ABSTRACT

Conventional drug delivery systems were insufficient to treat more fish diseases. Hydrophilic and hydrophobic polymeric nanoconjugates have been developed for therapeutic administration to overcome the disadvantages of conventional therapy.

At the same time, polymeric nanoconjugates offer more effective treatment and their adverse effects are reduced.

Cyclodextrins are more favorable natural polymers to prepare conjugated nanoparticle delivery systems due to their physical and chemichal properties. The other advantages of cyclodextrins are: to incease bioavailability and stability of drug molecules and protect them from physical factors, including pH, temperature and some enzymes. These single polymers have the ability to form nanoconjugates in aquous medium spontaneously.

The doctoral thesis entitled “Nanoconjugates used in prophylaxis and treatment of certain fish diseases”, according to the rules in force, is divided in two parts, which contain a number of 152 pages; Part I, under the name of “Current State of Knowledge” a number of 35 pages in which data on cyclodextrins and emulsions are summarized and Part II entitlet “Personal Contribution” presenting the results obtained during the doctoral period. To these parts are added, according to the norms, the introduction, the abstract and the list of scientific references as well as the integral parts of the thesis such as contents, cover/covers, list of abbreviations and the list of tables of figures.

The first part, "Current state of knowledge", is deployed in four chapters and associated subchapters, starting the main groups of parasites are of concern both for vertebrates and invertebrates, and indicate the most important publications for these groups; Subsequently, international specialized literature on nanoconjugates of cyclodextrins, how they form, their structure and, most importantly, how they form inclusion compounds with certain active principles are presented. Then follow the complexing methodologies with these cyclodextrins as well as some examples of pharmaceutical products containing these polymers. Designing the creation of a nanoscale bonded nanoconjugate with cyclodextrins for the oral route target is largely based on the knowledge of clinical requirements and biochemichal parameters that alter the pharmacokinetic profile of the drug of interest and allows a deductive manipulation of its physicochemical properties for an effective delivery and biochemical interaction *in vivo*. Particle sizes, surface properties, molecular weight, hydrophobicity, and particle charge have been demonstrated as important and adjustable physicochemical properties for manipulating the clinical applicability of orally administered nanoconjugates.

In Chapter Four, which is called “Techniques and Methods of Stufying the Functional Characteristics of Emulsion Systems”, there are some general notions about emulsions. Then, there were recent data on emulsion stability, emulsion appearance and morphology, and finallysome extracts.

The Second Part of the doctoral thesis called “Personal Controbution” is carried out in eight chapters (V-XII), the first chapter is assigned to the description of the purpose, objectives and activities of the proposed study, followed by three chapters in which all parts of each study, and finally the final chapter is of course the final conclusions in which the remarks are reproduced by analyzing all the results obtained.

Chapter Five – “The Purpose and Objectives of Research”. The rationale for the importance of the researches that have been carried out concerns several aspects: finding a complex drug with a cyclodextrin, obtaining a therapeutic effect on a parasitic disease of the fish, namely the ichthyophtiriosis, and finally making an emulsified composition based on praziquantel and florfenicol with antibacterial and antiparasitic properties for fish treatment.

In Romania, infestations with fish parasites have beed documented in a limited number of studies and conducted mainly in the Danube Delta area and more rarely in other areas of the country. Therefore, the purpose of this thesis was to evaluate the therapeutic potential of a drug composition in cyprinid culture in Romania, through research designed to improve prophylaxis and fight against *I.multifiliis* infestation. In this regard, the proposed objectives were to: select a polymer capable of delivering an antiprotozoar drug, select the active principle to be complexed with the polymer, characterize the complex, evaluate an *in vitro* study at different concentrations, and establish efficacy by testing *in vivo*. For this, a series of activities were carried out for the proposed objectives and some of them with a preliminary or secondary aspects in a successive order. The activities performed covered a series of physico-chemical analyzes as well as statistical analyzes.

Chapter Six " Ichthiofauna Pathology Of Intensive Fish Breeding Systems " reflects the main factors leading to the occurrence of aquaculture disease, and then some parasitic diseases of particular importance in our country are described in two studies.

A study conducted by us was based on the laboratory diagnosis of cyprinids (*Cyprinus carpio*) parasitic diseases in a polyculture growing system in Iasi County for a period of six months. This study is an interpretation of parasitic co-infections, recorded in winter and spring (from December 2014 to May 2015) in cyprinid ground ponds, from a semiintensive polyculture system. A total of 55 carp fish were collected to detect the stages of parasites that resist during winter. The fish were examined daily for clinical signs. The parasitological diagnosis was performed by microscopic examination of gills and dermal scrapes from dead fish. Our investigations have led to the identification of a protozoan - *Ichthyophtirius multifiliis*, two monogenic trematodes - *Gyrodactylus spp*., *Dactylogyrus spp*. And a *Lernea cyprinacea* copepod with different intensity depending on the water temperature. A case study has also been carried out describing an episode of ichthyophtiriosis in trout (*Salvelinus fontinalis*) from a fish farm in Neamț County. Using optical microscopy, parasites were identified in 100% of the examined trout, at an intensity of 13 trophons / fish. The emergence of this disease was a surprise to us because the water that supplies the reservoir comes from an underground source and is considered free of parasitic entities. Anamnestic

information shows that the source of the invasive elements (*Ichthyophthirius multifiliis*) was represented by three fish from the family of Cyprinides (*Alburnus alburnus*) introduced as living fish food.

In Chapter Seven – "Synthesis and Characterization of Diminazene Aceturate Inclusion Complex", the complex inclusion compound with a cyclodextrin is characterized and discussed. Cyclodextrins - are macrocyclic biopolymers with potential applications in targeted delivery of therapeutically active micro/macro-molecular active principles. Despite the host-guest strong inclusion property, the inherent lack of cellular binding capacity has limited applications in drug delivery. In this scientific paper, β-cyclodextrin (β-CD) was chosen to form a complex inclusion compound with diminazene aceturate (DA), which are bioactive molecules, widely distributed in some cells and responsible for antiprotozoal activity. The inclusion complex of diminazene aceturate with β-cyclodextrin was performed and then confirmed by textural, thermogravimetric, calorimetric (DSC), spectroscopic (FT-IR) and microscopic techniques (TEM-transmission electron microscopy and AFM-atomic force microscopy) . Besides these analyzes, some theoretical and molecular modeling analyzes were carried out using Material Studio 4.0 sowware. Thus, the proposed β-Cyclodextrin/Diminazene Aceturate inclusion system could be used as a delivery carrier for the drug-specific distribution area. This nanoconjugate was obtained by the equimolar ratio of co-evaporation of 1: 1. Then a series of characterizations of the complex formed (nanoconjugate) were made and its purpose was to obtain an effect on a parasite (*I. multifiliis*) in farmed carp.

Chaper Eight entitled "*In vitro* Study of Complexed Diminazen with B-Cyclodextrin for *Ichthyophthirius Multifiliis*" - reveals the expected effect of the complex DM-β-CD inclusion compound by *in vitro* testing on the *I. multifiliis* protozoa. Here again, some details about cyclodextrins are then focused on diminazen aceturate, an antiparasitic compound. Diminazene aceturate(DA) is the active ingredient of trypanocid drugs used to treat animals infected with tripanosomiasis and babesiosis. It was selected to form the β-Cyclodextrin inclusion complex to establish the effect on the *I. multifiliis* protozoa. Test steps have been set and the results were promising.
Tests in filtered water were performed to allow automatic exclusion of the possibility of partial or total inactivation of the test substance under the given conditions. This substance was considered to be effective *in vitro* by this procedure, and it was further concluded that it was effective in administering the bath or in medicated feed for infested fish. The final doses tested were: 100, 50, 25, 12.5, 6.2, 3.1, 1.5 and 0.8 ppm (mg/l) for diminazene aceturate administered as such and for the complex β-cyclodextrin/diminazen aceturate.

In Chapter Nine, called "Studies Of *In Vivo* Investigation Of Beta-Cyclodextrin Diminazene Aceturate Complex Used In Ciliatosis Treatment ". Oral administration of many therapeutic chemical agents remains challenging due to poor gastric insolubility and/or weak dissolution, inefficient intestinal permeability and pre-systemic inactivation. These issues limit the benefits of comfort and increase the degree of compliance they offer in the treatment of many diseases. Cyclodextrin nanoconjugates

have emerged as promising agents for the drug-specific construction of these oral administration nanosystems capable of optimizing the desired physicochemical properties and pharmacokinetic parameters without compromising safety.

This part of the thesis focuses on recent and encouraging advances in the application of β-cyclodextrin nanoconjugates for the oral administration of some drugs for fish ichthyohptiriasis. A general overview of cyclodextrins and pharmaceutical nanotechnology in oral administration systems is provided. Some strategies being exploited for the implemented nanoconjugate synthesis as well as the smart gastrointestinal tract (TGI) navigation potential for bioavailability and optimal biodistribution are also illustrated.

Perspectives are also discussed to translate these nanoconjugates from clinically useful drug-friendly formulations.

Three groups of 7 *Cyprinus carpio* fish were used for testing. A control group, a lot to which I was given a simple medication feed, a group of medicated feed with the diminazen/β-cyclodextrin complex. The result was a therapeutically satisfactory, meaning that a reduction in the number of trophons was achieved at the end of the 10 days of treatment. After testing, a set of biochemical analyzes was performed on 3 fish from each lot, with insignificant differences.

Chapter Ten is titled “Conditioning of Praziquantel and Florfenicol for Some Heterologous Coinfections in Farmed Carp” and discusses a new pharmaceutical system of emulsions with commonly used active principles, namely praziquantel and florfenicol. We have directed our attention to a system that treats some fish coinfectives because many scientists are focusing on just one pathogen ignoring others. An emulsion can be defined as a two-phase system consisting of two immiscible liquids, one of which (dispersed phase) is dispersed finely and uniformly in the second phase (continuous phase). Since emulsions of thermodynamically unstable systems, a third agent, the emulsifier is added to stabilize the system. Their small size results in useful properties, such as the large surface area per unit volume, robust stability, clear transparent appearance and adjustable rheology. Emulsions are found in various fields, such as drug delivery, food, cosmetics, pharmaceuticals and materials synthesis. In addition, they serve as model systems to understand nanoscale colloidal dispersions. High and low energy methods are used to prepare emulsions, including high pressure homogenization, ultrasonication, phase reversal temperature and emulsion inversion point, as well as recently developed approaches. In this study, we summarize emulsion preparation methods, theories to predict droplet size and chemical additives that affect droplet stability and recent applications in the field of fish diseases. Emulsions were prepared for each of the two drugs, and the target for which they were formed was to obtain a controlled release of each of the active principles. For characterization dynal light scattering (DLS) was performed.

The realization of these double emulsions led to the creation of a patent application.

Chapter Eleven called "Results and Discussions" - summarizes the corroboration of the observations and remarks in this thesis.

The last chapter, Chapter Twelve, “Final Conclusions”, where 13 concluding observations of the research are conceived and some remarks are made after an overall analysis of the data presented in the previous chapters. The results of this study provide new data on treatments used in aquaculture by introducing new nanoconjugates. Observations made during this study signaled the possibility of treatments and the need to implement a new technique for the prevention and control of infestations with *I. multifiliis* in farm carp (*Cyprinus carpio*).