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BUCHAREST

PHARMACY FACULTY

FORMULATION AND CARACTERISATION OF ORODISPERSABLE TABLETS CONTAINING CALCIUM CHANNELS BLOKERS CYCLODEXTRINS INCLUSION COMPLEXES

SUMMARY OF THE DOCTORAL THESIS

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According to the *World health statistics 2012* report, released in Geneva in May 2012, one in three adults worldwide has raised blood pressure – a condition that causes around half of all deaths from stroke and heart disease. Globally cardiovascular disease accounts for approximately 17 million deaths a year, nearly one-third of the total. Of these, complications of hypertension account for 9.4 million deaths worldwide every year. Hypertension is responsible for at least 45% of deaths due to heart disease, and 51% of deaths due to stroke.

In the early 1970s, a new class of Calcium channel blockers (CCB), calcium channel antagonists or calcium antagonists, dihydropyridines (nifedipine, amlodipine, felodipine, nimodipine) were discovered and introduced in the therapy of high blood pressure.

In our days, dihydropyridine (DHP) calcium channel blockers are often used to reduce systemic vascular resistance and arterial pressure. Sometimes when they are used to treat angina, the vasodilation and hypotension can lead to reflex tachycardia, which can be detrimental for patients with ischemic symptoms because of the resulting increase in myocardial oxygen demand.

Calcium channel blockers prevent the opening of calcium channels and thereby reduce the concentration of intracellular calcium. They mainly affect arterial vascular smooth muscle and lower blood pressure by causing vasodilation.

For the present study, we selected nifedipine, amlodipine, felodipine, and nimodipine, as active ingredients to be included in the cavity of three different beta-cyclodextrins.

Nifedipine (NIF), dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate, and Amlodipine (AML), 2-[(2-aminoethoxy)methyl]4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5- pyridinedicarboxylic acid 3-ethyl 5-methyl ester, are characterized by a high tendency to degradation when exposed to light. Oxidative aromatization of dihydropyridine fragment to the pyridine moiety is one of the main degradation pathways of amlodipine and related molecules of 1,4- dihydropyridine family (such as nifedipine) and occurs both in solution and in solid state and is promoted by light. These drugs absorb intensively in the UV-A (some derivates also in the visible) and are known to be photolabile. When amlodipine and corresponding besylate

were irradiated in a solution, both in the presence and in the absence of air, it was found to give the aromatized pyridine as the main product. Under exposition of nifedipine to daylight or to certain wavelengths of artificial light, it is converted to a nitrosophenylpyridine derivative, while exposure to ultraviolet light leads to the formation of nitrophenylpyridine derivative.
Photo-degradation products of amlodipine and nifedipine do not have pharmacological activity thus prevention of photo-degradation of their formulations is very important. For this purpose different kinds of protective agents are used. Several formulations including cyclodextrins, liposomes and microspheres have been prepared and characterized.

Also, the bioavailability of Nif is relatively low after oral administration of crystalline form due to the fact that it is practically insoluble in water and aqueous fluids.

Nimodipine, isopropyl (2-methoxyethyl) 1, 4– dihydro–2, 6-dimethyl–4–(3–nitro phenyl) 3, 5-pyridine-dicarboxylate, is a highly crystalline and practically insoluble in water and in aqueous fluids both acidic and alkaline. The aqueous solubility of nimodipine was reported to be 0.23mg/100ml3. Because of its limited aqueous solubility, it exhibits poor dissolution characteristics and its oral absorption is dissolution rate limited.
According to Biopharmaceutical Classification System, nifedipine, felodipine, and nimodipine are included in class II drugs (low solubility and high permeability), and amlodipine is a class I drug (high solubility and high permeability). (10)
In the last years, various oral formulations were developed in order to improve the solubility and bioavailability of nifedipine, felodipine, and nimodipine, and also to increase the stability of amlodipine besilat, and also of nifedipine. Among them: solid dispersions, nanocrystals, micronations of drugs are the most commonly used, leading to different results. Our attention is oriented in including the selected dihydropyridines in the cavity of different beta-cyclodextrins (natural and semisynthetic ones) in order to improve their disadvantages and increase the ability to formulate them in tablets for oral dispersion.
It is well known that cyclodextrins are a family of three industrially produced cyclic oligosaccharides, and several minor, rare ones; obtained by the enzymatic degradation of starch by the cyclodextrin glucosyl transferase. The three major natural cyclodextrins are crystalline, homogeneous, non-hygroscopic substances that have a torus-like macroring shape built up from glucopyranose units. Depending on the number of α-1,4-D(+)-glucosidic linked glucopyranose

units, several natural cyclodextrins have been described among which α-cyclodextrin (6 units), β-cyclodextrin (7 units) and γ-cyclodextrin (8 units) are of particular interest from the pharmaceutical point of view.

Crystal structure analyses of natural cyclodextrins have demonstrated that all glucose residues in the ring possess the thermodynamically favored $^4C_1$ chair conformation because all substituents are in the equatorial position. Cyclodextrins behave more or less like rigid compounds with two degrees of freedom: rotation at the glycosidic links C(4)-O(4) and C(1)-O(4) and rotations at the O(6) primary hydroxyl groups at the C(5)-C(6) band. As a consequence of this chair conformation, all secondary hydroxyl groups C(2) and C(3) are located at the broader side of the CD torus in the equatorial position. Hydroxyl groups on C(2) point towards the cavity and hydroxyl groups on C(3) point outwards. The primary hydroxyl groups at the C6 position are located at the narrower side of the torus. These hydroxyl groups ensure good water solubility for the natural cyclodextrins. The cavity of the torus is lined with a ring of C-H groups (C3), a ring of glycosidic oxygen atoms and another ring of C-H groups (C5). Thus, the cavity of cyclodextrins exhibits an apolar character. This is accompanied by a high electron density and Lewis base property. The physico-chemical characteristics and inclusion behavior of cyclodextrins are a direct consequence of these special binding conditions.

Contrary to the advantageous structure of cyclodextrins for molecular inclusion, their hydrophilic external surface makes it more difficult for the highly hydrophilic cyclodextrin molecule to react with lipophilic biological membranes. For this reason, natural cyclodextrins have been chemically modified to alter their water solubility, interaction with biological membranes and drug release properties.

Modification of the natural cyclodextrins has been the aim of many research groups to improve safety while maintaining the ability to form inclusion complexes with various substrates. Some groups have also focused on improving the interaction between pharmaceuticals and cyclodextrins while others have attempted to prepare materials that can be chemically defined more precisely.

In the present study, to complex the selected dihydropyridines, we chose two different beta-cyclodextrins: hydroxypropyl-β-cyclodextrin (HP-β-CD) and methyl- β-cyclodextrin (Me-β-CD), due to the fact that the molecular dimensions of the active ingredients are suitable to encapsulation in the cavity of α- or β-cyclodextrins, forming inclusion complexes, the cavity of γ-cyclodextrin being too large and not being able to create a stable complex with them.

To improve compliance and make the administration convenient, the design of new dosage forms gained significant importance. Conventional oral drug delivery presents a drug with a quick and full release that may go as such without producing the desired effect may be due to the presence of food, pH of the stomach, enzymatic degradation, change in GIT motility as so forth, giving not enough time to get absorbed. Recently much light is being put on the area of designing drug delivery systems bearing organoleptic elegancy and maximum patient acceptability in pediatrics and geriatric groups. A lot of innovative work is being done on drug delivery in which the oral route is preferred because of ease of administration, cost-effective therapy, self-medication and noninvasive method leading to patient compliance to a higher level.

The tablets for oral dispersion are designed to disintegrate in the mouth without the aid of water. Different methods are adopted to manufacture the orodispersible tablets with the aim of giving fast disintegration to the dosage form as it gets in contact with saliva with a good agreeable moth feeling.

These are tablets that get dispersed or disintegrate when gets in a contact with saliva with the release of the active ingredient, providing maximum drug bioavailability as compared to the conventional dosage form. This dispersible property is given by the addition of superdisintegrants to the dosage form, which releases the drug in the mouth increasing the bioavailability. The best time for an orodispersible tablet to get dispersed is considered to be less than a minute, but mostly the disintegration times vary from 5 to 30 seconds.

Due to the fact that the tablets for oral dispersion are considered to be the future of oral medication, in the current study, we decided to formulate the inclusion complexes of the three selected dihydropyridines with the three mentioned beta-cyclodextrins in orodispersible tablets using modern superdisintegrants and direct compression technique.

Another challenge for our study is the characterization of the release of active ingredients from the obtained tablets for oral dispersion. In the biopharmaceutical phase of the study is very important to establish the release or the dissolution rate, this being the reflection of the absorption and bioavailability of the active ingredient.

The objectives of the present study are:

- The preparation of the inclusion complexes of nifedipine, amlodipine, and nimodipine with each of the two cyclodextrins (hydroxypropyl-β-cyclodextrin, and methyl- β-cyclodextrin) using three different techniques: two in solution – lyophilization and coprecipitation methods, and one in the solid state: kneading method. By these, 36 solid binary systems are obtained.
- The confirmation of the formation of all inclusion complexes in a molar ratio of 1:1 (active ingredient: cyclodextrin) and the characterization of their properties in comparison with their corresponding simple physical mixtures (prepared in the same molar ratio) using different techniques such as: Fourier transform-infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), X-ray diffraction (XRD) and thermal measurements (TG/DTG and DSC).
- The mixing of all the studied inclusion complexes with the excipients used for the direct compression method, in order to obtain the direct compressible composed powders.
- The establishing of the flowing and compressible characteristics of the composed powders by determination of the morphological and mechanical properties of particles, such as: the flow rate, the angle of repose, the volumetric characteristics, the bulk properties of powders (compressibility, permeability, and bulk density), the Hausner ratio, the Carr index, the particle size, and the moisture content.
- The direct compression of the complex powders in order to obtain tablets for oral dispersion.
- The specific qualitative and quantitative control of the manufactured tablets for oral dispersion according to European Pharmacopoeia, EMEA guidelines, and specialized literature, following the organoleptic characteristics including the measure of physical attributes (size – height and diameter), the weight and content uniformity, the pharmaceutical properties (hardness, friability, disintegration time, moisture content), identification and assay of active ingredients, the chemical purity tests.
- The study of *in vitro* release of active ingredients and the comparison of dissolution profiles obtained for the tested products and the reference products, showing the influence of the

type of beta-cyclodextrin used, of the method applied at the preparation of the complex, and of different excipients selected for the formulation of the orodisperabile tablets.

In this doctoral thesis, we aimed to formulate inclusion complexes of three calcium channel blockers widely used in current therapeutic practice (nimodipine, amlodipine and nifedipine) in hydroxypropyl-β-cyclodextrin and methyl-β-cyclodextrin, in order to further process them in the form of orodispersible tablets that ensure the disaggregation of the pharmaceutical form and the rapid release of the active ingredients, which leads to the improvement of pharmaceutical availability.

The thesis is structured in two parts:

1. the general part
2. own contributions.

The first part of the doctoral thesis, the theoretical part, presents the current state of knowledge contained in three chapters, in which general notions about cyclodextrins and their use in the pharmaceutical industry, data about calcium channel blockers and their role in antihypertensive medication, are presented, some generalities about orodispersible tablets, detailing the advantages for which they are the focus of current scientific research.

The theoretical part includes the essential characteristics of the calcium channel blockers used in the present study, as well as the rationale for their inclusion in the cavity of cyclodextrins. Also, in the general part, the information on the motivation for the selection of the two cyclodextrins is provided.

The second part of the thesis, the experimental part, presents the own scientific contributions regarding the influence of cyclodextrins on the direct compression process and the essential pharmaceutical and biopharmaceutical properties of orodispersible tablets with calcium channel blockers and includes three chapters from which they can be formulated the following conclusions:

→ A first direction of research was the preparation, confirmation of formation and characterization of inclusion complexes between nimodipine, amlodipine and nifedipine and HP-β-CD and Me-β-CD, in a molar ratio of 1:1, both in aqueous solution, as well as in the solid state. Complexation in solution was achieved by two methods: coprecipitation and lyophilization. The inclusion complexes in the solid state were prepared by the trituration method. The binary systems made

were subjected to physico-chemical characterization by comparison with the simple physical mixtures prepared in the same molar ratio of 1:1. The complexes were evaluated by scanning electron microscopy (SEM), X-ray diffraction, dynamic differential calorimetry (DSC) and IR spectroscopy (FT-IR).

Following the evaluation of the morphology of the inclusion complexes by comparison with the substances as such and with their simple physical mixtures, through SEM, a significant change in the shape, size and structure of the binary systems was highlighted, which leads to the conclusion that the phenomenon of inclusion of the medicinal substance in cavity of cyclodextrins occurred. It was highlighted that the active ingredients are characterized by the presence of crystalline particles, HP-β-CD and Me-β-CD are amorphous powders, and in the case of physical mixtures, the active ingredients adhere to the surface of the cyclodextrins, confirming the affinity between the substrates. The morphologies of the inclusion complexes differ between samples depending on the type of active ingredient encapsulated and the preparation method chosen. Thus, in the case of nimodipine, the complex obtained by coprecipitation shows the fragments of cyclodextrin and smaller particles of nimodipine, for the one obtained by lyophilization, the SEM micrographs indicate a heterogeneous mixture between the two components. The cyclodextrin shells in the triturated sample appear to be filled with nimodipine, while smaller nimodipine particles and cyclodextrin fragments are also present in this sample.

In the amlodipine case in the system obtain by trituration method, Aml crystal are observed that adhere on the cyclodextrin surface. In return, by using the lyophilization and coprecipitation methods, there are drastic changes in the morphology of the finished product.

In this case it is not possible to differentiate the two initial compounds.

This is the proof of interaction between Aml and the cyclodextrin used. If in the coprecipitation compound it is still possible to observe the initial isolated compounds, in the lyophilization system a new solid phase is formed. In this case it is observed the amorpha aggregate is formed.

In case of Nif-HP-β-CD and Nif-Me-β-CD obtained complexes through trituration and coprecipitation methods, blocks are observed but the particle distribution it is more homogenous in the lyophilization obtain compounds.

Through the use of the three complexion methods new solid phases were formed with amorph structures in all the binary cases studied.

Observing that coprecipitation method and trituration method, the complexation was just partial in the lyophilization method was total.

If some small nanoparticles of amlodipine are still present in the systems obtain by trituration and coprecipitation, the lyophilization method has a new amorph compound.

In case of Nif-HP-β-CD and Nif-Me-β-CD obtained through trituration and coprecipitation methods big blocks are observed, while the particles distribution is more homogenous in case of the complexes obtained through lyophilization method.(cap. 4.2.).

While the melting top of nimodipine is almost gone in the lyophilization methods and trituration it was still visible in the physical compound obtained through coprecipitation but at lower temperatures. The move of the endothermic peak of Nim to a lower temperature indicates a certain type of interaction between the active ingredient and HP-β-CD. One can assume that the physical compounds formed by coprecipitation of Nim with HP-β-CD contained some quantity of crystalized Nim, but the lyophilization processes and trituration samples contained small quantities of none of crystalized nimodipine.

Thermograms DSC registered between 25°C and 250°C showed a characteristic look for the active ingredients a crystallized look, peak found in the physical compounds, but smaller intensity then the main substance, thus demonstrating the affinity of the two substances, but not forming the inclusion complex.

While the peak melting of nimodipine is almost gone in the lyophilization and trituration methods, this was still visible in the physical compound for the coprecipitation method, but it was moved in lower temperatures.

The move of the endothermic peak of Nim to a lower temperature indicated ta certain type of interaction between the active ingredient HP-β-CD. One ca assume that the physical compounds and the compound formed through coprecipitation contained crystalized Nim, but the lyophilization and trituration samples did not contain crystallized nimodipine. Based on the DSC results it was observed that the lyophilization method it is the best method to obtain inclusion complexes nimodipine and HP-β-CD, respectively, Me-β-CD, raport molar 1:1.

The melting peak for Aml it is shown at a lower temperature, with different intensities in the physical compound and the coprecipitation one, proof that the inclusion complex is not formed, The absence of melting temperature of 140,2°C was observed in the case of the compounds formed

through coprecipitation and lyophilization method. These hypothesis indicated the formation of the inclusion complexes in a raport molar de 1:1 using the lyophilization and trituration methods.

DSC curves of Nif and both physical compounds have demonstrated the presence of the pointed endothermic peak 172oC corresponding to the melting of the drug. This melting point is typical for Nif and was not observed at the same intensity and temperature as the DSC curves of inclusion complexes Nif-HP-β-CD and Nif-Me-β-CD. These data suggests that an amorphization took place (cap. 4.3).

Regarding the partial inclusion of the active ingredient in the physical compounds between Nim and the two CD, this proved a good affinity of Nim and CD, even though in the physical blending raport molar 1: 1 was used. Diffractograms X rays obtained for the inclusion complex formed through coprecipitation have shown a polymorph change of nimodipine. In the case of the lyophilization methods and trituration methods the disappearance of the peaks for nimodipine supports the conclusion of a powerful interaction between the formation of the inclusion complex in case of lyophilization. In the X ray diffractograms of the sample in case of trituration method, some spikes of Nim overlap the amorph model of CD, that indicates a partial inclusion. The coprecipitation method favors the polymorph change from I to II of Nim. The proofs of the complex formation Nim-CD are more obvious through lyophilization confirming DSC and SEM results. For the amlodipine compounds. In the physical mixture, the diffraction spikes for Aml are present with a small decrease of their intensity confirming that this method there is no interaction. The diffractogram of the compounds obtained through coprecipitation have shown a weak interaction between the two compounds, iterating the presence of the most important diffractions peaks of Aml at smaller intensities. On the contrary, the trituration and lyophilization methods support the formation of the inclusion complexes due to the fact that the diffraction peaks of Aml are gone, and the new formed compounds have an amorph model.

The Nif-CD inclusion complexes obtained by the trituration and lyophilization methods do not show the crystalline pattern of Nif that was verified both for the pure active ingredient and in the case of the inclusion complex obtained by the coprecipitation method between Nif and CD. At the same time, the amorphization of the active ingredient was also observed for the Nif-CD inclusion complexes obtained by trituration and lyophilization.

For the physical mixtures both for Nif-HP-β-CD, and Nif-Me-β-CD, Xray diffractograms shows a decrease of the peaks intensities and by result a decrease of the crystalline nature typical to polymorph A of Nif.

In addition, it could be observed the presence of all peaks characteristic to pure compounds in the diffractograms for the coprecipitation mixture, this demonstrates that the complexation between the compounds did not take place.

Contrasting with the compounds NIF-HP-β-CD și NIF-Me-β-CD obtained through lyophilization and trituration, where it was observed a decrease in the degree of crystallinity of the compounds although some peaks of pure Nif are still detectable.

The results through XRD and DSC and the low crystallinity observed in the systems obtained through lyophilization confirms the interactions in the solid state, reiterating the formation of the inclusion complexes (cap. 4.4).

The spectral analyses FT-IR have demonstrated in case of the amlodipine inclusion complexes that the peaks characteristic to Nim are almost not visible or they were shifted to a higher frequency and only the CD peaks were observed at approximately the same wavelength. Thus we may conclude that the characteristic functional groups from the active ingredient molecule were involved in the inclusion process. This demonstrates that the active ingredient was included in the CD cavity even partial inclusion, was noted in the physical mixture. In the FTIR spectrum of the inclusion complex Nim-CD formed through coprecipitation almost all the nimodipine and CD spikes were observed with no significant differences, demonstrating in this case a partial inclusion for a molar report of 1:1. In case of trituration inclusion complexes, the FTIR spectrum shown some modifications comparing to previous specters. More spikes from nimodipine are still present in the spectrum of the inclusion complex that demonstrates that the inclusion process was not complete. The characteristic C=O absorption band of nimodipine in case of lyophilization is shifted to a higher frequency (1701 cm-1) and it is due to the rupture of the hydrogen links of nimodipine.

FTIR spectrums of the physical mixture Aml-HP-β-CD and of the inclusion complexes obtained though coprecipitation are comparable. The characteristic peaks of amlodipine are shifted to different frequencies. In care of the compound obtained through trituration and lyophilization there is no record of the bands whose position, shape and intensity are characteristic to amlodipine. Also comparint the spectrums FTIR of the two CD with the FTIR spectrums corresponding to the

inclusion complexes obtained through lyophilization complex bands can be seen at 1685 cm-1 and 1465 cm-1 that are not present in the host molecule. This proves the interaction between the active ingredient and the CD molecule through N-H. In the inclusion complexes spectrums, not only that the peaks shifted, but some of them specifically disappeared when comparing the spectra of the compounds Aml and CD pure and the resulted complexes. This is due to the blocking of the active ingredient in the cyclodextrin cavity through the creation of hydrogen connection.

In the FTIR spectrums of the physical mixtures Nif-CD and the complexes Nif-CD obtained through coprecipitation, there were no new peaks observed. The main peaks were maintained connected to the functional groups of the two compounds. In case of the inclusion complexes Nif-CD through trituration and lyophilization a shift of the main absorption bands was observed for Nif and CD. These results indicates the involvement of these functional groups in some interactions connected to the formed inclusion complexes (cap. 5.5.).

Through all these studies was shown that Nim, Aml and Nif form stable inclusion complexes with both cyclodextrins HP–β–CD and Me–β–CD, in a molar ratio of 1:1, but through trituration and coprecipitation methods the complexation was partial although using lyophilization was complete.

→ The second direction of the research was the reformulation of orodisperable tablets containing inclusion complexes of Nimodipine, Amlodipine and Nifedipine in HP-β-CD şi Me-β-CD. Taking into account the results of physio chemical characteristics of the complexes , the inclusion of active ingredients was at its maximum when we used the method of lyophilization for preparation, which signifies that these systems will show a better performance in vivo.

Lyophilized complexes has an amorphous character and it's well known the fact that, though this is beneficial for dissolution performance, it induces a lower flow and compressibility characteristic. These attributes are essential for tablets manufacturing, especially for direct compression process. Processing of lyophilized powder is a challenge and requires a good formulation and an adequate selection of right process parameters. Excipient selection is a key stage in tablets manufacturing, and obtaining and adequate flow and compressibility of a tablet mixture is crucial for assuring a high quality manufacturing process for solid forms medicines.

The quantities of inclusion complexes and the excipients were calculated in a such way that the dosage for active ingredients for a tablet not to exceed 30 mg for nimodipine, 5 mg for amlodipine and10 mg for nifedipine, that corresponds to usual therapeutic dosages.

We obtained 18 powder mixtures, corresponding to those 3 active ingredients and two cyclodextrin, through mixing them with direct compressible excipients which have good properties, having suitable aspects, they were subject to future testing of flow property and compressibility, in order to evaluate and decide and final formula for production.

The obtained powder presented likewise organoleptic characteristics, fine, homogeneous, no particle agglomerations, F1-F12 formula have white color, and F13-F18 have white-yellow (chapter. 5.1.).

In a first stage, there was an evaluation of the particles of the obtained mixtures. The distribution of particles of the different formulations follow heterodisperse characteristics. All the six formulations corresponding to Nimodipine have a large distribution of particles dimension, majority of them are situated in interval between 80-600 μm, while over 800 μm there were nearly none found. The registered results are typical for materials that contain different components and may have the tendency to segregate, which will influence negatively the uniformity of the mixture. Also, due to differences in particles dimension, each of the components may act as individual particles. F1 and F4 show similar characteristics, with the smallest variability, having a small portion of the particles in interval outside the range of 160-600 μm, while F2, F3, F5 și F6 have particles with a high variety of diameter interval, but with a big fraction that have particles more smaller in the interval of 80-315 μm, with no significant differences usually between these four samples.

In the case of complexes with Amlodipine, it can be observed that all systems of particles show a large distribution of particles dimension, but differences between materials are as well important to be taken into consideration. For all six formulations of Amlodipine, majority of the particles have dimensions that fit in interval 160-600 μm, at a first glance predicting a similar behavior. Although, F7 and F10 present a narrow distribution, with low quantities of particles below 160 μm or over 600 μm, meanwhile other formulations (F8, F9, F11 și F12) have a larger arrangement. In this way, F7 and F10, based on silicified microcrystalline cellulose, present the desired distribution due to granulometry, more suitable than other formulations for consistency of manufacturing process as well as for tablet quality.

For complexes of nifedipine, the studied formulations present different granulometric properties, with similar behavior between F15 and F18 on one side, and the other four (F13, F14, F16 și F17) on the other side. Materials based on silicified microcrystalline cellulose and Disintequik™ ODT

have a considerable proportion of particles with dimensions between 160 and 600 µm, over 90% for F15 and almost 87% for F18, showing a more restricted range of all proposed formulations. Meanwhile, F14 and F17, which contain as excipients F-Melt® and Disintequik™ ODT, it holds the greatest part of particles in teh range of 80-160 µm of all formulations.

The majority of the particles have dimensions in the range of 160-600 µm, but the variability within the batches marks the differences in flow performance, especially for formulations that contain a larger ratio of particles smaller than 160 µm (chapter. 5.2.).

The characterization of compounded powder flow was realized through direct methods (determining the time and the speed of the flow) and through indirect methods (identifying the angle of rest and apparent density).

The flow behavior is an essential characteristic for filling entirely the matrix and inherent for unformal dosage of the tablets, being in the same time relevant for predicting manufacturing issues. All the six mixtures containing Nimodipine showed a slow flow, because it was only possible to measure it only by agitating it with a speed of 10 rpm through a nozzle of 15 mm, meanwhile there was no constant and consistent flow through a nozzle of 10 mm, even agitated, nor through a nozzle of 15 mm at a lower speed. This behavior is characteristic to amorphous systems with particles of small dimensions. Nevertheless, the value of the rest angle obtained for F1 and F4 fits within the range 25-30, considered by pharmacopeia specifications as to be corresponding to an excellent flow, but because of the experimental conditions, it can not be stated that they present a good flow, but it's suitable for compression process and it's better than the other four formulations. Meanwhile, F5 presents best value for rest angle (39,42), followed closely by F6 and F3 (38,96, respectively 38,71), then, F2 with 36,22, showing an unsatisfying quality for these materials.

The time of flow was around 12 seconds for F1 and F4 and over 13 seconds for the rest of the formulations, proving the influence of the selected excipients over the compressible mixture.

The longest flowing time (over 17 sec.) and the slowest speed (3,428 g/s) were registered for F5, but similar values were observed and for F6, F2 și F3, demonstrating the week flowing characteristic for these formulations. Though all used excipients are specially created for the technology of direct compression, having fluidity characteristics and high compressibility , the fact that the inclusion complex represents between 24 and 29 % of the final formulations affects directly the mechanical behavior of the mixture.

One can notice that the powder that contains the inclusion complex Me-β-CD have a reduced flowing attribute in comparison to powder containing the inclusion complex HP-β-CD, but also the incorporation of F-MELT in the formula, it conducts to a slower and reduced flowing speed.

Neither in the case of formulations based on Amlodipine (F7-F12) none of the formulations doesn't flow through a nozzle of 10 mm, but its flow could have been measured after it was changed to a nozzle of 15 mm, with no need of agitation. The behavior is not surprising, because active ingredients are amorphous components, and the mixture is formed from small dimension particles. The registered results confirm a proper flow for F7 and F10, as it was supposed to from previous result tests. Only F7 and F10 presented pause angles under 30 and speed of 6,451 g/s for F7 and 5,882 g/s for F10. As in the case of Nimodipine, also for Amlodipine the flow is weaker for materials containing Me-β-CD, than with HP-β-CD, but also F-Melt and Ludiflesh determined a reduction of flowing capability of the powders.

In the case of nifedipine, the flow varies considerably between formulations, indifferent of the type of cyclodextrin utilized. F15 presents the highest speed of 6,185 g/ml, followed by F18 with a speed of 5,825 g/ml. F13, F14, F16 and F17 registered a flowing time higher, nearly double in comparison to F15 and F18. According to specifications of European Pharmacopoeia regarding to the values of teh pause angle, F15 and F18 are classified ah having an excellent free flowing , meanwhile F14 and F17 present a good flow, and F13 and F16 juts a normal flow. Taking into consideration that the analysis couldn't be operated on nozzles with smaller diameter because the samples didn't flow, and the results are only registered for 15 mm nozzle, one can admit that only F15 and F18 hold am adequate flow for materials with direct compression.

The speed and the time of the flow, as well as the angle of the pause are influenced by the type of utilized cyclodextrin, HP-β-CD creates a better flow of the powder mixture in comparison with Me-β-CD. As well, diluent excipients influence significantly flow of materials, taking into account that the lubricant used is always the same, Magnesium stearate. We can conclude the fact that silicified microcrystalline cellulose induced the best flow for inclusion complexes, meanwhile the F-Melt and Ludiflesh resulted and induced a slower flow (chapter. 5.3.).

The ability to be compressed shows indications over powder ability to reduce its volume under the action of an external pressure (characteristic named compressibility).

Volumetric characteristics offers useful information about the flow and the cohesiveness of the powder.

HR values confirmed the registered results when testing the flow, with a characteristic of the flow obviously better than for F1 and F4 than the other four formulations. Through tests developed previously , F5 registered the best HR value (1,44), followed by F2 (1,36), F3 (1,32) and F6 (1,29). The reduced bulk density registered for F1 and F4 is specific to porous structures which facilitates compressibility, as it shows in CI results. Regarding CI, one can notice significant differences between formulations, proving that a reduced density improves compression characteristics. Calculation values of CI confirm results of HR, F5 presents the highest value, maintaining the same order for performance for compressibility as for the flow. It's obvious that the density (in bulk or in compression) grows together with the reduction of particle dimension. A larger distribution of particle dimension through compressible mixtures induced a denser consolidation of the particles.

One can observe the fact that the type of cyclodextrin does not influence the volumetric attributes of the mixture, meanwhile the excipients play an important role. The results show that using F-MELT powder formulations results in a notable reduction of essential compression characteristics, in comparison with the association of silicified microcrystalline cellulose with DISINTEQUIK.

In case of amlodipine, the bulk density is not different very much between formulations, but compression density variable values, with a significant increase for mixtures based F-Melt (F8 and F11) and Ludiflash (F9 and F12). High values of HR (1,29 and 1,24) and CI (22,68 and 19,50) calculated for formulation containing excipient Ludiflash prove the cohesive character of the materials, inadequate for direct compression process. Also, mixtures F-Melt, F8 and F11, presented HR values and CI values typical for materials where take place interaction between particles. However, HR and CI values registered for F7 (1.14 and 12.48) and F10 (1.12 and 14.01), based on silicified microcrystalline cellulose, demonstrates a porous structure of the powder which takes to better flow performance and compressibility.

What concerns nifedipine, volumetric characteristics showed more precisely the differences between batches and the influence cyclodextrin type on flowing parameters and compressibility. There was registered a clear difference between formulations that contain complex Nif-HP-β-CD (F13-F15) and those with Nif-Me-β-CD (F16-F18). The batch F16-F18 presents higher values for CI and HR than batches F13-F15 which include the same excipients, cyclodextrin being the only variable. This thing proves that Me-β-CD reduced compressibility and flow characteristics of the mixtures. However, F18 presents a CI of 9,3 and 1,10 HR, values which, according to European

Pharmacopoeia [68], signifies an excellent compressibility and flowing character. This things is due to the chosen excipients and there proportions (silicified microcrystalline cellulose and Disintequik™ ODT). F15 registered best values for CI (8,6) and HR (1,09) of all formulations, due to the usage of same excipients and in teh same proportions as for F18, plus including complex Nif-HP-β-CD which proved to possess better flowing and compressibility characteristic. In return, F14 and F17, formulas based on F-MELT® and Disintequik™ ODT, presented lower mechanical attributes, with high values for CI (16,3 – F14 and 18,3 – F17), as well as for HR (1,19-F14 and 1,22- F17). However, highest CI values (19,3) HR values (1,24) were highlighted for F16 which contains silicified microcrystalline cellulose mixed with F-MELT®. Even so, according to European Pharmacopoeia, F13, F14, F16 and F18 have a fluidity and a compressibility quite all right.

In conclusion, we can observe, that also in the study of fluidity of complex mixtures, the type of used cyclodextrin influences the flow of final powder, the slowest flow was presented by mixtures that contain Me-β-CD (chapter. 5.4.).

The humidity content is a very important characteristic of the powder that influences very much the behavior of the mixture during compression process but also its consistency , as well as final quality of the tablets. Taking into account that the water was used in the manufacturing of the active ingredient through lyophilization method, it's expected to find a certain level of humidity final mixtures.

F3, F5 and F6 contain the highest amount of water, over 8,5%, up to 9,22% in F5, nearly double versus 5,23% found in F1, proving than the degree of humidity does not depend of the type of cyclodextrin (HP-β-CD or Me-p-CD), nor the used silicified microcrystalline cellulose, but rather the added excipient F-MELT. F1 and F4 presents the lowest contain in humidity, with adequate values for direct compression process. An increase of humidity induces powder aggregation and to a lack of uniformity in filling the matrix of compression equipment. Powder humidity affects directly the flow depending of the water content and its distribution

For powder containing Amlodipine one can observe a big difference of humidity between materials, proving that not only the manufacturing process influences the water quantity. However, F7 and F10 show the lowest humidity degree (3,52% for F7 and 4,03% for F10), confirming that not the type of cyclodextrin affects water absorption. It stands out that the humidity content depends of the diluent excipient selected, more precisely the mode in which the wettability of the

mixture grows. The highest humidity content is registered for F12 (7,35%) and F9 (7,11), the formulations that include Ludiflash®.

F8, F9, F11 and F12 contain double quantities of humidity versus the F7, and their flowing characteristic and compressibility are significantly lower.

In case of F7 and F10, humidity assures the best plasticity, in comparison to formulations that contain excipients F-Melt or Ludiflash.

The results show that there's no significant difference between batches that contain Nif-HP-β-CD and those based on Nif-Me-β-CD, showing that the cyclodextrin doesn't influence humidity retention in the material. On the other way, obviously, types and excipient quantities for direct compression induce notable variations between formulas concerning the humidity content. The lowest humidity quantities were detected in F15 (2,45%) and F18 (2,7%), the samples containing silicified microcrystalline cellulose and Disintequik™ ODT. In the same time, the other four formulations include double quantities of water, 4,69% in F13, 5,13% in F14, 4,85% in F16, and the biggest content of 5,76% is included in F17 (chapter. 5.5.).

→ The studies from the third direction of the research had the objective of formulation, preparation and specific quality control of orodispersable tablets prepared by incorporation of studied inclusion complexes, utilizing the method of direct compression, determinations done according to stipulations described în Pharmacopeia, in official norms and specialty literature , and consist in determine the dimensions (diameter and height), the uniformity of the product, mechanical resistance, a friability, the necessary time for disintegration and dissolution speed .

The choice of excipients was done from technological consideration and also with the reason to obtain a final product with good disintegration and dissolution time, such that to have a therapeutic efficient product.

Preformulation studies showed that powder corresponding to formulas F1 and F4 for complexes of the two CD with nimodipine, F7 and F10 amlodipine complexes, and F15 and F18 for those of nifedipine, have adequate attributes for being processed through the method of direct compression.

The method of preparation assumes presupune direct compression of inclusion complexes in presence of diluent excipients and disaggregates (silicified microcrystalline cellulose, Disintequik™ ODT and starch of glycolate natrium), lubrication being done with magnesium stearate.

The chosen formulations assures powder mixtures that are due to be compressed a good fluidity and compressibility ,a precise dosage of the active ingredient , easy administration and quick release.

The previous prepared powder mixtures have beed subject to direct compression with different forces, as: 30 kN and 60 kN for F1, F4, F7 and F10, and 35 kN for F15 andF18, using a monopost equipment with exocentric Erweka EP-1 Tablet Press produced by Erweka, Germany. Flat poisons had a diameter of 12 mm for F1 and F4, and of 10 mm for F7, F10, F15 and F18. The equipment was adjusted for obtaining tablets with an average weight of 500 mg for F1 and F4, corresponding to a tablet of 30 mg Nimodipine/ tablet, with an average weight of 350 mg for F7 and F10, corresponding to a tablet of 5 mg Amlodipine/ tablet and with an average weight of 300 mg for F15 and F18, corresponding to a tablet of 10 mg Nifedipine/tablet.

All the four formulations nimodipine complexes resulted in round shape tablets, with smooth, plane surfaces uniform aspect, without any noticed defects and white coloured. Orodispersable tablets with amlodipine have also a round shape, white, with homogeneous aspect and with plane and smooth surfaces. Orodispersable tablets with nifedipine are round, white-yellow, with homogeneous aspect and smooth surfaces (chap. 6.2.).

Tablets with nimodipine have an average diameter of 12 mm, ± 1%. Dimension uniformity corresponds to dimension standard in Pharmacopoeia. Small unconformities of diameter of the tablets are due either to a nonuniform filling up of the matrix with powder, or because an irregular movement of inferior ponson . The uniformity of registered dimension for studied tablets confirms the pharmatechnical characteristics of powder mixture and parameters of compression well fixed. As far as the thickness of the tablets, it was noticed a slight difference between the batches depending of the type of cyclodextrin used in inclusion complex, thus, for HP-β-CD the height is smaller than for Me-β-CD, a predictable result taking into account - touching CI values , which were bigger than for material Me-β-CD, the mixture HP-β-CD presents a compressibility better in this specific.

And for amlodipine, all formulations result in similar dimensions tablets, a diameter of 10 mm with variability less than 1% within companies and thickness of approximately 4 mm with small differences between batches and type of de cyclodextrin(the tablets containing HP-β-CD being more pressed than Me-β-CD) and the compression force (a pressure higher than 60 kN inducing advanced compaction). Dimension corresponds requirements of Pharmacopoeia for all batches of

tablets. The results are in line with registered values for volumetric properties of the powder, especially with taxation and compression density.

Tablets dimension uniformity and weight within all batches confirmed that the accuracy of formulation lead to a full fill up of the matrix due to an optimum flow of materials and density consistency without manifestation of segregation. One can observe that dimensional characteristics of Amlodipine tablets are not influenced by compression force applied, but by powder compression attributes.

Also in nifedipine case, one can observe uniformity of tablet dimension (thickness and diameter). Registered variabilities intra and inter-batches are low, showing that the type of cyclodextrin does not affect dimension or tablets weight. Tablets thickness is approximately 4 mm and diameter is 10 mm for both formulations.

The registered values for dimensions of all studied batches of tablets meet the requirements of Europenean Farmacopeea, revealing an adequate selection of excipients and requirements of the compression process. In the case of nimodipine and amlodipine it stand out more pronounced the influence of the type of CD, so Me-β-CD conducts to a higher height of final tablets, indicating a lower compressibility degree. Also for Nimodipine and Amlodipine one can observe the significant influence of the compression force, stronger than (60 kN) leading to a more powerful compaction of the material and implicit to a lower hight(chap. 6.3.1.).

The average weight of all studied orodispersable tablets fits in the limits provided in Europenean Pharmacopoeia (± 5%), which proves that the materials have a good fluidity that determine a uniform filling of the matrix (chap. 6.3.2).

Regarding the hardness of the tablets, the impact of compression force is obvious. One can observe easily that F1b, F4b, F7b and F10b, manufactured at high force of compression, it presents an increased hardness. Because no other factor was altered, in composition or in process, the influence of the compression force is significant. For tablets that contain the inclusion complex Nim-HP-β-CD, the mechanical resistance was 40,2 N for a compression force of 30 N and 53,9 N for force of 60 N. Simillar values are obtained for tablets that contain inclusion complex Nim-Me- β -CD. Taking into consideration that the hardness is much more than just assuring mechanical integrity, because it’s directly connected with all other pharmaceutical attributes, important information were offered regarding differences in disintegration timing.

In the case of Amlodpine tablets, the hardness varies very much writing the batches and decreases in the following order F10b>F7b>F7a>F10a. Naturally, tablets hardness is profoundly influenced by utilized compression force, an increased value of it determine a higher resistance to tablets traction .The type of ciclodextrine does not affect very much the mechanical resistance of tablets, because F7a have 44 N and F10a have a hardness of 42,2 N. However, even though the tablets show a higher hardness, they might show and unsatisfying inclination at rolling. Consequently, friability must be analyzed for establishing tablets capacity to resist to manipulation before usage.
In the case of nifedipine, mechanical resistance of tablets is also satisfying , but one can notice a clear difference between formulations, and that means that the type of cyclodextrin is responsible for modifications of tablet's compactness. Me-β-CD induces a higher hardness (67 N) in comparison to HP-β-CD (55N). Nevertheless, excipients proved that they show adequate plasticity and elasticity in materials.
Even though the type of cyclodextrin clearly affects mechanical resistance of tablets with Nifedipine, with higher values for complex Nif-Me-β-CD, selected excipients and compression force conducted to tablets with optimal resistance .
All types of studied orodispersable tablets present a satisfactory mechanical resistance , they are hard enough to resist to pressure during manipulation. For tablets with Nimedipine and Amlodipine there are no significant variations of tablet hardness with contain different ciclodextrine, proving that it's not influenced by the nature or structure of the active ingredient. In return , for Nifedipe is obvious the influence of the type of cyclodextrin, Me-β-CD inducing a higher mechanical resistance comparting to HP-β-CD (chap. 6.3.3.).
Even though the test for friability it is tightly bound to tablet hardness, regarding friability results are similar between all studied formulations, in comparison to hardness values which are remarkable different, depending on the applied force. All batches of orodispersable tablets present a friability and ideal stability, hard to obtain for this type of form dosage and according to approved regulations. Friability depends totally of formulation and physical characteristics of the mixture for direct compression.
Studied tablets manifested an extreme low friability with values that comply to cu Pharmacopoeia specifications. Both compression force, and also physical characteristics of materials influence tablet friability. The friability of all batches of tablets it is much less than the imposed limit of 1%, with the highest value of 0,34% for F10b.

The fact that can be ascertained is that the percentage lost after the friability test situates itself in normal limits, under 1%, for all batches of tablets for oral dispersion studied (chap. 6.3.4.).

The behavior at disintegration of studied tablets was determined in two different environment: distilled water at 37 ± 0,5oC, according to standard compendium and in phosphate buffer medium which represents saliva medium and simulates a pH of 6,8 la 37 ± 2oC. The necessary time for complete disintegration, without residual product remained on sieve, was measure in seconds.

Disintegration time for all studied formulations presents ideal values for orodispersable tablets, strengthening the idea that the selection of type and quantities of excipients was correct.

In the case of tablets with Nimodipine were registered some differences, but they look like more depending on applied compression force than the type of cyclodextrin utilized. As it was predictable, taking into account the hardness results, disintegration time was higher for tablets manufactured at a higher force, but still comfortable with the norms in place.

Being able to maintain the balance between the hardness, friability and disintegration characteristics is a real success of the formulation process and manufacturing, taking into account the fact that that disintegration time is a critical quality attribute of orodispersable tablets.

Also, there was no significance difference of the disintegration time in the two medium used, but we can observe that for simulated saliva the values a bit a higher. The satisfying disintegration time is due to the pair of super disintegrating agents, DISINTEQUIK™ ODT and EXPLOTAB®, chosen in the studies for formulations.

For tablets with Amlodipine one can notice that a higher compression force (60kN) prolongs the disintegration time with maximum 7 seconds. Meanwhile, disintegration time varies with one second within batches which contain HP-β-CD and those with Me-β-CD.

One can observe also a variation of the results on different mediums used, with a disintegration surprisingly better on simulated saliva medium, for all formulations with Amlodipine. Remarkable disintegration of the tablets was realized through using sodium starch glycolate as a super disintegration agent, as well as, using the diluent agent (silicified microcrystalline cellulose).

The batches which showed a better mechanical resistance, compressed with a higher force, presented and a longer time for disintegration, proving that the importance of process parameters and the correct selection of them. Surprisingly, type of cyclodextrin does not affect too much disintegration behavior, which means that Amlodipine make inclusion complexes with similar characteristics with HP-β-CD, and as well as with Me-β-CD.

In the case of Nifedipine tablets, according to the results of hardness, time of disintegration in both environment for the two formulations proves to be quicker for tablets Nif-HP-β-CD in comparison with tablets Nif-Me-β-CD. Also , for both batches, time for disintegration in water (13 s for F15 and 21 s for F18) is much smaller than in simulated saliva environment (26 s for F15 and 34 s for F18). The inclusion complex HP-β-CD led to tablets with lower hardness and quicker disintegration performance. All tablets present and excellent capacity of disintegration due to excipient's super disintegration capabilities, specific to silicified microcrystalline cellulose, Disintequik™ ODT and specially to sodium starch glycolate.

The specific parameter of orodispersable tablets is the disintegration time which should be lower than 3 minutes according to Europenean Pharmacopoeia.

But, because a quick disintegration of orodispersable tablets does not always indicate a quick dissolution, establishing the dissolution rate in different environment is very important (chap. 6.3.5.).

The dissolution test was performed on two different dissolution medium: compendial dissolution environment (900 mL de 0,01-N HCl) and a more biorelevant environment which simulates the composition of oral saliva which contain 8-g/L NaCl, 0,19-g/L KH_2PO_4 and 2,38-g/L Na_2HPO_4 with a pH of 6,8.

For tablets with nimodipine one can observe that the releasing time has similar values for all four formulations and does not depend of compression force, but on the type of cyclodextrin used or the nature of dissolution medium. The obtained values match Pharmacopeia standards, after 30 minutes the release of nimodipine being nearly complete. The dissolution was quick with a low variability inter- and intraindividual. Regardless of the dissolution environment or the type of formulation, dissolution speed registered values above 95% after 30 de minutes, for all studied batches. This performance is due to the process of including nimodipine in the cavity of cyclodextrin, but is also due to selected excipients. It's well known the fact that nimodipine has a hydrophobic character which determines its floating at the surface of the dissolution medium, but this behavior was not identified in current study when it was incapsulated in cyclodextrin.

Regarding the performance of releasing the active ingredient of amlodipine tablets, all batches proved to have high rates of dissolution in agreement with compendial requirements. Values higher than 85% after 30 de minutes were detected, regardless of variabil formulation or process factors. But, the rates show significant differences depending on the nature of dissolution

environment. The highest values are registered in acid medium, with a release of over 98% for F7a, followed by F10a with a dissolution rate of 98,11%. In the same time, in biorelevant simulated saliva environment, dissolved quantities decreased by some 10% after the same period, the lowest value of 85,15% for F10b.

Similar with the other characteristics of the tablet, the release rate does not depend on the type of cyclodextrin not the processes' parameters. By including amlodipine in cyclodextrin's cavity, as well as the selected excipients, are responsible for this exceptional performance.

After 30 minutes, tablets with nifedipine release the whole quantity of nifedipine (99,11% for F15 and 98,79% for F18), proving that both formulas lead to tablets with quick dissolution time. Nevertheless, the differences between formulas are obvious. Even after the first 5 minutes, one can observe that F15 has a dissolution higher than 26,15% versus 18,57% for F18, and this behavior is maintained for the first 20 minutes, then F18 achieves similar release ratio.

The influence of the type of cyclodextrin is highlighted best in in vitro studies, disaggregation and dissolution. It was proven that HP-β-CD assures a better performance in vitro of tablets, but also the usage of Me-β-CD in tablets with nifedipine leads to tablets for oral dispersion quite satisfactory.

Tablets have a behavior in vitro typically for orodispersable tablets with quick dissolution, proving the correct selection of technique for obtaining inclusion complexes, of direct excipients and teh right manufacturing process (chap. VI.3.6.).

Through all this , we can conclude that cyclodextrin, through increasing dissolution of nimodipine, amlodipine and nifedipine, influence a lot its release from tablets, in the direction of increasing the concentration of available active ingredient that can be used by the patient.

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