Available online at www.sciencedirect.com

SCIENCE DIRECT

Bioelectrochemistry 65 (2004) 1–7

Bioelectrochemistry

www.elsevier.com/locate/bioelechem

Indirect electrochemical reduction of nicotinamide coenzymes

K. Vuorilehto*, S. Lütz, C. Wandrey

Institute of Biotechnology 2, Research Centre of Jülich, D-52425 Jülich, Germany

Received 22 March 2004; received in revised form 10 May 2004; accepted 17 May 2004
Available online 27 July 2004

Abstract

Nicotinamide coenzymes nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$) were electrochemically reduced to NADH and NADPH, respectively. As direct reduction of nicotinamide coenzymes leads to inactive by-products, an indirect method using (pentamethylcyclopentadienyl-2,2′ -bipyridine aqua) rhodium (III) as the mediator, was applied. A phosphate buffer solution, pH 8, with 1–10 mM $NAD(P)^+$ and 2.5–200 μM mediator, was pumped through a glassy carbon packed bed cathode. Virtually all the $NAD(P)^+$ was reduced to NAD(P)H in the cell. No sign of mediator loss due to side-reactions was detected though the mediator molecules shuttled hundreds of times between the oxidised and the reduced form. Adsorption of mediator molecules on the surface of the carbon cathode was found to be important for the reduction process. Due to strong adsorption, only minute amounts of mediator were consumed.

Keywords: Coenzyme reduction; Indirect reduction; Mediator; NAD; NADP; Rhodium

1. Introduction

The nicotinamide coenzymes, nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$) are ubiquitous in all living systems as they are required for the reactions of more than 400 oxidoreductase enzymes [1]. These coenzymes serve as acceptors and donors of hydrogen in enzyme catalysed reactions. In addition to their biosynthetic role, they play a key part in bioenergetics, transporting electrons (in the form of hydrogen) in the mitochondria. The structures of the coenzymes are presented in Fig. 1.

Nicotinamide coenzymes are widely applied in bioanalytical chemistry, but due to their high cost they have found only limited use in preparative organic synthesis. NAD^+ is prepared by isolation from yeast, $NADP^+$ by phosphorylation of NAD^+. The reduced coenzymes NADH and NADPH are produced by reduction of the corresponding oxidised coenzymes. For this reduction, there are chemical [2], enzymatic [2,3] and microbial [2,4] methods, all of which are complicated.

Electrochemistry could, at least theoretically, provide a simple and inexpensive means of reducing nicotinamide coenzymes. Ideally, an oxidised coenzyme $NAD(P)^+$ would need two electrons from the cathode and a hydrogen ion from the solution in order to be reduced to NAD(P)H. As the equilibrium potential of both coenzymes is −315 mV vs. SHE at pH 7 (pH dependence −30 mV/pH unit) [1], a permissible potential range of approximately 100 mV occurs between reduction of the coenzymes and evolution of hydrogen at pH 7–8.

However, according to the literature [1], the direct reduction of $NAD(P)^+$ leads to 1 e^- charge transfer and formation of a radical NAD(P)•, followed by dimerization to an enzymatically inactive 4,4′ dimer. To overcome this problem, various mediators have been suggested for the transport of the charge between the cathode and the coenzyme [1,5,6].

In the present study, NAD^+ and $NADP^+$ were reduced indirectly to NADH and NADPH using (pentamethylcyclopentadienyl-2,2′ -bipyridine aqua) rhodium (III) as the mediator. The aim was to produce these valuable reduced coenzymes in a simple electrochemical flow reactor without enzymes or other biological components.

2. Experimental details

2.1. Method of indirect reduction

The principle of indirect nicotinamide reduction is shown in Fig. 2. The oxidised mediator, (pentamethylcyclopenta-

* Corresponding author. Helsinki Bioenergetics Group, Institute of Biotechnology, P.O. Box 65, FIN-00014 University of Helsinki, Finland. Tel.: +358-40-7501797; fax: +358-9-19159920.
E-mail address: kai.vuorilehto@helsinki.fi (K. Vuorilehto).

1567-5394/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bioelechem.2004.05.006

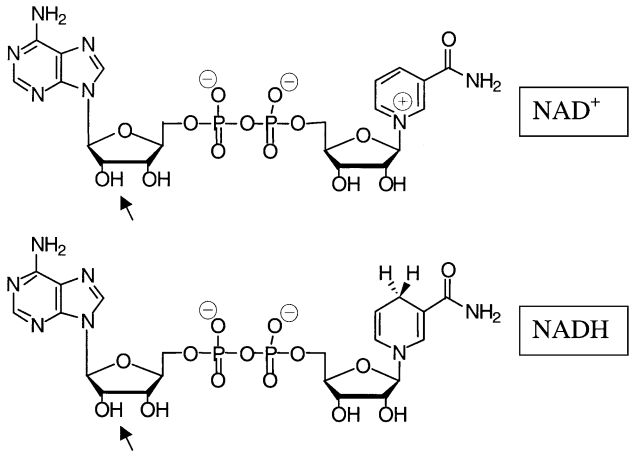

Fig. 1. NAD^+ (nicotinamide adenine dinucleotide) and its reduced form NADH. In $NADP^+$ and NADPH the hydrogen atoms indicated by arrows are replaced by a $PO(OH)_2$ group.

dienyl-2,2′ -bipyridine aqua) rhodium (III), is reduced to (pentamethylcyclopentadienyl-2,2′ -bipyridine hydrogen) rhodium (I) on the cathode surface [6]. This reduced rhodium mediator then reduces the nicotinamide $NAD(P)^+$ to NAD(P)H and is re-oxidised to its original form. The mediator can then be reduced on the cathode surface again. One mediator molecule can perform this loop repetitively until it is flushed out of the cell or is destroyed in a side-reaction. The mediator was not specifically bound to the cathode surface, but was dissolved in the phosphate buffer together with the nicotinamide coenzyme. However, part of the mediator was adsorbed on the carbon particles during the reduction process.

2.2. Chemicals

NAD^+ and $NADP^+$ were purchased from Jülich Fine Chemicals (Germany) and had a chemical purity of >98%. NADH for reference purposes was purchased from the same company. NAD^+ was in the free acid form, while NADH and $NADP^+$ were sodium salts. The rhodium mediator, (pentamethylcyclopentadienyl-2,2′ -bipyridine aqua) rhodium (III), was synthesized according to methods from the literature [7] by Jülich Fine Chemicals (Germany).

A 100 mM sodium phosphate buffer, pH 8, was prepared by dissolving 94.5 mM Na_2HPO_4 and 5.5 mM NaH_2PO_4

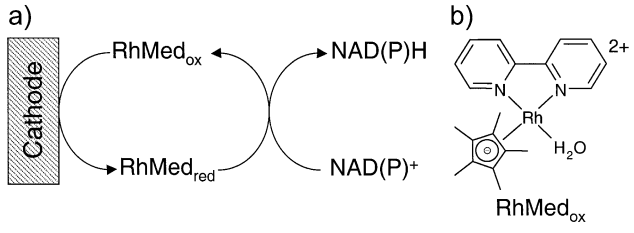

Fig. 2. (a) Principle of the indirect reduction of nicotinamide coenzymes. (b) The rhodium mediator.

Table 1
Buffers tested in preliminary experiments

Buffer	Concentration/ mol dm^{-3}	pH/mS cm^{-1}	Conductivity	NADH deactivation rate/h^{-1} (%)
K-phosphate	0.1	7	14.0	2.0
Na-phosphate	0.1	8	12.8	0.25
Tris + HCl	0.1	8	5.1	0.06
Na-borate	0.1	8	1.9	0.25

(both pro analysi, Merck, Germany) in deionized Milli-Q® water. The choice of this buffer was a compromise between electrochemical demands, stability of the coenzymes and ease of downstream processing (Table 1). 100 mM sodium phosphate has reasonable electrochemical conductivity and pH 8 provides optimum stability for the coenzymes [2]. As NAD(P)H is mostly used as sodium salt, sodium is a suitable cation for the buffer solution. The stability of the nicotinamide coenzymes would be better in some organic buffers, e.g. Tris [2], but these buffers have rather low conductivities.

2.3. Laboratory-scale flow cell

For the electrochemical reduction of the rhodium mediator and the subsequent chemical reduction of the nicotinamide coenzyme, a two-compartment cell was constructed of acrylic plastic (Fig. 3). The cell consisted of a packed bed cathode, an anode net and an ion exchange membrane between the electrodes. The buffer solution flowed vertically downwards through the cathode and the current flowed horizontally.

The cathode was a 10 cm^3 three-dimensional packed bed electrode, consisting of Sigradur® G glassy carbon spheres (HTW, Germany). Its dimensions were 10 cm (height) × 1 cm (width) × 1 cm (depth). Two particle diameters were tested: 80–200 μm and 200–400 μm. Current was fed to the cathode bed through a stainless steel sheet covered by carbon foil. Glassy carbon and carbon foil were chosen as materials to prevent production of hydrogen; at carbon

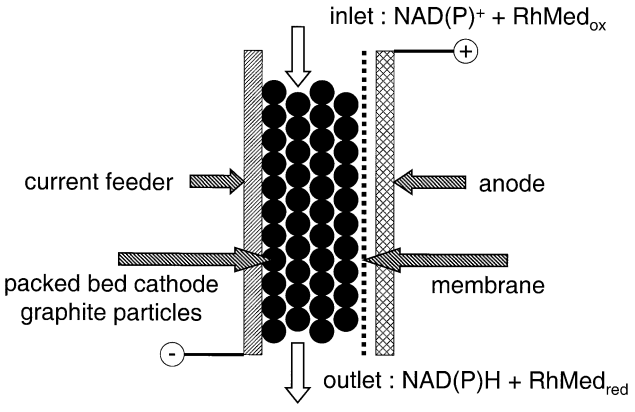

Fig. 3. Cross-section of the electrochemical cell.

surfaces hardly any hydrogen evolution is detected at potentials positive to −600 mV vs. SHE at pH 8.

The anode was a 10 cm^2 platinized titanium net. It was placed in a separate anode compartment in contact with the membrane. Oxygen gas, evolved at the anode, was released into the atmosphere from the top of the anode compartment. A 400 mM sodium phosphate buffer was used as the anolyte to ensure good conductivity.

A cation exchange membrane IONAC® MC-3470 was placed between the electrodes to prevent re-oxidation of the products. Sodium ions carried the charge through the membrane.

A saturated Ag/AgCl electrode (Kurt-Schwabe-Institut, Germany) was used as reference electrode. It was connected to the three-dimensional cathode by a plastic tube. However, all potentials are given here in the standard hydrogen electrode scale (SHE).

2.4. Experimental procedure

The experimental set-up, including auxiliaries, is shown in Fig. 4. The experiments were carried out at room temperature, 20–25 °C.

The buffer solution, with $NAD(P)^+$ and mediator, was bubbled with argon in a glass bottle to remove dissolved oxygen. Thorough removal of oxygen was important, as it could be reduced to hydrogen peroxide in the cathode bed, and hydrogen peroxide could further oxidize and deactivate the coenzymes. From the glass bottle the solution was pumped to the cell using a Masterflex® (Cole-Parmer, USA) tubing pump and Tygon® tubing. Pump speed was set to 2.1 cm^3/min in all experiments. The solution flowing out of the cell was analysed for $NAD(P)^+$ and NAD(P)H. To prevent re-oxidation by air, samples were taken by pipetting directly from the outlet tube. Cathode potential and cell current were controlled by a BAS100B potentiostat (Bioanalytical Systems, USA) and Fluke® (USA) multimeters.

2.5. Measurement of coenzyme concentration

$NAD(P)^+$ and NAD(P)H concentrations were measured enzymatically. This ensured that the result was the enzymatically active coenzyme, and not one of its enzymatically non-active isomers.

The method was based on the fact that NAD(P)H strongly adsorbs 340 nm light with an extinction coefficient of 6220 $M^{-1}\,cm^{-1}$, but $NAD(P)^+$ does not adsorb at this wavelength. When an alcohol dehydrogenase enzyme and a suitable substrate (acetaldehyde for NADH or acetophenone for NADPH) are added to a NAD(P)H solution, NAD(P)H is oxidised to $NAD(P)^+$ and the absorbance is decreased. If a formate dehydrogenase enzyme and a suitable substrate (sodium formate) are added to a $NAD(P)^+$ solution, $NAD(P)^+$ is reduced to NAD(P)H and the absorbance is increased [8].

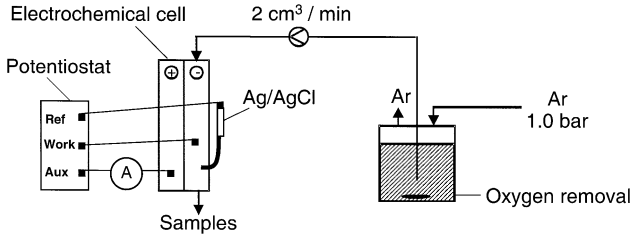

Fig. 4. The experimental set-up.

Most of the $NAD(P)^+$ solutions were prepared by weighing to have a concentration of 1 mM, but the analysis result was only about 0.95 mM. The reason for this is the hygroscopic nature of the coenzymes. Commercial nicotinamide coenzymes include some crystal water, the amount of which is not exactly known. In addition, their water content tends to increase during handling and storage. For the same reason, the NAD(P)H concentrations were often about 0.95 mM when a "1 mM" $NAD(P)^+$ solution had been completely reduced.

3. Results and discussion

3.1. Suitable potential range

The equilibrium potential of $NAD(P)^+$/NAD(P)H at pH 8 is −345 mV vs. SHE [1]. The equilibrium potential of $Rhmed_{ox}/Rhmed_{red}$, investigated by measuring the redox potential of a buffer solution containing 0.5 mM $Rhmed_{ox}$ and 0.5 mM $Rhmed_{red}$, was found to be -430 ± 10 mV vs. SHE. Thus, it can be expected that a suitable potential for indirect coenzyme reduction is close to these values.

To find the optimum potential range for indirect reduction, a series of linear potential sweeps from −300 to −600 mV vs. SHE was measured using the laboratory scale flow cell. As seen in Fig. 5a, the reduction current is constant at potentials negative to −500 mV but decreases fast at potentials positive to −500 mV. Therefore, the potential should be −500 mV or more negative.

However, it is known that at very negative potentials NAD^+ can be directly reduced, and the reduction product is not the enzymatically active NADH [1]. To avoid the risk of this direct reduction, a test was carried out with 1 mM NAD^+ without mediator (Fig. 5b). As can be seen, direct reduction takes place at potentials more negative than −550 mV. Thus, the optimum potential range for indirect $NAD(P)^+$ reduction is approximately −500–550 mV vs. SHE. To thoroughly investigate the influence of the cathode potential on indirect NAD^+ reduction, the outlet concentrations of NAD^+ and NADH were measured on various cathode potentials. The results are shown in Fig. 6.

At −350 mV no reduction of NAD^+ was detected though the equilibrium potential of NAD^+/NADH (pH 8) is −345 mV. Even at −400 mV only 10% was reduced. Finally, at −500 mV, virtually all NAD^+ was reduced to NADH. The reason for the high "overpotential" lies in the mediator. Its rather negative equilibrium potential of −430 mV prevents efficient indirect reduction at potentials

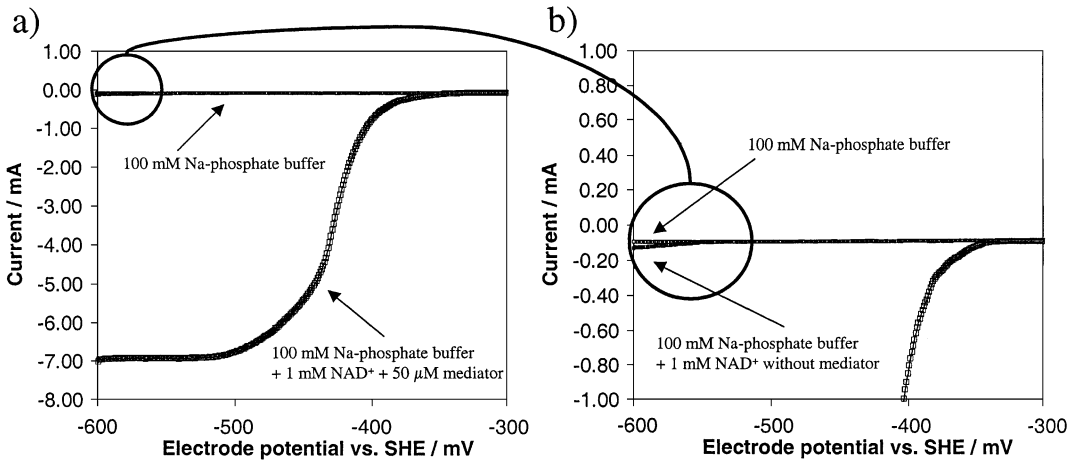

Fig. 5. (a) Potential sweep 0.1 mV/s from −300 to −600 mV vs. SHE. Particle diameter 200–400 μm. (b) Magnification showing direct reduction.

positive to −430 mV. A potential of −500 mV is needed to reduce all mediator molecules that reach the electrode surface and by this means to achieve an effective indirect reduction of the coenzyme. On the other hand, the 85 mV difference in equilibrium potentials ensures that the reduced mediator reduces NAD^+ almost irreversibly. The opposite reaction has a negligible rate.

It should be pointed out that the potential of a three-dimensional cathode, measured against the reference electrode, is a somewhat diffuse concept. Due to IR drop in the electrolyte, the real local cathode potential differed ±50 mV from the measured one. It was most negative near the membrane (ionic pathway = 0 cm) and most positive near the current feeder (ionic pathway = 1 cm). The membrane area, cathode thickness, buffer conductivity and $NAD(P)^+$ concentration were chosen so that the IR drop should not exceed 50 mV in the experiments.

3.2. Mediator consumption

The viability of this indirect reduction method depends considerably on the mediator consumption. If the mediator cannot be recycled, one mediator molecule should be able to reduce as many NAD^+ molecules as possible (have a maximum total turnover number, TTN) before it leaves the cell or is destroyed. Therefore, an experiment with various mediator concentrations was carried out (Fig. 7).

The "NADH curves" of Fig. 7 all have the same shape as that in Fig. 6, but the maximum level of NADH was strongly dependent on mediator concentration. It is obvious that a mediator concentration of 10 μM (TTN = 100) was too low as only 60% of NAD^+ was reduced. 50 μM (TTN = 20) worked well, and even 25 μM (TTN = 40) was satisfactory, but only if the cathode potential was set to −550 mV. The maximum TTN achievable with this set-up thus seemed to be near 40.

It may be assumed that a cell with more electrode surface area would reduce the mediator faster and thus require less mediator. To test the influence of the surface area, the cell was filled with smaller glassy carbon particles having a diameter of 80–200 μm. Different mediator concentrations were tested keeping the electrode potential constant at −500 mV. The results are shown in Fig. 8.

Using these smaller particles, 2.5 μM of rhodium mediator was able to reduce 1 mM of NAD^+. This TTN value of 400 was clearly better than the 40 achieved with the 200–400 μm particles.

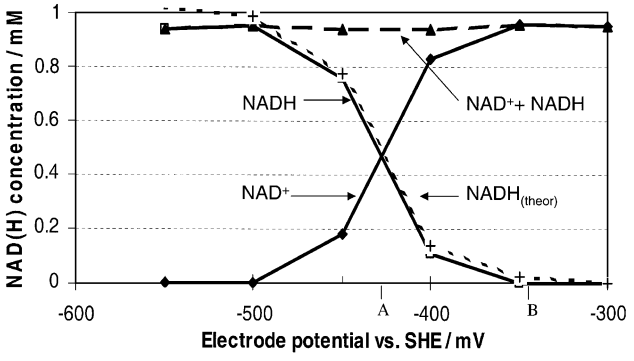

Fig. 6. Influence of cathode potential on the reduction of 1 mM NAD^+ using 50 μM mediator. Particle diameter 200–400 μm. NADH(theor) is the calculated concentration of NADH that should be found if all the current was used to reduce NAD^+. A = equilibrium potential of the mediator (−430 mV), B = equilibrium potential of NAD^+/NADH (−345 mV).

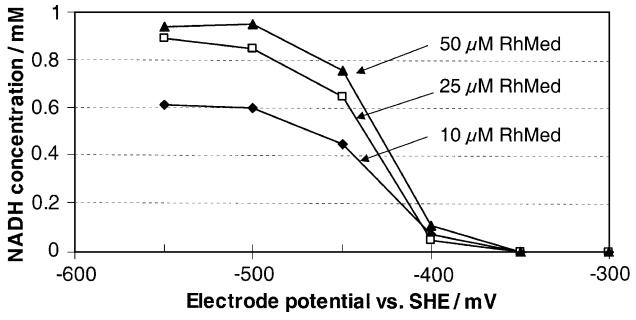

Fig. 7. Influence of mediator concentration on the reduction of 1 mM NAD^+. Particle diameter 200–400 μm.

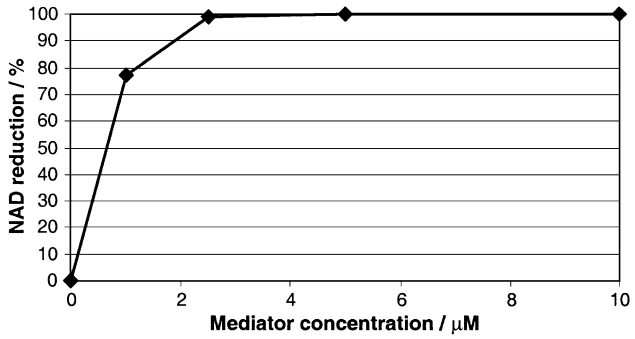

Fig. 8. Influence of mediator concentration on the reduction of 1 mM NAD^+. Particle diameter 80–200 μm, cathode potential −500 mV vs. SHE.

A TTN value of 400 is high enough to make recycling of the mediator superfluous. However, the product includes 0.25 mol% mediator if it is not removed by a downstream process. In an aqueous solution in an open vessel, this trace amount of mediator is oxidised by air, and then further slowly oxidises the reduced coenzyme NADH. During prolonged storage (hours to days), a few percent of the NADH are converted to NAD^+ and other oxidation products. For comparison, sodium phosphate buffer, pH 8, without any mediator, deactivates NADH at a rate of 0.25%/h, which is comparable to the loss caused by the trace mediator. If the TTN value could be increased, the detrimental oxidation reactions would become correspondingly slower. When using 80–200 μm particles, the hydrodynamic pressure drop in the cell was only 0.035 bar, which indicates that it is possible to use even smaller particles to further increase the TTN values.

3.3. Conversion, purity and current efficiency

As can be seen in Fig. 6, the total concentration of coenzymes was constant, which means that NAD^+ was really oxidised to NADH, not to other products. At −500 mV, 99.5 ± 0.5% of the NAD^+ was reduced, 99 ± 1% of which was detected as NADH. The uncertainty arises from the analysis method.

When the cathode potential was positive to −500 mV, the conversion decreased. At potentials −500–600 mV the conversion stayed at 99.5 ± 0.5%. At potentials negative to −550 mV direct reduction of NAD was possible, at least theoretically, but could not be detected due to the minute concentrations.

The current efficiency was close to 100%. Part of the current was consumed by the mediator that was in the oxidised form at the inlet and in the reduced form at the outlet. In the case of 50 μM mediator and 1 mM NAD^+ this effect was 5%. When 2.5 μM mediator was used, the effect was only 0.25%. Additionally, small amounts of oxygen were able to leak into the system. Reduction of this oxygen at the cathode consumed 1–2% of the cell current. Leaking oxygen could cause production of hydrogen peroxide at the cathode and consequently deactivation of the coenzymes. However, moderate changes in the oxygen concentrations were not found to influence the conversion or the purity of the product. Possibly, oxygen was reduced directly to water, or hydrogen peroxide was reduced further to water before it reacted with the coenzymes.

3.4. Reduction of $NADP^+$ to NADPH

The additional phosphate group of $NADP^+$ and NADPH is situated far from the electrochemically active part of the nicotinamide coenzyme (Fig. 1). Thus, it can be expected that $NADP^+$ could be reduced using the same method that was used for NAD^+. To test this hypothesis, 1 mM $NADP^+$ solution was reduced using the laboratory-scale flow cell. The results are shown in Fig. 9.

The reduction behaviour of $NADP^+$ was very similar to that of NAD^+, shown in Fig. 6. A potential of −550 mV vs. SHE was needed to reduce all $NADP^+$, but this phenomenon was probably caused by the IR drop and potential variation, not by the additional phosphate group of $NADP^+$.

When smaller glassy carbon particles with a diameter of 80–200 μm were used, a TTN value of 200 was achieved at the potential of −500 mV. This TTN value is slightly less than the 400 achieved for NAD^+. The difference may be caused by the additional phosphate group slowing down the reaction rate. However, the difference lies within the limits of experimental error.

Conversion, purity and current efficiency were similar to those achieved for reduction of NAD^+. The phosphorylated coenzymes $NADP^+$ and NADPH are known to decompose faster than NAD^+ and NADH [2], but this effect did not influence the results due to the short residence time of the solutions in the cell.

3.5. Scale-up

Using the laboratory scale flow cell, 1 mM $NAD(P)^+$ solution was reduced with a throughput of 2.1 cm^3/min. A space–time yield (STY) as high as 500 g(product) dm^{-3} day^{-1} was reached. However, as the solution volume inside

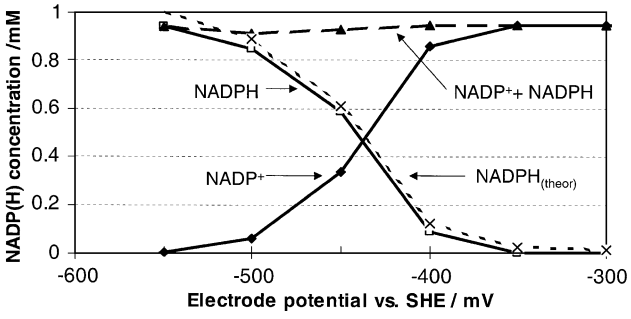

Fig. 9. Reduction of 1 mM $NADP^+$ using 50 μM rhodium mediator. Particle diameter 200–400 μm. NADPH(theor) is the calculated concentration of NADPH that should be found if all current was used to reduce $NADP^+$.

the cell was only 4 cm^3, the effective production rate was only about 2 g day^{-1}.

One simple way to increase the production rate may be to increase the concentrations. To find out if this was possible, the NAD^+ concentration was increased to 2 mM and the mediator concentration was increased to 10 μM (TTN = 200). The test failed: only 90% of the NAD^+ was reduced. The conductivity of the solution was not high enough for the double current density, and the amount of mediator was not sufficient for the double reaction velocity. When mediator concentration was increased to 20 μM (TTN = 100) and the conductivity was increased to 19.6 mS cm^{-1} by adding 50 mM Na_2SO_4, it was possible to reduce 98% of the coenzyme.

Thus, using the higher concentrations, a production rate of 4 g day^{-1} and a space–time yield of 1000 g dm^{-3} day^{-1} were achieved, but at the cost of higher mediator consumption, lower conversion and addition of salt.

A straightforward way to increase the production rate would be the use of a bigger cell that could support the higher current needed for the higher concentration. For this purpose, an existing cell, designed and optimised for reduction of dissolved oxygen [9], was applied. Its working principle was the same as that of the laboratory scale cell, but it was much wider: the cathode dimensions were 8 cm (height) × 9 cm (width) × 1 cm (depth), the volume of the cathode being 72 cm^3.

After filling the cell with 200–400 μm carbon particles, it was tested for a solution of 10 mM NAD^+, keeping the electrode potential constant at −500 mV and the pump speed at 2.1 cm^3/min. The results are shown in Fig. 10. Using this scale-up cell, 100 μm of rhodium mediator was able to reduce 10 mM of NAD^+. This TTN value of 100 is better than the 40 achieved in the laboratory-scale cell, using the same particle size.

The scale-up cell was not optimal for this purpose. This became evident when it was tested with the 80–200 μm carbon particles. Complete reduction of NAD^+ was not possible, probably due to bad contact between the particles and the channelling of the flow. By increasing the width of the cell from 1 to 9 cm without increasing the pump speed, the linear flow velocity of the solution was decreased

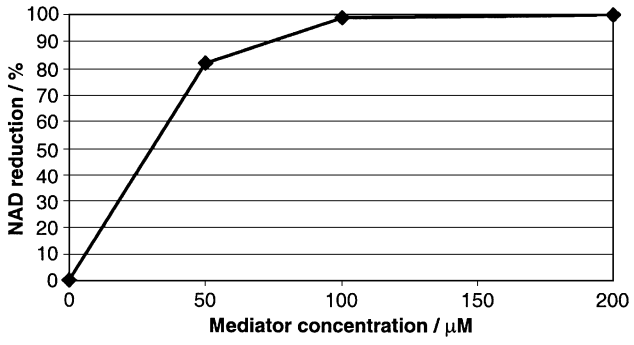

Fig. 10. Reduction of 10 mM NAD^+ in the scale-up cell using various mediator concentrations. Particle diameter 200–400 μm.

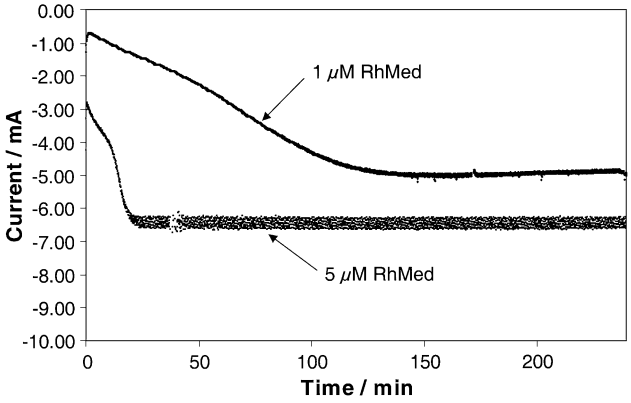

Fig. 11. Adsorption at the beginning of the reduction process. Reduction of 1 mM NAD^+, potential −500 mV vs. SHE, particle diameter 80–200 μm.

drastically. The slow flow was not able to press the very small particles together properly. Instead of scaling-up in width, the cell should be scaled up in height. (Scaling-up in depth is not possible as it would increase the IR drop by making the ionic pathway longer).

In spite of the less optimal design of the cell, the scale-up experiment showed that indirect reduction of the nicotinamide coenzymes is not limited to laboratory scale. An effective production rate of 20 g day^{-1} in a flow-through-once system is a good basis for further scale-up.

3.6. Significance of adsorption

When particle size was decreased from 200–400 to 80–200 μm, the total geometric surface area of the cathode increased from 1200 to 2600 cm^2. It could be assumed that this increase in surface area would roughly double the reduction rate of the mediator and decrease the required mediator concentration by about 50%. In practice, however, the required mediator concentration decreased by 90%.

This obvious contradiction can be solved by taking into account the adsorption of the mediator on the carbon surface. Double surface area adsorbs a double amount of mediator, if other parameters are kept constant. On the other hand, decreasing mediator concentration by 90% does not decrease the absorption by 90% if the carbon surface is nearly saturated with mediator molecules. If it is assumed that adsorption is decreased by 50% due to lower concentration, this would be compensated by the double surface area. In this case, the total amount of adsorbed mediator would be the same if 25 μM mediator was used on 200–400 μm particles or 2.5 μM mediator was used on 80–200 μm particles.

To test the adsorption hypothesis, the starting period of the reduction process was investigated in more detail. The result is shown in Fig. 11.

It can be seen that the cell did not work properly at the beginning. A current of 6.5 mA is needed to reduce 1 mM NAD^+ at a rate of 2.1 cm^3/min. When using 5 μM mediator, the current was only about 3 mA at start, but it increased

continuously. Finally after about 20 min, enough mediator had been adsorbed to reduce all NAD^+. At this point, less than 0.1 μM rhodium was found in the outlet solution. In other words, for a period of at least 20 min all the mediator pumped into the cathode stayed there, though the residence time of the solution was less than 2 min. After 240 min, the outlet concentration was found to be equal to the inlet concentration of 5 μM.

When using 1 μM mediator, the initial current was lower and the increase was slower due to the lower amount of mediator that was pumped into the cell. For a period of 2 h, the current increased continuously, but finally stayed at a level of 5 mA. At that point, the desorption rate of the mediator equalled the adsorption rate. Less than 80% of NAD^+ was reduced.

Separate adsorption experiments showed that the reduced mediator is adsorbed more strongly than the oxidised mediator. The reduced mediator is probably oxidised by $NAD(P)^+$ on the cathode surface, and is then again reduced by the cathode before it can escape.

For high TTN values, strong adsorption of the rhodium mediator is indispensable. The stronger the adsorption, the lower the required mediator concentration. At steady state, the same mediator concentration is flushed out that is pumped into the cell. No sign of mediator loss due to side reactions was detected in the experiments, but mediator loss seemed to be entirely caused by flushing out of the cell.

4. Conclusions

The indirect reduction of nicotinamide coenzymes in a three-dimensional cathode is a convenient tool for the production of NADH and NADPH. The conversion and the selectivity, both of virtually 100%, show the potential of this method. The space–time yield (STY) of 500–1000 g dm^{-3} day^{-1}, calculated for the solution volume in the cathode, is exceptionally high for this kind of valuable product (Table 2).

The mediator, (pentamethylcyclopentadienyl-2,2′ -bipyridine aqua) rhodium (III), is not commercially available but has to be synthesized. This fact together with the high value of rhodium makes it important to obtain high TTN values for the mediator. The molar mass of the mediator, 412 g/mol, is so near to that of the NAD(P)H, about 700 g/mol, that separation by ultrafiltration is scarcely possible. This complicates the recycling of the mediator. However, the TTN values up to 400, achieved in a single pass through the electrochemical cell, are so high that recycling is not obligatory.

Table 2
Summary of the productivity results for $NAD(P)^+$ reduction

Cell	Particle size/μm	TTN	Production rate/g day^{-1}	STY/g dm^{-3} day^{-1}
Lab. scale	200–400	40	2	500
Lab. scale	80–200	400	2	500
Lab. scale + Na_2SO_4	80–200	100	4	1000
Scale-up	200–400	100	20	700

Downstream processing of the product solution could include ion-exchange to remove the mediator, nanofiltration to remove the buffer and finally freeze-drying of the reduced nicotinamide coenzyme.

Acknowledgements

The authors wish to thank Dr. Dieter Sell and Prof. Klaus Jüttner (Dechema, Frankfurt) for kindly providing electrochemical cells and electrodes, and Umicore (Hanau) for supplying rhodium chloride.

References

[1] L. Gorton, E. Domiinguez, Electrochemistry of $NAD(P)^+$/NAD(P)H, in: A.J. Bard, M. Stratmann (Eds.), Encyclopedia of Electrochemistry, vol. 9, Wiley-VCH, Weinheim, 2002, pp. 69–143.
[2] H.K. Chenault, G.M. Whitesides, Regeneration of nicotinamide cofactors for use in organic synthesis, Appl. Biochem. Biotechnol. 14 (1987) 147–197.
[3] R. Mertens, L. Greiner, E.C.D van den Ban, H.B.C.M. Haaker, A. Liese, Practical applications of hydrogenase I from *Pyrococcus furiosus* for NADPH generation and regeneration, J. Mol. Catal., B 24–25 (2003) 39–52.
[4] P.K. Nath, Y. Izumi, H. Yamada, NADH production from NAD+ with a formate dehydrogenase system involving immobilized cells of a methylotrophic *Arthrobacter* strain, Enzyme Microb. Technol. 12 (1990) 28–32.
[5] R. Ruppert, S. Herrmann, E. Steckhan, Efficient indirect electrochemical in-situ regeneration of NADH: electrochemically driven enzymatic reduction of pyruvate catalyzed by D-LDH, Tetrahedron Lett. 28 (1987) 6583–6586.
[6] F. Hollmann, B. Witholt, A. Schmid, $[Cp^*Rh(bpy)(H_2O)]^{2+}$: a versatile tool for efficient and non-enzymatic regeneration of nicotinamide and flavin coenzymes, J. Mol. Catal., B. 19–20 (2003) 167–176.
[7] U. Kölle, B.-S. Kang, P. Infelta, P. Comte, M. Grätzel, Elektrochemische und pulsradiolytische Reduktion von (Pentamethyl–cyclopentadienyl)(polypyridyl)rhodium-Komplexen, Chem. Ber. 122 (1989) 1869.
[8] M. Villela, Enantioselective reduction of hydrophobic keto compounds in multiphase bioreactor, PhD thesis, University of Bonn, 2003.
[9] K. Vuorilehto, A. Tamminen, S. Yläsaari, Electrochemical removal of dissolved oxygen from water, J. Appl. Electrochem. 25 (1995) 973–977.