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From Current Research to Chemistry Education: Preparation of Polysaccharide-Based Nanoparticles by Dialysis

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Abstract

"Approximately 20 % of all-cause global deaths are due to sepsis and are largely preventable" [1]. This shows that there is the necessity for a change from conventional medical treatments using drugs with a mainly systemic mode of action towards a targeted, individual, flexible, and effective therapy. Other inflammatory diseases, as well as the fight against numerous cancers, also require alternative approaches. The use of nanotechnology in medicine offers promising potential. The aim is to formulate nanoparticles, enclose the active substances, transport them reliably to the required cells and finally to release them in a targeted way by using special strategies or stimuli (= drug delivery) [2]. For the realization, nanoparticle carrier materials made of natural or synthetic polymers bring suitable properties to function as an "active ingredient shell" [3–5]. In order to make this complex, current and relevant area of nanotechnology accessible to students, there are numerous requirements to adapt the scientific research to the educational context. Based on the Model of Didactic Reconstruction [6], various simplifications were made to (1) develop experiments and teaching materials and (2) to extend the classical subject of polymers by this current research context. In this contribution, we present an experiment where nanoparticles are formulated with the biopolymer derivate cellulose acetate phthalate by using a dialysis process.

Keywords: *drug delivery, medicine, nanomedicine, nanotechnology*

1. Introduction

In the treatment of diseases, not least the viral disease SARS-Cov2, scientists are constantly facing new challenges. While conventional therapies are mainly based on drugs with systemic modes of action, numerous current disease patterns, such as the fight against infectious diseases and sepsis, but also many cancers, require alternative treatment approaches. In this context, the use of nanotechnology in medicine offers promising potential [7]. Nano-scaled carrier materials transport the pharmaceutical active substances specifically to the corresponding tissues or diseased cells and release them at the desired place and at the desired time (frame) – this concept is called targeted drug delivery. This approach offers the advantage of higher dose of active ingredient at the targeted area, while fewer side effects occur in the surrounding tissue. Synthetic as well as biopolymers provide the necessary properties to act as drug carriers.

This publication focuses on modified biopolymers, in particular cellulose derivatives, to formulate nanoparticles by dialysis. After briefly summarizing the scientific backgrounds, we present an experiment for students in which a nanoparticulate carrier system is formulated based on cellulose acetate phthalate (CAPh).

2. Scientific background

Various methods exist for the formulation of nanoparticles. The focus of this publication is on the solvent exchange method, specifically by dialysis [8,9]. This method is based on the exchange of a

solvent with a "non-solvent" [9] and requires that the hydrophobic polymer, *e.g.*, cellulose derivatives, is soluble in an organic solvent, *e.g.*, *N,N*-dimethylacetamide (DMA) or dimethyl sulfoxide (DMSO). Further, it is important to ensure that the solvent is miscible with water. The polymer, however, has to be insoluble in the non-solvent, *e.g.*, water. In the dialysis process, the polymer solution is first placed in a dialysis tube. Consequently, spatial separation of the polymer solution and the non-solvent water is the starting point of the dialyses process [10]. As the dialysis tubing is a semipermeable membrane, a slow mixing of water and polymer solution follows [11]. During this process, only particles smaller than the exclusion limit of the dialysis tubing can pass through the pores of the membrane. The direction of flow is concentration-driven. At the beginning, there is no water in the dialysis tubing, therefore it flows in during the experiment. The opposite is true for the solvent: At the beginning, it is exclusively inside the tube and flows out during the course of the experiment. The polymer remains in the dialysis tubing because the pore size is deliberately selected to prevent the outflow of the polymer. The continuous loss of solubility due to solvent exchange eventually leads to the formation of the particle suspension [9]. To specifically formulate nanoparticles in this process, the polymer concentration must be below a critical overlap concentration, otherwise irregular aggregation will occur [12]. During the mixing of solvent and non-solvent, concentration fluctuations at the interface are responsible for particle formation [8,13]. This process is shown in the so-called ternary phase diagram and described in detail in the literature [10,14,15].

3. Implementation in Chemistry Education

Targeted drug delivery is undeniably highly relevant in research and in everyday life - in addition, it also offers great didactic potential for transfer to chemistry education [16]. In this context, the field allows students to gain insights into scientific research branches, but also to reflect on social discourses and critical issues, such as side effects and vaccinations [17]. Compared to synthetic polymers, drug delivery systems using polysaccharide-based nanocarriers are particularly interesting in terms of “sustainable chemistry [4]. In order to make the complex topic of polysaccharide-based carrier accessible to students, scientists in the fields of organic and macromolecular chemistry as well as chemistry education bundled their expertise within the DFG collaborative research center POLYTARGET [18].

Regarding the rather complex experiments, numerous simplifications have to be made for the implementation in the chemistry education:

1. **Solvent:** Starting from the publication by WONDRACZEK ET AL. [8], an alternative, less hazardous solvent compared to DMA which is used in scientific publications was sought. The solvent searched for should also be miscible with water and capable of dissolving the cellulose derivative. Samples prepared using DMSO as an alternative show no influence on the optical effect.
2. **Time:** In addition, different intervals were tested to save time. Visible effects can already be seen after 20 minutes, so that the experiment can be temporally integrated into a regular chemistry lesson.
3. **Particle purification:** Another simplification point for the school context was tested regarding particle purification. Within the chemistry research context, the polymer solution is usually centrifuged before dialysis to remove suspended particles such as dust. Omitting centrifugation did not show any differences with respect to the observation in the further course of the experiment compared to samples with centrifugation.
4. **Tap water:** To keep the financial cost for schools as low as possible, the experiment was performed using tap water instead of demineralized water. The optical effect is not affected using tap water.
5. **Freezer bag clamps:** For cost reasons, we also tested whether the experiment could be performed using commercially available freezer bag clamps instead of expensive dialysis clamps. The results show that the method of dialysis is not affected by the use of freezer bag clamps during a twenty minute dialysis process.

The adaptation options (1) to (5) were tested experimentally and the results of the series of experiments show that in particular the optical effect of the precipitation of solids in the form of nanoparticles is not affected. For the characterization of nanoparticles with their typical properties, the hydrodynamic diameter (d_H), polydispersity index (PDI), and zeta potential (ζ-potential) are mainly

determined by dynamic light scattering (DLS) in the specialized sciences. Furthermore, scanning electron microscopy (SEM) images give information about the particle shape present in addition to the particle size. In the school context, the required DLS device or scanning electron microscope is not available and instead of the exact sizes it is more important to know whether nanoparticles have been formulated or not. Therefore, the (6) **Tyndall effect** can be used for this purpose as a semi-qualitative nanoparticle detection method. For this, a sample is examined with the beam of a laser. The light will be visible from the side in the medium if nanoparticles have been formulated during the process. The effect is based on the light being scattered by particles that have a similar dimension as the wavelength of the used laser light. However, if there are no nanoparticles or other particles in this dimensional range, the light from the laser is not scattered and is therefore not visible from the side. Figure 1 summarizes the didactic simplifications.

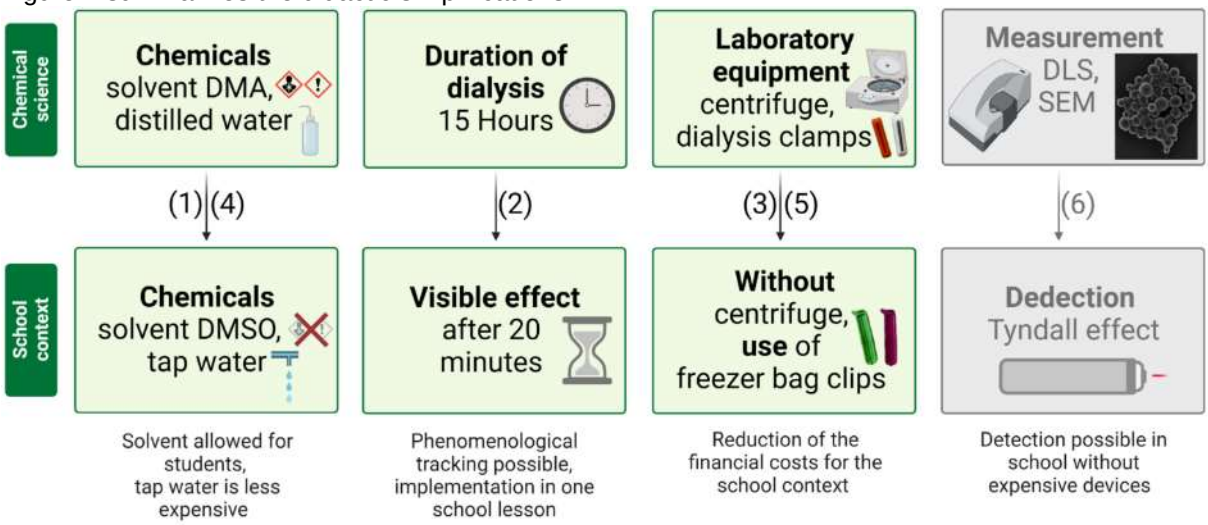

Fig. 1. Didactic simplifications made for implementation in chemistry education.

4. Experimental: Synthesis of nanoparticles via dialysis

Based on the simplifications made, in the following an experimental description of the formulation of nanoparticles by dialysis for High School students as well as the semi-qualitative detection by the Tyndall effect are shown.

4.1 Equipment and chemicals

Cellulose acetate phthalate (CAPh), dimethyl sulfoxide (DMSO, 99.5 %), tap water, dialysis tubes (18 mm, MWCO: 3.5 kD), 2 freezer bag clips, 2 glass vials, beaker, tweezers, measuring cylinder, stirring plate and fish, laser pointer

4.2 Implementation

In preparation, a beaker is filled with approximately one liter of tap water. For the polymer solution, 20 mg CAPh are dissolved in 5 mL DMSO on a stirring plate. The mixture is tested for the Tyndall effect. To do this, the laser beam is pointed sideways through the sample. Then, 17-18 cm of the dialysis tubing is placed in the beaker filled with water and soaked for 2 minutes. After that, the dialysis tubing is taken out, closed with a freezer bag clip on one side and filled with the polymer solution. Then, once the top end of the tubing is closed, it is placed in the beaker for 20 minutes. Finally, the contents of the dialysis tubing are transferred to a glass vial and tested again for the Tyndall effect.

4.3 Observation

CAPh dissolves in DMSO (Fig. 2A and Fig. 2B). The mixture is colorless, and the Tyndall effect is negative (Fig. 2C).

Fig. 2. (A): CAPh in DMSO before dissolving, (B): CAPh in DMSO after dissolving by stirring, (C): Negative Tyndall effect of CAPh in DMSO.

During dialysis, precipitation of a slightly white substance can be observed. At the end of dialysis, it is visible in the tube from top to bottom (Fig. 3A). In addition, the sample scatters the light of the laser pointer (Fig. 3B).

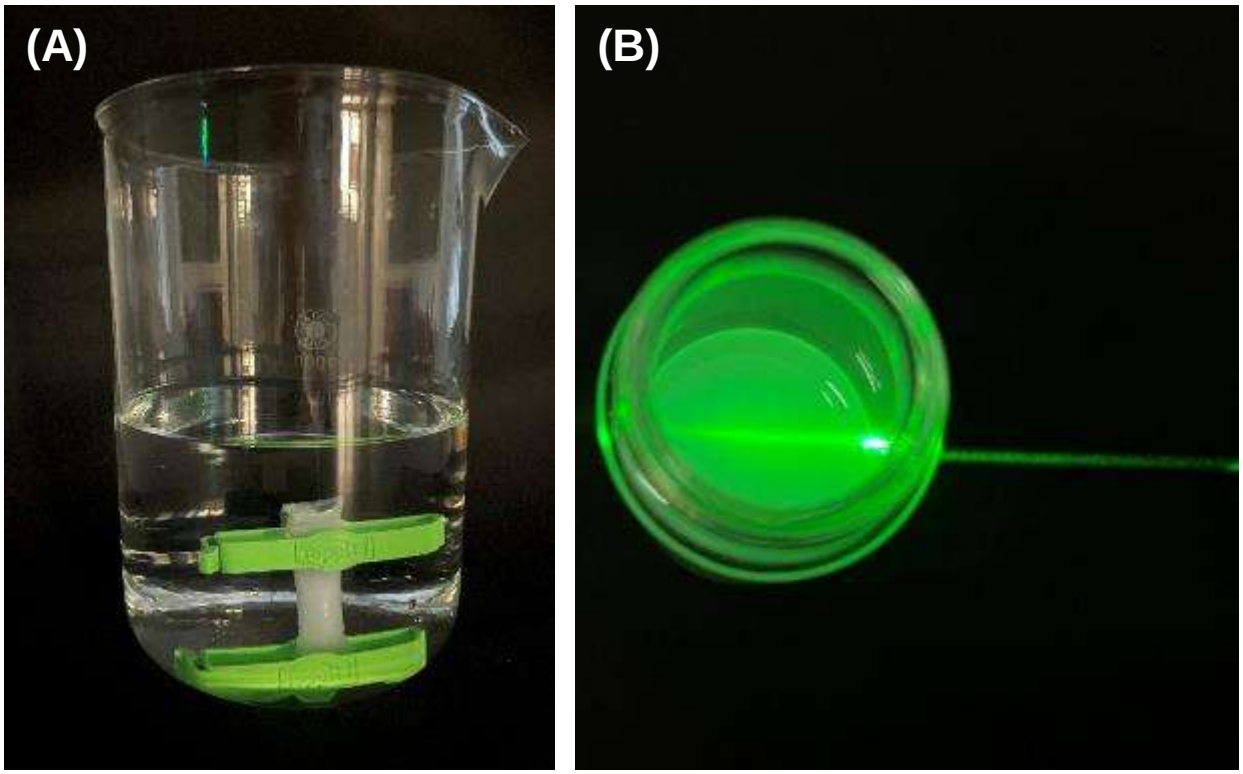

Fig. 3. (A): Dialysis after 20 minutes, (B): Positive Tyndall effect of nanoparticle sample from dialysis tubing.

4.4 Evaluation

While no nanoparticles are present at the beginning of the experiment (negative Tyndall effect), the increasing turbidity shows the formulation of solid particles. These represent the desired polymer nanoparticles, which can be confirmed semi-qualitatively via the Tyndall effect by scattering the light from the laser.

5. Conclusion & Outlook

The experiment enables students to formulate nanoparticles via dialysis simply and inexpensively. Further work is planned to teach drug delivery systems from the particle synthesis over loading them with active ingredients (in the school context: dyes) to a targeted release through a given stimulus. In addition, practical implementation including evaluation and optimization with school classes is pending.

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