Revista de Chimie
https://revistadechimie.ro
https://doi.org/10.37358/Rev. Chim.1949

Characterization *of* β-cyclodextrin-diminazene Aceturate Complex Used to Treat *Ichthyophthirius multifiliis* Infection in Common Carp

ANDREI-CRISTIAN LUPU[1], SIMONA CIOBOTARESCU[2,3], GABRIELA LISA[2], NICOLAE HURDUC[2], LIVIU DAN MIRON[1], ELENA-LUIZA EPURE[2]

[1]University of Agricultural Sciences and Veterinary Medicine Ion Ionescu de la Brad, Faculty of Veterinary Medicine, 8 Al. M. Sadoveanu,700489, Iasi, Romania

[2]Gheorghe Asachi Technical University of Iaşi, Faculty of Chemical Engineering and Environmental Protection, 73 Prof. Dimitrie Mangeron, 700050, Iasi, Romania

[3]Laboratoire de Photonique d'Angers EA 4464, University of Angers, Physics Department, 2 Bd Lavoisier, 49045, Angers, France

Abstract*: This study was carried out to explore the efficacy of a veterinary drug, diminazene aceturate (DA) (4,4'-(1-triazene-1,3- dyil)bis-benzenecarboximidamide), in an inclusion complex with β-cyclodextrin (β-CD) as a suitable treatment for parasitic disease caused by Ichthyophthirius multifiliis in farmed carp. The efficacy was determined by the reduction in the infection intensity. The complexes were characterized through thermogravimetric analysis and molecular modeling. The selected stoichiometry for the chosen drug was 1:1. The geometry of inclusion complexes has been investigated by molecular modeling at semi-empirical level in order to find the most favourable structure. Administration of DA in its simple form and included in β-CD was carried out by embedding appropriate doses in the animal feed. Our studies suggest that when DA was used in the complex structure, a reduction in the infection degree and decrease in the trophont size in the treated fish was observed. From these first results of our study, the oral treatment with the complex of DA included in β-CD(DA–β-CD) is found to be a suitable alternative to bath treatments in carp diseases.*

Keywords*: carp, cyclodextrin, diminazeneaceturate, molecular modeling*

1.Introduction

Cyclodextrins (CDs) are nontoxic compounds with the ability to complex and stabilize a wide range of substances. Due to their low toxicity and low immunogenicity, CDs have extremely attractive pharmaceutical applications[1]. In recent years, the inclusion of CDs in drugs formulation aimed to improve the drugs properties, such as low solubility and slow dissolution rate, in order to enhance bioavailability and to reduce adverse effects. A large number of studies have been published on the inclusion complexes of CD, many of which to establish suitable preparation procedures. Thus, previous studies in our laboratory and those of other groups in this field aimed to assess the usefulness of the formed complexes with regard to aspects such as stability[2], bioavailability [3]restrainof unwantedeffects e.g. gastrointestinal ulcer[4] or undesirable organoleptic properties [2-5]. Most studies focus on medicines in the form of complexes for human use. Concerning the public health problems that have recently risen in livestock farms and in aquaculture, increasing attention is paid to control and formulation of medicinal products used in the treatment of animals destined to human consumption. The typical high propagation density of infections in aquaculture promotes the emergence of all types of infectious diseases (viral, bacterial, fungal and parasitic). Parasitic diseases pose a particular challenge, especially those caused by life-cycle species, including fast-spread ectoparasites. Such infections can have serious economic consequences, mainly due to outbreaks that have caused high mortality rates. For many such diseases, effective vaccines and treatments are not available yet.

**email: lepure@tuiasi.ro*

One of the most representative species in freshwater aquaculture is the carp - *Cyprinus carpio*. Parasites that affect this cyprinid include *Gyrodactylus sp.*, *Ichthyobodo necator* and *Hexamita salmonis* and histophagus ciliate *Ichthyophthirius multifiliis*. Ichthyophthiriasis is one of the most important ciliate fish diseases and results in significant losses in aquaculture industry [6,7]. This parasitic disease is commonly named "white spot disease" and is caused by *Ichthyophthirius multifiliis*, a ciliated parasite that has a global distribution and induces a devastating effect especially on juvenile fish [8.9]. The life cycle of *I. multifiliis* consists of an infective theront, a parasitic trophont, and a reproductive tomont [10,11]. The tomont stage includes non-encysted tomonts and encysted tomonts [12]. Chemotherapeutic agents used to control *I. multifiliis* cause food safety concerns and have potential mammalian and environmental toxicities [13]. For example, malachite green has been banned for use in food fish by many countries because its carcinogenic and teratogenic effects on humans [14]. Vaccination has been regarded as a potential prevention measure against ichthyophthiriasis in aquaculture [9.10]. However, immobilizing antibodies are serotype specific, and only protect fish against *I. multifiliis* with the same serotype of i-antigens [15]. Currently, there are no commercially vaccines against *I. multifiliis* available yet [7]. An alternate approach to control *I. multifiliis* is to use natural products [12,16,17].In the following study, we evaluated the efficacy of DA in the form of a complex with β-CD compared to its simple form. The drug is usually orally administered to other animals to treat parasitic infections [18]. In general, oral administration of drugs is preferred over administration by immersion for aquatic animals which often leads to environmental contamination.

In the oral treatment study, we evaluated the efficacy of DAand DA–β-CD complex, which were administered by incorporating them into the animal feed. Medicines, such as DA, have poor organoleptic properties and some animals reject the food that contains it. Thus, efforts to mask these properties are justified. When administered by immersion in water, its low solubility poses problems poor absorption in the intestines on one hand, and water contamination and waste container problems, on the other hand. Concerning the objective of medication uses, all these drawbacks cause a decrease in the treatment efficiency.

In the present study, we evaluated the use of CD as an inclusion host for delivery of the active substance to *I. multifiliis* infected farmed carp (*Cyprinus carpio*). We also examined how the taste of feed containing DA–β-CD complexis perceived by fish. The goal of this article was to increase the efficacy of a veterinary medicinal product, DA,in the treatment of this parasitic disease caused by *Ichthyophthirius multifiliis* in farmed carp. For this purpose, we modified the drug by complexation with β-CD and we embedded the DA–β-CD complex in the animal feed, and then monitored the infection's evolution. Efficacy is determined by evaluation of reduction in infection intensity. The DA–β-CD complex was prepared by the rotary evaporation and was characterized by thermal analysis and molecular modeling.There are some theoretical studies that address the inclusion process of some drugs in α, β or γ-CD.They focus on anticipating or clarifying experimental data, e.g. finding the most favourable positioning in the complex structures. The methods used are quite diverse, ranging from semi-empiric to molecular dynamics and Monte Carlo[19 - 23].

The selected stoichiometry was 1:1 between the β-CD and the DA. Administration of the complex was performed by including the appropriate dose in animal feed. Our results suggest that ingestion of feed mixed with the complexed drug leads to reduction of infestation intensity, along with the decrease in the trophont number in the treated animals (fish). Oral treatment with this drug complex may serve as an alternative to conventional medicine used in treating fish.

2.Materials

The drug diminazene aceturate andβ-cylodextrin were bought from Sigma-Aldrich. The commercial feed used was Aller Aqua A/S® for carp (blood products, DDGS, feather meal, fish meal, poultry meal, rapeseed, rapeseed oil, soya, sunflower protein concentrate, triticale, vitamins, minerals and premix, wheat).

Host used for complexation

CDs are a class of molecules shaped like narrow conical cylinder with two open unequal sides (one opening is sligthly wider in diameter than the other). By enfolding smaller molecules like in a loop, CDs act as hosts for the included molecules.CDs are substances composed of sugar molecules (D-(+) glucopyranose) linked together in a ring form, known as cyclic oligosaccharides. They are also called cycloamylose, cyclomaltose or Schardinger's dextrins and are non-reductive in nature. The CD's nomenclature is based on the number of units of glucose in its structure, so that the CD with 6 units of glucose is called α-CD, the 7-linkage CD is called β-CD and the one with 8 units is called γ-CD. The CDs glucopyranose units are linked via 1-4 bonds. The formation of glucopyranose unit linkages gives the CDs their conical shape with one side a bit larger than the other [24]. There are hydrogen bonds between the 2-OH and 3-OH groups around the side. These links are the weakest in α-CD and the strongest in γ-CD. Around the narrowerside, the 6-OH groups can also form hydrogen bonds, but the bonds are destabilized by dipolar effects and are not normally present in the CD crystals. In α-CD, the hydrogen bond is pure 3-OH (donor), 2-OH (acceptor). But in β and γ-CD, the bonds changeby swapping 3-OH (acceptor) with 2-OH (donor). The CDs are amphiphilic structures in which the 3-OH and 2-OH groups are exposed on the larger edge and on the narrower side of the 6-OH group. The wider edge is lined with these hydrophilic groups and the inner surface is etched by the ether as anomeric oxygen atoms. Thus CDs have a hydrophobic inner cavity and an outer hydrophilic surface [11].

For our study, we considered working with β-CD which was found to behave best for medicinal purposes, as α and γ forms showed considerable toxicity levels. We choose β-CD due to several factors determining drug/cyclodextrin interactions. For example, ionic interactions between drug molecules and cyclodextrin can improve the loading efficacy and stability of the complexes compared to negatively charged or uncharged drug conjugates. Secondly, increased temperatures can help destabilize the drug/ cyclodextrin conjugation[25].

Preparation of DA-β-CD complex

The inclusion complex with a molar ratio of 1: 1 was prepared by dissolving 1.7 g of DA and 3.74 g of β-CD in 40 mL of water. The solution was left to stir for 3h at room temperature. After that, the mixture was concentrated under vacuum and dried in the oven for 48 h to obtain the complex.

Thermal analysis

Thermogravimetric analysis (TGA) and derivative thermogravimetric analysis (DTG) were performed under nitrogen flow (20 cm^3/min) with a heating rate of 10°C/min from 25 to 800°C using a Mettler Toledo model TGA/SDTA 851. The initial mass of the samples was 2.2 - 3.5 mg.

Molecular modeling

Theoretical calculations were conducted to obtain overall information on the geometrical arrangement of structure of DA-β-CD inclusion complexes, and also to highlight the intermolecular interactions between host and guest. While in experimental studies and treatment efficiency evaluation the drug is used in the corresponding diaceturate salt form, simulations took into consideration only the therapeutic part of the drug, diminazene (DM) [26].

The geometry of inclusion complexes has been optimized with VAMP module from Materials Studio 4.0 package (Materials Studio). The Pub Chem (Pub Chem) and PDB databases (Protein Data Bank) respectively provided the initial structures of DM and β-CD.

Optimization of potential energies was performed to bring the complexes to a stable geometry. For this purpose, the VAMP module was used, a semi-empirical molecular orbital package capable of predicting geometries, heats of formation, dipole and quadruple moments, etc. The semi-empirical method was preferred due to its reduced calculation time for the studied system. Convergence tolerance was chosen *Medium* with 0,4 kcal/mol/Å | *Gradient norm convergence criterion*. PM3[27]NDDO

Hamiltonian was selected for calculation of potential energy, as recommended in biological systems, where mimicking the hydrogen bonds bears great importance due to the high number of these bonds and their directionality. PM3 has been shown to be a superior method of assessing hydrogen bonding compared to AM1[28]. Moreover, PM3seems to be more suitable for modeling of cyclodextrins and their complexes [29]. The equilibrium molecular geometries were established *in vacuo*.

Preparation and characterization of animal feed and the drug containing feed

Firstly, a homogeneous mixture was prepared by pestling together the drug complex and the commercial feed.

The feed is previously ground in a blade mill. The mixture is moistened with water to obtain a suitable consistency for subsequent extrusion. The blend is processed with an extruder with2-mm orifice cylinder. The obtained pellets are dried in a furnace for 24 h. They have an average weight of 0.2g and 1 kg of feed contains about 5000 pellets. The fish feed is mixed with the drug and the complexed drug. In order to prevent drug loss in water by full dissolution of the feed, release tests were performed. The maximum time necessary for feed ingestion is 15 minutes. The fish were fed once a day and ate the feed in the first 5 min, before pellet disintegration.

In vivo studies

Fish stock

The test used fish saplings of the *Cyprinus carpio* species with a body mass between 78 - 101 g.

The fish stock (*Cyprinus carpio*) was obtained from a local fish farm (Research and Development Center for Aquaculture and Ecology Iaşi from Al. I. Cuza University) and has been acclimated for at least 36 h in a 200-litre tank, with aeration and constant temperature (19 ± 1°C, *p*H 6,5). The natural light-dark cycle is simulated (14-16 light hours, 8-10 h dark). The fish were fed on a daily basis with commercial food.

Infection

The fish used for the analysis are naturally infected with *I. multifiliis* (Figure 1), but also with other parasites. They show clinical signs, which are then confirmed by dermal examinationon microscope (Motic x20).

Figure 1. Trophont of*I. multifiliis*collected from the skin of an infested carp (20x)

Drugs and treatment

The treatment trials were performed on groups of 7 infected fish, immediately after infection has been detected. Duration of *I. multifiliis* infection varies between 9 and 10 days and is temperature dependent [18-30].

The fish were kept in 180 litre tanks. The tank conditions (water source, aeration, pH, temperature, light/dark cycle) are the same as those of the acclimatization period.

The treatment followed a consistent scheme: 5g drug/kg feed for 10 days. The feed provided is calculated to be 1.7-2% of body weight per day (given all at once).

Simultaneous control tests were performed on identical groups fed with non-medicated feed. Throughout the test period, fish have been regularly monitored to ensure that they are eating and to check for signs of toxicity.

The efficacy of simple drug containing feed and complex drug containing to treat *I. multifilius* infection was compared.

Determining intensity of the infection

The intensity of the infection is evaluated at the end of the 10 days treatment. In accordance, fish were anesthetized by immersion in water with MS-222 (0.3 mg/l) in a 150 l separate tank until the breathing became weak. A mucus sample was taken by gently scraping a part of the body surface of the fish (skin and fins). The mucus sample was then microscopically examined (100x). In the case of naturally infected fish, the intensity of the infection was recorded on a five-point scale after examination of the entire skin surface.

3.Results and discussions

Thermal analysis

Thermograms were evaluated for the samples: β-cyclodextrin (β-CD), diminazene aceturate drug (DA), β-cyclodextrin – drug physical mixture (Mixture) and of DA–β-CD solid complex (COMP). The TG and DTG curves depicturedin figures 3 and 4 allowed establishing the main thermo-gravimetrical characteristics shown in Table 1.

Table 1
THERMOGRAVIMETRICAL CHARACTERISTICS

Sample	Stage	T_{onset}	T_{peak}	T_{endset}	W%	Residue %
β-CD	I	46	89	103	12.90	13.30
	II	317	329	340	73.80	
DA	I	42	106	146	10.47	25.91
	II	216	228	234	8.88	
	III	234	253	277	28.94	
	IV	342	372	550	25.80	
Mixture	I	49	95	117	12.56	22.16
	II	214	227	235	5.80	
	III	235	250	270	21.24	
	IV	304	322	440	38.24	
COMP	I	205	239	295	18.27	30.90
	II	295	320	390	50.83	

T_{onset} – onset temperature at which thermal degradation starts, T_{endset} – temperature at which the degradation process ends, T_{peak} – temperature at which the degradation rate reaches its maximum, W%,– quantity of degraded sample, residue – sample quantity that is not degraded till 800° C.

The obtained results indicate that if the mass losses within the range 40 – 140°C, caused by the presence of humidity and traces of solvents, are neglected, it can be stated that the thermal decomposition of β-CD takes place in one stage, with T_{peak}= 329°C. We encounter this stage both in the case of DA – β-CD physical mixture and DA – β-CD complex (COMP). The DA decomposition takes place through a series of three stages with T_{peak}= 228°C, 253°C and 372°C. As revealed in Figure 2, the first two decomposition stages can also be found in the physical mixture. The formation of COMP complex is confirmed by the different way in which the thermal decomposition takes place in the temperature range

200 – 300°C, compared to the DA and physical mixture (Figure 2 and 3). For the complex, we identify that the residue left at 800°C has the highest value from all samples.

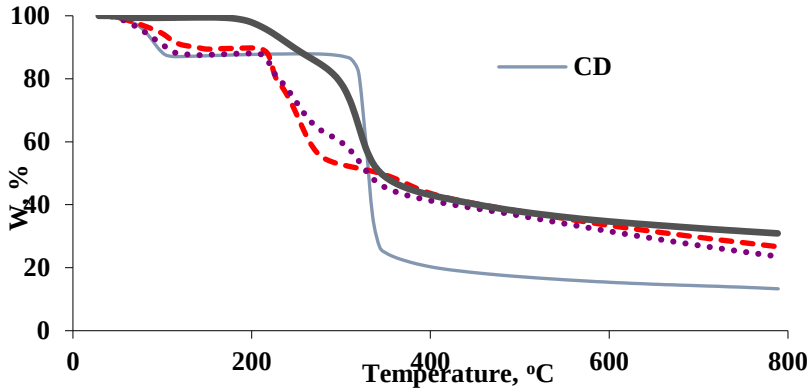


Figure 2. TG curves of analysed samples

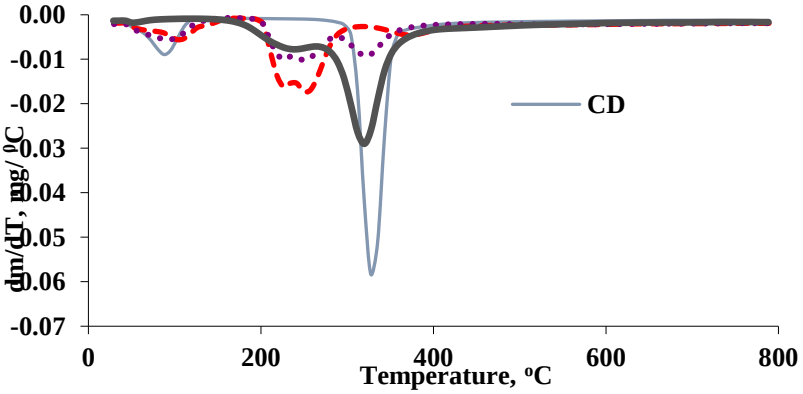


Figure 3. DTG curves of analysed samples

Theoretical analysis

In order to build the initial systems, the Best Fit Plane option was used (Figure 5). The centre of the reference system was considered the centre of plane of the β-CD. The geometrical centres of the two molecules were defined with the "Centroid" option and were used as reference points in our distance calculations.

Built of the inclusion complex assumes the DM's entry into β-CD as a linear sliding. Once the two molecules centres are overlapped, a rotation of DM is pursued, looking for the best orientation.

The insertion path of the guest molecule (DM) into the host (β-CD), which depicts the formation process of inclusion complexes β-CD:DM=1:1,has been accomplished taking into account two motion paths, similarly to a method previously described by another group [31].

a) The guest molecule enters the host through the larger opening, and enters through the narrow edgewithH1 part (Figure 4).This linear displacement along the OX axis defines different distances between DM and β-CD

b)When the host and guest molecules are positioned with their centroids overlapped, the guest rotates around its OX axis. This rotating shift generates different angular orientations of the host molecule included in the CD's cavity).

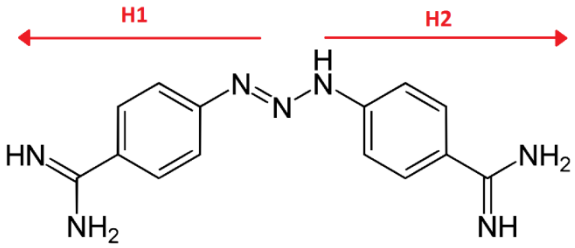


Figure 4. Diminazene molecule depicting the notation used to distinguish the two parts

Equilibrium geometries were obtained by varying the X (Å) distance between the of CD's and DM's geometrical centres. The X distance was considered in the range(-16, 18) Å. In figure 6, the variation of energy minima is observed. When the distance between centroids is in the range (-10, 10) Å, one DM end (or the centre) is within the CD cavity. It was found that at -12 and respectively12Å, the energy variation is not favourable to a stable complex formation. The fact that theydo not approach 0 indicates the presence of sufficient intramolecular bonds between DM and CD, even when the DM molecule is outside the CD cavity. If the centroids are at distances greater than 14 Å, then the molecules are sufficiently far so that there are no intramolecular interactions. The binding energy for these cases approaches the 0 value.

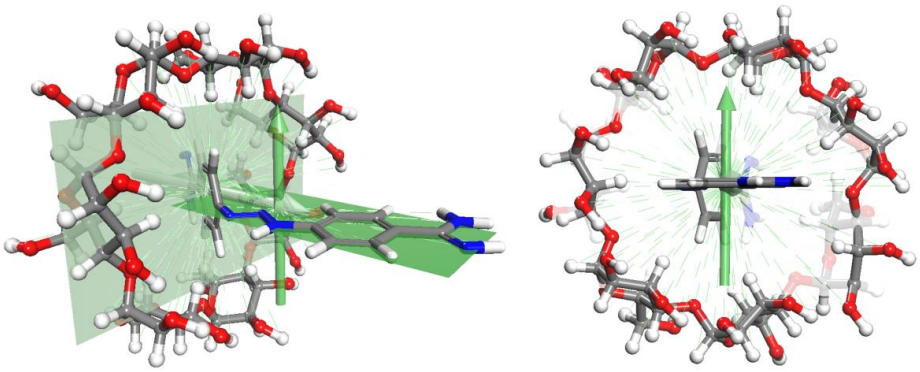


Figure 5. Representations of the inclusion CD-DM(H1) at 4Å distance (side-left view, perpendicular view on the right CD plane; CD- CPK representation, DM-stick representation)

Negative values of binding confirm the formation of inclusion complexes with various levels of stability. The binding energy value of the complex was calculated as the difference between the formation heats of the complex and of the guest and host molecule:

$$\Delta E = E_{COMP} - (E_{CD} + E_{DM})$$

where, E_{COMP} is the value of the formation heat of the complex and E_{CD}, E_{DM} represent the values of the formation heat of CD, respectively DM.

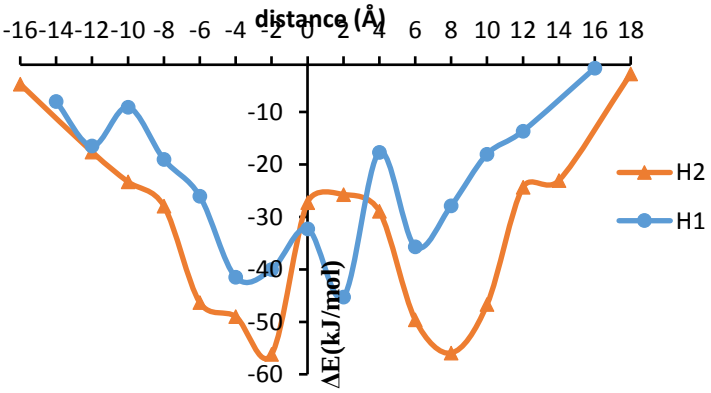
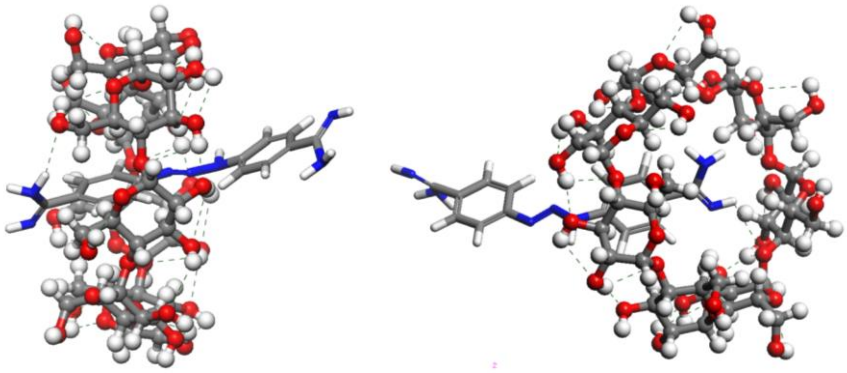


Figure 6. Dependence of binding energy of the complex on the distance between the centroids of host and guest for both inclusion directions of DM in the CD's secondary edge

For both orientations, H1 and H2, the most stable complexes(with lowest binding energy) are those that have the DM centroid within the CD at a distance of 2 and -2Å (the energy values are -45.2 and -56.19 kJ/mol) relative to the reference system (Figure 6). The next low energy complexes are those in which the guest molecule is partially included in the host molecule, having the H2 end inside the CD and H1 outside the large base (-55.9 kJ/mol) or the small base.

Although it is possible to form hydrogen bonds between DM and CD, this type of connection only occurs once in complexes with energies of -45.2 and -55.9 kJ/mol (the hydrogen bond was calculated for a maximum distance of 3Å) (Figure 7). In almost all cases, no hydrogen bonds were identified between CD and DM. Moreover, the number of hydrogen bonds formed in CDs in the complexes does not differ from that of isolated CD. This leads to the conclusion that these inclusion complexes are stabilized by van der Waals interactions.



a) b)

Figure 7. Complex structures corresponding to the energy minima a) -45.2 and b) -55.9 kJ/mol. Hydrogen bonds (represented by dotted line) formed between a) H from DM and a primary O andb) H from DM and a glycosidic O

The second way to search the energy minimum was by rotating the DM molecule plane around the OX axis, starting from the two structures with overlapping centroids. Angle Θ is defined by the axis perpendicular to the plane of DM and the OY axis is varied by 30˚ in the range 0-330˚, considering that the DM molecule penetrates the CD' cavity with the ends H1 or H2 (Figure 8).

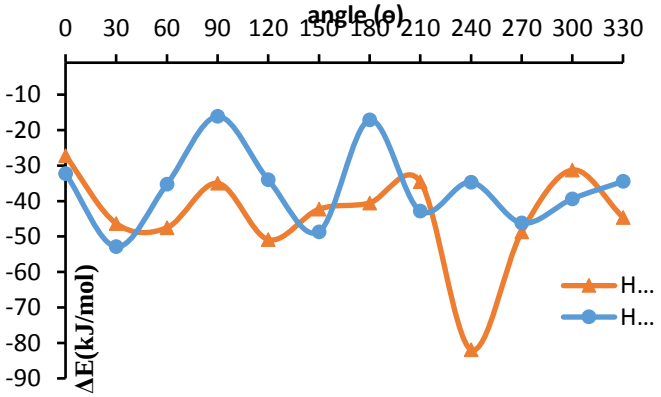
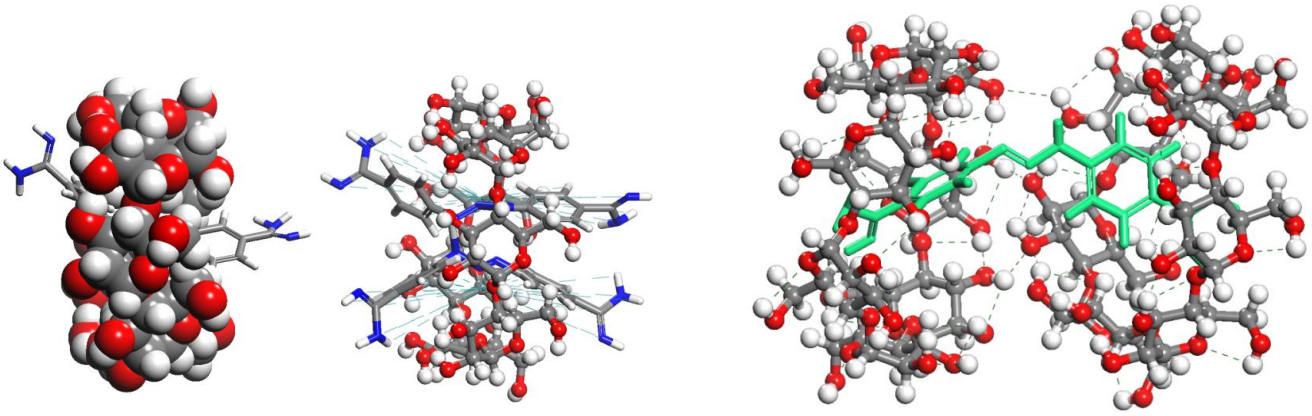


Figure 8. Dependence of binding energy of complex on the angular orientation of DM molecule (centroids overlap, both possibilities for inclusion through the large base of CD were considered)

Even if a slight stabilization is observed for H1 by varying the Θ, the most stable complex (formation energy -81.8 kJ/mol) is obtained for H2 with the orientation of 240 ° compared to the starting structure (Fig. 9.a)-left).



a b

Figure 9. Minimum energy structures ofa) – right CD:DM= 1:1molar ratio with CD – CPK and DM – stick representation, a) – left CD:DM= 1:2 with CD – ball and stick and DM – stick representation, b) CD:DM= 2:1 with CD – ball and stick, DM – stick and hydrogen bonds – dotted line representation

Since the β-CD cavity is large enough to include two DM molecules, the energy of such complex has also been calculated. Thus, the three molecules complex was considered having the centroids on the same line, so that there was no close contact between them. The two DM molecules are introduced with the opposite end through the large base. After minimization, the formation energy of the complex is 175.4 kJ/mol, indicating that the assembly is unstable because of steric repulsion effects (Figure 9.a) – right).

Another considered case was that of a CD:DM=2:1molar ratio of complexation, the DM molecule penetrating the large base of the two CDs (Fig. 9. b). The energetic minimumis -84.4 kJ/mol, indicate a slightly greater stabilization relative to the CD:DM=1:2 structure (Figure 9. a)-left). In this case, several intra- and intermolecular hydrogen bonds are formed between CDs, but none between DM and CD.

In vivo results

The selected dosage form (the drug containing food) requires that the feed pellets remain in water until ingested. Under these circumstances, it is very important to have minimal medication loss from the dosage form in the first few minutes in contact with water: this would lead not only to drug loss, but also to water contamination. The main feed components (flour, proteins, oils, etc.) and the procedures used to make it (typical extrusion) favour negligible or very slow release in the tank water. The DA–β-CD complex containing feed is eaten in the first 5 minutes from its release in water. Therefore, most of the pellets are in a compact form at the ingestion time, contributing to drug loss minimization.

Once ingested, the gastrointestinal environment favours the feed disintegration and digestion, which results in the release of the soluble components contained in the pellet. The tests searched for differences between the two drug forms: unmodified DA and DA – β-CD complex.The food that contained the complex was quickly eaten and there was no evidence of unsatisfactory flavour, indicating good palatability. By contrast, feed containing only the medicine, DA, was sometimes rejected, leading to a lower quantity of eaten food, therefore of drug. This is, of course, an important aspect of a treatment administered through the animal feed. CD sare known for their ability to hide the unwanted taste of drugs, such as bitterness, as DA has.

Table2
Infection evaluation of infected carp treated with feed containing da and da–β-cd complex compared to the control group

Carp no.	*DA – β-CD*	*DA*	*Control*
1	+	+++	+++
2	+	+++	+++
3	±	+++	+++
4	±	+++	+++
5	-	++	++
6	-	+++	**M**
7	-	++	**M**

Fish stock (7fish /group) naturally infested with*I. multifiliis*. Legend: zero (-) no trophond detected; very low (±), a single trophont; low (+) 2-10 trophonts; moderate (++) 11-50 trophonts; high (+++) >50 trophonts; (M) dead fish.

The test results show that the control group of infected untreated fish have maintained high infections, and 2 out of 7 fish died (Table 2) during the 10-day test period. In the fish group that received DA containing feed, no mortality occurred, but the intensity of infections remained high for whole group throughout the test period. For the fish group treated with DA – β-CD complex, the infection intensity completely diminished to zero in three out of seven fish and decreased at very low levels in the other four fish.

Table 2 shows the very high intensity of the parasite load in the first two groups, compared to the group treated with DA – β-CD complex in the feed.

Currently, more studies are conducted in our laboratory to investigate this phenomenon and to determine the doses and time needed for the treatment to completely eliminate the parasite.

Certainly, a subsequent challenge with artificial infected fish requires a further experiment, since naturally infected fish have a different degree of infection and it is not appropriate to test the efficacy of the drug using only naturally infected fish.

4.Conclusions

β-CD has a sufficiently large cavity for the formation of inclusion complexes with various drugs. The formation of the DM – β-CDinclusion complex, without fully incorporation of the guest, was highlighted by thermal analysis and also by molecular modeling. Comparison of the energy values of the equilibrium geometries indicates the formation of stable complexes for bothCD: DM molar ratio 2: 1 and 1: 1.

Preliminary results suggest that these inclusion complexes of β-CD with an antiparasitic drug are a promising option for the treatment of ichthyophtiriosis in farmed carp. Because *I. multifiliis* is a skin localized parasite, it is not easily accessed by oral drugs. β-CD inclusion complexes embedded in feed seem to improve the drug access to fish intestines and avoid the need to use immersion treatments, which often cannot be authorized because it raises concerns regarding public health and environment.

References

1.MARCOS B. L., *Strategies to Modify the Drug Release from Pharmaceutical Systems* - textbook-, 1st ed., Biopharmaceuticals (2015), pp 87-194.
2. SZEJTLI, J., *Cyclodextrin Technology*. Kluwer Academic Publishers (1988), Dordrecht.
3. VILA-JATO J. L., BLANCO J., VILAR A., *Spironolactone/h cyclodextrin complex: oral bioavailability in humans.* Acta Pharm. Technol. 32(1986), 82–85.
4.OTERO-ESPINAR F. J., ANGUIANO-IGEA S., BLANCO-MENDEZ J., VILA-JATO J.L., *Reduction in the ulcerogenicity of naproxen by complexation with h-cyclodextrin*. Int. J. Pharm. 70(1991), 35–41.

5.ANGUIANO-IGEA S., OTERO-ESPINAR F. J., VILA-JATO J. L., BLANCO-MENDEZ J., *Improvement of clofibrate dissolution by complexation with cyclodextrin*., Int. J. Pharm. 135 (1996), 161–166.
6.BUCHMANN K., SIGH J., NIELSEN C. V., DALGAARD. M., *Host responses against the fish parasitizing ciliate Ichthyophthirius multifiliis*., Vet. Parasitol., 100 (2001), pp. 105-116.
7.MATTHEWS R. A., *Ichthyophthirius multifiliis Fouquet and ichthyophthiriosis in freshwater teleosts*., Adv. Parasitol., 59 (2005), p. 159-241.
8.DICKERSON H.W., FINDLY R.C., *Immunity to ichthyophthirius infections in fish: a synopsis*, Dev. Comp. Immunol., 43 (2014), pp. 290-299.
9.XU D. H., KLESIUS P. H., SHOEMAKER C. A., *Effect of immunization of channel catfish with inactivated trophonts on serum and cutaneous antibody titers and survival against Ichthyophthirius multifiliis*., Fish. Shellfish. Immunol., 26 (2009), p. 614-618.
10.JØRGENSEN L.V.G., *The fish parasite Ichthyophthirius multifiliis - host immunology, vaccines and novel treatments*, Fish. Shellfish. Immunol., 67 (2017), pp. 586-595.
11.ZHOU J., RITTER H., *Cyclodextrin functionalized polymers as drug delivery systems*. Polym. Chem. 1; 2010, 1552–1559.
12.FU Y.W., ZHANG Q.Z., D.H. XU, LIANG J.H., WANG B., *Antiparasitic effect of cynatratoside-C from Cynanchum atratum against Ichthyophthirius multifiliis on grass carp*, J. Agric. Food Chem., 62 (2014), p. 7183-7189.
13.REVERTER M., BONTEMPS N., LECCHINI D., BANAIGS B., SASAL P., *Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives*., Aquaculture, 433 (2014), p. 50-61.
14.SHINN A. P., PICÓN-CAMACHO S. M., BRON J. E., CONWAY D., YOON G. H., GUO F. C., TAYLOR N. G., *The anti-protozoal activity of bronopol on the key life-stages of Ichthyophthirius multifiliis* Fouquet, 1876 (Ciliophora) Vet. Parasitol., 186 (2012), p. 229-236.
15.WANG X., DICKERSON H.W., *Surface immobilization antigen of the parasitic ciliate Ichthyophthirius multifiliis elicits protective immunity in channel catfish (Ictalurus punctatus)*, Clin. Vaccine Immunol., 9 (2002), p. 176-181.
16.LIU Y. M., ZHANG Q. Z., XU D.H., FU Y.W., LIN D.J., ZHOU S.Y., LI J.P., *Antiparasitic efficacy of curcumin from Curcuma longa against Ichthyophthirius multifiliis in grass carp*, Vet. Parasitol., 236 (2017), p. 128-136.
17.ZHANG Q.Z., XU D.H., KLESIUS P.H., *Evaluation of an antiparasitic compound extracted from Galla chinensis against fish parasite Ichthyophthirius multifiliis*, Vet. Parasitol., 198 (2013), p. 45-53.
18.TOJO J. L., SANTAMARINA M. T., UBEIRA F. M., LEIRO J., SANMARTIN M. L., *Trials for the control of ichthyophthiriosis in rainbow trout (Oncorhynchus mykiss).* Bull. Eur. Assoc. Fish Pathol. 14(1994b), 148–152.
19.CAIRO P., ORTUSO F., ALCARO S., FONTANANOVA E., TOCCI E., DRIOLI E., *β-Cyclodextrin interactions with three drugs used in inflammatory pathologies: An experimental and theoretical study*, Chemical Physics Letters, 454(2008), 4–6, 374-381.
20.GRANADERO D., BORDELLO J., PÉREZ-ALVITE M. J., NOVO M., AL-SOUFI W., *Host Guest Complexation Studied by Fluorescence Correlation Spectroscopy: Adamantane–Cyclodextrin Inclusion*, Int. J. Mol. Sci., 11 (2010), 173-188.
21.NAGARAJU M., NARAHARI SASTRY G., *TheoreticalStudies on Inclusion Complexes of Cyclodextrins*, J. Phys. Chem. A, 113(2009), 9533–9542.
22.RINIKER S., DAURA X., VAN GUNSTEREN W.F., *α-Cyclodextrin Host – Guest Binding: A Computational Study of the Different Driving Forces*, Helvetica Chimica Acta, 93(2010), 2318-2325.
23.SONG L. X., WANG H. M., XU P., YANG Y., ZHANG Z. Q., *Experimental and theoretical studies on the inclusion complexation of syringic acid with α-, β-, γ-, and heptakis(2,6-di-O-methyl)-β-cyclodextrin*, Chem. Pharm. Bull.(2008),56, 4, 468-474.

24.ARUN, R., ASHOK KUMAR C. K., SRAVANTHI V. N. S. S., *Cyclodextrins as drug carrier molecule: a review*. Sci. Pharm. 76(2008), 567–598.
25.NURUNNABI M., REVURI V., HUH K. M, LEE Y., *Polysaccharide based nano/micro formulation: an effective and versatile oral drug delivery system*. Nanostructures for Oral Medicine, 409-433(2017) (Elsevier Press).
26.PEGREGINE, *Pharmacology of diminazene: a review* , (1993), Acta Tropica 54 (3-4): 185-203.
27.STEWART J. J. P., *Optimizationof Parameters for Semiempirical Methods II. Application*s, J. Comput. Chem. (1989), 10, 2, 209–220.
28.DEWAR M. J. S., ZOEBISCH E. G., HEALY E. F., STEWART J. J. P., *Development and Use of Quantum Mechanical Molecular Models. 76. AM1: A New General Purpose Quantum Mechanical Molecular Model*., J. Am. Chem. Soc. 107(1985), 3902–3909.
29.LI X-S., LIU L., MU T-W., GUO Q-X., *A Systematic Quantum Chemistry Study on Cyclodextrins*, Monatshefte fur Chemie, 131(2000), 849-855.
30.TOJO J., SANTAMARINA, M. T., *Attempts at oral pharmacological treatment of Ichthyophthirius multifiliis in rainbow trout Oncorhynchus mykiss*. J. Fish Dis. 24(2001), 249–252.
31.FIFERE A., MARANGOCI N., MAIER S., COROABAA., MAFTEI D., PINTEALA M., *Theoretical study on β-cyclodextrin inclusion complexes with propiconazole and protonated propiconazole*, Beilstein J. Org. Chem., 8 (2012), p.2191-2201.

Manuscript received: 2.08.2019