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NMR Relaxometry Applied to Pharmaceutical Forms

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Abstract NMR relaxometry proved to be a powerful tool that provides useful information on the molecular dynamics of different pharmaceutical materials. The proton NMR relaxometry was measured using low-field NMR equipment. The aim of this work was to evaluate the interaction of different drugs in pharmaceutical systems, such as: propranolol (POP) and atorvastatin (ATV) in tablets with Tapioca Starch (TS); POP in *Rosa Mosqueta* oil nanoemulsion (NE); and papain in carboxymethylcelulose (CMC) hydrogel, using the proton spin-lattice relaxation time parameter. The spin-lattice relaxation measurements (T_1H) were done through the inversion-recovery pulse sequence and the spin-spin relaxation measurements (T_2H) were done by two pulse sequences, one was Magic-Sandwich Eco (MSE) and the other was Carr-Purcell-Meiboom-Gill (CPMG). From the analyses of T_1H data it was concluded that there was an interaction between the drugs and TS in the tablets, which is explained by the increase of T_1H values. The evaluation of T_2H relaxation time of NE confirmed a correlation between NE phases and it was observed that POP was effectively distributed in water phase. From the structure of CMC hydrogel it was observed that papain interacted with free water molecules of the hydrogel. This study confirmed that NMR relaxometry can be used to characterize the drug delivery systems.

Keywords Relaxometry, spin-lattice relaxation time, spin-spin relaxation time, Pharmaceutical forms

1. Introduction

Nuclear magnetic resonance (NMR) is a completely noninvasive technique, which is successfully used to investigate both structure and molecular dynamics of different aggregation states. Consequently, NMR techniques are also very useful in materials science, biology, chemistry and physics, especially in the pharmaceutical field [1]. There is a great interest in understanding the molecular dynamics of new materials, since the molecular dynamics can influence several conditions like: biological activity, manufacturing process and storage conditions chosen for pharmaceutical systems [2].

Among different NMR techniques, the NMR relaxometry has proved to be a powerful tool that provides useful information on molecular dynamics in different materials, such as pharmaceutical and polymer systems. The proton NMR relaxometry can be measured in a low-field NMR employing the inversion-recovery pulse sequence [1,3].

Proton NMR relaxometry measures the energy exchange between proton spin systems of the hydrogen nuclei and the energy exchange between the proton spin systems and the lattice. Spin-lattice relaxation is highly sensitive, not only to local molecular rotations/reorientations but also to translational self-diffusion of molecules, because the ^1H spin-lattice relaxation depends on the dipolar spin-lattice interactions that can occur between inter or intramolecular spins [2]. NMR relaxometry technique has been used by several researchers to measure the nuclear relaxation times, such as: spin-lattice relaxation time in the laboratory frame (T_1H) and spin-spin relaxation time in the laboratory frame (T_2H). The spin-lattice relaxation time in laboratory frame promotes evaluation of the samples in the MHz scale, due to collective and/or segmental movements [4,5].

The use of relaxometry opens new opportunities for the pharmaceutical sciences, especially under the point of view of molecular interactions and molecular dynamics between excipients and the drug in different pharmaceutical forms (tablets, emulsions and gels) [6]. The excipients can affect two main drug features: its pharmacokinetic properties and the durability of dosage forms. Those molecular interactions depend not only on the quantity and quality of the components, but also on production parameters. The analysis of the interaction between excipients and drugs by the molecular dynamics analysis can help to simulate and optimize the properties of pharmaceutical forms [7].

Wilczyńki *et al* (2017) applied ^1H spin-lattice Nuclear Magnetic Resonance (NMR) relaxometry to differentiate original and counterfeit Viagra®. The relaxation studies have been performed in a frequency range covering four orders of magnitude, from 4 kHz to 40 MHz. It has been shown that for the counterfeit product, the relaxation is bi-exponential in the whole frequency range, while for the original Viagra® the relaxation process is always single exponential. Thus, even a qualitative analysis of the relaxation data makes it possible to identify the falsified medicine [7].

Rodrigues *et al* (2016) developed poly(vinyl alcohol) (PVA) based cross-linked xerogels, neat and loaded with nanoparticles of hydrophilic silica (SiO_2) and it were characterized through time domain NMR experiments, using the spin-lattice relaxation constant values. It was shown that the SiO_2 nanoparticles influenced the PVA matrix, affecting its molecular mobility [8].

Further, there is an increased demand for the implementation of new analytical techniques in the pharmaceutical field due to the concept of process analytical technology and the advantages are the enhancement of formulation and understanding the production process [9]. Summarizing, NMR relaxometry is a method that has great potential in the pharmaceutical field, especially for the analysis of different pharmaceutical forms.

The aim of this work was to study the interaction of different drugs, such as propranolol (POP) and atorvastatin (ATV) in tablets with Tapioca Starch (TS); the interaction of POP in *Rosa Mosqueta* oil nanoemulsion (NE); and the interaction of papain in a carboxymethylcelulose (CMC) hydrogel, using NMR relaxometry. In this work the longitudinal relaxation time of the drugs, polymer and tablets were analyzed from the distribution of relaxation domains obtained by the Laplace Inverse Transform from the inversion-recovery curves acquired in low-field NMR.

2. Materials

The materials used for the tablets development were: tapioca starch from Sarfam Comercial Ltd-Brasil, propranolol hydrochloride from All Chemistry Ltd-Brazil, Atorvastatin from Pharma Nostra Ltd-Brazil. The materials used in the NEs development were: Propranolol hydrochloride from All Chemistry Ltd-Brazil, nonionic surfactants polyoxyethylenesorbitanmonooleate (Tween 80®) from Mapric Ltd-Brazil, which presents 20 moles of ethylene oxide, and sorbitanmonooleate (Span 80®) from Sigma Aldrich Ltd-Brazil, *Rosa Mosqueta* oil from Galena Ltd-Brazil. The materials used in the hydrogel development were: Carboxymethylcelulose from Farmos, conserve novamit® from Farmos, glycerin from Pharma Nostra and papain from Pharma Nostra.

2.1. Methods

2.1.1. Development of Tablets

The tablets were prepared by compressing 550 mg of a binary mixture of TS powder and POP. Each of the 550 mg tablets contained POP and 15:85 wt % TS powder, which were homogenized with the aid of a mortar and pestle,

using geometric progression, before compression. The tablets were prepared by compressing the mixture, which was manually filled into the die cavity of 11 mm flat punches, by means of a single-punch press tablet compression machine Monopress LM1, model CFW 08, (Lemaq/LM). The compression pressure was manually adjusted. ATV tablets were developed with the same excipient ratios and with the same procedures.

2.1.2. Development of Nanoemulsions

Two formulations were developed: NE vehicle with *Rosa Mosqueta* oil (NE RM), NE with *Rosa Mosqueta* oil and 1 wt% of POP (NE RM – POP) (Table I). The drug was added to the internal phase of the NE and the oil phase was slowly poured into to the aqueous phase. Then, the system was processed using an ultrasonic processor (model UP 100 H, power maximum = 100 W, equipped with a 7-mm-diameter tip, Dr Hielscher GmbH, Germany) and the input power level was 100 W, with continuous cycle. The processing time of NE was 10 minutes. The temperature was kept at 5^{o}C by cold bath during the formulation processing (Figure 1). The total amount obtained for each formulation was 10 g.

The ultrasonication method depends on high-frequency sound waves (20 kHz and higher). It can be used to reduce size of a pre-formed emulsion. Bench-top sonicators consist of a piezoelectric probe which generates intense disruptive force at its tip. When dipped in a sample, ultrasonic waves produce cavitation bubbles which continue to grow until they implode. This implosion sets up shock waves, which in turn create a jet stream of surrounding liquid, pressurizing dispersed droplets and effecting their size reduction [10].

Table 1: Components of Nanoemulsions

Samples	Water %	Oil %	Surfactants %		Pop %
			Tween 80®	Span 80®	
NE RM	8 %	64 %	21 %	8 %	-
NE RM - POP	8 %	63 %	21 %	8 %	1 %

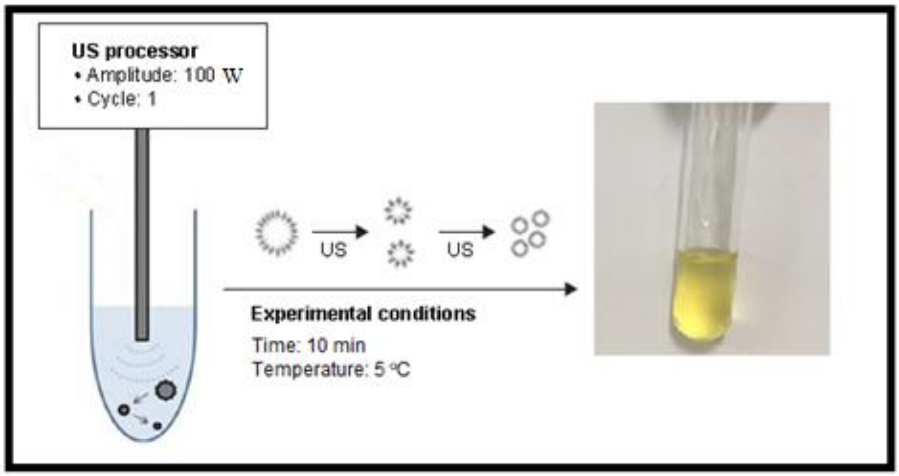

Figure 1: Production of Nanoemulsions using ultrasound (US)

2.1.3. Development of Hydrogel

The carboxymethylcellulose (CMC) hydrogel was prepared by dissolving 5% of CMC, 10 % glycerin, and 0.3% of conserve novamit®, by continuous mechanical stirring into distilled water, using a Fisaton machine, model 713 ds, at 60 ^{o}C for 2 h. The papain was incorporated into the CMC hydrogel, after being ground in a mortar and pestle with glycerin (Table II).

Table 2: Hydrogels components

Samples	CMC %	Conserve Novamit %	Glycerin %	Papain %	Water %
CMC hydrogel	5 %	0.3 %	-	-	94.7%
Papain Hydrogel	5 %	0.3 %	10%	10%	74.7%

2.2. Time-Domain nuclear magnetic resonance (TD-NMR) analysis

Relaxometry was employed to access molecular dynamics of samples, through the determination of spin-lattice and spin-spin relaxation processes. All measurements were obtained in a Maran Ultra spectrometer (Oxford Instruments, Oxford, UK) with 18 mm magnet bore, operating at 0.54 T (23.4 MHz for ^{1}H). The masses of each sample were determined to be similar to each other, so that the results could be better compared.

2.2.1. Spin-Lattice Relaxation Time

The spin-lattice relaxation time, with a constant T_1H, of TS, POP, ATV and tablets were analyzed using an inversion-recovery pulse sequence (recycle delay – 180° – τ – 90° – acquisition data) and the 90° pulse of 7.5 μs was calibrated automatically by the instrument´s software. The amplitude of FID was sampled for forty τ data points, ranging from 0.1 to 40.000 ms, with 4 scans for each point, 10 s of recycle delay and 4% of recycle gain. The data were processed in commercial software packages, namely WinFit version 2.4.0.0 and Origin version 8.5. Table III shows the analytical parameters used to measure samples relaxation times [3,8].

The T_1H values displayed correspond to average of results from two runs. This mean value was extracted from fitting the experimental points using Equation 1.

$$M(\tau) = A_0 + A_1 e^{(-\tau/T_1H)} \quad \text{Equation 1}$$

Where M (τ) is the magnetization as a function of time between 180° and 90° pulses; A_0 is the baseline adjustment constant, which corrects the noise associated with the relaxation signal; $A_1$is a factor proportional to the number of relaxing proton nuclei in each interval and with relaxation interval between each pulse that necessarily needs to be 5 times higher than the highest value of T_1H; τ is the time interval between 180° and 90° pulses; and T_1H is the spin-lattice relaxation time constant [3,8].

Table 3: Parameters employed in the inversion-recovery pulse sequence

Parameters	Values
Pulse sequence	180° – τ – 90°
90° pulse - μs	Automatically set. Typically 7.5
180° pulse - μs	Automatically set. Typically 15
Number of scans	4
Number of τ (logarithmically spaced)	40
Tau sweep - μs	10 – 4E7
Recycle delay – s	10

2.2.2. Spin-Spin relaxation time

The spin-spin relaxation time T_2H, of TS, POP, ATV and tablets was measured using Magic Sandwich Eco (MSE) pulse sequence and the 90° pulse of 7.5 μs was calibrated automatically by the instrument´s software. The amplitude of FID was sampled for 2048 τ data points; interval between each point of 1 μs, with 16 scans for each point and 3 s of recycle delay and receptor gain of 4%.

The NE vehicle and NEs with POP; CMC hydrogel and CMC-papain hydrogel were evaluated with Carr-Purcell–Meiboom-Gil (CPMG) pulse sequence [p90x – (t – p180y – t)n], the 90° pulse of 7.5 μs was calibrated automatically by the instrument´s software, and τ was 800 μs, numbers of echo of 2048, points per echo of 1, with 8 scans for each point, 3 s of recycle time and 2% receptor gain.

Analysis temperature was 25 °C. Data were processed in commercial software packages, namely WinFit version 2.4.0.0 and Origin version 8.5. Table V shows the analytical parameters used to measure samples relaxation times. The NMR samples tube of 18 mm diameter were filled with 3 mL of material. The acquired signal decay (M(t)) for NEs samples was better fitted using 3 simple exponentials decay that returned a minor chi-square value, according to the function in Equation 2.

$$M_{(t)} = M_1\exp(-t/T_{2,1}) + M_2\exp(-t/T_{2,2}) + M_3\exp(-t/T_{2,3}) + k \quad \text{Equation 2}$$

Where component 1 refers to protons of water confined into the nanodroplets, component 2 refers to protons of oil phase and component 3 refers to protons of surfactants. M_1, M_2 and M_3 indicate proton intensity of each component and the percentage values were obtained by the Equation 3.

$$X\ \% = \frac{M_x . 100}{M_1 + M_2 + M_3} \qquad \text{Equation 3}$$

The acquired signal decay (M(t)) for CMC hydrogel and CMC-papain hydrogel samples was better fitted using 4 and 3 simple exponentials decay that returned a minor chi-square value, respectively.

3. Results

3.1. NMR Measurements of Tablets

Tablet is a solid dosage form containing medicinal substances with suitable diluents. Tapioca starch (TS) is produced from *Cassaca roots* and it is differentiated from other starches because contains about 17–20% amylose, with low level of residual materials, such as proteins, lipids, phosphorous and ash [11]. Propranolol (POP) is a non-selective beta-adrenergic blocking agent, which is in the World Health Organization's (WHO) List of Essential Medicines, as one of the most effective and safe medicines needed in a health system [12]. Atorvastatin (ATR) is a selective, competitive inhibitor of HMG-CoA reductase and a widely prescribed drug in case of hyperlipidemia [13].

In this work, proton NMR relaxometry was used to measure the behavior of spin-lattice relaxation time with a time constant, T_1H, and also the spin-spin relaxation with a time constant, T_2H. The T_1H values are relatively long due to the increase in the rigidity of sample, which makes the relaxation process long; in this process, the movement of collective proton nuclei takes longer to relax due to the rigidity. In T_2H relaxation process, which is mainly entropy driven, the relaxation change occurs between neighboring nuclei [3, 14, 15].

The experimental relaxometry relaxation data were determined to better understand the interaction between drug and polymer into tablets, due to the changes caused by the intermolecular interaction between components, generating new molecular arrangements, which are observed by changes in the values of proton spin-lattice relaxation times and T_1H domain curves. The proton spin-lattice relaxation time is strongly affected by the spin diffusion process, which tends to be an average of the spin diffusion process to a single value throughout the sample. For this reason, it is a measurable time constant for detecting the homogeneity in solid dispersions, because the value of the relaxation time is often sufficiently different between pure small molecule, such as an active pharmaceutical component, and pure polymer. In general, if the samples are homogeneous it is expected a uniform behavior of relaxation value and a single T_1H detected for drug and polymer [15].

In our previous work, Nevirapine was incorporated into polycaprolactone (PCL) hybrids containing layered silicates (S7), silica (SiO_2), and dioxide titanium (TiO_2) and the T_1H relaxation times were measured at varied Larmor frequencies, by a customized FFC and inversion recovery T_1H dispersion curve. The PCL showed a three-exponential behavior, described by $T_{11}H$, $T_{12}H$, and $T_{13}H$, which were related to the crystalline, rigid amorphous, and flexible amorphous portions of PCL, respectively. The $T_{11}H$ and $T_{12}H$ were higher for PCL with S7 nanoparticles suggesting more ordered structure with this particles introduction. The $T_{13}H$was more affected in PCL/SiO_2 system. The PCL chain motions slowed down in PCL/TiO_2 system. All PCL $T_{11}H$, $T_{12}H$, and $T_{13}H$ relaxation values were affected with nevirapine introduction, suggesting the drug distribution in all domains, but the amorphous region was the most affected [16].

The MSE-FID pulse sequence was applied to determine the rigid and amorphous fraction of the TS and the tablets developed. It is clear that the T_1H and T_2*H values characterize two different processes and therefore, in the common case, T_1H is different from T_2*H.

TD-NMR allowed the determination of spin-lattice relaxation times and domains distributions curves for TS, POP, ATV, POP tablets and ATV tablets (Table IV and Figure 2). The TS presented two domains or segments: T_1H at 4.3ms, which is related to less rigid molecular domain and it has higher molecular mobility, corresponding to the water hydrogen nuclei presents in the sample; T_1H at 86.3ms, which is related to more rigid domain and it has lower molecular mobility, corresponding to a region with restricted movement, attributed to the relaxation of

polysaccharide hydrogen nuclei. The domain with greater intensity, T_1H of 86.3 ms, controlled the relaxation process and this can be verified by the larger area under the distribution curve [3,17].

POP showed one domain and one relaxation time, T_1H, at 496ms, corresponding to its crystalline domain. This result was expected since POP is a small molecule, which has a high crystalline organization [3,15]. ATV presented two domains with distinct relaxation times, T_1H, one at 7.2 ms probably related to a less rigid domain, with higher mobility, corresponding to the CH_3 and COOH groups in the ATV molecule; and another T_1H at 250.1ms related to a more rigid domain, with lower molecular mobility, corresponding to hydrogen of pyrrole ring and hydrophobic groups [13]. The domain with higher intensity, T_1H of 250.1ms, controlled the relaxation process and it can be verified by the larger area under the curve. Although both drugs are crystalline, POP showed a higher T_1H value than ATV and this can be attributed to the POP arrangement in the crystal lattice, which causes a major restriction to the hydrogen nuclei [13,14,15].

For the POP tablets, three molecular domains with distinct T_1H values at 5.3 ms, 88.9 ms and 1597.4ms were observed. Also, the ATV tablets presented three molecular domains with distinct T_1H values at 4.7 ms, 81.2 ms and 693ms. The T_1H values around 5.3ms and 88.9mscorrespond to TS protons and the T_1H value at 1597.4ms corresponds to POP protons and the T_1H values at 4.7 ms and 81.2ms correspond to the TS protons and the T_1H value at 693ms corresponds to ATV protons. The domains at 88.9msand 81.2mspresented a higher intensity compared to the others domains. The increase of T_1H relaxation time from 496 ms to 1597.4 ms indicates that POP is not only entrapped between the TS chains, but also establishing hydrogen bonds between hydroxyls and the NH group of the drug with the polymer segments. Consequently, the increase of T_1H relaxation time was due to loss of mobility of POP when incorporated into polymer matrix after the compression process (Figure 3). Probably, in ATV tablets the same phenomenon occurred because there was an increase in T_1H relaxation time attributed to the ATV. The increase of T_1H relaxation time from 250.1 ms to 693 ms indicates that the drug, ATV, is not only entrapped between the TS chains but also performing hydrogen bonds with polymer segments. This increase in T_1H relaxation time was due to loss of mobility of ATV when incorporated into the polymer matrix after the compression process [15,16].

Another important point is that molecular mobility of hydrogen nuclei of TS matrix exhibited the same behavior after the compression process, even using two drugs with different biopharmaceutical classifications. Thus, it is a suitable excipient for tablets production.

Table 4: Spin-lattice relaxation time (T_1H), spin-spin lattice relaxation time (T_2H) attributed to rigid (T_2H_r*) and mobile (T_2H_m*) domains, including your respective percentage values and residual second moment of the dipolar interaction (M_2) for TS, POP and tablets

Sample	T_1H (ms) ± 2%	T_2H_r* (us)/ percentual (%) ± 2%	T_2H_m* (us) / percentual (%) ± 2%
TS	4.3	26 / 61	394 / 39
	86.3		
POP	496	13.64 / 100	----
POP Tablets	5.3	22 / 68	645 / 32
	88.9		
	1597.4		
ATV	7.2	-	-
	250.1		
ATV Tablets	4.7	-	-
	81.2		
	693		

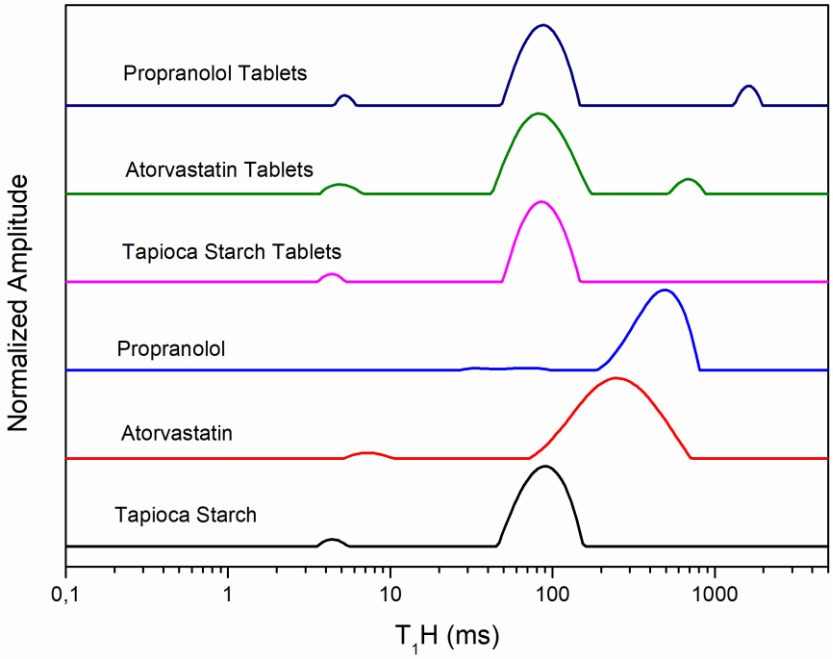

Figure 2: Distribution curves obtained by low-field nuclear magnetic resonance of tapioca starch, propranolol, propranolol tablets, atorvastatin and atorvastatin tablets

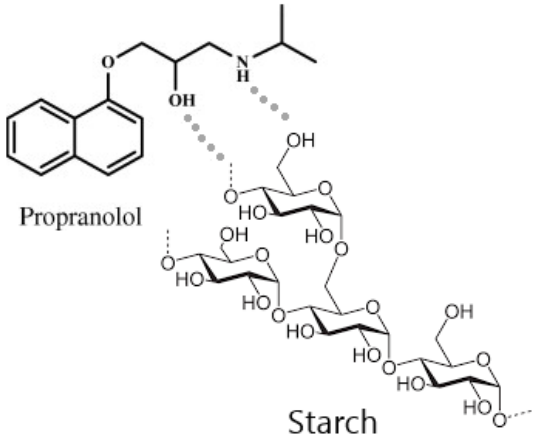

Figure 3: Propranolol and Tapioca starch interaction

MSE-FID pulse sequence was used to investigate the spin-spin relaxation time (T_2H) and percentage of each fraction. Thus, it was possible to evaluate each individual fraction (Table VI). The signal obtained for TS was composed of two distinct regions: the first related to rigid nuclear region ($T_2H_r^*$), at 26 µs, which corresponds to the polymer crystalline phase, equivalent to 61% of the material, following an Abragamian function; the second region with the highest molecular mobility of the hydrogen nucleus ($T_2H^*_m$), at 394 µs, corresponds to the amorphous phase, equivalent to 39% of the material. In the crystalline phase, polymer chains are more rigid or restrict, thus the hydrogen's of the polymeric chains have lower T_2H values. It was also observed that the rigid component has a great contribution to the T_2H values, according to the percentage of domains intensity, showing that the TS relaxation mechanism is controlled by its rigid phase [18]. POP presents only one T_2H^* value at 13.64 µs, which corresponds to its crystalline phase. Since a lower value of T_2H corresponds a higher crystalline material.

The tablets presented two T_2H^* values: one at 22 µs, which corresponds to the crystalline domain, equivalent to 68% of the material; and another at 645 µs, corresponding to the most amorphous domain, equivalent to 32% of the

material. Consequently, POP interacted with both crystalline and amorphous phase of TS. Nevertheless, in the amorphous part of TS there was major interaction with POP, because there was a significant increase in the $T_2H_m^*$value, indicating that the drug increased the molecular mobility of the polymer chains in the amorphous part. Besides, it was found that the major rigid component contributes to the T_2H values, according to the percentage of domains intensity, showing that the TS relaxation mechanism is controlled by its rigid phase [18].

3.2. NMR Measurements of NEs

Nanoemulsion (NE) is a dispersion system of nanodroplets, with a size between 20 and 250 nm, of one liquid in another immiscible one, surrounded by surfactant. These surfactants are amphiphilic molecules, which means that they are composed of hydrophobic and hydrophilic parts (usually a hydrophobic hydrocarbon with hydrophilic groups attached to it). These distinct regions allow such compounds to be soluble in both phases of the emulsion and therefore act at the interface between oil and water [19,20].

Rosa Mosqueta oil is extracted from the seed of the fruit of the wild plant and it is characterized by high concentrations of antioxidants and α-linolenic acid. The *Rosa Mosqueta* oil has been used for decades to treat wounds and/or scars. Due to its anti-inflammatory activity, evidenced in several studies, this oil was selected for the development of NE, as it may be useful in the cicatricial process of hemangiomas [21]. In recent years, some studies have reported the use of non-selective beta-blocker, such as POP, as a potential new topical agent for the treatment of superficial hemangioma [22].

In spin-spin lattice relaxation, the decay to equilibrium is based on the spins of the nuclei in neighboring molecules. Because all nuclei in the sample have varying spin, the spin of the nuclei in neighboring molecules produce magnetic fields that affect the spin of the nuclei in other molecules. So, the relaxation time T_2H is related to the molecular dynamics of the system, this means that any change in the molecular mobility in the system may be reflected in this parameter. The rigid and restricted systems present a smaller and less intense signal of T_2H [18].

The variation of the apparent spin-spin relaxation time (T_2H) of the emulsions is based on the difference between the spin-spin relaxation times of water molecules inside a water droplet [23].

Table V and Figure 4 show the T_2H measurements and distributions curves for NEs vehicle and POP, respectively. The NMR analysis showed that the NEs with *Rosa Mosqueta* oil presented four domains with different T_2H values. It can be correlated to the NEs phases: water, surfactants mixture (polyoxyethylene sorbitanmonooleate and sorbitanmonooleate) and oil, and each phase have an intrinsic relaxation time. It is possible to associate the highest $T_{2,4}H$ value (347.9ms) to the hydrogen's with high molecular mobility present in the structure of *Rosa Mosqueta* oil phase; the $T_{2,3}$ value (107.8 ms) corresponds to the hydrogen's of Span80®, which is a lipophilic surfactant and the more restricted hydrogen's of *Rosa Mosqueta* oil; $T_{2,2}$ value (38.8 ms) corresponds to the hydrogen of Tween® 80, which is a hydrophilic surfactant and it has great affinity for water; $T_{2,1}$ value (2 ms) corresponds to the hydrogen's present in the water molecules confined into the nanodroplets and some Tween 80® protons located at the interface of the nanodroplets.

Usually, the water molecules have T_2H above 1 s, however, when water molecules are confined to nanodroplets, as in the case of NEs developed, the value of T_2H is inversely proportional to the droplet volume. Thus, a small droplet of water has a large surface area and presents a greater effect of superficial relaxation, reducing its T_2H values. Moreover, the shift of the T_2H relaxation time distribution towards lower values is proportional to the increase of shear intensity during production and the decreasing size of the droplet [23, 24]. As mentioned before, the NEs were developed using the ultrasonication method and it is a high-power processor, which produces violently and asymmetrically imploding vacuum bubbles, creating micro-jets that break up the original droplets around nanometer size [10,22]. This confirmed the lowest $T_{2,1}H$ value for the water confined into the nanodroplets which have a mean diameter around 250 nm (Figure 5).

Moreover, when the molecules have high viscosity, they have slow movements and their magnetic fields oscillate slowly, presenting lower values of T_2H. Although not directly related, viscosity is the easiest extrinsic property of a liquid to relate T_2H. So, the NMR relaxation correlates well with emulsion viscoelastic characteristics, consequently, as viscosity increases the value of T_2H decreases [18]. The viscosity of *Rosa Mosqueta* oil is 77 mPAs, the viscosity

of Tween® 80 varies from 375 to 480 mPas and the viscosity of Span® 80 varies from 1000 to 2000 mPas. So it is possible to associate the highest value of T_2H (347.9 ms) to the hydrogen molecules present in the structure of oil, the intermediate value of T_2H (107.8 ms) to the hydrogen molecules present in the surfactants chains of Span® 80 and the lowest value of T_2H (38.8ms) corresponds to the hydrogen molecules present in the surfactants chains of Tween® 80. The Tween® 80 is a hydrophilic surfactant and interacts with water nanodroplets, where it is confined.

After the addition of 1% POP into *Rosa Mosqueta* NE, it was possible to verify an increase in the $T_{2,1}$ value from 2 ms to 4 ms and other peak at 38.8 ms. Since POP is a water-soluble drug, it interacts predominantly with the hydrogen's of water and Tween® 80, which is a hydrophilic surfactant. Therefore, there have been changes in the relaxation time and in domains that corresponds to the protons of these components. After POP incorporation there was an increase in the droplets size from 250 nm to 330 nm, consequently the water molecules were less confined and the T_2H increased from 2 ms to 4 ms. Also the increase in droplet size influenced the molecular dynamics of the Tween® 80 hydrogen's, disorganizing the droplet interface, observed by the peak displacement, and also altered the molecular motility of the hydrogen nuclei of the oil phase, as seen by the reduction of T_2H. This showed that POP was more effectively distributed in the water and in hydrophilic surfactant.

Table 5: T_2H measurements of NEs, by low field NMR

Sample	$T_{2,1}$ (ms)/% ± 2%	$T_{2,2}$ (ms)/% ± 2%	$T_{2,3}$ (ms)/% ± 2%	$T_{2,4}$ (ms)/% ± 2%
NE RM	2	38.8	107.8	347.9
NE RM 1% POP	4.1	37.5	109.6	315

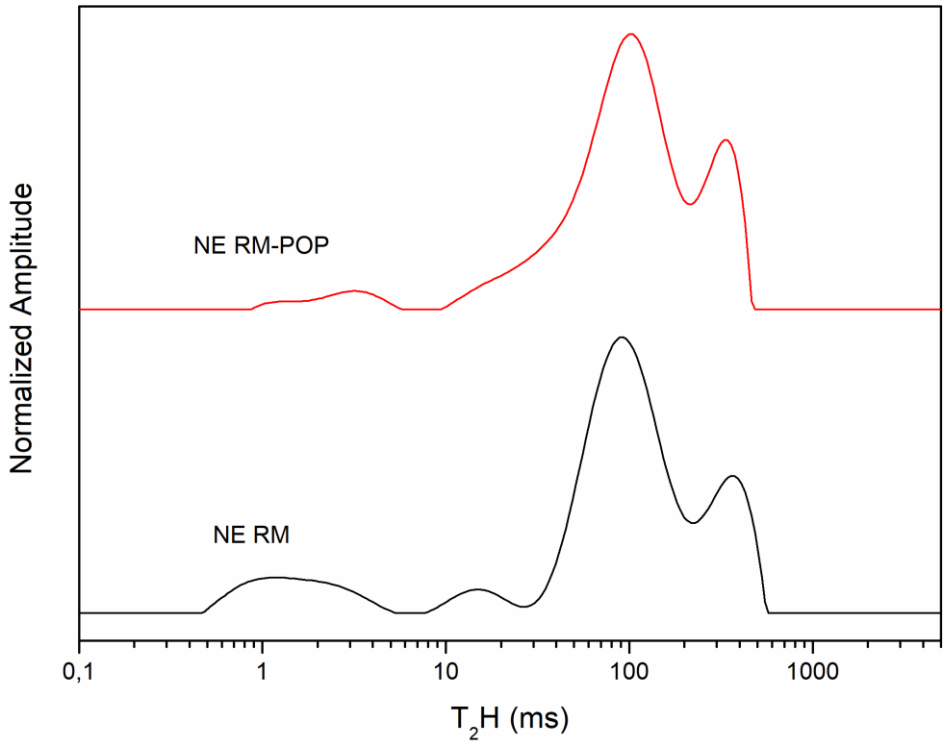

Figure 4: Distribution curves obtained by low-field nuclear magnetic resonance of almond oil NE vehicle and with 0.5% of POP

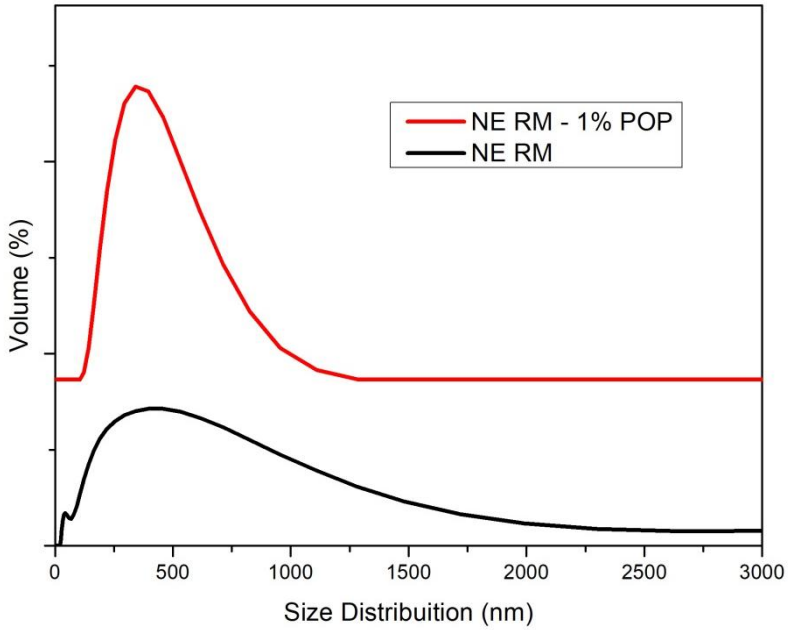

Figure 5: The droplet size distribution of NE RM and NM RM-POP

In our previous work, NMR analysis showed that after POP inclusion there was a reduction in T_2H values, indicating a possible interaction of the drug with the components of the system [25].

3.3. NMR Measurements of Hydrogel

Hydrogels are semisolid systems consisting of either suspensions made up of small inorganic particles or large organic molecules interpenetrated by a liquid. Hydrogels based on natural polysaccharide such as carboxymethylcellulose (CMC) are widely utilized in medical applications because of their high compatibility, biodegradability and non-toxicity [26]. Papain is a proteolytic enzyme extracted from green *Carica papaya* and/or latex of the leaves and it is widely known as a medical fruit with bacteriostatic and anti-inflammatory properties [27].

The hydrogel's properties and their dependence on drug incorporation were studied using spin-spin relaxation time. The relaxation time, T_2H, was used to investigate the water mobility within the hydrogel, to obtain information about its structure. Such information is important since the hydrogel's structures have an important role in drug release control [28].

Table 6 and Figure 6 show the T_2H measurements for pristine CMC hydrogel vehicle and vehicle with papain. The vehicle presented four domains with different T_2H values, which correspond to different proton populations, which can be correlated to the binding regions of the water molecules with the CMC polymer. The $T_{2,1}H$ value at 5.4 ms and the $T_{2,2}H$ value at 49 ms correspond to the most confined water molecules in the CMC structure, since a lower T_2H value represents a lower molecular mobility. When the polymer is added to water, the water molecules interact with it by hydrogen bonding and hence, the fluctuations of water molecules slow down. As a result, bound water molecules have smaller T_2H values. Consequently, both domains represent the water molecules confined and bound on free hydroxyl groups of basic glucopyranose unit, because this group presents a great steric barrier for the access of water molecules [29].

The third domain, $T_{2,3}H$, at 409 ms, controls the relaxation process, which can be verified by the larger area under the distribution curve, because it has a higher intensity. This relaxation time corresponds to the water molecules bound on carboxymethyl side groups ($-CH_2-COOH$), it is present only a minor steric barrier for the access of water molecules compared to the free hydroxyl groups. Consequently, it can be inferred that water molecules were interacting with the CMC gel network but not confined. The fourth domain, $T_{2,4}H$, at 1.5 s corresponds to free water molecules that are not bound to the CMC gel network. As water molecules in bulk water move very fast, the proton–

proton magnetic coupling of the water molecules is effectively averaged out, the transverse magnetization decays slowly in time, and T_2H is long [29].

This hydrogel dynamics was described by Shapiro (2011), where a three-state model for water in hydrogels was proposed, based on the observation of structured water near the water/solid interfaces and in natural macromolecular gels. It was suggested that water can behave dynamically and thermodynamically as a part of the polymer chains when the water molecules interact strongly with such specific sites as the hydroxyl or ester groups, and classified this type of water as the "bound" water. When the interaction between water molecules and polymer chains is weaker, or when the water molecules are preferentially structured around the polymer network, "intermediate" water is formed. The third type of water is "free" water where interaction between the water and the polymer chains is insignificant. The short component of T_2H was assigned to water molecules that strongly interact with polymer chains. The intermediate component, T_2H, was assigned to water residing in sample pores. The long component, T_2H, was shown to arise from water residing in cracks in the polymer network. The water molecules residing within finer pores decay faster (short T_2H) than those within larger (long T_2H) [28].

The incorporation of papain, which is water soluble drug, caused the suppression of the fourth domain, $T_{2,4}H$, at 1.5 s, indicating that the drug interacted preferentially with free water molecules. Also, the third domain, $T_{2,3}H$, continued to be majority, but its spin-spin relaxation time was reduced to 392 ms and it was enlarged due to the interaction of papain with this phase. This result agrees with the study of Mikac *et al* (2011), where it was shown that the drug tends to have interactions with polymer [30].

Dorozynski *et al*. (2012) observed that the T_1H and T_2H are intrinsic properties of the water molecules in the sample and, in general, depend on the water environment, such as the presence of other molecules that significantly decrease their rotational freedom and diffusion motion. For pure water, T_1H and T_2H relaxation times are roughly equal but even a small amount of macromolecules, such as agar, can significantly reduce both relaxation times. For example, for 2% agar solution (by weight) T_1H value was 1880 ± 10 ms and T_2H value was 75 ± 11 ms and these values linearly decrease with agar concentration [9].

Table 6: T_2H measurements of CMC hydrogel vehicle and vehicle with papain, by low field NMR.

Samples	**$T_{2,1}$(ms)± 2%**	**$T_{2,2}$ (ms)± 2%**	**$T_{2,3}$(ms)± 2%**	**$T_{2,4}$ (ms)± 2%**
CMC hydrogel	5.4	49	409	1400
Papain-CMC hydrogel	6.1	43.1	392	-

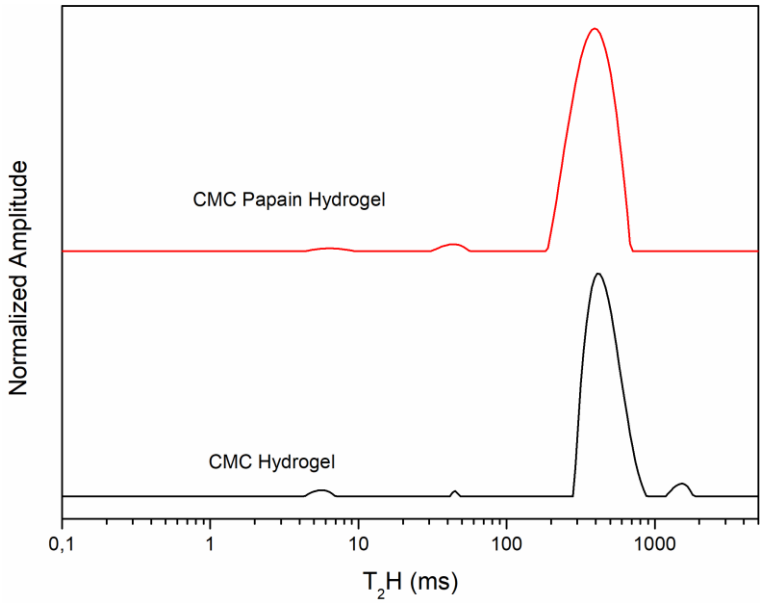

Figure 6: Distribution curves obtained by low-field nuclear magnetic resonance of CMC hydrogel vehicle and with papain

Conclusion

NMR relaxometry proved to be a tool that provides useful information on the molecular dynamics of different pharmaceutical materials, such as tablets, nanoemulsions and hydrogels. It was possible to study the interaction of different drugs, such POP and ATV in TS tablets; the interaction of POP in *Rosa Mosqueta* oil NE; and the interaction of papain in a CMC hydrogel, using NMR relaxometry.

Moreover, in the tablets analysis it was possible to conclude there was a physical interaction or dispersion between the tablets components. The TS, used as a tablet matrix, exhibited the same behavior after the compression process, even using two drugs with different biopharmaceutical classifications, indicating it is a suitable excipient. The T_2H relaxation times of NE allowed correlating the NEs phases: water, surfactants mixture and oil and it was also possible to observe through NMR relaxometry, that the drug POP was effectively distributed in the water and in surfactants. Furthermore, employing the T_2H relaxation times it was also possible to verify that the papain interacted preferentially with free water molecules of the hydrogel.

Declaration of interest statement

The authors declare no conflict of interest.

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