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Rate-limiting step and micellar catalysis of the non-classical nitro group nucleophilic substitution by thiols in 4-nitro-*N*-n-butyl-1,8-naphthalimide

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ABSTRACT: Nitronaphthalimides are a particularly interesting group of nitroaromatics used for staining cells in hypoxia and in photodynamic therapy. The kinetics of the aromatic nucleophilic substitution of the nitro-group of 4-nitro-*N*-n-butyl-1,8-naphthalimide (4-NBN) by n-heptanethiol (RSH), thiophenol (ArSH) and 2-hydroxy-1-ethanethiol (HESH) in aqueous solutions containing micelles and in water–methanol mixtures were analyzed. The substitution of the nitro group by aromatic or aliphatic thiols led to fluorescent substitution products. Hexadecyltrimethylammonium chloride micelles (CTAC) increased the rate of thiolysis of 4-NBN by 1×10^5 (RSH) and 4×10^5 (ArSH) fold. For the reaction of ArSH with 4-NBN the calculated second-order rate constant in the micellar phase, k_{2m}, is 1.4 times higher than that in the aqueous phase, k_{2w}. For the reaction of ArSH with 4-NBN, $k_{2m}/k_{2w} = 0.05$. Both k_{2w} and k_{2m} decreased with increasing pK_a of the thiol, suggesting that thiolate attack is not rate limiting in either the aqueous phase or micellar pseudophase. The high micellar rate acceleration is therefore largely due to concentration of the reagents in the micellar phase and effects on the thiol acid–base equilibrium. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: nitronaphthalimides; thiol substitution; nucleophilic substitution; micellar catalysis; kinetics

INTRODUCTION

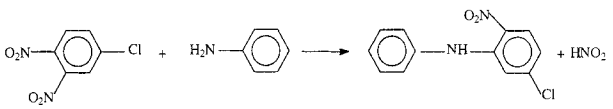
The substitution of the nitro group of 3,4-dinitrochlorobenzene by aniline in an aprotic dipolar solvent, DMSO, yielding 2-nitro-5-chlorodiphenylamine (Scheme 1) was demonstrated in 1876.[1,2] The reaction type shown in Scheme 1 is still considered a special case of nucleophilic aromatic substitution.

Nucleophilic aromatic displacements (S_NAr) of the nitro group, used as a synthetic tool, are currently employed in the synthesis of condensation polymers based on nitro-monomers.[3–5]

The S_NAr reaction of the nitro group from an activated system usually occurs by a two-stage addition–elimination pathway and the attack of the nucleophile is usually, but not always, the rate-limiting step.[4,5a]

Aromatic nitro groups can be displaced by nitrogen (amines), oxygen (alkoxides and phenoxides), carbon (carbanions) and sulfur (thiolate) nucleophiles.[6–8] The high reactivity of thiolates in aromatic nitro group displacement has been ascribed to the polarizability of both species.[8] The displacement of the nitro group, however, requires activation by electron-withdrawing groups and the relative efficiency for the activation is[5a] CN > OC—NR—CO > C=O ≈ S=O > RCOOR. The reaction rate is sensitive to conjugation of electron-withdrawing groups (in relation to the nitro group), steric hindrance and solvents.[6,9–11]

Nitronaphthalimides, a particularly interesting group of nitroaromatics, are used for staining cells in hypoxia[12,13] and are also important in photodynamic therapy.[14,15] The biologically selective reduction of the nitro group to amines, due to the presence of an electron-donating group in the naphthalene ring, induces high quantum yield fluorescence.[12,13] Nitro group displace-



Scheme 1

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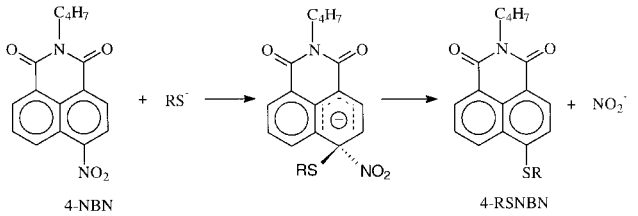


Scheme 2

ment has been used in the synthesis of alkylamino-*N*-alkylnaphthalen-1,8-imides from 4-nitro-*N*-n-butyl-1,8-naphthalimide and primary amines in aprotic solvents.[9]

Here we have investigated the kinetics of the aromatic nucleophilic substitution of the nitro-group of 4-nitro-*N*-n-butyl-1,8-naphthalimide (4-NBN) by n-heptanethiol (RSH) (Scheme 2), thiophenol (ArSH) and 2-hydroxy-1-ethanethiol (HESH) in water and hexadecyltrimethylammonium chloride (CTAC) micelles.

EXPERIMENTAL

Materials

The following compounds were of analytical grade and used as received: 4-nitro-1,8-naphthalic anhydride (Aldrich), n-butylamine (Carlo Erba), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), n-hepthanethiol and thiophenol (Aldrich), Tris and 2-hydroxy-1-ethanethiol (Sigma), boric acid (BDH) and $Hg(NO_3)_2$ (Riedel de Haën, Seelze-Hanńover, Germany). Methanol (Mallinckrodt) and acetonitrile (Merck) were of spectroscopic grade. Hexadecyltrimethylammonium chloride (CTAC) (ACROS Organics) was recrystallized (×3) from methanol–acetone (1:3, v/v). Distilled, deionized water was used throughout.

Methods

4-Nitro-*N*-n-butyl-1,8-naphthalimide (4-NBN) was synthesized as described.[9a,9b] Analytical data for 4-NBN: melting-point (uncorrected) = 103.5–104.0 °C (lit.[9b] 103.5–104.5 °C); ^{1}H NMR ($CDCl_3$), δ (ppm) = 0.96 (t, 3H, CH_3), 1.56 (sextet, 2H, CH_2), 1.72 (q, 2H, CH_2), 4.18 (t, 2H, CH_2), 7.96 (t, J = 8.0 Hz, 1H, Ar), 8.38 (d, J = 8.0 Hz, 1H, Ar), 8.67–8.86 (m, 3H, Ar); IR (KBr), ν (cm^{-1}) = 3074, 2961, 2872, 1706, 1656, 1624, 1530, 1347, 1231, 1082; GC–MS: *m/z* (%) = 298 (M^+, 100), 256, 243, 225, 209, 179, 151, 126, 75. The spectroscopic data for 4-NBN agreed with the literature.[9b]

The product of the reaction between 4-NBN and RSH was synthesized in acetonitrile–borate buffer (0.1 M), pH 10.8 (1.5:1, v/v) and a 10% molar excess of RSH. The reaction was maintained for 2 h under N_2 with stirring at room temperature and the precipitate was filtered and washed with water. Flash chromatography in a silica gel column (with chloroform as eluent) was necessary to extract the thiol oxidation side-product. Yield = 90%. Analytical data for 4-heptanothiolate-*N*-n-butyl-1,8-naphthalimide (4-RSNBN): melting-point (uncorrected) >300 °C; ^{1}H NMR ($CDCl_3$), δ (ppm) = 0.97 (t, 3H, CH_3), 1.30–1.55 (m, 4H, CH_2), 1.64–1.85 (m, 4H, CH_2), 3.15 (t, 2H, CH_2), 4.17 (t, 2H, CH_2), 7.53 (d, 1H, Ar), 7.74 (t, 1H, Ar), 8.45–8.63 (m, 3H, Ar); IR (KBr), ν (cm^{-1}) = 3048, 2953, 1698, 1654, 1590, 1363, 1273, 1073; GC–MS: *m/z* (%) = 383 (M^+, 100), 327, 284, 229, 212.

Melting-points were determined with an electrothermal apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AC-200 spectrometer in $CDCl_3$. IR spectra were registered on a BOMEM-FTIR MB 102 spectrophotometer in a KBr plate. Capillary GC–MS analyses were performed on an HP-5890 gas chromatograph, coupled to an MSD-5970 mass-selective detector. Fluorescence emission spectra were measured with a Hitachi 4500 spectrofluorimeter and absorption spectra with a Cary 3E spectrophotometer.

The critical micellar concentration (CMC) of CTAC in Tris–HCl buffer (0.01 M), pH 7.0, at 30 °C determined by conductivity measurement (Orion Model 160 conductivity meter) was 5.5×10^{-4} M.

Thiol concentrations were determined with DTNB. A sample of thiol (ca 0.01 ml of a 0.01 M solution in acetonitrile) was added to a cell containing 3.0 ml of 0.1 M Tris–HCl buffer, pH 8.0, and 0.05 ml of a 0.01 M solution of DTNB prepared in the same buffer. The absorbance was determined at 412 nm and the thiol concentration was calculated using 13600 M^{-1} cm^{-1} as the molar extinction coefficient for the 2-nitro-5-thiobenzoate anion.[16] CTAC concentrations were determined by chloride titration with $Hg(NO_3)_2$ using diphenylcarbazone as indicator.[17]

Kinetic measurements were performed with a Cary 3E spectrophotometer. All reactions were carried out in a quartz cell of 1.0 cm optical pathlength containing 2.0 ml of buffer at the desired pH at 30 °C.

Reaction products of the thiolysis of 4-NBN were measured at 391 nm for all the thiols. The reaction of 4-NBN with thiols, in the presence of CTAC, was initiated by the addition of an aliquot of a 0.10 M thiol solution in acetonitrile to 2.0 ml of buffer containing 3.0×10^{-5} M 4-NBN. The thiol concentration was, in all cases, at least 10 times greater than that of 4-NBN to assure first-order kinetics. All the reactions were followed at least for 10 half-lives and the rate constants were calculated from linear first-order plots. For the hydrolysis reaction an aliquot of 0.003 ml from a stock solution 0.01 M of 4-NBN in acetonitrile was added to 2.0 ml of buffer (0.01 M), final concentration 1.5×10^{-5} M, and the absorbance change was measured at 350 nm. No hydrolysis of 4-NBN was detected under the conditions studied.

The appearance of 4-RSNBN was also followed by measuring the fluorescence increase at the maximum emission wavelength at 460 nm ($\lambda_{exc} = 391$ nm).

RESULTS AND DISCUSSION

The UV absorption spectrum of 4-NBN in aqueous solution exhibits maxima at 356 and 233 nm [Fig. 1(A)]. Addition of CTAC leads to an increase in the absorbance of 4-NBN and a blue shift of the absorption maximum at 356 nm to 353 nm [Fig. 1(A)]. The spectral changes observed on CTAC addition to 4-NBN solutions [Fig. 1(B)] permitted the estimation of the micelle incorporation constant, K_{NBN}, [Scheme 3 (1)][18] using the equation

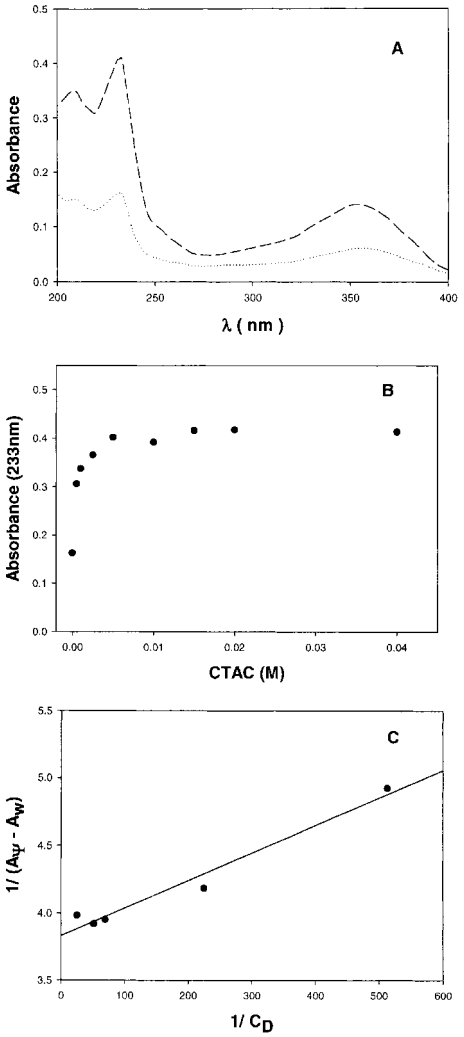
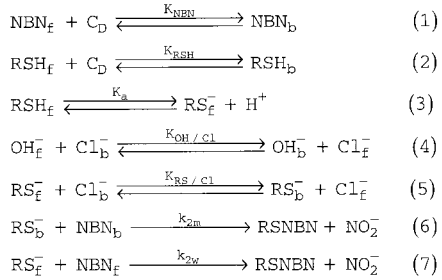


Figure 1. Effect of CTAC on the UV spectra of 4-NBN. (A) Absorption spectrum of 4-NBN (1.5×10^{-5} M) in Tris–HCl (0.01 M), pH 7.0: (dotted line) without micelles and (dashed line) with CTAC (0.004 M). (B) Effect of [CTAC] on the absorbance of 4-NBN (1.5×10^{-5} M) in Tris–HCl buffer (0.01 M), pH 7.0. (C) Plot of ($1/A_\psi - A_W$) vs $1/C_D$ [Eqn. (3)]



Scheme 3

$$K_{NBN} = \frac{NBN_b}{NBN_f C_D} \quad (1)$$

where NBN_b and NBN_f are the concentrations of 4-NBN bound to the micelles and in the aqueous phase, respectively, and C_D is the concentration of micellized detergent, given by

$$C_D = C_T - CMC \quad (2)$$

where C_T is the total concentration of CTAC and CMC is the critical micellar concentration. The value of K_{NBN} was determined using the following equation that relates the change in absorption of 4-NBN, at fixed wavelength, with CTAC concentration:[18]

$$\frac{1}{(A_\psi - A_w)} = \frac{1}{(A_m - A_w)} + \frac{1}{K_{NBN}(A_m - A_w)} \times \frac{1}{C_D} \quad (3)$$

where A_ψ is the measured absorbance at each C_D, A_w is the absorbance in the absence of micelles and A_m is that of totally micelle-incorporated 4-NBN. Using the data in Fig. 1(C) and Eqn. (3), the calculated value of K_{NBN} was 1.877 M^{-1}.

Alkyl-*N*-substituted naphthalimides react with hydroxide ion yielding amides with UV and fluorescence spectra different from those of the starting substrates.[19] In Tris–HCl buffer (0.01 M), pH 7.0, or in borate buffer (0.01 M), pH 10.8, with and without CTAC, no measurable changes in the absorption or fluorescence of 4-NBN was observed for several hours. The alkaline decomposition of 4-NBN, therefore, is not relevant on this timescale.

The fluorescence of 4-NBN was negligible [Fig. 2(B)]. The addition of RSH to CTAC-incorporated 4-NBN resulted in time-dependent UV [Fig. 2(A)] and fluorescence [Fig. 2(B)] changes. In CTAC, the reaction product exhibited fluorescence spectra with emission λ_{max} at 463nm (λ_{exc} at 391 nm) that was identical with synthetic 4-n-heptanothiolate-*N*-n-butyl-1,8-naphthalimide (4-RSNBN) (see Methods). The spectral changes were attributed, therefore, to substitution of the nitro group of 4-NBN by RSH yielding 4-RSNBN (Scheme 2).

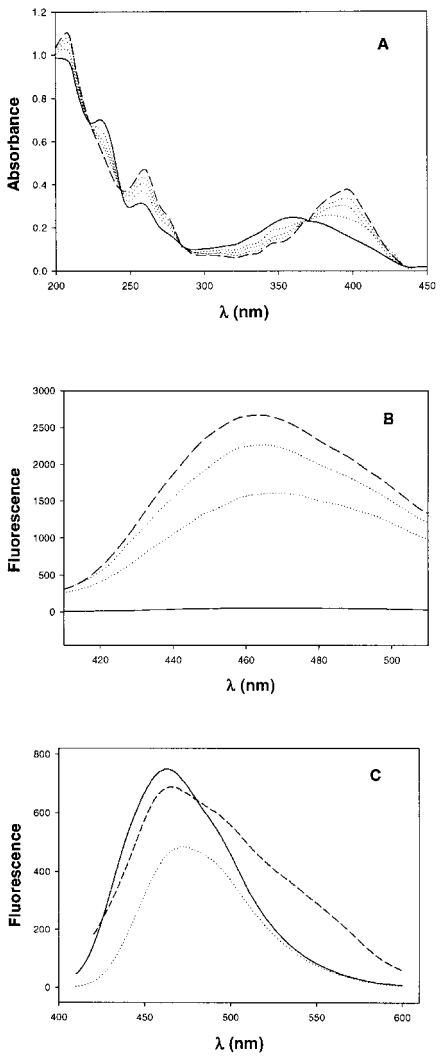


Figure 2. Absorption and fluorescence spectra of the reaction products of 4-NBN with thiols. (A) Absorption spectra of the reaction mixture of 4-NBN and RSH at different times: (solid line) $t = 0$, (dotted lines) at intermediate times and (dashed line) $t = \infty$. Conditions: Tris–HCl buffer (0.01 M), pH 7.0, $[RSH]_T = 2.5 \times 10^{-4}$ M, $[4\text{-NBN}] = 2.5 \times 10^{-5}$ M, [CTAC] = 0.004 M. (B) Fluorescence emission spectra of reaction mixture of RSH and 4-NBN at different times ($\lambda_{exc} = 391$ nm): (solid line) $t = 0$, (dotted lines) at intermediate times and (dashed line) $t = \infty$. Conditions as in (A). (C) Fluorescence emission spectra ($\lambda_{exc} = 391$ nm) of the reaction products of 4-NBN with: (solid line) RSH [Tris–HCl (0.01 M), pH 7, CTAC 0.004 M]; (dashed line) ArSH (HCl 0.0015 M, pH 2.82, CTAC 0.004 M); and (dotted line) HESH [borate buffer (0.01 M), pH 9.8]

Similar spectral changes were observed in the reaction of 4-NBN with other thiols. 4-NBN reacts with ArSH, in CTAC–0.0015 M HCl, leading to changes in UV and fluorescence spectra [Fig. 2(C)]. The reaction of 4-NBN with HESH, carried out in 0.004 M borate buffer (0.01 M), pH 9.64, without micelles led to spectral changes comparable to those observed with RSH and ArSH. In Fig. 2(C) the fluorescence spectra of the products of 4-NBN with the three thiols are presented to allow comparison. It is clear, therefore, that the three thiols used here react with 4-NBN and yield comparable substituted products.

In analyzing micellar effects, previous knowledge of the rate-limiting step of the reaction in aqueous solution is important.[18] A limited analysis of the displacement reaction of the nitro group of 4-NBN in water, using thiols with different pK_as, was undertaken. Since RSH and ArSH are sparingly soluble in water, it was necessary to add methanol to the reaction mixture in order to obtain a significant thiol solubilization.

The observed first-order rate constants, k_ψ, for the reaction of RSH and 4-NBN determined by absorbance or fluorescence changes were identical (not shown). The k_ψ of the reaction of 4-NBN with RSH was measured in borate buffer (0.01 M), pH 10.5, in a mixture containing water and methanol at a water molar fraction (X_{H_2O}) of 0.835. Under these conditions k_ψ increased linearly with the total concentration of thiol, $[RSH]_T$ (not shown). In order to estimate the value of k_ψ in water, k_ψ^w, X_{H_2O} was varied at fixed $[RSH]_T$ and $[4\text{-NBN}]_T$ (total concentration of 4-NBN) in borate buffer (0.01 M), pH 10.5. k_ψ decreased linearly with X_{H_2O} (between $X_{H_2O} = 0.82$ and 0.97) and the value of k_ψ^w was calculated by extrapolation to $X_{H_2O} = 1$ [Eqn. (4)]. The calculated value of the second-order rate constant in water for the reaction, k_{2w}, (50.8 M^{-1} min^{-1}) was calculated using k_ψ^w, the pK_a of RSH in water[20,21] (p$K_a = 10.7$), $[RSH]_T = 2.5 \times 10^{-4}$ M and Eqn. (5):

$$k_\psi = 0.5044 - 0.4967 X_{H_2O} \quad (4)$$

$$k_{2w} = k_\psi / [RS^-] \quad (5)$$

where $[RS^-]$ is the concentration of thiolate at pH 7.0.

The value of k_{2w} for the reaction of 4-NBN and ArSH was obtained at a single water: methanol ratio ($X_{H_2O} = 0.953$) using Tris–HCl (0.01 M), pH 8.0, $[4\text{-NBN}]_T = 1.25 \times 10^{-5}$ M, total thiophenol concentration $[ArSH]_T = 1.25 \times 10^{-4}$ M and p$K_a = 6.5$ for ArSH.[21] The value of k_{2w} calculated using Eqn. (5) was 187.6 M^{-1} min^{-1}.

The reaction of HESH and 4-NBN was carried out in borate buffer (0.01 M), pH 9.64. From the linear dependence of k_ψ with total concentration of HEHS, $[HESH]_T$, and using p$K_a = 9.64$ for the thiolate group,[22] a value of $k_{2w} = 90.9$ M^{-1} min^{-1} was calculated.

The k_{2w} value for thiolysis of 4-NBN, in aqueous solution, decreased with the pK_a of the thiol (Fig. 3), strongly suggesting that the rate-limiting step is not nucleophilic attack but decomposition of the intermediate. In a related reaction of the same and similar substrates, e.g. nitro group displacement by amine nucleophiles in aprotic polar solvents, the decomposition of the intermediate Meisenheimer complex can also be rate limiting.[5a,9a,9b]

CTAC micelles catalyzed the reactions of 4-NBN with

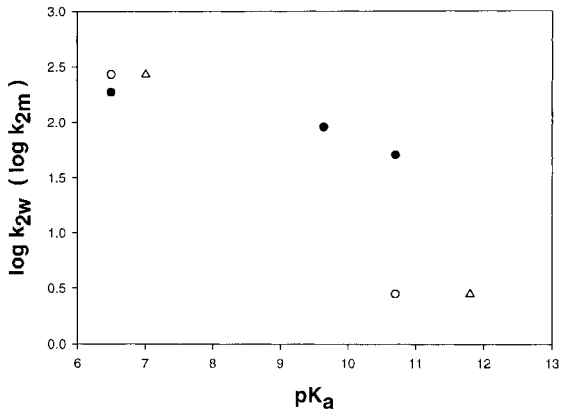


Figure 3. Effect of the pK_a of the thiol on (●) logk_{2w} and (○) logk_{2m}. Effect of pK_m of the thiols on logk_{2m} (△)

RSH and ArSH. Addition of micelles caused a large rate increase, and therefore the kinetics in the presence of CTAC were obtained at pHs lower than those used without the addition of detergent.

The rate of the reaction between RSH and 4-NBN was determined by varying [CTAC] in 0.01 M Tris–HCl buffer, pH 7.0. k_ψ increased with [CTAC], reaching a maximum, $k_{\psi max}$, and then decreased to a plateau, as observed for other micellar-modified bimolecular reactions [Fig. 4(A)].[18] The maximum micellar rate enhancement, $k_{\psi max}/k_{\psi 0}$, for the reaction was ca 4×10^5 at 1 mM CTAC (Table 1). This value was obtained by dividing the maximum observed rate constant, $k_{\psi max}$, obtained at [CTAC] = 1 mM, by the calculated observed rate constant in the aqueous phase, $k_{\psi 0}$. The value of $k_{\psi 0}$ was calculated taking into account the concentration of the thiolate, [RS^-], at pH 7.0 and k_{2w} [Eqn. (5)].

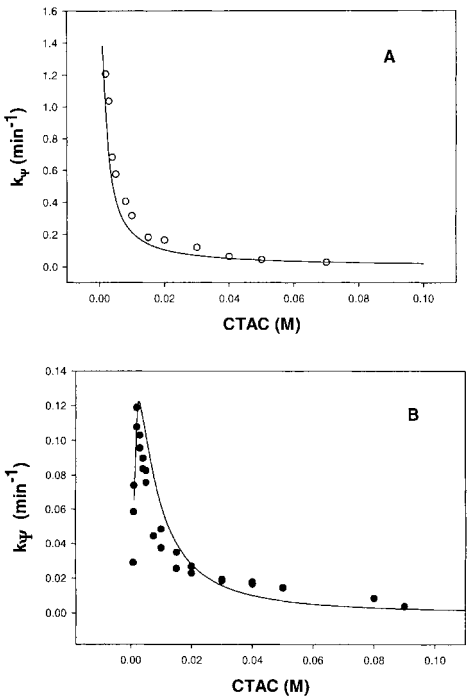


Figure 4. Effect of CTAC on k_ψ for the reaction of 4-NBN with thiols. Circles are experimental values and solid lines were obtained by using Eqn. (9) and the parameters in Table 1 to fit the data. (A) Tris–HCl (0.01 M), pH 7.0, [4-NBN] = 3.0×10^{-5} M, $[RSH]_T = 2.5 \times 10^{-4}$ M. (B) HCl 0.00154 M, pH 2.86, [4-NBN] = 3.0×10^{-5} M, $[ArSH]_T = 2.5 \times 10^{-4}$ M

The rate of the reaction of ArSH with 4-NBN in CTAC micelles was determined in 0.00154 M HCl, pH 2.81 [Fig. 4(B)]. The effect of CTAC in the reaction rate was qualitatively similar to that in the reaction of 4-NBN and RSH and at 0.002 M CTAC and the value of $k_{\psi max}/k_{\psi 0}$ was ca 1×10^5.

The kinetic effect of micelles in bimolecular reactions is seldom due to catalysis.[21,23] The observed rate enhancements are generally related to the concentrations of the reagents in the micellar pseudophase and, in this particular case, to the micellar effect on the extent of acid–base dissociation generating the reactive nucleophile, e.g. the thiolate anion.[21,23] The pK_as of RSH and ArSH are 10.7 and 6.5, respectively, and the kinetic data in micelles were obtained at pHs about 4 pH units below the pK_as of the thiols. Separation of local concentration and acid–base dissociation effects from catalysis in micellar-modified reactions requires quantitative analysis of the effect of detergent concentration on the reaction rates.[23,24]

The effect of CTAC on k_ψ was analyzed quantitatively using an ion-exchange formalism, as applied previously for other bimolecular reactions where the nucleophile is negatively charged and is formed by dissociation of a protonated acid.[24] The equilibrium and kinetic steps involved are described in Scheme 3. In Scheme 3 the subscripts f and b refer to the substrate free in the aqueous phase and bound to the micellar phase, respectively, and K_a is the thiol dissociation constant in the aqueous phase.

The binding of the RSH to the micelles is given by

$$K_{RSH} = \frac{RSH_b}{RSH_f C_D} \quad (6)$$

Table 1. Parameters used in Eqn. (9) to fit k_ψ data of the reactions of 4-NBN with n-heptanethiol and thiophenol[a]

Parameter	n-Heptanethiol	Thiophenol
$K_{RS/Cl}$ or $K_{ArS/Cl}$	122	16.3
K_{RSH} or K_{ArSH} (M^{-1})[24]	3.700	100
k_{2w} (M^{-1} min^{-1})	50	189 ± 9
k_{2m} (M^{-1} min^{-1})	2.3	270 ± 13
$k_{\psi max}/k_{\psi w}$	4.03×10^5	1.04×10^5
pK_a[24]	10.70	6.50
pK_m[21]	11.80	7.01
Conditions, pH	Tris–HCl (0.01 M), pH 7.0	HCl 0.00154 M, pH 2.81

[a] The following values were used in both fitting procedures: $\alpha = 0.27$; $CMC = 5.5 \times 10^{-4}$ M; $K_{OH/Cl} = 0.13$;[25] $K_{Cl/Br} = 0.615$;[25] $K_{NBN} = 1.877\ M^{-1}$; $V = 0.32$ l mol^{-1}; pK_w = 13.833 at 30 °C.[22]

The binding of RS^-, OH^- and Cl^- to CTAC micelles can be described through the ion-exchange constants $K_{RS/Cl}$ and $K_{OH/Cl}$ as in Eqns (7) and (8) and Scheme 3:[23,24]

$$K_{RS/Cl} = \frac{RS_b Cl_f}{RS_f Cl_b} \quad (7)$$

$$K_{OH/Cl} = \frac{OH_b Cl_f}{OH_f Cl_b} \quad (8)$$

The observed rate constant, k_ψ, for the reaction of RS^- with 4-NBN in the presence of micelles [Tris–HCl buffer (0.01 M), pH 7.0] is given by[23,24]

$$k_\psi = \frac{[RSH]_T K_a \{(k_{2m}/V)\left[K_{NBN}K_{RS/Cl}(Cl_b/Cl_f)\right] + k_{2w}\}}{\{(K_w/OH_f)(1 + K_{RSH}C_D) + K_a\left[1 + K_{RS/Cl}(Cl_b/Cl_f)\right]\}(1 + K_{NBN}C_D)} \quad (9)$$

where K_w is the ionization constant for water (pK_w = 13.833 at 30 °C),[22] OH_f is the hydroxide concentration in the aqueous phase and Cl_b and Cl_f are given by

$$Cl_f = \alpha C_D + RS_b + OH_b \quad (10)$$

$$Cl_b = (1 - \alpha)\ C_D - OH_b - RS_b \quad (11)$$

Equations for solving for OH_b and RS_b are given elsewhere.[21,23–25] k_{2m} is the second-order rate constant for the reaction in the micellar phase, [RSH] is the total concentration of RSH used in the experiment, V is the molar volume of micellar phase and α is the degree of micellar dissociation. The detailed calculation and fitting procedures have been extensively discussed.[23,24]

The values of $K_{OH/Cl}$, $K_{Cl/Br}$, $K_{RS/Br}$, K_{RSH}, α and V were taken from previous work and are given in Table 1.[24,25] $K_{RS/Cl}$ was calculated from the ratio $K_{RS/Br}/K_{Cl/Br}$ and CMC, k_{2w} and K_{NBN} were determined here (Table 1). Using these parameters, the best fit of the experimental data with Eqn. (9) was obtained with a value of k_{2m} = 2.8 $min^{-1}\ M^{-1}$ and a ratio k_{2m}/k_{2w} = 0.056. According to this calculation, the rate constant in the micellar pseudophase is lower than that in the aqueous phase, as observed for a variety of bimolecular reactions.[24] Therefore, the rate enhancement observed upon transferring the reagents to the micellar phase was due mainly to reagent concentration.

The same analysis was performed with the data obtained in the reaction of ArSH and 4-NBN. The binding constant of ArSH to CTAC, k_{ArSH}, and the ion-exchange constant for ArS^-/Cl^- exchange, $K_{ArS/Cl}$ are defined in Eqns (6) and (7). The values of k_{ArSH} and $K_{ArS/Br}$ in CTAB micelles have been experimentally determined elsewhere.[21] $K_{ArS/Cl}$ was calculated from the ratio $K_{ArS/Br}/K_{Cl/Br}$ and K_{ArSH} used in the fit was 100 M^{-1}, very close to that determined in CTAB (120 M^{-1}).[21] The value of k_{2m} obtained from fitting the data to Eqn. (9) was 270 $M^{-1}\ min^{-1}$, ca 1.4 times higher than k_{2w} (Table 1).

The relationship between log k_{2w} (or log k_{2m}) and pK_a shows a decrease in both second-order rate constants with increasing pK_as of the thiols (Fig. 3). It is likely, therefore, that both in the aqueous phase and in the micellar pseudophase thiolates attack is not rate limiting.

The rate enhancement promoted by CTAC micelles of the reactions of ARSH and RSH with 4-NBN can be attributed to a change in the pK_a of the thiol in the micelles or to different site locations of the thiols in the micellar phase. Correia *et al.*, in a study of the thiolysis of substituted benzoate esters, calculated the pK_a of the thiols in the micellar phase, pK_m.[21] The calculated pK_m values for ArSH and RSH in CTAB micelles were 7.01 and 11.8, respectively.[21] Using either pK_a or pK_m, rate constants decrease with pK and the slopes do not change significantly (Fig. 3).

The environment of different reacting molecules in the micellar phase involves polar and non-polar interactions and the solubilized molecules are relatively mobile.[18] The nucleophiles used here are hydrophobic and negatively charged thiols. The positive charge of CTAC micelles allows preferential solubilization of both thiols with the negative charge located at the surface of the micelles and the hydrophobic moiety at the micellar interior. 4-NBN is probably more mobile than the thiols and it can be assumed that it is more homogeneously distributed in the micelle. On the other hand, the transition state of both reactions has a more diffuse negative charge, implying a less defined location. As the structures of the transition states for the reactions of RSH and ArSH with 4-NBN are different, the average localizations can also be different. The effect of methanol on the reactivity of the reaction of RSH and 4-NBN indicates that differences in solvation can lead to a decrease in the rate constant of the reaction. Hence it is reasonable to assume that differential site locations from both transition states, and/or substrates, can be responsible for the diverse effects on k_{2m} for ArSH and RSH reactions with 4-NBN.

In conclusion we have shown that thiolates react with 4-NBN producing substitution products. CTAC micelles significantly increase the reaction rate and quantitative analysis of micellar effects ascribe this effect almost exclusively to reagent concentration and local effects on thiol acid–base equilibrium. In addition, the reaction products of thiolysis of 4-NBN were shown to be highly fluorescent and this reaction can be used to determine quantitatively the concentration of thiol groups.

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