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Nanomedicine: Development of a series of experiments on the synthesis of smart nanocarriers

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Abstract

The treatment of infections and inflammatory reactions is a current and ubiquitous topic worldwide, especially considering the current SARS-CoV-2 pandemic. The local and appropriate immune response is critical in combating these diseases since an overshooting immune response outside the infected area can cause severe and systemic damage to the body as a result of adverse reactions. A promising strategy for targeted drug delivery is based on the development of functionalized polymers that act as a specific carrier for pharmaceutical agents and thus enable a targeted release at the desired location. This topic shows great didactic potential for teaching in chemistry education because of its current media coverage and relevance to everyday life. In addition, it links up with several subject areas at the secondary level, e.g., organic and polymer chemistry. Furthermore, the interdisciplinarity of the topics nanotechnology, medicine and pharmacy offers motivating learning opportunities for students, especially for young women [1,2]. In this article, we present an experimental approach on how current scientific findings on targeted drug delivery can be made accessible for K-12 chemistry education and school laboratories through didactic reconstruction. The experiments focus on the preparation of polyester nanocarriers for drugs through the polymerization of δ-valerolactone, followed by a simple nanoprecipitation.
They provide a good opportunity to link classic topics in chemistry education with a current research and contemporary contexts. In addition, the series of experiments can also be used to either introduce or deepen scientific skills and methods.

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

1. Introduction

The fight against inflammatory and viral diseases is currently of great importance in light of the corona pandemic. SARS-CoV-2 in particular highlights the need of effective treatment with targeted strategies for site- and time-specific activation of the immune response. Active ingredients are needed in high concentrations at the site of infection, but they can cause severe damage outside and lead to the development of sepsis and ultimately death, in the worst case. The development of smart nanomaterials can resolve this contradiction by encapsulation of active substances in functionalized nanocarriers transporting them to the targeted site and releasing them at the right time. In the DFG collaborative research center PolyTarget, these questions are being researched in an equally fundamental, application-oriented, and interdisciplinary manner. For the development of suitable polymer-based nanocarriers for targeted drug delivery, several systems for the treatment of inflammatory responses are being investigated [3]. The objectives, research questions and central findings of PolyTarget offer great opportunities for chemistry education, as they allow to synergistically link current research contexts with classical chemistry education contents. With respect to the SARS-CoV-2 pandemic, they also provide opportunities for discussion and critical reflective evaluation. In this contribution, we present experimental approaches that allow the topics of nanomedicine and smart drug delivery to be studied in the chemistry classroom. Within a series of didactically reconstructed experiments, the formulation and characterization of polymer-based nanocarriers is made accessible to students. Starting from the polymerization of δ-valerolactone, subsequent nanoprecipitation yields nanocarriers as potential drug carriers using simple devices and chemicals.

2. Didactic opportunities

From a didactic point of view, teaching nanomedical content in chemistry classes offers numerous opportunities [4]. In principle, various links are already existent in chemistry curricula. The subject area of polymer chemistry offers a strong starting point and is anchored in all upper secondary school curricula in Germany. The learning goals listed there, such as imparting knowledge of syntheses, properties, modifications and the degradation of polymers, can be ideally taught using the example of nanomedicine, thus creating learning opportunities for a future-oriented chemistry education. In addition, pharmaceutical and biochemical processes can be treated in an interdisciplinary way with biology education.

In recent years, nanosciences and nanotechnology have been included in almost all curricula in Germany and offer equally good opportunities for curricular innovation research due to their widespread use [5]. Empirical studies on students' attitude and previous knowledge on "nano" confirm a high interest in a classroom treatment of this topic: 87% of the surveyed students indicated a "high" or "very high" interest [1]. By expanding (nano-) chemistry to include medical application areas, this motivation shall be taken up and consolidated. Based on further research results [6], it can further be expected that the expansion of the subject area of (nano-) chemistry to include medical applications will promote the interest of young women in particular.

3. Experimental: Synthesis of nanocarriers

The series of experiments presented in this paper was developed in collaboration between researchers in chemistry and chemistry didactics. The aim was to didactically reconstruct a synthesis concept from current research [7], so that it can be conducted by students in chemistry class with simple and inexpensive chemicals in a short time with reliable results. Despite the restriction to common school (laboratory) equipment and conditions as well as the use of non-toxic and inexpensive chemicals, good product qualities have to be achieved to enable further use of the materials in follow-up experiments by loading and unloading with active substances.

3.1 Scientific background

Different approaches to the synthesis of polyesters exist. The ring-opening polymerization (ROP) of lactones has prevailed over polycondensation in the context of polymers for biomedical use. Typical polyesters obtained *via* that method include polylactide as an exemplary homopolymer and poly(lactide-*co*-glycolide) as an exemplary copolymer. Typically, such polyesters are obtained by ROP using Sn(II) catalysts. However, modern organocatalysts such as triazabicyclodecene (TBD) or diazabicycloundecene (DBU) facilitate shorter reaction times at milder reaction conditions. Moreover, less by-products are formed [8]. The monomers δ-valerolactone and δ-caprolactone were selected here as δ-lactones are naturally occurring, cheap and non-toxic. The respective amount of a stock solution, containing the catalyst TBD and the initiator benzylalcohol, was added to the 500 mg of monomer in toluene to ensure a monomer/initiator/catalyst ratio of 100/1/1 [7]. These syntheses were performed in a glovebox as well as under semi-inert conditions. The obtained polyesters were then used to prepare nanoparticles by nanoprecipitation [9]. For this purpose, the hydrophobic polyester was dissolved in acetone and the obtained solution was added dropwise to water under stirring. Thereupon, the nanoparticles precipitated due to the abrupt decrease in solubility as well as the evaporation of the organic solvent. Compared to other methods, nanoprecipitation is very advantageous – it is a simple, direct, and inexpensive method to produce nanoparticles with defined properties [10].

3.2 Didactic reconstruction and results

The aim of the experiments was to transfer the syntheses of potential polymeric nanocarriers from research laboratories with professional equipment under inert conditions to chemistry classes, using only student laboratory equipment and simple chemicals. Based on the results as well as the observations during the reaction, the monomer δ-valerolactone is more suitable for schools and student laboratories than δ-caprolactone, in particular due to fast reaction times. In terms of stability, the results showed that, the poly(δ-valerolactone) was still usable even after one week which is particularly relevant in terms of lesson planning. In addition, all experiments can be performed without an inert gas atmosphere and in bulk, avoiding the use of toluene as a solvent.

In the further course, the quantities of chemicals were increased for the procedure, so that precision balances are no longer required. Additionally, the amount of polymer is thus larger, so that optical changes are more easily perceived with the naked eye. By using low-cost medical equipment (syringes), microliter pipettes are no longer necessary for precise addition. The experiment was further simplified in that the catalyst and initiator can be weighed in directly and reacted with the monomer,

eliminating the need to prepare stock solutions. In terms of chemicals, the initiator benzyl alcohol can be replaced by ethanol, which is cheaper and more widely available. In a final step, it was tested whether TBD could be replaced by a cheaper catalyst such as DBU. However, much lower conversions and lower average molar masses were obtained under the same reaction conditions using the less reactive catalyst, so TBD was kept.
Nanocarriers could be then obtained by nanoprecipitation. An examination of the particles showed that their size range even meets scientific requirements of a suitable drug carrier [11]. Since the duration of the experiment does not exceed 20 minutes, it can be easily performed within one chemistry lesson.

3.3 Implementation results and practical experience

Based on the adjustments on the experiments mentioned above, a procedure for the nanocarrier synthesis was developed and piloted with a group of students during the Jena Summer School. In this context, the feasibility and robustness of the experiments was confirmed. The students independently synthesized poly(δ-valerolactone), whereby an optical change from a clear colorless liquid to a white, highly viscous liquid could be observed within a few minutes. The polyester was then used for nanoprecipitation, which takes advantage of the insolubility of the hydrophobic polymer in an aqueous medium (Fig. 1). It can be deduced that the experimental procedure can be utilized by students, as no difficulties were encountered during the performance.
Thus, it is possible to produce suitable biocompatible nanocarriers within a student experiment, despite the limitation to common school (laboratory) equipment and conditions involving the use of non-toxic and inexpensive chemicals.

Fig. 1: Piloting of the experiments during Jena Summer School (2021). Start of reaction (ROP, left), Reaction mixture after 5 minutes (ROP, middle), student conducting the nanoprecipitation (right).

4. Outlook

The series of experiments will be further piloted, evaluated, and optimized in a cyclic design. Further, it will be extended in subsequent work to include the encapsulation of a fluorescent dye during nanoprecipitation and its subsequent release. Within first preliminary tests, the loading as well as the release could be realized *via* a simple nanocarrier degradation through ester cleavage with diluted sodium hydroxide solution.

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