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Research Article

Determination of the Total Circulating Blood Volume using Magnetic Particle Spectroscopy

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Abstract

The knowledge of the patient's total circulating blood volume is essential in clinical routine. Due to its extend volume and its dispersion throughout the body a direct measurement seems unfeasible. A variety of methods have been studied over the past decades, but only few of them result in accurate total circulating blood volume determination. The most promising approaches are based on the dilution measurement of applied tracers. In this work we present a method for measuring the total circulating blood volume based on Magnetic Particle Spectroscopy. The presented method consists of three major steps. First, from the signal-to-noise ratio estimation of the undiluted tracer and a chosen minimum accuracy of the total circulating blood volume determination combined with a rough estimation of it, a bolus volume for injection is calculated. This step has to be performed only once per used tracer. In a second step, the bolus is injected into the subject. Last, a small amount of blood is taken after a mixing time. From the measured concentration, the total circulating blood volume is calculated with at minimum the prior given accuracy from step one.

I. Introduction

The knowledge of the patient's total blood volume is essential in clinical routine. A variety of methods have been studied over the past decades but due to its extend volume and dispersion throughout the body only few of them result in accurate total circulating blood volume determination. Nuclear medicine techniques, most likely the dual-isotope blood volume method, are the gold standard, but are time consuming and suffer from high technical demand [1, 2].

The existing methods are commonly based on the tracer dilution theory [3] as a direct measurement of the total circulating blood volume seems unfeasible. In this approach a well-known tracer volume is injected in the vascular system and after complete mixing with the blood a sample is taken and measured. From the tracer dilution, the total circulating blood volume is determined. This measurement can be done for example with a radioisotope blood product as red blood cells, albumin, dye like Evans blue, or Indocyanine green [1]. Recent work also presented a method using a magnetic resonance imaging (MRI) contrast agent combined with a MRI concentration measurement based on T1 changes of the blood [4].

For an accurate measurement, it is of essential importance that the used indicator is a sufficient blood pool agent, not leaving the blood vessels during the procedure and ideally not taken up by the macrophages of the reticuloendothelial system (RES) in the timeframe of the measurement. As this is the case for tagged blood products like red blood cells, there are only a few tracers known to match this requirement. On the other hand, an accurate concentration measurement of the used indicator is necessary. In this work, we present a method for measuring the total circulating blood volume based on Magnetic Particle Spectroscopy (MPS). MPS is a sensitive tool to monitor the magnetization response of a particle system to an applied alternating magnetic field. In case of superparamagnetic nanoparticles, this response is in general nonlinear to the applied field, leading to harmonic distortion. The measured signal is a spectral answer of the particle system to the excitation field consisting of the magnetization at the excitation frequency and its odd harmonics (if no offset field is present). In general from the amplitude and phase of all spectral lines the magnetization curve of the particle system can be reconstructed. Only those particles and materials with a nonlinear response can generate signal on frequencies other than the excitation frequency. Using appropriate suppression or filter techniques of the excitation field combined with a sensitive detector, the higher harmonics can be detected with a large dynamic range and high sensitivity. Suitable magnetic nanoparticles show a strong nonlinear behavior even for low excitation fields, still providing a high magnetic moment. Despite the nonlinear magnetization response, the signal amplitude is highly linear to the concentration and virtually background free, as those nanoparticles are absent in the body.

Clinical approved particles suitable for this measurement have been established for other modalities in the past. Feraheme® (ferumoxytol), as an example for a blood pool agent consisting of superparamagnetic nanoparticles, has been developed for enhanced iron uptake of the body [5]. Other particle suspensions like Resovist® were also used as an MRI contrast agent optimized for liver imaging [6].

Since Gleich und Weizenecker have shown a setup to use these nanoparticles for imaging, known as magnetic particle imaging, a vast number of new particles have been developed with a great variety of properties [7]. In general these particles consist of a small magnetic core with a biocompatible coating. These properties can be tailored for the desired application.

The presented method consists of three major steps. First, from the signal-to-noise ratio (SNR) estimation of the undiluted tracer and a chosen minimum accuracy of the blood volume determination combined with a rough estimation of it, a bolus volume for injection is calculated. This step has to be performed only once per used tracer. In a second step, the bolus is injected. Last, a small amount of blood is taken after a mixing time. From the measured concentration, the total circulating blood volume is calculated with at minimum the prior given accuracy.

II. Methods

II.I. Theory

The basic concept used for analysis of the total circulating blood volume is the indicator dilution technique. This method is based on a quantitative determination of the particle concentration of the dilution of a well-known indicator. Injecting a well-known particle volume to an unknown blood pool causes a composition of blood and particles. By sampling and analyzing the concentration change it is possible to calculate the active blood volume, as the quotient of the concentrations behaves proportional to the quotient of the active pool volume and the volume of the given bolus. As the measured signal is linear with the concentration for convenience the determination of the concentration can be replaced by measured signal intensities. Additionally this can be corrected for a more precise description of the measurement with the volume of the bolus and the sampled volume. It is possible to determine the blood volume directly by applying the following relation:

$$V_{\text{blood}} = \frac{S_1}{S_2} V_{\text{bolus}} - V_{\text{bolus}}\,. \tag{1}$$

Consequently, the total circulating blood volume V_{blood} results of the quotient of the signals given by the injected particle concentration S_1 and by the diluted particle concentration S_2 multiplied by the added particle volume V_{bolus}. Obviously, the given volume V_{bolus} does not belong to the total circulating blood volume and has to be subtracted. To calculate an accurate blood volume after the procedure one has to add the injected volume of tracers and to subtract the sampled volume. If these volumes are equal and/or preferable small compared to the total blood volume they can be neglected. Additionally, it is also possible to sample a small amount of blood before injecting particles in order to investigate the influence of individual blood on the determination of particle concentration, depending on the used particles. This sample of known volume can be mixed with a well-known amount of tracers and quickly measured. This control measurement, analog to the blood volume determination, can be used for correcting this influence. For particles with a significant dependence on the individual blood composition this step is obligatory, otherwise it is optional.

By analyzing the signal-noise-ratio of the injected solution in advance, appreciating the expected SNR of the diluted sample, it is possible to estimate the expected

measurement uncertainty of the total circulating blood volume determination for a given injected tracer volume and a given blood pool volume. A sufficient (over-)estimation of the blood volume of the subject allows minimizing the amount of used particles but still guaranteeing a sufficient accuracy.

II.II. Measurements

Magnetic Particle Spectroscopy is based on the nonlinear magnetization response of superparamagnetic iron oxide nanoparticles. During the measurement, the particles are exposed to a time varying, sinusoidal magnetic field and the magnetization of the particles is recorded. The nonlinear response of the magnetic nanoparticles is detected as higher harmonics of the excitation frequency with amplitude proportional to the particle concentration. In general, the third harmonic exhibits the best SNR, due to the symmetric signal no even harmonics are present. In this study all MPS measurements were performed with a commercially available MPS spectrometer (MPS unit, Pure Devices GmbH, Würzburg, Germany). The measurement volume used was 50 μl.

As a suitable particle suspension, FeraSpin™ R (nanoPET Pharma GmbH, Berlin, Germany) was chosen, but can be replaced with other particles and the results can easily be transferred. FeraSpin™ R consists of the identical iron oxide core particles and coating material as Resovist®, but is specifically formulated for preclinical imaging in small animals. Therefore, the composition and iron concentration of FeraSpin™ R differs from that of Resovist®. The hydrodynamic diameter of the particles is 60 nm and the blood half-life in rat is about 15 min. Regarding the MPS signal, it was shown that FeraSpin™ R exhibits a comparable harmonic spectrum as Resovist® [8]. For a prolonged blood half-life a product from the FeraSpin™ Series, which are narrowly size-distributed particles of identical composition extracted from FeraSpin™ R, can be used. FeraSpin™ XS, with a hydrodynamic diameter of 15 nm, exhibits already a blood half-life of about 30 min in rat. It should be noted that with decreasing particle size the MPS signal also decreases. Therefore, a reasonable compromise between blood half-life and MPS signal should be found depending on the individual application. The iron concentration was 0.5 mmol/ml, which is the typical clinical concentration of Resovist® [9].

The ability of MPS spectroscopy to deliver a highly linear signal to the particle concentrations was shown by measuring a dilution series.

MPS signal in general depends on the excitation frequency, for low frequencies the particles can rotate mechanically, called Brown relaxation, for high frequencies only the magnetization of the magnetic core changes its direction, called Néel relaxation. As in the Brownian regime the signal depends on mechanical parameters like the viscosity, a measurement frequency far above is preferable [10, 11]. A frequency of 20 kHz was chosen, which is clearly in the Néel regime for the used particles. The excitation field strength was set to 20 mT, sufficient for a nonlinear response of the particles. The signal was detected in all measurements on the third harmonic frequency.

As the viscosity and other mechanical parameters of the individual blood are not known in advance, a high excitation frequency is preferable. To simulate blood with various viscosities, which is a major parameter for signal alterations in the Brownian regime, we measured the signal from various glycerin concentration from 0 % to 80 %, covering a range of dynamic viscosities from 0.0007 Ns/m^2 to 0.035 Ns/m^2, containing the same amount of particles to check that there is no dependency on the measured signal. This covers typical dynamic viscosities of blood ranging from approximately 0.003 Ns/m^2 to 0.004 Ns/m^2 [12].

The first step of the proposed procedure is to measure the signal and the SNR of an undiluted sample. The signal amplitude is used for the calculation of the total circulating blood volume and the SNR for calculating the maximal allowed dilution still delivering a sufficient SNR in the diluted samples delivering the necessary accuracy. The SNR achieved in this measurement was 4504.0 using 100 averages. To ensure a correct preparation of the 50 μl volume, the samples were checked with an analytical scale unless otherwise noted.

According to Wolfensohn et. al. typical female rat with a body weight ranging from 250 g to 300 g, respectively a male rat with a body weight from 450 g to 520 g, has a blood volume of 54 ml/kg weight to 70 ml/kg weight, resulting in a total blood volume from 13.5 ml to 36.4 ml typically [13]. To ensure the coverage of all typical total circulating blood volumes for each gender, male and female, the smallest blood volume was calculated using the smallest weight and blood volume per kg weight, the largest respectively by using the largest body weight and largest blood volume per kg weight. To cover the full range of animals this is completed by the blood volume using the averaged blood volume per kg weight and the minimum, averaged and maximum body weight. The resulting maximum blood volume is used as estimation for the bolus calculation.

The bolus amount was calculated based on the SNR of the undiluted particle solution measurement and a given accuracy of 3 %, respectively this means a minimum SNR of 33.33 in the diluted samples. Therefore the maximal dilution factor of the bolus is in this case 135.1. The estimated blood volume for this calculation was assumed to be 21 ml for female rats and 36.4 ml for male rats, covering generously all typical animals. From this the bolus for the female rat model was set to 156.5 μl, representing a dilution factor of 134.2, and for the male

rat model to 271.1 μl, representing a dilution factor of 134.3. As in general the total blood volume is unknown in advance it is necessary to estimate the largest possible blood volume. If the value is estimated too large, this will result in a larger bolus, but on the other hand in a lower uncertainty, if estimated to low, the bolus given is less, but the result will have a higher uncertainty, which may result in the necessity to repeat the measurement in the worst case. In this case, the total blood volume is roughly known for the recalculation of the correct bolus.

The bolus injection and volume determination was performed for 10 fluid volumes and is shown in Tab. 1, covering typical female and male rats. Five of these samples cover the typical range of total blood volume of female rats, another five the typical range of male rats. From these measurements, the total fluid volumes were determined from the measured signal intensities by using Eq. (1). The measured volumes are compared to the given volume. The bolus amount given is also stated in Tab. 1.

Additionally a variety of available particle solutions was compared concerning its SNR of the undiluted substance. This data allows one to assess the necessary amount in advance or to compare other spectrometers or particles for suitability.

Table 1: Weight and total blood volume of typical female and male rats.

Female rat model					
Weight [g]	250	250	275	300	300
Blood volume [ml]	13.50	15.50	17.05	18.60	21.00
Bolus [μl]	156.5	156.5	156.5	156.5	156.5

Male rat model					
Weight [g]	450	450	485	520	520
Blood volume [ml]	24.30	27.90	30.07	32.24	36.40
Bolus [μl]	271.1	271.1	271.1	271.1	271.1

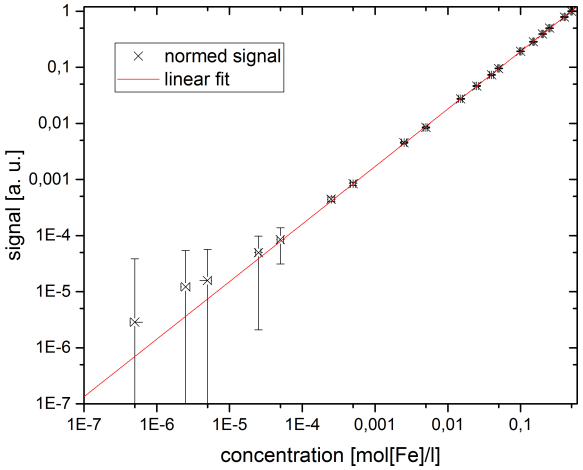

Figure 1: Measured Signal of the third harmonic versus the concentration of FeraSpin™ R. The error bars represent both the measurement uncertainty and a pipette error of 2 %.

The results are shown in Fig. 2. The measured signal shows no significant signal change based on the viscosity.

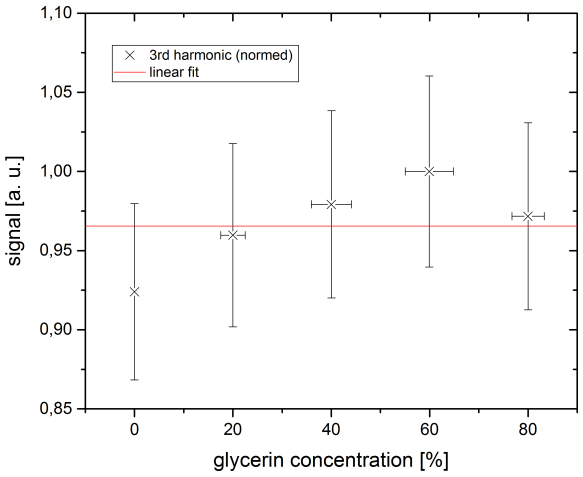

Figure 2: Measured signal for the same amount of particle solution mixed with different glycerin/water solutions resulting in a glycerin content from 0 % to 80 %.

III. Results

To demonstrate the linear signal dependence of the concentration for the used particles, the acquired signal was recorded for a wide range of particle concentrations resulting in a linear correlation of the third harmonic signal and the concentration. The results are shown in Fig. 1.

To simulate different blood viscosities, the particles were mixed with different glycerin concentrations ranging from 0 % to 80 % glycerin. In all cases, 20 % of the measured solution was diluted particle suspension. The error bars cover all steps of pipetting the substances with a pipette error of 2 %, which is in this case the dominating source of error.

For the total circulating blood volume determination ten different amounts of water were prepared, representing typical blood volumes of female and male rats as given in Tab. 1. The corresponding bolus of particle solution was injected and after mixing a small amount of 50 μl was removed and measured in the magnetic particle spectrometer.

The calculated blood volume and given blood volume show a good agreement with $r > 0.999$ from the linear fit (Fig. 3). The error bars represent the uncertainty of the prepared volume and of the calculated blood volume based on the measurement uncertainty given by the spectrometer. In this measurements the amount of the bolus was fixed to the maximum possible total blood volume,

as this is the case for a priori unknown total blood volumes. The relative uncertainty, as listed in Tab. 2, for the female rat samples varied from 2 % (for smaller blood volume) to 3 % (for the largest female sample). Similar for male rat (see Tab. 2) it varied from 2 % to 2.8 % for the four of five samples with the lower weight, the largest sample leads to an uncertainty of 4 %, even though 3 % were expected. The lower uncertainty for the smaller total blood volumes result from the lower dilution of the bolus, as it is obvious that measurement SNR increase with higher concentrations.

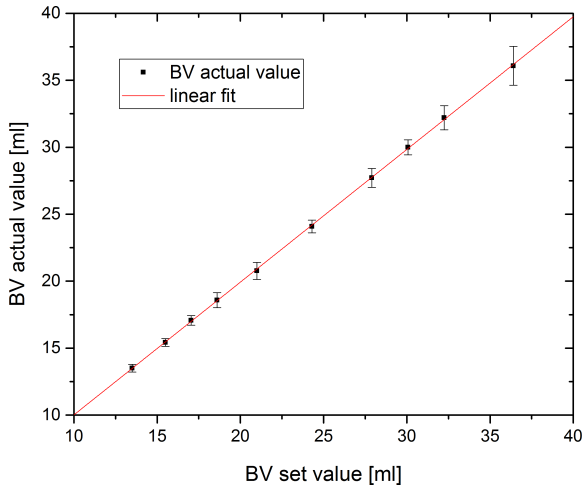

Figure 3: The measured total circulating blood volume (BV actual value) versus the given total circulating blood volume (BV set value). The linear fit is shown in red.

Table 2: Measured total blood volume and resulting measurement uncertainty.

Female rat model		Male rat model	
BV actual value [ml]	**Measurement uncertainty [%]**	**BV actual value [ml]**	**Measurement uncertainty [%]**
13.5	2.2	24.1	2.0
15.4	1.9	27.7	2.6
17.1	2.1	30.0	1.9
18.6	3.1	32.2	2.8
20.8	3.1	36.1	4.0

The SNR of other undiluted tracer material of a variety of iron oxide nanoparticles is given in Tab. 3. The measured SNR is scaled to the iron content of the particle suspension. The SNR from FeraSpin™ R is set as reference to 100 %.

This data allows comparing the necessary bolus size for different available particle solutions. Ferucarbotran (DDM128N/511, Meito Sangyo Co. Ltd., Nagoya, Japan) and FeraSpin™ R are used frequently in MPI for its magnetic behavior similar to Resovist®. Feraheme shows a significant lower SNR, due its high amount of small particles, as it is an iron replacement product indicated for the treatment of iron deficiency in adult patient with chronic kidney disease and contains a large distribution of particle diameters.

Table 3: Achievable SNR (scaled to FeraSpin™ R) and particle sizes from a variety of iron oxide particles.

Particle	**Concentration [mg(Fe)/ml]**	**SNR (scaled to Feraspin™ R) [1/mg(Fe)]**
Feraspin™ R	27.9	100
DDM128N/511	55.9	78.6
Feraheme	30.0	26.7

IV. Discussion

Magnetic particle spectroscopy is a highly sensitive method to measure iron oxide nanoparticles. The measured signal is linear to the particle concentration, allowing measuring the dilution of these tracers.

The shown setup detects the tracer material virtually background free, similar to nuclear medicine techniques, which are the gold standard for the determination of the total circulating blood volume. Suitable tracers that stay in the blood pool for a sufficient time are available today. It has been shown that the exemplarily chosen Feraheme, as an available blood pool tracer, is less sensitive than specially designed MPI tracers, but data from other tracers like FeraSpin indicate that those particles stay sufficiently long enough in the blood pool. To overcome this, by the effort of taking multiple blood samples, the blood volume can be extrapolated from the measured decay of the tracers. Similar techniques are used in nuclear medicine methods as well [2].

The proposed procedure consists of two steps. First, the SNR of the undiluted tracer material is measured. From this, based on a given necessary accuracy of the total circulating blood volume, the amount of tracer material is calculated. In a second step the tracer material is injected as a bolus into the subject. Last, a small amount of blood is sampled after a mixing time and from the measured concentration the total circulating blood volume is calculated with at minimum the prior given accuracy from step one. The presented data showed in 9 of 10 cases an equal or higher accuracy than predicted, only in one case the accuracy was slightly lower.

Additionally it has to be noted that in principle signal from any harmonic can be chosen for data evaluation and that it is not limited to the third harmonic used in this work. The actual selection depends on the used spectrometer, measured data from the undiluted probe can show best SNR for other harmonics depending on the used hardware. It can clearly be noted that a higher SNR

reduces the amount of particles that are injected, which is beneficial. This SNR can be optimized by dedicated hardware or extend averaging. This may reduce the necessary amount of particles in the future.

The selection of the third harmonic was done as most MPS spectrometers do not allow the detection of the first harmonic. The used MPS is able to detect this. Especially smaller tracers, which typically stay longer in the blood pool, with their rapid decrease of higher harmonic signal can benefit from this. For Feraheme the achievable SNR is 533 % at the first harmonic compared to the third harmonic. Even though the first harmonic signal is detectable with an increased SNR, one should keep in mind, that the signal also includes the signal generated by dia- and paramagnetic material, which only generates signal on the first harmonic due to the absence of nonlinear magnetization response. Fortunately, the amplitude of this additional signal is orders of magnitude smaller compared to the particle signal, and in case of blood, one can correct for this difference.

The accuracy can be set by choosing the bolus amount in advance. The resulting uncertainty can be estimated from the achieved SNR from the undiluted tracer material measured in advance and the roughly overestimated blood volume.

Using an MPS excitation frequency high enough to reduce Brownian relaxation, typically for nanoparticles above a few kilohertz, the mechanical properties of the blood as viscosity do not alter the signal and therefore the signal is only a function of tracer amount.

Even though the target for this work was to demonstrate that the total circulating blood volume in rat can be detected in principle using MPS with a given accuracy, it is not limited to small animals. The same procedure can be applied in principle without change to patients. Especially in those cases where particles are administered for other reasons, e.g. Feraheme for iron uptake, it can be used to determine the total circulating blood volume simply by sampling blood after injection. If one administers 17 ml tracer material, which corresponds to the standard dose of Feraheme, one is able to measure the total circulating blood volume with an accuracy of 7.8 %. As this estimation is based on a maximum total circulating blood volume of 6 l, representing the maximum value found in male humans, in general the accuracy will be higher. Obviously, the amount of the injected bolus has to be adapted to the larger total circulating blood volume based on the necessary accuracy and the SNR measurement of the used particles, but the proposed procedure is still able to measure with a comparable accuracy to competing methods [1, 4]. The major advantage is the lack of radioactive tracers, but still providing a background free measurement setup with a linear dependency of the tracer signal and its concentration. As the used spectrometer is optimized for a measured volume of 50 μl, for use of this technique in humans this volume can be chosen larger, as sampled blood in general is not limited to this volume. This will increase the sensitivity of the measurement again.

The presented data shows a good agreement of the given and the measured total circulating blood volume model.

V. Conclusion

The presented method is suitable to measure the total circulating blood volume with a high accuracy. Suitable tracers are non-toxic, radiation free and can be measured virtually background free using magnetic particle spectroscopy.

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