H NMR in Solution of Nanocomposites from Polycaprolactone – Nevirapine

Journal of Nanotechnology and Nano Science Research Review Article

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Submitted : 20 Mar 2021 ; Published : 5 Apr 2021

Abstract

The major understanding of molecular and cells basis of pathophysiological states has allowed the development of new drugs. However, this required a new and complex release system. Polymers offer the most promising way for controlled release and polymeric nanocomposite is a new perspective in the pharmaceutical area. NMR techniques employed supply a vision of the molecular dynamic processes and give information of structural level. NMR experiments like, inversion-recovery pulse sequence, have the advantage of detecting intermolecular interaction between a drug and polymeric nanocomposite matrix.

Keywords: nanocomposites, PCL, Nevirapine, NMR

Introduction

The major understanding of molecular and cell basis of the pathophysiological states has allowed the development of new drugs either deriving from the extraction of natural sources and/or synthetic ones, thus new therapeutic strategies. The applications however, require new and complex release systems aiming at improving the specificity by a certain organ and/or improving the biocompatibility processes. In this context, polymers offer the most promising way for controlled release of drugs due to its adaptation characteristics and possibility of subsequent changes [1].

Nevirapine (NVP) (Figure 1a) is an antiretroviral nucleoside reverse transcriptase inhibitor used in treatment of Acquired Immunodeficiency Syndrome (Aids) caused by the HIV virus. The World Health Organization (WHO) recommends an only daily dose. However, in many cases of the antiviral therapy, it is desirable to develop a long duration dosage to improve the patient's compliance and accordingly maximize the therapeutic effect [2] [3].

Polycaprolactone (PCL) (Figure 1b) is synthetized by the polymerization of the E-caprolactone ring using a variety of catalyzing of anionic and cationic coordination or by free radical through polymerization of the 2-methylene-1,3 dioxepine [4] [5]. PCL has become commercially available following the efforts to identify the synthetic polymers which could be degraded by microorganisms [6]. PCL is a hydrophobic polymer and semi-crystalline, its crystallinity tends to decrease with the increase of the molar mass, low melting point (59 – 64°) and its exceptional compatibility with blends has encouraged an extensive research in the biomedicine field [7]. The advantages which have been assigned to PCL over other biopolymers include kinetic custom, solution degradation and mechanic properties. Thus, it is easy to manufacture and shape them allowing to establish the size of suitable conducting pores to the target tissue or organ and controlled release of drugs contained in its matrix. Functional groups may be added so that they turn the polymer more hydrophilic, adhesive and biocompatible letting a favorable answer of the cell. A disadvantage is that PCL degrades at a lower speed than other polymers such as polyglycols (PGA) and poly D, L lactides on the average of 3 to 4 years [8].

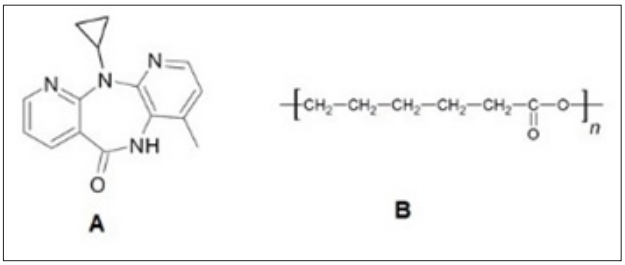

Figure 1: Chemical structure of Nevirapine and PCL

The inclusion complex (IC) of a drug with crystalline polymer or semi crystalline is not explored enough as a solid pharmaceutical form in which the drugs molecules form the host structure as ways and the polymer linear chains reside in such ways as hosts. It has been shown that when hydrophobic polymers (eg. PCL) are used as hosts, the solubility and the dissolution speed of the drugs – PCL inclusion complex is significantly

lower than that to the drugs crystals themselves which provides a new method to develop medicine of controlled release [9].

A new perspective in the pharmaceutical area arises with the introduction of polymeric nanocomposites [10]. This new type is formed by organic and inorganic materials distributed by the polymeric matrix which may act as excipients, improving series of properties such as formulation process, organoleptic characteristics (color, scent and flavor) as well as physical-chemical properties among them. The viscosity which can also act as dissolution facilitators in oral formulations [11].

Nanoparticles for controlled release must be steady from the psycho-chemical point of view have high loading capacity be able to incorporate drugs molecules either hydrophilic and/or hydrophobic and be feasible to several ways of managing them (oral, parenteral, nasal and topical application). Furthermore, the benefits of the nanoparticles go beyond conducting the drugs to its target since they allow the drugs sustainable release from a matrix, leading to a bioavailability improvement, lower dosage frequency, side-effect reduction resulting in better acceptance by the patient [12].

The polymer- drugs complex may be studied by a variety of techniques including UV-visible spectroscopy, split techniques, fluorescence measures, X-Ray crystallography, calorimetry, NMR, mass spectrometry among others. From these techniques, only X-Ray crystallography and NMR may provide a static view of the complex in an atomic level and it depends on the quality of a single crystal. As it may provide measures in solution, the NMR offers the benefit of supplying a dynamic process view as well as information in a structural level [13].

Experimental

Materials

The PCL and NVP nanocomposite samples in the form of films were prepared during Mariana B. Sato's doctoral thesis [14] and they are related in Table 1 being used as they were received. Nevirapine standard was obtained from Instituto de Tecnologia em Fármacos (Farmanguinhos/FioCruz- RJ), being used without any previous treatment. The CDCI3 (99,8 % d) was obtained from CIL (USA) and the NMR 5mm OD tubes used in all samples were Wilmad 527-PP-7.

Sample Code	Composition	%Nanoparticle	%NVP
NVP-PCL1	PCL/NVP	-	5
NVP-PCL2	PCL/NVP/A200	0.15	5
NVP-PCL3	PCL/NVP/S7	3	5

Notes: A200 – unmodified silica; S7-Viscogel®

Table 1: Samples of NVP-PCL and related nanocomposites.

Sample preparations for NMR

20 mg of NVP standard and NVP nanocomposites were weighed and transferred to a NMR tube, 0.8 ml of CDCI3 and the samples were shake until their complete dissolution. All tubes were closed with Teflon caps (WILMAD WG-1264-5) and sealed with Parafilm™.

NMR analysis

All NMR ranges were obtained in the Varian Mercury VX 300 spectrometer operating at 299.99 MHz to the hydrogen nucleus using a 5-mm probe with gradient. The chemical displacements were referenced by the solvent signal at 7.27 ppm and the temperature acquisition was 30° Celsius (303 K) in which the samples were left for five minutes in the probe without acquisition in order to obtain thermal equilibrium. The acquisition parameters to the H spectra were: spectral window (sw) = 4800 Hz; acquisition time (at) = 2.5; interval between pulses (d1) = 20 seconds and pulse of 90° calibrated for 15.90 us. Spin-lattice relaxation time value measurements (T1) were obtained using the device standard inversion-recovery pulse sequence [recycle delay 180° - τ - 90° acquisition)] n in which the acquisition parameters were kept to all samples in order to evaluate the Nevirapine interaction in the nanocomposites, sw= 4800 Hz; at= 2.5; pw 90=15.90 us; nt=20; t1 minimum (t1 min.) = 0.5 s; t1 maximum (t1 max.) = 80s and d1=80 s. The relaxation times were extracted by plotting the intensity of signals experimentally obtained against the recovery period (Tm) on the basis of the equation **M(t)= (M(o) – Mo) * exp (-t /T1) + Mo**, in which Mo is the Z magnetization balance and M(o) is the time zero magnetization immediately after the 180 pulse. In this equation, an adjust is done to data inversion-recovery for which M(o) is approximately the same as – Mo [15] [16].

Results and Discussion

Figure 2 shows the hydrogen spectrum (^{1}H) of Nevirapine Standard (NVP-STD) with the corresponding assignments and the structure with the numbering used in this study. In Figure 3 A and B, we have the NMR ^{1}H of NVP-STD comparative spectra, Nevirapine and pure (NVP-PCL1) polycaprolactone (PCL), and the complexes with nanoparticles addition NVP/PCL/A200 (NVP-PCL 2) and NVP/PCL/S7 (NVP-PCL 3). One of the observed aspects in the study of inclusion complexes is the chemical displacement variation. Changes in chemical displacement can be used to identify the interactions between drugs-polymer and the degree of affinity [17]. However, for many complexes, the spectra may present a high level of complexity and/or the change of the chemical displacement is very short, thus it is not allowed to get a real estimation of the interaction [18]. In Table 2, there have been listed the Standard-Nevirapine chemical displacements and the other complexes and also the displacement differences among the signs of the analyzed samples. As it is observed, in the NMR ^{1}H spectra, the sign of H-8 in NVP-PCL1 and NVP-PCL 3 samples are covered by the sign of PCL.

The listed values were obtained from the spectrum of COSY from the samples shown in Figures 4 A and B.

N° Hdg	NVP-STD	NVP-PCL1	Diff-STD-PCL1	NVP-PCL2	Diff-STD-PCL2	NVP-PCL3	Diff-STD-PCL3
H-1	8.51	8.60	0.09	8.48	-0.03	8.59	0.08
H-2	7.03	7.16	0.13	7.03	0	7.16	0.13
H-3	8.07	8.16	0.09	8.07	0	8.17	0.10
H-4	8.13	8.32	0.19	8.12	-0.01	8.26	0.13
H-5	6.90	7.14	0.24	6.89	-0.01	7.09	0.19
H-6	1.01	1.17	0.16	0.95	-0.06	1.12	0.01
H-7	0.53	0.52	-0.01	0.45	-0.08	0.54	0.01
H-8	3.79	4.00(#)	0.03	3.71	-0.08	3.97(#)	0.03
CH_3	2.39	2.51	0.12	2.35	-0.04	2.46	0.07
NH	8.64	8.37	-0.27	8.37	-0.27	8.31	-0.33

Table 2: Chemical shifts of NVP standart and its nanocomposites.
(#) Values obtained from the COSY spectrum

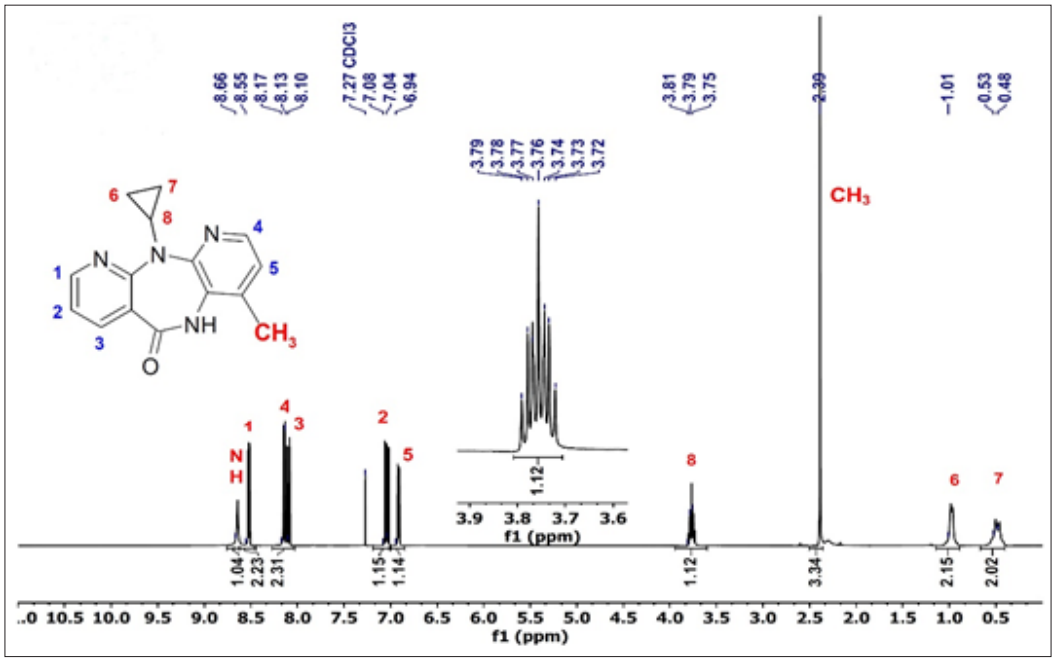

Figure 2 : ^{1}H NNR spectrum of NVP standard at 300 MHz Chemical structure and assignments

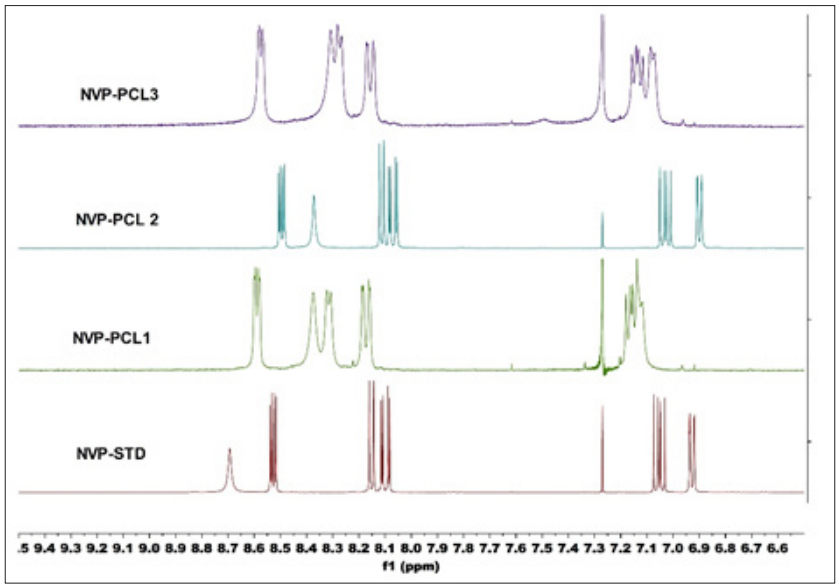

Figure 3 A : ^{1}H NMR comparative spectra of NVT STD and related nanocomposites. Aromatic region.

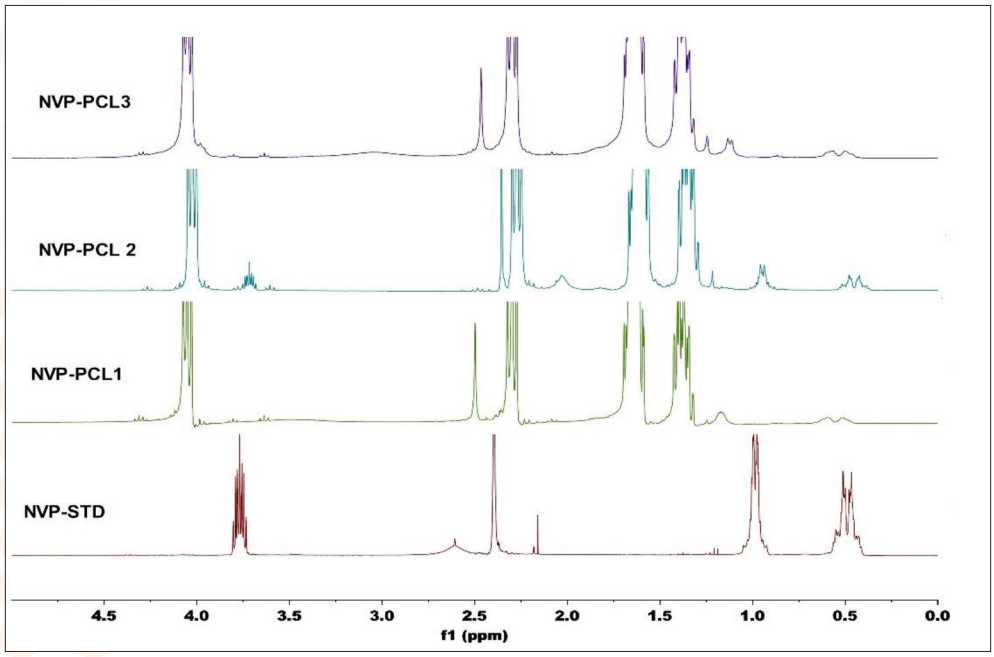

Figure 3 B : ^{1}H NMR comparative spectra of NVT STD and related anocomposites. Aliphatic region.

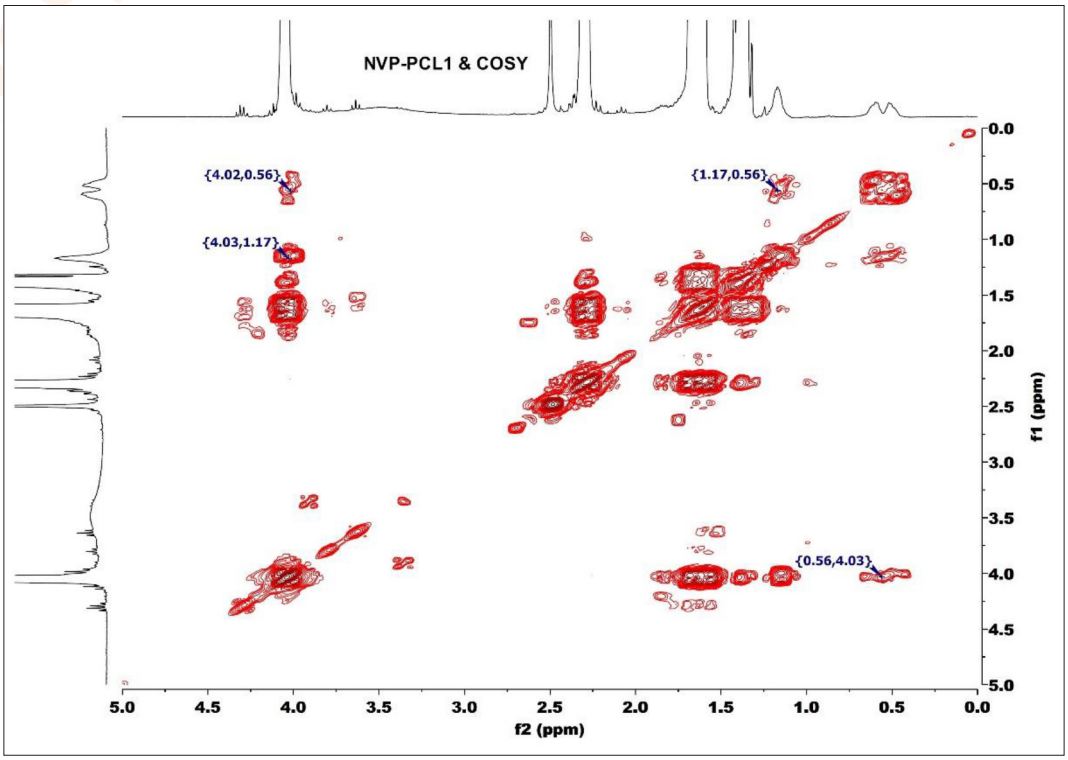

Figure 4 A : COSY spectrumof NVP-PCL1showing overlay of H-8(4,0ppm) by the signal of PCL. See the correlation with H-6 e H-7 (1.17 and 0.56 ppm)

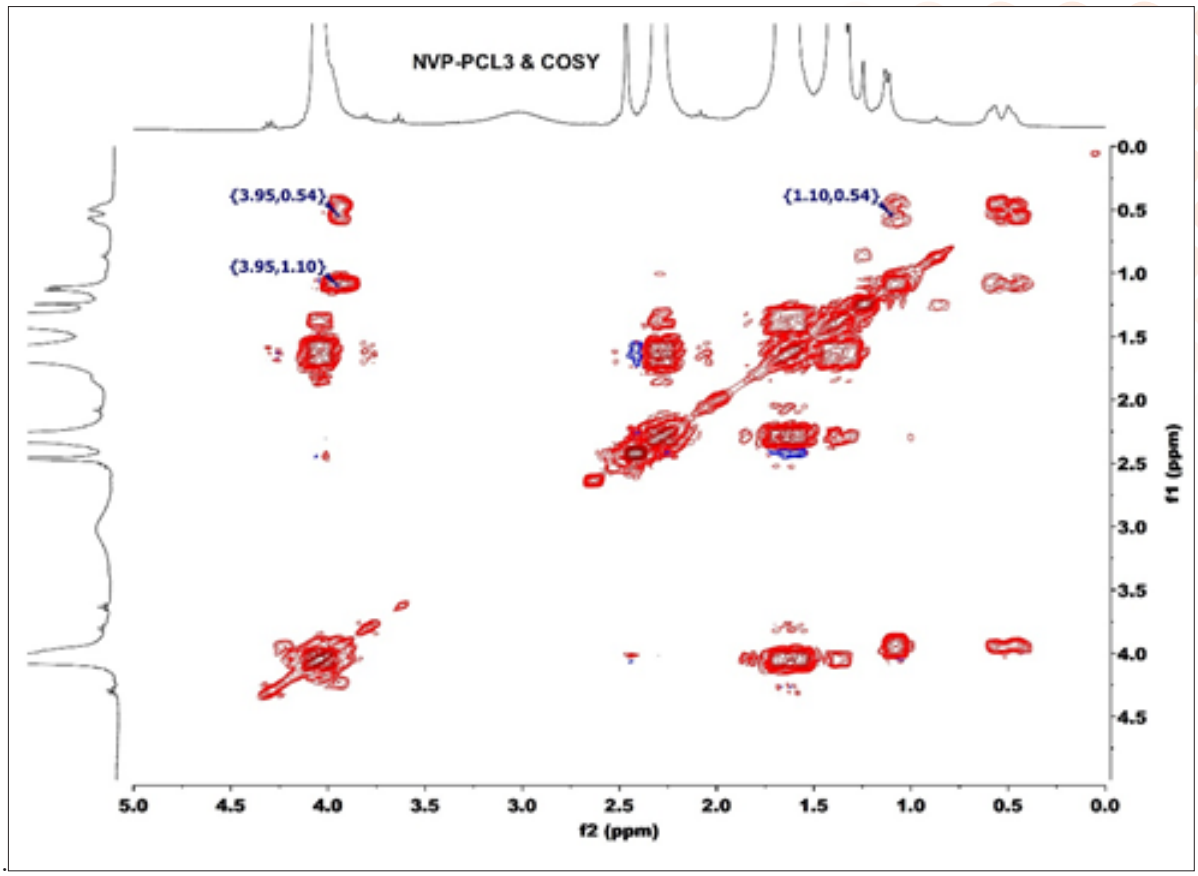

Figure 4 B : COSY spectrum of NVP-PCL3 showing overlay of H-8(3.95 ppm) by the signal of PCL. See the correlation with H-6 e H-7 (1.10 and 0.54 ppm).

It has been observed chemical displacement variations in the three studied complexes when compared to Nevirapine Standard. The complexes of NVP-PCL 3 present the highest values of chemical displacement and NVP-PCL 1 and NVP-PCL3 presents the lowest values. The average of the differences is small. In NVP-PCL1, it was 0,13 mm, NVP-PCL 2, it was -0,04 and NVP-PCL3, it was + 0,13 mm. IN NVP-PCL1, the highest chemical displacement variations are observed in aromatic hydrogen H4 and H-5 as in hydrogen H-6 and H-8 of the cyclopropane, the same observed for NVP-PCL3. In NVP-PCL2 the displacement to lower levels is not only observed in hydrogen H-2 and H-3 which keep themselves with no alteration in relation to Nevirapine Standard. The highest chemical displacement variations were observed in NVP-PCL2 system. In the three complexes, the signal of NH presents lower levels of displacement in relation to Nevirapine Standard in absolute terms as NH is a label hydrogen which may suffer chemical displacement variations of the environment conditions such as concentration, temperature, pKa of the solution and hydrogen relations. The variations of time of spin-lattice relaxation (T1) and the variations in the standard profiles of NVP coupling in the inclusion complexes, which were studied, are also indicatives of different levels of NVP interaction with the pure polymeric matrix (PCL) and/or with added nanoparticles.

The NVP structure has a butterfly shape with pyridyl planar rings which may be folded up and down, having as a starting point, the central diazepine distortion in which its intersection plans are in an angle of 121 degrees. The cyclopropylamine nitrogen atom has its pair of electrons mismatched with adjacent relocation to the pyridyl rings. Being very pyramid-shaped, that makes the cyclopropane to adopt an almost perpendicular position to the diazepine plane in opposite to both pyridyl rings (Figure 5A). This conformation is confirmed by X-Rays analysis [19] in which a similar conformation is obtained in solution according to molecular modeling and vibrational spectroscopy studies [20]. This conformation does not have plane of symmetry that enables to convert in its mirror image due to the flexing of the ring with the inversion of the nitrogen atom, causing the movement of the pyridyl rings as well as the cyclopropane which comes closer to the pyridyl ring (Figure 5B) [21].

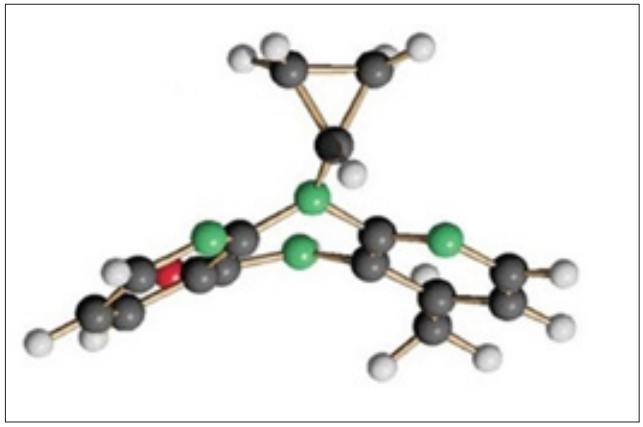

Figure 5A

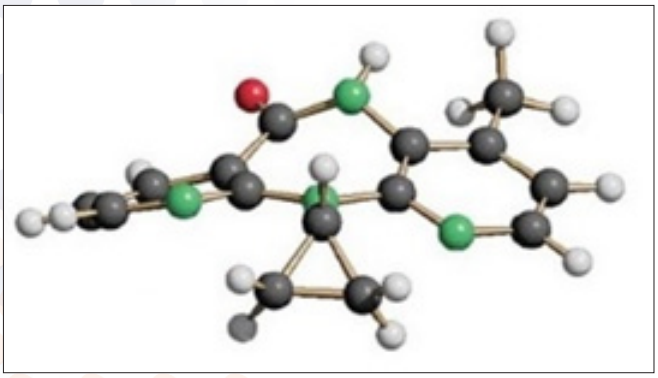

Figure 5B

Figure 5: Preferential conformation of NVP (A). Conformation with nitrogen inversion of diazepona.

The non-planarity of NVP can be observed in the spectrum of NMR ^{1}H through the analysis of the cyclopropane hydrogen that in CDCI3 presents multipletes at 3,79 ppm (H-8; 1H), 1,01 ppm (H-6; 2H) and 0,53 ppm (H-7; 2H). The H6 and H7 hydrogen pairs present a symmetrical shape which indicates a coupling standard type ABCD of a system ABCDX. As the standard, the coupling constant cannot be measured as usual (Pascal Triangle) and it is necessary to use computer simulation in order to get it [22]. In Table 3, there are the chemical displacements listed and the coupling constant obtained by MSpin (Mestre lab- 2018, Spain) in the condition of Karplus [23].

Hdg	δ(ppm)	H-6(Hz)	H-6'(Hz)	H-7(Hz)	H-7'(Hz)
H-6	1.01	X	-4.8	7.5	2.6
H-6'	0.96	-4.8	X	7.0	7.5
H-7	0.53	7.5	2.6	X	-4.8
H-7'	0.49	2.6	7.5	-4.8	X
H-8	3.79	8.1	4.6	7.5	2.6

Table 3: Coupling constant of cyclopropane of NVP. Simulation made by MSpin.

The H-6 and H-7 pair of hydrogen proximity to the nitrogen of cyclopropylamine and non-chemical equivalence excludes the plane of symmetry in the rest of the molecule, suggesting the formation in solution of a pair of enantiomers shown in Figure 6. In NVP standard (NVP- STD) the lowest values of T1 relaxation data (Table 4) were obtained for the H6-H7 pair of hydrogen of the cyclopropane in relation to the other hydrogen of the molecule. This lowest value may be attributed to the cyclopropane rotation through the connection C-N. The highest value obtained by H-8 may be attributed to a larger interaction with the orbital of the nitrogen, which has a load distribution that is not symmetrically spherical, interfering in the relaxation process [24].

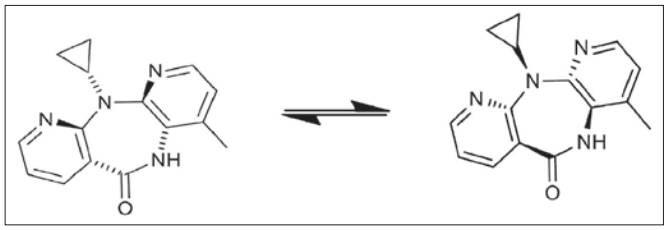

Figure 6: Enantiomers of NVP.

N° Hdg	NVP-STD	NVP-PCL1	NVP-PCL2	NVP-PCL3
H-1	2.31	0.42	3.08	0.69
H-2	1.60	0.51	1.95	0.57
H-3	1.95	0.37	2.49	0.63
H-4	1.75	0.15	2.62	0.26
H-5	1.41	0.18	2.14	0.21
H-6	0.59	0.70	0.70	0.43
H-7	0.63	0.74	0.74	0.20
H-8	1.76	Overlaid	2.55	Overlaid
CH_3	0.90	0.20	1.44	0.39
NH	0.89	0.27	3	0.65

Table 4: Relaxation time T_1(ms) of NVP by inversion-recovery sequence.

Relaxometry studies of low field in solid state of NVP-PCL1 (PCL- NVP) indicate that the introduction of NVP in pure PCL increases the value T1 parameter of PCL generating less mobility material with distribution of NVP as much in the amorphous phase as in the crystalline one [25]. The values of T1 for PCL-NVP complex (NVP-PCL1) in solution indicate that the hydrogen of the pyridyl rings is closer to the polymeric chain, since, in solution, the spin-lattice relaxation depends on the proximity level of the kernels involved. The H6-H7 pair of hydrogen presents extended signs and T1 values slightly larger than the observed ones for Nevirapine Standard (NVP-STD) (Table 4). The less mobility of the PCL chains might have led to a larger interaction, occasioning an inversion of the diazepine ring, resulting chemical change of the hydrogen pairs H-6/ H-6' and H-7/H-7' which results the formation of a spin system A, A', B, B'X (Figure 7). We can consider the chemical change as an important random modulation in the analysis of the relaxation times. The chemical change means that the nuclear spins have changed their environment (interaction with PCL) leading to a chemical process which, in this case, is intramolecular, that is, configuration change [26]. The pyridyl ring proximity of the polymeric matrix is evidenced by profile change of the coupling standard.

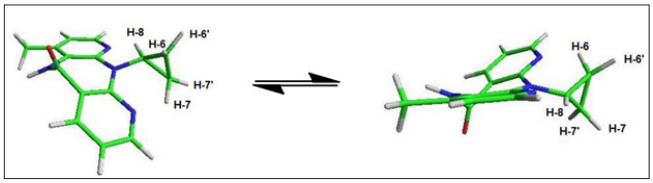

Figure 7: Conformational inversion of NVP and chemical exchange

The formation of hybrid systems polymers-nanoparticles leads to an alteration in the intermolecular interactions of the polymeric matrix, causing changes in the molecular dynamics. The introduction of the A200 nanoparticle (non-modified silica) causes an increase of the molecular rigidity with a consequent raise of the T1 of PCL value, as it was observed by low field relaxometry which is explained by the increase of the hydrogen links between the silica and the PCL. The relaxometry also indicates that the A200 effect is more evidenced in the amorphous-flexible part, having connection with the silica concentration in PCL [27]. After the NVP dispersion in the PCL/A200 system, the amorphous region presents lack of mobility which added to a larger interaction of A200, facilitated by the silica small size that leads to a more homogeneous distribution of the nanoparticle in PCL [28]. This larger homogeneity of the PCL/ A200/ NVP-PCL 2) system can be observed by the NMR ^{1}H spectrum in the complex (Figure 3A and B). In NVP-PCL 2 there is no variation of the profile in the pyridyl ring coupling and cyclopropane standard when compared to the spectrum of NVP-STD hydrogen, unless by the slight difference in the chemical displacement, they are virtually alike. The obtained T_1 values are higher in comparison with NVP-STD and two other complexes. "This increase of the T_1 values may be assigned to the strong NVP intermolecular interaction in the PCL/A200 system, leading to restriction of the BVP molecule rotation in the complex, since the efficiency of the relaxation" process, in solution, is linked to the molecule movement properties [29]. This fact allows to infer that the PCL/A200 system produces a better NVP molecule encapsulation which can be supported by the controlled kinetic release where NVP-PCL2 (PCL/ A200/ NVP) was the one which presented the lower release tax of the NVP [30].

The low field relaxometry data for the PCL/ Viscogel S7 system indicate a reduction of T1 relaxation time in relation to a pure PCL, since that Viscogel S7 provides the formation of exfoliated morphology of nanocomposites. The decrease in the relaxation time value suggests a strong dipole-dipole intermolecular interaction among the polymeric chains [31] [32]. Due to the presence of organic modifier, a surfactant, the molecule acquires polarity degree allowing a better interaction with the existent polar groups in PCL [33]. When NVP is introduced in the PCL/ Viscogel S7 system, there is a decrease of the crystallinity level with changes in the semi-crystalline structure, resulting in a compound of lower PCL sort string due to the electrostatic interaction and/or hydrogen links between the PCL and NVP [34]. These interactions can be observed in the profile of pyridyl rings of the coupling standard and the cyclopropane hydrogen in the NVP-PCL3 sample (PCL/ Viscogel S7/ NVP) indicating a more direct NVP interaction with the PCL polymeric matrix, similar behavior in NVP-PCL1 (PCL/ NVP). The main difference is in the polymeric matrix, since in NVP/PCL1 there is a simple dispersion process of NVP while in NVP- PCL3 occurs exfoliation by the introduction of the nanoparticle. In the NMR ^{1}H spectrum in solution, the difference between the interactions can be seen by the difference of the profile in the hydrogen coupling H-2 and H-5 (Figure 3A). The mentioned hydrogen pairs are placed in each pyridyl rings and the change of the coupling standard profile can be influenced by the diazepine inversion. The profile difference in NVP-PCL1 and NVP-PCL3 indicates that the inversion degree is not equivalent in both samples, since we have distinct environment with different interactions of NVP molecule in the polymeric matrix. The H-6 pair of hydrogen profile which presents coalesced in NVP-PCL1 shows a more defined profile in NVP-PCL3 (Figure 3B). The T1 values obtained to NVP-PCL3 (Table 4) are lower when compared with NVP-PCL1 in which H-6 and H-7 hydrogen pair of cyclopropane that presents the lowest values of T1, not only with regard to NVP-STD but also to the other two complexes (Table 3). The difference may be explained by the proximity of cyclopropane with the PCL chains containing organically modified clay (Viscogel S7) with an increase in the polarity system and the Fe^{3+} presence in their composition.

Conclusion

In NVP molecule the cyclopropane is the mobile portion due to free rotation by the simple C-N is an indicator of NVP molecule setting changes. The cyclopropane position with regard to the rest of NVP molecule is of great importance to achieve an adequate NVP conformational structure in the including complexes, since NVP does not have any other flexible portion. The reversal of the pyridyl rings in the shape of a butterfly is an indicator of interaction with the polymeric matrix.

In the preparation of drugs-polymer nanocomposites, having as a purpose to drug controlled release, the choice of polymer, especially the nanoparticle, the study of the mechanism interaction (dispersion, intercalation and exfoliation) has important role due to the influence which can have sequences as the observed ones in drugs release to the biological setting.

Acknowledgements

The authors are grateful to CNPQ, FAPERJ and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

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