^{-4} M naproxen with β-CD at pH 7.4 in the presence of 1% ethanol. The solid line is a fit of the data to Eq. (7).

Table 1
Equilibrium constants, K_{app}, for the binding of naproxen to β-CD at pH 7.4 in the presence of various linear alcohols

Alcohol[a]	K_{app} (M^{-1})
None	475 ± 35
Ethanol	444 ± 27
1-Propanol	331 ± 34
1-Butanol	214 ± 27
1-Pentanol	10 ± 27

[a] [Alcohol] = 1% v/v.

$$\Delta I = K_{\text{app}} \left(\frac{\Delta i [\text{NX}]_0 [\beta\text{-CD}]}{1 + K_{\text{app}}[\beta\text{-CD}]} \right) \quad (7)$$

(Again [β-CD] is replaced by [BSA] for binding of NX to the protein.) The integrated emission intensities were treated according to this equation. ΔI refers to the difference between the fluorescence intensity at a particular [β-CD] and that in the absence of β-CD. $[\text{NX}]_0$ in all the measurements was about 100 μM, and Δi reflects the maximum value of ΔI. Fig. 2 shows non-linear regression fits of Eq. (7) to the binding isotherm data for the aqueous system in the presence of 1% ethanol. Similar plots were obtained for the other systems examined. The K_{app} values obtained are shown in Table 1.

In the absence of added alcohols the value of K_{app} corresponds to that of K_1. The value of $475 \pm 35\ \text{M}^{-1}$ obtained in this study corresponds closely to the value of 620 reported at pH 9 by Sadlej-Sosnowska et al. [65]. The K_{app} data in Table 1 for the linear alcohols follow the same trend as that observed for the influence of linear alcohols on the binding of 2-naphthol [25] and of naphthalene [27]. A similar pattern has been reported for the influence of sodium *n*-alkyl sulfate surfactants on naphthalene binding to β-CD [39]. That is, the apparent binding of naphthalenes to β-CD decreases systematically as the chain length of the linear alcohol increases. This is usually interpreted as reflecting the reduction in the concentration of free CD when the alcohol is present which in turn inhibits binding of the NX to the CD cavity environment [25,27,55]. However, it has also been proposed that such effects may be due to the alteration in the nature of the bulk solvent when alcohol is present, at least with salicylic acid as guest [71].

To gain additional insight into the β-CD:NX system we determined the value of K_{app} at various concentrations of 1-pentanol (Table 2, Fig. 3). These measurements allow estimation of the three equilibrium constants K_1, K_2 and K_3 (Eqs. (1), (3) and (4), respectively). The relation between K_{app} and these three equilibrium constants is given by [53]

$$K_{\text{app}} = \frac{K_1 + K_2 K_3 [\text{ROH}]}{1 + K_2 [\text{ROH}]} \quad (8)$$

where K_1 is the equilibrium constant for binding of NX to β-CD in the absence of alcohols and can be determined independently. We prefer, however, to use it as a variable which provides an internal check of the reliability of the fit.

Table 2
Variation of K_{app} as a function of [1-pentanol] at pH 7.4

[1-Pentanol] (mM)	K_{app} (M^{-1})
0	475 ± 35
5	310 ± 26
9	177 ± 31
25	178 ± 32
46	177 ± 30
70	157 ± 66

Fig. 3 shows K_{app} values for the β-CD:NX system as a function of the 1-pentanol concentration. The data were fit by Eq. (8) and yielded an excellent correlation. The equilibrium constants obtained from the fit were $K_1 = 477 \pm 38\,M^{-1}$, $K_2 = 31 \pm 18\,M^{-1}$ and $K_3 = 141 \pm 32\,M^{-1}$.

The K_1 value determined from Fig. 3 closely matches that obtained by fitting the variation in NX fluorescence intensity in the absence of alcohol to Eq. (7). The value of K_2, corresponding to the binding constant for the association of pentanol with β-CD, is in good agreement with the literature value of $63\,M^{-1}$ [72]. This gives us confidence that our model and fitting procedure are reliable.

The results of our modeling procedures support the view that the β-CD:NX system forms ternary complexes in the presence of linear alcohols, as does the β-CD/naphthalene system [27]. By contrast, the β-CD complexes of 2-naphthol do not [25]. 2-Naphthol, NX and naphthalene are similar in size but nonetheless exhibit quite different complex stoichiometries in CD/alcohol solutions. Clearly it is insufficient to attribute additive effects on binding to steric issues alone at least in some cases.

Water-soluble fluorescence quenchers have also been used to gain insight into the relative distribution of a guest between the aqueous and CD environments [25,27,35,62]. NaI is a good choice of fluorescence quencher in this context. Iodide salts are often used to quench excited singlet states

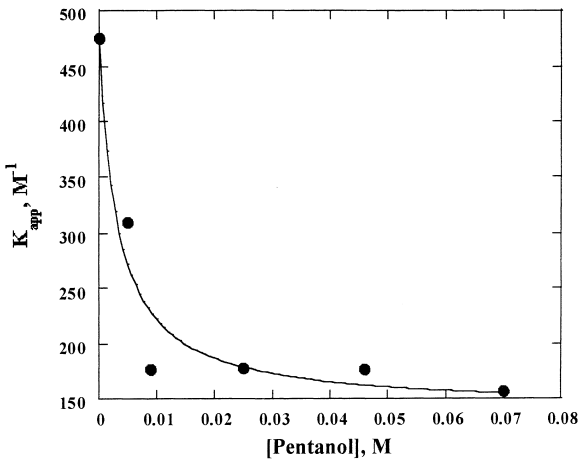

Fig. 3. Fit of K_{app} values as a function of [pentanol] to Eq. (8) for 1×10^{-4} M naproxen at pH 7.4.

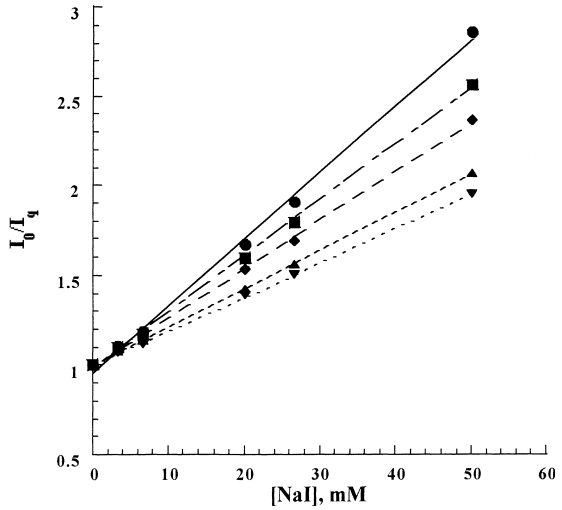

Fig. 4. Stern–Volmer plots for NaI quenching of naproxen at pH 7.4 in the presence of 1% pentanol at [β-CD] concentrations of 0 mM (●), 2 mM (■), 4 mM (◆), 8 mM (▲) and 10 mM (▼).

[20,73] and NaI has at most a very weak association ($K = 18\,M^{-1}$) with the β-CD cavity [74].

We performed a series of experiments in which we used NaI to quench the fluorescence of NX in the presence of varying [CD] either in the absence or presence of 1-pentanol. Stern–Volmer plots (I_0/I_q versus [NaI]) were measured up to 50 mM NaI. Under these conditions the plots are linear (Fig. 4) in all cases and the resulting Stern–Volmer constants, K_{SV}, are presented in Table 3. The table also shows the concentration of free NX under the different experimental conditions as calculated from the values of K_1 and K_{app}. The trends in K_{SV} are consistent with those reported previously for NaI quenching of naphthalene derivatives in the presence of β-CD [25,27]. In the absence of added pentanol there is a gradual reduction in K_{SV} as the β-CD concentration is increased. This simply reflects the encapsulation of the NX by the β-CD thereby partially isolating it from the primarily aqueous phase NaI quencher. When pentanol is present the binding of NX to β-CD is strongly suppressed and this is reflected in a very gradual and very limited reduction in the K_{SV} value as [β-CD] is raised. In essence the alcohol forces

Table 3
Stern–Volmer slopes (K_{SV}) for NaI quenching of naproxen fluorescence at pH 7.4 under various conditions and bound to free naproxen ratio[a] under the same conditions

[β-CD] (mM)	No pentanol		1% pentanol	
	K_{SV} (M^{-1})	$[NX]_{bound}/[NX]_{free}$	K_{SV} (M^{-1})	$[NX]_{bound}/[NX]_{free}$
0	42.1	0	37.3	0
2	21.4	0.95	31.4	0.02
4	18.8	1.9	27.3	0.04
8	17.2	3.8	21.4	0.08
10	15.5	4.8	20.1	0.1

[a] Based on the values of K_1 and K_{app} and using Eq. (6).

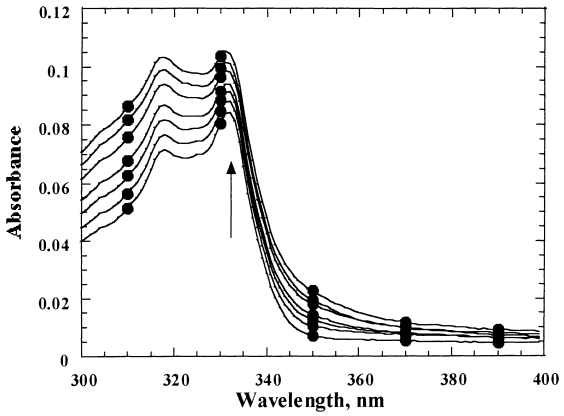

Fig. 5. Absorption spectra for 5×10^{-4} M naproxen at pH 7.4 photolyzed ($\lambda_{ex} > 310$ nm) for 5 min intervals from 0 to 30 min. Arrow shows increasing time.

the NX to reside in the aqueous environment where it is more readily quenched than in the β-CD environment.

The results of our fluorescence experiments clearly indicate that linear alcohol additives can weaken the association between NX and the β-CD cavity. Will this be reflected in the photochemistry of NX? Fig. 5 shows a series of absorption spectra for 5×10^{-4} M NX in pH 7.4 buffer containing 10 mM β-CD. The different spectra represent different irradiation times in the photochemical reactor under air saturated conditions. As photolysis time increases there is clear formation of photoproduct(s) absorbing in the 300–350 nm region with some tailing out to longer wavelengths. We used the variation in absorbance 330 nm to evaluate the initial rate of photoconversion. Plots of absorbance versus time were linear for the first 30 min of irradiation and the slopes of these plots (Table 4) reflect the efficiency of photoconversion.

From Table 4 it is clear that photoconversion is strongly suppressed in the presence of 10 mM β-CD as expected from previous work on similar NSAIDs [24,29]. In the absence of pentanol the efficiency of the photoreaction is suppressed by a factor of $2.0/0.7 = 2.9$ upon addition of 10 mM β-CD. This closely matches the inhibition of NaI quenching by 10 mM β-CD ($42.12/15.50 = 2.7$, Table 3) as expressed by the ratio of Stern–Volmer slopes. Based on recent literature reports on the photolysis of similar carboxylic acid type NSAIDs [2,24,29], we attribute the suppression of the photoreaction to a reduction in the quantum yield for photodecarboxylation when NX is complexed by β-CD.

Table 4
Initial slopes of photolysis curves (absorbance vs. time) for various irradiation conditions under air saturation[a]

[β-CD] (mM)	Initial photolysis slope (min^{-1})	
	No pentanol	1% pentanol
None	2.0×10^{-3}	2.8×10^{-3}
10	0.7×10^{-3}	2.0×10^{-3}

[a] [NX] = 5×10^{-4} M and pH 7.4 in each case.

Upon irradiation of 5×10^{-4} M NX at 1% pentanol, 0 mM β-CD the photoconversion slope is very similar to that in buffer alone indicating that the mere presence of the alcohol has no impact on the photoreaction. However, under the 1% pentanol, 10 mM β-CD conditions only minor inhibition of the photoreaction is observed. The photoconversion slope ratio in this case is $2.8/2.0 = 1.4$. Again, this is in good agreement with the ratio of Stern–Volmer constants under these conditions ($37.28/19.10 = 1.9$, Table 3). Thus, we can conclude that the effect of adding pentanol to the β-CD:NX system is to cause redistribution of the NX into the aqueous environment thus reducing any control that the CD cavity had over the course of the NX photoreaction.

Experiments with the β-CD/naproxen system in the presence of protein (BSA, pH 7.4) show that BSA is also effective at disrupting the complex between β-CD and NX. Fig. 6 shows the variation in the NX fluorescence spectrum induced by addition of BSA in the absence of β-CD. The samples were excited at 320 nm to avoid light absorption by the protein. The protein alone exhibits no fluorescence under these conditions. As BSA is added the NX fluorescence spectrum is shifted slightly to the blue and acquires vibronic structure. In the presence of 8 μM BSA and 10 mM β-CD the observed fluorescence of NX was also blue-shifted and structured.

The spectral changes described in Fig. 6 indicate an interaction between the fluorophore and the protein. Based in the recorded peak heights at 355 nm we calculate a K_{app} for binding of NX to BSA of $(3.2 \pm 1.6) \times 10^5$ M^{-1} assuming a 1:1 binding model. Although the error in this value is large, the magnitude of K_{app} indicates that NX binds much more strongly to BSA than to β-CD in buffered aqueous solution.

The fluorescence spectra of NX buffered to pH 7.4 in the presence of 10 mM β-CD with or without 8 μM BSA were recorded. The former sample exhibited the "normal" (i.e. red-shifted, unstructured) fluorescence spectrum of NX while the latter exhibited the blue-shifted, structured spectrum. These samples were centrifuged through a Centricon

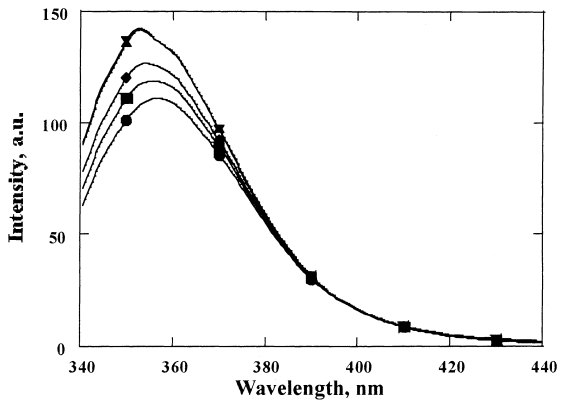

Fig. 6. Changes in naproxen fluorescence as a function of [BSA] at pH 7.4. Protein concentrations were 0 μM (●), 1 μM (■), 2 μM (◆), 4 μM (▲) and 8 μM (▼).

YM-10 device designed to cut-off a MW = 10,000 or higher. The filter should pass NX and β-CD but not the protein. Both filtrates were collected and the fluorescence recorded. The filtrate fluorescence intensity of the sample containing no BSA was within 20% of the intensity of the unfiltered sample containing no BSA. By contrast, the filtrate when BSA was present exhibited no measurable fluorescence. This clearly shows that the NX is entirely associated with the protein, even if CD is also present. Further, this interaction does not involve association of CD/NX complexes with the BSA. As noted above the fluorescence spectrum of NX is blue-shifted and structured in the presence of BSA even if β-CD is present. This indicates NX is no longer probing a CD cavity environment when the protein is present. This, together with the large NX/BSA binding constant, strongly suggests that the protein disrupts the CD/NX complexes such that uncomplexed NX is associated primarily with the BSA.

Recent work, reviewed by Stella et al. [15], indicates that in vivo drug release from CD complexes is typically rapid and quantitative. Dissociation is driven by dilution upon i.v. administration (the dilution factor would be about 10,000-fold for a 70 kg individual). In cases where the drug is strongly CD bound or where dilution is minimal drug release is facilitated by competitive displacement by endogenous materials, binding to plasma protein, binding to tissue components, etc. Our present results with model materials (alcohols and BSA) fit well with this hypothesis of efficient drug displacement from CD complexes in vivo.

The entire premise of using CDs as drug carriers is based on the concept that the CD will release the drug once the CD/drug complex comes in contact with a hydrophobic domain such as a bio-membrane or with a target bio-polymer such as a protein [12,15]. The use of CDs to suppress the photosensitizing side effects of NSAIDs like NX, ketoprofen, suprofen and tolmetin is, by contrast, based on the premise that the CD-mediated inhibition of drug photochemistry observed in vitro will be maintained in vivo. That is, it is based on the drug remaining CD complexed even in the presence of bio-membranes, tissue and proteins. Clearly, given the high concentrations of lipid like domains and bio-polymers in the body, the association of drug and CD is likely to be undermined. Even so, there is clear evidence that CDs do suppress the photosensitizing power of the NSAIDs mentioned [2,5,24,29]. In their recent paper dealing with tolmetin [2], Sortino and Scaiano suggest that the observed reduction in bio-damage cannot be entirely attributed to a decrease in NSAID photoconversion in the presence of CD. They propose that the inhibition of phototoxicity may be due in part to CD complexation of toxic photoproducts, thereby rendering them inactive, and in part to the possibility of CD trapping of radical species formed during tolmetin photolysis. The products of tolmetin photolysis are structurally similar to tolmetin itself. A similar situation pertains to photoproducts of the NX system [8]. Conditions which inhibit CD binding of tolmetin or NX should also inhibit binding of their photoproducts. CD trapping of radicals, on the other hand, remains a possible mechanism for the in vivo impact of CDs on reduction of the photosensitizing effects of NSAIDs.

In conclusion, our results on model systems involving β-CD, NX and alcohols or protein support the view [15] that CD complexation of drugs will be significantly weaker in vivo than in vitro. It thus seems unlikely that the reduction in phototoxicity observed for the NSAIDs in the presence of CDs can be attributed solely to the ability of the CD to complex the drugs or their photoproducts. Clearly this is an area which needs additional work both on model systems and in vivo.

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References

[1] J.N. Delgado, W.A. Remers (Eds.), Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry, 10th Edition, Lippincott-Raven, New York, 1998.
[2] S. Sortino, J.C. Scaiano, G. de Guidi, S. Monti, Photochem. Photobiol. 70 (1999) 549.
[3] B.L. Diffey, T.J. Daymond, H. Fairgreaves, Br. J. Rheumatol. 22 (1984) 239.
[4] B. Ljunggren, K. Lundberg, Photodermatology 2 (1985) 377.
[5] G. de Guidi, G. Condorelli, S. Giuffrida, L.L. Constanzo, J. Incl. Phenom. 15 (1993) 43.
[6] T. Artuso, J. Bernadou, B. Meunier, N. Paillous, Biochem. Pharmacol. 39 (1990) 407.
[7] T. Artuso, J. Bernadou, B. Meunier, J. Piette, N. Paillous, Photochem. Photobiol. 54 (1991) 205.
[8] D.E. Moore, P.P. Chappuis, Photochem. Photobiol. 47 (1988) 173.
[9] L.L. Costanzo, G. de Guidi, G. Condorelli, A. Cambria, M. Fama, J. Photochem. Photobiol. B: Biol. 3 (1989) 223.
[10] L.L. Costanzo, G. de Guidi, S. Giuffrida, S. Sortino, G. Condorelli, G. Pappalardo, J. Inorg. Biochem. 57 (1995) 115.
[11] S. Giuffrida, G. de Guidi, P. Miano, S. Sortino, G. Condorelli, L.L. Costanzo, J. Inorg. Biochem. 63 (1996) 253.
[12] K. Uekama, F. Hirayama, T. Irie, Chem. Rev. 98 (1998) 2045.
[13] K.-H. Fromming, J. Szejtli, Cyclodextrins in Pharmacy, Kluwer Academic Publishers, Dordrecht, 1994.
[14] T. Loftsson, M.E. Brewster, J. Pharm. Sci. 85 (1996) 1017.
[15] V.J. Stella, V.M. Rao, E.A. Zabbou, V. Zia, Adv. Drug Delivery Rev. 36 (1999) 3.
[16] T. Loftsson, T. Jarvinen, Adv. Drug Delivery Rev. 36 (1999) 59.
[17] J. Szejtli, Cyclodextrins and their Inclusion Complexes, Akademiai Kiado, Budapest, 1982.
[18] G. Wenz, Angew. Chem. Int. Ed. Engl. 33 (1994) 803.
[19] J. Horsky, J. Pitha, J. Incl. Phenom. Mol. Recog. Chem. 18 (1994) 291.
[20] K. Kalyanasundaram, Photochemistry in Microheterogeneous Systems, Academic Press, New York, 1987.
[21] V. Rammamurthy (Ed.), Photochemistry in Organized and Constrained Media, VCH, Weinheim, 1991.

[22] V. Ramamurthy, R.G. Weiss, G.S. Hammond, Adv. Photochem. 18 (1993) 67.
[23] P. Bortolus, S. Monti, in: D.C. Neckers, D.H. Volman, G. von Bünau (Eds.), Advances in Photochemistry, Vol. 27, Wiley, New York, 1996, p. 1.
[24] S. Monti, S. Sortino, G. de Guidi, G. Marconi, New J. Chem. 22 (1998) 599.
[25] J. van Stam, S. De Feyter, F.C. De Schryver, C.H. Evans, J. Phys. Chem. 100 (1996) 19959.
[26] C.H. Evans, S. De Feyter, L. Viaene, J. van Stam, F.C. De Schryver, J. Phys. Chem. 100 (1996) 2129.
[27] C.H. Evans, M. Partyka, J. van Stam, J. Incl. Phenom. 38 (2000) 381.
[28] M. Christoff, L.T. Okano, C. Bohne, J. Photochem. Photobiol. A: Chem. 134 (2000) 169.
[29] S. Sortino, G. De Duidi, G. Marconi, S. Monti, Photochem. Photobiol. 67 (1998) 603.
[30] L.W. Chan, T.R.R. Kurup, A. Muthaiah, J.C. Thenmozhiyal, Int. J. Pharm. 195 (2000) 71.
[31] K. Kano, M. Matsumoto, S. Hashimoto, J. Am. Chem. Soc. 107 (1985) 6117.
[32] S. De Feyter, J. van Stam, F. Imans, L. Viaene, F.C. De Schryver, C.H. Evans, Chem. Phys. Lett. 277 (1997) 44.
[33] T.C. Barros, K. Stefaniak, J.F. Holzwarth, C. Bohne, J. Phys. Chem. A 102 (1998) 5639.
[34] G. Grabner, K. Rechthaler, B. Mayer, G. Kohler, J. Phys. Chem. A 104 (2000) 1365.
[35] M.V. Encinas, E.A. Lissi, A.M. Rufs, Photochem. Photobiol. 57 (1993) 603.
[36] E. Harris, N.R. Russell, J. Incl. Phenom. Mol. Recog. Chem. 16 (1993) 355.
[37] E.K. Fraiji, T.R. Cregan, T.C. Werner, Appl. Spectrosc. 48 (1994) 79.
[38] D.J. Jobe, V.C. Reinsborough, S.D. Wetmore, Langmuir 11 (1995) 2476.
[39] S. Hashimoto, J.K. Thomas, J. Am. Chem. Soc. 107 (1985) 4655.
[40] E.A. Cummings, T.T. Ndou, V.K. Smith, I.M. Warner, Appl. Spectrosc. 47 (1993) 2129.
[41] E. Junquera, G. Tardajos, E. Aicart, Langmuir 9 (1993) 1213.
[42] T. Tominaga, D. Hachisu, M. Kamado, Langmuir 10 (1994) 4676.
[43] L. Garcia-Rio, J.R. Leis, J.C. Mejuto, J. Perez-Juste, J. Phys. Chem. B 101 (1997) 7383.
[44] H.C.B. Yang, J. Phys. Chem. 100 (1996) 14533.
[45] T. Loftsson, A.M. Sigurdardottir, Eur. J. Pharm. Sci. 2 (1994) 297.
[46] T. Loftsson, H. Fridriksdottir, A.M. Sigurdardottir, Int. J. Pharm. 110 (1994) 169.
[47] A. Muñoz de la Peña, T.T. Ndou, J.B. Zung, K.L. Greene, D.H. Live, I.M. Warner, J. Am. Chem. Soc. 113 (1991) 1572.
[48] J.B. Zung, A. Muñoz de la Peña, T.T. Ndou, I.M. Warner, J. Phys. Chem. 95 (1991) 6701.
[49] N.B. Elliott, T.T. Ndou, I.M. Warner, J. Incl. Phenom. Mol. Recog. Chem. 16 (1993) 99.
[50] J. Schuette, T. Ndou, A. Muños de la Peña, S. Mukundan, I. Warner, J. Am. Chem. Soc. 115 (1993) 292.
[51] A. Ponce, P. Wong, J.J. Way, D.G. Nocera, J. Phys, Chem. 97 (1993) 11137.
[52] J. Schuette, Y. Will, R. Agbaria, I. Warner, Appl. Spectrosc. 48 (1994) 581.
[53] Y. Liao, C. Bohne, J. Phys. Chem. 100 (1996) 734.
[54] M. Mileewski, W. Augustyniak, A. Maciejewski, J. Phys. Chem. A 102 (1998) 7427.
[55] O.S. Tee, M.I. Hussein, I.E. Turner, O.J. Yaxbeck, Can. J. Chem. 78 (2000) 436.
[56] M. Sbai, A. Ait Lyazidi, D.A. Lerner, B. del Castillo, M.A. Martin, Anal. Chim. Acta 303 (1995) 47.
[57] Y.-B. Jiang, X.-J. Wang, Appl. Spectrosc. 48 (1994) 1428.
[58] S. De Feyter, J. van Stam, N. Boens, F.C. De Schryver, Chem. Phys. Lett. 249 (1995) 46.
[59] T. Soujanya, T.S.R. Krishna, A. Samanta, J. Phys. Chem. 96 (1992) 8544.
[60] A. Muñoz de la Peña, F. Salinas, M.J. Gómez, M.I. Acedo, M. Sánchez Peña, J. Incl. Phenom. Mol. Recog. Chem. 15 (1993) 131.
[61] S. Hamai, Bull. Chem. Soc. Jpn. 55 (1982) 2721.
[62] G. Nelson, I. Warner, J. Phys. Chem. 94 (1990) 576.
[63] X. Wang, M.L. Brusseau, Environ. Sci. Tech. 29 (1995) 2346.
[64] I. Velaz, M. Sanchez, A. Zornoza, N. Goyenechea, Biomed. Chromatogr. 13 (1999) 155.
[65] N. Sadlej-Sosnowska, L. Kozerski, E. Bednarek, J. Sitowski, J. Incl. Phenom. 37 (2000) 383.
[66] Handbook of Chemistry and Physics, 63rd Edition, CRC Press, Boca Raton, 1982.
[67] A.C. Moffat (Ed.), Clarke's Isolation and Identification of Drugs, Pharmaceutical Press, London, 1986.
[68] K.A. Connors, Binding Constants, Wiley, New York, 1987.
[69] K.A. Connors, Chem. Rev. 97 (1997) 1325.
[70] M. Barra, C. Bohne, J.C. Scaiano, J. Am. Chem. Soc. 112 (1990) 8075.
[71] E. Junquera, D. Ruiz, E. Aicart, J. Colloid Interface Sci. 216 (1999) 154.
[72] Y. Matsui, K. Mochida, Bull. Chem. Soc. Jpn. 52 (1979) 2808.
[73] S.L. Murov, I. Carmicheal, G.L. Hug, Handbook of Photochemistry, 2nd Edition, Marcel Dekker, New York, 1993.
[74] R.P. Rohrbach, L.J. Rodriguez, E.M. Eryring, J.F. Wojcik, J. Phys. Chem. 81 (1977) 944.