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OPTIMIZATION IN NANOPARTICLE SYNTHESIS METHODS FOR DRUG DELIVERY SYSTEMS

OPTIMIZAREA METODELOR DE SINTEZĂ A NANOPARTICULELOR PENTRU SISTEME CU ELIBERARE CONTROLATĂ DE MEDICAMENTE

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Summary

The current years are witnessing the evolution of the third generation of controlled-release drug delivery systems, whose main research directions include the use of nanoparticles for the targeted and controlled release of bioactive compounds. In this context, the main aim is to achieve optimized synthesis procedures that allow for the control of nanoparticle size and size distribution, shape, surface chemistry, and functionality. Thus, the principal goal of the current PhD thesis is in accordance with the requirements of the pharmaceutical industry regarding drug formulation development. Specifically, in the process of developing nanoparticle-based drug delivery systems for the controlled release of drug molecules, a series of unconventional synthesis methods and the associated synthesis parameters were investigated for their potential to achieve uniformity and controllability of nanoparticle size and size distribution, high drug loading capacity, sustained release of drug molecules, and process reproducibility. Therefore, the synthesis processes were optimized in order to meet the previously described criteria that are required for obtaining suitable and efficient drug delivery systems. Consequently, the objectives established within the current thesis were formulated in accordance with the shortcomings identified within the previously available research through a comprehensive process of literature review.

The PhD thesis is divided into two main parts, the first one focusing on a literature survey that provides the general aspects associated with the concepts introduced within the thesis, namely drug delivery, controlled release of bioactive compounds, and nanomaterials for drug delivery within Chapter 1, and magnetite structure, properties, non-conventional synthesis methods, and applications for drug delivery systems. The second part of the thesis focuses on the experimental aspects of the thesis, which were divided into three distinct chapters that focus on three synthesis methods. In the following paragraphs, the general conclusions drawn from the experimental chapters of the thesis will be presented in accordance with the objectives established at the beginning of the doctoral studies.

The first established objective was to identify and select suitable, non-conventional synthesis methods for obtaining magnetite nanoparticles that allow for synthesis parameter variation and, consequently, the tunability of the outcome properties in terms of size and size distribution. The first synthesis method involved the solvothermal technique, which allowed for varying a series of parameters during the synthesis process, namely the reaction temperature and time, the type of solvent used for

the reaction, and the use of a surfactant within the reaction mixture. While the available literature has demonstrated that the nanoparticle size increases with temperature and time and it is directly dependent on the type of solvent, e.g., the use of diethylene glycol reduces nanoparticle size in comparison to ethylene glycol, limited attention was directed towards the influence of the surfactant. In this manner, Chapter 4 describes a comparative study between the synthesis of two types of magnetite nanoparticle-based carriers using two types of surfactants, i.e., PEG with different molecular weights. Three main conclusions were drawn from this study, specifically (i) the surfactant used in the synthesis protocol also acts as a coating agent, thus emphasizing the importance of using non-toxic compounds, (ii) the use of a higher molecular weight leads to higher nanoparticle sizes with slightly narrower size distributions, (iii) the diameter of the obtained MNPs were at the limit between the nanoscale and microscale, thus proving the unsuitability of the synthesis protocol for obtaining nanostructured DDSs and the need to select a different method. Nevertheless, the solvothermal direction could also be exploited by reducing the concentration of the iron ions, the use of a different solvent for the reduction of iron(III), and the ensuring of a faster heating process of the autoclave (considering the slow thermal gradient from the outside to the reaction medium due to the large thickness of the stainless-steel autoclave).

The second synthesis was based on the microwave-assisted hydrothermal method, which allowed for varying the imposed pressure, temperature, and time of the reaction. The synthesis protocol involved the co-precipitation of the iron ions into an alkaline medium, followed by the microwave-assisted hydrothermal treatment of the obtained precipitate. In this context, Chapter 5 describes three studies focusing on the comparison between four types of magnetite nanoparticles obtained through different treatment parameters and between the co-precipitation and the microwave-assisted hydrothermal synthesis with either post-synthesis drug loading within the silica layer or *in-situ* drug loading. In the first study, the synthesis parameters involved an imposed pressure of 10 and 80 bar, temperature of 60 °C, and reaction time of 30 and 60 min. The main conclusion of this study is that although high-pressure reaction conditions lead to the formation of secondary crystalline phases, i.e., goethite, the size distribution is significantly narrower. In this context, the following two studies focused on the optimization of the hydrothermal treatment parameters that could ensure a narrow size distribution while also preventing the formation of secondary phases. Thus, the parameters involved an imposed pressure of 60 bar, temperature of 80 °C, and reaction

time of 30 min. While both studies focused on the comparison between the co-precipitation and the microwave-assisted hydrothermal synthesis, the protocols differed as one study involved the post-synthesis silica coating and drug loading, while in the other study, the bioactive compound was added to the iron precursor solution. The current synthesis parameters did not cause the formation of goethite and the obtained magnetite nanoparticles were characterized by a narrow size distribution. However, performing the *in-situ* drug loading, increasing the drug concentration resulted in narrower size distributions but larger nanoparticle sizes. Another important aspect observed within this chapter resides on the need to perform the concomitant synthesis, coating, and drug loading in order to avoid the formation of multi-core-shell particles. In this manner, this synthesis method is not suitable for polymeric shells, as they are not resistant to the high-pressure and high-temperature conditions imposed.

The third and final synthesis method involved the microfluidic technique, through which the concentration of the iron precursors and the flow of the iron precursor and precipitating agent solutions could be varied. Thus, Chapter 6 involves three studies describing the synthesis of magnetite nanoparticles, their functionalization, and their drug loading. The first study investigated the influence of iron(III) and iron(II) concentrations and the flow of the precursor solutions. The microfluidic method ensures the narrowest size distribution among the three types of synthesis methods described, especially when using higher concentrations. Therefore, the second study involved a total mass concentration of 1% for the precursor solution, while the flow was varied for both the precursor and the precipitating agent solutions. In addition, two types of functionalization agents were added to the precipitating agent solution at three different concentrations. Optimal results in terms of functionalization were achieved for the samples with higher flows, as they result in turbulences within the microfluidic channels. The presence of the functionalization agents did not increase the nanoparticle size as it did in the case of microwave-assisted hydrothermal method and the narrow size distribution was maintained. Thus, the final study described the concomitant synthesis and drug loading, which compared two types of antibiotics for antimicrobial purposes. The main conclusion that could be drawn from this study is that the drug loading capacity does not solely depend on the synthesis parameters, but also on the type of drug molecule used. The microfluidic direction seems to be the most promising in the goal of developing drug delivery systems with a high degree of uniformity and reproducibility. In this context, the future step should involve the use of a microfluidic platform that

allows for the concomitant synthesis, coating, and drug loading. In this manner, an increased drug loading capacity could be achieved due to the presence of a polymeric layer on the surface of the nanoparticles that could enhance the interactions with the drug molecules.