Low-Cost Tracking of A Nanoscopically Compartmentalized Photocatalytic System - an Experiment for High School Students

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Due to their sustainability potential and versatility, photochemical reactions are becoming more and more important in the context of research, chemical production and also teaching at schools. In the research project CataLight new methods are investigated to increase the efficiency of photochemical reactions. One possibility is to ensure the spatial proximity of the reactants in order to increase the efficiency. To demonstrate this relationship in the presented experiment, the molecules are confined in a sphere consisting of lipids, so called liposomes. The conversion of NADH to NAD^+ within this sphere by the ruthenium complex acting as a photosensitizer can be followed by spectroscopic methods. First, simplifications of the experiment are addressed using the concept of didactic reconstruction. Second, it is shown that by slight adaptations to the photometer of the low-cost measuring station LabPi an adequate tracking of the degradation of NADH can be ensured, enabling future experiments in schools.

Keywords: *photocatalysis, efficiency of reactions, spectroscopy, liposome-nanoreactors*

1. Introduction

Even if we do not always perceive them directly, we cannot imagine our everyday life and environment without light-driven chemical reactions. The most prominent example from nature is photosynthesis, which forms the basis for the life of many species. However, in everyday life, this kind of reaction will have a greater influence over time. In Germany, the conversion of some industrial processes from gas to hydrogen must be mentioned. Classically, it can be obtained via electrolysis and photochemical processes. For example, the photocatalytic process of water splitting into oxygen and hydrogen using titanium dioxide under UV light was described by Fujishima and Honda already in 1972 [1]. Despite this long awareness and the increasing relevance for students' everyday lives, a transfer to German schools has not yet taken place on a larger scale. The first approach already teaches the basics of photochemistry and simplified processes for the generation of hydrogen [2]. In the here reported study, another photochemical system involving nanoscope photoreactors is presented, which is based on nature's models in order to significantly increase the efficiency of photochemical processes. Such nanoreactors have been investigated within the CataLight research network and allow for novel approaches to improve photochemical processes [3]. Such a specialized scientific system is simplified with the concept of didactic reconstruction and adapted for application in a student laboratory [4,5]. In addition to preparative and content reductions, the problem of tracking using suitable measuring instruments is further addressed.

2. Scientific Background

When considering the efficiency of light-driven reactions, it should always be noted that the excited state of the involved photosensitizer and oxygen species (here: $^1O_2$) is very short-lived and, therefore, often turns out to be a limiting factor. This is because the probability of a collision, and thus of a chemical reaction, is significantly reduced in this case. To address this problem, a liposome nanoreactor was formed in a study in which the reactants involved could be confined and thus kept in local proximity [4]. These nanoreactors consist of a lipid bilayer and can be formed from the phospholipid DPPC (Figure 1).

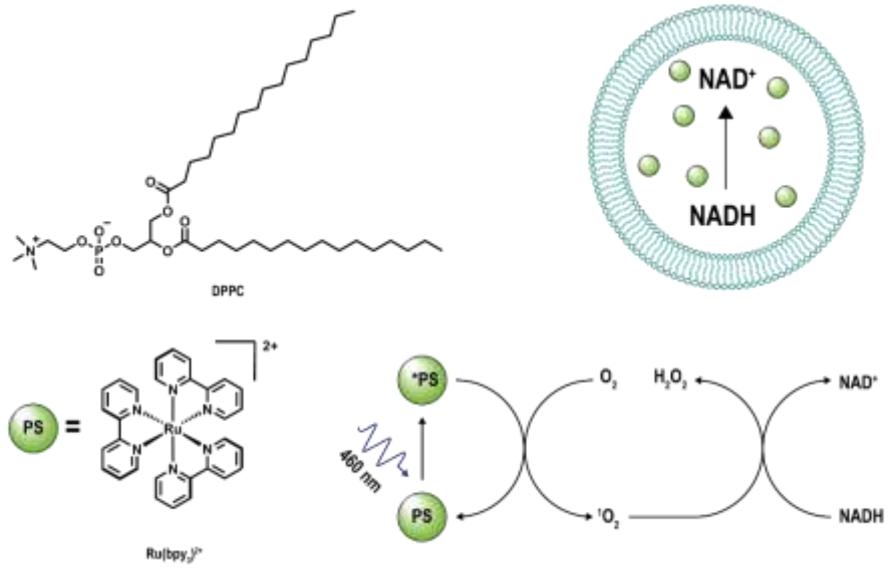

Figure 1: Schematic representation of the photochemical process with a light absorber (bottom) and the integration of this system in a liposome nanoreactor (top right) consisting of DPPC (top left).

This principle was investigated using a model system for photochemical oxidation of NADH to NAD^+. In this process, the ruthenium photosensitizer is excited by blue light and forms reactive singlet oxygen, which is capable of oxidizing NADH. The conversion was monitored photometrically by observing the typical absorbance band for NADH at λ = 340 nm. When using such a system in research, acceleration of the reaction by a factor of 21 compared to a classical aqueous bulk solution can be achieved by locally combining the photosensitizer and NADH in an air saturated sample.

3. Didactic reconstruction of the research system

When looking at the research system, procedural obstacles arise at first glance, which represent hurdles for the transfer to the school, and were therefore approached step by step. In the first step, the focus was on an application in the student laboratory.

Preparation of the lipid solution: To evaporate the solvent, research involves the use of a vacuum pump for three hours. This step was successfully reduced using nitrogen stream for 6 min.

Synthesis of liposome nanoreactors: Instead of three freeze-thaw cycles between liquid nitrogen and a 55 °C water bath, it was shown that reproducible synthesis of liposomes is also possible with stirring and heating in a water bath.

Homogenization of liposomes was carried out by extrusion eleven times through a membrane filter. This point of execution can be replaced with the use of an inexpensive syringe filter.

Spectroscopic tracking: The progress of oxidation was observed using an emission or UVvis spectrometer. The first instrument is not found in any school and the second in only a few schools. Alternatively, an inexpensive LabPi photometer [6] was adapted, and an LED with a wavelength of 340 nm. This simple adaptation of the available instrument allows cost-efficient quantitative tracking of the reaction course.

4. Experimental Setting

Chemicals: Ruthenium complex $(Ru(bpy_3)^{2+}$ (c = 0.05 mM in 10 mM phosphate buffer) NADH (97%), lipid stock solution of DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine, c = 5 mM, Avanti Polar Lipids) and 14: 0 PEG 2000 PE (1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt), Avanti Polar Lipids) in chloroform (GHS06, GHS08) as solvent, compressed nitrogen gas (GHS04), 10 mM phosphate buffer.

Equipment: brown glass vial, small magnetic stirrer, microliter pipette (100-1.000 µL), gas tubing, water bath, heating plate, stand with clamps, ultrasonic bath, UV lamp (λ = 365 nm), size exclusion column, UVvis spectrometer, LabPi photometer, cuvettes (fluorescence UV transparent) blue LED (λ = 460 nm), precision balance (d = 0.0001 g), glass vials (3 mL), precision dosing syringe (1 mL), Luer stop caps, syringe filter (pore size 0.45 µm), safety reaction vessel (1 mL), gloves, photoreactor with blue LED

Experiment: 1 mL of lipid stock solution is poured into a brown glass vial and evaporated under a stream of nitrogen. Meanwhile, 10 mg of NADH is weighed into a glass vial and taken up in 0.468 mL of ruthenium solution along with 32 µL of phosphate buffer. This is then transferred to a brown glass vial and heated while stirring at 55°C for 10 min. The solution is treated in an ultrasonic bath for 20 s, drawn up into the syringe, and a syringe filter with a cap saturated in buffer is placed on. The syringe is again heated for 10 min in a water bath. The liquid is then extruded, and the filtrate is applied to the column. A phosphate buffer is used as the mobile phase for the column. To be able to collect the desired fastest phase, this must be made visible by using a UV lamp. The fraction is collected in small reaction vessels with five drops each, and the most intense phases are combined and made up to 3 mL with buffer. Illumination is performed in a photoreactor with blue LED. After 0, 0.5, 1, 2, 5, 10, 15, 30, 60, and 65 min, the sample is measured using a LabPi photometer and a UVvis spectrometer.

5. Observations and Results

Liposomes are synthesized via lipid film hydration [6]. In this technique, it can be assumed that the concentration in the liposome corresponds to that in the prepared medium. Based on this assumption, it is possible to make statements about the molar ratio between the photosensitizer and the model substrate. The concentration used was approximately 1–600 mmol/L. The first indication of successful imaging of liposomes was observed when they were drawn into the syringe. The liquid appeared slightly milky when liposome nanoreactors were formed. The spectrum shown in Figure 2 can be observed if the isolated fraction is examined using the UVvis spectrometer. The noise that can be seen here can be explained by the liposomes themselves. These interfere with the spectroscopic method due to the Tyndall effect and thus cause a "background noise" to appear in the spectrum. This noise can be removed by creating a difference spectrum. For this purpose, the spectrum after t=65 min was subtracted from all previous spectra. The reference spectrum was selected in such a way that no further change in the spectrum could be observed, and it can therefore be assumed that all NADH has been oxidized.

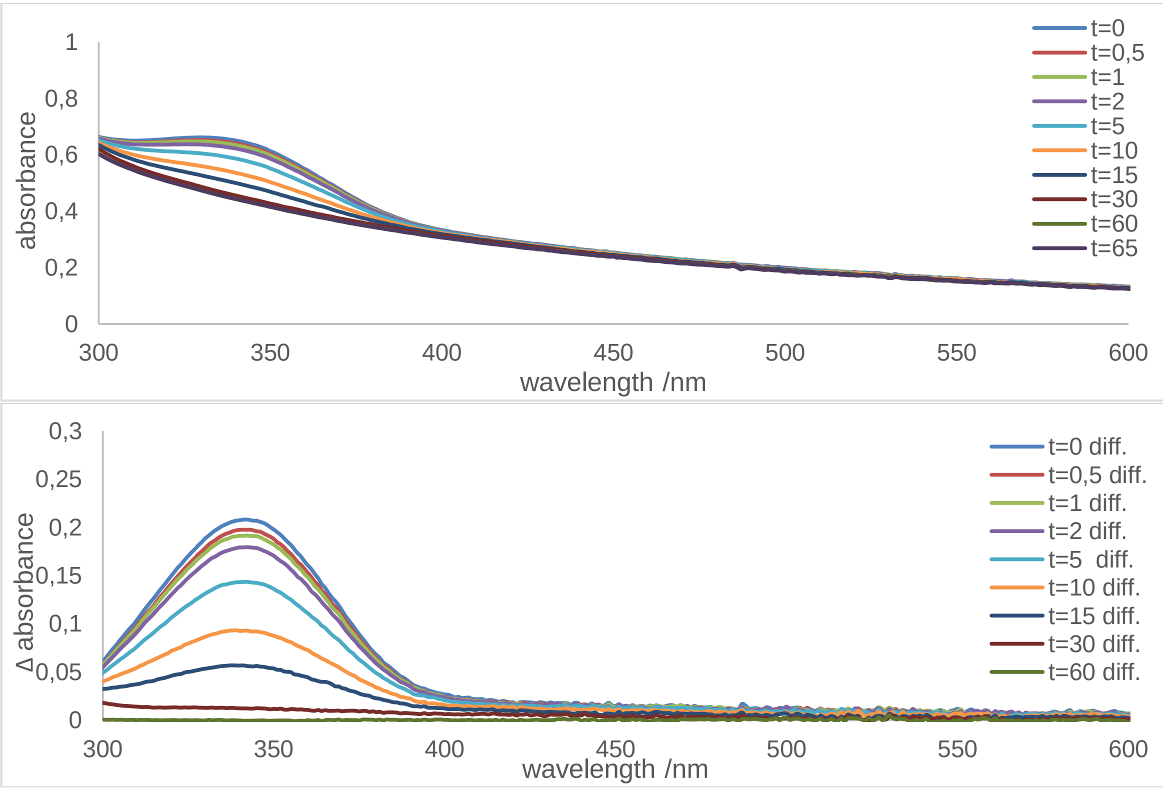

Figure 2: UVvis spectrum (top) and difference spectrum (bottom) over time.

The same principle was used for acquisition using the LabPi photometer. The values of the band at 340 nm were acquired, and then the difference values were determined. A comparison between the professional and low-cost acquisition methods is presented in Figure 3.

	UVvis-Spektrometer		LabPi-Photometer	
Time / min	$A_{\lambda=340\ nm,\ Differenz}$	NADH / %	$A_{\lambda=340\ nm,\ Differenz}$	NADH / %
0	0,207878	100	0,123091	100
0,5	0,197497	95,01	0,117851	95,74
1	0,190994	91,88	0,113705	92,38
2	0,179089	86,15	0,106543	86,56
5	0,143294	68,93	0,085740	69,66
10	0,092705	44,60	0,056746	46,10
15	0,05681	27,33	0,034695	28,19
30	0,012198	5,87	0,007205	5,85
60	0,000603	0,29	0,000794	0,65

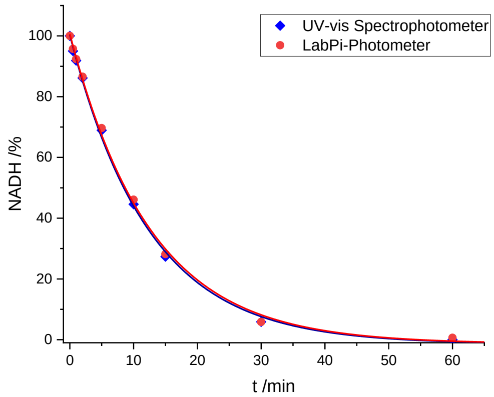

Figure 3: Table with the adjusted data of the spectroscopic investigations and the corresponding percentage values (left) and graphical plot of the percent degradation of NADH over time (right).

6. Conclusion and Outlook

This study shows that with the didactically adapted version, the same effect as with the research system can be observed. Through local holding in a liposome nanoreactor, an increase in the efficiency of the system is possible compared to a conventional solution. This first transfer into a school context is supported by the use of the low-cost photometer LabPi during the follow-up of the experiment. When comparing the two measurement methods, the curves obtained from the percentage values were almost identical (Figure 3).

It remains to be said that this first conception of the school context can currently only be realized in a student laboratory or in a very defined setting. Not only is the topic of photochemistry new to students, but the chemicals used also have limitations in their applicability in school and will have to be further subdivided in future contributions [7]. The high cost factor of purchasing the chemicals and the necessary column for isolating them, demands for further adaptations. Even if these points still stand in the way of a broad transfer to schools, the possibility of conveying this central insight from an efficiency increase of a reaction by clustering of the reactants in local proximity is an interesting methodology that should be brought closer to the students.

Acknowledgments &

We thank the Deutsche Forschungsgemeinschaft (DFG) for funding this work through the Sonderforschungsbereich/Transregio 234 CataLight, project number 364549901 (projects Ö1 and B8).

Conflict of Interest

The authors Manuel Wejner and Timm Wilke have affiliations with the organization iTUBS mbH which distributes the measuring system LabPi for educational institutions and training laboratories at cost price. No profits were generated.

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