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REDOX REACTIONS INVOLVING N-ALKYL-DIHYDRONICOTINAMIDES

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ABSTRACT

Electron transfer reactions involving a series of N-alkyl-dihydronicotinamides (R-NAH) as donors were studied in homogenous solvents and in micellar media. In particular the redox chemistry and kinetics of the reduction of methylene blue and cytochrome-C, by varying the alkyl chain length (R= C_4, C_8, C_{12}) were investigated. The schemes proposed for functionalized surfactants of nicotinamide suggest photochemical conversion based on light-induced electron-transfer reactions.

Keywords: Dihydronicotinamide derivatives, functionalized surfactants, photo-redox mechanisms.

RESUMO

Reações de transferência de elétrons utilizando sistemas modelo, tais como a redução do azul de metileno (AM) e citocromo-C (CC) por N-alquil-dihidronicotinamidas (R-NAH) foram estudados em solventes homogeneos e meios micelares. Os mecanismos redox e a cinética química, determinada em função do comprimento da cadeia carbonada (R= C_4, C_8, C_{12}), permitem concluir que os sistemas investigados apresentam características de reações de transferência de elétrons por indução foto-química.

INTRODUCTION

Nicotinamide adenine dinucleotide (NAD+) is an important cofactor taking part in numerous cellular processes, particularly in the intermediary metabolism and energy conversion processes (e.g., electron transport and oxidative phosphorylation). It has been firmly established that the nicotinamide chromophore is the portion that undergoes reversible redox reactions during the numerous processes involving NAD+ or NADP+. The reduction of NAD+ to NADH (particularly to the 1:4-isomer) is a complex, stereospecific process involving transfer of two electrons and a proton reaction [1,2]:

$$\mathbf{NAD^+ + H^+ + 2e^- \rightarrow NADH} \qquad \mathbf{(I)}$$

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Assisted by enzymes, the reduction occurs very efficiently as a one-step process 2. The reduced form, NADH is a good electron donor and undergoes thermal and light induced electron transfer reactions. Various systems studied include, for exemple:

(a) reductive irreversible quenching of metalloporphyrins 3,4 (Equation II), where S = Zn(II) or Sn(IV)-tetramethylpyridylporphyrin, as follows:

$$NADH + S^* \rightarrow SH^- + NAD^+ \qquad (II)$$

(b) The irreversible photoreduction of viologens by photoexcited NADH 5, (Equation III), as follows:

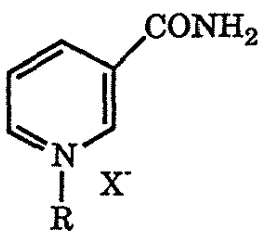
$$NADH^* + 2MV^{2+} \rightarrow NAD^+ + 2MV^+ + H^+ \qquad (III)$$

(c) As a "sacrificial" reductant of photooxidized sensitizer dyes or for thermal reduction of organic dyes 6, (Equation IV), as follows:

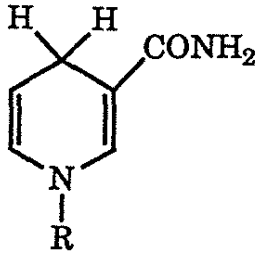
$$NADH + S^+ \rightarrow S + NAD^+ + H^+ \qquad (IV)$$

where S is ruthenium bypiridyl [$Ru(bpy)_3^{+2}$], chlorophyl or methylene blue. The mechanism of action of NADH continues to be controversial 7 wheter the reaction involves a direct hydride (H^-) transfer or a sequential transfer of an electron, a proton and an electron, (e^-, H^+, e^-) 7.

As regards to redox potentials, representative E_0' values in water, relative to NHE at pH 7.0 are as follows: E_0' (NADH/NAD+) = -0.315 V; E_0'(Benzyl-NAH/Benzyl-NA+) = -0.361 V and E_0'(C_3-NAH/C_3-NA+) = -0.387 V. The one electron oxidation potential of NADH closely related 1,4-dihydronicotinamides has been established to be ≈ -1.30 V vs. NHE by indirect methods 8. This implies that only very powerful oxidants can oxidize NADH by one electron pathway. Accordingly, thermal oxidation of NADH and analogs have been found to occur by hydride exchange, thereby avoiding the high energy intermediates by a stepwise pathway.



1-N-alkyl-nicotinamide surfactant (RNA^+X^-)



1-N-alkyl-1,4-dihydro nicotinamide (R-NAH)

MATERIALS AND METHODS

N-alkyl-1,4-dihydronicotinamides were prepared from the corresponding n-alkyl-nicotinamides by chemical reduction using sodium dithionite as a reductant, following the procedures described in the literature [9, 11, 12]. Sodium lauryl sulfate (SLS) (Fluka) was used after single recrystallization. Phosphate buffer solution of pH= 7.41 from Fluka, containing 0.0087 M KH_2PO_4 and 0.03 M of Na_2HPO_4 was used as supplied. Methylene blue (MB) (Fluka) was recrystallized once from ethanol/water mixture. Cytochrome-C (Sigma-Chemical Company) was used as supplied.

Kinetic studies of reduction of methylene blue (MB) and cytochrome-C (CC) by N-alkyl-1,4-dihydronicotinamides were performed by monitoring the UV-Visible spectra, recorded on a 8000 Diode Array Spectrophotometer (Hewlett-Packard). Known amounts of C_8-NAH and C_{12}-NAH were solubilized in methanol or micellar solutions of sodium lauryl sulfate (SLS). These were then mixed with different concentrations of MB or CC in a buffer solution. C_4-NAH (being water soluble) was studied in an aqueous buffer solution. Dihydronicotinamide solutions were freshly prepared and used the same week. A cuvette containing 1.8 ml of a MB or CC buffer solution was placed in a 1-cm cell holder and a solution of R-NAH was injected into the sample solution. Degassing of solutions was made by helium bubbling, during 15 minutes. Solution were illuminated under continuous stirring using a 450 nm Xe arc lamp, light filtered using a solution filter that passes light in the wavelength range of 300-400 nm , a 10 cm solution filter containing 2:1 mixture of $CoSO_4$ (0.30 M) and $CuSO_4$ (0.20 M) [13].

RESULTS AND DISCUSSION

Some current work has been devoted to systematic studies of some of the above basic chemistry of the nicotinamide chromophore using a series of functionalized surfactants, (viz., long chain derivatives of nicotinamide / dihydronicotinamide) as model compounds [9, 10]. As mentioned, functionalized surfactants allow model system studies to be carried out both in homogenous solvents and in organized assemblies.

An important finding has been the ability to tune the solubility of the neutral molecule R-NAH by varying the chain length. The shorter chain derivatives of dihydronicotinamides (R-NAH, $R \leq C_6$), are fairly water soluble. The longer chain derivatives are totally insoluble in water and hence can serve as excellent hydrophobic donors for studies in reversed micellar systems.

In this work, as a model system, the reduction of two water-soluble substrates *methylene blue* (abbreviated hereafter as MB) and *cytochrome-C* (abbreviated hereafter as CC) by a series of 1,4-dihydronicotinamide surfactants has been studied (Equations V and VI):

$$RNAH + MB \rightarrow RNA^+ + MB\text{-}H \quad (V)$$

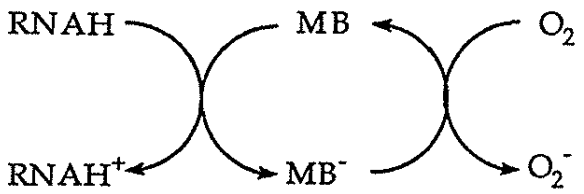
$$RNAH + 2Fe^{+3}(CC) \rightarrow RNA^+ + 2Fe^{+2}(CC) + H^+ \quad (VI)$$

It is known that methylene blue (MB) can be reduced to leucomethylene blue (MB-H) ($E_0' = 0.011$ V vs. NHE) [11]. The reduction generally proceeds as a two-electron process, although at low pH, semiquinone formation has been reported [14]. The potentiality of 1,4-dihydronicotinamides to reduce MB+ was noted by Karrer and his coworkers in the late 1930's [15]. Since then only a few studies have been reported that deal with the reactivity of MB+ towards 1,4-dihydropyridines, which are those of Engbersen and Verhoeven and their coworkers [16, 17]., with important kinetic studies in homogeneous solutions. If molecular oxygen is present in the system involving MB, the leuco-MB produced in Equation V is rapidly re-oxidized to MB (Equation VII) , see Figure 1:

$$\text{MB-H} + O_2 \rightarrow \text{MB} + O_2^- + \text{H+} \qquad \text{(VII)}$$

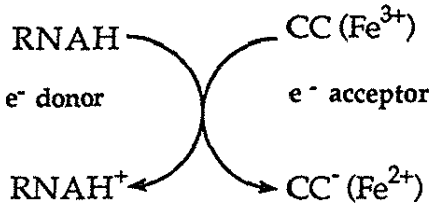
In homogenous solution, the reaction described by Eq. (VII) is 100 times faster than the rate of R-NAH oxidation (Eq. VI). Under such conditions, *Scheme 1* represents a sensitized reaction and MB serves as a reversible electron relay.

Scheme 1 :



Cytochrome-C (CC) is an electron-transferring heme-protein (mol.wt.: 13000), involved in the respiratory chain. Its chromophore unit, Fe-protoporphyrin undergoes reversible Fe(II) - Fe(III) valence changes (E_0') = 0.254 V). In the reduced form, the heme group has its characteristic absorption bands α, β and Soret located at 550, 521 and 415 nm respectively ($\varepsilon_{550nm} = 2.95 \times 10^4$ M-1.s-1) [1-c, 1-d]. The reduced form of the CC is not readily reoxidized by molecular oxygen, except in the presence of enzymes such as oxidase, peroxidase or catalase. Thus reduction of cytochrome-C via Scheme 2 is essentially irreversible.

Scheme 2 :



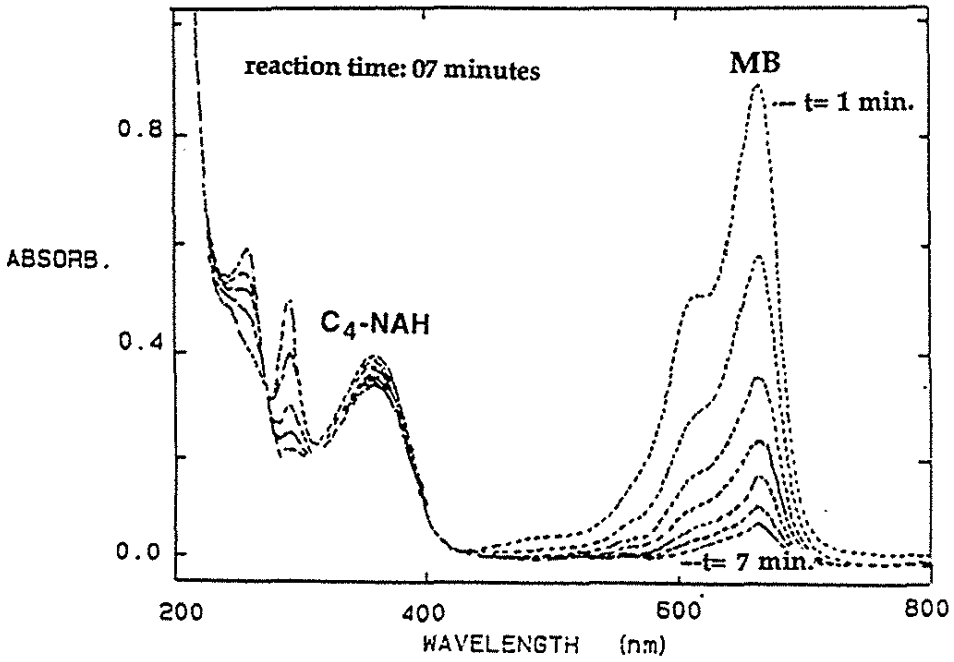


FIGURE 1. SPECTRAL CHANGES OBSERVED DURING THE REDUCTION OF METHYLENE BLUE BY C_4-NAH IN AQUEOUS DEAERATED - (He DEGASSING) BUFFER SOLUTIONS. CONDITIONS: [C_4-NAH] = 14.43 x 10-5 M AND [MB] = 1.12 x 10-5 M. Δt FOR EACH REACTION = 1 MINUTE. 1/10 V/V PHOSPHATE/PHOSPHATE BUFFER, pH = 7.41 AND T = 22 ° C.

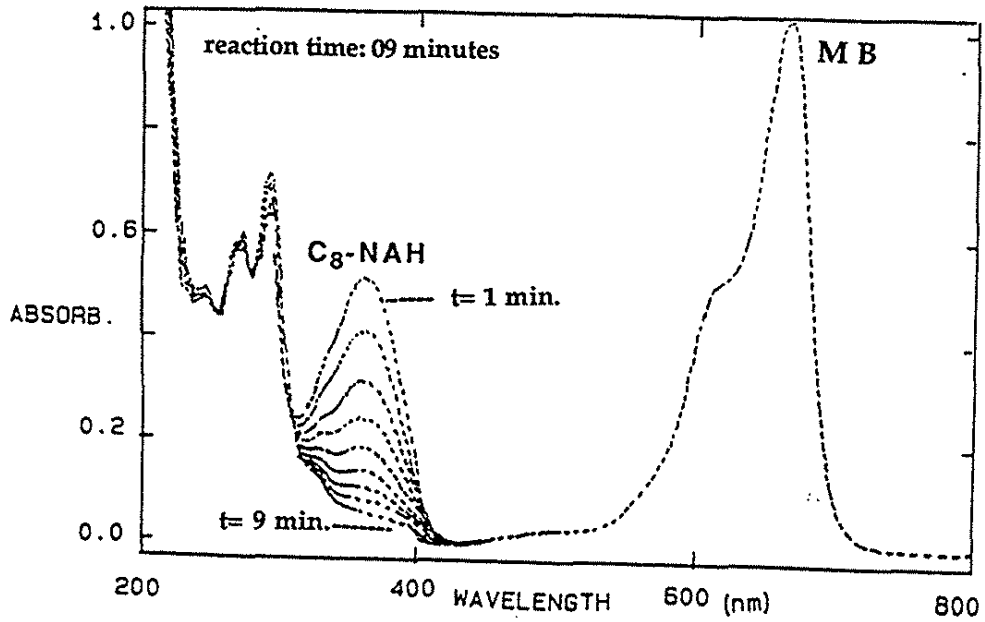


FIGURE 2. SPECTRAL CHANGES OBSERVED DURING THE REDUCTION OF METHYLENE BLUE BY C_8-NAH IN METHANOL. CONDITIONS: [C_8-NAH] = 8.13 x 10-5 M AND [MB] = 1.12 x 10-5 M. Δt FOR EACH REACTION = 1 MINUTE. 1/10 V/V - METHANOL/AQ. PHOSPHATE BUFFER, pH = 7.41 AND T = 22 ° C.

Variations in the alkyl chain length of R-NAH allowed the reduction reactions to be followed under a variety of conditions: C_4 derivative in aqueous medium (being very soluble in water); C_8, C_{12} derivatives either in solvents as methanol or solubilized molecules in host micelles of SLS (sodium lauryl sulfate), concentration of the donor and acceptor(s), temperature, molecular oxygen and reaction medium (homogenous solvent vs. micelles). The organized assembly consists of a neutral hydrophobic donor (R-NAH) solubilized in the anionic micelles with cationic acceptor (MB+ or CC) adsorbed on the surface.

The redox reactions involving dihydronicotinamides are conveniently monitored via their absorption spectral changes. In the oxidized form the nicotinamides do not have any absorption in the visible light region. In the reduced form (1:4-isomer), there is an intense absorption band at ≈ 355 nm. In our studies, reduction processes under scheme 1 and 2 have been studied under pseudo first-order conditions, i.e., {[R-NAH] >> [MB] or [CC]} [18].

Figure 1 presents typical absorption spectral changes observed during the thermal reduction of MB by C_4-NAH under homogenous solvent conditions (aqueous aerated solutions). Figure 2 and 3 present similar spectra in methanol as solvent for the C_8 and C_{12} derivatives respectively. In all cases at time(t) =0 , the absorption spectrum of the reaction mixture is composed of absorption of the two reactants (R-NAH at 360 nm and MB at 660 nm). Subsequent to the mixing of R-NAH and MB, the absorption maximum corresponding to R-NAH at 360 nm decreases with smaller absorption changes occurring at the MB maximum.

Kinetics of oxidation of R-NAH has been followed by monitoring the disappearance of the 360 nm band. Figure 4 shows representative first-order kinetic plots for the disappearance of R-NAH as a function of time.In all cases the disappearance of R-NAH follows first order kinetics as expected. Table I presents a collection of derived rate constants data for MB-reduction reaction measured under various conditions. Figure 5 and 6 shows the variation of the observed rate constant K_{obs}, with the concentrations of MB+ for both C_8 and C_{12}-NAH. The second order rate constants (k_2) derived from such plots are summarized in Table II.

The hydrophobic long chain derivative of dihydronicotinamide (C_{12} derivative for example) can be readily solubilized in anionic micelles of sodium lauryl sulfate. With the cationic acceptor adsorbed on the surface, the reduction process takes place as an intramicellar process. Table II also contains kinetic data for methylene blue reduction carried out in the presence of anionic micelles SLS. Complex formation between the electron donor and acceptor methylene blue has been reported earlier [16]. Under our experimental conditions we could not detect any such complexation spectroscopically.

Figure 7 and 8 presents some representative absorption spectral changes observed during the reduction of cytochrome-C (CC) by C_8 and C_{12} derivatives solubilized in host micelles of SLS (10^{-2} M). At t = 0 the absorption spectrum is composed of absorption maxima characteristic of the two components, R-NAH at 360 nm and CC at 530 nm (their typical spectrum). Subsequent to the mixing, kinetics of R-NAH oxidation was followed by monitoring the disappeareance of the 360 nm band, after correcting for the absorption of CC. The disappearance of R-NAH follows first order kinetics in non-irradiated systems.

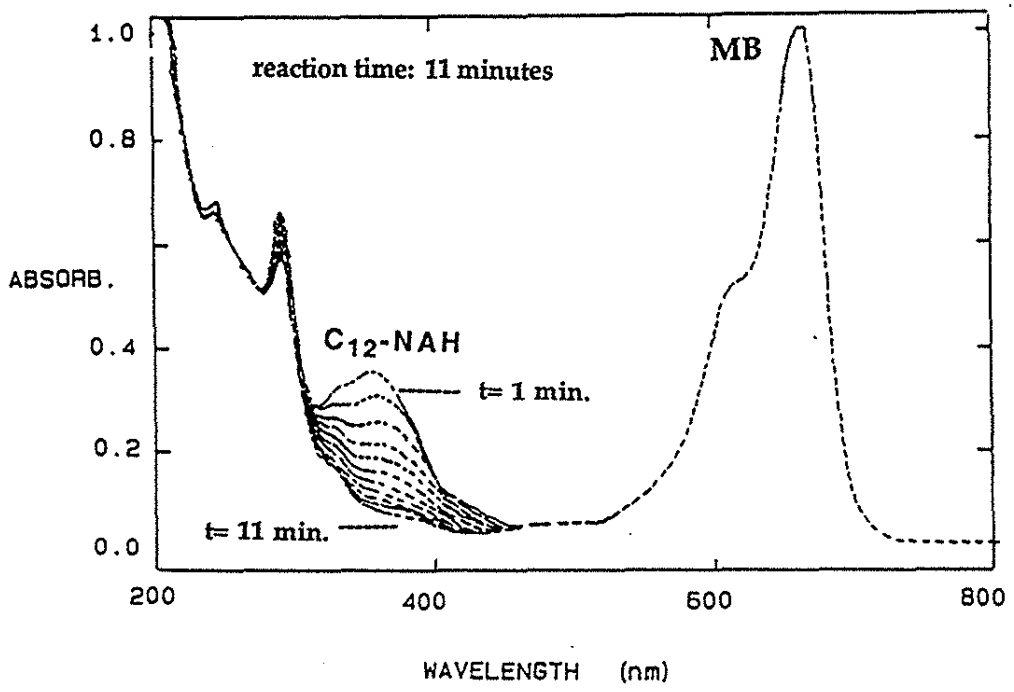


FIGURE 3. SPECTRAL CHANGES OBSERVED DURING THE REDUCTION OF METHYLENE BLUE BY C_{12}-NAH IN METHANOL. CONDITIONS: [C_{12}-NAH] = 8.91 x 10-5 M AND [MB] = 1.12 x 10-5 M. Δt FOR EACH SPECTRUM = 1 MINUTE. 1/10 V/V - METHANOL/ PHOSPHATE BUFFER, pH = 7.41 AND T = 22 °C.

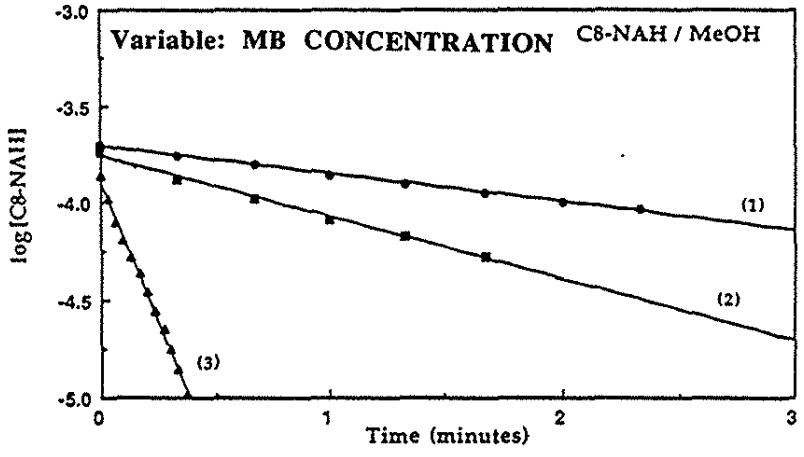


FIGURE 4. PLOT OF LOG OF [C_8-NAH] (in M) vs. TIME (in MINUTES). REACTION OF C_8-NAH (ε = 6350) MONITORED AT λ = 360 nm WITH MB. (CURVE 1 = 5 x 10-5 M, K_{obs} = 0.3342 min-1; CURVE 2 = 2 x 10-4 M, K_{obs} = 0.7283 min-1; CURVE 3 = 5 x 10-4 M, $K_{obs.}$ = 6.7457 min-1)

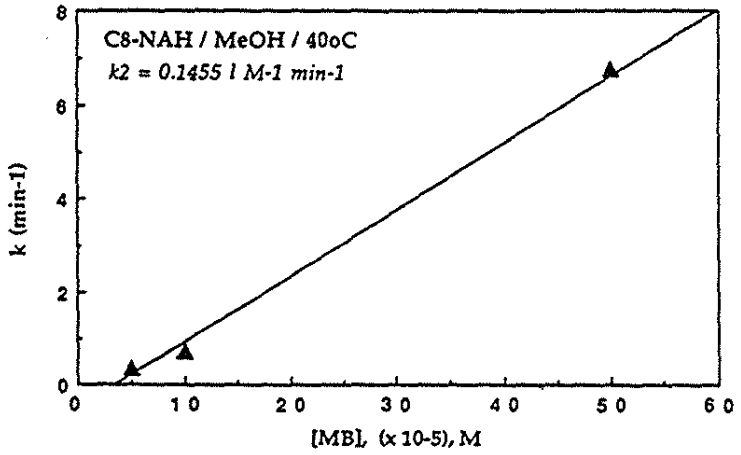


FIGURE 5. VARIATION OF THE OBSERVED RATE CONSTANTS FOR C_8-NAH REACTION WITH [MB] IN METHANOL OVER THE CONCENTRATION RANGE 5 x 10-5 M TO 50 x 10-5 M AT 40 °C.

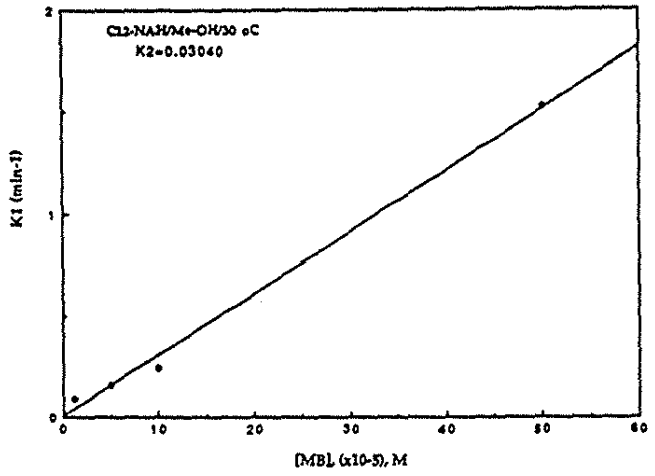


FIGURE 6. VARIATION OF THE OBSERVED RATE CONSTANTS FOR C_{12}-NAH REACTION WITH [MB] IN METHANOL OVER THE CONCENTRATION RANGE OF 0.5 TO 50 x 10-5 M AT 30 oC.

TABLE I : FIRST ORDER RATE CONSTANTS (K_1) FOR THE REDUCTION OF METHYLENE BLUE (MB) BY VARIOUS N-ALKYL DIHYDRONICOTINAMIDES UNDER VARIOUS CONDITIONS

Experiment	RNAH M ($x10^{-5}$)	M.Blue M($x10^{-5}$)	Temp. ^{o} C	Experimental Conditions#	k (min^{-1})
C_4-NAH					
1	14,43	1,12	22	1	0,0898
2	6,48	1,12	22	3a	0,0614
3	12,9	1,12	22	3a	0,1095
C_8-NAH					
4	7,52	1,12	10	2	0,0497
5	8,84	1,12	15	2	0,0648
6	8,13	1,12	22	2	0,1303
7	6,2	1,00	22	2,5	0,6565
8	20.5	50,0	30	2	5,7890
9	20.3	1,15	38	2	0,1429
10	20.63	5,0	40	2	0,3342
11	20.8	10,0	40	2	0,7283
12	24.7	50,0	40	2	6,7457
13	20.0	1,19	45	2	0,1701
14	5,15	1,12	22	3a	0,1647
15	14.0	5,0	35	3c	0,1738
16	17.2	5,0	35	3b	0,1892
17	9.7	5,0	35	3a	0,5029
18	8,66	1,12	22	2	0,0560

Experimental Conditions: (1) - Buffer: 0,03 M Na_2HPO_4; 0,0087 M KH_2PO_4;

(2) MeOH ; (3) a)SLS- 10^{-2} M; b) 5x 10^{-2} M ; - c) 10^{-1} M

(4) Irradiation: 300 to 400 nm (filter solution: 2 $CoSO_4$ + 1 $CuSO_4$), Xe arc lamp - 140 mW/ cm^2; (5)Degassing:. 15minutes with.He.or.N_2

TABLE I. FIRST ORDER RATE CONSTANTS (K_1) FOR THE REDUCTION OF METHYLENE BLUE (MB) BY VARIOUS N-ALKYL DIHYDRONICOTINAMIDES UNDER VARIOUS CONDITIONS

Experiment	RNAH M(x 10-5)	M.Blue M (x 10-5)	Temp. o C	Conditions#	k (min -1)
C_{12}-NAH					
19	8,91	1,12	22	2	0,0715
20	32.5	50,0	22	2	1,0579
21	12,73	1,12	22	2,4	0,2253
22	20.5	0,5	30	2	0,04187
23	7,43	1,12	30	2	0,1223
24	22.75	5,0	30	2	0,1600
25	26.5	10,0	30	2	0,2445
26	31.25	50,0	30	2	1,5241
27	21.25	10,0	40	2	0,4246
28	23.25	50,0	40	2	3,0989
29	9,77	1,12	22	3a	0,0926
30	17.45	5,0	35	3c	0,1271
31	15.02	5,0	35	3b	0,2065
32	12.60	5,0	35	3a	0,4260

#**Experimental Conditions:** (1)aqueous buffer of 0.03 M Na_2HPO_4; 0.0087 M KH_2PO_4; (2) MeOH ; (3) a) SDS- 10^{-2} M; b) 5x 10^{-2} M ; c) 10^{-1} M; (4) Irradiation: 300 to 400 nm using filter solution of $CoSO_4$ +$CuSO_4$ (2:1), Xe arc lamp - 140 mW/ cm^2; (5) Degassing: 15minutes with He or N_2.

TABLE II. SECOND-ORDER RATE CONSTANTS, K_2, AND ACTIVATION PARAMETERS FOR THE OXIDATION OF C_4-NAH, C_8-NAH and C_{12}-NAH DERIVATIVES BY METHYLENE BLUE

Compound	Temp. ^oC	k_2 ℓ $M^{-1}min^{-1}$	$E_{act.}$ $KJ.M^{-1}$	$\Delta H^{\#}$ $KJ.M^{-1}$	$\Delta S^{\#}$ $JK^{-1}min^{-1}$
C_4-NAH	22	$2,14 \times 10^4$ *			
C_8-NAH	22	$1,76 \times 10^4$ *	44,7	39,8	-208,5
"	40	0,1455			
C_{12}-NAH	22	$0,54 \times 10^4$*	19,6	14,7	-77,0
	30	0,03040			

*in O_2-purged solution.

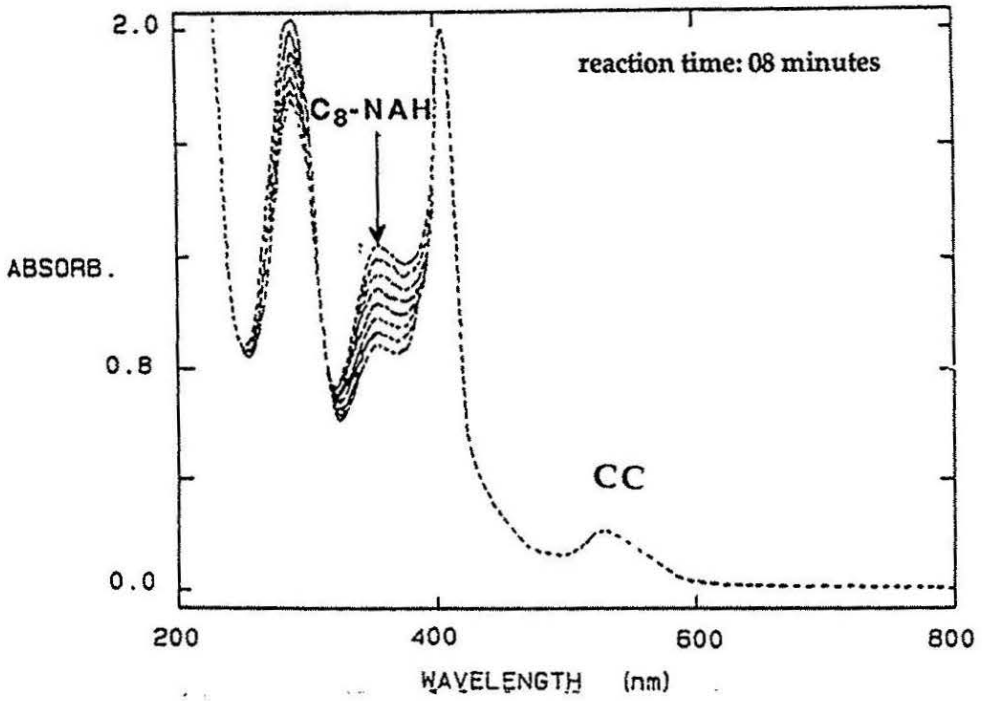


FIGURE 7. SPECTRAL CHANGES OBSERVED DURING THE REDUCTION OF CYTOCHROME-C BY C_8-NAH IN MICELLAR SOLUTIONS OF SDS. CONDITIONS: [C_8-NAH]= 11.06 x 10^{-5} M in SDS, ε = 6350; [CC] = 2.05 x 10^{-5} M IN BUFFER SOLUTION. Δt FOR EACH SPECTRUM IS 1 MINUTE; 1/10 V/V - [SDS] = 10^{-2} M, PHOSPHATE BUFFER, pH = 7.41 AND T = 30 °C.

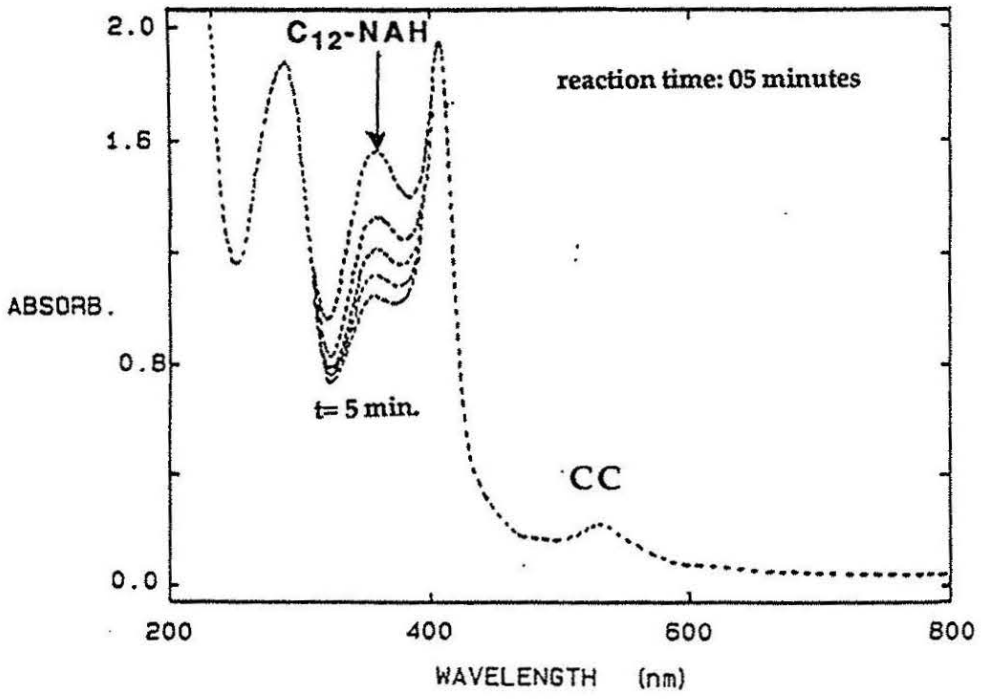


FIGURE 8. SPECTRAL CHANGES OBSERVED DURING THE REDUCTION OF CYTOCHROME-C BY C_{12}-NAH IN MICELLAR SOLUTIONS OF SDS. CONDITIONS: [C_{12}-NAH] = 25.22 x 10^{-5} M IN [SDS] = 10^{-2} M, ε = 4000, [CC] = 2.05 x 10^{-5} M IN BUFFER SOLUTION . Δt FOR EACH SPECTRUM IS 1 MINUTE; 1/10 V/V - SDS SOLUTION (10^{-2}M); PHOSPHATE BUFFER, pH = 7.41 AND T = 22 °C.

Table I - III presents rate data for the reduction of methylene blue and cytochrome-C with different R-NAH (R= C_4, C_8, C_{12}), under various conditions: concentration of R-NAH, temperature, oxygen presence and variation of number of SLS micelles.Table II presents data on the sencond order rate constants (k_2) and the activation parameters for the reaction of C_4, C_8 and C_{12} derivatives with MB.

The second order rate constant derived from the concentration dependence of MB is enhanced once, stacked dimers are known to be formed at high MB concentration [14]. Data from Table I and II for different R-NAH compounds indicate that dimers exhibit greater reactivity on the oxidation of R-NAH substrate as compared to the monomers.

The rate of reduction of CC is much less sensitive to the R-NAH concentration. High concentration of the donor leads to decrease the observed rate constant K_{obs}. One possibility would be formation of micelles when R-NAH+ concentration increases. Due to experimental limitations, the second order constant for CC reduction could not be determined.

Temperature dependence of the reduction was studied over the range of 22-38 °C. (A thermal decomposition of R-NAH coumpounds was observed when temperature rises to 40-45 °C. At temperatures below 15 °C non-reproducible results were obtained). The classical expression for the rate constant for a non-adiabatic electron exchange reaction contains a pre-exponential term which considers essentially the electron frequency, assuming that the reaction proceeds via thermal excitation [9]. According values presented in the Tables I - III, we can conclude that MB systems are much more thermally influenced with the additional variable of the chain length favouring the electron transfer processes in all systems.

For CC reduction, a slight increase on the reaction rate was observed with increasing temperature, which is in accordance to the idea that most biological electron-transfer processes are non-adiabatic [9].

TABLE III. RATE CONSTANTS (K_1) FOR R-NAH AND CYTOCHROME-C; WITH VARIED CONCENTRATIONS AND TEMPERATURES; LISTED BELLOW:

Experiment	C_4NAH M, (x 10^{-5})	Cyt.C M, (x 10^{-5})	Temp. °C	Conditions#	k (min^{-1})
1	10,18	2,05	22	1	0,0592
2	23,86	2,05	30	1	0,0439
3	19,09	2,05	22	1,4	0,0443
4	20,82	2,05	22	3	0,0303
5	16,21	2,05	30	3	0,0330
6	18,28	2,05	22	3	0,0468

#Conditions: (1) Buffer: 0,03 M Na_2HPO_4; 0,0087 M KH_2PO_4; (2) MeOH; (3) SLS= 0,01 M - (3a) SLS=0,1 M; (4) Irradiation: 300 to 400 nm (filter solution: 2 $CoSO_4$ + 1 $CuSO_4$); Xe arc lamp - 140 mW/ cm^2; (5) Degassing: 15 minutes with N_2 or He.

TABLE III. RATE CONSTANTS (K_1) FOR R-NAH AND CYTOCHROME-C; WITH VARIED CONCENTRATIONS AND TEMPERATURES; LISTED BELLOW:

Experiment	RNAH M, (x 10^{-5})	Cyt.C M, (x 10^{-5})	Temp. ^{o}C	Conditions#	k (min^{-1})
C8NAH					
7	12,0	2,05	15	2	0,0462
8	11,99	2,05	30	2	0,0780
9	12,11	2,05	22	2,4	0,0763
10	21,46	2,05	22	2,4	0,0318
11	13,16	2,05	22	2,4	0,0646
12	10,55	2,05	22	2,5	0,0445
13	16,2	3,76	22	2	0,0340
14	15,6	8,36	22	2	0,0355
15	10,75	2,05	22	3	0,0327
16	11,06	2,05	30	3	0,0513
C12NAH					
17	25,88	2,05	22	2	0,0349
18	16,81	2,05	22	2	0,0376
19	16,44	2,05	30	2	0,0353
20	25,22	2,05	22	3	0,0834
21	26,14	2,05	30	3	0,0633
22	30,02	2,05	22	3,4	0,1466
23	13,08	4,91	30	2	0,0805
24	14,32	4,91	22	2,4	0,8486
25	13,02	4,91	22	2,4	0,7945
26	55.8	8,36	22	3a,4	no reaction
27	42.5	4,91	22	3a	no reaction
28	56.2	8,36	30	3a	no reaction

#Conditions : (1) Buffer: 0,03 M Na_2HPO_4; 0,0087 M KH_2P0_4;(2) MeOH; (3) SLS= 0,01 M - (3a) SLS=0,1 M; (4) Irradiation: 300 to 400 nm using a filter solution of $CoSO_4$ + $CuSO_4$ (2:1);Xe arc lamp, 140 mW/cm^2;(5) Degassing: 15 minutes with N_2 or He.

As described in the literature [8] solubilization of donor and/or acceptor molecules in micellar assemblies provides control on solute distribution generating mainly monomers and as consequence enhancing efficiencies of photoredox processes. Data on MB reduction in the presence of SLS micelles confirm such possibilities.Table I shows (exp. 30-32) that k_{obs} decreases with increasing SLS concentration. For CC case, compartimentalization has also a negative effect. As shown in Table III (exp. 26 to 28) in the presence of SLS (concentration 10^{-1} M), no reduction is observed. The reaction may be blocked because of solubilization of the electron donor in the micellar assemblies.

The majority of the photochemical conversion schemes are based on light-induced electron-transfer reactions. When a molecule absorbs a photon of suitable energy, an electronically excited state is obtained that is a better oxidant and reductant than the ground-state [1-c, 9]. An electron-transfer reaction between such an excited state and a suitable reaction partner may convert a fraction of the absorbed light into chemical energy. Experimentally, the reduction of MB by R-NAH is enhanced upon photolysis. By comparison of the extend of reduction of two similar solutions (one kept in the dark and one irradiated) , an increase of approximately 80% of reaction yield by photolysis has been estimated. Photolysis by CC containing solutions however leads to non-reproducible results and first order kinetics is observed no longer due to decomposition of the central heme.

We may conclude that the reduction of methylene blue and cytochrome-C by the dihydronicotinamides follows first order kinetics in all systems. The electron transfer process is enhanced in the presence of MB (sensitized reaction), under irradiation and by increasing the chain length ($C_{12}>C_8>C_4$). The slow electron transfer of CC is due to restrictions of reorientation of its dipoles, the molecule shows differences in the equilibrium structure when the heme changes from ferric to ferrous state.

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