THE OBTENTION AND ANALYSIS OF INCLUSION COMPLEXES OF SOME DIURETICS WITH CYCLODEXTRINS

Thesis

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Cyclodextrins are cyclic oligosaccharides, with a toroidal shape, formed by units of glycopyranose, linked by 1, 4-α-glycosidic bonds, capable of forming inclusion complexes with a large number of substances, including pharmaceuticals. It is known that the inclusion compound does not invariably enter, or it is not completely contained in the cavity. In many instances adducts are formed, which improves the possibility of interactions between cyclodextrins and drugs.

Diuretics are pharmaceutical substances that stimulate urine formation, by increasing the elimination of water and electrolytes through the kidneys. By complexation with cyclodextrins, diuretics are molecularly dispersed in a hydrophilic matrix and become water soluble, which leads to a faster dissolution and a better bioavailability.

In the present work the guest molecules are represented by 5 sulphonamidic diuretics: chlorthalidone, furosemide, acetazolamide, hydrochlorothiazide and indapamide; the cyclodextrins used were β-cyclodextrin and two of its soluble derivatives: 2-hydroxypropyl-β-cyclodextrin (HPBCD) and randomly methylated-β-cyclodextrin (RAMEB). The inclusion complexes were prepared by physical mixture, kneading, ultrasonication and spray-drying method, in molar ratio of 1:1 and 1:2. Ternary complexes of furosemide with RAMEB and polyvinylpirrolidone were also prepared in order to increase the stability of the final product.

The binary and ternary diuretic-cyclodextrin products were analyzed by different methods, mentioned in various papers: thin-layer chromatography, in vitro dissolution tests, IR, Raman and NMR spectroscopy, differential scanning calorimetry, electronic scanning microscopy. Measurements of particle size distribution were also accomplished.

Thin-layer chromatography revealed the diminution of the Rf value of the inclusion complex when compared with the active substance alone, due to the retention of the diuretic into the cyclodextrin cavity.

In vitro dissolution tests show a significant increase of water solubility of diuretics in the presence of cyclodextrins, the best result being obtained by semisynthetic cyclodextrins (HPBCD, RAMEB). The solubility increase depends on the preparation method and the molar ratio: the spray-drying method and the ultrasonication in molar ratio of 1:2 led to optimal results. The ternary complexes of furosemide exhibited a better solubility compared to binary complexes, which illustrates the major role played by polyvinylpirrolidone in stabilizing the final complex. On the other part, complexation of furosemide with a hydrophobic cyclodextrin (tri-acetyl-β-cyclodextrin, TABCD) has caused the reduction of water solubility of the diuretic.

The Raman spectra of inclusion complexes of chlorthalidone and furosemide resemble generally to the characteristic profiles of the cyclodextrines involved, suggesting that the inclusion in the cyclodextrin cavity prohibits the normal vibration of the diuretic. IR spectroscopy is not conclusive in what concerns the nature of the final product because the spectra are identical with those of the pure cyclodextrins.

Differential scanning calorimetry reveals that when inclusion complexes appear the endothermic or exothermic peaks produced by the melting (with or without decomposition) of the active substance disappear due to inclusion in the cyclodextrin cavity. For chlorthalidone and indapamide, the kneading process leads to partial inclusion complexes formation (the endothermic peaks do not disappear completely). Spray-drying method has been proved to be
efficient in preparing real inclusion complexes. It is interesting to notice that in case of furosemide and acetazolamide even the physical mixtures do not present the exo- or endothermic peaks; the explanation can be the formation of an inclusion complex by melting, during the thermal analysis.

The thermogravimetric method confirms the results of DSC; along with the increase of the temperature a loss of mass takes place in case of furosemide, acetazolamide and indapamide.

The X-ray diffraction analysis shows that all of the diuretics are crystalline substances, presenting characteristic peaks. For the kneaded products, one can notice an amorphization which becomes complete for the spray-dried and ultrasonicated products. The formation of an amorphous phase proves the appearance of a real inclusion complex between the cyclodextrin and the diuretic.

NMR \(^{13}\)C spectra show the appearance or disappearance of some diuretic characteristic peaks along with the shifting of the cyclodextrin peaks; the shifts are the result of interactions between the two substances and lead us to the conclusion that a real inclusion complex is obtained.

\(n\)-Octanol / water partition coefficient of the diuretics involved, calculated according to Nernst'law, diminishes significantly in the presence of cyclodextrins, proving the existence of an interaction between the drug and the cyclodextrin.

Scanning electron microscopy was used for the pure diuretics, cyclodextrins and their spray-dried inclusion complexes. The crystalline form can be easily observed for the diuretics and \(\beta\)-cyclodextrin; RAMEB in an amorphous substance, with beautiful smooth and spherical particles. The spray-dried products present an amorphous nature, with spherical, crumpled particles which prove the existence of only one phase that can be an inclusion complex.

Particle size distribution shows the diminution of particle sizes after the spray-drying process, especially for acetazolamide, phenomenon which significantly influences the solubility and dissolution rate of the active substance.

In vitro bioavailability tests led to the conclusion that the presence of cyclodextrins strongly influences membrane diffusion of diuretics. For chlorthalidone, kneading and physical mixture do not modify considerably the diffusion process; the diffusion rate does modify by spray-drying, the best result being obtained for the 1:1 molar ratio.

For furosemide, an increase of membrane diffusion takes place in the presence of RAMEB, in binary or ternary products in molar ratio of 1:1 or 1:2. The complexation with the hydrophobic cyclodextrin produces the diminution of membrane diffusion, which offers the future possibility of delayed release pharmaceutical forms, by using low water soluble cyclodextrins. For acetazolamide and indapamide, an increase of membrane diffusion is also noticed.

The furosemide bioavailability was also tested in vivo, by HPLC analysis of rat plasma after oral administration of furosemide, pure or complexed by ultrasonication with RAMEB, in molar ratio of 1:2. The presence of complexed furosemide tends to increase the seric concentration and the bioavailability of the diuretic, the results being of statistical significance.

By using molecular modeling programs, I was able to calculate the difference between the sterical energy of the complex compared to the sum of sterical energies of individual components, which proves the natural tendency of diuretics to form inclusion complexes with cyclodextrins. The docking program FRED allowed me to establish the maximum affinity of diuretics for natural / semisynthetic cyclodextrins; the theoretical results concorded in general with the experimental ones.

As a conclusion, cyclodextrins are capable of forming inclusion complexes with diuretics, complexes that show the advantage of an increased water solubility and a superior bioavailability. An objective of future researches is the obtention of delayed release pharmaceutical forms, by using hydrophobic cyclodextrins; also, by combining hydrophilic and hydrophobic cyclodextrins, in a specific ratio, one can produce controlled release pharmaceutical preparations.