Photodegradation of Riboflavin – Model Experiments on Photo Processes in Chemistry Class

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Abstract

Interaction between electromagnetic radiation and matter influences numerous chemical reactions and often leads to phenomena already known from everyday life. Despite their relevance, little attention has been paid to these interactions in chemistry education, especially due to a lack of conceptually embedded experiments.

The presented experiments show how fluorescence can be applied as a detection method for reaction components by using the example of photodegradation of the vitamin riboflavin. These experiments also illustrate structure-property relationship by means of fluorescence and colour.

In the first experiment presented, the orange-yellow vitamin is extracted from custard powder and identified by its typical yellow fluorescence as well as by forming a non-fluorescent red complex when silver ions added. [1]

In a second experiment, an aqueous riboflavin solution is exposed to UV radiation for a few minutes. Thus, riboflavin degrades, among other photoproducts, into lumichrome, showing blue fluorescence. [2]. By thin-layer chromatography, riboflavin and lumichrome can be distinguished by their respective fluorescence colour.

Based on these experiments, a third experiment focuses on factors that influence riboflavin degradation: temperature and solvent as well as duration and wavelength of radiation are being varied and the influence of these factors on reaction speed is being examined. For this purpose, a semi-quantitative analysis of the riboflavin/lumichrome ratio is performed by thin-layer chromatography.

The experiment illustrates the formation of a particularly large quantity of photoproducts at elevated temperature and long radiation time when using the solvents ethanol, 1-propanol or acetone, and when irradiating with visible short-wave or UV light.

The presented experiments can be theoretically and practically expanded, for example, by measuring absorption and emission spectra, or by comparing the fluorescence behaviour of riboflavin and other vitamins such as thiamine.

Keywords: *riboflavin, photodegradation, fluorescence, model experiment*

1. Introduction

Light and colour have always fascinated people and especially the generation of cold glow (luminescence) has a high relevance for everyday life, from fireflies to LED lamps or OLED TV displays. Fluorescence phenomena are particularly motivating because "invisible" UV light is applied to generate "visible" light. They are used intensively in biochemistry and medicine, for example, to examine cell components or metabolic processes by fluorescence microscopy. Fluorescence phenomena can also be integrated in chemistry class at all ages [3].

Flavoproteins such as flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN) play an important role as coenzymes as electron transporters in metabolism, for example in cellular respiration, and at the same time have a central function in photosynthesis due to their photocatalytic properties.

Riboflavin (RF) is a moderately water-soluble, non-toxic vitamin known from everyday life (vitamin B2) that is sensitive to light. It belongs to the dye group of flavins and differs from other flavins by its ribityl group in position 10 [1].

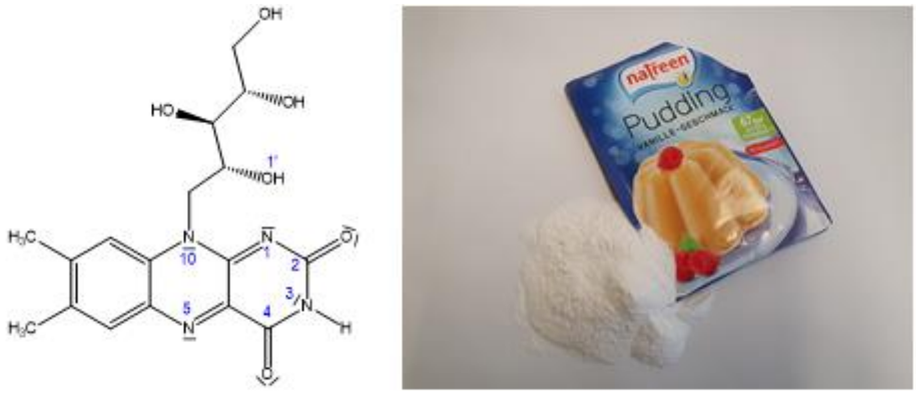

Figure 1 Structural formula of riboflavin and custard powder.

Like most flavins, RF shows a yellow colour with absorption maxima at 223, 267, 373 and 444 nm due to its extended conjugated double-binding system [2]. RF also fluoresces yellowish, with emission maxima at about 520 nm [1].
RF reacts with monovalent silver ions to form a red silver complex (absorption maxima: 378 and 492 nm). The complex formation between the fluorophore and the quencher molecule ensures that this complex cannot be excited and does not fluoresce [4].
The fluorescence of RF and its fluorescence quenching by reduction with dithionite is also known in the educational literature [5] and represents the starting point of the investigations presented here [6].

2. Photodegradation of riboflavin

An aqueous solution of RF is not light stable and is degraded upon exposure primarily to lumichrome (LC) as well as to side chain fragments. Lumichrome (LC) shows a blue fluorescence under UV-irradiation with an emission maximum at 471 nm [1].

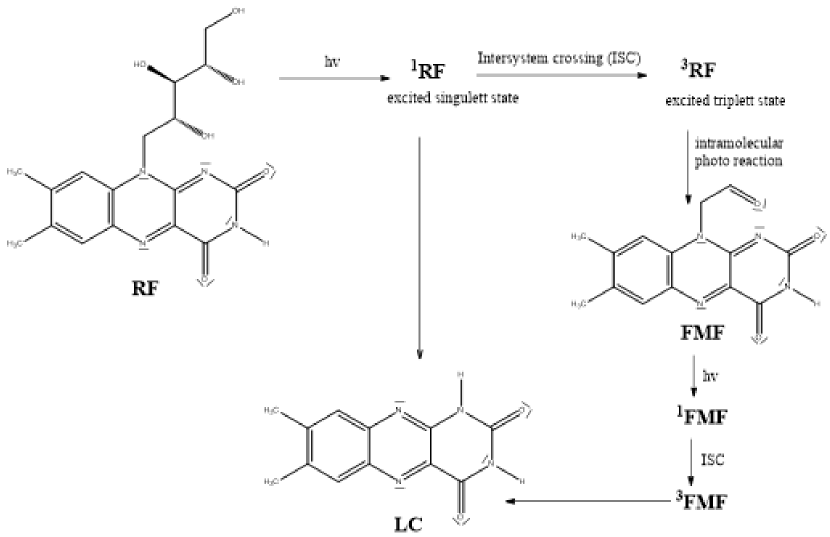

Figure 2 Scheme of the photodecomposition of RF [2, 6].
The degradation of RF occurs by irradiation with UV or short-wave visible light and is accelerated by higher temperature, longer irradiation time or by polar, low-viscosity solvents and solvents with a high dielectric constant (e.g. ethanol, acetone or propan-1-ol).

2.1 Experiment 1: Detection of riboflavin in custard powder by fluorescence and complexation

About 25 g custard powder (with RF dye, cf. the information on the packaging) are stirred with 50 mL demineralised water for a few minutes and filtered. About 5 mL of the clear yellow filtrate is poured into each of two test tubes.
For comparison, a solution of pure RF is prepared by dissolving a small spatula tip of RF in about 10 mL of demineralised water in one test tube and adding half of the solution to a fourth test tube.
A small spatula tip of silver nitrate (hazard: GHS03, GHS05, GHS09) is added to one of each of the glasses with the custard powder filtrate and the RF solution.

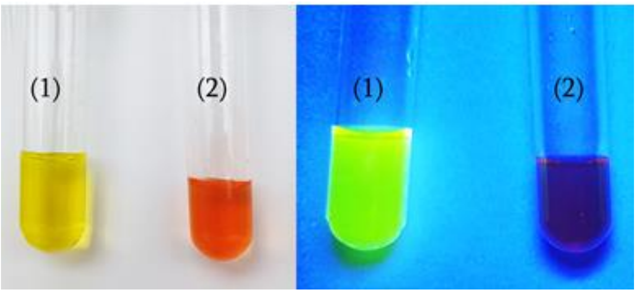

Figure 3 Left: RF solution (1) and RF solution after adding silver nitrate (2) under daylight; Right: RF solution (1) and RF solution with silver nitrate added (2) under UV illumination.

In daylight, the pure RF solution as well as the custard powder filtrate appear yellowish orange. Under UV light, both samples show yellowish fluorescence. The addition of silver nitrate solution causes a strong red colouration of the filtrate as well as of the reference solution and an extinction of the fluorescence under UV light. The colour impression and the fluorescence of the solutions are used in this experiment to detect RF and the silver-RF complex.

2.2 Experiment 2: Photodegradation of riboflavin and detection of a photoproduct by fluorescence

About 25 mL of a RF solution from experiment 1 is divided among four test tubes and illuminated with the irradiation lamp for 5, 15, 30 and 60 minutes respectively. Another test tube is positioned in a dark place as a reference sample for this and the following experiment (tube 0). Under UV light it can be observed that the exposed samples fluoresce yellow-green (at 5 minutes), greenish (at 15 minutes), green-blue (at 30 minutes) or blue (at 60 minutes) depending on the irradiation time.

The irradiated solutions and the reference sample are analysed by thin-layer chromatography (TLC): A water-acetone mixture in a ratio of 2:1 is used as eluent (hazard: GHS02, GHS07), and a cellulose TLC plate is used as the stationary phase.

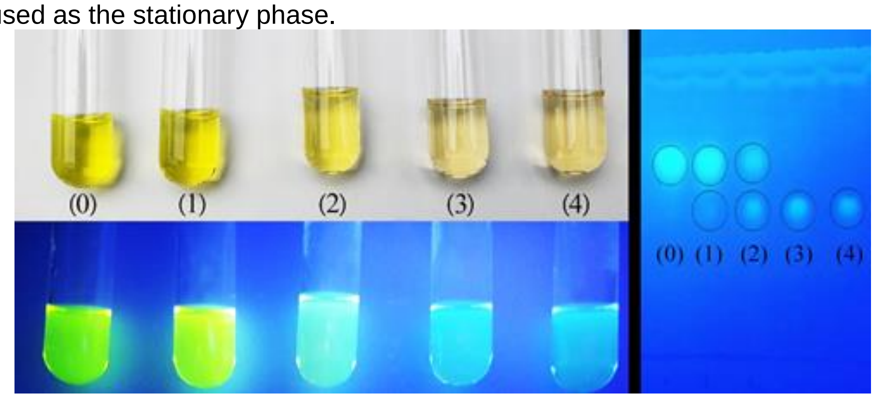

Figure 4 Left: RF solution with increasing irradiation time from (0) to (4); top in daylight, bottom in UV illumination; right: TLC of samples (0) to (4) under UV illumination.

Separate yellow and blue fluorescent substance spots are visible on the chromatogram. The yellow fluorescent ones are located above the blue fluorescent spots and decrease in intensity from left to right. The yellowish fluorescent areas of the TLC are due to the presence of RF, the bluish fluorescent areas are from lumichrome. The fluorescent colours of the solutions or the spot intensities in the TLC thus indicate the amount of photoproducts and correspondingly the degree of degradation. The TLC investigation thus shows the dependence of the product concentration on the reaction time.

2.3 Experiment 3: Factors influencing the photodegradation of riboflavin

This experiment investigates how temperature, solvent and colour of the irradiation light influence the photoreaction.

a) Four tubes with RF solutions are irradiated in an ice bath (temperature about 0°C), at room temperature or on a hot plate (temperature about 80°C) for 5 minutes (tubes 1-3).

The observation of the solutions under UV light as well as a TLC of the solutions (carried out analogously to experiment 2) show that the higher the temperature during irradiation, the faster the reaction takes place.

b) RF solutions with about 5 mL of demineralised water, methanol, ethanol, propan-1-ol, acetonitrile or acetone (tubes 4-9; hazard: GHS02, GHS05, GHS06, GHS07, GHS08) added were irradiated for 5 minutes. The fluorescent colours and the results of the TLC show that the photoreaction is particularly fast when the solvents ethanol, propan-1-ol or acetone are used, whereas the solvents methanol, acetonitrile and especially water slow down the degradation.

c) RF solutions are irradiated with red, green, blue and UV light for 30 minutes (tubes 10-13). The solutions irradiated with red and green light show practically no changes compared to the unexposed reference sample when viewed under UV light and TLC. Only the other two solutions show a yellow and a blue fluorescent spot in the chromatogram. The observations reveal that the energy of the long-wave red and green light is not sufficient for the photoreaction to take place. Only when irradiated with more energetic blue and ultraviolet light the degradation and thus a change in the fluorescent colours take place.

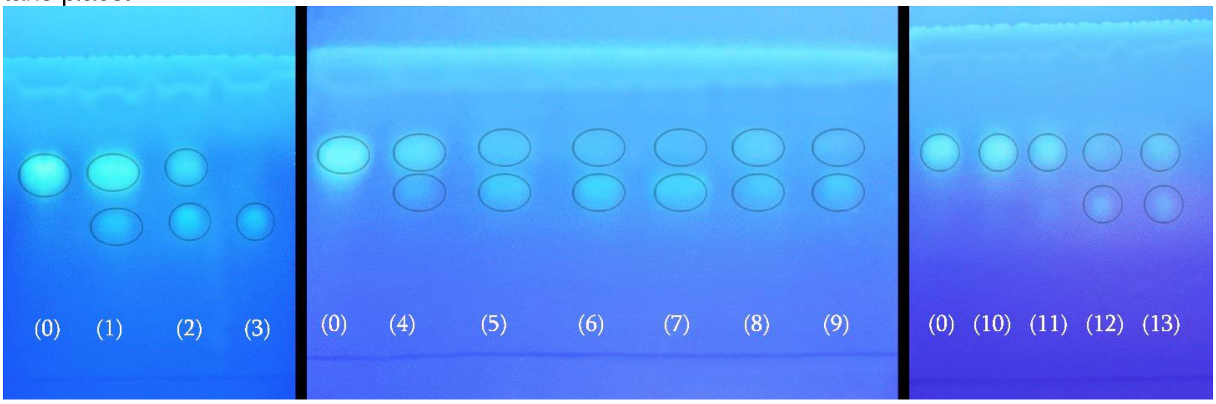

Figure 5 TLC of the samples of the photodegradation of RF under UV illumination; assignment of the numbers see in the text.

3. Didactic aspects

For the integration of new, innovative contents into existing curricular structures, these are often shifted into a "niche", a linkage with contents that are to be dealt with obligatorily often does not take place or only rather arbitrarily. We would like to present a simple and memorable model that can be used to clarify the functions of such content that is not (yet) at the centre of the curriculum: the mnemonic RACE stands for **r**epetition, **a**pplication, **c**onsolidation, **e**xtension. In German, the mnemonic WAVE is used instead for ***W****iederholung,* ***A****nwendung,* ***V****ertiefung,* ***E****rweiterung*. In the following table, we will show how this model works using the example of experiments on the fluorescence of riboflavin.

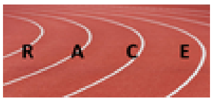	Repetition	Atomic structure (electron shell and energy level model); Absorption and emission of energy, energy diagrams; Thin layer chromatography as a separation method; Solvents and their properties, structure-property relationships
	Application	Light resistance of food or drugs, food ingredients, food colouring, photosynthesis (comparison with chlorophyll)
	Consolidation	Mesomerism and delocalisation, solvatochromism
	Extension	Photocatalysts and electrochemistry, Complexes and colouring, orbital model

Table 1 Application of the RACE model to the fluorescence of riboflavin

The experiments can be used at different age levels, phenomena-oriented even in lower secondary school. For the experiments on influencing factors (experiment 3), students should have in-depth knowledge of solvent influences.

The topic is also suitable for showing cross-connections to biological or physical questions. Keywords may suffice here: Wave optics, light, quantum model, atomic structure, energy transfer through radiationless desactivation, photonics in physics, photoactive substances, role of flavins in nature as energy transfer agents, comparison with chlorophyll, photocatalysts in biology.

Experiences with a group of pupils at a science camp on the topic of "Light and colour in chemistry - How do chemists use the interaction of light and matter?" in autumn 2019 at the XLAB - Experimental laboratory for young people in Göttingen have shown that the topic of riboflavin fluorescence can play an extremely motivating role and is very well suited for explorative learning. With the task of finding out which factors influence the photodegradation of riboflavin, it was possible to achieve a high degree of autonomy in performing the experiments.

References

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