Preparation and characterization of nanocapsules containing 2-nitrate and 1,3-diisobutoxypropane (NDIBP) nanocapsules and NDIBP/cyclodextrin inclusion complexes

https://doi.org/10.56238/sevened2024.007-054

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ABSTRACT

2-nitrate-1,3-diisobutoxypropane (NDIBP) is a synthetic organic nitrate derived from glycerin, with low solubility in water. The objective of this work was to prepare and characterize inclusion complexes and nanocapsules with NDIBP. The inclusion complexes were prepared by lyophilization and the nanocapsules by the preformed polymer interfacial deposition method. The solubility isotherms determined for NDIBP showed Al-type profiles (K1:1 = 33.0 M-1). The formation of the inclusion complex provided a 12.5-fold increase in the solubility of NDIBP. The inclusion complexes showed changes in crystal structure and stability up to 150 days at 4ºC. The nanocapsules measured diameters between 100 and 300 nm, and exhibited polydispersion index below 0.2, and zeta potential < -30 mV. In addition, the nanosystems studied showed fungicidal and vasorelaxant activity of the mesenteric artery, thus showing that the nanotechnological strategies employed are promising in increasing desirable characteristics to the NDIPB.

Keywords: Nanosystems, Organic nitrates, Fungicidal activity, Vasorelaxant activity in mesenteric artery.

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INTRODUCTION

The pharmaceutical sciences encourage research that promotes the control of the release of drugs in a specific place where the action of the molecule is desired, for which vectors such as microparticles and colloidal systems such as liposomes and nanoparticles can be used. Currently, nanocarriers have become especially attractive in conventional drug delivery systems, in addition to the delivery of therapeutic molecules such as proteins, peptides, and nucleic acids (Ramsden, 2018; Sousa *et al*., 2019).

Polymeric nanoparticles are drug carrier systems with a diameter of less than 1 μm and this term applies to nanosystems consisting of a polymeric envelope arranged around an oily core, and the drug may be dissolved in this core and/or adsorbed to the polymeric wall (Gnach *et al*., 2015; Rao; Geckeler, 2011). The use of these nanostructures has some advantages, such as the protection of the encapsulated molecule against degradation in the body, better tissue absorption of the drug, as well as changes in its pharmacokinetics (Güven, 2021; Scallop; Gamarra, 2016;).

Some therapeutic compounds have limitations in their use due to their insolubility in water and low bioavailability, stimulating the development of new drug carrier systems, to which cyclodextrins gain prominence (Matioli, 2000). Cyclodextrins form inclusion complexes with drugs, which can influence their stability, resulting in a reduction, increase or unalteration of drug degradation (Popielec; Loftsson, 2017).

Cyclodextrins (DCs) are cyclic oligosaccharides formed by D-glucose molecules joined by means of glycosidic bonds, obtained from the enzymatic degradation of starch from various plant species (Loftsson; Masson, 2001; Marques, 2010). The DCs are in the form of cones, with the wider side formed by the secondary hydroxyls in C2 and C3 and the narrower side formed by the primary hydroxyls bound in C6. The oxygen atoms involved in the glycosidic bonds (in C1 and C4) and the hydrogen atoms bonded in C3 and C5 determine the hydrophobic character of the cavity inside, while the presence of free hydroxyls on the outside confers a hydrophilic character to cyclodextrins (Britto et al., 2004).

However, the increase in solubility as well as the reduction in toxicity of CDs can be improved by chemical modifications, such as 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) (Gould; Scott, 2005; Veiga *et al*., 2006). HP-β-CD forms relatively non-specific complexes with a wide variety of substrates and has therefore been used as a prototype for the investigation of non-covalent interactions involving different compounds (Saenger *et al*., 1998; Van de Manakker *et al*., 2009; Yergey *et al*., 2017).

NDIBP (2-nitrate-1,3-diisobutoxypropane; C11H23NO5, molar mass = 249.3) is a synthetic organic nitrate, with low solubility in water, whose mechanism consists of the release of nitric oxide (NO) thus promoting the relaxation of the endothelium (MAEDA et al, 2004). In addition, antifungal

action is observed in organic nitrates by inhibiting the synthesis of cholesterol found in the membrane (Phillips, 2005). Glycerin trinitrate (TNG) is a precursor of NDIBP, as well as other organic nitrates, such as 2-nitrate-1,3-dimethoxypropane (NDMP), 2-nitrate-1,3-dietoxypropane (NDEP) and 2-nitrate-1,3-dipropoxypropane (NDPP), all of which have vasorelaxant activity in isolated rat cranial mesenteric artery rings, induced by NO release (França-Silva *et al.,* 2012).

Based on this, this work aimed to prepare and characterize nanocapsules (NC) containing NDIBP and NDIBP-2-hydroxypropyl-β-cyclodextrin (NDIBP/HP-β-CD) inclusion complexes, aiming to increase the characteristics of the complexed compound, as a form of controlled release and to increase its solubility in water.

MATERIAL AND METHODS

ACQUISITION AND CHARACTERIZATION OF THE NDIBP/HP-B-CD INCLUSION COMPLEX

The inclusion complexes were prepared by the *Freeze-Drying lyophilization method*, which consists of the complete solubilization of HP-β-CD in distilled water and subsequent addition of the NDIBP molecule. The sample remained under magnetic stirring at room temperature for 48 hours. It was then stored at -80 ºC and freeze-dried for approximately 72 hours. The complexes were obtained using equimolar concentrations (1:1) of the molecule and HP-β-CD.

SOLUBILITY STUDY

The phase solubility study was performed according to the method of Higuchi and Connors (1965). An excess of NDIBP was added to a series of microtubes containing distilled water and increasing concentrations of HP-β-CD. The suspensions were kept under agitation for 72 hours at room temperature. Then, the samples were diluted and the amount of NDIBP was determined by spectrophotometric reading at a wavelength of 240 nm.

CHARACTERIZATION OF INCLUSION COMPLEXES

The following tests were performed: scanning electron microscopy – SEM (JEOL J-210), of samples metallized with colloidal gold for 30 seconds; X-ray diffraction (Bruker, D8 Advance), with LYNXEYE detector and TWIN-TWIN optics. Data were collected in the angular interval between 10 and 70º 2θ, with an angular velocity of 0.2 seconds, a step size of 0.02º 2θ, voltage conditions equivalent to 40 kW and a tube current of 40 μA; infrared spectroscopy (BOMEM), in the spectral range of 4000-400cm-1 with 32 scans/min and resolution of 4 $^{cm-1}$.

PREPARATION OF POLYMERIC NANOPARTICLES CONTAINING NDIBP

The preparation of the Nanocapsules (NC) was carried out according to the method of interfacial deposition of the preformed polymer, initially described by Fessi et al. (1989). This method involves mixing an organic phase into an aqueous phase. The organic phase consisted of polymer, organic solvent (acetone), sorbitan monostearate, oil and NDIBP. The aqueous phase was composed of polysorbate 80 and deionized water. The resulting suspension was kept under agitation for 10 minutes, then the organic solvent was eliminated and the suspension was concentrated to the final volume of 10 mL, with the aid of a rotary evaporator.

Long-Term Stability Test

The objective of the test was to establish the durability of the prepared CNs. In these tests, the conditions of the CN were evaluated immediately after manufacture and at regular intervals (0, 7, 15 and 30 days) or until stability was maintained, always at a temperature of 4°C.

Macroscopic Aspects

It was observed if there were alterations in the general appearance of the preparations: homogeneity, color, viscosity, material deposition, lump formation, cremage, flocculation, coalescence and phase separation.

Microscopic Aspects

It was observed whether there were changes in the microscopic aspect of the CN as a result of the formation of lumps due to the degradation of the polymer.

PH VARIATIONS

The formulations were analyzed by a digital potentiometer containing a glass electrode and a temperature sensor, and the tests were performed at 20°C. An aliquot of the formulation will be placed in a beaker, the electrode and probe will be placed in contact with the beaker sample and the pH reading will be performed.

ZETA POTENTIAL

The zeta potential value, given in mV, was determined using a zeta potential analyzer, Zetasizer Nano ZS 90 (Malvern). The analyses were performed by diluting the CN suspensions in water (Milli-Q) 100 times and the results were expressed as averages of three determinations.

EFFICIENCY OF NDIBP ASSOCIATION IN POLYMERIC CNS

The amount of encapsulated NDIBP was determined by diluting the suspension in methanol. The result of the diluted suspension was analyzed in a spectrophotometer at a wavelength of 240 nm and the negative control used to zero the equipment is methanol.

Bioassays

Antifungal activity

The antimicrobial activity of CN was tested with *Candida albicans* ATCC 60193 by the microdilution method. In 24-well plates, arranged in 4 columns (1 to 4) and 6 rows (A to F). In line A, the CN containing NDIBP was inserted, at concentrations ranging from 3.5 mg/mL (column 1) to 0.43 mg/mL (column 4). Rows B, C and D (24, 48 and 72 hours, respectively) were used to evaluate yeast growth, where every 24 hours 10 µL was inoculated from the wells of row A into wells of row B and later C and D. In row E, there were the positive controls, located in columns 1 and 2 and the negative controls located in columns 3 and 4. In row F, columns 1 and 2 were the color controls and in columns 3 and 4 the controls for the delivery of the drug candidate.

In all wells, 500 µL of Sabouraud broth and 500 µL of a solution containing the yeasts were inserted, diluted in isotonic solution, at a concentration of 4.5x107 cells/mL. In row A column 1, 1 ml of NC containing NDIBP was inserted, and serial dilutions were made from column 1 to 4 to obtain the desired concentration. In the positive control, Sabouraud broth and the solution containing yeasts were inserted. Sabouraud broth and a solution containing yeasts and nystatin 7.4 IU/mL, the reference antibiotic used in the treatment of *Candida albicans* (Nenoff *et al*., 2016). The color control had Sabouraud broth and 10 µL of nanocapsule, while in the vehicle control, Sabouraud broth and nanocapsule without the drug were inserted. The yeast growth was read visually.

VASORELAXATION IN MESENTERIC ARTERY INDUCED BY NDIBP/HP-B-CD AND NC INCLUSION COMPLEX CONTAINING NDIBP

Wistar Kyoto rats weighing between 250 and 300g from the Vivarium Prof. George Thomas (CEUA-UFPB, process no. 305/2009) were used. The animals were kept under controlled temperature (21±1º C) and a light-dark cycle of 12:12 h (6-18 h), with free access to water and feed (Labina®, Purina).

The NDIBP was obtained through organic synthesis and kindly provided by Prof. Dr. Petrônio Athayde Filho. The molecule was solubilized in Cremophor® and distilled water (1:1). The NDIBP/HP-β-CD inclusion complex was solubilized in the same way. The experiment also evaluated the action of CN containing NDIBP and NC without drug, following the same methodology, without dilution in Cremophor®.

The following drugs were used: acetylcholine hydrochloride (ACh); L(-) phenylephrine hydrochloride (FEN); Cremophor®, obtained from Sigma-Aldrich® (USA). The nutrient solution used was Tyrode, aerated with a carbonogenic mixture (95% O2 and 5% CO2), with temperature maintained at 37°C, and pH ≈ 7.4. In the preparation of this solution, the following salts were used: KCl, NaCl, MgCl2.6H2O, glucose, sodium bicarbonate, NaH2PO4.H2O and CaCl2.2H2O.

After euthanasia of the animals, an incision was made in the abdomen for the identification and removal of the cranial mesenteric artery, which was immediately transferred to Tyrode's solution. Next, the artery was dissected and sectioned into 2-5 mm long rings. Some of the already dissected rings, free of connective tissue and adipose, had the endothelium removed by mechanical friction between the inner walls of the vessel and a metal rod. Each ring was immersed in vats containing 10 mL of Tyrode solution at 37ºC and carbonated with a carbogenic mixture (95 % O2 and 5 % CO2) to keep the pH constant at 7.4. The rings were suspended by cotton lines fixed to a force transducer (DATAQ,® 2008, Insight, Brazil) coupled to a PowerLab™ data acquisition system (software version 4.2, ADInstruments, MA, USA), so that isometric stress recordings could be obtained. Each of the rings was subjected to a baseline tension of 0.75 g for a stabilization period of 60 minutes. During this time, Tyrode's solution was changed every 15 minutes to prevent metabolite interference and the baseline was adjusted when necessary. Changes in isometric tension were captured by the LabChart Pro® acquisition system (ADInstruments, Australia).

VERIFICATION OF THE PRESENCE OF FUNCTIONAL ENDOTHELIUM

The presence of functional endothelium was verified by relaxing the pre-contracted rings with 10 mM phenylephrine (FEN, 10 M) with subsequent addition of 10 mM acetylcholine (10 M ACh). The rings that obtained a relaxation greater than 80% over the pre-contraction with FEN were considered to have functional endothelium (E+). On the other hand, the rings with a relaxation of less than 10% were considered without functional endothelium (E-). For this study, rings without functional endothelium were used.

EVALUATION OF THE VASORELAXANT ACTIVITY OF NDIBP AND NDIBP/HP-B-CD AND NC INCLUSION COMPLEX CONTAINING NDIBP ON VASCULAR TISSUE

The protocol for the evaluation of vasorelaxant activity induced by the NDIBP/HP-β-CD and NC inclusion complex containing NDIBP was performed according to Daiber et al. (2004). After the stabilization period, a contraction was induced with FEN (10 M) and increasing concentrations of the complex (10-8, 3 x 10-8, 10-7, 3 x 10-7, 10-6, 3 x 10-6, 10-5, 3 x 10-5 and 10-4 M) were applied cumulatively to obtain a concentration-response curve. The vasorelaxant activity of NDIBP was evaluated according to the same protocol. Relaxation was expressed as a reverse percentage of the

FEN-induced contraction. After obtaining the concentration-response curves, the values of pD2 (-log of EC50) and Emmax (maximum effect) of the individual curves were calculated. The relaxation induced by NDIBP in mesenteric artery rings was compared with the relaxation of the rings induced by the NDIBP/HP-β-CD inclusion complex and by the NDIBP-containing NC.

Statistical analysis

The results were expressed as mean ± SE (standard error of the mean). Two-way ANOVA and the Student's t-test were applied for statistical analysis, and the differences were considered significant when $p<0.05$. Relaxation was expressed as a reverse percentage of the contraction induced by NSF and the curves were obtained by nonlinear regression, through which pD2 was obtained. The statistical program used was GraphPad Prism version 6.00®.

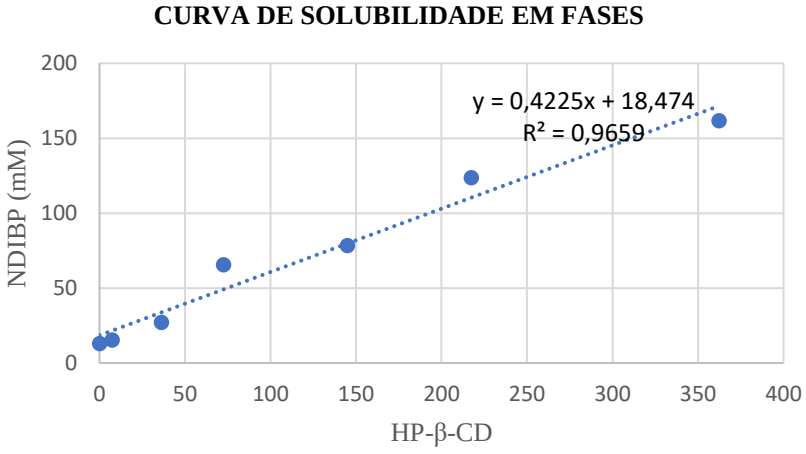
RESULTS AND DISCUSSION

CHARACTERIZATION OF THE INCLUSION COMPLEX

Phased Solubility Diagram

The phased solubility diagram of the NDIBP in HP-β-CD in aqueous solution behaved like an AL curve (Figure 1), because it showed a linear increase in NDIBP solubility as a function of increasing concentrations of HP-β-CD. Based on the diagram obtained, the stability constant (KC) of the NDIBP/HP-β-CD complex was calculated.

Figure 1 - Study of the solubility diagram in phases.



Source: Author

KC's interest is to characterize the molecular interaction between the constituents of the complex and its practical feasibility (Aguiar *et al*., 2014; *VALENTINE*, 2006). The KC of the system NDIBP/HP-β-CD was 33.0 M-1, this result is in agreement with other inclusion complexes obtained. Melo *et al.* (2007), Preparing a Nitrofurazone Inclusion Complex/HP-β-CD, determined the stability

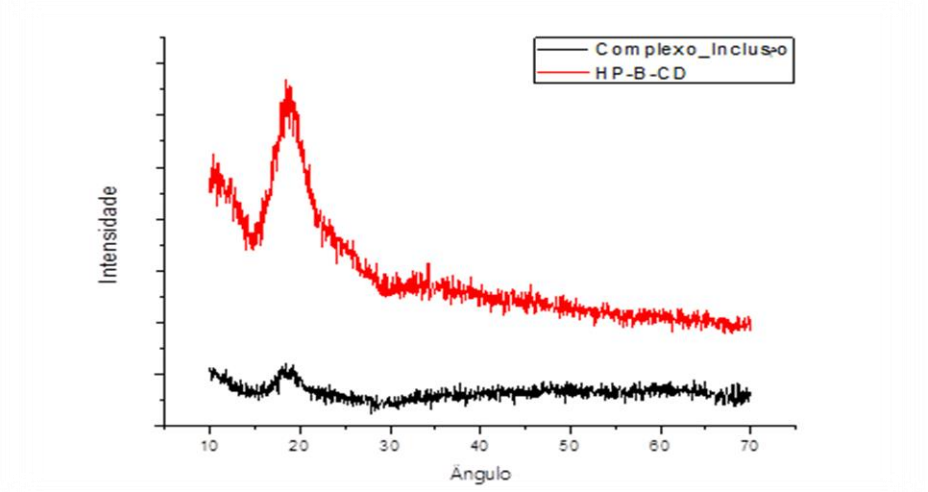
constant of 14.9 M-1, on the other hand, Messner *et al*. (2011) determined the stability constant of the hydrocortisone/HP-β-CD complex to be 1450.0 M-1, thus indicating that the interaction force that will occur between the host molecule and HP-β-CD is specific, generating complexes of greater or lesser stability. Complexes with low Kc values (less than 200 M-1) lead to a faster release of the active ingredient, this is due to the lower affinity between the host molecule and HP-β-CD, whereas inclusion complexes that have a high Kc (above 5000 M-1) have a delay in the release of the active ingredient, given by the high affinity that occurs between the host molecule and HP-β-CD.

The results of the phased solubility diagram showed that there is an interaction between NDIBP and HP-β-CD, through the use of the complexation technique it was possible to increase the water solubility of the drug candidate (NDIBP) 12.5 times.

X-Ray Diffraction

From the analysis of diffraction patterns, it can be observed that HP-β-CD has an amorphous structure, or non-crystalline structure, as previously described in the literature (SPAMER et al., 2002). The X-ray analysis of the complex sample reveals that the NDIBP/HP-β-CD complex presents a different curve from that presented by the free HP-β-CD (Figure 2). These differences in diffraction patterns may be due to the formation of the inclusion complex, which generate changes in the structure of HP-β-CD and this leads to a change in the pattern of X-ray diffraction. These results are in agreement with others reported in the literature, in which HP-β-CD is used as a host in the inclusion complex, the same pattern of the pure matrix is observed in the complex (Liu *et al*., 2006; Zingone; Rubessa, 2005). In addition, HP-β-CD showed an amorphous diffraction pattern, without the presence of crystalline peaks, where two wide diffraction halos around 10 and 20º can be observed, which are characteristic of these cyclodextrins (Huang *et al*., 2016; Qiu *et al.,* 2014).

Figure 1 - X-ray diffraction, Inclusion complex intensity, and HP-β-CD molecule intensity.



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Infrared Spectroscopy

HP-β-CD in its free form and the NDIBP: HP-β-CD inclusion complex (in a ratio of 1:1) were submitted to infrared spectroscopy in order to observe changes due to the NDIBP/HP-β-CD interaction. The Figure containing the spectroscopy results shows the main vibration frequencies of the HP-β-CD and the inclusion complex.

In Figures 3 and 4 it is possible to observe that the spectrum of HP-β-CD reveals typical absorptions of β-cyclodextrin (EGYED, 1990), the band at 1155 cm-1 is attributed to the vibration of the pyranose ring and to the asymmetric stretching of the glycosidic bonds, this marking is also observed for most saccharides, at 3410 cm-1 it is still possible to observe the stretching referring to the OH group (Egyed, 1990).

There is a decrease in the intensity of the band in the region of 3000 to 3500 cm–1 of the free HP-β-CD compared to the inclusion complex, this fact can be explained by the breaking of hydrogen bonds after the interaction of the molecule in question with the NDIBP, as a consequence of the release of the inclusion water. These water molecules normally occupy the cavity and are displaced when some less polar compound approaches the cavity. In addition, the peaks of free HP-β-CD resemble those of the NDIBP/HP-β-CD complex, which denotes a probable encapsulation (Chaves et al, 2010). In addition, the spectra referring to free HP-β-CD and IC showed absence, displacement, or changes in the intensity of certain absorption bands, suggesting the complexation of NDIBP by CD (Garrido *et al*., 2018; Pan *et al*., 2017).

Figure 3 – IV referring to the free HP-β-CD molecule

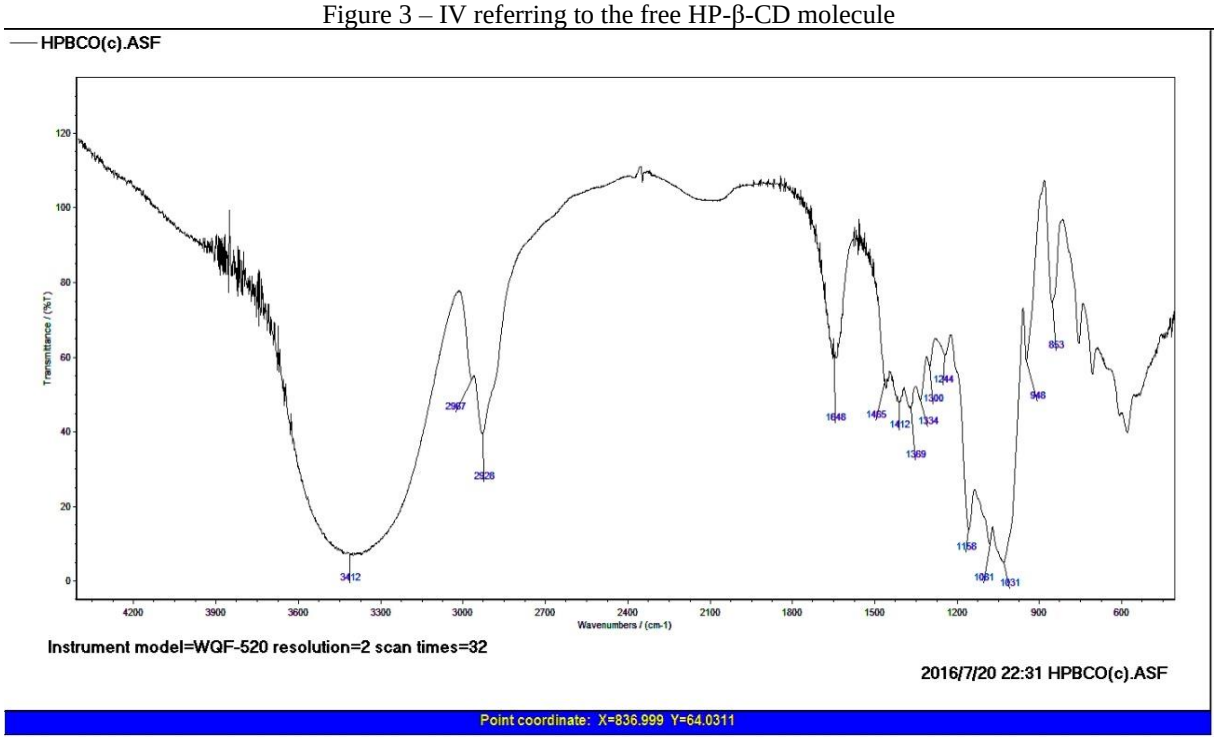

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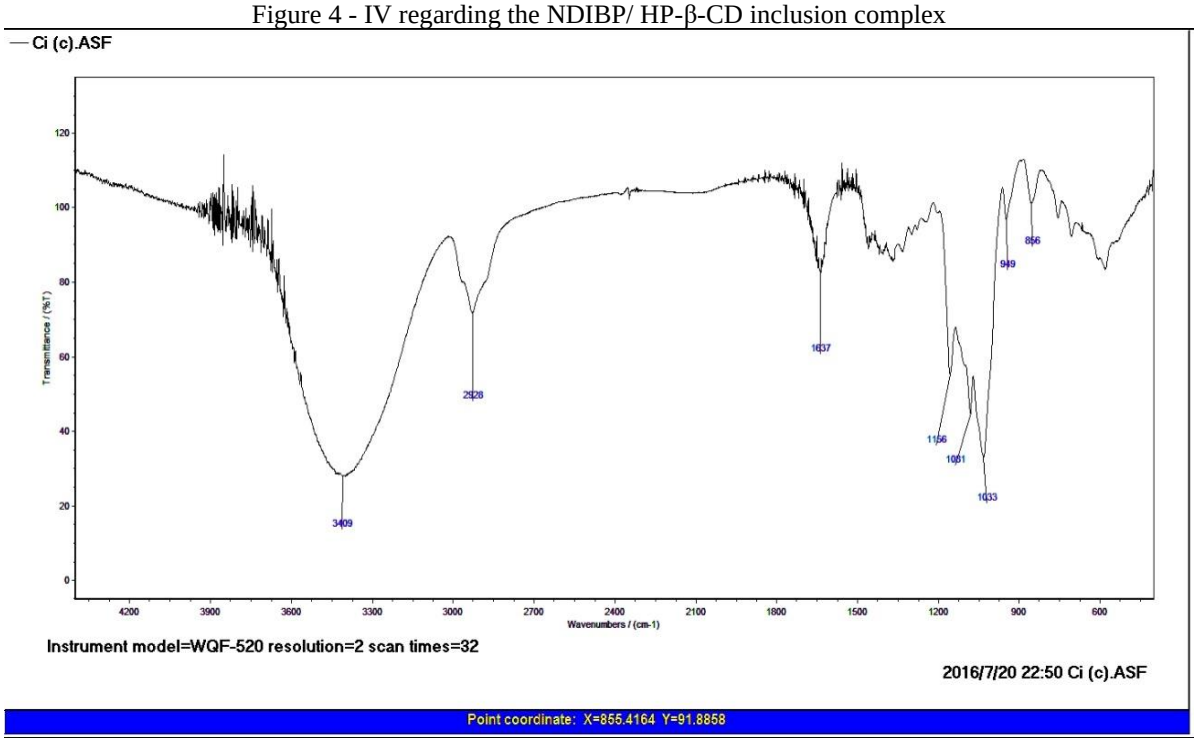
Figure 4 - IV regarding the NDIBP/ HP-β-CD inclusion complex



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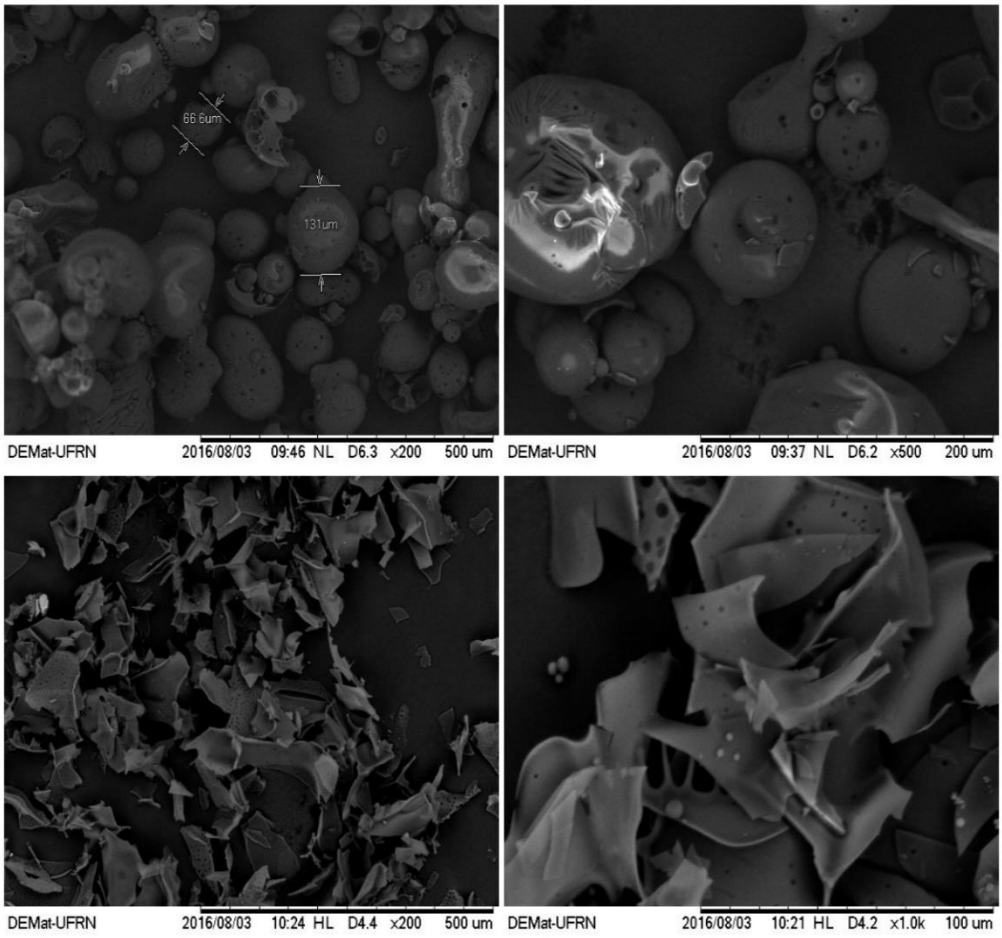
Scanning Electron Microscopy

SEM is a tool that can be used to observe interaction between molecules, as long as the change in their structure can be observed, drastic changes in the shape, appearance and size of the particles, are strong indicators of the complexation of substances in CDs (Lyra *et al.,* 2010; Qiu *et al*., 2014). The SEM analysis facilitated the observation of the crystals of the HP-β-CD sample and the inclusion complex between the NDIBP:HP-β-CD. Figure 5 shows changes in the structure of HP-β-CD when compared to the inclusion complex. This alteration indicates that the interaction that occurs between NDIBP and HP-β-CD generates a change in the crystal structure of HP-β-CD, possibly due to the formation of the inclusion complex (Moraes *et al*., 2007). Several authors have reported that these morphological changes are indicative of the formation of inclusion complexes, mainly due to the interaction between the compound under study and HP-β-CD (Wei *et al.*, 2017; Yao *et al.,* 2014).

Figure 5 – Scanning electron microscopy. Images A and B represent the structure of the free HP-β-CD, while images C and D represent the structure of the inclusion complex formed.



Source**:** Author

Characterization of Nanocapsules

Particle Size, Polydispersion Index, Zeta Potential, and pH

Initially, NC containing the drug candidate NDIBP and NC were prepared, which were prepared without NDIBP increment. Parameters such as particle size, polydispersion index, zeta potential and particle pH can indicate the stability of the formulated NC in suspension. Table 1 lists the measured values of these parameters for the prepared NC containing or not containing the NDIBP.

Table 1 - Values of medium size (nm), zeta potential (mV) and polydispersion (PDI) of polymeric nanocapsule suspensions

Samples	Stability (time)	Particle Size	Zeta Potential	PDI
NDIBP – 7mg	T 0 days	191.93 nm	- 29.1 mV	0,116
NDIBP – 7mg	T 30 days	197.03 nm	- 30.96 mV	0,125
NDIBP – 7mg	T: 90 days	202.16 nm	- 21.63 mV	0,125

Source: author

The results presented in Table 2 indicate that polymeric CN suspensions containing NDIBP and those without the drug had diameter and polydispersion index compatible with colloidal suspensions (Guterres *et al.,* 1995).

From Table 1, it is possible to observe that the mean particle diameter for the analyzed formulations remained practically constant in the period from zero to 150 days, indicating that these formulations are stable in size for at least 150 days, with no formation of aggregates that would be evidenced by the increase in particle size.

According to Mohanraj and Chen (2006), polydispersion values, which indicate the size distribution of NC, less than 0.2 for colloidal suspensions are ideal. Regarding polydispersion, Table 1 shows that the formulations have values lower than 0.2, which indicates that the formulations have an acceptable size distribution. Large variations in the polydispersion value as a function of time may indicate aggregate formation (Mohanraj; Chen, 2006). Table 1 shows that small variations in polydispersion were observed for all formulations as a function of time, thus showing that the formulations have adequate stability. Thus, according to the literature, the results indicate the formation of monodispersed systems with successful production (Balest, 2013).

The zeta potential values of the formulations indicate a good stability in suspension, since the values were close to -30 mV, values that indicate a good colloidal stability due to an electrostatic repulsion between the nanoparticles, so the nanoparticles tend not to aggregate. Nanoparticles with zeta potential values of approximately (±) 30 mV are more stable in suspension (Camargo *et al*., 2023; Mohanraj; Chen, 2006).

Table 2 -*ph* of nanocapsules containing NDIBP and without the compound

Time	**NC – NDIBP (7 mg)**	**NC – as NDIBP**
T 0 days	5,55	3,23
T: 7 days	5,51	3,22
T: 15 days	5,46	3,40
T 30 days	5,44	3,30

Source: Author

The last stability parameter investigated for NC was pH. Table 2 shows the pH behavior of NC and NDIBP-containing formulations as a function of time. The analysis of pH as a function of time is important to ensure the stability of NC suspensions, as pH change may indicate polymer degradation. This is due to the hydrolysis of the polymer, releasing some of its components (Schaffazick *et al*., 2003). In all formulations, variations in pH values were observed as a function of

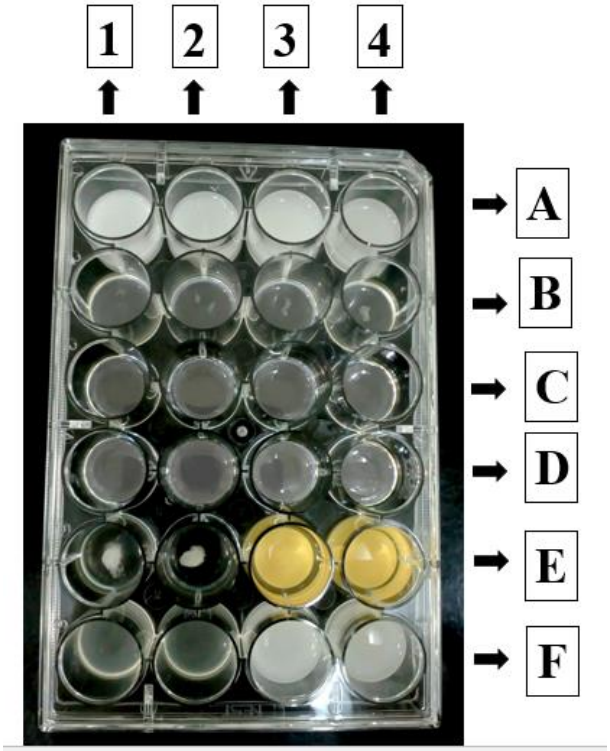
time, these variations may be associated with the degradation of the polymer producing free lactic acid causing a decrease in pH (Guterres *et al.,* 1995).

EFFICIENCY OF NDIBP ASSOCIATION IN NC POLYMERS

Bioassays of Nanocapsules containing NDIBP as an Antifungal Agent

The susceptibility test with *Candida albicans* 60193 by the broth microdilution method, revealed that the nanocapsule containing NDIBP presents biocidal activity when incubated in the medium containing the yeasts, this action was obtained after a period of 48 hours. The results of the analysis can be seen in Figure 6. To determine the susceptibility test, the microdilution test was used, which is in accordance with the protocol used by the National Health Surveillance Agency (ANVISA) (Barry *et al*., 2000).

Figure 6 - Method of microdilution in Sabouraud broth, evaluation of the activity of the nanocapsule containing NDIBP on Candida albicans ATCC 60193.

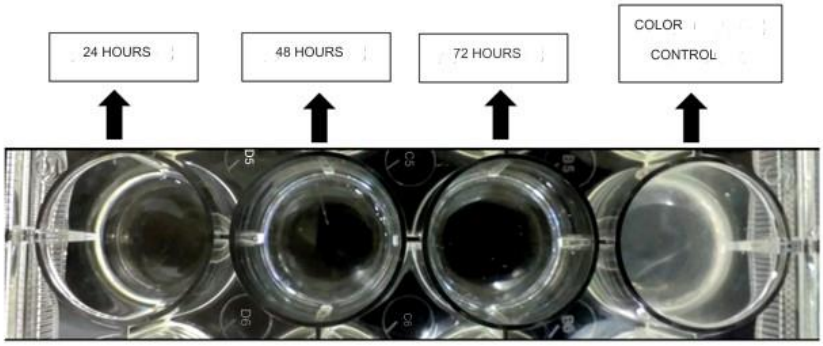


Source: Author

In the period of 24 hours after incubation of the nanocapsule in the middle, line B, the yeast grew in all wells with different concentrations of nanocapsule, indicating that the yeast-like strain remained viable. After a period of 48 hours from the incubation of the nanocapsule containing NDIBP, no growth of the microorganism was seen in the wells, this result can be seen in line C, columns 1 to 4, thus showing that all concentrations of nanocapsule added to the wells were able to inhibit the growth of *Candida albicans*. The result of line D, referring to the period of 72 hours of incubation of the nanocapsule containing the drug candidate, was similar to those presented in line C.

From the results, it was not possible to define whether the nanoencapsulated NDIBP had a biocidal or biostatic activity in relation to the yeast *Candida albicans* ATCC 60193, since the growth of the microorganism was observed in the period of 24 hours, not being repeated in the subsequent analyses. In order to evaluate the effect of the nanocapsule containing NDIBP on the yeast, new assays were carried out, where 10 µL was removed from the wells containing the highest concentration of NC in the periods of 24, 48 and 72 hours, i.e., an aliquot was removed from wells 1-B, 1-C and 1-D and transferred to a new culture medium. In order to evaluate the growth of the yeast, the result can be seen in Figure 7.

Figure 7 - Assessment of the feasibility of Candida albicans ATCC 60193 after 72 hours of incubation in the nanocapsule containing NDIBP



Source: Author

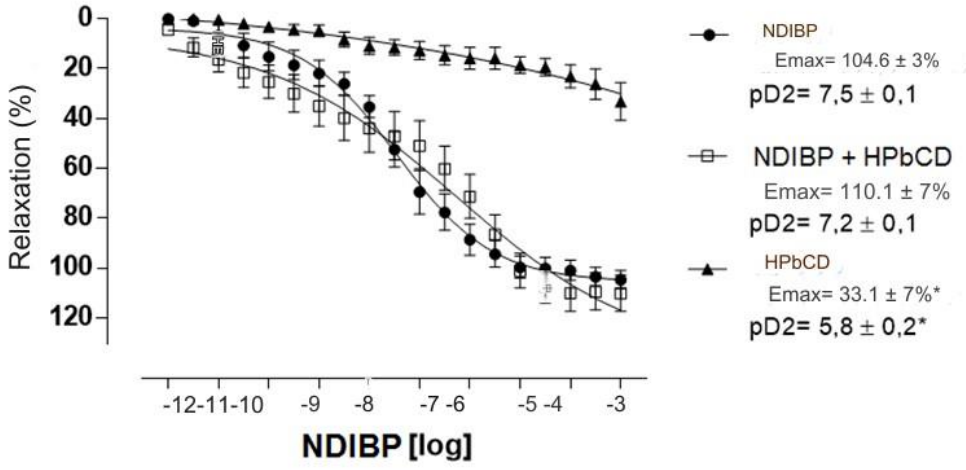
Through the result obtained in this test, it was observed that the yeast did not grow in the wells, thus suggesting that the nanocapsule containing NDIBP has biocidal activity in relation to the yeast *Candida albicans* ATCC 60193. Probably, in the 24-hour period the polymeric matrix, which forms the nanocapsule, did not degrade and thus the NDIBP was not available in the medium, this may explain why the yeast growth in the wells referring to the 24-hour period, line A. On the other hand, the growth of the microorganism was not observed after 48 hours, thus suggesting that after this time the polymeric matrix, shape and structure of the nanocapsule, suffered degradation and promoted the release of NDIBP in the medium.

Vasorrelaxation in the Mesenteric Artery Induced by NDIBP/HP-β-CD Inclusion Complex and by NC containing NDIBP

In superior mesenteric artery rings isolated from Kyoto Wistar rat without functional endothelium and precontracted with FEN (10µM), increasing concentrations of 2-nitrate-1,3-diisobutoxypropane (10-8⁻10-4 M) promoted vasorelaxation concentration dependent (Emax = 104.6 ± 3/7.5 ± 0.1) as shown in Figure 8. This suggests that NDIBP is a compound with a potential vasodilator effect. In mesenteric artery rings without functional endothelium and precontracted with

FEN (10µM), increasing concentrations of 2-nitrate-1,3-diisobutoxypropane (10-8–10-4 M) + hydroxypropyl-β-cyclodextrin (inclusion complex) also promoted vasorelaxation concentration dependent (Emmax = 110.1 ± 7/7.2 ± 0.1). The result shows that the complexation of NDIBP by the HP-β-CD molecule does not interfere with the vasorelaxant effect caused by the compound.

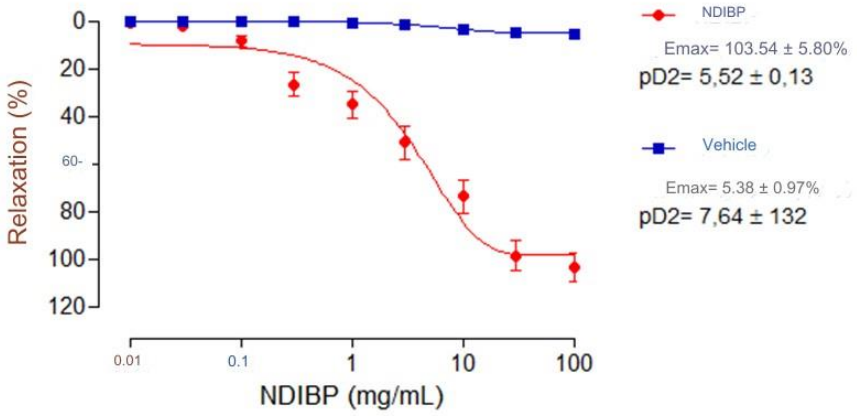
Figure 8 – Vasorelaxant activity of the inclusion complex and NDIBP (10^{-8} at $3x10^{-4}$ M) in isolated superior mesenteric artery rings of rats precontracted with FEN (10 µM) without functional endothelium. The effect was evaluated in vascular tissue (n=6). |average ±e.p.m|



Source: Author

In mesenteric artery rings without functional endothelium and precontracted with FEN (10µM), increasing concentrations of nanoencapsulated 2-nitrate-1,3-diisobutoxypropane (10-8 – 10-4 M) also promoted vasorelaxation concentration dependent (Emax = 103.54 ± 5.8/5.52 ± 0.13), the result can be observed in Figure 9. From the analysis of the graph we can observe that the nanocapsule without the compound (vehicle) did not interfere in the relaxation of the mesenteric artery, this proves that the constituents that form the CN do not have vasorelaxant potential and the effect we see related to the nanoencapsulated NDIBP was due to the action of the NDIBP released by the nanocapsule.

Figure 9 - Vasorelaxant activity of NDIBP-containing nanocapsules (10-8 to 3x10-4 M) in superior mesenteric artery rings isolated from rats precontracted with FEN (10 µM) without functional endothelium. The effect was evaluated in vascular tissue (n=4). Values expressed as mean ± e.p.m.



Source: Author (2017).

Similar results for Emax and pD2 were observed by Franca-Silva *et al.* (2012), when they evaluated the vasorelaxant effect on mesenteric artery rings in the absence of functional endothelium of glycerin-derived organic nitrates. The NDIBP values, as well as the values of other organic nitrates, can be seen in Table 3.

Table 3 - Values (%) of Emmax and pD2 referring to the vasorelaxatory effect of NDIBP and other glycerin-derived organic nitrates in rat isolated superior mesenteric artery rings precontracted with FEN, in the absence of functional endothelium

COMPOUND	$E_{máx}$ ± WITHOUT	pD_2 ± WITHOUT
NDIBP	104.6 ± 3.0	7.5 ± 0.1
NDMP	93.8 ± 11.7	4.4 ± 0.07
NDEP	108.8 ± 5.4	4.8 ± 0.06
NDPP	111.1 ± 8.5	5.4 ± 0.08
NDBP	105.4 ± 2.7	5.9 ± 0.06

Fonte: Adapted from Franca-Silva et al. (2012)

Polymeric CNs containing NDIBP were used in the experiments *in vitro*, referring to the evaluation of vasorelaxation in the mesenteric artery, without the addition of the diluent agent Cremophor(R), which is a non-ionic surfactant derived from castor oil, which is an oil obtained from the seeds of *Ricinus communis.* Cremophor® has been used in the solubilization of a number of hydrophobic drugs, including sedatives, anesthetics, and anticancer drugs (Zuylen *et al*., 2001).

Some adverse effects of Cremophor® have been observed in clinical medicine, an example is the acute hypersensitivity reaction, which is characterized by dyspnea, tachycardia, and hypotension (Gelderblomm *et al.,* 2001), also has an action on the endothelium, causing vasodilation (Singla *et al*., 2002). Thus, this compound can interfere with experimental results if it is not used in the ideal concentrations so as not to present effects.

In addition to having unwanted adverse effects, Cremophor® can also alter results of experiments *in vitro*, for these reasons, the search for diluents or inert carriers of drugs that have hydrophobic characteristics has been growing. In the results shown above, we can observe that the NC served as carriers for the NDIBP and did not interfere in its action, since the relaxation of the mesenteric artery had an Emmax of 103.5± 5.8%. Thus suggesting that this strategy may be a way out for encapsulation of hydrophobic drugs that are currently being diluted in Cremophor®.

CONCLUSIONS

The present work showed evidence of the complexation of NDIBP by the HP-β-CD molecule, the analyses performed from the data obtained by methods such as X-ray diffraction, infrared spectroscopy and scanning electron microscopy show us the alterations undergone by the HP-β-CD compound show that the complexation actually occurred. The complexation promotes an improvement in the solubility of NDIBP in the order of 12.5 times.

Considering the results generated by the polymeric CNs containing the NDIBP, the analyses performed from the data obtained by methods such as photon autocorrelation spectroscopy show us the changes undergone by the NC samples over time and show that the nanoparticles have a good stability. The same was confirmed by the surface potential and polydispersibility analyses.

The nanocapsule containing NDIBP proved to be efficient in biological assays with *Candida albicans* ATCC 60193, the work suggests based on the results presented that the compound presented a biocidal potential when inoculated with the yeast in Sabouraud broth.

The NDIBP showed a vasorelaxant effect on superior mesenteric artery rings isolated from Wistar Kyoto rats without functional endothelium. The vasorelaxant effect was observed both in the NDIBP/HP-β-CD inclusion complex and in the NC containing the NDIBP.

NDIBP encapsulated in NC had highly relevant effects on vasorelaxation results, since it was not necessary to dilute NDIBP in Cremophor®. This factor highlights the importance of the formulation, since it made it possible to carry out in vitro experiments without the use of Cremophor®, a diluent that has toxic characteristics.

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