# PHOTOCHEMICAL GENERATION OF REDUCED β-NICOTINAMIDE-ADENINE DINUCLEOTIDE (INDUCED BY VISIBLE LIGHT)

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## Summary

In this study we report evidence for the photochemical generation of β-nicotinamide-adenine dinucleotide reduced (NADH) in a homogeneous solution. NADH is the major product of the reaction using β-nicotinamide-adenine dinucleotide (NAD$^+$) as the starting material. The system contains tris(2,2'-bipyridine)ruthenium(II) (Ru(bipy)$_3^{2+}$) as a sensitizer, triethylamine (TEA) as an electron donor and NAD$^+$ as a reducible substrate in a CH$_3$CN–H$_2$O solution. The utilization of radiant energy in the irreversible electron flow from TEA to NAD$^+$ ($E_0' = -0.320$ V at pH 7 with respect to a normal hydrogen electrode) shows that the strong reducing potential of the photoredox intermediate Ru(bipy)$_3^+$ can be used efficiently to mediate this process. Optimum conditions for the system under continuous illumination are elaborated and implications for an energy conversion system are discussed.

## 1. Introduction

Work carried out previously in this laboratory [1 - 3] has shown that chemical processes are capable of storage of light energy. Light-driven redox processes have led to hydrogen ion reduction and consequent hydrogen gas production as a fuel. In these processes a transition metal complex tris(2,2'-bipyridine)ruthenium(II) (Ru(bipy)$_3^{2+}$) has been used as a sensitizer. It has been shown to have excellent properties as a sensitizer and to participate directly in redox processes [4], leading to energy storage through electron transfer reactions [5, 6].

In the present study we are concerned with β-nicotinamide-adenine nucleotide (NAD$^+$) reduced to NADH and the coupling of this process with the reduction of hydrogen ions. Visible light-induced processes with good quantum efficiency and turn-over (in terms of moles of NADH produced per quantum of light absorbed) are desired. This approach has been employed in the photoreduction of NAD$^+$ with a sensitizer (Ru(bipy)$_3^{2+}$) and triethylamine (TEA) under visible light in non-aqueous solution [5]. The purpose of

this paper is to discuss the nature of the redox events leading to NADH production by means of steady state and laser spectroscopy techniques and to determine the optimum conditions necessary for this process. The advantage of the system employed here is its simplicity, even though $H_2$ and NADH are produced simultaneously. An aprotic solvent such as acetonitrile ($CH_3CN$) and a protic solvent such as $H_2O$ are combined in such a way that the least amount of $H_2$ is produced. This system can store energy in the form of NADH where the protons are provided by the $H_2O$.

## 2. Experimental details

### 2.1. Materials

Experiments were carried out using $NAD^+$ obtained from Serva (catalogue number 30311, p.a.) without further purification. $NAD^+$ solutions were stable when stored in cold conditions (a 1 wt.% solution keeps for about 1 week at 4 °C), although above pH 7 the rate of decomposition increases with pH. At pH 10.7 in buffered solutions ($NaHCO_3$–NaOH buffer) where irradiations were carried out, the reagent remained stable for at least 1 day at room temperature. The NADH (the reduced form of $NAD^+$) used was Serva (catalogue number 30314, p.a.). The disodium salt of $\beta$-nicotinamide-adenine dinucleotide phosphate (NADP) and its reduced form disodium NADPH that were utilized were Fluka (p.a.) and were used as received. The complex tris(2,2'-bipyridine)ruthenium(II) chloride hexahydrate (Strem Inc.) and TEA (BDH 30502) were also used as received.

### 2.2. Procedure

Spectrophotometric detection of the products NADH and NADPH, as well as the measurement of the initial concentrations of the reagents, were carried out with a Hitachi–Perkin–Elmer 340 recording spectrophotometer. Determinations of $H_2$ were carried out on a molecular sieve 5A gas chromatographic column as reported previously [1]. Laser photolysis experiments were performed with a JK 2000 frequency-doubled neodymium laser which generated 20 ns pulses at 530 nm. Transient absorptions were detected by fast kinetic spectroscopy. The details of this method have been published elsewhere [7]. Continuous irradiation experiments were carried out using an Osram XBO-450 W lamp as a light source. A 400 nm cut-off filter eliminated the UV radiation and a 15 cm $H_2O$ filter was used as a cut-off for near IR light. The colloidal catalyst Pt–polyvinyl alcohol (PVA) was tested for its effect on NADH and $H_2$ production. This system has previously been used to evolve $H_2$ [2]. This catalyst was prepared as reported before [3] and the sample was centrifuged for 1 h at $5 \times 10^4$ gf. The catalyst contained 1.0 (g Pt) $l^{-1}$ as determined by atomic absorption and contained 1% PVA. The particle radius was found to be 410 A with a Chromatix KMX-6 autocorrelator. Ag–PVA catalyst was prepared in the following way: 2 g of PVA were dissolved in 100 cm³ of $H_2O$ and the pH was brought up to 9.0. $AgNO_3$

(170 mg) was added and the solution was reduced for 2 h by bubbling $H_2$ through it. It was left to stand overnight, leading to a brownish yellow colour (the reaction seemed to take some time to go to completion) which, on centrifugation (2 h at $5 \times 10^4$ gf), gave a clear yellow solution of transparent Ag–PVA suspension.

## 3. Results and discussion

The mechanism of the reduction of $NAD^+$ to its reduced form NADH has attracted much attention for many years, generally utilizing hydrogenases as transfer agents [8]. The reverse reaction, namely the oxidation of NADH to $NAD^+$, has also been reported to be mediated by dehydrogenases and peroxidases [9, 10]. Figure 1, curve a, is the absorption spectrum of a solution consisting of $10^{-4}$ M $Ru(bipy)_3{}^{2+}$, 0.12 M TEA and $5 \times 10^{-3}$ M $NAD^+$ in a $70\%CH_3CN-30\%H_2O$ solvent after irradiation for 6 h ($\lambda \geqslant$ 400 nm). The absorption cell had a path length of 0.2 cm and the spectrum of the photolysed solution was always measured against an unphotolysed solution as reference. The pH remained constant over a period of 6 h. In Fig. 1, curve a, it is possible to observe the depletion of $Ru(bipy)_3{}^{2+}$ with a negative absorption change relative to the unphotolysed solution centred around 460 nm. From Fig. 1 it is inferred that $Ru(bipy)_3{}^{2+}$ does not cycle throughout the process but is depleted with time. $Ru(bipy)_3{}^+$ apparently is recycled during the process since no absorption was observed at its charac-
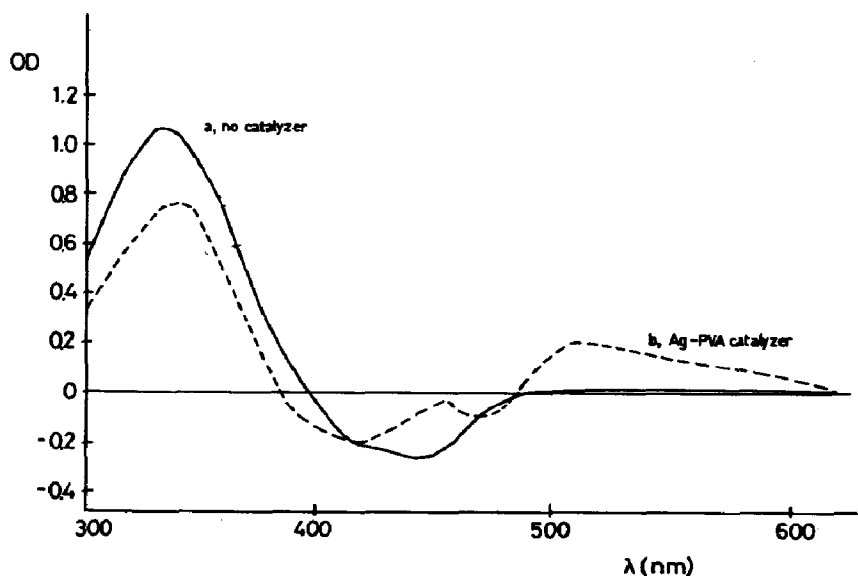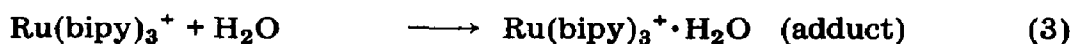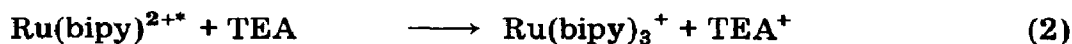


Fig. 1. Difference absorption spectrum in a 0.2 cm cell of a solution ($10^{-4}$ M ($Ru(bipy)_3{}^{2+}$, $5 \times 10^{-3}$ M $NAD^+$ and 0.12 M in a $70\%CH_3CN-30\%H_2O$ solvent) which had been photolysed for 6 h (measured with respect to an unphotolysed solution) (the light below 400 nm was filtered out): curve a, no catalyst added; curve b, Ag–PVA catalyst added to give a solution containing 170 (mg Ag) $l^{-1}$.

teristic peak wavelength (515 nm) [5], as shown in Fig. 1, curve a. The growth of the band at 340 nm shows the characteristic spectrum of NADH with $\epsilon$ = 6220 M$^{-1}$ cm$^{-1}$, all in agreement with results reported already [11]. It is not possible to observe the second band in the UV which is centred at 260 nm since its absorption position coincides with the NAD$^+$ band and the extinction coefficient is too large (about $14 \times 10^3$ M$^{-1}$ cm$^{-1}$) to make meaningful spectral observations [12]. (Spectroscopic observations of effects induced by 530 nm laser pulses (duration, 25 ns) at 510 nm are shown in Fig. 3.) The growth of the characteristic absorption peak of NADH at 340 nm as a function of time is shown in Fig. 2(a) in which experimental results for an irradiation time of up to 6 h are shown. The fact that the sensitizer is indeed directly involved in the generation of NADH is shown in Fig. 2(b).

## 3.1. Proposed mechanism of reaction

According to the experimental observations, the following mechanism for NADH generation is postulated:

$$\text{Ru(bipy)}_3{}^{2+} \xrightarrow{h\nu} \text{Ru(bipy)}^{2+*} \tag{1}$$

$$\text{Ru(bipy)}^{2+*} + \text{TEA} \longrightarrow \text{Ru(bipy)}_3{}^+ + \text{TEA}^+ \tag{2}$$

$$\text{Ru(bipy)}_3{}^+ + \text{H}_2\text{O} \longrightarrow \text{Ru(bipy)}_3{}^+ \cdot \text{H}_2\text{O} \quad \text{(adduct)} \tag{3}$$

$$\text{Ru(bipy)}_3{}^+ \cdot \text{H}_2\text{O} + \text{NAD}^+ \longrightarrow \text{NAD}^{\cdot} + \text{Ru(bipy)}_3{}^{2+} \cdot \text{H}_2\text{O} \tag{4}$$

$$\text{H}^+ + 2\text{NAD}^{\cdot} \longrightarrow \text{NAD}^+ + \text{NADH} \tag{5}$$

Ru(bipy)$_3{}^+$ has a lifetime in the presence of H$_2$O (in 70%CH$_3$CN–30%H$_2$O solutions) of about 1 $\mu$s, as shown in Fig. 3. In this experiment the photo-
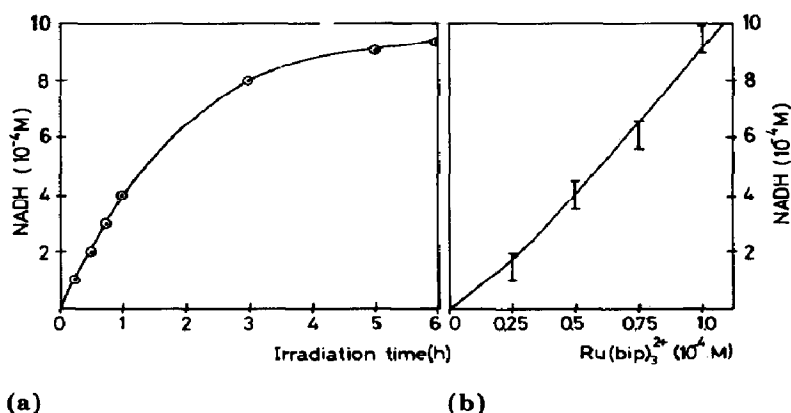


(a)                                    (b)

Fig. 2. (a) Growth of the photolytically induced NADH measured at 340 nm as a function of irradiation ($\lambda \geqslant 400$ nm) time in a solution containing $10^{-4}$ M Ru(bipy)$_3{}^{2+}$, 5 × $10^{-3}$ M NAD$^+$ and 0.12 M TEA in a 70%CH$_3$CN–30%H$_2$O solvent; (b) growth of NADH photolytically induced as a function of the initial concentration of Ru(bipy)$_3{}^{2+}$ (the photolysis cell has an optical path length of 3.0 cm; other reagents in the solution are 5 × $10^{-3}$ M NAD$^+$ and 0.12 M TEA in a 70%CH$_3$CN–30%H$_2$O solvent.
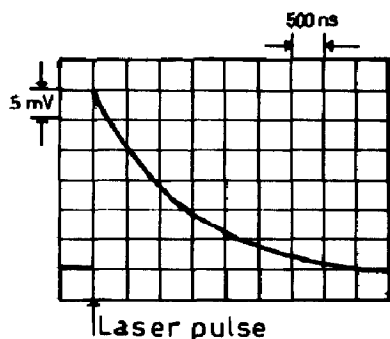
Fig. 3. Oscilloscope trace at 515 nm for the lifetime of the transient species induced by 530 nm laser flash photolysis on the solution measured in Fig. 1, curve a. The induced absorption is shown on the vertical axis and the time after the 25 ns pulse is shown on the horizontal axis.

chemistry was induced by a 25 ns laser pulse (530 nm). The adduct formed $Ru(bipy)_3^+ \cdot H_2O$ then reduces $NAD^+$ to the radical $NAD^\cdot$ which subsequently disproportionates to give NADH and $NAD^+$ as reported previously [13].

The principal results of these studies involve a system in which the precursor $Ru(bipy)_3^+$ is directly and efficiently coupled with $NAD^+$ as noted in reaction (4) of the proposed scheme. The spectrum of this oxidation state of $Ru^+$, which has a peak at 515 nm, is stable in pure $CH_3CN$ [14]. $Ru(bipy)_3^+$ reverts to $Ru(bipy)_3^{2+}$ when $H_2O$ is introduced into the system. The reaction of $Ru(bipy)_3^+$ with $NAD^+$ in Fig. 1, curve a, is apparently so efficient that no accumulation of $Ru^+$ is observed, i.e. there is no appreciable peak in the difference spectrum at 515 nm. In Fig. 3 the trace shows a lifetime of about 1 $\mu$s for the $Ru(bipy)_3^+$ species in the presence of $5 \times 10^{-3}$ M $NAD^+$ and 0.12 M TEA with 70% $CH_3CN$ in $H_2O$ as solvent at pH 10.0. The kinetics of Fig. 3 shows an approximate first-order decay, with a lifetime of 1 $\mu$s which is due to the scattering effects in this multicomponent solution; this is probably responsible for the deviation from first-order kinetics that is observed. Under the experimental conditions stated, the lifetime determined by laser-induced spectroscopy is quite different from that of $Ru(bipy)_3^+$ in dry $CH_3CN$ which remained stable for several days. The efficient coupling of the $Ru(bipy)_3^+$ intermediate towards NADH production indicates that electron transfer with a neutral species such as TEA leads to a pronounced diminution in the possible back electron transfer that can take place in reaction (2). The addition of different concentrations of $NAD^+$ does not influence the lifetime of the $Ru(bipy)_3^+$ shown in Fig. 3, apparently because $Ru(bipy)_3^+$ does not react directly with $NAD^+$ in the presence of $H_2O$ but through a water adduct as postulated in reaction (3).

The role of TEA depicted in reaction (2) is to produce $Ru(bipy)_3^+$ which subsequently affords, through reactions (3) and (4), the reduction of $NAD^+$. TEA is consumed irreversibly in the reaction described by reaction (2) and makes the photoinduced NADH production a sacrificial system. In this way we provided a route effectively to bypass the energy-wasting reverse

electron transfer step and to store energy through the resulting redox product. In our case light-induced reaction of the $Ru(bipy)^{2+}$ complex with TEA was found to be relatively straightforward. The fate of the TEA radical cation probably follows the same scheme as that proposed for tertiary amines in general. Extensive studies of photochemical [15] and electrochemical [16] oxidation of tertiary amines indicate that, in aqueous solution, cleavage of the N—C bond occurs with hydrolysis and formation of a secondary amine and aldehyde via an iminium salt. When reaction (2) takes place in the presence of $H_2O$, acetaldehyde ($CH_3CHO$) is formed as a product. The mechanism of reaction (2) leads to a net photoreduction of $Ru(bipy)_3^+$ which is shown in more detail by [17]

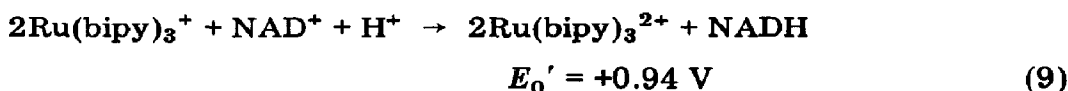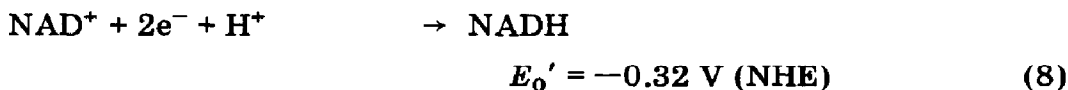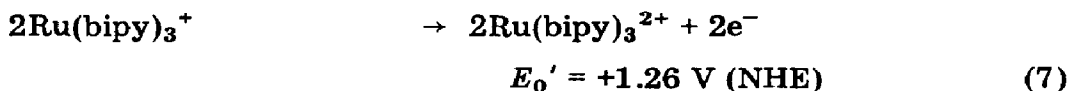$$2Ru(bipy)_3^{2+} + 2(C_2H_5)_3\text{—NH} \rightarrow 2Ru(bipy)_3^+ + (C_2H_5)_3\text{—NH}^+ +$$
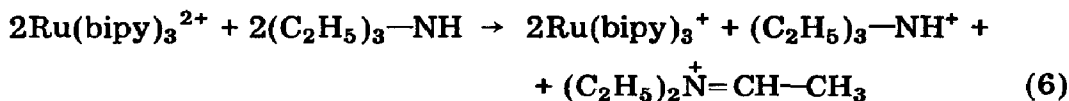
$$+ (C_2H_5)_2\overset{+}{N}=CH\text{—}CH_3 \qquad (6)$$

The $CH_3CHO$ is derived from hydrolysis of the $(C_2H_5)\overset{+}{N}=CH\text{—}CH_3$ [18]. The higher limit of 70% $CH_3CN$ in our mixtures is set by the solubility necessary for the other components of the mixture. Experiments were also carried out with NADP using $5 \times 10^{-3}$ M of this reagent in the usual photochemical system $10^{-4}$ M $Ru(bipy)_3^{2+}$ and 0.12 M TEA in a 70%$CH_3CN$–30%$H_2O$ solvent. An increase in absorbance was also observed at 340 nm as in the NAD–NADH system. To assess the concentrations of NADPH obtained, we used the same extinction coefficient reported for NADH [19].

Control experiments omitting separately light, $NAD^+$, TEA, the ruthenium complex, $CH_3CN$ and $H_2O$ all resulted in no generation of NADH. The synthesis of NADH occurred only in the light and was dependent on $NAD^+$, $Ru(bipy)_3^{2+}$ and an electron donor (TEA) as well as on a proper choice of the $CH_3CN$:$H_2O$ ratio in the solvent.
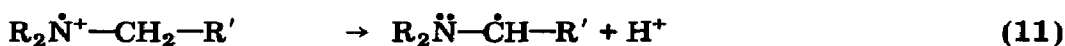
## 3.2. Energetics

The energetics of $NAD^+$ reduction by $Ru^+$ can be derived from formulating two half-cell reactions as follows (the potentials are measured with respect to a normal hydrogen electrode (NHE)):

$$2Ru(bipy)_3^+ \rightarrow 2Ru(bipy)_3^{2+} + 2e^-$$

$$E_0' = +1.26 \text{ V (NHE)} \qquad (7)$$

$$NAD^+ + 2e^- + H^+ \rightarrow NADH$$

$$E_0' = -0.32 \text{ V (NHE)} \qquad (8)$$

$$2Ru(bipy)_3^+ + NAD^+ + H^+ \rightarrow 2Ru(bipy)_3^{2+} + NADH$$

$$E_0' = +0.94 \text{ V} \qquad (9)$$

The potentials for reactions (7) and (8) have been reported [20, 21]. The large energy released in reaction (9) may have consequences in the coupling observed in this system. However, it is also possible that, when relatively

large molecules such as NAD$^+$ interact with Ru(bipy)$_3$$^+$, the reduction is retarded by steric factors. These considerations show that there is enough energy to drive the reduction process at high speed. These high rates possibly approach the diffusion-controlled rate in a reaction where the presence of redox sites both on the metal ions and on the NAD$^+$ substrate are necessary. The efficiency of the process depicted by reaction (9) affords conversions up to 20% under irradiation for 6 h with visible light ($\lambda \geqslant 400$ nm), since with $5 \times 10^{-3}$ M NAD$^+$ as the initial concentration a $10^{-3}$ M solution in NADH was attained as shown in Fig. 2(a). The irradiation flask was 25 cm$^3$ under conditions of full light absorption. The limit of 6 h was set because of the following experimental observation: prolonged irradiation of Ru(bipy)$_3$$^{2+}$ in aqueous CH$_3$CN containing TEA led to a slow decomposition of the metal complex [22]. The quantum yield for the production of NADH is about 0.10 with an incident light intensity of $1.60 \times 10^{17}$ quanta min$^{-1}$ using a K-45 Balzers cut-off filter for the incident light. If light in the visible can be as high as 40% efficient [23], the maximum efficiency for solar photons is then about $0.40 \times 0.10 = 4\%$, leading to the stable photoproduct NADH. $\Delta G^\circ$ for reaction (2) is $(-0.84) - (+0.82) = -0.02$ V (NHE) and this favourable potential makes the coupling between Ru$^{2+}$ and TEA possible [15]. The decomposition of TEA may be written as a two-electron sequence:

$$R_2\overset{..}{N}-CH_2-R' \quad\quad \rightarrow \; R_2\overset{.}{N}{}^+-CH_2-R' + e^- \quad\quad (10)$$

$$R_2\overset{.}{N}{}^+-CH_2-R' \quad\quad \rightarrow \; R_2\overset{..}{N}-\overset{.}{C}H-R' + H^+ \quad\quad (11)$$

$$R_2\overset{..}{N}-\overset{.}{C}H-R' \quad\quad \rightarrow \; R_2N^+{=}CH-R' + e^- \quad\quad (12)$$

$$R_2N^+{=}CH-R' + H_2O \; \rightarrow \; R_2NH + R'-CHO + H^+ \quad\quad (13)$$

The overall oxidative decomposition process may be written as

$$N(C_2H_5)_3 + H_2O \; \rightarrow \; HN(C_2H_5)_2 + H_3-CHO + 2H^+ + 2e^- \quad\quad (14)$$
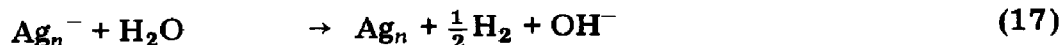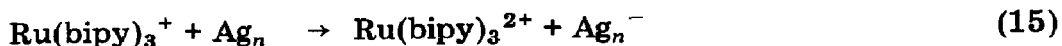
It should be noted that the oxidation potential of TEA (+0.82 V (NHE)) is about the same as that of H$_2$O. Taking into consideration reaction (8) with a reduction potential of $-0.32$ V and reaction (14) with a reduction potential of $-0.82$ V, we have a negative $\Delta G$ for the addition of the reactions which are postulated to lead to NADH (reactions (1) - (5)). The facts that TEA is a suitable donor but that H$_2$O is not should therefore be ascribed not to thermodynamic but to mechanistic reasons. The critical step in the sequence of reactions (10) - (13) is probably the deprotonation in reaction (11). The loss of the proton prevents reoxidation of the reduced ruthenium species by TEA$^+$; furthermore, the neutral radical formed has reducing properties, making a second electron available in reaction (12), a property which may explain the rapid generation of the Ru$^+$. The existence of a pH optimum for NADH generation, at about p$K_a$(TEA) 10.7 [24], suggests that in fact opposite effects are occurring: at low pH, H$_2$O reduction is easier (reaction (10)) and possibly will not take place when the amine is protonated and the deprotonation step (reaction (11)) is slowed down. At high enough

pH (for instance greater than 10) the reduction potential of water is too low (less than $-0.6$ V) and the proton loss (reaction (11)) is fast enough to prevent back electron transfer in reaction (2).

### 3.3. Action of the catalyst

We saw in Fig. 1 the $Ru^{2+}$-complex-induced production of NADH, utilizing radiant energy. Attempts were made to see whether the reduction of $NAD^+$ to NADH could be affected by a highly active Pt–polymer catalyst. Pt–PVA catalysts [3] with the characteristics already outlined in Section 2.2 did not change the yields obtained for NADH and shown in Fig. 1, curve a. Nevertheless, the presence of Ag–PVA catalyst caused a decrease in the yield of NADH obtained in the photolysis (provided that all other conditions were identical) as shown in Fig. 1, curve b. Silver has been used widely in organic chemistry as a partial hydrogenation catalyst [25]. A less efficient production of NADH is observed accompanied by a growth in absorption with a peak between 500 and 600 nm. Also the observed yield of $H_2$ in the presence of the Ag–PVA catalyst was close to the yields observed for $H_2$ production when Pt–PVA was present. The Ag–PVA catalyst induces a decrease in the yield of NADH and an increase in $H_2$ compared with the situation where no Ag–PVA is present. This experimental observation confirms previous work [3] that shows that organic radicals charge the platinum or silver colloid catalysts by electron transfer. These particles assume the role of a pool of electrons which can reduce either $NAD^+$ or $H^+$ ions. If the catalyst is absent, the $NAD^+$ reduction by $Ru(bipy)_3{}^+$ proceeds more efficiently since it is the only reaction taking place, compared with the two species which are reduced when a catalyst is present. The following reactions account for these experimental observations:

$$Ru(bipy)_3{}^+ + Ag_n \quad \rightarrow \quad Ru(bipy)_3{}^{2+} + Ag_n{}^- \tag{15}$$

$$Ag_n{}^- + NAD^+ + H^+ \rightarrow Ag_n + NADH \tag{16}$$

$$Ag_n{}^- + H_2O \quad \rightarrow Ag_n + \tfrac{1}{2}H_2 + OH^- \tag{17}$$

The negative absorption at 450 nm is due to the $Ru^{2+}$ complex that intervenes in the photolytic process and the band at 340 nm reflects the NADH produced. The possibility that NADH in the presence of the Pt–PVA catalyst would spontaneously release hydrogen and reconvert to $NAD^+$ was checked. From redox potentials such a process is thermodynamically favourable at pH $\leqslant$ 5. However, the instability of NADH precluded work at an acidic pH where a rapid loss of activity was observed.

### 3.4. Effects of light absorption and time of irradiation

The amount of NADH synthesized is proportional to the amount of $Ru^{2+}$ complex added up to about $10^{-4}$ M, as shown in Fig. 2. The optical absorption and the concentration of the active precursor increase with the $Ru^{2+}$ complex, as indicated in the reaction mechanism. Figure 2(a) shows that after 6 h about $10^{-3}$ M NADH is produced and little increase was ob-

served for the formation of this product after this time. This experimental observation agrees with the fact [26] that $H_2O$–$CH_3CN$ mixtures containing amines lead to a slow decomposition of the metal complex.

### 3.5. Variation in $H_2O$ in $H_2O$–$CH_3CN$ mixtures

In Fig. 4 the yields of NADH and $H_2$ are plotted against the $H_2O$ content of the solvent mixture used. Details for the system are given in the caption to Fig. 4; the important modification in this case is that Pt–PVA is used as a catalyst to obtain significant yields of $H_2$ compared with the $H_2$ yield when no catalyst is added. Stable solutions with more than 70% $CH_3CN$ in the mixture could not be obtained without the presence of $H_2O$. As the amount of $CH_3CN$ present in the mixture is increased, the amount of NADH formed increases; this shows that the $Ru^+$ complex in solution probably forms an adduct and leads to product formation. Both the products NADH and $H_2$ need, for their formation, the $Ru^+$ complex and protons ($H_2O$ in the net sense) which have different effects on their yield.
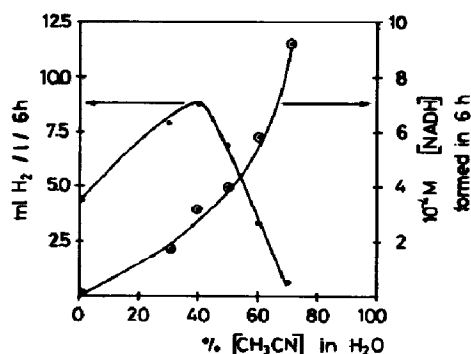


Fig. 4. Yields of hydrogen and NADH produced in the photolysis of a solution containing $Ru(bipy)_3^{2+}$, $5 \times 10^{-3}$ M $NAD^+$ and 0.12 M TEA in a 70%$CH_3CN$–30%$H_2O$ solvent. Pt–PVA was also added (1 mg of platinum per 25 $cm^3$ of photolysed solution for $H_2$ production).

## 4. Conclusion

The present results show that $NAD^+$ can be reduced photocatalytically in an exergonic reaction with local conditions of pH and with ionic species present. Metal complexes afford possible control of redox rates that could be used successfully for other biochemical systems.

# References

1  K. Kalyanasundaram, J. Kiwi and M. Grätzel, *Helv. Chim. Acta, 61* (1978) 2720.
2  J. Kiwi and M. Grätzel, *Nature (London), 281* (1979) 657.
3  J. Kiwi and M. Grätzel, *J. Am. Chem. Soc., 101* (1979) 7214.
4  V. Balzani, F. Boletta, M. Gandolfi and M. Maestri, *Top. Curr. Chem., 75* (1978) 1.
5  N. Sutin and C. Creutz, *Adv. Chem. Ser., 168* (1978) 1.
6  H. D. Abruña, A. Y. Teng, G. J. Samuels and T. J. Meyer, *J. Am. Chem. Soc., 101* (1979) 6745.
7  J. Kiwi and M. Grätzel, *J. Am. Chem. Soc., 100* (1978) 6314.
8  H. F. Fisher, E. E. Conn, B. Vennesland and F. H. Westheimer, *J. Biol. Chem., 202* (1953) 681.
9  K. Yokota and I. Yamazaki, *Biochemistry, 16* (1977) 1913.
10  A. Nadezhdin and H. B. Dunford, *J. Phys. Chem., 83* (1979) 1957.
11  R. B. McComb and R. J. Gav, *Clin. Chem., 14* (1968) 754.
12  A. L. Lehninger, *Biochemistry*, Worth, New York, 1970, p. 730.
13  E. J. Land and A. J. Swallow, *Biochemistry, 8* (1963) 2117.
14  R. C. Young, C. P. Anderson, D. J. Salmon and T. J. Meyer, *J. Am. Chem. Soc., 99* (1977) 1980.
15  S. G. Cohen, A. Parola and G. H. Parsons, *Chem. Rev., 73* (1973) 141.
16  P. G. Smith and G. K. Mann, *J. Org. Chem., 34* (1969) 1821.
17  P. Delaive, J. T. Lee, H. Abruña, H. W. Sprintschnik, T. J. Meyer and D. G. Whitten, *Adv. Chem. Ser., 168* (1978) 28.
18  D. G. Whitten, personal communication, 1980.
19  M. Grassl, M. Nelbock-Hochstetter and K. Beaucamp, *Z. Anal. Chem., 252* (1970) 331.
20  W. Mansfield Clark, *Oxidation–Reduction Potential of Organic Systems*, R. Krieger, New York, 1972, p. 494.
21  C. R. Bock, J. A. Connor, A. R. Guterrez, T. J. Meyer, D. G. Whitten, B. P. Sullivan and J. K. Nagle, *J. Am. Chem. Soc., 101* (1979) 4815.
22  P. Delaive, B. P. Sullivan, T. J. Meyer and D. G. Whitten, *J. Am. Chem. Soc., 101* (1979) 4007.
23  S. Claeson and L. Engström, *Project Results NE 1977*, Vol. II, Li Bev Tryck, Stockholm, 6th edn., 1977, p. 57.
24  A. Krasna, *Photochem. Photobiol., 29* (1979) 267.
25  *Engelhard Catalysis*, Engelhard Industries, Newark, NJ, 1977.
26  M. Kirch, J. M. Lehn and J. P. Sauvage, *Helv. Chim. Acta, 62* (1979) 1345.