Vol.66: e23220370, 2023
https://doi.org/10.1590/1678-4324-2023220370
ISSN 1678-4324 Online Edition

Article - Engineering, Technology and Techniques

Isosorbide Dinitrate Orally Disintegrating Films Cyclodextrins/HPMC Based: Mechanical, Optical, and Physicochemical Studies

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Editor-in-Chief: Paulo Vitor Farago
Associate Editor: Paulo Vitor Farago

Received: 17-May-2022; Accepted: 07-Jun-2023

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HIGHLIGHTS

- Orally films Cyclodextrins/HPMC based films were prepared by solvent casting.
- Films for oral delivery of ISDN were prepared by 3^2 factorial designs.
- Mechanical, optical, and dissolution properties were evaluated.
- The experimental design allowed to optimize the formulations.
- The best formulation was obtained by means of the desirability function.

Abstract: Orally disintegrating films (ODFs) were prepared from hydroxypropyl methylcellulose (HPMC E6 1.5%, 2.0%, and 2.5%), plasticizers ((glycerin (Gly), propylene glycol (PP), or polyethylene glycol (PEG)), and isosorbide dinitrate using the solvent casting method. Design of experiments (DoE) was used considering the amount of film-forming agent (HPMC) and the nature of the plasticizers as independent variables and thickness, mechanical properties, disintegration time, and dissolution efficiency as dependent variables. The best formulation was selected based on the desirability function (f_D). Color analysis was performed using CIE-Lab coordinates. DSC curves and XRPD diffractograms showed ISDN amorphization, probably due to complexation with polymers (HPMC E6 and HPβCD). The polymer (HPMC E6) and plasticizer played a critical role in the mechanical and optical properties of the films; however, these factors had no significant effect on the dissolution efficiency of ISDN. Principal component analysis revealed a more defined distinction

of the ODFs according to their chromatic characteristics. ODFs prepared with Gly and PEG 400 were translucent, whereas the other films were transparent. The dissolution efficiency values of the ODFs (F1–9) were higher than those observed for the reference product (88.35±0.01%), indicating increased solubility of ISDN, probably due to complexation with polymers (HPMC E6 and HPβCD). The maximum value obtained for desirability function (f_D) was 0.86, which corresponded to the use of 1.5% to 2.0% polymer (HPMC E6) and Gly or PP as plasticizers. The F5 formulation showed adequate thickness (85.00±6.38 μm), elongation at break (4.82±0.01%), adhesiveness (0.23±0.09 mJ), dissolution efficiency (95.97±0.01%), and high transparency.

Keywords: isosorbide dinitrate; cyclodextrins; orally disintegrating films; mechanical properties; desirability function.

INTRODUCTION

Isosorbide dinitrate (D-GLUCITOL, 1,4:3,6-dianhydro-, 2,5-dinitrate, ISDN) is commonly used for the treatment and prophylaxis of angina pectoris and is traditionally administered orally or sublingually. However, loss of consciousness appears in patients when angina pectoris breaks out; thus, it is difficult for patients to take medicine by themselves. Additionally, orally administered ISDN has low bioavailability (22%–29%) due to its high first-pass metabolism in the gastrointestinal tract and liver. Moreover, the critical point of antianginal therapy depends, to a certain extent, on the ability of the drug to produce an immediate effect [1].

Orally disintegrating films (ODFs) have emerged as promising and prominent pharmaceutical dosage forms. The oral cavity presents many advantages for drug delivery, aside from its acceptable acceptance by patients. The oral mucosa, which is generally divided into sublingual, gingival, buccal, and soft palatal mucosa, is relatively permeable, allowing systemic transmucosal drug delivery. Absorption through the mouth prevents the degradation of drugs by the action of stomach acid, bile, and first-pass metabolism. As a result, these thin films have the potential to accelerate the onset of the action of the drugs, lower the drug strength, and enhance the efficacy and safety profile of some drugs. From the market perspective, new drug delivery technologies offer opportunities to extend revenue life cycles for pharmaceutical companies whose drug patents are about to expire, and who are vulnerable to generic competition [1–7].

Hydrodispersible polymers are the main components of the oral films. Both natural and synthetic polymers, alone or in blends, are used to fabricate films with the desired properties [6–9].

Cyclodextrins (CDs) are cyclic oligosaccharides that usually contain six to eight glucose units. They can be used to improve the various properties of drugs, such as solubility [10–12], stability [13], and bioavailability [14,15], as well as reduce the side effects associated with some drugs [16,17].

Oral films can be produced by the solvent casting method, hot melt extrusion (HME), solid dispersion extrusion, and, more recently, by electrospinning and 3D printing [1,2,18–20]. The solvent casting method is undoubtedly the most widely used method because it forms films with good physical properties, uniform thickness, ease of processing, and low cost [6]. The main critical issues in developing these dosage forms are dissolution in the oral cavity, tensile properties required for packaging and handling procedures, and taste masking [4,21].

There is an increasing number of published studies on the application of statistically based optimization processes in the field of pharmaceutical technology. Design of experiments (DoE) is a statistical tool capable of facilitating the interpretation of experimental data, which ultimately allows the identification of optimal factor levels for maximum performance [22,23].

The purpose of this work was to develop orally disintegrating films containing ISDN, using HPβCD as a solubilizing agent, and HPMC, a hydrophilic non-ionic polymer, as a matrix, using the solvent casting method. The ODFs were developed using design of experiments (DoE) and characterized based on their mechanical, optical, and physicochemical properties, and dissolution characteristics. The responses were optimized using the *desirability function* (f_D).

MATERIAL AND METHODS

Materials

ISDN:lactose (25:75, w/w) was kindly provided by EMS Sigma Pharma Ltda. (Hortolandia, São Paulo, Brazil). 2-hydroxypropyl)-β-cyclodextrin (2-HPβCD, average Mw ~ 1,460) produced by Wacker Chemie AG (Burghausen, Germany) was purchased by Merck/Sigma-Aldrich (São Paulo, Brazil). Hydroxypropyl methylcellulose (HPMC E6 Premium LV, Dow Chimica; 4.8-7.2 mPa.s 2% in water at 20°C, 28.0-30.0%

methoxyl substitution, and 7.0-12.0% hydroxypropoxyl substitution) was kindly provided by Colorcon Inc. (Cotia, São Paulo, Brazil). Glycerin (Gly), propylene glycol (PP), polyethylene glycol 400 (PEG) were obtained from LabSynth (Diadema, São Paulo, Brazil).

Tablets containing 5 mg of ISDN (Isordil® Sublingual 5 mg tablets, EMS Sigma Pharma Ltda., Brazil) and Listerine PocketPaks® Oral Care Strips (Pfizer - Warner-Lambert consumer healthcare division) were obtained from the pharmaceutical market. Methanol (HPLC grade) was obtained from J. T. Baker. Ultra-purified water was obtained using a reverse osmosis system Gehaka model OS10LXE (São Paulo, Brazil). All other reagents were of analytical grade.

Preparations of ODFs

ODFs were prepared using a full factorial DoE approach employing two independent variables and three levels (3^2) using the Statistica version 13.1 software (TIBCO Statistica Inc., CA, USA), resulting in nine formulations (Table 1). The films were prepared by the solvent casting method using HPMC E6 [1.5% (-1), 2.0% (0), and 2.5% (+1)] as a film-forming agent, and Gly (-1), PP (0), or PEG 400 (+1) as plasticizers (10% mass polymer, w/w) [20,24]. 2-HPβCD was added as a solubilizing agent (Table 2).

To prepare the formulations 120 mg of ISDN base (480 mg ISDN/lactose 25/75, w/w) was weighed, dispersed in 5 mL of 10% HPβCD and subjected to stirring on a 10-position magnetic stirrer. Then the plasticizer (GLY, or PP, or PEG) in the proportion of 10% of the polymer mass and an appropriate volume 7% HPMC E6 were added; and finally, the volume was made up to 100 mL with purified water (Table 2). After 30 min, stirring was stopped, and the dispersions were kept at rest for 30 min for deaeration [6]. The dispersions thus obtained were transferred to a polystyrene mold (120x120mm, 144 cm^2 of area), and dried in an oven with forced air circulation and renewal (40.0±0.5°C) for 24 h. The films were removed from the molds, wrapped in an aluminum foil, and placed in a desiccator.

Table 1. Statistical planning using full factorial DoE approach with two independent variables and three levels (3^2)

Variables	Levels								
	F8	F5	F6	F9	F4	F1	F7	F2	F3
HPMC E6	+1 (2.5)	0 (2.0)	0 (2.0)	+1 (2.5)	0 (2.0)	-1 (1.5)	+1 (2.5)	-1 (1.5)	-1 (1.5)
Plasticizer	0	0	+1	+1	-1	-1	-1	0	+1

The values in parentheses represent the amounts of HPMC E6 used in the preparation of the films, expressed in percentage.

Table 2. Composition of ODFs

Composition	ODFs								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
ISDN base (mg)	120	120	120	120	120	120	120	120	120
HPMC E6 (%)	1.5	1.5	1.5	2.0	2.0	2.0	2.5	2.5	2.5
2-HPβCD (%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Plasticizer[a]	GLY	PP	PEG	GLY	PP	PEG	GLY	PP	PEG
Water, qsp (mL)	100	100	100	100	100	100	100	100	100

[a]10% mass polymer, w/w

The dispersions obtained were transferred to a polystyrene mold (120x120 mm, 144 cm^2 area), and dried in an oven with forced air circulating and renewal (40.0±0.5 °C) for 24 h. The films were removed from the mold, wrapped in an aluminum foil, and placed in a desiccator.

The amount of ISDN/lactose (25/75, w/w) in ODFs was calculated according to Ali [25], such that each unit of ODFs (6 cm^2) contains 5 mg of ISDN base. Films without plasticizer (F1-WP, F4-WP and F7-WP) were prepared for comparison.

Characterization and optimization

Thermal analysis

ISDN:lactose (25:75, w/w), lactose monoidratada, HPMC E6, 2-HPβCD, and ODFs (F1-9) were evaluated using differential scanning calorimetry (DSC) (~2-4 mg) and thermogravimetric analysis (TGA) (~4-8 mg).

DSC curves were obtained using a DSC Shimadzu model DSC-60 at a heating rate of 10 °C min^{-1} over a temperature range of 40 – 600 °C. Samples of 2 – 4 mg were used in sealed aluminum pans under a dynamic nitrogen atmosphere (100 mL min^{-1}). The system was previously calibrated with metallic indium (99.99% purity, $T_{melting}$ = 156.4 °C, $\Delta H_{melting}$ = 28.7 J g^{-1}).

TGA curves were obtained using a TGA Shimadzu model DTG-60 at a heating rate of 20 °C min^{-1} over a temperature range of 40–900 °C. Sample (5 – 7 mg) was used in a platinum pan under a dynamic nitrogen atmosphere (100 mL min^{-1}). System calibration was performed using a calcium oxalate monohydrate standard. The curves were analysed using the TA-60WS software.

X-ray Powder Diffraction (XRPD)

X-ray diffraction patterns of ISDN:lactose (25:75, w/w), lactose monoidratada, HPMC E6, 2-HPβCD and ODFs (F1-9) were obtained using a diffractometer (Bruker D8 Advance X-ray powder diffractometer) employing a radiation source Cu K_α operating at 40 KV, 40 mA, scan range from 5 to 60° (2 theta), and scan rate of 2°C min^{-1}. Polycrystalline silicon (Si) was used as the standard.

Physical and mechanical properties

The thickness of the ODFs was measured in continuous mode using a Defelsko Inspection Instruments model PosiTector Standard 200 [26] with an accuracy of 0.001 ± 0.0001 μm.

The mechanical properties of the ODFs were evaluated using a puncture test (ASTM D2582-16 (2016)) and pull-off adhesion test (ASTM D4541–17(2017)), using a Brookfield CT3 Texture Analyser with a 50 kg load cell at room temperature. The tests were performed using the Texture ProCT software.

For the puncture test, the samples were fixed in the TA-FSF accessory, placed on the TA-BT-kit (fixture base table), and subjected to puncture strength using a TA39 probe (2 mm D, 200 mm L, stainless steel). Load *vs.* displacement data were recorded from the point of contact of the probe with the film until the film ruptured. The puncture strength, elongation at break, and puncture to energy were calculated according to the method described by Radebaugh *et al.* [27]. The nature of the test did not allow the calculation of Young's modulus [6,27].

In the adhesion test, an epithelium segment of the pig oral mucosa (Animal Ethics Committee n° 1352120520) was fixed on the mucus adhesion text fixture (TA-MA) accessory, submitted in a borosilicate glass flask containing 0.9% physiological solution to reach the lower surface of the mucosa, with stirring at 37.5 °C. On the probe TA5 (12.7 mm D, 35 mm L; Black Delrin), a piece of double-sided adhesive tape was applied, and the sample was deposited on it. The parameters of hardness, adhesive force, and adhesiveness were evaluated [28].

Listerine PocketPaks® Oral Care Strips dissolve instantly, have excellent palatability and appearance, and have been used as a standard film.

The experiment was repeated in triplicate and the mean value reported.

Optical properties

Color determination of the ODFs (F1-9) was carried out using a CR-400 colorimeter (Konica-Minolta, Co. Ltd., Japan) calibrated with white backing, using standard D65 illumination and a 10° absorber. The CIELAB reading system was represented by coordinate L^*C^*h, where L^* $(\mathrm{lighntness})$ $(0 = \mathrm{black}, \mathrm{and}\ 100 = \mathrm{white})$, $C^*(\mathrm{Chromaticity})$, and h (tone angle).

The optical properties of ODFs were compared with standard polystyrene film (SPF), represented as a standard of transparency. The final values are the averages of three measurements.

Surface characteristics and morphology

The films were placed in a light booth under a daylight source (D65 lamp, 6500K) and compared visually.

The morphology of the ODFs (F1-9) was evaluated using SEM JEOL model JSM-6610. The samples were fixed on a metallic support with the aid of a 12 mm thick double-sided carbon tape and subjected to metallization under vacuum to make them electrically conductive. The visualization was performed with an increase of 1.000 x with an excitation voltage of 10 – 15 kV.

Desintegration time (DT)

Disintegration time was analyzed by taking film strip of 6 cm^2 area and employing Petri dish method. The film was placed in a petri dish containing 5 mL of simulated salivary fluid (pH 6.8 phosphate buffer). The time

was noted down when the film strip was disintegrated completely. Tests were performed at room temperature. The disintegration time of ODFs were compared with standard film (Listerine PocketPaks® Oral Care Strips). The experiment was repeated in triplicate and the mean value reported [29].

Water activity (A_w)

A_w was evaluated using the FA-st Water Activity Meter (GBX Instruments, France) previously calibrated with k_2SO_4 ($A_w = 0.970 \pm 0.003$) at room temperature. The final values are the averages of three measurements.

HPLC analysis of ISDN

HPLC system equipped with UV-vis detector (SPD-20A photo-diode array), DGU-20A degassing system, LC-20AT pump, CBM-20A controller, and SIL-20A/HT automatic sampler from Shimadzu, Japan, was used for ISDN analysis. The data were processed using LC-Solution software. The separation was carried on a Kromasil C18 HPLC column (250 mm×4.6 mm×5 µm) using the mobile phase containing metanol:ammonium sulfate 0.1M pH 3.0 (50:50, v/v); this was delivered isocratically at a flow rate of 1 mL/min. The injection volume was 20 µL and the run time was 10 min. The temperatures of the column and autosampler were maintained at 25 °C [30].

ISDN standard solution (25 µg mL^{-1}) was prepared by dissolving 5 mg ISDN:lactose (25/75, w/w) in ultra-purified water using a 50 mL volumetric flask. Analytical curves (1 to 25 µg mL^{-1} ISDN base) were constructed by diluting the standard solution (25 µg mL^{-1}) in mobile phase, using a 5 mL volumetric flask. Linear relations were obtained in the concentration range from 1.00 to 25.00 µg mL^{-1} of ISDN base, according to the equation: y = 12626.781 C (µg mL^{-1}) - 39.457 and Pearson's correlation coefficient (r = 0.9997). The detection limits (LOD) and limit of determination (LOQ) obtained were 0.804 and 2.436 µg mL^{-1}, respectively [31].

Content uniformity

ODFs (F1-9) were transferred to Falcon 15 mL conical tubes, 5 mL of purified water was added, stirred on a vortex-type agitator for 60 s, and filtered through filter paper. Aliquots of 100 µL were diluted 1:100 (v/v) and quantified using HPLC method. The final values are the averages of three measurements. Content uniformity results were used to calculate the amount of dissolved ISDN in the dissolution profiles.

Dissolution profile

Dissolution profiles were obtained using the dissolution equipment Ethik Technology model 299/TTS. A total of three units of each dosage forms (ODFs, or reference product) were subjected to the dissolution tests using the following conditions:

ODFs (F1-9), 6 cm^2: Apparatus 5 (paddle over disc), stirring speed 50 rpm, medium volume 400 mL, degassed water at 37 ± 0.5°C, and analyzed by HPLC method.

Reference product, Isordil® Sublingual 5 mg tablets: Apparatus 2 (paddle), stirring speed 50 rpm, medium volume 900 mL, degassed water at 37 ± 0.5°C, and analyzed by HPLC method [32].

The dissolution efficiency (DE) was obtained from the average dissolution profile for each formulation according to the equation [33]:

$$\text{DE, \%} = [\int_0^T (y\ x\ dt)/y_{100}\ x\ (t_t - t_0)]\ x\ 100, \quad (1)$$

where y_t is percent of drug dissolved at any time t, y_{100} denotes 100% dissolution, and the integral represents the area under dissolution curve between time zero and T.

The model-independent approach employing the similarity factor (f_2) was used to compare the dissolution profiles. According to this approach, f_2 values greater than 50 (50–100) ensure sameness or equivalence of the two curves [34].

Statistical analysis

Statistica version 13.1 software (TIBCO Software Inc., CA, USA) was used for the statistical analysis of the data. The results of thickness, mechanical properties, opacity, and dissolution efficiency were analyzed using DoE for the models without interaction, and with two-level interactions, (linear, linear) or (linear, quadratic) [35]. The desirability method was used to optimize the formulations [36]. Principal component analysis (PCA) and hierarchical cluster analysis were used to explain the optical properties of CIE-LCh.

RESULTS

Thermal Analysis

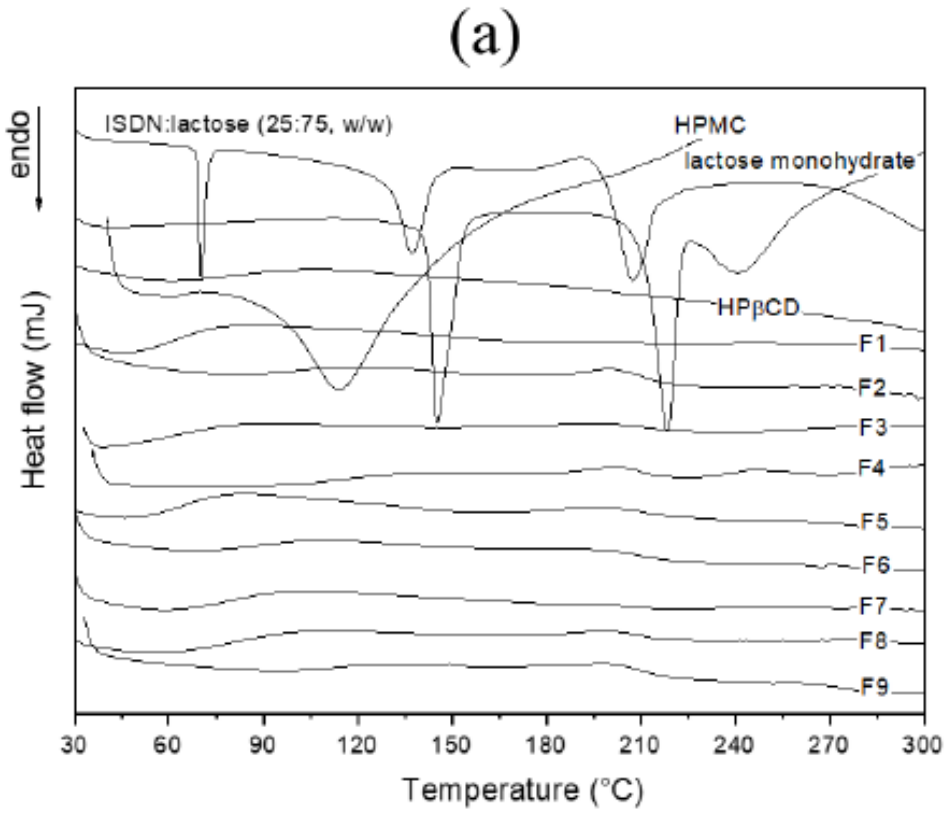
The thermal properties of ISDN:lactose (25:75, w/w), HPβCD, HPMC, and ODFs (F1–9) were evaluated using DSC and TGA.

The DSC curve of ISDN/lactose (25/75, w/w) (Figure 1, panel "a") showed an endothermic event at 70.37 ºC (ΔH = 28.58 J g^{-1}) relative to the melting point of ISDN. The endothermic event at 137.30 °C (ΔH = 78.98 J g^{-1}) was attributed to the loss of water of crystallization of lactose. The endothermic event at 207.57 °C (ΔH = 125.47 J g^{-1}) was attributed to the melting of the α-lactose form, followed by decomposition at this temperature [37]. The overlap of the thermal events of ISDN and lactose monohydrate confirmed the compatibility between ISDN and excipient, as observed by Reddy and Rao [38]. The TG/DTG curves of ISDN/lactose (25/75, w/w) showed decomposition in three events: the first event occurred with fast kinetics between 118.29 °C and 144.96 °C, with a mass loss of approximately 8.08%; the second and third events occurred more slowly and gradually, with mass losses of 24.78% and 51.71%, respectively, and a residual content of approximately 7.93% (Figure 1, panel "b").

ISDN has high explosive power and is commercially available in 25% and 40% concentrations, both diluted with α-lactose monohydrate, for better safety in handling, transport, and storage [38]. According to Reddy and Rao [38], the dilution of ISDN with lactose monohydrate did not influence the thermal stability of ISDN, but considerably reduced its decomposition energy (exothermicity), acting as a heat sink and increasing the safety of its handling.

HPMC is a hygroscopic material that inevitably contains a measurable quantity of water. The DSC curve of HPMC showed an endotherm between 40 and 160 °C (T_{peak} = 114.11 °C, Δm_1 = 0.53%) due to the elimination of surface water, followed by an exothermic decomposition at this temperature. As shown in the TG/DTG curves, the material remained stable up to 300 °C, undergoing a two-step decomposition with mass losses of 83.18% and 13.07%, respectively. The DSC curve of HPβCD showed a wide endothermic event between 24.86 °C and 105.64 °C, which was attributed to loss of water (Δm_1 = 3.05%).

The DSC curves of ODFs (F1–9) showed that the event related to the melting of ISDN/lactose (25/75, w/w) was suppressed, probably due to conversion into its amorphous form [39]. The TG/DTG curves of the ODFs showed a two-step decomposition: the first stage occurred at a temperature below 100°C attributed to water loss (3-5%), followed by slow and gradual decomposition of the material (Figure 1, panel "b").



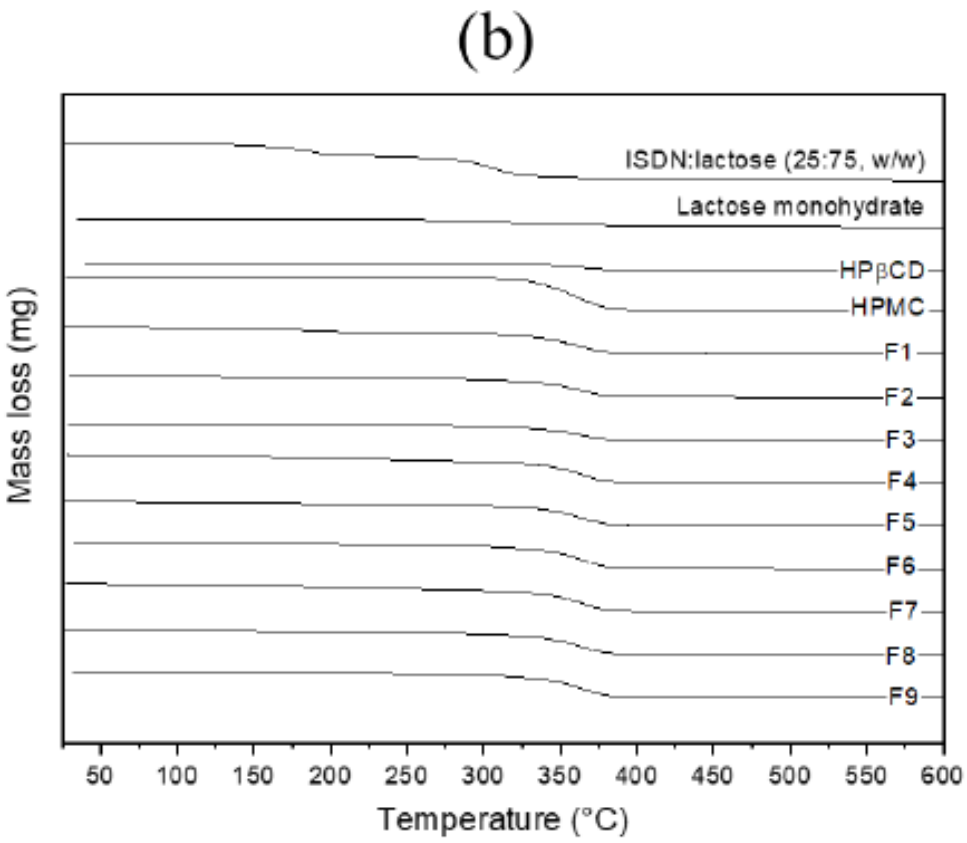


Figure 1. DSC (panel "a") and TG/DTG (panel "b") curves of ISDN/lactose (25/75, w/w), lactose monohydrate, HPβCD, HPMC E6 and ODFs (F1-9)

XRPD

Figure 2, panel "a", shows the XRPD patterns of ISDN:lactose (25:75, w/w), HPβCD, HPMC, HPβCD, and ODFs (F1–9). Reflections of the crystalline planes of ISDN/lactose (25/75, w/w) were observed at 9.99°, 12.50°, 16.37°, 17.27°, 19.42°, 19.98°, 21.23°, 23.75° and 25.46° (2θ). The lactose monohydrate exhibited main reflections at 12.50°, 16.37°, 19.42°, 19.98° and 21.23° (2θ) [37]. A hollow pattern was recorded for HPβCD indicating their amorphous state. The HPMC E6 cellulose derivative exhibited a characteristic of a semi-crystalline structure, revealing two main broad peaks at approximately 9.14° and 19.5° (2θ), and three very low-intensity sharp peaks (*) at 27.3°, 31.6°, and 45.4°.

XRPD diffractograms exhibited ISDN amorphization, probably due to complexation with polymers (HPMC E6 and HPβCD), suggesting the formation of a multi-component complex [(ISDN:HPβCD)-HPMC].

An important characteristic of cyclodextrins is the formation of inclusion complexes, both in the liquid and solid states, through van der Waals interactions and hydrogen bonds. The complexation efficiency increase by adding a small amount of water-soluble polymer, resulting in the formation of multi-component complexes [(drug:CD)-polymer], also called co-complexes. In solution, the polymers decrease the mobility of the cyclodextrins and increase the solubility of the complexes formed [40,41].

The formation of amorphous solid dispersions (ASDs), in which active pharmaceutical ingredient (API) is dispersed in a polymer matrix, thus forming an amorphous one-phase system, is one of the main strategies employed in the pharmaceutical industry to increase the solubility and stability of drugs [42]. Water-soluble polymers can interact with drug molecules through ion-ion, ion-dipole, and dipole-dipole electrostatic bonds, van der Waals forces, and hydrogen bonds, resulting in polymer-(drug)n complexes [43]. According to Usui *et al.* [44], the increased stability in dispersed systems can be attributed not only to the increased viscosity of the systems, but also to the establishment of interactions between the components.

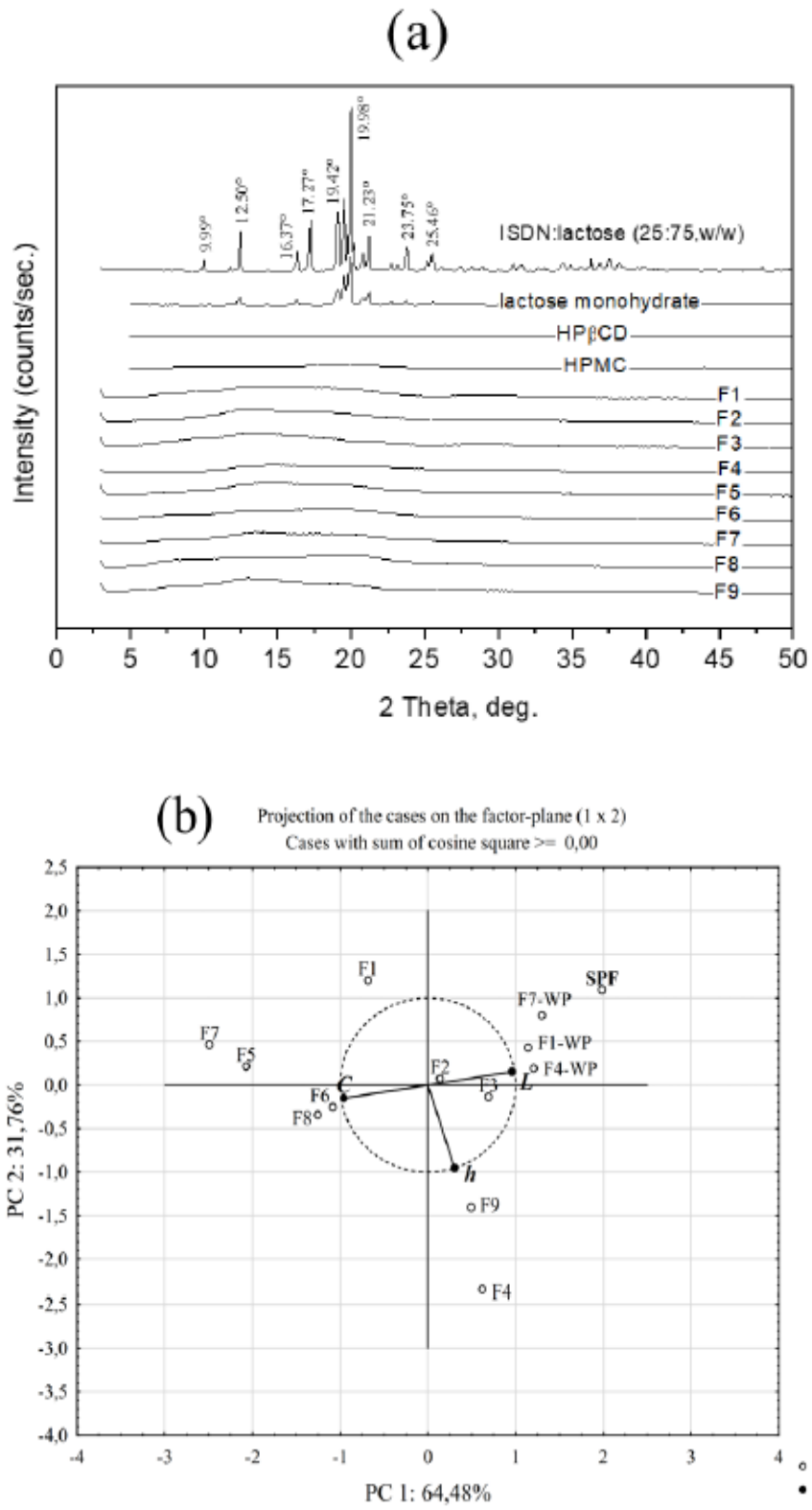


Figure 2. XRPD diffractograms (panel “a”) and PCA: Bi plot based on CIE-L^*C^*h space color (panel “b”)

Physical and mechanical properties

The thickness of oral films is important in achieving convenient dosage form administration and is related to the amount of polymer and drug present in the preparation. It is known that the amount of plasticizer can slightly increase the thickness of oral films [6].

The thicknesses obtained in this study agreed with the ideal values described in the literature [6]. The high standard deviations observed in some ODFs could be attributed to the equipment used for ODF drying, where the irregularity of the tray level prevented the homogeneous distribution of the dispersions in the molds (Table 3).

The disintegration time increased proportionally with the thickness of the ODFs (F1–9) (Table 3). According to Speer et al. [45], when the film reacts with liquid, hydration of the film-forming polymer surface occurs, forming a gel, which makes it difficult for the liquid to diffuse through the film; thus, thicker films have higher disintegration time values.

DoE is a statistical technique used to plan experiments and analyze data using a controlled set of tests designed to model and explore the relationship between factors and observed responses. This technique allows the researcher to use the minimum number of experiments in which the experimental parameters can be varied simultaneously to make evidence-based decisions. It uses a mathematical model to analyze process data. The model allows a researcher to understand the influence of the experimental parameters (inputs) on the response (outputs) and to identify an optimum process [22].

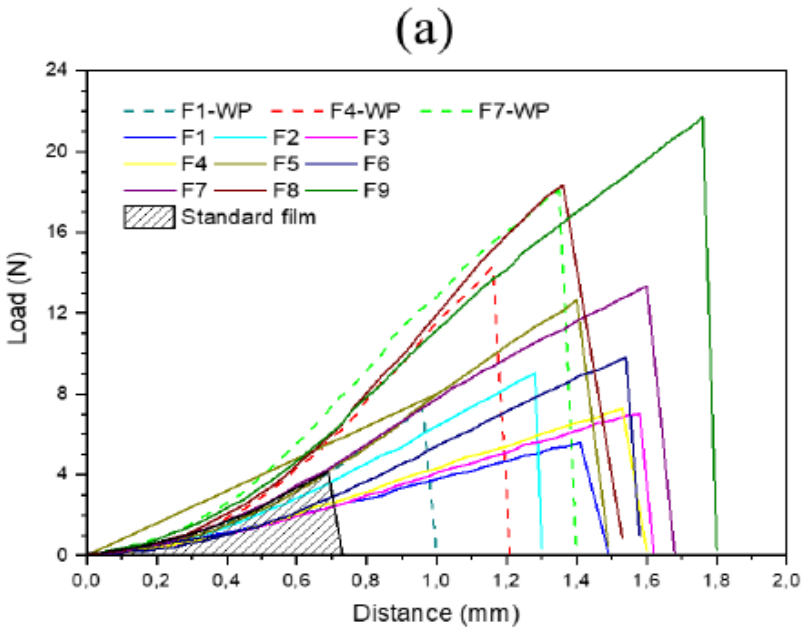
The responses of the dependent variables (physical and mechanical properties) for the nine batches and the film standard are shown in Table 3 and 4.

The films prepared without plasticizer showed high fragility and difficult extrusion from the molds. For both parameters, thickness (R-sqr = 0.99637; Adj = 0.99032) and disintegration time (R-sqr = 0.98757; Adj = 0.96685), only the polymer variable (linear) showed a significant effect ($p < 0.05$), and the parameters increased as a function of the amount of polymer (HPMC) present in the formulations (1.5%, 2.0%, and 2.5%) (Table 3).

The mechanical properties of the films were affected by the composition of the formulation and preparation method. Oral films must have adequate mechanical strength to avoid or minimize damage during handling and, have sufficient tension to be easily removed from the mold after drying; however, they must not be too flexible to elongate during cutting and packaging, compromising the content uniformity of pharmaceutical units [9].

Although stress-strain testing is commonly used in the evaluation of films, it has limitations because it is designed for tough materials, and therefore has limited sensitivity for polymers [27]. The puncture test is an alternative method for evaluating the mechanical properties of oral films, which are capable of overcoming these disadvantages [6].

For puncture strength (R-sq = 0.92308; Adj = 0.79487), only the polymer variable (linear) exhibited significant influence ($p < 0.05$); thus, generally, this parameter varied according to the amount of polymer. For plasticizer, propylene glycol (PP) showed higher values. For displacement (R-sq = 0.9915; Adj = 0.97734) and elongation at break (R-sqr = 0.98659; Adj = 0.96424), polymer (linear) and plasticizer (linear, quadratic), exhibited significant influence ($p < 0.05$), in which the elongation at break increased proportionally with polymer concentration (HPMC). The addition of plasticizer increased the ductility of the films in the following order: PEG400 > Gly > PP. In addition, puncture energy (R-sqr = 0.67967; Adj = 0.14578) was not significantly influenced by the amount of polymer and nature of plasticizer (Table 4; Figure 3, panel “a”).



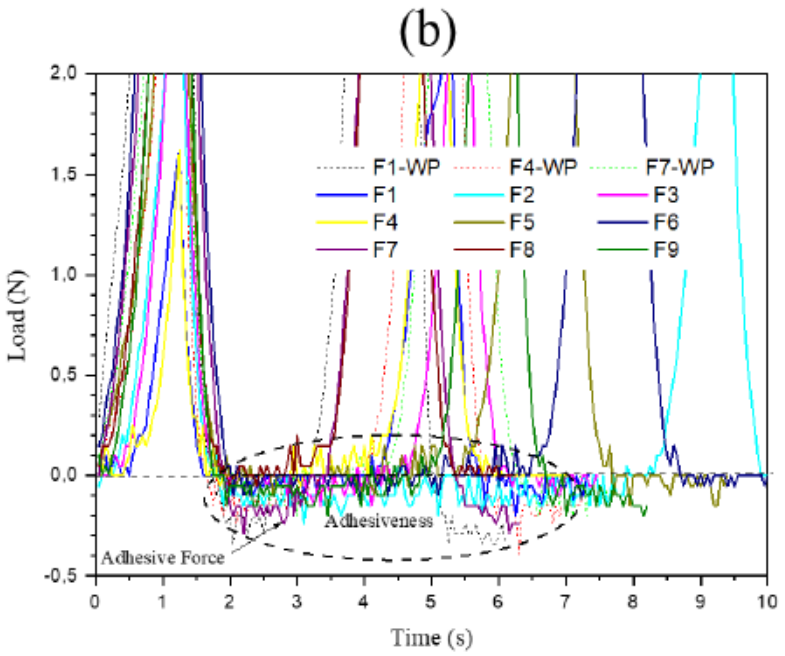


Figure 3. Curves obtained for ODFs with plasticizer (F1-9) and without plasticizer (F1-WP, F4-WP and F7-WP), and standard film (Listerine PocketPaks® Oral Care Strips, cross-hatched area) by means of puncture test (panel "a") and pull-off adhesive test (panel "b")

The elongation at break is a measure of polymer ductility. Plasticizers are molecules with low molecular weight and low volatility, which reduce intermolecular interactions by coupling between the polymer chains, increasing their mobility and reducing the glass transition temperature, viscosity, Young's modulus, and fragility of the films [46]. For this reason, films prepared with plasticizers deform more than films prepared without plasticizers [6].

The physical and chemical properties of the plasticizers, such as chemical structure, shape, polarity, chain length, physical state, and number of active functional groups, determine their ability to plasticize a polymer network. The differences in the plasticizer effect can be attributed to the different availability of oxygen atoms for the hydrogen bond. The spacing of the O atoms in PEG can allow more space for the formation of hydrogen bonds with biopolymer chains [47]. Mahadevaiah et al. [48] evaluated the addition of PEG (0.01%–0.04%) or glycerin (0.01%–0.05%) to HPMC E6 Premium LV films (5%) and found that the addition of plasticizers decreased the Young's modulus and tensile strength and that glycerin was a more efficient plasticizer than PEG.

The use of plasticizers can overcome the brittleness and soften the rigidity of the film structure by reducing the intermolecular forces. However, using an excessive amount of plasticizer can decrease the adhesive strength of the films by over-hydrating the film formulations [6].

Bioadhesion describes the adhesion between a biological surface and another synthetic surface by means of interfacial forces [35]. The majority of oral films are not necessarily designed to be mucoadhesive; however, they may exhibit some degree of mucoadhesiveness because of the inherent characteristics of the polymers used. In general, non-ionic polymers have lower mucosal adhesive strength than ionic polymers (anionic or cationic). Although weak, non-ionic polymers can exhibit bioadhesive properties through non-covalent interactions with the surrounding fluids [6,28]. Mucoadhesion may also vary depending on the chemical properties and molecular weight (Mw) of the film-forming polymer used. The molecular weights of the most common polymers used for mucoadhesive films are usually below 200,000 Da, whereas non-adhesive fast-dissolving films are normally composed of low molecular weight hydrophilic polymers (approximately between 1.000 and 9.000 Da) [5].

The ODFs (F1–9) exhibited values of hardness, adhesive force, and adhesiveness lower than those of a standard film (Listerine PocketPaks® Oral Care Strip) (Table 4; Figure 3, panel "b").

For hardness (R-sqr = 0.92744; Adj = 0.8065), only the plasticizer (linear) had a significant effect ($p < 0.05$) and varied in the following order: PEG400 > PP ≅ Gly. For adhesive force (R-sqr = 0.93039; Adj = 0.81438), polymer (linear) and plasticizer (linear) showed significant effects ($p < 0.05$). For low polymer concentration (HPMC 1.5% and 2%), the adhesive force increased proportionally with the amount of polymer, and the addition of plasticizer increased the adhesiveness of the films in the following order: PEG400 > PP > Gly. In addition, for adhesiveness (R-sqr = 0.98451; Adj = 0.95869), only plasticizer (linear, quadratic), and

the interaction between them, showed significant effect ($p < 0.05$) and varied in the following order: PP > Gly > PEG400.

Pullulan is the main film-forming polymer of the Listerine PocketPaks® Oral Care Strips; however, it also contains xantham gum and carrageenan. Pullulan is a modified starch with good film-forming properties and is one of the preferred polymers used in the preparation of oral polymeric matrices; however, its low availability results in high-cost pullulan products. Therefore, pullulan is normally mixed with other more abundant and less expensive compatible polymers. Anionic polymers can naturally be used as bioadhesive materials because they tend to adhere to the mucosa through non-covalent secondary interactions, normally hydrogen bonds between the charged polymer chains and the oligosaccharide side chains of mucosal proteins. Xanthan gum is an anionic polysaccharide with exceptional mucosaladhesive properties, formed by 1.4-linked residues β-D-glucose with a trisaccharide chain linked to alternating D-glucosyl residues. Additionally, carrageenans constitute a group of anionic polymers that are widely used for the formation of films, owing to their excellent mucosal-adhesive properties; they contain sulfated functional groups capable of forming non-covalent bonds with the lateral chains of the oligosaccharides of mucosal proteins [5].

Table 3. Average values (and standard deviation) of physical and physicochemical properties obtained for ODFs with plasticizers (F1-9) and ODFs without plasticizers (F1-WP, F4-WP and F7-WP), and standard film (SF, Listerine PocketPaks® Oral Care Strips) (n=3)

Parameters	Thickness (μm)	DT (s)	a_w (%)	UC (mg)	DE (%)	f_2*
SF	39.44±1.20	42±0.02	0.07±0.03	NA	NA	NA
F1-WP	61.00±2.94	35±0.89	0.56±0.01	NA	NA	NA
F4-WP	88.00±2.00	54±1.12	0.56±0.00	NA	NA	NA
F7-WP	124.67±3.40	132±0.95	0.55±0.00	NA	NA	NA
F1	55.98±4.58	38±1.20	0.54±0.01	4.86±0.12	97.95±0.02	72.32
F2	55.50±4.26	32±1.64	0.71±0.01	4.49±0.42	73.42±0.01	50.99
F3	67.77±5.56	42±0.67	0.55±0.01	4.84±0.08	97.11±0.01	63.85
F4	86.53±1.51	55±0.89	0.55±0.01	4.51±0.10	88.34±0.02	34.39
F5	85.00±6.38	52±1.97	0.76±0.02	4.89±0.15	95.97±0.01	53.03
F6	88.77±0.83	58±2.01	0.56±0.00	4.89±0.10	94.61±0.01	63.55
F7	125.00±2.94	131±3.32	0.55±0.00	4.94±0.08	95.05±0.01	66.50
F8	125.93±0.90	134±1.89	0.62±0.00	4.90±0.07	97.21±0.01	69.37
F9	123.93±3.42	135±1.72	0.56±0.01	4.86±0.10	97.65±0.01	58.29

DT, disintegration test; a_w, water activity; UC, uniformity content; DE, dissolution efficiency; f_2, similarity factor. *NA not applied.

Table 4. Average values (and standard deviation) of mechanical properties obtained for ODFs with plasticizers (F1-9) and ODFs without plasticizers (F1-WP, F4-WP and F7-WP), and standard film (SF, Listerine PocketPaks® Oral Care Strips) (n=3)

Parameters	Puncture test				Pull-off adhesive test		
	D (mm)	PS (MPA)	E (%)	PE (N/mm^3)	H (N)	AF (N)	AD (mJ)
SF	0.70±0.07	1.12±0.16	1.16±0.25	0.30±0.04	4213±136	70.00±7.07	2.70±0.16
F1-WP	1.13±0.27	2.75±0.78	3.31±0.02	0.95±0.56	6.23±2.92	0.27±0.11	0.10±0.08
F4-WP	1.34±0.18	5.31±0.76	4.44±0.01	1.43±0.49	4.74±0.88	0.18±0.13	0.10±0.08
F7-WP	1.57±0.16	5.90±0.81	5.32±0.01	1.37±0.35	3.30±1.98	0.16±0.14	0.07±0.09
F1	1.39±0.02	1.82±0.04	4.69±0.00	0.85±0.09	3.07±0.89	0.00±0.00	0.17±0.09
F2	1.25±0.06	3.59±0.21	3.80±0.00	1.94±0.20	3.63±1.54	0.10±0.00	0.27±0.05
F3	1.45±0.13	2.04±0.62	5.13±0.01	0.82±0.19	10.34±0.24	0.21±0.06	0.13±0.05
F4	1.51±0.30	2.36±0.39	5.69±0.02	0.60±0.44	7.37±1.12	0.16±0.11	0.20±0.16
F5	1.41±0.08	5.38±0.67	4.82±0.01	1.88±0.13	7.77±2.92	0.17±0.02	0.23±0.09
F6	1.59±0.26	2.83±0.00	6.12±0.25	0.73±0.79	10.74±5.53	0.36±0.06	0.10±0.14
F7	1.59±0.08	5.03±0.06	6.08±0.01	1.17±0.01	6.24±1.47	0.19±0.14	0.25±0.05
F8	1.56±0.20	6.35±0.26	5.98±0.02	1.42±0.31	6.83±4.17	0.28±0.27	0.30±0.10
F9	1.78±0.18	7.31±3.00	7.65±0.01	1.94±.95	6.86±0.12	0.29±0.27	0.10±0.00

D, displacement, PS, puncture strength; E, elongation at break; PE, puncture to energy; H, hardness (cycle I); AF, adhesive force; AD, adhesiveness.

Optical Properties

The color is often used to make judgments on its quality of products. The color evaluation can be performed visually by trained panels of experts using preselected attributes or charts. This approach is characterized by a high degree of subjectivity and to overcome such limit, color should be evaluated using more objective methods. Instrumental techniques that use spectrophotometers or colorimeters which require less time and provide easily comparable results, have been used more often for research and routine daily analysis. Among the different spectrophotometric systems, the tristimulus CIE (Commission Internationale de l'Eclairage, International Commission on Illumination) space defines the color spaces in CIE XYZ, CIE Lab and CIE LCh using chromatic coordinates. These systems allow users to evaluate color attributes, identify inconsistencies, and accurately express their findings to others in numerical terms. Currently, color space L^*C^*h is preferred by some industry professionals because it correlates best with how the human eye perceives color [49]. In this color space, L^* indicates lighntness, C^* represents chroma, and h is the hue angle.

Slight color difference was observed between the standard polystyrene film (SPF) and the ODFs. The high L^* values indicated high luminosity, C^* shows the saturation level, and the h values showed yellow hue (Table 5).

The first two principal components were selected from the observed eigenvalues (Table 5). Based on the absolute values of coefficients found for the eigenvectors it was possible to observe that the first principal component (PC1) had a positive association with L^* and a negative association with C^*, while the second principal component (PC2) was negatively associated with h (Table 5):

$$\text{PC1: } \mathbf{+\ 0.6897\ } \boldsymbol{L^*} \mathbf{\ -\ 0.6900\ } \boldsymbol{C^*} + 0.2194\ h \qquad (2)$$

$$\text{PC2: } +\ 0.1569\ L^* - 0.1534\ C^* \mathbf{-\ 0.9756\ } \boldsymbol{h} \qquad (3)$$

Table 5. Mean values and standard deviation of the chromaticity coordinates obtained for the color space CIE-LCh

Samples	L^*	C^*	h
SPF	104.33±0.07	2.50±0.02	101.92±0.19
F1-WP	104.29±0.09	3.00±0.12	102.32±0.28
F4-WP	104.16±0.11	2.59±0.08	102.53±0.35
F7-WP	104.11±0.16	2.67±0.07	102.03±0.39
F1	103.66±0.14	3.22±0.12	101.32±0.27
F2	103.74±0.16	3.00±0.17	102.43±0.49
F3	104.00±0.42	3.00±0.09	102.71±0.35
F4	103.74±0.10	3.02±0.08	104.57±0.35
F5	103.36±0.23	3.78±0.45	101.89±0.97
F6	103.53±0.04	3.48±0.07	102.48±0.28
F7	103.13±0.07	3.73±0.24	101.60±0.52
F8	103.61±0.74	3.68±0.27	102.52±0.60
F9	103.90±0.17	3.16±0.07	103.75±0.25

The factorial loadings plot allows us you to identify the variables that have the greatest effect on each component. The graph of factorial loadings allowed us to infer that the variables L^* and C^* had a strong influence on PC1, and h on PC2 (Figure 2, panel "b").

The score plots indicate the color similarities between the samples. Figure 2, panel "b", shows the projection of the samples in a two-dimensional space, formed by the first two principal components (PC1 vs. PC2), which explain 96.24% of the data (Table 5). Standard polystyrene film (SPF), ODFs without plasticizers (F1-WP, F4-WP and F7-WP) and ODFs with plasticizers (F2, F3, F4 and F9) occupied the same region in the score plot, to the right of PC1, with a positive score (region with strong influence of L^*), suggesting similarity between them. When analyzing the samples located at the extremities of PC1 (x-axis), we verified that on the left side was F1, with a negative score, and F4 and F9, with a positive score, indicating a color difference between them. When analyzing PC2 (y-axis), at the bottom of the graph, we found F4, F6, F8 and F9, with negative score (region with strong influence of h), and at the top, SPF, F1, F2, F3, F5 and F7, with a positive score, indicating opposite behavior among the samples in relation to PC2 (Figure 2, panel "b").

Surface characteristics and morphology

Optical media that interact with light can be classified as transparent, which allows the passage of light in a defined way, translucent, where the passage of light occurs in an irregular way, and opaque, where the light does not propagate, causing reflection and absorption [46]. Through the visual evaluation it was possible to see that the films prepared with glycerin (F1, F4, and F7) and PEG 400 (F3) were translucent, whereas the other films were transparent (Figure 4).

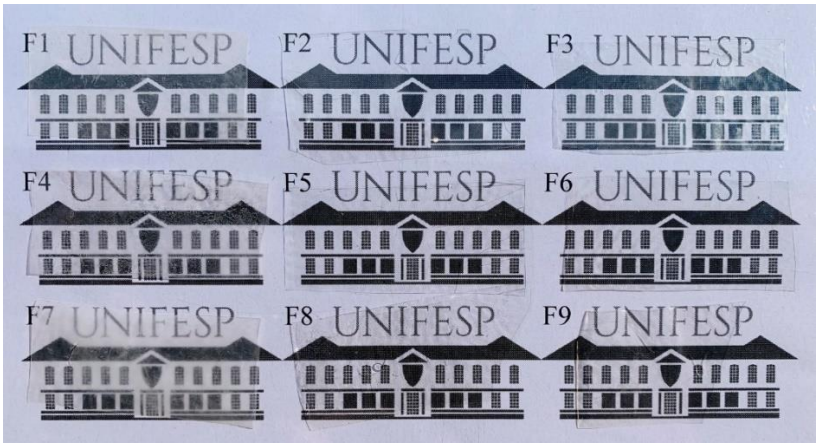


Figure 4. Photographs of ODFs (F1-9). Background image has been added to make it easier to see the transparency.

In the microscope, ODFs prepared without a plasticizer (F1-WP, F4-WP, and F7-WP) had a rough and grooved surface. Films prepared with Gly (F1) or PEG 400 (F3) and low concentrations of HPMC E6 (1.5%) exhibited the presence of fat droplets dispersed in the systems, suggesting greater difficulty of these plasticizers in dispersing ISDN:lactose. Homogeneity in these systems increased proportionally with polymer concentration. In contrast, the films prepared with prolylene glycol (PP) were more homogeneous, showing better drug solubilization capacity (Figure 5).

Glycerol, polyethylene glycol and propylene glycol are hydrophilic plasticizers most commonly added to films. The efficiency of each plasticizer to function in the film network depends on molecular size, shape, structure, and water binding. Propylene glycol has two -OH groups (76.1 g/mol), glycerol has three -OH groups (92.0 g/mol), and, finally, PEG is a polyether that has no fixed value for molecular weight. According to miscibility, plasticizers with low molar mass have higher entropy for mixing than those with high molar mass. Thus, PEG and Glycerol are considered more difficult to disperse than propylene glycol [50,51].

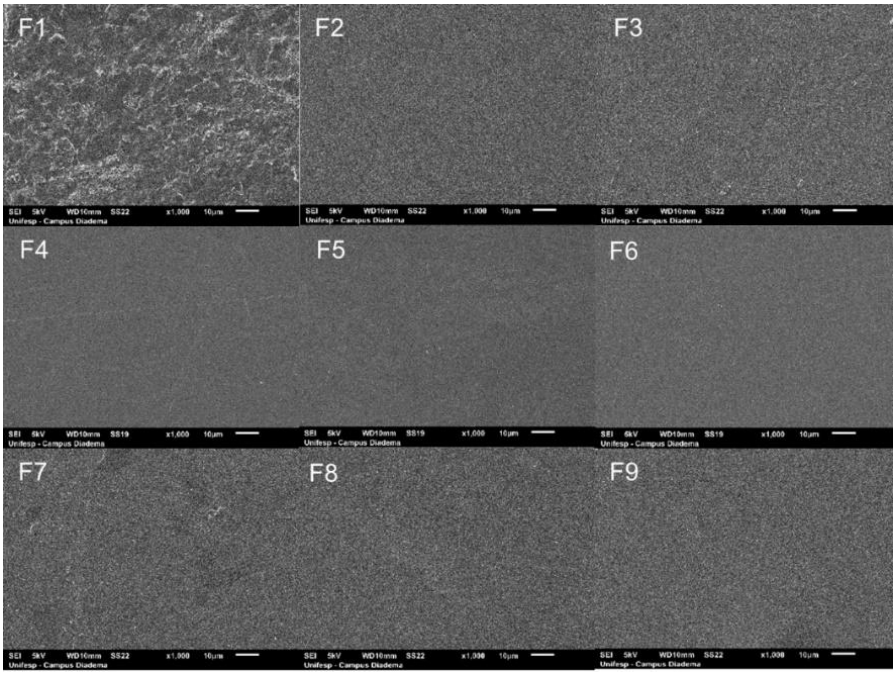


Figure 5. Photomicrographs of ODFs obtained using scanning electron microscopy (SEM) with an increase of 1,000x

Dissolution profile

Saliva plays a critical role in the absorption of drugs by ODFs. Among the parameters that may have an impact on the dissolution of drugs, pH (6.97–7.40), viscosity (1.5–1.6 mPa s) and salivary flow (0.58–1.51 mL min^{-1}) must be considered [5,52,53]. ISDN has good solubility (0.98 mg/mL) and permeability ($\log P_{O/A}$ = 1.31) in the gastrointestinal tract and is classified as class I in the Biopharmaceutical Classification System (BCS) [54]. However, its administration in the oral cavity is preferred over the gastrointestinal tract because of its relatively faster initial action [55]. Although less permeable, the buccal mucosa is well vascularized, and drugs can be rapidly absorbed into the venous system underneath the oral mucosa [7].

Except for F4, the dissolution profiles of the films were similar (f_2, similarity factor) (Table 3) to the reference product, Isordil® sublingual 5 mg (Figure 6). The dissolution efficiency values of the ODFs (F1–9) were higher than that observed for the reference product (88.35±0.01%), suggesting increased solubility of ISDN by hydroxypropyl-β-cyclodextrin (HPβCD) (Table 3).

Dissolution efficiency (DE) is a parameter used to compare dissolution profiles and may correlate with *in vivo* data [33]. The analysis of the DE values of the films using DoE, showed no significant influence of the amount of polymer and nature of plasticizer (Table 3).

In recent years, industries have successfully applied experimental planning to improve production efficiency and reduce processing costs without sacrificing the quality of their products [56]. According to Goethals and Cho [57], one of the main difficulties in solving problems of multiple characteristics (multivariate) is to optimize each characteristic simultaneously, and one of the most widely used methods to solve problems of multiple response optimization is the *desirability function* (f_D). This function is based on the idea that the quality of a product or process that has many variables is completely unacceptable if any of them is outside a "desirable" limit. Its goal is to find conditions that ensure compliance with the criteria of all, or the main responses involved, and at the same time provide the best value in the joint response, this value being the most desirable. That is, to be able to convert the multiple response variables into one, by combining the individual responses into a composite function, followed by their optimization [36].

Based on the effects of thickness, mechanical properties (elongation at break and adhesiveness), and dissolution efficiency (DE) of the ODFs (F1-9), it was possible to select the best formulation based on the *desirability function* ($0 \leq f_D \leq 1$), using the optimal values of all the factors [36,58]. The optimum values adopted were thickness (40 to 100 μm), elongation at break (3.8 to 5.7%), adhesiveness (0.2 to 0.4 mJ), and dissolution efficiency (>85%). Thickness and break elongation are important to facilitate extrusion of the films from the molds, the adhesiveness should be sufficient to prevent removal of the films by the patient, and the efficiency should be as high as possible (>85%) for maximum product efficiency.

The maximum value for the *desirability function* (f_D) was 0.86 (Figure 7), corresponding to 1.5% to 2.0% of the polymer (HPMC E6) and Gly or PP as plasticizers. The F5 formulation showed adequate thickness (85.00±6.38 μm), elongation at break (4.82±0.01%), adhesiveness (0.23±0.09 mJ), and dissolution efficiency (95.97±0.01%) (Table 3 and 4). In parallel, showed transparency close to the standard polystyrene film (SD).

Dinge and Nagarsenker [39] developed oral films of triclosan using HPMC as a matrix, and Poloxamer and HPβCD as solubilizing agents. According to the authors, HPMC (Methocel E5) can form films with excellent palatability, good mechanical properties, and adequate dissolution speed at a concentration of 2.2% (w/v).

Water activity (a_w) is a measure of the energy state of the water in a system and is a more effective indicator of stability than humidity. In general, the higher the water activity ($0 \leq a_w \leq 1$) of a material, the greater its risk of contamination and degradation; therefore, the importance of a_w is related to the stability of the final product [28,59]. All ODFs showed a_w values above the optimal conditions to inhibit microbial growth (a_w > 0.86); therefore, the use of preservatives is recommended (Table 3).

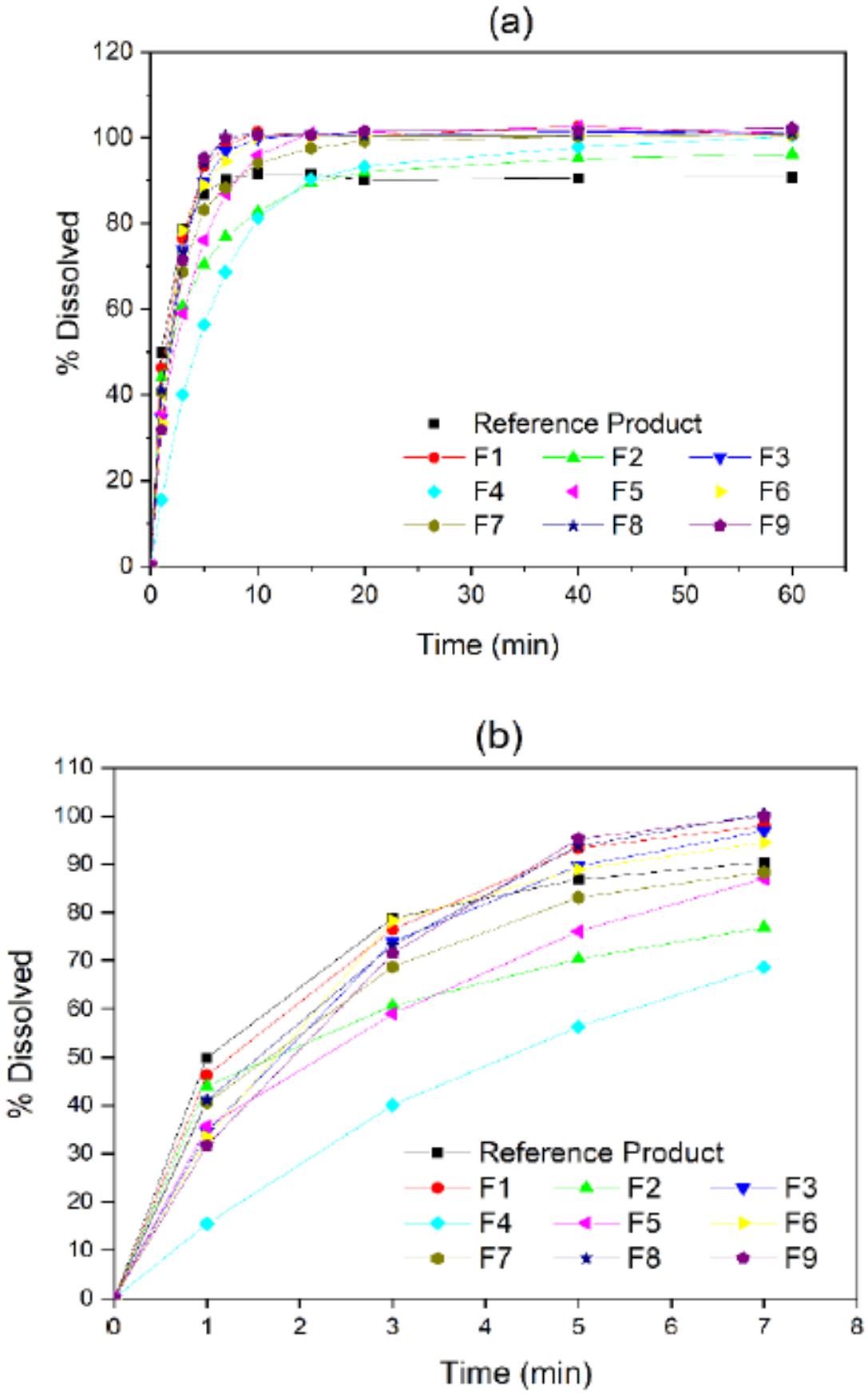


Figure 6. Dissolution profile of reference product and ODFs (F1-9) containing 5 mg ISDN base (f_2 = 54.09) obtained in the range from (panel “a”) 0 to 60 min and (panel “b”) 0 to 7 min.

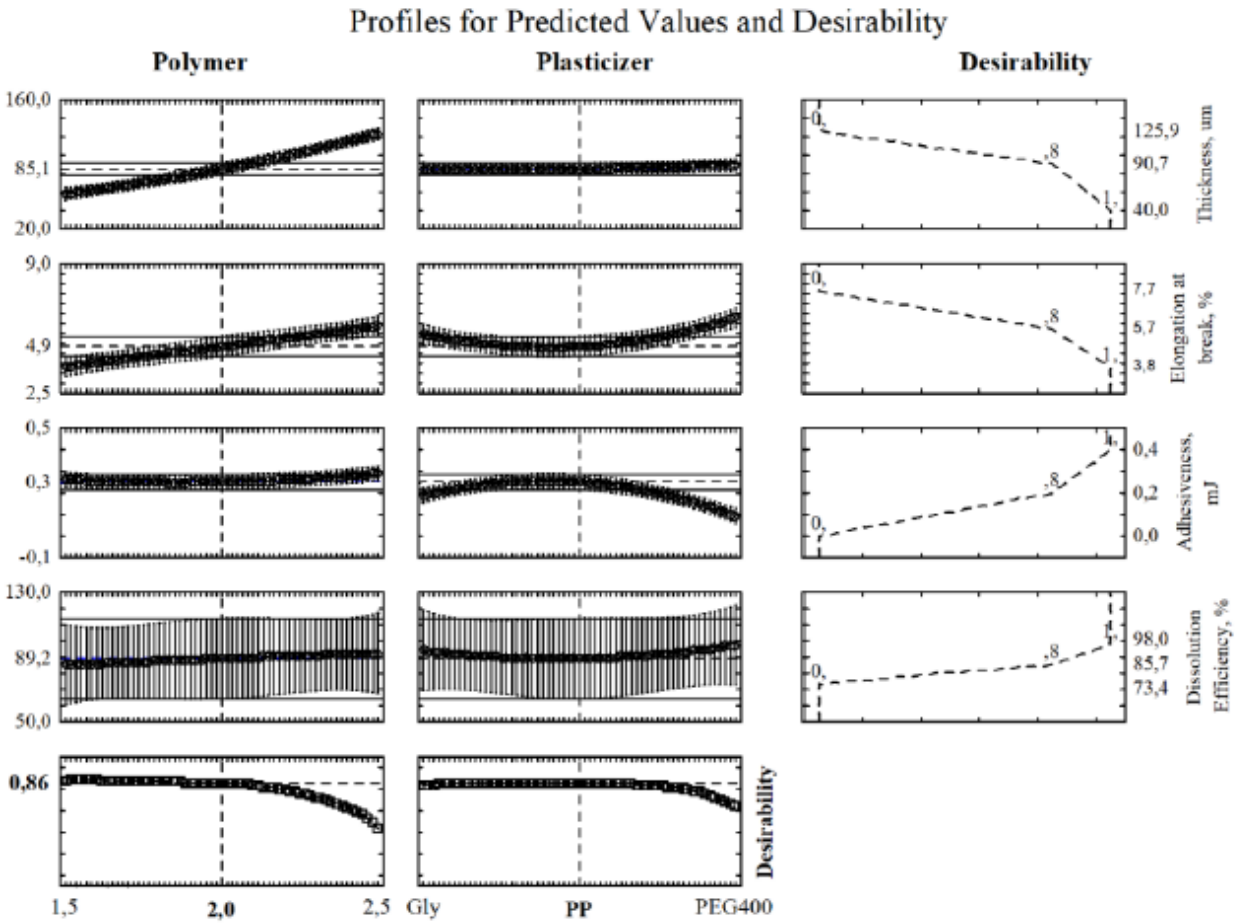


Figure 7. Global desirability function (f_D = 0.86)

CONCLUSION

In this study, an orally disintegrating film of ISDN was developed and evaluated for mechanical, optical, and physicochemical properties.

DSC curves and XRPD diffractograms showed ISDN amorphization in the presence of HPβCD and HPMC, probably due to complexation with polymers, suggesting the formation of a multi-component complex [(ISDN:HPβCD)-HPMC].

The DE values of the ODFs (F1–9) were higher than those observed for the reference product, suggesting increased solubility of ISDN:lactose monohydrate (25:75, w/w), which contributed to obtaining films with a good aspect and high transparency.

DoE successfully helped in understanding the interaction effects between the analyzed factors. Polymer (HPMC E6) and plasticizers played a critical role in the mechanical properties of the films; however, these factors had no significant effect on the speed of dissolution of the orally disintegrating films, which had similar performance to the reference drug.

Based on the effects of thickness, mechanical properties (elongation at break and adhesiveness), and dissolution efficiency of the films, it was possible to select the best formulation based on the *desirability function* ($0 \leq f_D \leq 1$), using the optimal values of all the factors. The maximum value for the *desirability function* was 0.86, corresponding to the application of 1.5% to 2.0 % polymer (HPMC E6), and Gly or PP as plasticizers. The F5 formulation showed adequate thickness (85.00±6.38 μm), elongation at break (4.82±0.01%), adhesiveness (0.23±0.09 mJ), and dissolution efficiency (95.97±0.01%). In parallel, showed transparency close to the standard polystyrene film (SD).

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgments: We would like to thank NATEP and NIPE for allowing the use of the Brookfield CT3 Texture Analyzer and Scanning Electron Microscope (SEM) and Editage (www.editage.com) for English language editing.

Conflicts of Interest: The authors claim that there is no conflict of interest.

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