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EFFICIENT INDIRECT ELECTROCHEMICAL IN SITU REGENERATION OF NAD$^+$ AND NADP$^+$ FOR ENZYMATIC OXIDATIONS USING IRON BIPYRIDINE AND PHENANTHROLINE COMPLEXES AS REDOX CATALYSTS

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Summary: Using iron bipyridine or phenanthroline complexes **1** as redox catalysts it was possible to electrochemically generate NAD(P)$^+$ from NAD(P)H very efficiently. Electrochemically driven enzymatic oxidations of the test systems 2-hexen-1-ol and 2-butanol could be performed with **1e** as redox catalyst in presence of yeast alcohol dehydrogenase (YADH) or the alcohol dehydrogenase of thermoanaerobium brockii (TADH) with turnover numbers of about 40/h.

NAD$^+$ and NADP$^+$ are most important cofactors for enzymatic oxidations of great synthetic and technical importance making a regeneration of oxidized pyridine nucleotides indispensable. A number of chemical[1], enzyme coupled[2], and electrochemical[3] regeneration procedures have been described. Electrochemical regeneration is especially valuable as it is reagent free. However, even at relatively high overpotentials only relatively low current densities are obtained, except in some special cases. To increase the current density and to lower the overpotential it is favorable to apply an indirect electrochemical regeneration procedure for NAD(P)$^+$ under use of a redox catalyst. Because of stability and mass transfer problems for preparative scale enzymatic oxidations it is more promising to use a redox catalyst in homogeneous solution than fixed to an electrode surface[3]. Out of thermodynamic reasons, two-electron, respectively hydride atom transfer mediators should be effective at lower potentials than one-electron transfer agents[4]. However, the systems studied so far are decomposing during prolonged synthetic applications at pH $\geq$ 9, where most oxidoreductases possess their maximal activity in the oxidative direction.

Table 1: Redox potentials of iron bipyridine and phenanthroline complexes **1** in the absence and presence of NADH[a]

Complex[d]		E_{pa}[b] [mV]	$E_{pa(cat)}$[c] [mV]
$Fe(bpy)_3SO_4$	**1a**	915	705
$Fe(dmbpy)_3SO_4$	**1b**	745	670
$Fe(phen)_3SO_4$	**1c**	980	730
$Fe(mphen)_3SO_4$	**1d**	920	720
$Fe(tmphen)_3SO_4$	**1e**	730	610

[a] In tris-H_2SO_4 buffer(0.1 M) of pH 9 at a glassy carbon working electrode vs. Ag/AgCl-reference electrode; [b] anodic peak potential of **1** in the absence of NADH; [c] anodic peak potential of the additional catalytic peak in the presence of NADH (**1**/NADH = 1:1); [d] abbreviations: bpy = 2,2'-bipyridine, dmbpy = 4,4'-dimethyl-2,2'-bipyridine, phen = 1,10-phenanthroline, mphen = 5-methyl-1,10-phenanthroline, tmphen = 3,4,7,8-tetramethyl-1,10-phenanthroline.

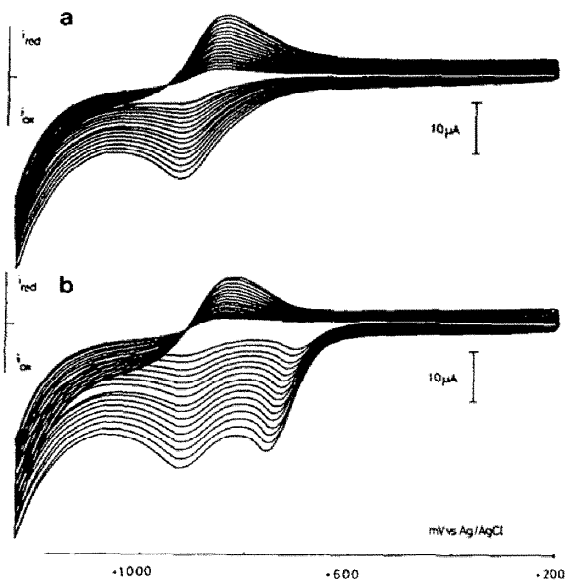
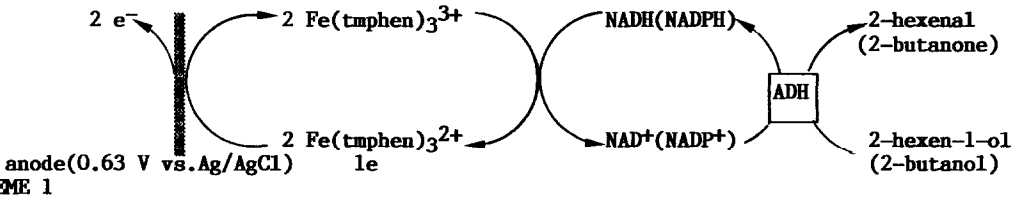


Fig. 1: Cyclovoltammograms **a:** **1a**(0.5x10^{-3} M); **b:** **1a**/NADH = 1:1 (25,36,49.....400 mV/s)

We now found that iron bipyridine and phenanthroline complexes **1** (**Table 1**) are highly effective and very stable as mediators for the $NAD(P)^+$ regeneration. This is clearly demonstrated by cyclic voltammetry (**Fig. 1**). As expected, the cyclovoltammogram of NADH alone in tris-H_2SO_4 buffer of pH 9 at a glassy carbon electrode only shows an electrochemically very irreversible character with a very low current density. $Fe(bpy)_3SO_4$, on the other hand,demonstrates a totally reversible one-electron behavior (**Fig. 1a**). In presence of equimolar amounts of NADH a catalytic pre-peak is observed at a 210 mV more negative potential (**Fig. 1b**). The peak current of the catalytic pre-wave is proportional to the NADH concentration and the square root of the scan rate. The peak potential shifts to more positive values by 30 mV for a tenfold increase of the scan rate. This behaviour was theoretically predicted by Savéant[5] for extremely fast redox catalytic systems, in which the homogeneous electron transfer step is diffusion controlled. All complexes, **1a** - **1e**, behave similarly. Potential-controlled electrolyses of NADH ($1.5x10^{-3}$ M) using a divided beaker-type glass cell in presence of **1a** ($1.5x10^{-3}$ M) in 150 mL tris-H_2SO_4-buffer (0.1 M) of pH 9 at a carbon foil electrode (Sigraflex^R; 5x10 cm) after 2.5 h gave a current efficiency of more than 90 % for enzymatically active NAD^+. Effective electrochemical enzymatic oxidations by in-situ regeneration of NAD^+ and $NADP^+$ by indirect electrolysis with **1e** could be performed under potential control at 630 mV in presence of either YADH (EC 1.1.1.1) or TADH (EC 1.1.1.2) as enzymes and 2-hexen-1-ol or 2-butanol as test systems according to **Scheme 1**. The application of a carbon felt anode with a high surface area (4x12 cm geometrical surface with a thickness of 0.5 cm) is especially efficient. The results are summarized in **Table 2**.



SCHEME 1

Table 2. Electrochemically driven ADH catalyzed enzymatic oxidation of 2-hexen-1-ol and 2-butanol to 2-hexenal and 2-butanone[a]

Exp.Conditions	t	Charge	Product	Turnovers w.r.t.		CY[b]
	[min]	[As]	[M]	**1e**	cofactor	[%]
2-hexen-1-ol [$7x10^{-2}$ M]	15	12	$1.1x10^{-3}$	22	11	90
1e [$1x10^{-4}$ M]	30	16	$1.47x10^{-3}$	30	15	90
NADH [$1x10^{-4}$ M]	45	17	$1.6x10^{-3}$	32	16	91
YADH: 5000 U; 21°C	60	19	$1.77x10^{-3}$	36	18	90
2-butanol [$7x10^{-2}$ M]	30	18	$1.8x10^{-3}$	36	18	96
1e [$1x10^{-4}$]	60	29	$2.4x10^{-3}$	48	24	83
NADPH [$1x10^{-4}$ M]	90	36	$3.5x10^{-3}$	70	35	95
TADH: 110 U; 37°C	120	39	$3.8x10^{-3}$	76	38	95
	150	42	$4.1x10^{-3}$	82	41	95

[a] In 50 mL 0.2 M tris-H_2SO_4 buffer pH 9, divided cell, carbon felt anode, 0.63 V vs. Ag/AgCl; [b] CY = current efficiency

It is shown that an initial turnover number per hour of about 40 can be obtained with both systems. The time dependence of both reactions can be deduced from **Table 2**. We were able to demonstrate that the decrease in reaction rate with time is due to product inhibition of the enzyme and not to cofactor or mediator decomposition. For technical application we are currently working on a continuous system with extraction of the product.

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