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ORIGINAL ARTICLE

# Quantitation of naproxen by quenching of phosphorescence from a ternary complex of 2-bromo-6-methoxynaphthalene and α-cyclodextrin

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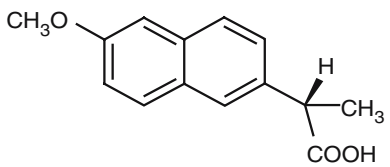
**Abstract** A unique method, based on the measurement of phosphorescence lifetimes, is reported for use in analytically detecting the widely used anti-inflammatory drug naproxen. The reciprocal phosphorescence lifetime of a ternary complex containing α-cyclodextrin and 2-bromo-6-methoxynaphthalene increases linearly with naproxen concentration and has a quenching constant of $2.1 \pm 0.05 \times 10^6$ $M^{-1}$ $s^{-1}$. Similar behavior is observed for quenching by the sodium salt of naproxen that has a quenching constant of $1.8 \times 10^6 \pm 0.05$ $M^{-1}$ $s^{-1}$. The experimental sensitivity is sufficient to permit measurements of naproxen concentrations differing by 11 μg/ml. Phosphorescence lifetime measurements reveal that the quenching mechanism does not involve displacement of the unrelaxed triplet-state guest molecule by naproxen from the complex and may occur by energy transfer or electron transfer.

**Keywords** Cyclodextrin · Inclusion complexes · Phosphorescence lifetime measurement

## Introduction

Naproxen [(*S*)-(+)-6-methoxy-α-methyl-2-naphthaleneacetic acid] is a non-steroidal, anti-inflammatory drug that also possesses analgesic and antipyretic properties [1, 2] and has been used in the treatment of rheumatoid arthritis and other rheumatic or musculoskeletal disorders, dysmenorrheal, and acute gout. The drug has become suspected of increasing the likelihood of heart attack, and ongoing research has tested the validity of this suspicion [3].




naproxen

If in some patients naproxen or naproxen derivatives can be administered only under controlled circumstances in the future, it likely will have to be possible for it to be monitored analytically, conveniently, and quickly.

Several methods for analytical detection of naproxen in solution, formulations, and/or biological fluids have been described that are based on the intensities of fluorescence or phosphorescence [4–9]. Generally, phosphorescence methods require the presence in solution of a heavy atom to enhance the phosphorescence signal, such as Tl (0.02 M) [7] and an oxygen scavenger such as sulfite ion to minimize quenching of the phosphorescence. Tl and some other heavy-atom containing molecules are toxic, and the use of sulfite can require a significant equilibration time to achieve a constant oxygen concentration in solution. A convenient analytical method for detection of naproxen, based on shortening by naproxen of the phosphorescence lifetime of 2-bromo-6-methoxynaphthalene (N) encapsulated by two α-cyclodextrin (CD) molecules, is presented herein. The method has

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the advantages of requiring no oxygen scavenging and only a small concentration of heavy-atom containing molecule.

The method reported herein is based on the fact that 2-bromo-6-hydroxynaphthalene (guest molecule) forms a ternary complex with two CD molecules in which it is strongly phosphorescent even in solutions that have not been deoxygenated [10, 11]. Similar results have been obtained with other brominated naphthalenes, notably 2-bromo-6-methoxynaphthalene, used as guest molecules (M. D. Schuh et al. Manuscript submitted for publication). It is believed that the guest molecule is encapsulated in the cavity between the two CD molecules, which are assumed to have their edges containing the secondary hydrogens close together. This view is consistent with the theoretical structure calculated for the ternary complex of naphthalene with CD [12].

## Experimental

### Kinetics model

The experimental method and kinetics treatment have been described in detail [13, 14]. The formation of complexes between CD and N is proposed to occur by the following mechanism. $^3$N designates the triplet state molecule. $k_a$, $k_{-a}$, $k_+$, and $k_-$ designate the rate constants for formation and dissociation of the binary and ternary complexes involving $^3$N, respectively. $K_1$ and $K_2$ are the binding constants for formation of the ground-state binary complex and ternary complex, respectively. $k_o$, $k'$, and $k''$ are the unimolecular (or pseudo-unimolecular) decay rates of $^3$N, $^3$N·CD and $^3$N·CD$_2$, respectively. $k''$ includes the phosphorescence decay rate of $^3$N·CD$_2$, which is the only phosphorescent species in aerated solutions.

$$\begin{array}{ccccccc} & & & \overset{k_a}{} & & \overset{k_+}{} & \\ 2\mathrm{CD} + {}^3\mathrm{N} & \rightleftarrows & {}^3\mathrm{N{\cdot}CD} + \mathrm{CD} & \rightleftarrows & {}^3\mathrm{N{\cdot}CD_2} & & \\ & & & \underset{k_{-a}}{} & & \underset{k_-}{} & \\ k_o\downarrow\uparrow h\nu & & k'\downarrow\uparrow h\nu & & & k''\downarrow\uparrow h\nu & \\ & & & K_1 & & K_2 & \\ 2\mathrm{CD} + \mathrm{N} & \rightleftarrows & \mathrm{N{\cdot}CD} + \mathrm{C} & \rightleftarrows & \mathrm{N{\cdot}CD_2} & & \end{array}$$

Since the laser pulse produces a population of excited-state guest molecules for which more than 95% of the $^3$N molecules are bound in either the binary or ternary complex in aerated solutions, the rate equations for only the bound forms of $^3$N need to be solved. Application of the steady-state approximation to the $^3$N·CD concentration, [$^3$N·CD], and integration of the rate equation for [$^3$N·CD$_2$] shows that the phosphorescence decay of $^3$N·CD$_2$ should be single-exponential with a lifetime $\tau$ given by Eq. (1) [13].

$$1/\tau = k'' + k_- - \frac{k_-[\mathrm{CD}]}{(k_{-a} + k')/k_+ + [\mathrm{CD}]} \quad (1)$$

If a quencher, Q (naproxen), deactivates $^3$N·CD and $^3$N·CD$_2$ by causing $^3$N to relax to the ground state (either as a free *N* molecule or bound in a complex, N·CD or N·CD$_2$), then it can be shown that Eq. (2) governs $1/\tau$. $k_q'$ and $k_q''$ are the rate constants for quenching of the binary and ternary complexes, respectively.

$$1/\tau = k' + k_- + k_q''[\mathrm{Q}] - \frac{k_-[\mathrm{CD}]}{\left(k_{-a} + k' + k_q'[\mathrm{Q}]\right)/k_+ + [\mathrm{CD}]} \quad (2)$$

If [CD] is sufficiently small, then $k'' + k_- + k_q''$ [Q] dominates the right-hand side of Eq. (2), and a plot of $1/\tau$ vs. [Q] should be linear with a slope equal to $k_q''$

### Materials

2-Bromo-6-methoxynaphthalene (stated purity 97%) and (*S*)-naproxen and its sodium salt, both of USP grade, were obtained from Sigma-Aldrich and used without further purification. CD of highest purity was supplied as a generous gift by Cerestar USA, Inc. of Indianapolis, IN (lot no. G 8071-2) and was used without further purification. Water was deionized with a Barnstead EASY pure water purification system and typically had a resistivity of >18.0 MΩ cm.

### Method

All solutions were aqueous, contained pH 7, 0.05 M phosphate buffer, and were not deoxygenated, which favored strong quenching of both uncomplexed and binary-complexed triplet-state guest molecules and made phosphorescence from the ternary complex dominant [13, 14]. A circulating constant-temperature bath maintained sample temperatures at 25.0 ± 0.1 °C.

Solutions of stock mixture were prepared by combining fixed volumes of solutions containing CD and the phosphorescent probe 2-bromo-6-methoxynaphthalene. Different volumes of naproxen stock solution

and different volumes of water were added to produce individual 2-ml samples, each containing a different naproxen concentration. CD and 2-bromo-6-methoxynaphthalene concentrations were 0.05 M and $2 \times 10^{-5}$ M, respectively, in each sample. The same procedure was used to prepare 2-ml samples containing different concentrations of the sodium salt of naproxen.

Pulsed laser excitation (7 ns) was at 345 nm and was produced as the frequency doubled output of a dye laser that was pumped by a Spectra Physics model 150 Nd:YAG laser, and phosphorescence was recorded at 512 nm, the spectral peak. Guest molecule fluorescence and scattered laser light were greatly reduced in intensity relative to that of phosphorescence by using an electronic gating circuit that reduced the photomultiplier voltage during the time interval of the laser pulse. Phosphorescence signals were acquired by signal averaging on a Tektronix model TDS 620 digital oscilloscope and was found to decay single-exponentially over two to three orders of magnitude.

## Results

The linear plot of $1/\tau$ vs. [naproxen] in Fig. 1 is consistent with the kinetics model and has a slope of $2.1 \pm 0.05 \times 10^6$ $M^{-1}$ $s^{-1}$. A similar plot was obtained for the sodium salt of naproxen, with slope of $1.8 \pm 0.05 \times 10^6$ $M^{-1}$ $s^{-1}$. The plots of $1/\tau$ vs. concentration of 2-bromo–6-methoxynaphthalene in Fig. 2 are used to test the mechanism for quenching of $^3NC_2$ by naproxen (*vide infra*).

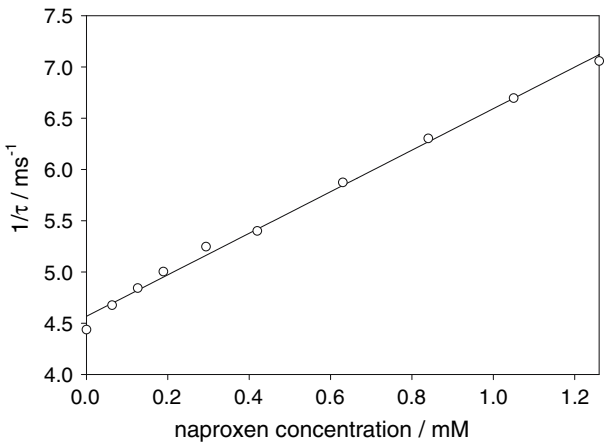


**Fig. 1** Plot of $1/\tau$ vs. naproxen concentration. The slope gives $k_q'' = 2.1 \times 10^6$ $M^{-1}$ $s^{-1}$. The concentrations of CD and 2-bromo-6-methoxynaphthalene were 0.050 M and $2 \times 10^{-5}$ M, respectively

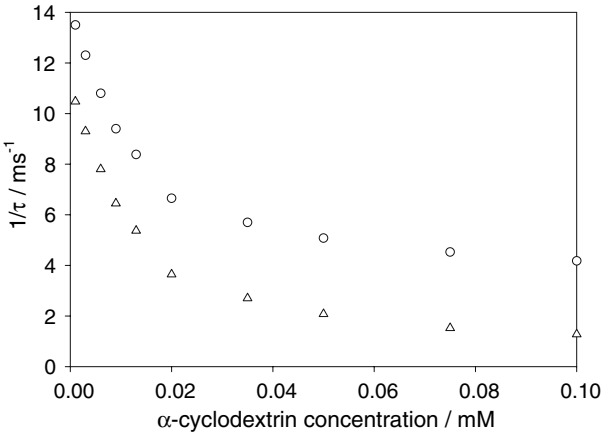


**Fig. 2** Plots of $1/\tau$ vs. CD concentration for solutions containing 0.0 mM naproxen (Δ) and 1.26 mM naproxen (○). The difference between values of $1/\tau$ for CD extrapolated to infinity equals $k_q''$ [naproxen] and yields $k_q'' = 2.3 \times 10^6$ $M^{-1}$ $s^{-1}$, which is in agreement with the value obtained from Fig. 1

## Discussion

The linearity of plots of $1/\tau$ vs. [Q] for both naproxen and its sodium salt makes the analytical detection of naproxen convenient. The slopes of these plots are sufficiently large, and the phosphorescence lifetime resolution is sufficiently great to permit measurements of naproxen (naproxen salt) concentrations differing by as little as 0.05 mM, which corresponds to a sensitivity of 11 $\mu$g/ml. Although this sensitivity is not as good as that reported for other methods, the method described here has several advantages over methods based on measurements of fluorescence or phosphorescence intensities. First, the method requires only a very low concentration of N, which reduces the toxicity of solutions. Second, the solutions need not be deoxygenated. Third, it is not necessary to add an oxygen scavenger, which obviates the need of an equilibration time period during which the phosphorescence intensity reaches a steady level. Fourth, lifetime measurements are more convenient than intensity measurements because they do not require correction for typical scattered light signals and inner filter effects. Fifth, since phosphorescence is such a rare occurrence, there is only a small chance that the emission, especially with its long lifetime of >100 $\mu$s, will be mistaken for that from other molecules (impurities) in the solvent. So, in general the method described herein requires less time to complete measurements than is required for most fluorescence measurements and does not involve the time-consuming sample preparation required for typical phosphorescence measurements.

The method shows promise for use with biological solutions and has been successfully applied to samples of human urine doped with naproxen. The phosphorescence decay curves are biexponential, and experiments are in progress to understand this somewhat more complicated system and to increase the sensitivity of the measurements.

Next, the mechanism for quenching of phosphorescence by naproxen is considered. The similar structure of naproxen and 2-bromo-6-methoxynaphthalene suggests a possible quenching mechanism in which a naproxen molecule displaces one CD molecule from either or both the binary complex and ternary complex, while leaving the guest molecule in its triplet state. For such a quenching mechanism it can be shown that the limiting value of $1/\tau$, as [CD] is extrapolated to infinity, should be the same value regardless whether or not naproxen is present in solution. Instead, as is seen in Fig. 2, in accordance with Eq. (2) the entire plot is shifted when solutions contain naproxen. This result is consistent with quenching of $^3$N directly to the ground state perhaps by energy transfer from $^3$N to naproxen or by electron transfer.

The parameters have been fully characterized for the triplet state of naproxen in ethanol glass [15], and the triplet state energy has been reported to be 260 kJ/mol. The triplet state energy corresponding to the wavelength of maximum phosphorescence intensity for 2-bromo-6-methoxynaphthalene (512 nm) is 234 kJ/mol. Hence, quenching of phosphorescence by energy transfer to naproxen would seem to be endergonic and would suggest that the quenching mechanism involves electron transfer instead. However, no firm conclusion can be drawn because it is conceivable that the triplet-state, 0,0 transition peak for 2-bromo-6-methoxynaphthalene lies within the broad spectral envelope of the first phosphorescence peak. Also, the 260 kJ/mol energy was measured for naproxen in an ethanol glass and not water, which might lower the energy somewhat.

Finally, it should be noted that the proposed kinetics model is supported by the self-consistency between the lifetime data and Eq. (2). Comparison of Eqs. (1) and (2) shows that the entire plot of $1/\tau$ vs [CD] should be shifted higher by an amount $k$ [naproxen] when the naproxen concentration is changed from zero to a finite value. Consistent with this expectation, the numerical difference between the values of $1/\tau$ for [CD] = 0.1 M gives a value of $k'' = 2.3 \times 10^6\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$ which is in excellent agreement with the slope of the plot in Fig. 1.

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