Self-assembly of Bioactive Polymers in Aqueous Media and Their Interactions with Lipid Membranes

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This dissertation consists of three chapters. The first focuses on the behavior of amphiphilic derivatives of chondroitin sulfate in aqueous media and the possibility of using such biopolymers as carriers for hydrophobic drugs. At the beginning of the chapter, a theoretical introduction was included, describing the growing interest in nanomaterials in medicine and the reason for this phenomenon, namely the search for carriers for controlled delivery of bioactive substances. The advantages of using such formulations over conventional pharmaceutical delivery are presented, as well as the types of nanoparticles that are being studied for use for such purposes. The advantages of biopolymers as materials for the design of drug delivery systems are then introduced, and the self-assembly of amphiphilic polysaccharide derivatives in aqueous media is discussed. The origin, functions and properties of chondroitin sulfate (CS) are also described. Examples of carriers reported in the literature that have been based on this polysaccharide are then presented. At the end of the theoretical part, curcumin, used as a model drug with hydrophobic properties in this thesis, was briefly characterized. In the experimental section, studies on hydrophobically modified chondroitin sulfate, obtained by attachment of octadecyl or oleyl groups along the polysaccharide chain, are presented. The derivatives differed in the degrees of substitution. First, the critical aggregation concentration of these compounds was determined, as well as the size, zeta potential, and morphology of the structures they formed. Next, the effect of curcumin on the self-assembly of amphiphilic CS derivatives was checked, and the so-called binding constant (K_b), which quantifies the partitioning of the drug into the polymer and water phases, was calculated. For selected polysaccharides, DLC and EE parameters (which characterize their ability to accumulate curcumin) were determined, as well as the in vitro release profile of the drug from the carriers.

The second chapter concerned the study of interactions of amphiphilic derivatives of chondroitin sulfate with lipid membranes. In the theoretical part to this chapter, the structure

and properties of biological membranes were presented, focusing on their lipid composition. Aggregates formed by lipid molecules are also described, with particular emphasis on lipid bilayers and liposomes, as these are used in this work as simple models of cell membranes. The thermotropic behavior of lipid membranes is then discussed, as well as the method of differential scanning calorimetry, which is a useful technique for analyzing them. At the end of the theoretical introduction, the interactions of polymers with model membranes and examples of how to study them are introduced. In the experimental part, research to determine the effect of CS derivatives on the size and zeta potential of liposomes made of phosphatidylcholine are described. It was also investigated whether interaction with polymers causes an increase in membrane permeability and changes in vesicle morphology. The effect of polysaccharides on the thermotropic behavior of the lipid bilayer was also examined using differential scanning calorimetry.

The final chapter focused on determining the interactions between strong polycations containing quaternary ammonium groups and model biological membranes, which in this case were negatively charged membranes composed of phosphatidylcholine and phosphatidylserine. The study concerned two polymers with different chain lengths. The introduction describes the applications of polycations and their interactions with lipid membranes. The research consisted of experimental methods combined with computer simulations. Experimental methods were used to determine the effect of the polymers on the vesicle size and zeta potential, as well as on the membrane permeability. Molecular dynamics simulations shed light on the nature of the interactions of macromolecules with the negatively charged lipid membrane. The effect of the polyelectrolytes on the thickness and fluidity of the bilayer and on the distribution of anionic lipids in it was also determined.

The presented results provided information on the self-assembly of amphiphilic derivatives of chondroitin sulfate in an aqueous environment and demonstrated their potential in applications as carriers of bioactive substances. It was proved that the size and morphology of the polymeric structures depended on the hydrophobic group that the polysaccharide macromolecules were modified with, the degree of chain substitution and the addition of a hydrophobic drug. The nanoparticles showed good curcumin accumulation capacity and prolonged cargo release in vitro. The more substituted derivatives showed a disintegrating effect on model lipid bilayers, which may indicate their cytotoxicity. However, the magnitude of this effect varied depending on the type of hydrophobic chains. The knowledge presented here may be useful in the design of chondroitin sulfate-based drug delivery systems.

The last chapter confirmed the interactions of the studied polycations with the model membrane and showed that they depend on the polymer chain length. Macromolecules consisting of more repeating units interacted more strongly with the lipid bilayer and affected its properties to a larger extent. The results presented here will be helpful in further investigating the mechanism of the cytotoxicity of polycations and its relationship to their structure.