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SCHOOL OF DOCTORAL STUDIES

The study of the cyclodextrin complexes

ABSTRACT OF THE DOCTORAL THESIS

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Introduction

The cyclodextrins (CD) are cyclic oligosaccharides, with a unique distribution of the hydrophobic/hydrophilic zones, which results in well-defined arrangement of amphiphile. These properties offer for the CD some distinctive advantages at forming host-guest complexes with small lipophilic molecules, which are included all the way or just some part of the molecule into the cavity of the cyclodextrin or with the lipofil part of a protein, which enter partially into the cavity of the cyclodextrin. With these complexes the solubility and stability of the guest molecule can be improved or the taste, odor and other physical properties of the pharmaceutically active ingredients can be masked, enriching the domain of application of the CDs.

The most efficient drug for the treatment of diabetes is the insulin molecule. The insulin is a small protein, composed of 2 polypeptide chains: A and B; these chains are linked with two disulfide bonds. Structural modification can appear during deposition of the product or utilization of it. These can be divided in two categories, in physical and chemical modification. The insulin degradation results in aggregation, precipitation and amiloid fibril formation, in consequence of these events the insulin will become inactive. For the solution of this problem, there can be found many attempts in the scientific literature, where the insulin is complexed with different types of excipients, inclusive CDs.

In the last decade an increased tendency in the research of polymeric nanofibers can be observed, which are functionalized with CDs or are obtained just from CDs, without any polymer. These nanofibers have some particular properties, because they can form host-guest complexes with an active substance. These nanofiber-complexes have the ability to increase the solubility, bioavailability, thermic resistance and the stability of the complexed active substances.

Objectives

In this thesis our goal was to develop a simple, cost-effective method for the production of CD nanofibers and the incorporation of the insulin molecule into this nanofiber to increase the solubility of the insulin molecule. The experimental part is divided into three studies, in which the three necessary steps for obtaining the insulin-CD nanofibers are presented: complexation of the insulin with 5 groups of CD, development of a methodology for producing the CD nanofibers, then producing and studying the insulin-CD nanofibers.

Study 1: Cyclodextrin Complexation with Insulin, using Molecular Modeling

The aim of this study is to form different insulin complexes with all the CDs used for pharmaceutical excipients (native CDs, methyl-β-cyclodextrin, hydroxyethyl-β-cyclodextrin, hydroxypropyl-β-cyclodextrin (HPβCD) and sulfobutylether-β-cyclodextrin (SBEβCD) with different degrees of substitution (DS)), in silico condition, with the AutoDock molecular modeling program, to determine the best type of CD or CD derivate to form a complex with an insulin monomer.

In conclusion, the insulin molecule can form a complex with the native CDs, HPβCD (DS 1-4), SBEβCD (DS 1-3), usually in molar ratios of 1:5–7 insulin:CD. The best complex with the insulin molecule was formed with SBEβCD, with a DS of 2, and the best binding energy was −18.70 kcal/mol around the residue B:LYS29 and B:THR30. These two residues were entered in most of the cases in the cavity of the cyclodextrins, with another four insulin residues (A:LEU13, B:PHE1, B:HIS10, and B:TYR16).

From the number of the hydrogen bonds appearing in the complex and the binding energy calculated by the AutoDock program, it can be predicted that insulin can make a stable complex with 5–7 molecules of HPβCD or SBEβCD, and by forming a complex it can potentially prevent or delay the amyloid fibrillation of the insulin and increase the stability of the molecule.

Study 2: Obtaining CD-based nanofibers

The aim to this study was to obtain polymer-free nanofibers, using HPβCD and SBEβCD. For this reason, four-four electrospinning solutions were prepared with different concentrations from both types of CDs. With HPβCD the following concentrations were used: 170%, 180%, 190% and 200% (v/w – water solution) and with SBEβCD the following concentrations were made: 210%, 220%, 230% and 240% (v/w – water solution).

Based on our results we can declare, that by using the “in house” developed electrospinning machine, HPβCD and SBEβCD nanofibers can be obtained, using a flow of 1.5 ml/h, a voltage of 25 kV, at a

temperature of 25-28°C, with a humidity of 45-50%, using a single-use needle of 24G in the case of HPβCD or a single-use needle of 22 G in the case of SBEβCD. The fibers were collected on an aluminum foil placed on the grounded collector, at the distance of 13-15 cm in the case of HPβCD and at the distance of 9-11 cm in the case of SBEβCD. The nanofibers obtained from HPβCD had a great quality, whilst the nanofibers obtained from SBEβCD also had a sufficiently good quality.

For the nanofibers obtained from HPβCD, the ideal concentration was the 200% electrospinning solution. From the electrospinning solution with a concentration of 170-180%, in the defined time unit, less nanofibers was formed and with an unacceptable aspect, meaning that the fibers contained some drops from the electrospinning solution. This fact was observed especially during the dissolution and disintegration tests, having a negative influence on the quality of the obtained nanofibers. From the electrospinning solution with a concentration of 200%, in the defined time unit a great quantity of nanofiber was obtained, forming a thick film on the collector, with a great volume, having appropriate dissolution and disintegration properties.

In the case of the SBEβCD nanofibers, the nanofiber formation wasn`t that efficient compared to the HPβCD nanofibers forming process. The nanofiber formation was discontinuous, probably because the available voltage wasn`t sufficient. This fact was also mentioned in the scientific literature. From these data it can be concluded that the minimum voltage used for producing SBEβCD nanofibers was 40 kV. Nanofibers from SBEβCD were obtained using a voltage of 25 kV, but these fibers were wet, adherent and due to these properties became difficult to handle them. In the same time, these fibers contained drops from the electrospinning solution, resulting in inadequate dissolution and disintegration properties.

Study 3: Preparation and examination of the nanofibers obtained from the complexes of CD-insulin

The aim of this study was to develop a cost-effective method for incorporating the insulin molecules in the polymer-free HPβCD nanofibers, without using any other adjuvant substances. Then the morphological, desolvation, dissolution properties were investigated and formation of the host-guest complexes, finally the insulin concentration of the fibers was determined. For evaluating the quality of insulin-CD nanofibers, CD nanofibers were made without complexation, as a comparison to the insulin-CD nanofibers.

The cost of the method was reduced by using the electrospinning machine developed by the author. This electrospinning box was made of Plexiglas with accessible and low cost components. This innovation had an important influence over the electrospinning method, which was modified by eliminating the tube connecting the syringe with the needle, thus simplifying the installation and the cleaning of the electrospinning system.

We were using HPβCD based nanofibers for the complexation of the insulin, with success. The HPβCD has a good capability to form complexes with the insulin molecule, because the insulin is totally incorporated in the nanofibers and cannot be observed any morphological difference between the insulin-CD nanofibers and the “empty” nanofibers. With the help of HPβCD the insulin molecule resisted the electrospinning process without degradation, and did not lose from the incorporated quantity. The HPβCD improved the insulin dissolution in water and the disintegration in the phosphate buffer solution with pH=6.8.

The originality of the thesis

This thesis has three important aspects from the originality point of view . The first aspect was the elaboration of the docking methodology of the insulin molecules with multiple cyclodextrin molecules to form a complex with 6-8 molecules. The second aspect was the development of the electrospinning method for producing the HPβCD nanofibers with the construction of the improved “in house” electrospinning box. The third aspect was the forming of the inclusion complexes of the insulin-CD and the transformation of these in nanofibers.

In Study 1 complexes were obtained of the insulin molecule with native CD and CD substituted with different functional groups and substitution degrees. The docking process was realized by three innovative steps, these steps helped to eliminate the limitation of the AutoDock program, because this program can only calculate with limited number of flexible ligands and just one ligand can be added to the

receptor molecule. These innovative steps, with the blind docking, short docking and flexible docking and saving the best complex for the next cycle can help to obtain an insulin complex with multiple ciclodextrin molecules.

In Study 2 an “in house”, custom made electrospinning box was developed for producing nanofibers from HPβCD and SBEβCD. This machine is made from simple, cost-effective components, resolving the problems of the electrospinning process with some simple solutions, like the monitoring of the temperature and controlling the humidity of the system.

In Study 3 complexes of the insulin with HPβCD were obtained and transformed into nanofibers without using any other auxiliary substances. The morphological, dissolution and disintegration properties of host-guest complexes of the ins-CD nanofibers were examined. Then with a thermal study was demonstrated the formation of the complexes and with the capillary electrophoresis were determined the quantity of the insulin incorporated in the nanofiber.