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NMR structural analysis of epigallocatechin gallate loaded polysaccharide nanoparticles

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ABSTRACT

Nuclear magnetic resonance (NMR) spectroscopy has been employed for structural characterization of epigallocatechin gallate loaded maltodextrin/gum arabic nanoparticles (EGCG-MD/GA). Measurements of the nuclear relaxation times (T_1) and application of diffusion ordered spectroscopy (DOSY), obtained through pulsed field gradient (PFG) NMR experiments, have been performed to determine the structure of the epigallocatechin gallate–polysaccharide conjugates and to clarify the mechanisms of drug immobilization into the polymer matrix. The results suggest the entrapment of EGCG into the polysaccharide matrix of maltodextrin/gum arabic (MD/GA) and support the potential of these vehicles for their sustained delivery and release.

1. Introduction

Catechins are a class of polyphenolic flavonoids predominantly found in foods and beverages, such as apples, chocolate, red wine and green tea (Hackman et al., 2008; Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995). The main catechin species include epicatechin (EC), catechin (C), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). Among them, EGCG is the most abundant catechin found in the green tea. The catechins possess antioxidant activity due to their ability of trapping free radicals by donation of the phenolic hydrogen atoms (Mendoza-Wilson & Glossman-Mitnik, 2006). As radical scavengers, catechins show interesting medicinal properties, including anti-inflammatory, antiviral, anti-cancer and antifungal activities (Hirasawa & Takada, 2004; Kuzuhara, Suganuma, & Fujiki, 2008; Zaveri, 2006). However, their pharmaceutical application is limited by several factors like their poor solubility, inefficient permeability, instability, first pass effect and gastrointestinal (GI) tract degradation (Mochizuki, Yamazaki, Kano, & Ikeda, 2002; Neilson et al., 2007; Zhu, Zhang, Tsang, Huang, & Chen, 1997). Therefore, there is a need to develop strategies for new medical tools for more effective protection and delivery of catechins, considering the performance and maintaining of their original physical properties.

Nanoparticles made of polysaccharides, due to their unique properties are promising carriers to deliver and protect the physiological properties of hydrophilic drugs and have been successfully applied as drug-delivery systems (Gonçalves, Pereira, & Gama, 2010; Hu et al., 2008; Liu, Jiao, Wang, Zhou, & Zhang, 2008; Vauthier & Couvreur, 2000). As natural biomaterials, polysaccharides are stable, safe, non-toxic, hydrophilic and biodegradable. In addition, polysaccharides have abundant resources in nature and low cost in their processing. Recently, polysaccharides nanoparticles based on maltodextrin and gum arabic have been reported as a delivery system for catechins (Ferreira, Rocha, & Coelho, 2007; Gomes et al., 2010).

The nanoparticle structure and properties are critical to understand and develop novel therapeutic agents. The knowledge of the nature of the intermolecular interactions between the species presented in these nanostructures is of fundamental importance in the understanding of the factors that determine their biological activity. It is therefore essential to elucidate the structure of drug-loaded nanoparticles and to clarify the mechanisms of drug immobilization in the polymer matrix.

Nuclear magnetic resonance (NMR) spectroscopy is one of the most powerful experimental methods for investigation of the structure and intermolecular interactions of multicomponent systems

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(Evans, 1995; Teng, 2005). The combined application of diffusion ordered NMR spectroscopy (DOSY) and measurement of the nuclear spin-lattice relaxation times (T_1) offers a chance to gain insight into the structure of intermolecular aggregates in solution (Bakhmutov, 2004; Cohen, Avram, & Frish, 2005). DOSY is nowadays a well-established technique for characterizing the structure and dynamics of complex systems. The self-diffusion coefficients and structural properties of the molecules are connected by the dependence of their self-diffusion coefficients on the molecular size, weight, shape, etc. (Brand, Cabrita, & Berger, 2005; Cohen et al., 2005; Johnson, 1999). Therefore, DOSY experimental technique has become a valuable tool for studies of molecular interactions in solution.

The aim of the present study was to investigate the structure of EGCG-loaded maltodextrin/gum arabic nanoparticles (EGCG-MD/GA) and to estimate the origin of the intermolecular interactions responsible for the EGCG–polymer particulate formation on the base of high-resolution NMR spectroscopy. The structure and intermolecular interactions of drug-loaded systems determine their capability and biological activity as drug-delivery nanomedicines.

2. Experimental part

2.1. Sample preparation

2.1.1. Preparation of unloaded and EGCG-loaded polysaccharide nanoparticles

Maltodextrin/gum arabic nanoparticles (MD/GA) were obtained by homogenization of gum arabic (GA, M_W = 250 kDa, Sigma–Aldrich Co.) and maltodextrin (MD, 1 kDa, DE 16.5–19.5, Grain Processing Corporation) dissolved in ultrapure water (Nanopure Diamond Water Purification, Barnstead Thermo Scientific, USA, resistivity = 18.2 MΩ cm) at 50–60 °C under magnetic stirring. The suspension was homogenized at a constant speed of 9500 rpm with a dispersing device IKA DI25 Basic. Further, the suspension was spray-dried in a pilot spray-dryer (designed by Niro A/S) wherein the inlet and outlet air temperature were 160 ± 5 and 60 ± 5 °C, respectively. After leaving the drying chamber, the nanoparticles were recovered using a cyclone and finally collected in a powder collector vessel.

EGCG-loaded nanoparticles (EGCG-MD/GA) were prepared by the same methodology, but in this case, EGCG was added to the MD/GA suspension in a ratio of 5 wt.% before the homogenization. The constant speed of homogenization and inlet and outlet air temperature of spray-drying were kept the same.

2.1.2. Preparation of physical mixtures

Physical mixtures of free EGCG with MD/GA nanoparticles (EGCG + MD/GA) and free EGCG with MD and GA (EGCG + MD + GA) were prepared by mixing the components in the same proportion employed in the preparation of EGCG-MD/GA particles. Each of the mixtures (20 mg) was dissolved in 600 μL of D_2O under magnetic stirring.

2.2. NMR spectroscopy

All NMR experiments have been recorded on a Bruker Avance III 400 spectrometer, operating at 400.15 MHz for protons, equipped with pulse gradient units, capable of producing magnetic field pulsed gradients in the *z*-direction of 50 G/cm. The spectra have been acquired in D_2O solution at 30 °C in 5 mm tubes. Sodium trimethylsilyl-[2,2,3,3-d4]-propionate (TSP) has been used as an internal standard for both the chemical shift and the diffusion measurements. Unloaded (MD/GA) and EGCG-loaded nanoparticles (EGCG-MD/GA), physical mixture of EGCG and unloaded nanoparticles (EGCG + MD/GA) and physical mixtures of EGCG, MD and GA (EGCG + MD + GA) were prepared for NMR measurements as described above (see Section 2.1). With the exception of EGCG, in all cases 20 mg of solute, 600 μL D_2O and 20 μL 0.05 mM solution of TSP in D_2O were used. NMR spectra of EGCG were recorded in D_2O at concentration of 10 mg/mL. All NMR measurements have been done with standard Bruker pulse sequences.

Two-dimensional $^1H/^1H$ correlation spectra (COSY) and gradient-selected $^1H/^{13}C$ heteronuclear single quantum coherence (HSQC) spectra were recorded using the standard Bruker software. 2D COSY spectra were acquired with a multiple quantum filter, gradient pulses for selection, a gradient ratio of 16:12:40 and a relaxation delay of 1.5 s. A total of 2048 data points in F2 and 512 data points in F1 over a spectral width of 6500 Hz were collected. 2D $^1H/^{13}C$ HSQC experiments via double inept transfer, using sensitivity improvement and decoupling during the acquisition were carried out with a spectral width of ca. 6000 Hz for ^1H and 28,000 Hz for ^{13}C, a relaxation delay of 1.5 s, FT size 2 K × 256 W.

The two-dimensional nuclear Overhauser effect spectroscopy (NOESY) experiments were acquired in phase-sensitive mode with gradient pulses in the mixing time, using the standard pulse sequences with optimized mixing time of 400 ms. Generally, 64 scans and 512 F1 slices were obtained and the spectral width in both dimensions was 6500 Hz.

The spin-lattice relaxation rates were measured using the inversion-recovery pulse sequence, (180°–τ–90°). Thirty-two τ increments were used for the experiments, with values between 0.01 and 13.0 s and relaxation delay of 13 s. Relaxation times were calculated by exponential regression analysis of recovery curves of longitudinal magnetization components.

The DOSY experiments were performed using the bipolar longitudinal eddy current delay (BPPLED – Bipolar Pulsed Field Gradient Longitudinal Eddy Delay) pulse sequence (Wu, Chen, & Johnson, 1995). The experimental conditions (amount of the solute and the solvent, temperature, air flow, sample rotation) for all DOSY experiments were kept constant. Before all NMR experiments, the temperature was equilibrated and maintained at 30 °C, as measured using the spectrometer thermocouple system. The measurements were carried out in D_2O and the solutions were prepared at constant concentration of 20 mg/0.60 mL. The diffusion coefficient of TSP (6.68×10^{-10} m^2 s^{-1}, calculated standard deviation of 3.1×10^{-3}) obtained from ^1H DOSY experiments was used as an internal reference for the diffusion measurements. The spectra were recorded in 5 mm NMR tubes with an air flow of 535 L/h. Typically, in each experiment a number of 32 spectra of 16K data points and 64 scans were collected, with values for the duration of the magnetic field pulse gradients (δ) of 4 or/and 5 ms, diffusion times (Δ) of 160–200 ms and an eddy current delay set to 5 ms. The pulse gradient (g) was incremented from 2 to 95% of the maximum gradient strength in a linear ramp. The spectra were first processed in the F2 dimension by standard Fourier transform and after baseline correction the diffusion dimension was processed with the Bruker Topspin software package (version 2.1). The diffusion coefficients are calculated by exponential fitting of the data belonging to individual columns of the 2D matrix. The diffusion coefficients (D) were obtained by measuring the signal intensity at more than one place in the spectra. Between 5 and 10 different measurements were done for the determination of each diffusion coefficient. The TSP was used as an internal diffusion reference for the DOSY measurements and all diffusion coefficients are given as a ratio D/D_{TSP} according procedure published before (Cabrita & Berger, 2001) and average values have been calculated.

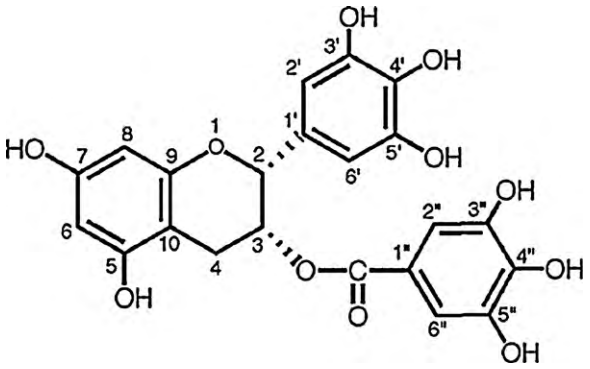

Fig. 1. Chemical structure of EGCG.

2.3. Dynamic light scattering (DLS)

The hydrodynamic diameter of EGCG-MD/GA and MD/GA nanoparticles was determined by DLS at a scattering angle of 173°, using a Malvern Zetasizer Nano ZS (Malvern Ltd., UK). Each sample was measured at 0.3 wt.% concentration in ultrapure water at pH 5, at room temperature. The average hydrodynamic diameter values were obtained from three independent experiments at similar conditions.

3. Results and discussion

The structure of EGCG-MD/GA was estimated by studying the behaviour and intermolecular interactions of EGCG with the polysaccharides on a number of NMR parameters, such as chemical shifts, spectroscopic line shape, spin-lattice relaxation (T_1) and translational diffusion. For comparison, NMR spectroscopic investigations of EGCG (chemical structure depicted in Fig. 1), MD/GA, physical mixtures of EGCG + MD/GA and EGCG + MD + GA were also performed. Additionally, the hydrodynamic diameters of MD/GA and EGCG-MD/GA were determined and correlated to the self-diffusion coefficients of the corresponding species.

3.1. Structure and NMR spectral characterization of EGCG-MD/GA

Typical 400 MHz ^{1}H NMR spectrum of EGCG-MD/GA is presented in Fig. 2. The spectra obtained for EGCG, MD/GA and their physical mixtures (EGCG + MD/GA and EGCG + MD + GA) are included for comparison. The assignment of the proton resonances of the samples was based on the analysis of the one- (1D) and two-dimensional (2D: ^{1}H/^{1}H COSY, and ^{1}H/^{13}C HSQC) NMR spectroscopic data and is consistent with the data published before (Dror, Cohen, & Yerushalmi-Rozen, 2006; German, Blumenfeld, Yuryev, & Tolstoguzov, 1989; Mclntyre, Ceri, & Vogel, 1996; Ninni, Meirelles, & Maurer, 2005; Pigman, Horton, & Herp, 1970; Xu, Tan, Janson, Kenne, & Sandström, 2007). The assignments of the resonance signals in the spectra of EGCG and polysaccharides are shown in Fig. 2.

The ^{1}H NMR spectra of EGCG-MD/GA, EGCG + MD/GA and EGCG + MD + GA, are dominated by the intense, broad and overlapped resonance signals characteristic for the polysaccharide structures of maltodextrin (MD) and gum arabic (GA). MD is a polysaccharide consisting mainly of α(1 → 4) and α(1 → 6) D-glucose units connected in chains of variable length (from 3 to 19 glucose units) (German et al., 1989; Ninni et al., 2005; Pigman et al., 1970). However, GA is a complex multifraction material consisting mainly of highly branched polysaccharides and small fractions of high molecular weight protein–polysaccharide complex and glycoproteins. The GA polysaccharides consist of β-(1 → 3) galactose backbone with linked branches of arabinose and rhamnose, which terminate in glucuronic acid as it was confirmed by NMR spectroscopy and chromatography (Mclntyre et al., 1996). Recently, X-ray and neutron scattering investigation of the overall structure and inner heterogeneity of GA in aqueous solution have been reported (Dror et al., 2006).

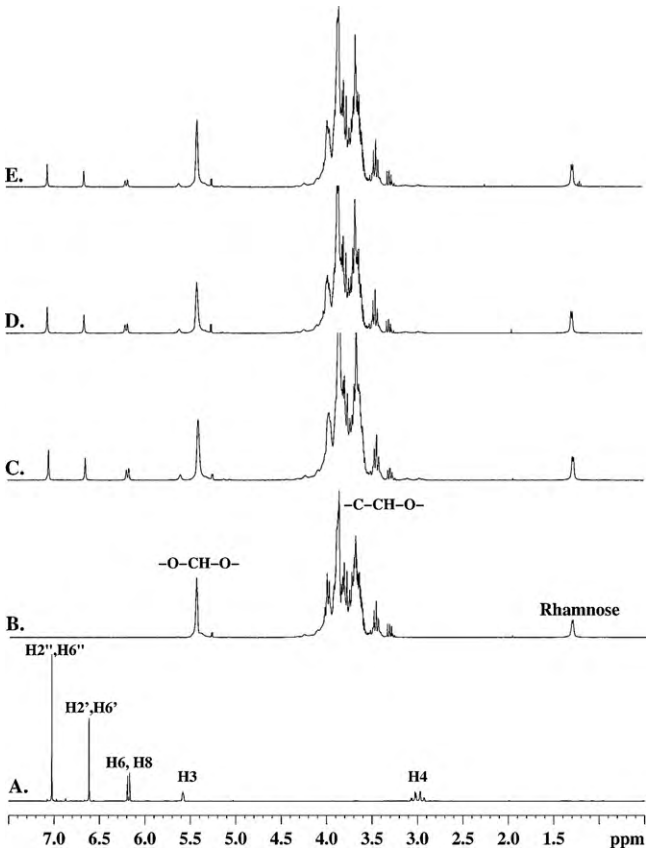

Fig. 2. 400.15 MHz ^{1}H NMR spectra of EGCG (A), MD/GA (B), EGCG + MD/GA (C), EGCG + MD + GA (D) and EGCG-MD/GA (E). The assignment of ^{1}H resonances of EGCG and MD/GA is included.

Resonance signals of the anomeric and hydroxymethine protons of saccharide residues of the polysaccharide structures of MD and GA in the ^{1}H NMR spectra of EGCG-MD/GA, EGCG + MD/GA and EGCG + MD + GA were observed at 5.40–5.20 and 4.5–3.2 ppm. The broad signal at 5.40 ppm was attributed to the anomeric protons of α(1 → 4)- and α(1 → 6)-linked glucose units of MD. The corresponding resonance signals of the glucose methine protons appeared in the spectral area 4.15–3.5 ppm. The resonances at 5.23, 5.04, 4.70 and 4.50 ppm were assigned to the anomeric protons of arabinose, α-glucoronic acid, rhamnose and β-glucoronic acid, respectively, in agreement with the data published before (Mclntyre et al., 1996). The analysis of 2D COSY and TOCSY spectra and the distinct resonance signal at 1.27 ppm of C6 methyl protons (doublet, J = 5.7 Hz) also confirmed the presence of rhamnose moieties. The observed broadening of the resonance lines suggests involvement of the saccharide residues into high molecular polysaccharide structures with low mobility and variation in the spin–spin relaxation time (T_2).

In the ^{1}H NMR spectra of EGCG-MD/GA, EGCG + MD/GA and EGCG + MD + GA, an appearance of signals belonging to the protons of EGCG were clearly observed (Fig. 2). A down field chemical shift of 0.03–0.04 ppm was detected for H3, H4, H2′, H6′, H2″ and H6″ protons of EGCG in the spectra of EGCG-MD/GA and EGCG + MD + GA but smaller in EGCG + MD/GA (Table 1). The stronger downfield chemical shift of these protons in EGCG-MD/GA and EGCG + MD + GA was attributed to more effective H-bonding of

Table 1
Selected ^{1}H chemical shifts (in ppm) and chemical shift differences with respect to pure EGCG (in italic) of EGCG, EGCG + MD/GA, EGCG + MD + GA and EGCG-MD/GA.

Proton	EGCG	EGCG + MD/GA	EGCG + MD + GA	EGCG-MD/GA
H2″, H6″	7.018	7.046 *0.028*	7.047 *0.029*	7.048 *0.030*
H2′, H6′	6.604	6.637 *0.033*	6.643 *0.039*	6.645 *0.041*
H6, H8	6.17	6.184 *0.014*	6.177 *0.007*	6.175 *0.005*
H3	5.567	5.581 *0.014*	5.597 *0.030*	5.598 *0.031*
H4	2.984	3.017 *0.033*	3.033 *0.049*	3.033 *0.049*

EGCG included into the polysaccharide matrix. A possible explanation for the smaller chemical shifts in EGCG + MD/GA is the fact that the EGCG molecules are located on the surface of the MD/GA nanoparticles and thus have less effective H-bonding.

Despite the ^{1}H/^{1}H NOESY spectra of EGCG-MD/GA (Fig. 3) being dominated by nuclear Overhauser effects (NOE) between the protons belonging to the polysaccharide chains, weak signals were detected due to intermolecular dipole–dipole interactions between H2″, H6″ protons of EGCG (7.05 ppm) and glucose methine protons of MD/GA in the spectral area 4.15–3.5 ppm. The dipole–dipole interactions between EGCG and MD/GA protons confirm the entrapment of EGCG into the polysaccharide matrix of maltodextrin/gum arabic of EGCG-MD/GA.

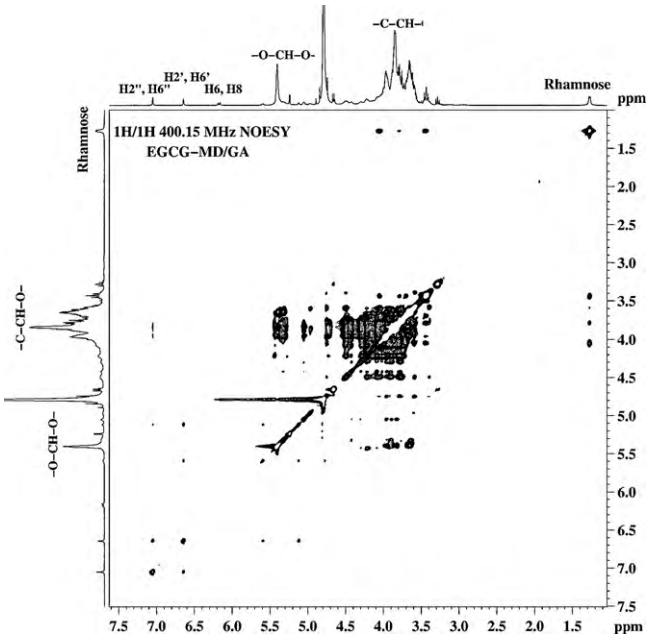

Fig. 3. 400.15 MHz 2D ^{1}H/^{1}H NOESY spectra of EGCG-MD/GA in D_2O.

3.2. T_1 relaxation investigation

The NMR relaxation measurements can provide structural and dynamic information about molecules and molecular aggregates (Bakhmutov, 2004). For protons in solution there are three principal mechanisms of spin-lattice relaxation: dipole–dipole interaction, chemical shift anisotropy and spin–rotational interaction, however, the first one tends to be dominant. Spin-lattice relaxation times are related via correlation times to the mobility of the molecules and may be used to estimate the possibility for intermolecular aggregate formation (Bakhmutov, 2004; Freeman, Hill, & Tomlinson, 1974; Freeman, Wittekoek, & Ernst, 1970).

Examples of spin-lattice relaxation times (T_1) determined for protons belonging to EGCG and carbohydrate polymer matrix for the samples studied are shown in Table 2.

The relaxation times of protons from the individual functional groups of EGCG and polysaccharides MD and GA vary according to their internal mobility but the overall trends observed were identical for all groups. Only insignificant T_1s alterations were detected for MD and GA protons in the presence of EGCG probably due to more effective dipole–dipole interaction between the rigid polymer molecules and EGCG. The relaxation times of EGCG protons were significantly longer than when it was encapsulated or physically mixed with MD/GA or MD + GA. Considering the complex structures and the high molecular weight of the species presented into MD and GA, the decreased T_1s of EGCG were attributed mainly to the increased viscosity of the medium. It is significant to note that the measured relaxation times of EGCG in both physical mixtures were closely similar but longer than those in EGCG-MD/GA, even though the samples have been prepared at the same concentration and no difference in the viscosity could be assumed. This indicates that the microenvironments of the protons of EGCG in the EGCG-MD/GA and both physical mixtures (EGCG + MD/GA and EGCG + MD + GA) are different. The results strongly suggest that in the case of EGCG-MD/GA the EGCG molecules are entrapped into the MD/GA matrix of the nanoparticles.

3.3. Diffusion NMR spectroscopy

Diffusion ordered NMR spectroscopy (DOSY) was used to analyse overall structure of EGCG-MD/GA, to clarify the origin of the EGCG–polysaccharide interactions and to locate the EGCG molecules into the MD/GA matrix. The pulsed field gradient spin echo (PFGSE) NMR technique is a straightforward procedure for measuring the diffusion coefficients of individual species. Particularly, useful information can be obtained regarding the size, molecular weight and shape of the species in solution under given conditions. In order to get insight into the structural modifications of the species presented in the EGCG-MD/GA and to account for changes in the solvent viscosity and minor fluctuations of samples temperature, we found it useful to use an internal diffusion reference (Brand et al., 2005; Cabrita & Berger, 2001; Cohen et al., 2005). Since modifications of the solution composition could induced changes in the viscosity and these changes affect equally all species in the solution, the use of reference allows one to take into account

Table 2
Selected spin-lattice relaxation times (T_1, in s) determined for selected ^{1}H chemical shifts (in ppm) belonging to EGCG, MD and GA in D_2O at 30 °C.

Sample	EGCG (δ, ppm)			MD and GA (δ, ppm)					
	7.03	6.6	6.17	5.40	5.23	4.1–3.5	3.4	3.3	1.28
EGCG	1.691	1.242	1.833						
MD/GA				0.907	1.958	0.558	1.339	2.017	0.715
EGCG + MD/GA	1.384	1.149	1.379	0.885	1.908	0.576	1.323	2.002	0.693
EGCG + MD + GA	1.321	1.115	1.316	0.875	1.828	0.559	1.323	2.142	0.701
EGCG-MD/GA	1.26	1.021	1.275	0.889	1.818	0.56	1.304	1.941	0.666

Table 3
Relative diffusion coefficients (related to TSP), with calculated standard deviations (in italics) of EGCG and MD/GA in D_2O at 30 °C for the samples studied.

Sample	EGCG (δ, ppm)	MD and GA (δ, ppm)					
	7.03	5.42	5.25	4.1–3.5	3.43	3.3	1.28
EGCG	0.62 *0.01*						
MD/GA		0.40 *0.04*	0.55 *0.01*	0.28 *0.03*	0.37 *0.02*	0.52 *0.02*	0.04 *0.03*
EGCG + MD/GA	0.49 *0.03*	0.45 *0.08*	0.57 *0.03*	0.37 *0.07*	0.42 *0.04*	0.61 *0.04*	0.07 *0.03*
EGCG + MD + GA	0.49 *0.02*	0.49 *0.04*	0.58 *0.02*	0.35 *0.04*	0.46 *0.03*	0.61 *0.05*	0.09 *0.01*
EGCG-MD/GA	0.45 *0.03*	0.37 *0.04*	0.52 *0.05*	0.30 *0.05*	0.37 *0.06*	0.50 *0.04*	0.06 *0.03*

the variation in solvent properties and correct the measured diffusion values (Cabrita & Berger, 2001). The reference compounds should have chemical inertness, well resolved signals, negligible diffusion coefficient dependence on the solutes concentration and ideally presented already in the solution as either reference for chemical shifts or quantitative determination. Therefore, we found it useful to use TSP not only as chemical shift reference but also as an internal diffusion reference and all diffusion coefficients presented in Table 3 are given as a ratio D/D_{TSP} according procedure published before (Cabrita & Berger, 2001).

As it was mentioned above, MD and GA are complex structures that mainly consist of saccharide units connected in chains of variable length. Typically, EGCG is at least one half of the average size of MD and much less portion of those of GA. Therefore, it was found reasonable to compare the diffusion coefficient of EGCG, MD and GA in EGCG-MD/GA with that of the pure EGCG, MD/GA and both physical mixtures (EGCG + MD/GA and EGCG + MD + GA).

Special care was taken to perform all experiments under identical conditions of concentration and temperature. Self-diffusion coefficients (D) of EGCG molecules present in EGCG-MD/GA, EGCG + MD/GA and EGCG + MD + GA were determined from the resonance signals of H2″ and H6″ protons of gallate moiety (at 7.03 ppm). The corresponding values of *D* for MD and GA were determined from the resonance signals of the anomeric protons at 5.40 (MD) and 5.23 (GA) ppm, saccharide hydroxymethine protons at 4.15–3.50, 3.40 and 3.30 ppm, and methyl protons of rhamnose moieties at 1.27 ppm. The relative diffusion coefficients to TSP (D/D_{TSP}) and the calculated standard deviations are presented in Table 3.

The D/D_{TSP} for MD and GA in EGCG-MD/GA and MD/GA were found to be similar but slightly lower than the corresponding values in the physical mixtures (EGCG + MD/GA and EGCG + MD + GA). The higher D/D_{TSP} values determined for the components of MD and GA in EGCG + MD/GA and EGCG + MD + GA can be a result of EGCG initiated distortion of the branched polysaccharide structures of MD/GA. The decreased diffusion of MD and GA in EGCG-MD/GA is an indication for intermolecular interactions between EGCG and these species in the loaded nanoparticles.

The relative diffusion coefficient of the pure EGCG was found to be significantly higher than the corresponding values in the EGCG-MD/GA, EGCG + MD/GA and EGCG + MD + GA formulations. This was attributed mainly to the increased viscosity of the solution in the presence of MD and GA. However, the corresponding D/D_{TSP} values of EGCG were lower in EGCG-MD/GA than the corresponding values in both physical mixtures that reproduce the composition of EGCG-MD/GA. This reduction is a strong indication of an interaction between EGCG and the polysaccharide matrix. Typical DOSY spectrum of EGCG-MD/GA is shown in Fig. 4.

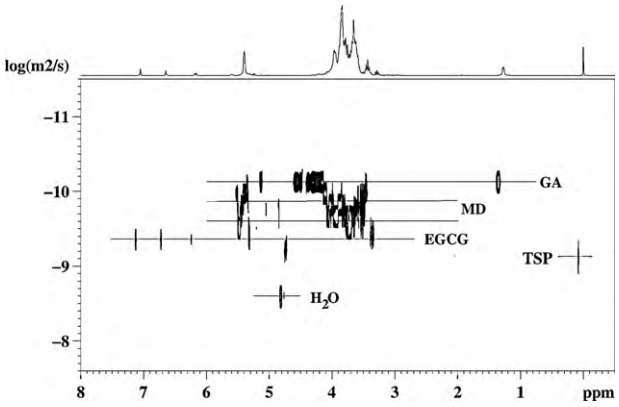

Fig. 4. 400.15 MHz 2D DOSY spectra of EGCG-MD/GA in D_2O, at 30 °C.

3.4. Dynamic light scattering (DLS)

The hydrodynamic diameters of MD/GA and EGCG-MD/GA determined by DLS revealed a bimodal size distribution by intensity as depicted in Fig. 5. Stable dispersions were obtained due to the nanoparticle high negative zeta potential (−36 ± 6 mV). The size distributions were almost similar for both the samples. EGCG-MD/GA have mean diameters of 40 and 400 nm and MD/GA show mean diameters of 60 and 400 nm. A bigger intensity ratio observed by DLS between big and small nanoparticles for EGCG-MD/GA, can explain the results of DOSY that show lower diffusion coefficients measured for the EGCG-loaded nanoparticles when compared to unloaded ones (see Section 3.3).

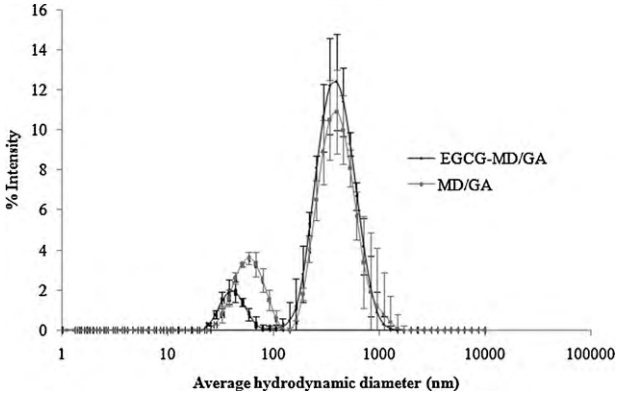

Fig. 5. Average hydrodynamic diameter distribution of EGCG-loaded (EGCG-MD/GA) and unloaded nanoparticles (MD/GA). Average value ± standard deviation, number of experiments = 3.

4. Conclusion

NMR spectroscopy has been applied to study the structure and composition of EGCG-MD/GA polysaccharide nanoparticles. Diffusion ordered NMR spectroscopy combined with spin-lattice relaxation time determinations provided valuable information on the structure and intermolecular interactions of EGCG-MD/GA.

The decreased translation diffusivity and spin-lattice relaxation times (T_1) of the species presented in the EGCG-MD/GA suggest the entrapment of EGCG in the polysaccharide matrix and predict the potential of these vehicles for sustained delivery/release of EGCG. These results are supported by the significant changes observed for the dynamic parameters (diffusivity and spin-lattice relaxation times) of EGCG.

The present study shows how a synergistic combination of NMR spectroscopic techniques can be used to characterize the structural behaviour of complex nano-scaled intermolecular aggregates and better understand this EGCG polymer based vehicle.

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