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Physical-Chemical Characterization and Polymorphism Determination of Two Nimodipine Samples Deriving from Distinct Laboratories

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ABSTRACT Knowing the characteristics of raw materials in pharmaceutical practice is both important and useful. Firstly, evaluating the physical-chemical properties of the substances that will be used must be the primary step for quality control in the pharmacy industry. This work aims at analyzing the physical-chemical characteristics of two nimodipine samples I and II derived from distinct laboratories through thermal analysis (DSC and TG/DTG), HPLC, crystallography, and microscopy. Thermal analysis showed that sample II was more unstable than I. Morphological differences concerning shape, size, and crystallinity of particles were visualized by scanning electron microscopy (SEM) and X-ray powder diffraction. To sum up, the techniques used in this study can be said to have been efficient in the characterization and evaluation of quality control of the raw material.

KEYWORDS Nimodipine, Quality control, Thermal and morphological analysis, Polymorphism, Non-isothermal kinetic analysis

INTRODUCTION

The properties of the solid-state of drug candidates are critical factors in the pharmaceutical formulation development. The most relevant properties can affect the therapeutic efficacy, toxicity, bioavailability, pharmaceutical processing, and stability (Giron, 2003).

Most drug molecules can adopt a variety of conformations. This feature can give rise to solid structures, differing from each other in their space lattice type, in molecular conformation or simultaneously in both features when it is associated with specific interactions between polar groups, often found in those molecules (Leito et al., 2002).

The interrelationship between the presence of amorphous material and drug degradation has caused continuous problems for the development of pharmaceutical formulations (Saklatvala et al., 1999).

The thermal analysis method has been used for drug quality control whenever possible. Combining differential scanning calorimetry (DSC) and

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thermogravimetry/derivative thermogravimetry (TG/DTG) with scanning electron microscopy (SEM) and X-ray powder diffraction is a state-of-the-art technique. They offer quick and proper interpretations, offering the possibility of analytical quantification (Becket et al., 1993; Giron, 2001; Medeiros et al., 2001). The DSC method particularly gives us some quantitative information about the purity of the compounds, making it possible not only to establish the melting temperature interval for the drug and but also to study the phenomenon of polymorphism characteristic of this drug.

Thermogravimetry is an analytical, quantitative, and comparative method capable of producing fast and reproducible results. It can be used in drug quality control for improving the final product through the determination of drug stability by isothermal and nonisothermal kinetic methods. These methods are somewhat easier to quantify (Ford & Timmins, 1989).

High-performance liquid chromatography (HPLC) is a consistent method for qualitative and quantitative drug characterization. It is largely used for determining the purity and impurity of pharmaceutical products (Lacroix et al., 1998).

X-ray powder diffraction (XRPD) of the physical-chemical methods will definitely confirm the different molecular organization within the solid, while thermal analytical methods will provide useful additional information (Ford & Timmins, 1989).

Scanning electron microscopy has found widespread use for the characterization of polymorphs and solvates. It can be performed at extraordinarily high magnification levels, and the images that can be obtained contain a considerable amount of three-dimensional information. The particle size distributions of small samples may be assessed by this technique (Brittain, 1999).

Nimodipine [isopropyl(2-methoxyethyl)-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine-dicarboxylate] (Fig. 1) is a 1,4-dihydropyridine type of drug substance. It is a calcium channel blocker that acts by relaxing the arterial smooth muscle. As it is able to cross the blood-brain barrier, nimodipine can dilate the cerebral arterioles; thus, it is used currently to prevent and treat the ischemic damage caused by cerebral arterial spasm in subarachnoid hemorrhage. Nimodipine has also been used in other cerebrovascular disorders, such as ischemic stroke and multi-infarct dementia (Qiu et al., 2004).

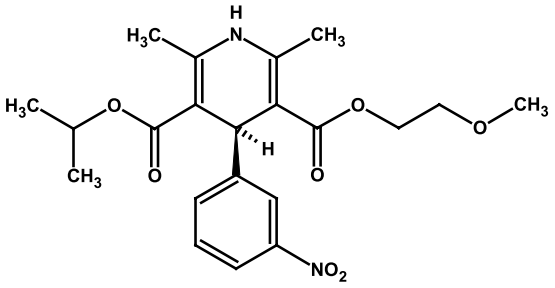

FIGURE 1 Chemical Structure of Nimodipine.

This drug substance can show polymorphic forms (Monographs, 2002). Grunenberg et al. (1995) investigated the polymorphic forms of nimodipine. These authors characterized two specific polymorphic forms: a racemic mixture and a conglomerate one. The substances showed differences concerning some physical-chemical characteristics such as solubility, density, melting point, and enthalpies fusion beyond crystal forms. Therefore, the development of new formulations in which nimodipine is present needs a detailed polymorphism investigation (Grunenberg et al., 1995).

The aim of this work was to evaluate the nimodipine physical-chemical characterization derived from two distinct laboratories through thermal analysis (DSC and TG/DTG), XRD, and SEM. HPLC was used to evaluate the purity of samples.

MATERIALS AND METHODS

Materials

The nimodipine drugs were donated by laboratories designated I and II (for protect of laboratories). The standard was donated by Apsen Pharmaceutics (Brazil).

Differential Scanning Calorimetry

DSC curves were obtained in a DSC-50 cell (Shimadzu) using aluminum crucibles with about 2 mg of samples, under dynamic N_2 atmosphere (100 mL min^{-1}) and heating rate of 10°C min^{-1} in the temperature range from 25° to 600°C. The DSC cell was calibrated with indium (mp 156.6°C; ΔH_{fus}= 28.54 J g^{-1}), and zinc (mp 419.6°C). For purity determination, the heating rate was 2°C min^{-1} in the temperature range of 25–200°C.

High Performance Liquid Chromatography

For purity determination, the HPLC system used consisted of a Rheodyne 7125 injector (Cotati, CA), a model LC-10AD pump and UV/VIS SPD-10AVP, and a controller SCL-10AVP (Shimadzu Corporation, Kioto, Japan). Chromatographic separations were carried out with a Polaris C18-A column (150 × 4.6 mm, I.D.; particle size 5 μm: Metachem Technologies Inc., Canada) and a guard-column (20 × 2 mm, I.D.: Alltech Associates Inc., Deerfield, IL) at ambient temperature. The mobile phase consisted of methanol-water (75:25, v/v). The flow rate was 1 mL min^{-1}. The purity was determinate by comparison between standard and sample solutions.

Thermogravimetric

TG/DTG curves were obtained with a thermobalance model TGA-50 (Shimadzu) in the temperature range 25–900°C, and using platinum crucibles with ~4 mg of sample, under dynamic N_2 atmosphere (50 mL min^{-1}) and heating rate of 10°C min^{-1}. The nonisothermal kinetic study was accomplished under dynamic N_2 and air atmosphere (50 mL min^{-1}, with temperature range 25–400°C, heating rates of 2.5°, 5.0°, 10.0°, 15.0°, and 20.0°C min^{-1}. The results were treated by Ozawa's model in the Tasys software (Shimadzu).

X-Ray Powder Diffraction

For characterization of crystallinity, X-ray diffractions patterns were obtained on a Siemens model D5000, with tube of Cukα, voltage of 40 kw, and current of 40 mA, in the range of 3–65 (2θ) and 1 s of pass time, using the powder XRD method.

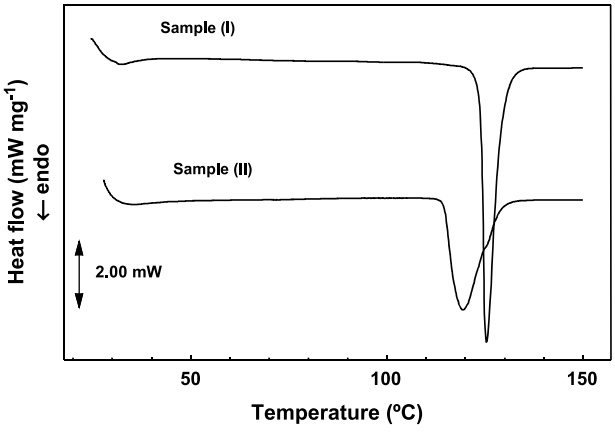

FIGURE 2 DSC Curves of Nimodipine Samples Obtained in Dynamic Nitrogen Atmosphere (ca. 100 mL min^{-1}) and Heating Rate of 10°C min^{-1}.

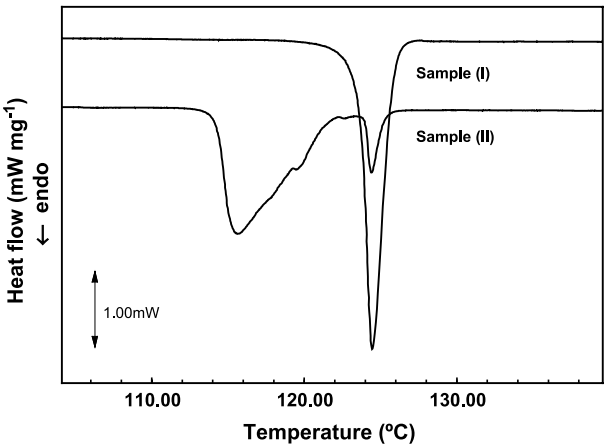

FIGURE 3 DSC Curves of Nimodipine Samples Obtained in Dynamic Nitrogen Atmosphere (ca. 100 mL min^{-1}) and Heating Rate of 2°C min^{-1}.

Microscopy

The morphology of nimodipine samples was observed by a scanning electron microscope (Phillips XL30). Samples were mounted onto metal stubs using double-side adhesive tape, vaccum-coated with gold (350 Å) in a Polaron E 5000 and directly analyzed under SEM (50×, 200×, and 1000×).

RESULTS AND DISCUSSION

Thermal Characteristics Studies

DSC curves (Fig. 2) show different melting points and enthalpies fusion for the samples. The temperature

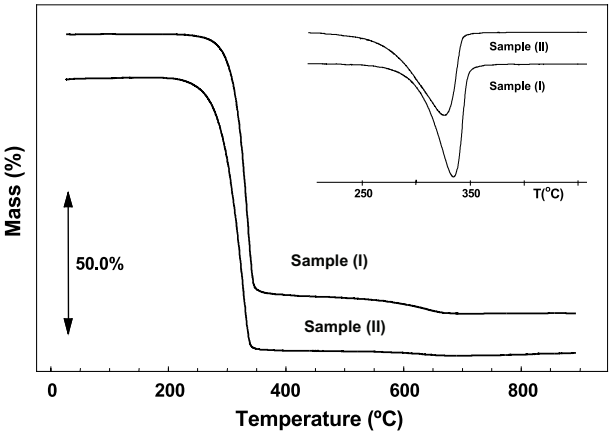

FIGURE 4 TG/DTG Curves of Nimodipine Samples Obtained in Dynamic Nitrogen Atmosphere (ca. 50 mL min^{-1}) and Heating Rate of 10°C min^{-1}.

TABLE 1 **Purity Determinations for DSC and HPLC**

	Purity (%)	
Samples	DSC[a]	HPLC[a]
I	99.4	101.4
II	–	99.5

[a]Assay accomplished through three determinations.

range fusion of sample I occurred between 121–134°C (T_{onset}=124.17°C and ΔH_{fusion}=84.9 J g^{-1}), while sample II evidenced a large temperature range of 112–134°C (T_{onset}=114.5°C and ΔH_{fusion}=123.79 J g^{-1}), in this case indicating the impurity presence in the sample or polymorphic forms existence. DSC curves obtained at 2°C min^{-1} confirmed the existence of polymorphic forms in sample II (Fig. 3).

The TG/DTG curves illustrated in Fig. 4 indicate that both samples are stable thermally up to ~200°C and that the thermal decomposition of sample I begins at least 15°C above in relation to sample II. The mass loss main event for both samples occurs between 200° and 350°C with a DTG peak at 326°C and Δm=97% for sample II and DTG peak at 334°C and Δm=93.2% for sample I. The smaller thermal stability of sample II can be due to either impurity presence or the existence of polymorphic forms. It was observed that sample I presented a thermal behavior similar to the polymorph containing a racemic mixture and sample II as a conglomerate when obtained values of melting point and enthalpies fusion of the appraised samples of nimodipine and the data of Grunenberg et al. (1995) were compared.

Determination of Purity

The purity samples percentage was determined simultaneously for both HPLC and DSC (Table 1). DSC curves were obtained at a heating rate of 2°C min^{-1}. Sample I showed similar purity in HPLC and

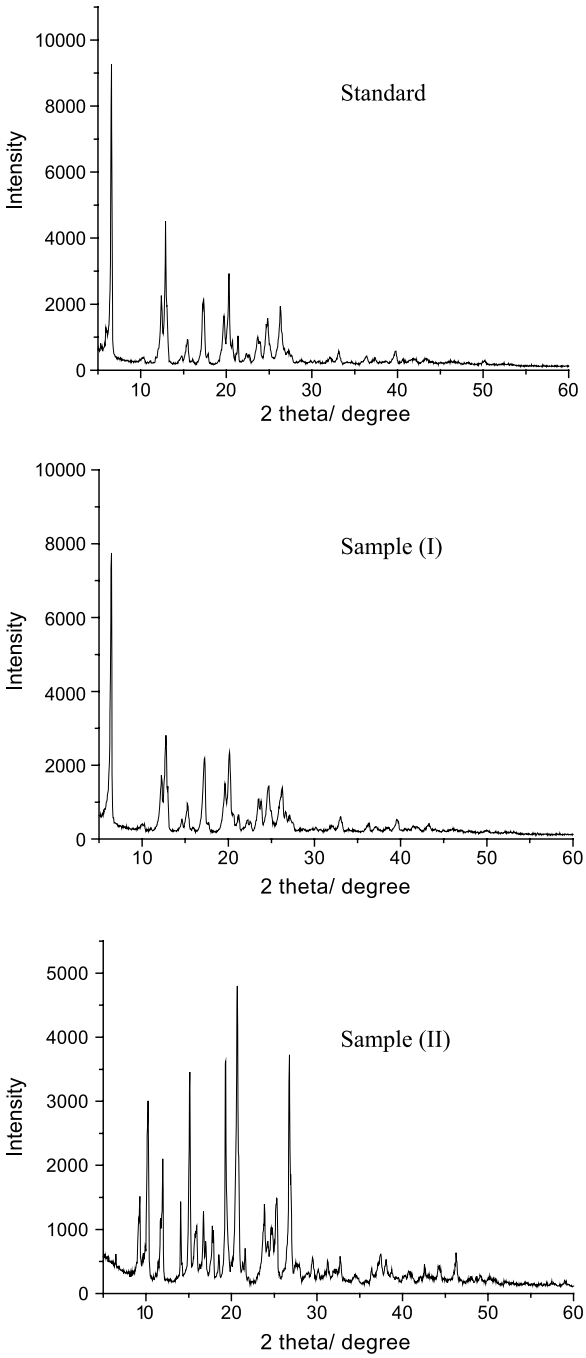

FIGURE 5 **X-Ray Powder Diffraction Patterns of Standard and Nimodipine Samples I and II.**

TABLE 2 **Thermal Decomposition Parameters of Nimodipine Samples Through Non-Isothermal TG Curves**

	Atmosphere					
	Air			Nitrogen		
Samples	E (KJ mol^{-1})	A (min^{-1})	n	E (KJ mol^{-1})	A (min^{-1})	n
I	108	5.2×10^{8}	0.1	122	7.6×10^{9}	0.1
II	103	2.9×10^{8}	0.3	89	1.6×10^{7}	0.1

Note: E=apparent activation energy, A=Arrhenius frequency factor, n=order of reaction.

TABLE 3 X-Ray Powder Diffraction Data for Nimodipine Standard and Samples

Standard			Sample I			Sample II		
2θ	*d*-Distance	*I*/Io (%)	2θ	*d*-Distance	*I*/Io (%)	2θ	*d*-Distance	*I*/Io (%)
6,563	13.46	100	6,440	13.72	100	9,280	9.53	31.39
12,901	6.86	48.46	12,777	6.92	36.44	10,226	8.65	61.62
15,452	5.73	9.64	15,246	5.81	12.63	11,954	7.40	43.35
17,345	5.11	23.34	17,263	5.13	27.91	14,053	6.30	28.87
20,308	4.37	31.97	20,143	4.41	30.83	15,123	5.86	71.67
23,682	3.76	10.91	23,518	3.78	14.48	15,946	5.56	22.58
24,834	3.59	16.99	24,711	3.60	18.60	16,728	5.30	26.97
26,316	3.39	20.29	26,275	3.39	18.60	17,757	5.00	22.58
						19,320	4.59	75.47
						20,678	4.30	100
						23,847	3.73	30.77
						25,287	3.52	30.77
						26,769	3.33	77.96

DSC methods; on the other hand, only HPLC enabled this quantification for sample II. The appearance of two peaks in the range of fusion may explain this result, which suggests the presence of different crystalline habits or impurities in the sample (like degradation products and/or another substances).

Nonisothermal Kinetic Study

It is necessary to evaluate the effect of temperature upon the reaction in order to rate the velocity of degradation that can be used in drug stability studies. One main purpose for the kinetic analysis of solid decomposition is to determine the reaction mechanism(s) calculated by Arrhenius parameters.

There are two ways to achieve this. One of them uses isothermal kinetic analysis while the other uses nonisothermal kinetic analysis (Huang et al., 2001). However, these studies may evaluate some parameters: Arrhenius frequency factor (A), apparent activation energy (E), and order of reaction (n). In this work was used Ozawa's nonisothermal method for the determination of the parameters mentioned above (Table 2). The method designed by Ozawa is an integral method for determining the activation energies in dynamic heating experiments. The activation energy can be obtained from a plot of logarithms of heating rates (β) as a function of the inverse of temperature (1/T) for a constant G(x), where G(x) is the integrated form of the conversion dependence function, $f(\alpha)$.

Table 2 shows that in air and nitrogen atmospheres, the apparent activation energy (E) and Arrhenius frequency factor (A) in sample II were smaller than in sample I. According to Ozawa's method, the thermal decomposition followed a zero-order kinetic. Sample II was more unstable despite the reaction order. This apparent instability was previously visualized in the DSC and TG analyses, as shown in Figs. 2 and 4 where the degradation of this sample was quicker.

Crystallinity Characterization

X-ray powder diffraction is a common technique for the qualitative and quantitative identification of crystallinity (Phadnis et al., 1997). The result shows that diffractograms of the standard and sample I are similar but different from that of sample II (Fig. 5). The majority of peaks from the sample II fail to correspond to peaks in the standard sample. Therefore, the former shows more peaks, which are needed to characterize other crystalline forms (polymorphism) in the same sample. Table 3 shows the *d*-Distances and relative intensities (I/Io) of the observed peaks in these patterns.

The USP general chapter (General Test, 1995) on X-ray diffraction states that identity is established if the scattering angles of the strongest reflections obtained from an analyte agree to about ±0.2 degrees with that of the reference material; and if the relative intensities of these reflections do not vary more than 20% (Brittain, 1999). The nimodipine sample X-ray diffraction patterns show substantial differences in the position of peaks, *d*-Distance, and relative intensities. The differences suggest that these two samples are completely different, according to the behavior of DSC curve visualized in Fig. 3.

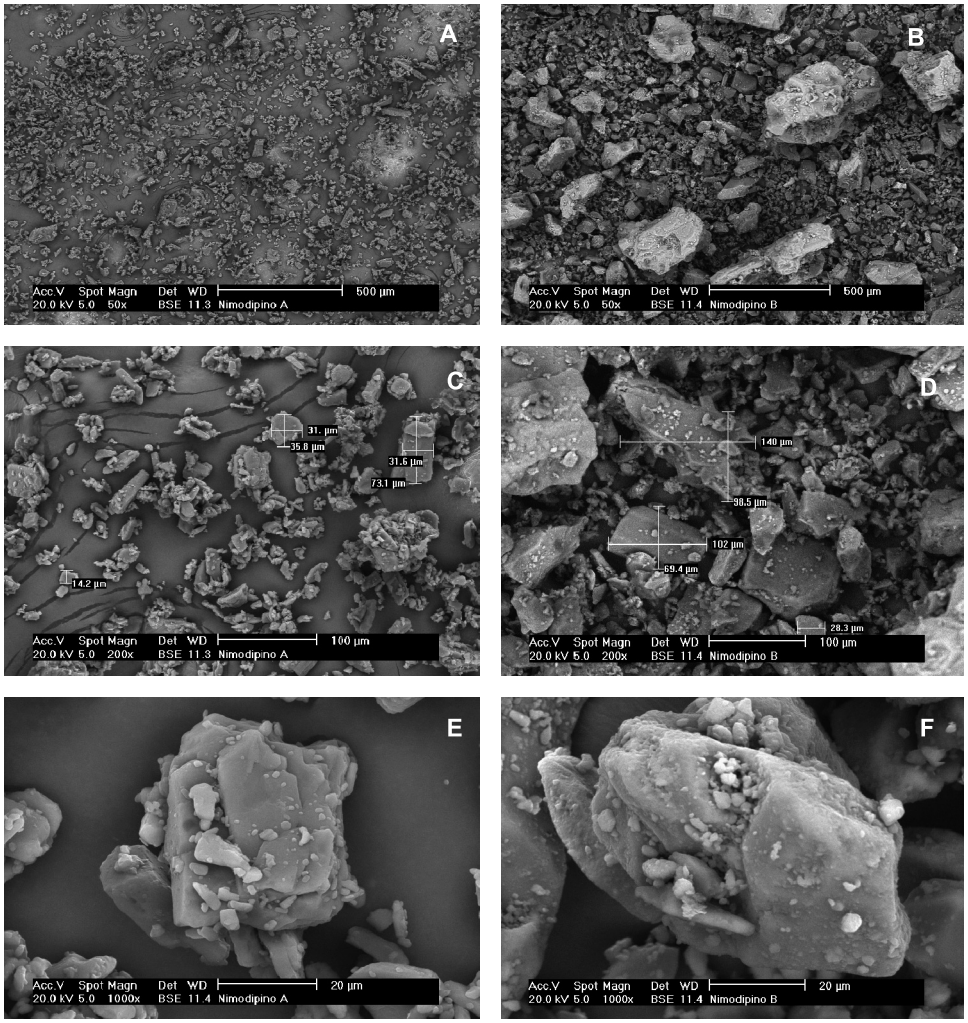

FIGURE 6 **Scanning Electron Microscopy of Nimodipine Samples I (A, C, and E) and II (B, D, and F). The Photomicrographs A and B were Taken at a Magnification of 50×, C and D of 200×, E and F of 1000×.**

Several investigators have used indices of relative crystallinity (X_c^{rel}) based on methods in which the area of the crystalline diffraction relative to the total area of the diffractogram is taken as a measure of crystallinity (Van Soest et al., 1995; Zimeri & Kokini, 2002). The indices of relative crystallinity (X_c^{rel}) of samples I and II were 26.42% and 18.86%, respectively. Considering that crystallinity degree is associated with stability, sample II is more instable than I, according to the kinetic parameters shown in Table 2.

Morphological Characterization by Scanning Electron Microscopy

Based on the micrographs obtained from scanning electron microscopy, greater homogeneity can be observed in sample I than in sample II in relation to particle size (Fig. 6a and b). The particle size of samples were different (Fig. 6c and d). Sample II showed a large particle size; while in sample I it was smaller. The characteristics of crystals can be visualized by increasing the approximation (1000×) (Fig. 6e and f). Orthorhombic crystals were observed in sample I. Sample II didn't show regular shape, which characterizes a polymorphism. The polymorphism of sample II explains this material's different physical-chemical characteristics when compared to the characteristics of sample I.

CONCLUSION

Refined quality control in the pharmaceutical industry is very important due to the possibility of variations in the production process causing changes to the final product. Purity degree and polymorphism are

factors that significantly influence stability, bioavailability, and processing of the considered pharmaceutical compound. In this work, the two distinct raw materials of nimodipine that were evaluated showed different characteristics as to the melting point, the enthalpy of fusion, the purity, some parameters about kinetic of degradation, the crystallinity degree, and the shape of crystals. The results highlight the importance of applying these techniques for a quality control practice in the pharmaceutical industry.

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