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NMR RELAXOMETRY TO EVALUATE THE *BAUHINIA FORFICATA* TEA INFUSION AND DECOCTION

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ABSTRACT

Nuclear magnetic resonance is a spectroscopic that permits analyzing samples by both solution and solid state. From solution and solid state NMR it is possible to obtain a good deal of information on sample molecular structure and dynamics, respectively. Plant extracts are often analyzed by solution NMR due to the complex compositions. The use of solid state, employing low-field NMR, such as spin-lattice relaxation time, gives response on the molecular dynamics. In this study, *Bauhinia forficata* parts and their extracts were evaluated to obtain tea with better properties to the health quality. Solution NMR techniques and relaxometry were used to evidence the therapeutic effects of this plant on diabetes, according to its chemical constitution. Studies revealed that its effectiveness is due to the presence of several classes of compounds as terpenes and flavonoids. The relaxometry was effective in characterizing the domains formed after the introduction of sugar in the tea.

Keywords: Food. Tea. NMR. Relaxometry.

AVALIAÇÃO POR RELAXOMETRIA DE RMN DO CHÁ DE *BAUHINIA FORFICATA* PREPARADO POR INFUSÃO E DECOCÇÃO

RESUMO

A ressonância magnética nuclear é uma técnica espectroscópica que permite a análise de amostras em solução e no estado sólido. A partir da análise RMN em estado sólido ou líquido é possível obter uma grande quantidade de informação sobre a estrutura molecular da amostra e dinâmica, respectivamente. Os extratos de plantas são muitas vezes analisados por RMN em solução, devido às composições complexas. O uso de estado sólido, empregando baixo campo RMN, tais como tempo de relaxação spin-estrutura, apresenta a resposta sobre a dinâmica molecular. Neste estudo, partes de *Bauhinia forficata* e seus extratos foram avaliados para obter chá com

melhores propriedades para melhorar a qualidade de vida. As técnicas de RMN e relaxometria foram usadas para evidenciar os efeitos terapêuticos da planta sobre a diabetes, de acordo com a sua constituição química. Os estudos revelaram que a sua eficácia é devida à presença de várias classes de compostos, com destaque para os terpenos e flavonóides. A relaxometria foi eficaz na caracterização dos domínios formados após a introdução do açúcar no chá.

Palavras-chave: Alimentos. Chá. RMN. Relaxometria.

1 INTRODUCTION

Bauhinia forficata is popularly called ion Brazil *pata-de-vaca* ("cow's foot") it belongs to the *Fabaceae* family. It is a medium-sized flowering tree that grows in some Brazil states such as Rio de Janeiro to the country's southernmost state, Rio Grande do Sul. Its tea made from its leaves has long been used as popular medicine especially to reduce blood sugar levels, since it acts as "natural insulin" for treatment of diabetes mellitus (CIPRIANE et al., 2008; SALGUEIRO et al., 2013). Diabetes mellitus is known as metabolic disorder with multiple etiologies, characterized by chronic hyperglycemia with disturbances caused by erratic carbohydrate and fat absorption and protein metabolism, resulting from defective secretion of insulin or action of insulin, or both (ANDRADE-CETTO; HEINRICH, 2005; NEGRI; SANTI; TABACH, 2012).

Some studies have reported of the hypoglycemic action of *Bauhinia forficate.* Silva and Cechinel Filho (2002) and Pessuto et al. (2009) made a comparative study of different plants of the *Bauhinia* genus (*B. manca, B. candicans, B. uruguayensis, B. purpurea, B. forficata* and *B. splendens*), found different classes of organic compounds, including lactones, flavonoids, terpenoids, steroids, triterpenes, tannins and quinones. The *forficata* species has been the subject of the largest number of studies regarding the effect of lowering blood sugar, and it is widely used to make teas and other phytotherapeutic preparations (HAMZA et al., 2010; FERRERES et al., 2012). PIZZOLATTI et al. (2003) described the isolation and identification of a kaempferol and various types of flavonoids from the leaves and flowers of *Bauhinia forficata* through chemical and spectroscopic methods. Other studies showed that solution nuclear magnetic resonance (NMR) can identify the types of flavonoids presented in the plant in lower percentages. It is Known that NMR techniques can indicate the presence of others tea components like polysaccharides. NMR has several advantages: it is precise; the analyses can be qualitative and quantitative; it does not destroy the sample; and it does not need sophisticated sample preparation or treatment before analysis (BORGES; BAUTISTA; GUILERA, 2008; NASCIMENTO; TAVARES, 2007).

One of the good NMR techniques that can be applied to evaluate solid samples is relaxometry techniques, through the relaxation times parameters; these give information to evaluate samples' components, especially in food areas, as a consequence of components intermolecular interaction and affinities. The relaxation parameters as proton spin-lattice relaxation times, which has time constant - T_1 (equation 1) and proton spin-spin relaxation time, with a time constant T_2 (equation 2), are able to identify the domains formed in the samples due to the affinity of components and its intermolecular interactions, according to their molecular organization (PRETO et al., 2013). The spin-lattice relaxation time is the time needs to the spins return to the equilibrium after being disturbed from the radiofrequency (is a thermal process) and the spin-spin relaxation time is required to the spins returns to its magnitude after being excited from the radiofrequency, this process does not involves

energy changes, is more entropic. The determination of these parameters, through low field NMR, is rapid and precise, and the values determined are representative of the whole sample, because they are influenced by all the compounds, proportion of them as well as the molecular organization and intermolecular interaction (COSTA et al., 2007; NASCIMENTO; TAVARES, 2007). Generally speaking an increase in the proton spin-lattice relaxation time means that the samples become more rigid due to the new intermolecular interactions, causing a long time to the components relaxing. A decrease in the proton spin-spin relaxation time means a strong intermolecular interaction, and the sample becomes more rigid, or with less molecular mobility due to the increase in the chains proximity, that restrict the molecular motions (PRETO et al., 2013; NASCIMENTO; TAVARES, 2007).

Equation 1: Proton spin-lattice relaxation time (T_1)

$$M_t = M_0 [1 - 2\, e^{-t/T_1}]$$

Equation 2: Proton spin-spin relaxation time (T_2)

$$M_y (t) = M_y (0) [1 - 2\, e^{-t/T_2}]$$

According to the statements before, in this study we decide to use both proton spin-lattice and proton spin-spin relaxation time to evaluate the behavior of *Bauhinia forficata* tea in the effective attraction of sugar from the blood, forming a complex with glucose or sugar, after being added to the tea.

2 EXPERIMENTAL

In this section we are showing the procedure to evaluate the initial plant samples and the two processes chosen of obtaining tea. Another point was the complex evaluation adding sugar in the tea samples. The final point in this section was the NMR characterization procedure.

2.1 Materials

The initial plant samples were then analyzed by low-field NMR spectroscopy to measure the T_1 relaxation parameter. This parameter allows evaluating the morphology and molecular organization of samples, as well as detection of the ranges of major classes of compounds present in the sample.

2.2 Extraction steps

The dehydrated plant material was subjected to extraction at room temperature using deuterated water, for analysis by NMR in solution, at a frequency of 300 MHz for the hydrogen-1 nucleus.

Two processes of obtaining tea from these plants were employed. In the first, the whole plant parts (without grinding) were mixed and subjected to an infusion process. In this process,

when the purified water (Milli-Q) reached the boiling point, the plant parts were added and allowed to boil for a few seconds, after which the mixture was removed from the heat and left at rest for 15 minutes.

In the second process, the whole plant parts were submitted to extraction by decoction, in which the parts were placed in purified water (Milli-Q) and then heated to boiling, after which the mixture was left at rest for 15 minutes.

2.3 Complex step

Complex is a step that forms aggregates with the components present in the extracts and different concentrations of glucose and/or sugar. The concentrations utilized were similar to those found in the digestive tract (see Table 1), and they were added to the extracts for subsequent examination by dynamic light scattering and also by NMR relaxometry through the measurement of spin-spin relaxation time, using a low-field NMR spectrometer. A concentration of 0.5 g/mL of glucose or sugar was chosen to verify if complexation occurred. The samples prepared for analyses are described in table 1 (where sample 1 refers to Milli-Q water alone).

Table 1: Types or extraction, aggregation agent and concentration of the samples

Sample	Type of extraction	Aggregation agent	Concentration (g/mL)
1	Water	-	-
2	Decoction	-	-
3	Infusion	-	-
4	Water	Glucose	0.07
5	Water	Glucose	0.1
6	Water	Glucose	0.2
7	Decoction	Glucose	0.07
8	Decoction	Glucose	0.1
9	Decoction	Glucose	0.2
10	Decoction	Glucose	0.5
11	Infusion	Glucose	0.07
12	Infusion	Glucose	0.1
13	Infusion	Glucose	0.2
14	Infusion	Glucose	0.5
15	Water	Sugar	0.07
16	Water	Sugar	0.1
17	Water	Sugar	0.2
18	Decoction	Sugar	0.07
19	Decoction	Sugar	0.1
20	Decoction	Sugar	0.2
21	Decoction	Sugar	0.5
22	Infusion	Sugar	0.07
23	Infusion	Sugar	0.1
24	Infusion	Sugar	0.2
25	Infusion	Sugar	0.5

Source: The authors.

All measurements of spin-spin and spin-lattice relaxation times were performed using a Resonance Instruments Maran Ultra 23 low-field NMR spectrometer operating at 23.4 MHz (for protons), equipped with an 18 mm variable temperature probe. The pulse sequence used

to obtain data on spin-spin relaxation time was Carr-Purcell-Meibom-Gil (CPMG), recycle time was 10s, t value was 40 and the 90° pulse was 4.1μs, which was calibrated automatically by the instrument's software. The same sample was analyzed at 27 ºC. The pulse sequence used to obtain data on spin-lattice relaxation time (T_1) was inversion-recovery (recycle delay - 180° - t - 90° - acquisition data) and the 90° pulse of 4.1 μs was calibrated automatically by the instrument's software. The amplitude of the FID was sampled for 20 t data points, ranging from 0.01 to 5000 ms, using 40 data points and 4 scans for each point. The same sample was analyzed at 27 ºC. The relaxation values and relative intensities were obtained by fitting the exponential data with the aid of the WINFIT program. Distributed exponential fits by plotting the relaxation amplitude versus relaxation time were performed by using the WINDXP software. Both WINFIT and WINDXP are commercial programs and come with the low-field NMR spectrometer.

3 RESULT AND DISCUSSION

The values of spin-lattice relaxation times are shown in Table 2. This parameter is the time necessary to transfer energy from excited nuclei to the fundamental state, through thermal energy. The response of the relaxation data is based on the molecular dynamic, molecular interaction and domain formation and the behavior of spin-lattice relaxation is used to verify the domains present in the samples due to the molecular organization, according to their distribution and intermolecular interaction.

The behavior of this parameter determined at room temperature shows that the leaves, flowers and stems had higher molecular mobility than those dehydrated at 50 °C. These changes in the molecular mobility are attributed to the higher moisture content at room temperature, which comes from the molecular distribution and diffusion of water in the sample domains, because it is responsible for the intermolecular interactions in the different sample parts.

Table 2: Values of T_1 for the samples dried at room temperature (25 ºC) and at 50 ºC, under circulating air

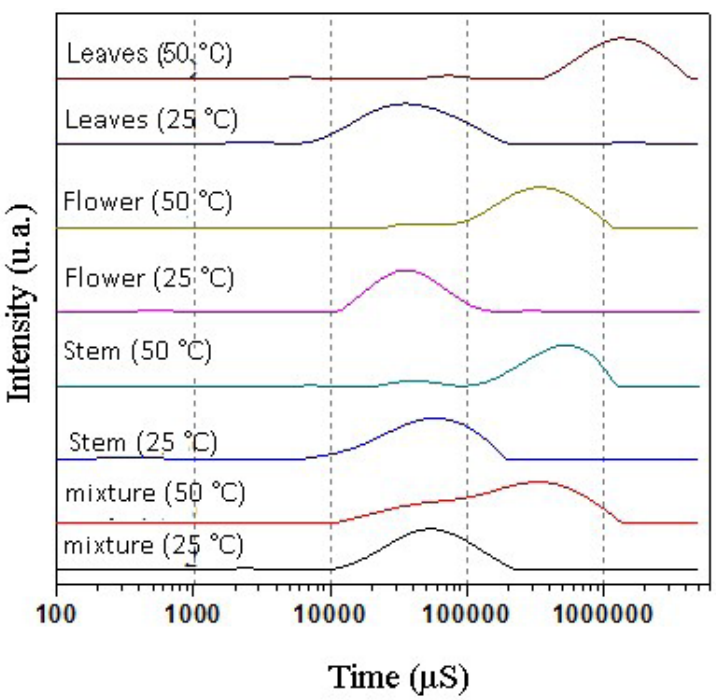
Samples	T_1 (ms)
Leaf 25°C	11 59
Leaf 50°C	22 104
Flower 25°C	21 57
Flower 50°C	35 134
Stem 25°C	14 68
Stem 50°C	18 114
Mix 25°C	23 80
Mix 50°C	29 121

Source: The authors.

The relaxation times of the samples dehydrated at 50 °C showed a reduction in molecular mobility of the materials, probably due to the formation of more rigid structures after moisture elimination, favoring the appearance of a new structural organization, such as hydrogen bonds and intra and intermolecular bonds. The removal of part of the water from the sample also permitted better examination of the domains in the material. Each type of material contained two domains. The domain with higher mobility and consequently low T_1 values probably referred to the presence of at least essential oils, fixed oils, terpenes and flavonoids, while the domain with lower mobility was related to polysaccharides and fibers, with high T_1 values. The forced-air dehydration did not degrade the samples because no new domain was observed. These domain formations have been observed by photochemical studies, which demonstrated that *Bauhinia forficata* contains alkaloids, tannins, mucilage, essential oils and fixed volatile acids (PEPATO et al., 2004). Various other studies have also confirmed that the plant contains high concentrations of free flavonoids and glycosides in the leaves and flowers, such as β-sitosterol and kaempferol 3, 7-dirhamnoside, the last substance only found in the leaves (FERRERES et al., 2012). Other classes of metabolic substances have also been mentioned as part of the chemical composition of this species, such as steroids, alkaloids, alcohols and polyalcohols (NASCIMENTO; TAVARES, 2007).

The domain curves of spin-lattice relaxation time are shown in figure 1 for each plan part and their mixture dehydrated at room temperature and 50 ˚C. From these Figure large domains containing both relaxation time values, because in these materials are heterogeneous and all types of intermolecular interactions that are presented in those samples promotes an enlargement of these domains caused by the different intermolecular forces due to water type.

Figure 1: Domain curves for the spin-lattice relaxation time values, each plan part and their mixture dehydrated at room temperature and 50 °C



Source: The authors.

4 SPIN-SPIN RELAXATION TIME

The objective of to determine the T_2 H relaxation parameter was to evaluate the complex process between tea and the sugar, which was added to the tea samples to simulate the tea action in the body. The spin-spin relaxation times of the *Bauhinia forficata* samples were determined for both extraction processes (decoction and infusion), using different concentrations of sugar or glucose, ranging from 0.07 to 0.5 g / mL.

The values of spin-spin relaxation times describe in tables 3 and 4 diminishing with an increase in the sugar or glucose concentration, reflecting the reduction in molecular mobility as a consequence of the formation of more rigid and/or big domains, probably due to the complexion between tea extracted components and the sugar or glucose added to them. These domains were confirmed by the formation of new interactions due to the extraction compounds with sugar or glucose, such as hydrogen bonds and intra and intermolecular interaction, causing changes in the molecular organization and also domain size.

Table 3: Spin-spin relaxation data for the samples extracted from the plant mixture at different glucose concentrations

Samples	Extraction Process	T_2 H (ms)	Glucose Concentration (g/mL)
1	Water	2458	-
2	Decoction	1979	-
3	Infusion	2002	-
4	Water	1843	0.07
5	Water	1803	0.1
6	Water	1350	0.2
7	Decoction	1882	0.07
8	Decoction	1777	0.1
9	Decoction	1508	0.2
10	Decoction	1046	0.5
11	Infusion	1945	0.07
12	Infusion	1828	0.1
13	Infusion	1570	0.2
14	Infusion	1057	0.5

Source: The authors.

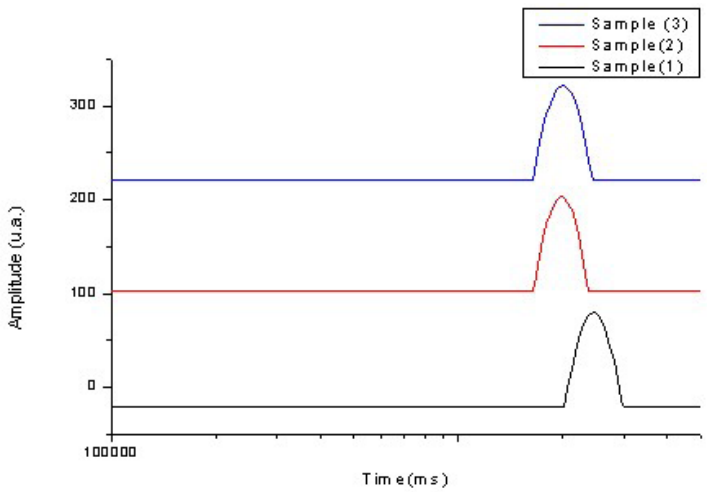
Table 4: Spin-spin relaxation data for the samples extracted from the plant mixture at different sugar concentrations

Samples	Extraction process	T_2 H (ms)	Sugar Concentration (g/mL)
15	Water	2239	0.07
16	Water	2170	0.1
17	Water	1968	0.2
18	Decoction	1755	0.07
19	Decoction	1691	0.1
20	Decoction	1514	0.2
21	Decoction	1272	0.5
22	Infusion	1827	0.07
23	Infusion	1732	0.1
24	Infusion	1469	0.2
25	Infusion	1261	0.5

Source: The authors.

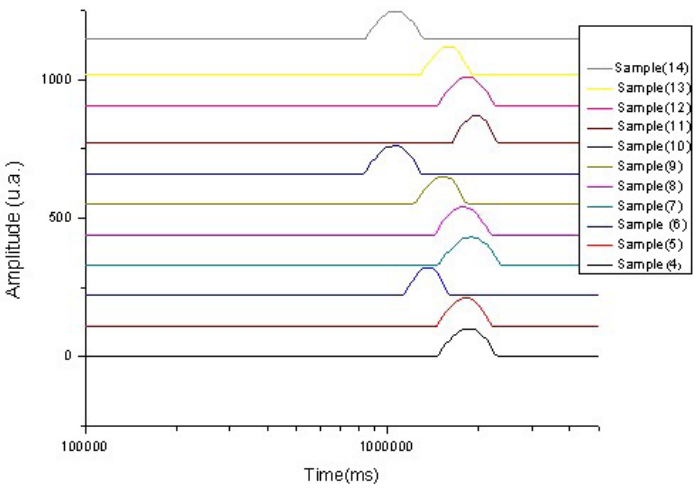
The domain curves of spin-spin relaxation times for the samples are shown in figures 2, 3 and 4. Figure 1 shows the domain curve behavior of the pure samples. Both treatment decoction and infusion presented the same domain distribution, which are shifted from water, but similar between them, therefore the infusion process shows a little narrow base line, which can be due to the less heterogeneous behavior of this sample.

Figure 2: Domain curves of the spin-spin relation of sample 1, 2 and 3
(Mili-q water - sample 1, Extraction decoction - sample 2 and Extraction infusion - sample 3)



Source: The authors.

Figure 3: Domain curves of the spin-spin relaxation data of sample named 4 to 14 (according to the Table 1)[1]



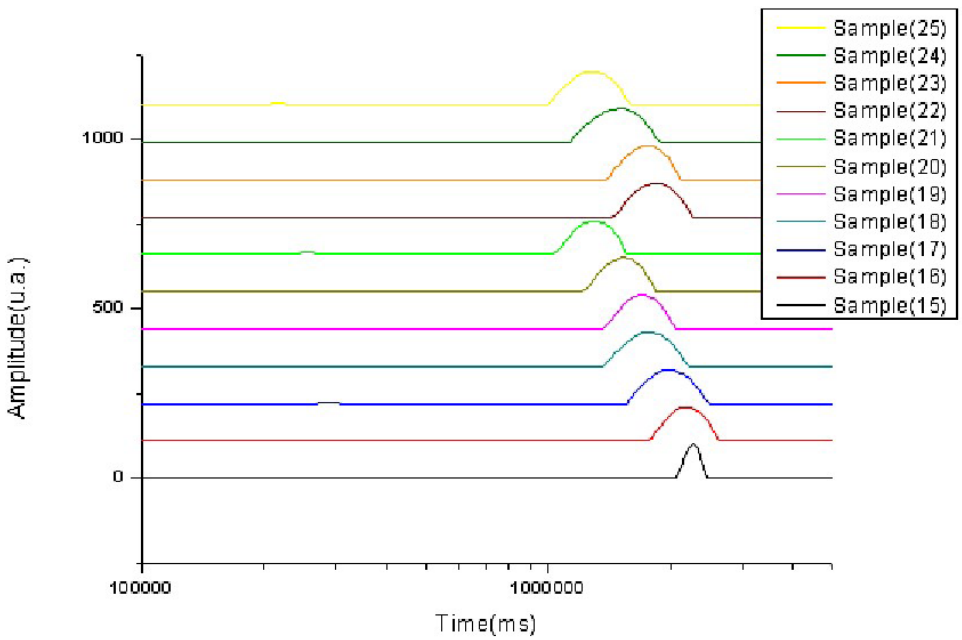
Source: The authors.

[1] Legend: water + glucose 0.07g - Sample 4; water + glucose 0.1g - Sample 5; water + glucose 0.2g - Sample 6; Extraction decoction + glucose 0.07g - Sample 7; Extraction decoction +glucose 0.1g - Sample 8; Extraction decoction +glucose 0.2g - Sample 9; Extraction decoction + glucose 0.5g - Sample 10; Extraction infusion + glucose 0.07g - Sample 11; Extraction infusion + glucose 0.1g - Sample 12; Extraction infusion + glucose 0.2g - Sample 13; Extraction infusion + glucose 0.5g Sample 14.

The values of spin-spin relaxation time for the samples 4 to 6 that only contains water and glucose shows a shift in the value of this parameter as well as for the domain curves to lower values with the of glucose quantity, showing that in this parameter there was a strong effect between both mixture components as hydrogen bonding.

Comparing the effect of glucose quantity in both extraction methods in general the relaxation parameter decrease with the increase of glucose up to lower values comparing to the samples containing only glucose, which confirms that in these extractions methods other substances reacts with the glucose compounds complexion with it.

Figure 4: Domain curves of the spin-spin relaxation data of sample named 15 to 25 (according to the Table 1)[2]



Source: The authors.

Analyzing the domain curves for the second sets of samples it can be seen that it contains two groups of samples. The three initial domain curves named 15 to 17 an enlargement of the domain curves was observed after sugar been added to this sample, due to a formation of heterogeneous samples. Observing the domain curves as the sugar quantity increases there was a shift in the curves to lower values of spin-spin parameter and an increase in the domain base line which is more accentuated for the samples prepared by infusion method. For infusion samples the domain base line is enlarged compared to the decoction process, proving that in both cases there was complexion between tea and sugar, due to the intermolecular interaction formed among tea components and sugar.

[2] Legend: Water + sugar 0,07g - Sample 15; Water + sugar 0,1g - Sample 16; Water + sugar 0,2g - Sample 17; Extraction decoction + sugar 0.07g - Sample 18; Extraction decoction + sugar 0.1g - Sample 19; Extraction decoction + sugar 0.2g - Sample 20; Extraction decoction + sugar 0.5g - Sample 21; Extraction infusion + sugar 0.07g - Sample 22; Extraction infusion + sugar 0.1g - Sample 23; Extraction infusion + sugar 0.2 g - Sample 24; Extraction infusion + sugar 0.5g - Sample 25.

5 CONCLUSION

The spin-lattice and spin-spin relaxation times measured indicated the presence of new molecular organization, with the formation of intra and intermolecular bonds, such as hydrogen bonds, in the samples dehydrated under forced air at 50 °C in comparison with those dried at room temperature.

The spin-spin relaxation measurement was an accurate and efficient technique to prove the complexation between the extraction components and the sugar or glucose samples.

This study can be applied to other systems.

The combination of both relaxometry techniques utilized in this work was effective in characterizing the main components present in the structure of *Bauhinia forficate.* It was also detected that there was components present in the plant that act to reduce the blood glucose level by complexion with sugar or glucose.

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