Herein, we report the application of nanoparticulate platinum (nPt) to enhancing the heterogeneous electron transfer between NAD$^+$ (nicotinamide adenine dinucleotide, oxidized form) and electrodes in the presence of an organometallic mediator. (Pentamethylcyclopentadienyl-2,2'-bipyridine-chloro)rhodium(III) ($M = \text{[Cp}^*\text{Rh(bpy)Cl]}^+$; $\text{Cp}^* = \text{C}_5\text{Me}_5$, bpy = 2,2'-bipyridine) was used as a primary mediator to shuttle electrons between NAD$^+$ and electrodes. nPt functioned as a homogeneous catalyst and also as a secondary mediator to improve the turnover kinetics of $M$.

Pyridine nucleotides (NAD(P)H) or their oxidized counterparts (NAD(P)$^+$) are used as cofactors that are critically required for redox reactions catalyzed by various oxidoreductases\cite{[1],[2]}. In biocatalytic reactions, NADH should be regenerated to allow the enzymes to continue their turnover. Electrochemical regeneration has been chosen as an attractive strategy that is an alternative to enzymatic regeneration\cite{[3]}. In electrochemical regeneration, however, the first drawback to overcome is the slow electron transfer between NAD$^+$ and the electrodes, even at a potential where the reduction of NAD$^+$ into NADH is thermodynamically favorable. The use of a homogeneous mediator to shuttle electrons between electrodes and NAD$^+$ can be one solution to solve the problem\cite{[4],[5]}.

The rhodium complex $M$ was successfully used as an electron shuttle for NAD$^+$ in electrolyte, which improved the kinetics of NADH regeneration\cite{[7],[8],[9]}. The active reduced form $M_{\text{red2}}$ that enables NADH to be generated is made through a typical electrochemical/chemical (EC) process (Scheme 1).

$M_{\text{ox}}$ is reduced to $M_{\text{red1}}$ by accepting two electrons from the electrodes (E step). Successively, $M_{\text{red1}}$ is chemically converted into $M_{\text{red2}}$ without any change in the total number of electrons, by taking up one proton from solution (C step). NADH is generated from NAD$^+$ with the active form $M_{\text{red2}}$ by accepting one proton plus two electrons from $M_{\text{red2}}$ and returning $M_{\text{red2}}$ to the initial state $M_{\text{ox}}$ (Scheme 2).

The chemical step from $M_{\text{red1}}$ to $M_{\text{red2}}$ (the uptake of a proton into the ligand sphere of $M_{\text{red1}}$) is the rate-determining step of NADH generation, specifically at high rates, even though it was reported to proceed quite fast\cite{[8]}. Figure 1a shows cyclic voltammograms obtained at various scan rates. The intensity of the anodic peak increased with scan rate, whereas the peak was not apparent at scan rates.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.
less than 100 mV s⁻¹. At slow anodic potential sweep, the C step proceeds faster than the E step so that there is no chance for M_{\text{red1}} to donate electrons to the electrode. In fast scans (rate above 500 mV s⁻¹), however, M_{\text{red1}} is electro-chemically oxidized to M_{\text{ox}} before the C step proceeds, as the electron-transfer rate between M_{\text{red1}} and electrodes is controlled by the scan rate and is faster than the rate of the chemical reaction. The anodic peak current \(i_a\) followed the dependency on scan rate of conventional faradaic processes (\(i_a \approx \text{scan rate}^{0.5}\)) only at scan rates higher than 500 mV s⁻¹, which indicates that M_{\text{red1}} was totally converted to M_{\text{ox}} by the E step (Figure 1b). The peak currents at scan rates less than 500 mV s⁻¹ deviated from the extrapolated lines fitting peak currents at the three largest scan rates, because M_{\text{red1}} was partly converted to M_{\text{red2}} by the C step. Therefore, the kinetics of proton uptake in the C step should be enhanced to achieve efficient formation of M_{\text{red2}}, which is active for NADH generation.

Platinum has been extensively used to reduce protons in electrolytes to hydrogen (hydrogen evolution reaction, HER) and also to oxidize hydrogen to protons in fuel cells[10,11]. The main reason that makes the platinum catalyst superior to other alternative metals is that protons are adsorbed onto platinum atoms. The intermediate state Pt-Hads makes the H²/H₂ reaction kinetically more favorable, which results in a decrease of overpotential.

Metal-H_{\text{ads}} species, including Pt-H_{\text{ads}}, were reported to be able to function as a reducing agent for organic molecules, markedly in their nanoparticulate form. Platinum nanoparticles with adsorbed hydrogen atoms (nPt-H_{\text{ads}}) were used to reduce the lucigenin cation to its monocation radical in the intermediate state Pt-Hads makes the H²/H₂ reaction kinetically more favorable, which results in a decrease of overpotential.

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The concentration of NADH decreased by 5 h after the potential was applied increased five times in the presence of 1.2 mm nPt when compared with that in the absence of nPt. The NADH generated with nPt and M proved to work effectively with glutamate dehydrogenase, an oxidoreductase, for the synthesis of l-glutamate from α-ketoglutarate in preliminary experiments.

To confirm the nanoparticulate contribution of nPt to the generation of NADH, various flat and bulky disk electrodes including platinum were tested as the working electrode for NADH generation in the presence of M. A very small amount of NADH was generated on these bulky disk electrodes (Figure 4a). For comparison, the amount of NADH generated on glassy carbon (GC) disk electrodes in the presence of Mox was not stirred). For electrodes and buffer solutions see Figure 2.

The platinum disk electrode was even less efficient for NADH generation than GC and gold disk electrodes. On the other hand, the addition of nPt to the solution including M and NAD⁺ resulted in an increase of the amount of NADH generated on the platinum disk electrode (Figure 4b). Therefore, the catalytic (proton donation) and reducing (electron donation) power of nPt can be said to originate from its nanoparticulate feature. The possibility that the increase in the amount of generated NADH is mainly a result of the increase in surface area through adsorption of nPt on the working electrodes should be rejected, because there were no significant differences in the amounts of NADH when using bare and nPt-adsorbed GC electrodes.

To confirm the roles of nPt in the proposed mechanism, prereduced solutions were used to generate NADH without applied potential (Figure 5). If Mox in solution were totally reduced to Mred, 0.5 mm NADH would be generated after addition of NAD⁺ to the prereduced solution. However, the amount of NADH generated in the solution of M was estimated as a very dilute concentration of less than 0.01 mm. nPt directly reduced NAD⁺ to NADH even if a very small amount of NADH was generated. There were no significant differences of the NADH concentration between solutions including only M and only nPt. On the other hand, a synergistic effect was observed in the mixture of nPt and M. The amount of NADH generated increased about 20 times (0.125 mm NADH), thus indicating that nPt enhanced the formation of Mred.

In conclusion, nPt was used as a homogeneous catalyst and simultaneously as a secondary mediator for NADH regeneration in the presence of the primary mediator M. It enhanced the rate of NADH generation by donating protons and electrons to M. We expect that the use of nPt could be extended to the reduction of other chemicals, even in proton-deficient environments (high pH).

**Experimental Section**

The rhodium complex M was synthesized by the method of Kolle and Gratzel. The nPt was prepared by citrate reduction of potassium tetrachloroplatinate. An aqueous solution of sodium citrate (30 mL, 680 mm) was added to a boiling aqueous mixture of K₂PtCl₄ (120 mL, 11.5 mm) and polyvinylpyrrolidone (3 g, molecular weight 10k) for 4 h.
To sweep the potential in cyclic voltammograms or to apply constant potential for generating NADH, a single-compartment cell was configured with three electrodes: a GC disk (working, 0.03 cm²), a platinum wire (counter), and an Ag/AgCl (reference, 0.197 V versus normal hydrogen electrode) connected to a potentiostat/galvanostat (EG&G, Model 273A). All potentials are reported versus Ag/AgCl. The concentration of NADH was estimated from the difference of the absorbance at 340 nm before and after adding NAD⁺.

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