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Detection of Iron Nanoparticles using Nuclear Magnetic Resonance Relaxometry and Inverse Laplace Transform

Seong Min Kim*

Department of Bioindustrial Machinery Engineering College of Agriculture and Life Sciences, Chonbuk National University, 567 Baekje-daero, Deokjin-gu, Jeonju-si 561-756, Republic of Korea

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Abstract

Purpose: Rapid detection of bacteria is very important in agricultural and food industries to prevent many foodborne illnesses. The objective of this study was to develop a portable nuclear magnetic resonance (NMR)-based system to detect foodborne pathogens (E. coli). This study was focused on developing a method to detect low concentrations of magnetic nanoparticles using NMR techniques. **Methods:** NMR relaxometry was performed to examine the NMR properties of iron nanoparticle mixtures with different concentrations by using a 1 T permanent magnet magnetic resonance imaging system. Exponential curve fitting (ECF) and inverse Laplace transform (ILT) methods were used to estimate the NMR relaxation time constants, T_1 and T_2, of guar gum solutions with different iron nanoparticle concentrations (0, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} M). **Results:** The ECF and ILT methods did not show much difference in these values. Analysis of the NMR relaxation data showed that the ILT method is comparable to the classical ECF method and is more sensitive to the presence of iron nanoparticles. This study also showed that the spin-spin relaxation time constants acquired by a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence are more useful for determining the concentration of iron nanoparticle solutions comparwith the spin-lattice relaxation time constants acquired by an inversion recovery pulse sequence. **Conclusions:** We conclude that NMR relaxometry that utilizes CPMG pulse sequence and ILT analysis is more suitable for detecting foodborne pathogens bound to magnetic nanoparticles in agricultural and food products than using inversion recovery pulse sequence and ECF analysis.

Keywords: Carr-Purcell-Meiboom-Gill, Inverse Laplace transform, Inversion recovery, Iron nanoparticle, NMR relaxometry

Introduction

The nuclear magnetic resonance (NMR) technique is uniquely suited to the measurements of chemical composition, rheological properties, particle size, and imaging of internal structure (Callaghan, 1991; McCarthy, 1994; Gadian, 1995). Because NMR relaxometry is noninvasive and nondestructive, it is widely used in quality evaluation of agricultural and food products. NMR relaxation time constant estimation using the exponential curve fitting (ECF) method was used to evaluate the quality of agricultural and food products (Kim and Kim, 2004; Kim and McCarthy, 2010). Inverse Laplace transform (ILT) is also another good alternative for calculating relaxation time constants of heterogeneous materials (Moldovan et al., 2010; Silva et al., 2012; Berman et al., 2013; Xu et al., 2014).

Rapid detection of bacteria is very important in agricultural and food industries to prevent many foodborne illnesses, To separate and detect foodborne bacteria from a dilute sample containing various background materials is very challenging. The immunoassay utilizing the binding of antigens to antibodies is one of the most popular methods. Recent researches show that immunomagnetic separation technique utilizing the antibody-coated magnetic particles is very useful in separation and detection of target pathogens (Sung et al., 2013).

The NMR technique can be used to detect foodborne

***Corresponding author:** Seong Min Kim
Tel: +82-63-270-2617; **Fax:** +82-63-270-2620
E-mail: smkim@jbnu.ac.kr

bacteria such as *E. coli*. When target pathogens are bound to magnetic nanoparticles, they easily form soluble nanoscale clusters, which lead to a corresponding decrease in relaxation time of the sample containing the target pathogens (Lee et al., 2008).

This study focused on developing a method to detect low concentration of magnetic nanoparticles. Several NMR experiments were performed to examine the NMR properties of mixtures with different concentrations of iron nanoparticles. ECF and ILT methods were used to estimate the NMR relaxation time constants, T_1 and T_2.

Materials and Methods

Samples

To determine the NMR properties of iron nanoparticles with diameter less than 50 nm (Iron (II, III) oxide, Sigma Aldrich), a set of samples with iron concentrations of 0, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} M was prepared. The iron particles were mixed with 0.7% guar gum solution to prepare samples.

NMR system

NMR signals were acquired using a magnetic resonance imaging system (M10, Aspect Imaging, Israel) with a 1 T permanent magnet, which is installed in the Department of Food Science and Technology, University of California at Davis. A 60 mm inner diameter solenoid coil was used for the NMR measurements. A sample holder was used to place the samples in the middle of the homogeneous magnetic field of the NMR coil.

Relaxation process

Because NMR methods are nondestructive, safe, and provide information about the physical properties of a sample, they are potential tools for sorting and grading agricultural products. The relaxation process is one such NMR method. Following an external excitation, the signal induced in the receiver coil decays exponentially with respect to time, which is called relaxation. Relaxation causes the spins to return to their equilibrium energy levels by releasing their surplus energy to the surroundings. Once the equilibrium conditions are attained, there is no further change unless the system is disturbed. Relaxation process can generally be used to determine the moisture content of a sample.

Relaxation can be described using two separate mechanisms. One is longitudinal relaxation (spin-lattice or T_1 relaxation), and the other is transverse relaxation (spin-spin or T_2 relaxation). Longitudinal relaxation can be expressed as the following equation:

$$M(t)_{lg} = M_{lg}^{0}(1 - e^{-t/T_1}) \quad (1)$$

where $M(t)_{lg}$ is the magnetic moment at time t, M_{lg}^{0} is the initial amount of longitudinal magnetization, and T_1 is the spin-lattice relaxation time constant and is equal to the time that the longitudinal magnetization, $M(t)_{lg}$, takes to decay to 67% of the initial longitudinal magnetization, M_{lg}^{0}. Transverse relaxation can be described as:

$$M(t)_{tr} = M_{tr}^{0}\, e^{-t/T_2^*} \quad (2)$$

where $M(t)_{tr}$ is the magnetic moment at time t, M_{tr}^{0} is the initial amount of transverse magnetization, and T_2* is the spin-spin relaxation time constant and is equal to the time that the transverse magnetization, $M(t)_{tr}$, takes to decay to 37% of the initial transverse magnetization, M_{tr}^{0}.

In a proton NMR test, these relaxation constants are influenced by the molecular correlation times, state of water (i.e., percentage of free and bound water), amount of water in a sample, strength of external magnetic field, and temperature.

NMR measurements and data analysis

The NMR measurements were performed three times on each sample. The inversion recovery pulse sequence was used to measure the spin-lattice time constant (T_1). Forty inversion times were set from 0.00105 ms to 15,096 ms. The Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence was used to measure the spin-spin time constant (T_2). The echo time (TE) was set to 1 ms, and 12,077 data points were acquired. The NMR relaxation time constants, T_1 and T_2, were calculated using a poly-exponential curve fitting method (Kim and McCarthy, 2010) and an inverse Laplace transform from the acquired NMR signals. Figure 1 and Figure 2 show the NMR measurement and data analysis of one sample of guar gum solution. Figure 1 shows the snap shot image of data measurement and analysis for inversion recovery signal and exponential curve fitting (left) and the T_1 distribution calculated using inverse Laplace transform. Figure 2 shows a snap shot image of data measurement and analysis for CPMG signal and exponential

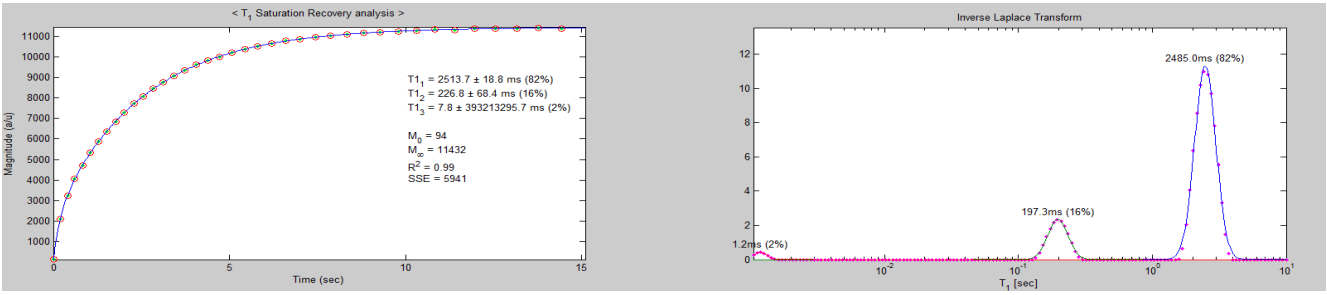


Figure 1. Snap shot image for inversion recovery signal (o) and exponential curve fitting (-) (left), and T_1 distribution calculated using inverse Laplace transform (right) of a sample of guar gum solution.

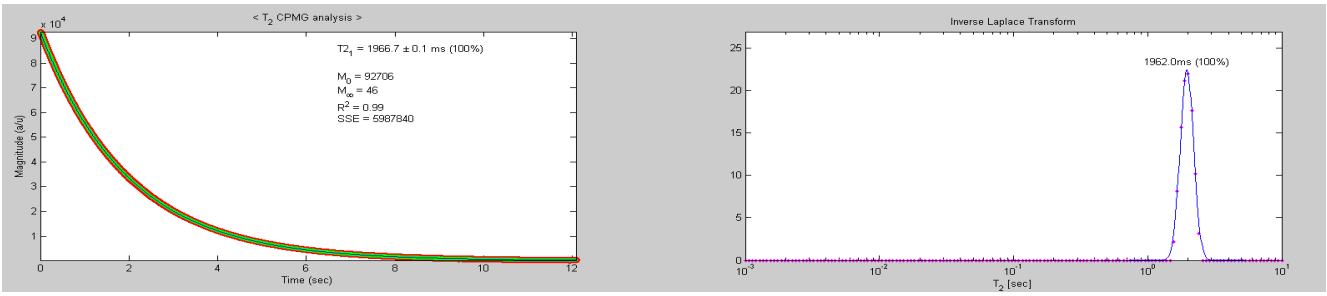


Figure 2. Snap shot image for CPMG signal (o) and exponential curve fitting (-) (left) and T_2 distribution calculated using inverse Laplace transform (right) of a sample of guar gum solution.

curve fitting (left) and the T_2 distribution calculated using inverse Laplace transform (right). The acquired NMR signals were processed using a commercial high level programming language (MATLAB R2013b, Mathworks Inc., USA).

Results and Discussion

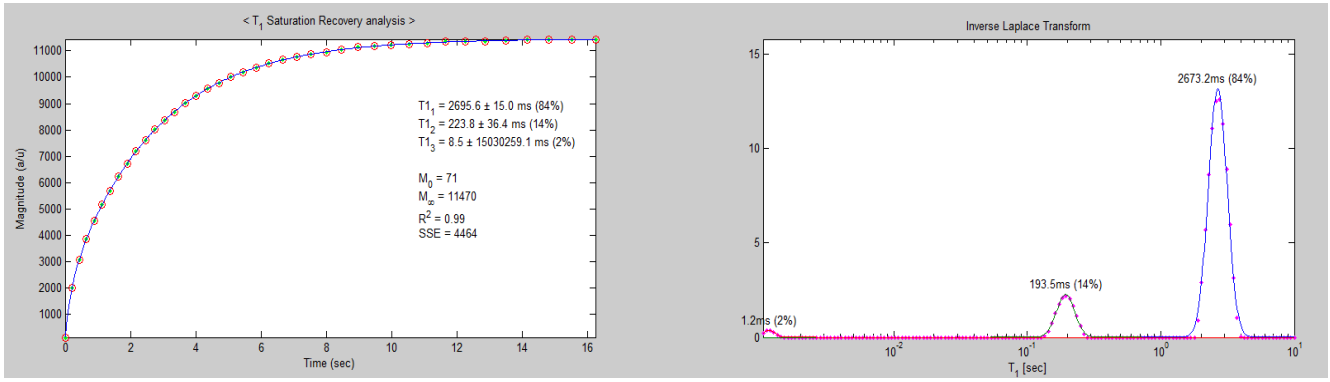
NMR measurement

When we use an inversion recovery pulse sequence to measure T_1, it is important to set a sufficiently long inversion time so that the inversion recovery signal attains the equilibrium state and reaches the maximum signal in the later part of the acquired signal. In Figure 1, we can see that the signal attains equilibrium state at an inversion time of approximately 12 seconds. In this experiment, we set the maximum inversion time to 15,096 ms, which was sufficient for the analysis.

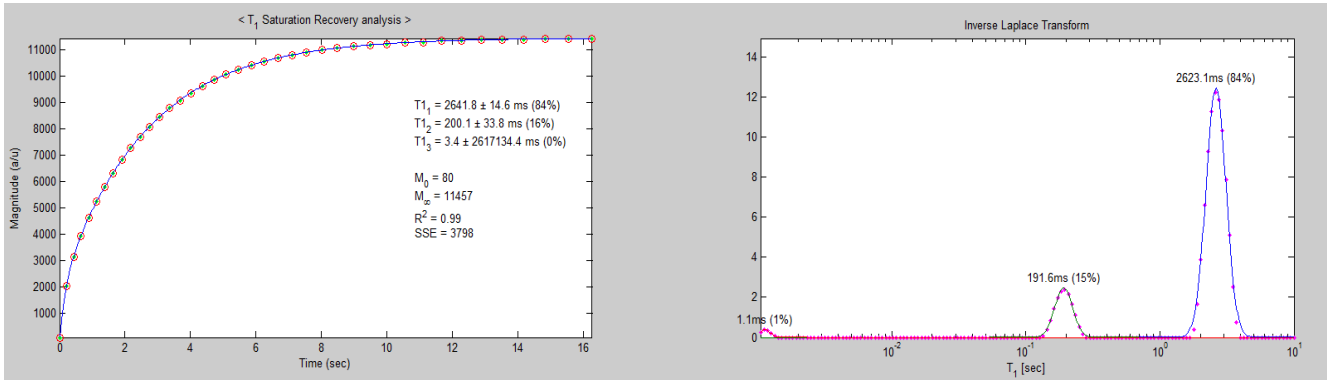
Similarly, when using a CPMG pulse sequence to measure T_2, it is important to set a sufficiently long data acquisition time so that the signal attains the equilibrium state and reaches the minimum signal, in the later part of the acquired signal. In Figure 2, we can see that the signal reaches the equilibrium state when the data acquisition time is approximately 10 seconds. In this experiment, the maximum data acquisition time was set to 12,077 ms, which was sufficient for the analysis. However, long inversion and data acquisition times lead to an increase in the experimental time. Therefore, choosing appropriate times is very important in the first stage of NMR relaxometry.

Exponential curve fitting analysis

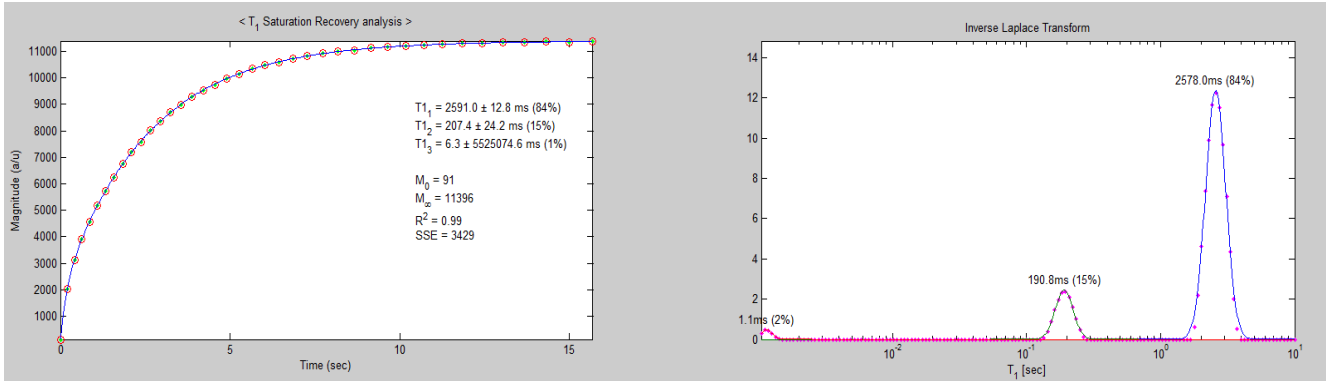
Figure 3 and Figure 4 show the snap shot images of T_1 and T_2 for different iron particle concentrations. The determination coefficient of exponential curve fitting is 0.99 for both the time constant measurements. It means that the acquired data accurately describes the state of samples and that the instrument was stable throughout the experiments. Table 1 shows the average values of relaxation time constants calculated using the ECF and ILT methods. Spin-lattice relaxation (T_1) analysis showed that there are three phases of water state. The values estimated using ECF are higher than those estimated using ILT. The spin-spin relaxation (T_2) analysis showed that there are two phases of water state when the iron particle concentration is higher than 1×10^{-4} M. Similar to T_1, the values estimated using the ECF method are higher than those estimated using the ILT method. The relaxation time constants of mixtures without iron particles were expected to be longer than those of the mixtures with iron particles,



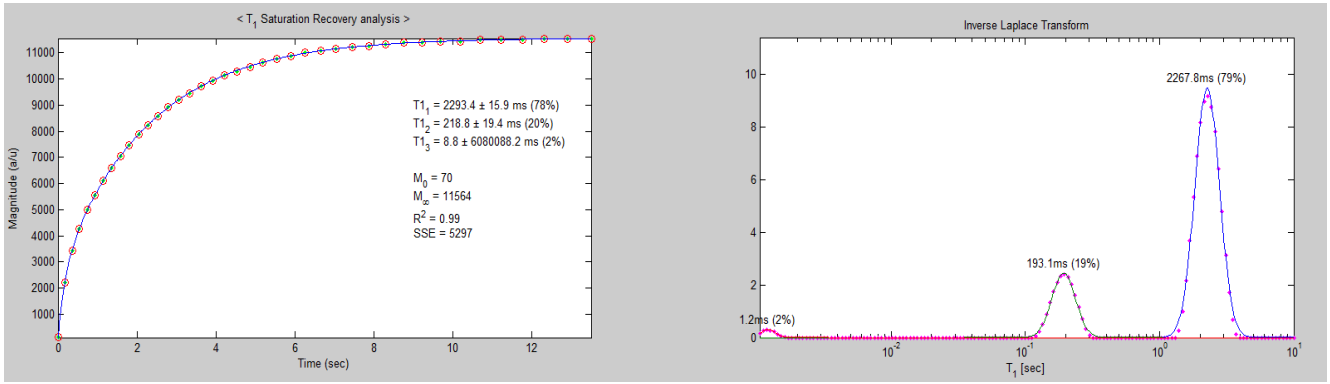
(a)



(b)



(c)



(d)

Figure 3. Snap shot images for T_1 analysis of mixtures with different iron particle concentration of 1×10^{-7} (a), 1×10^{-5} (b), 1×10^{-4} (c), and 1×10^{-3} M (d).

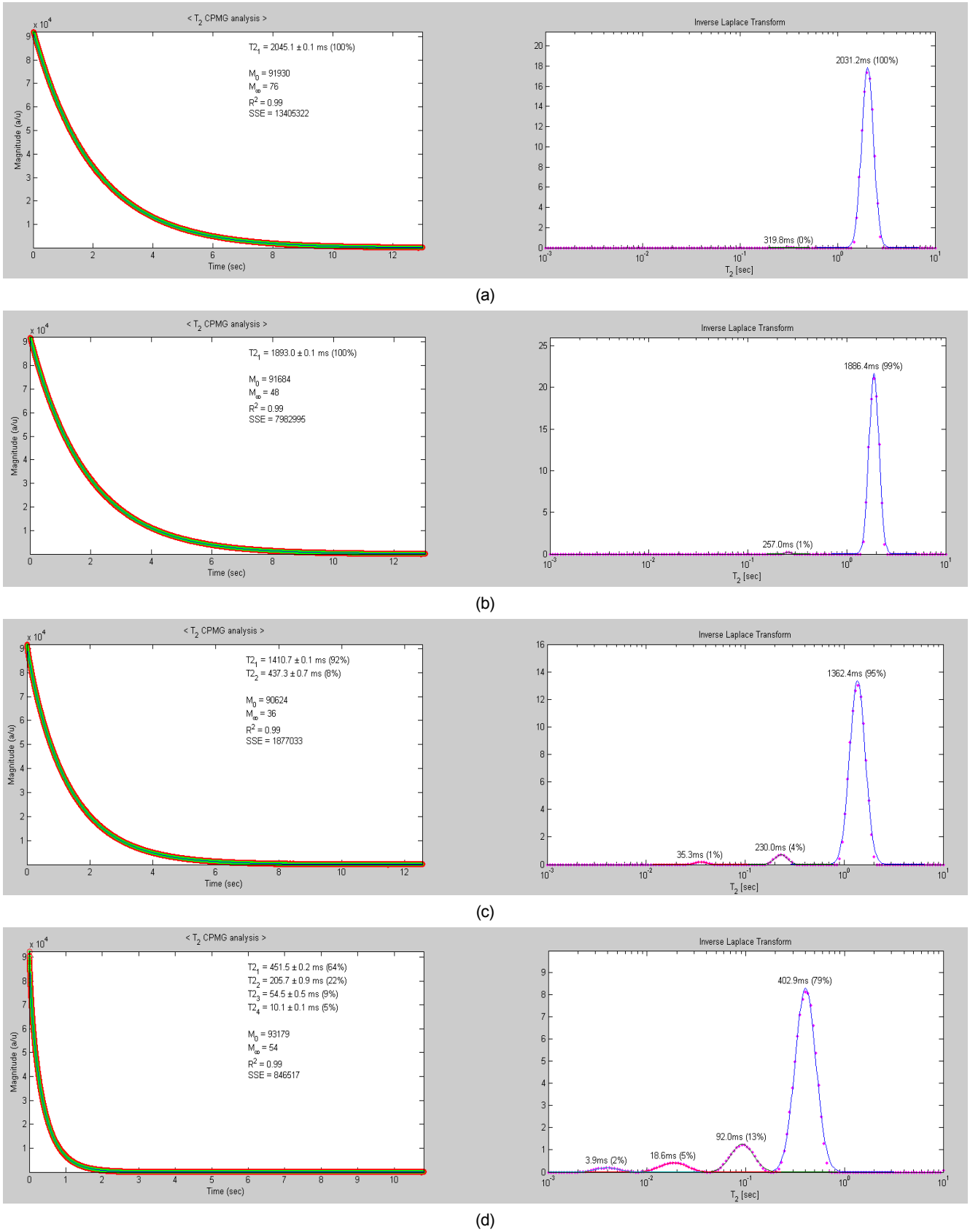


Figure 4. Snap shot images for T_2 analysis of mixtures with different iron particle concentration of 1×10^{-7} (a), 1×10^{-5} (b), 1×10^{-4} (c), and 1×10^{-3} M (d).

because of magnetic field inhomogeneity and the heterogeneity of the samples. However, this was observed to be different when the iron particle concentration was 1×10^{-7} M. Thus, further research is required to investigate this phenomenon. The estimated values of the major component of spin-spin time constants (T_{21}) are found to decrease with increasing concentration of iron particles (Figure 6). Thus, the feasibility of this approach in detecting microbes bound to magnetic nanoparticles is proven. However, the change in estimated spin-lattice time constants (T_{11}) with increasing iron particle concentration is not obvious (Figure 5).

Inverse laplace transform analysis

In Figure 3 and Figure 4, the ILT analysis displays the distribution of the average peak values of relaxation time constants. Spin-lattice relaxation (T_1) analysis shows that there are three phases of water state in all samples and there is no obvious change in the distribution with changing iron particle concentration. However, spin-spin relaxation (T_2) analysis shows two phases of water state on adding the iron particles, even though the portion is very small and the distribution is clearly changing according to the iron particle concentration variation. Thus, the feasibility of ILT approach in detecting microbes bound to magnetic nanoparticles is proved. In Table 1, the ILT analysis shows the average peak values of relaxation time constants. The T_1 values estimated using ILT are lower than the values estimated using ECF. However, the values calculated using ECF and ILT do not significantly differ. The change in estimated spin-lattice time constants (T_{11}) with increasing

Table 1. Average values of relaxation time constants calculated using exponential curve fitting and inverse Laplace transform. Each value represents the mean ± standard deviation of three replicates

Concentration of iron (mole)	Method	Spin-lattice time constant (ms)			Spin-spin time constant (ms)		
		T_{11}	T_{12}	T_{13}	T_{21}	T_{22}	T_{23}
0	ECF[1]	2684±208	226±14	7.5±1.6	2046±98.2	-	-
	ILT[2]	2648±185	192±5.2	1.2±0.0	2037±96.4		-
1×10^{-7}	ECF	2723±203	213±9.5	4.3±3.8	2081±84.8	-	-
	ILT	2705±208	195±3.2	1.2±0.1	2071±83.5	280.3±11.8	-
1×10^{-6}	ECF	2675±231	199±7.0	2.5±3.3	2072±84.3	-	-
	ILT	2660±229	193±2.6	1.1±0.0	2063±83.1	256.1±5.7	-
1×10^{-5}	ECF	2729±267	206±5.6	6.3±2.6	1941±71.2	-	-
	ILT	2703±279	188±7.9	1.1±0.1	1931±71.6	219.8±19.6	-
1×10^{-4}	ECF	2645±210	218±15	6.8±4.4	1434±83.1	394.8±59.3	-
	ILT	2624±208	190±6.6	1.3±0.2	1387±72.7	219.9±15.8	37.7±3.3
1×10^{-3}	ECF	2225±141	215±4.4	5.1±4.5	401±57.2	160.5±62.7	38.6±23.1
	ILT	2194±150	195±3.2	1.2±0.0	361±44.1	79.7±17.8	14.4±7.3

[1]ECF = Exponential curve fitting
[2]ILT = Inverse Laplace transform

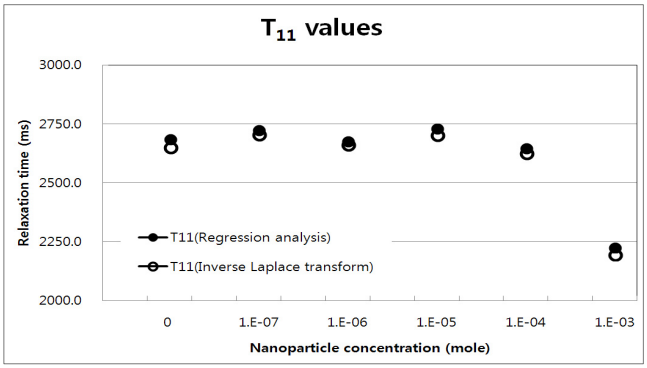


Figure 5. Average values of major spin-lattice relaxation time constants (T_{11}) of mixtures with different iron particle concentrations calculated using exponential regression analysis and inverse Laplace transform.

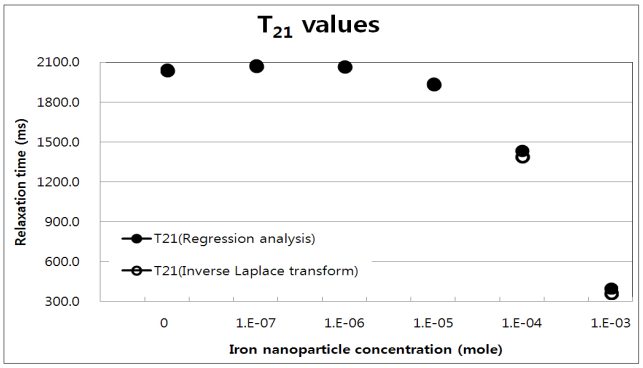


Figure 6. Average values of major spin-spin relaxation times (T_{21}) of samples with different iron concentrations calculated using exponential regression analysis and inverse Laplace transform.

iron particle concentration is not as evident as that in the ECF analysis (Figure 3 and Figure 5). Unlike the ECF analysis, T_2 analysis show two phases of water state, even when the concentration of iron particle was 1×10^{-7} M (Table 1 and Figure 4). This indicates the feasibility of ILT in detecting small amounts of iron particles in heterogeneous mixtures. This result can be utilized to detect bacteria bound to antibody-coated magnetic particles. Analysis of the NMR relaxation data shows that the ILT method is comparable to the ECF method. In addition, ILT is found to be more feasible in detecting the presence of iron nanoparticles than ECF.

Conclusions

This study focused on developing a method to detect low concentrations of magnetic nanoparticles using NMR techniques. NMR relaxometry was performed to examine the NMR properties of iron nanoparticle mixtures with different concentrations. Exponential curve fitting (ECF) and inverse Laplace transform (ILT) methods were used to estimate the NMR relaxation time constants, T_1 and T_2, of guar gum solutions with different iron nanoparticle concentrations. The values calculated using ECF and ILT do not differ significantly, implying that ILT is a comparable method to the classical ECF method. Moreover, the analysis of the NMR relaxation data shows that the ILT method is more sensitive to the presence of iron nanoparticles. This study showed that the variation in spin-spin relaxation time constants acquired using CPMG pulse sequence with variation in iron nanoparticle concentration in a gel mixture is larger than that of spin-lattice relaxation time constants acquired using inversion recovery pulse sequence. In conclusion, NMR relaxometry using CPMG pulse sequence and ILT analysis is more suitable for detecting foodborne pathogens bound to magnetic nanoparticles than using inversion recovery pulse sequence and ECF analysis.

Further studies for the development of a portable NMR system for detecting bacteria bound to magnetic nanoparticles will be performed in future.

Conflict of Interest

No potential conflicts of interest relevant to this study exist.

Acknowledgments

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