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$\mathcal{R}$: a Quantitative Measure of NMR Signal Receiving Efficiency

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Abstract

Recognizing that the sensitivity of NMR is influenced by factors such as conductance and dielectric constant of the sample, we propose the receiving efficiency $\mathcal{R}$ to characterize how efficiently the NMR signal can be observed from a unit transverse magnetization in a sample under optimal probe tuning and matching conditions. Conveniently, the relative receiving efficiency can be defined as the ratio of the NMR signal induced by a unit transverse magnetization in a sample of interest and a reference solution. Based on the reciprocal relationship between excitation and observation in NMR, the relative receiving efficiency can be correlated with the 90° pulse length (τ_{90}). In the special case of perfect probe tuning (impedance matched to 50 Ω), $\mathcal{R}$ is inversely proportional to τ_{90} . Application of the NMR receiving efficiency in quantitative analysis potentially enables a single external concentration reference for almost any sample, eliminating the need to know its exact chemical composition or detailed electromagnetic properties.

Keywords

NMR; receiving efficiency; 90° pulse length; probe tuning and matching; concentration reference

Introduction

Modern NMR is a robust quantitative and reproducible method with the output signal amplitude linearly proportional to the concentration for all concentration ranges. In a simple case, it has been demonstrated that such a linear relationship can be easily extended from 100 M (in the proton concentration) to less than 10 μ M. [1] The accuracy and precision of NMR have allowed determinations of site-specific ¹³C isotope content. [2] Additionally, NMR can be a universal quantification method, because almost all organic and biological molecules and many interesting inorganic compounds readily contain protons or other nuclei observable by NMR.

However, significant challenges still exist for accurate quantitation by NMR. One of the biggest issues is that the same signal source (typically an analyte proton) may produce NMR signals of difference sizes under different conditions. Those conditions include the solution composition, excitation angle, NMR tube size, sample volume, and other geometrical or

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experimental parameters. Without stringent control, signal size-based quantitation can be subject to large errors.

Hence quantitation by NMR is best performed using an accurate internal concentration standard. We recently demonstrated that protonated NMR solvents are excellent candidates for primary concentration standards, as an alternative to preparing a known reference and adding it to the solution of interest. [1] In cases of constant sample load, the ERETIC signal was suggested as an artificial reference without resorting to any physical internal concentration standard. [3] However, such approaches are limited by the assumption of a known solvent reference (or another added reference) or the ability to maintain constant sample load and probe tuning. The introduction of internal standards that may interact with analytes of interest or other sample components such as macromolecules is also a concern for a number of sample matrices.

An alternative approach is to recognize and analyze how the NMR signal amplitude is influenced by the aforementioned factors. With good laboratory practice, it is not difficult at all to control geometrical and experimental parameters so that they do not introduce errors larger than 0.5%. Thus sample load becomes the main influencing factor that is frequently beyond the operator's control. In theory, it is possible to calculate the change in sample load based on the knowledge of solution dielectric constant and conductivity. In reality, such an approach is tedious, inaccurate and not cost-effective.

Based on the principle of reciprocity, the efficiency in NMR signal detection is related to the efficiency of excitation.[4-7] Previously, quality factor (Q) and pulse length based methods have been proposed for NMR concentration determination. [8,9] Nevertheless, these methods may fail when the condition of perfect probe tuning (i.e., impedance matching to 50 Ω) is not met, due to either insufficient efforts by the operator or high sample conductance. Moreover, the application of reciprocity is only an approximation as it has been realized that NMR excitation and receiving circuits actually differ slightly (Fig 1b). [See for example, references 10 and 11] In fact, it has been argued that the probe is best tuned by reception. [12]

For quantitative analysis, we propose the definition of the NMR receiving efficiency $\mathcal{R}$ to characterize how efficiently a unit magnetization can be detected by an NMR spectrometer under the best probe tuning condition. The best tuning condition refers to tuning and matching the probe for minimal RF reflection at the observe frequency in the excitation circuit (Fig 1b). Furthermore, we argue that $\mathcal{R}$ can be correlated to the 90° pulse length for a set of fixed spectrometer parameters and conditions. Hence it is possible to use a *single external concentration reference for almost any realistic sample* once this correlation is pre-calibrated.

Experimental

We used a set of samples containing 7.20 mM NaOAc and varying amount of NaCl in 1.8% D₂O solution. Their preparation has been described elsewhere. [1] An additional sample containing approximately 0.3 M Na₂HPO₄ was also prepared (supplementary table 1).

500 μ l sample solutions were loaded into 5 mm NMR tubes (528-PP, Wilmad-Lab Glass, Buena, NJ) separately. Each sample was shimmed and the probe was tuned to the water resonance and matched for minimal reflected RF power. Receiver gain was kept constant throughout. For ease of operation, all proton pulses were centered on the water resonance. Nominal 90° pulse lengths were determined with constant transmitter power attenuation for all samples.[18]

One dimensional proton spectra were acquired on either a Bruker Avance 800 MHz or 500 MHz NMR spectrometer equipped with a 5 mm inverse single z-axis gradient probe operating at 25°C. The proton sweep width was 10 ppm and the FID (Free induction Decay) consisted

of 16 k complex data points. On the 800 MHz spectrometer, the acetate proton signal was observed after a 90° pulse excitation. The inter-scan delay was set to more than 60 s, which included a 4 s pre-saturation pulse (50 Hz RF field or slightly weaker). Three measurements were conducted at week 1, 2 and 7. On the 500 MHz spectrometer, three measurements were conducted within one day and the water proton was detected after a 2.25° pulse excitation with an inter-scan delay of 14 s. The slight difference in proton concentrations among different solutions was taken into account in receiving efficiency determination. [1,16]

All FID's were exponentially line-broadened by 0.1 Hz (800 MHz) or 0.3 Hz (500 MHz), Fourier transformed, manually phased and baseline corrected. The acetate proton signal (800 MHz) was integrated over ± 0.1 ppm and the water signal (500 MHz) over ± 1 ppm from the peak center.

Theory

We use the symbol $\mathcal{R}$ to represent the NMR receiving efficiency under fixed experimental conditions (including temperature, receiver gain and *best probe tuning*). $\mathcal{R}$ is analogous to the extinction coefficient used in UV-visible spectroscopy, and the sample volume V is analogous to the optical path length. If we consider all influential factors, the NMR signal amplitude (or more specifically, peak integral) A can be expressed as

$$A = A_0 * g(RG) * \mathcal{R} * c * V * \sin(\theta) * I(\theta) \quad (1)$$

In this equation, A_0 is an instrument constant, $g(RG)$ is the receiver gain function that describes the gain of FID amplitude through the receiver (which is solely dependent on the receiver gain parameter (RG) for a given spectrometer; see Fig 1a), c is the concentration of the observed nucleus or is proportional to the concentration of the transverse magnetization prior to detection (in case of insufficient relaxation delay and/or variable temperatures), θ is the excitation angle, and $I(\theta)$ is an inhomogeneity factor that describes the effect of the RF field distribution over the active sample volume V .

For simplicity, we can keep RG , θ , and V constant. Then the NMR signal amplitude normalized by $\mathcal{R}$ is directly proportional to the concentration. This forms the foundation of concentration determination for any interesting sample by a single external concentration reference, regardless of the analyte solution type or salt concentration.

In practice, it is beneficial to use the relative receiving efficiency $\mathcal{R}_r$, which is calculated as the signal size of unit magnetization normalized by that of a reference state

$$\mathcal{R}_r = \mathcal{R} / \mathcal{R}_{\text{ref}} = \frac{A/c}{A_{\text{ref}}/c_{\text{ref}}} \quad (2)$$

Such a reference state can be represented by a sample of minimal load (resistance R_s is close to zero in Fig 1b) and its receiving efficiency is 1. Essentially, the relative receiving efficiency $\mathcal{R}_r$ quantitatively characterizes how the NMR signal amplitude is influenced by the sample load, which is mainly contributed by the solution's dielectric constant and conductance. [13, 14] Hereafter, the NMR receiving efficiency refers to the relative receiving efficiency $\mathcal{R}_r$ unless otherwise stated.

For a modern commercial high resolution probe of single saddle coil design, pulse excitation and signal detection are reciprocal under a first order approximation. Hence a strong correlation between $\mathcal{R}$ and the 90° pulse length (τ_{90}) is expected. We would further argue that such a correlation can be empirically established and is highly reproducible under the best probe tuning condition.

The best probe tuning condition is necessary because both the pulse length and NMR signal amplitude depend on the probe tuning: a poorly tuned probe tends to have longer pulse lengths and small NMR signal amplitude and thus poorer sensitivity. In theory, if the probe is tuned for minimal RF reflection, then the sample load eventually determines the values of the tuning capacitor C_T and matching capacitor C_M (all other probe circuit components are assumed to remain constant). In some probe circuits, the interactive nature between C_T and C_M may add uncertainties to their experimental determinations. [15] However, the best probe tuning condition does guarantee that the RF reflection coefficient $(Z-50)/(Z+50)$ is as close to zero as possible (Z is the overall impedance of the probe circuit shown in Fig 1b). Then the NMR receiving efficiency can be regarded as an empirical function of τ_{90} :

$$\mathcal{R}=f(\tau_{90}) \quad (3)$$

In the special case of perfect probe tuning ($Z = 50 \Omega$ at the resonance frequency),

$$(\mathcal{R} * \tau_{90})|_{50\Omega} \propto 1/V_t \quad (4)$$

or

$$\mathcal{R}|_{50\Omega}=(\tau_{90})_{\text{ref}}/\tau_{90} \quad (5)$$

where V_t is the total transmitter voltage during excitation (Fig 1b).

If a sample is non-conductive, the probe can be typically tuned to 50Ω and V_t is a constant. For a highly conductive or salty sample, the probe circuit impedance (Z) may not be matched to 50Ω and V_t may become Z dependent. Nevertheless, as long as Z can be maintained constant and the spectrometer has stable performance characteristics (and without probe arcing), it is safe to assume that V_t in eq. (4) does not change. In other words, the product of the receiving efficiency and τ_{90} is expected to be a constant at or near a certain probe impedance value.

We choose the best probe tuning condition by the excitation for several considerations. First, it conforms to general good NMR practice and is easy to implement. Second, the sensitivity remains the same when the probe is tuned by excitation and reception, if the receiver gain is sufficient.[12] Third, the 90° degree pulse and signal amplitude can be reproduced with ease for the same sample.

We will demonstrate by experimental data and probe circuit simulations that the definition of $\mathcal{R}$ is meaningful and feasible, and its correlation with τ_{90} can be easily established for a given spectrometer.

Results

Because the acetate (or solvent) concentrations and relevant data acquisition parameters remained the same, $\mathcal{R}$ for all other samples or measurements could be simplified as the normalized signal size ratio according to eq.(2). Complete lists of $\mathcal{R}$ and τ_{90} can be found in the supplementary materials. In Figure 2, $\mathcal{R}$ is plotted against the corresponding $1/\tau_{90}$ for the 800 MHz (Fig 2a) and 500 MHz (Fig 2b) spectrometers. The solid line in each sub-plot connects one set of experimental data (black circles). Subsequent measurements conducted either 1 and 6 weeks later (for 800 MHz) or within the same day (for 500 MHz) gave $\mathcal{R}$ - τ_{90} data points (red squares and green diamonds) that are positioned on or very near the previously determined data (within 3% of expected $\mathcal{R}$ according to any measured τ_{90}), indicating that the correlation between $\mathcal{R}$ and τ_{90} not only can be determined but also is highly reproducible over reasonable periods of time.

For the same sample, the values of τ_{90} are shown to vary up to several percent (supplementary tables 2 & 3) among different measurements. Especially for the 800 MHz spectrometer, the τ_{90} 's at week 7 are consistently about 3 to 7% longer than those at week 1. Nevertheless, the product of τ_{90} and $\mathcal{R}$ remains constant for any given sample in this study, suggesting the NMR signal size corrected by $\mathcal{R}$ is a better representation of the concentration.

For quantitative analysis, we argue that the signal size should be weighted by a factor (receiving efficiency) that takes τ_{90} into account. This approach is especially useful when analyzing samples (i.e. bio-fluids) containing varying amount of electrolytes. In the current set of examples, if the 90° pulse length (or the receiving efficiency) difference is not considered, the concentration determination for the highly conductive sample (1.6 M NaCl) using the salt free external reference would easily lead to an underestimation of the proton concentration by more than 50%.

Overall, an inverse relationship between τ_{90} and signal size (or $\mathcal{R}$) can be seen in Fig. 2: larger τ_{90} corresponds to diminished $\mathcal{R}$. Under perfect probe tuning and matching conditions, such a relationship becomes strictly quantitative as eq. (5) suggests. The dashed lines in Fig. 2 correspond to the calculated $\mathcal{R}$ by eq. (5) under the assumption that it can be extended to all samples (pulse lengths). As expected, when the NaCl concentration is below 200 mM, the probe can be matched to 50 Ω . The dashed lines overlap the solid ones very well. On the other hand, when the NaCl concentration is higher than 200 mM, the probe circuit cannot be tuned to 50 Ω due to the limited range of the matching capacitor. A gradual deviation for the dashed curves is observed as the salt concentration increases. It is interesting to note that the measured $\mathcal{R}$'s are lower than eq. (5) suggests at 500 MHz and higher at 800 MHz. Presumably, the root cause is that the V_t term in eq.(4) is no longer a constant when the probe cannot be matched to 50 Ω . This observation is in disagreement with the literature suggestion that eq. (5) leads to lower concentration estimation for salty samples.[9] In our opinion, the establishment of the correlation between $\mathcal{R}$ and τ_{90} allows a more accurate concentration determination for a far wider range of samples (up to 1.6 M NaCl equivalent in the current study).

The utility of such a correlation can be further demonstrated by another sample containing a different salt (about 0.3 M Na₂HPO₄). While the total probe resistance $R_0 + R_s$ (Fig 1b) can be calculated from the solution's conductivity so that $\mathcal{R}$ can be determined by eq.(5) [17], such a sophisticated approach is not necessary. Instead, the receiving efficiency can be interpolated as 0.60 (800 MHz) and 0.62 (500 MHz) from the corresponding τ_{90} values (Fig 2 triangles). Both numbers are in excellent agreement with the experimentally determined averages of 0.60 (800 MHz) and 0.62 (500 MHz) (see supplementary tables 2 & 3). Hence a single external concentration reference is possible for almost any realistic NMR sample, and it is not necessary to know all the details concerning solution composition or electromagnetic properties.

Discussion

For the most popular commercial NMR probe circuits, the tuning and matching capacitors are not orthogonal: slight deviation of the probe circuit impedance from $50\ \Omega$ due to under / over tuning can be largely compensated by over / under matching and vice versa. [15] As such, even for the same sample (or constant $R_0 + R_s$ in Fig 1b), the actual positions of the tuning and matching capacitors may not be reproduced exactly every time. The question arises as to how much error can be caused by the probe tuning uncertainty when the same sample is measured multiple times. Consequently, the correlation between $\mathcal{R}$ and the 90° pulse length may become unstable in practice if τ_{90} cannot be reliably measured. Based on practical experience, we know that τ_{90} can be highly reproducible for the same sample. Furthermore, we can conduct probe circuit simulations and demonstrate that uncertainties in tuning and matching do not prevent reliable measurements of $\mathcal{R}$ and τ_{90} either, as long as the probe is tuned and matched for minimal RF reflection.

For simplicity, we will consider a representative equivalent probe circuit shown in Fig 1b with C_T parallel to L and C_M serial to the transmitter / receiver (other circuit types can be simulated similarly leading to the same conclusion). Typical circuit parameters are $L = 41\ \text{nH}$, $R_0 = 0.2\ \Omega$, and the resonance frequency is 500 MHz. For a non-conductive sample, R_s can be approximated as 0. Then $50\ \Omega$ impedance matching condition requires that tuning and matching capacitance as $C_T = 2.32\ \text{pF}$ and $C_M = 0.156\ \text{pF}$. In our experience, it is quite easy to achieve zero or near zero RF reflection with careful probe tuning and matching, though the mutual compensative interaction between C_T and C_M may cause some uncertainties in their exact values (Fig 3a blue region). Nevertheless, the overall impedance has to be very close to $50\ \Omega$. As a result, the power source (transmitter) voltage V_i in eq.(4) can be safely treated as a constant. Furthermore, the 90° pulse length variation (as a percentage deviation from matched condition) is well within 1% (Fig 3b blue region). The dark blue region in Fig 3b is much larger than that in Fig 3a, suggesting that the 90° pulse length is much more tolerant to the uncertainties in C_T and C_M . Similarly, the NMR signal detected by the receiver is expected to be highly reproducible too.

In the case of a highly conductive sample, the probe circuit may not be matched to $50\ \Omega$ due to increased sample load (R_s in Fig. 1b) and the limited adjustable range of the matching capacitor. While a conductive sample can introduce additional capacitance, the equivalent probe circuit shown in Fig 1b can still apply. For convenience, we can fix the matching capacitor to its limit before setting out to find the equivalent values for C_T , C_M , L and R_s at 500 MHz in Fig 1b. First, L remains as a constant, because the any realistic sample causes minimal change in magnetic permeability (or relative permeability can be treated as or very close to zero). R_s can be extrapolated from its dependence on conductivity (or NaCl concentration in the current study), the observed 90° pulse length, and R_0 . C_M can be estimated by tuning C_T to a nearby frequency at which RF reflection is about zero (we observed 497 MHz in our set-up). Those values are very similar to those calculated for the 200 mM NaCl sample ($R_0 = 0.2\ \Omega$ and $R_s = 0.22\ \Omega$), as we also observed little tuning or matching capacitor position changes for samples of higher conductivity under the best tuning conditions. Hence, the initial simulation parameters for the 0.6 M NaCl solution sample on our 500 MHz spectrometer are: $L = 41\ \text{nH}$, $R_0 = 0.2\ \Omega$, $R_s = 0.66\ \Omega$, $C_M = 0.225\ \text{pF}$ and $C_T = 2.25\ \text{pF}$.

The solid line in Fig. 4 is the calculated wobb RF reflection near the resonance frequency, which resembles the experimentally observed curve. The dashed line in the same figure represents the calculated 90° pulse length normalized by that of the load-free probe. Over a rather wide frequency range (from 499.9 to 500.1 MHz), the variation among calculated τ_{90} values is less than 1% from the median. Though the conductive sample does not allow probe impedance matching to $50\ \Omega$, the 90° pulse length can be reproduced with ease. Based on the

principle of reciprocity, the receiving efficiency can be treated as a constant near the resonance frequency as well.

The above two simulations explain why a stable pulse length and its correlation to the receiving efficiency can be readily reproduced over time. In our experience, up to 2% in τ_{90} variation can be observed for the same sample when two independent measurements are made back-to-back. The best tuning condition, however, guarantees that the probe circuit impedance Z approaches an optimal value such that the product of relative receiving efficiency and 90° pulse length remains a constant for any realistic sample load. We have obtained plots very similar to Fig 2 for other brands of NMR systems, including a Varian Inova 600 with an inverse probe, a Bruker Avance 500 with an inverse cryoprobe as well as another conventional inverse probe.

The concept of the NMR receiving efficiency and the determination of its dependence on τ_{90} enable accurate quantitative analysis without introduction of any additional internal or artificial standard. Since $\mathcal{R}$ only concerns the interaction between the transverse magnetization and the receiver, it is applicable in multidimensional NMR or when the signal is created in a non-conventional way. While the current study shows that the correlation between the 90° pulse length and the receiving efficiency can be established by a set of known standards, the latter can be calculated on the fly from a compound (such as the solvent) of a known or constant concentration for any interesting sample.

Nevertheless, it is imperative to remember that the experimentally determined receiving efficiency is not a “state function” (as used in thermodynamics) or intrinsic to a solution. Neither is it the exact NMR equivalent of extinction coefficient in UV spectroscopy. Instead, $\mathcal{R}$ depends on both internal factors such as sample compositions and external ones including the sample geometry, the probe's electromagnetic properties, and consistent performance of the transmitter and receiver. While the extinction coefficient of an analyte is fixed for a given sample, it cannot be accurately predicted with ease in different samples. On the other hand, the receiving efficiency in NMR may not be directly transferable from one spectrometer to another, even for the same sample. Fortunately, for most applications including concentration determinations, the combination of probe and transmitter/receiver is typically fixed. Hence a pre-calibrated $\mathcal{R}$ is possible and useful.

Other practical factors influencing the determination of $\mathcal{R}$ include uncertainties in reproducing the best tuning and matching conditions, variation in the NMR tube size and integration errors. We measured and confirmed that the high quality NMR tubes used in this study did not contribute to more than a 0.5% active volume variation from the mean. Because of the potential variation in inner or outer tube diameters, we would argue that utilization of a co-axial insert to introduce an internal reference in the interesting sample (that is then placed in the outer tube) may lead to higher volume variations (percentage-wise). Additionally, the inner tube may have a different receiving efficiency from the outer tube, due to the RF inhomogeneity in the transverse plane. [19]

Concentration determination through the use of $\mathcal{R}$ is complementary to an internal solvent reference method that we recently developed. [1] While the solvent reference method is very tolerant to probe tuning / matching, it frequently requires the knowledge of the solvent concentration and pulse angles. In the current method, the only requirement is to reach minimal RF reflection and careful calibration of the 90° pulse length. Modern high resolution spectrometers are now capable of automatic probe tuning and pulse calibrations, and hence concentration determination can be done with greater ease.

Conclusions

We have proposed the use of the receiving efficiency $\mathcal{R}$ to characterize how efficiently the NMR signal can be observed from a unit transverse magnetization under the best probe tuning and matching conditions. It quantitatively reflects the influence of sample load over the observed signal size. For all practical purposes, the relative receiving efficiency can be defined and obtained reproducibly from a set of known standards. We have demonstrated with experimental data and simulations that a simple correlation between $\mathcal{R}$ and the 90° pulse length can be reliably measured for samples containing low to modestly high concentrations of electrolytes. Benefits of using the NMR receiving efficiency approach outlined here include robust and accurate determinations of compound concentration in solutions of which we have limited knowledge, based on a single external reference. The quantification error by the τ_{90} -dependent $\mathcal{R}$ can be limited to be about 2% or better under favorable conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

a) A schematic flow chart of pulse excitation and signal observation in modern high resolution NMR; **b)** The equivalent probe circuit for a typical excitation and detection. The transmitter and receiver are separated by PIN-diodes of small however finite resistance. Under the best probe tuning condition, the probe circuit impedance Z (short dashed enclosure) is tuned so that $(Z-50)/(Z+50)$ approaches zero. The probe's RF coil can be represented by an inductance (L), load-free resistance (R_0) and sample load (R_s). The matching network (solid enclosure) includes tunable tuning (C_T) and matching (C_M) capacitors. For simulations shown in Figures 3 & 4, C_T is assumed to be parallel to L and C_M is assumed to be in a serial arrangement with respect to the transmitter / receiver.

Figure 2.

The proton NMR proton receiving efficiency $\mathcal{R}$ can be correlated to the 90° pulse length for an **a)** 800 MHz and **b)** 500 MHz NMR spectrometer. The short dashed lines are the calculated $\mathcal{R}$ under the assumption that eq. (5) can be extended to long τ_{90} 's. Experimental $\mathcal{R}$ was determined according to eq. (2), with sample 1 in measurement 1 used as the reference. The solid black lines simply connect the experimental data (black circles) from measurement 1 without smoothing. $\mathcal{R}$ values are highly reproducible as repeat measurements (green diamonds and red squares) fall right on or very near to the solid lines with a maximal deviation less than 3%. Additionally, $\mathcal{R}$ for sample 13 can be readily interpolated from the corresponding curve

as 0.60 (open triangles) for **a**) and 0.62 for **b**), which are in excellent agreement with the expected (supplementary tables 2 & 3).

Figure 3.

a) Simulated wobb RF reflection when the tuning and matching capacitors (C_T and C_M) were perturbed near the impedance match condition ($50\ \Omega$) for a typical RF circuit shown in Fig 1b. The sample load is minimal ($R_s = 0$). **b)** 90° pulse length deviation from the shortest value. Up to 0.8% and 1.2% changes for the optimum values are allowed for C_T and C_M . As long as RF reflection can be maintained at less than 1%, essentially the same pulse length can be obtained. Similarly, the same NMR signal amplitude can be expected. Hence, both τ_{90} and $\mathcal{R}$ measurements can be reproduced with ease for the same low load sample on a given spectrometer. Simulation parameters: $C_T = 2.32\ \text{pF}$, $C_M = 0.156\ \text{pF}$, $L = 41\ \text{nH}$, $R_0 + R_s = 0.2\ \Omega$.

Figure 4.

Simulated wobb RF reflection (blue solid line) and 90° pulse length (red dashed line; normalized by that of the load free sample) at 500 MHz for a typical RF circuit shown in Fig 1b with high sample load (0.6 M NaCl). The uncertainty in C_T roughly corresponds to the frequency shift for minimal RF reflection. The matching capacitor reaches its adjustable limit due to the high salt content. Between 499.9 MHz and 500.1 MHz, both the RF reflection and 90° pulse length essentially remain constant (less than 1% variation). Simulation parameters: $C_T = 2.25$ pF, $C_M = 0.225$ pF, $L = 41$ nH, $R_0 + R_s = 0.86 \Omega$.