DETERMINATION OF PERMEABILITY-SURFACE AREA PRODUCTS OF GD-DTPA AND PAMAM-TU-DTPA G = 4 MAGNETIC RESONANCE IMAGING CONTRAST AGENTS IN MAMMARY TUMORS

BY

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Abstract

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Michael Aref
Department of Nuclear, Plasma, and Radiological Engineering
University of Illinois at Urbana-Champaign, 2000
Dr. Erik Wiemer, Advisor

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Using the fitted parameters and equation of the concentration calibration curve we can convert tumor ROI signal intensities to contrast agent concentrations. The time dependent behavior of the contrast agent concentration in a tumor is governed by a two compartment model, composed of the plasma compartment and the extravascular extracellular space (EES) compartment. Following a bolus injection into the blood (i.e. the

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plasma compartment), the contrast agent is distributed into the EES compartment, essentially the leakage space of the normal and abnormal tissue of the body, or is excreted by the kidneys. In the model, influx and efflux is different in each region of interest, therefore there are different flow rates, for the same agent, in different tissues of the body. In the model, these flow rates are averaged for each compartment except for specific ROI being investigated, namely the mammary tumors. The ROI that we are interested in, the tumor compartment, is composed of volume fractions of the plasma and EES compartments.

The tumor compartment’s flow rate is related to the permeability-surface area product of that tumor. To find the flow rate, the data collected was fit by nonlinear least-squares data fitting by the Gauss-Newton method. The parameters of the plasma compartment were fitted (p < 0.001) for both agents and physiologically measurable values, such as the agent’s distribution half-life, excretion half-life, EES volume and plasma volume were calculated. The fitted plasma compartment values were substituted as constants in the tumor compartment equation. Then the EES volume fraction, $v_e$, of the tumor and the flow rate normalized to tumor volume, $K_{p*e}/V_T$, were fit for (p < 0.001).

For Gd-DTPA, $v_e = 9-13\%$ and $K_{p*e}/V_T = 0.01-0.06 \text{ min}^{-1}$ and for PAMAM-TU-DTPA $G = 4$, $v_e = 0.8-1\%$ and $K_{p*e}/V_T = 0.008-0.04 \text{ min}^{-1}$.

Our results show that the PAMAM-TU-DTPA $G = 4$ is better at differentiating tumor heterogeneity than the low molecular weight agent, Gd-DTPA. This is because the dendrimer agent is a blood pool agent, it will have the greatest contrast enhancement in the areas of greatest capillary density and the greatest permeability in regions of neovascularization. These areas of neovascularization are associated with angiogenesis which is induced by vascular endothelial growth factor (VEGF). High levels of VEGF in tumors is an indicator of metastatic potential. In this way macromolecular agents of differing size may be used as an indicator of metastasis or metastatic potential.
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- St. Francis Medical Center, Peoria, IL
- Department of Nuclear, Plasma, and Radiological Engineering, UIUC

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### List of Symbols

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<th>Meaning</th>
<th>Units</th>
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<tr>
<td>Cᵣ(t)</td>
<td>Concentration in the plasma compartment</td>
<td>mM</td>
</tr>
<tr>
<td>Cₑ(t)</td>
<td>Concentration in the extravascular extracellular space (EES) compartment</td>
<td>mM</td>
</tr>
<tr>
<td>Cₛ(t)</td>
<td>Concentration in the tumor compartment</td>
<td>mM</td>
</tr>
<tr>
<td>Kₑ→p</td>
<td>Flow rate per unit volume from the EES to the plasma compartment</td>
<td>L/kg•min</td>
</tr>
<tr>
<td>Kₑ→c</td>
<td>Flow rate per unit volume from the EES to the plasma compartment</td>
<td>L/kg•min</td>
</tr>
<tr>
<td>Kₑ→cₑ</td>
<td>Isodirectional flow rate per unit volume between the plasma and the EES compartments</td>
<td>L/kg•min</td>
</tr>
<tr>
<td>Kₑ→k</td>
<td>Flow rate per unit volume from the plasma compartment to the kidneys</td>
<td>L/kg•min</td>
</tr>
<tr>
<td>Kₑ→t</td>
<td>Flow rate per unit volume from the plasma to the tumor compartment</td>
<td>L/kg•min</td>
</tr>
<tr>
<td>Kₜ→p</td>
<td>Flow rate per unit volume from the tumor to the plasma compartment</td>
<td>L/kg•min</td>
</tr>
<tr>
<td>Kₑ→tₑ</td>
<td>Isodirectional flow rate per unit volume between the plasma and the tumor and the tumor compartments</td>
<td>L/kg•min</td>
</tr>
<tr>
<td>P</td>
<td>Permeability constant</td>
<td>m/min</td>
</tr>
<tr>
<td>S</td>
<td>Surface area per unit mass</td>
<td>m²/kg</td>
</tr>
<tr>
<td>Pₑ→Sₑₚ</td>
<td>Permeability surface area product per unit mass, transfer from the plasma to the tumor compartment</td>
<td>m³/kg•min</td>
</tr>
<tr>
<td>Pₑ→Sₑₚₚ</td>
<td>Permeability surface area product per unit mass, transfer from the plasma to the tumor compartment</td>
<td>m³/kg•min</td>
</tr>
<tr>
<td>Pₑ→Sₑₚₑ</td>
<td>Permeability surface area product per unit mass, isodirectional transfer between the plasma and the tumor compartments</td>
<td>m³/kg•min</td>
</tr>
<tr>
<td>Vₑ</td>
<td>EES volume (per unit mass of body)</td>
<td>L/kg</td>
</tr>
<tr>
<td>Vₑᵖ</td>
<td>Plasma volume (per unit mass of body)</td>
<td>L/kg</td>
</tr>
<tr>
<td>Vₑₜ</td>
<td>Tumor EES volume (per unit mass of tumor)</td>
<td>L/kg</td>
</tr>
<tr>
<td>Vₑₜ</td>
<td>Tumor volume (per unit mass of tumor) or inverse tumor density</td>
<td>L/kg</td>
</tr>
<tr>
<td>vₑ</td>
<td>EES volume fraction of the tumor volume</td>
<td></td>
</tr>
<tr>
<td>vₑᵖ</td>
<td>Plasma volume fraction of the tumor volume</td>
<td></td>
</tr>
<tr>
<td>Dₑ</td>
<td>Dose</td>
<td>mmole/kg</td>
</tr>
<tr>
<td>a₁,₂</td>
<td>Normalized concentration amplitudes for unit dose</td>
<td>kg/L</td>
</tr>
<tr>
<td>α</td>
<td>Distribution rate constant</td>
<td>1/min</td>
</tr>
<tr>
<td>β</td>
<td>Excretion rate constant</td>
<td>1/min</td>
</tr>
<tr>
<td>S/S₀</td>
<td>Normalized signal intensity</td>
<td>mM</td>
</tr>
<tr>
<td>[C]</td>
<td>Contrast agent concentration</td>
<td>radians</td>
</tr>
<tr>
<td>θ</td>
<td>Flip angle</td>
<td></td>
</tr>
<tr>
<td>TR</td>
<td>Repetition time</td>
<td>sec</td>
</tr>
<tr>
<td>T₁</td>
<td>Longitudinal relaxation time (spin-lattice relaxation time)</td>
<td>sec</td>
</tr>
<tr>
<td>T₂</td>
<td>Transverse relaxation time (spin-spin relaxation time)</td>
<td>sec</td>
</tr>
<tr>
<td>T₁₀</td>
<td>Longitudinal relaxation time in the absence of paramagnetic ion</td>
<td>sec</td>
</tr>
<tr>
<td>T₂₀</td>
<td>Transverse relaxation time in the absence of paramagnetic ion</td>
<td>sec</td>
</tr>
<tr>
<td>r₁</td>
<td>Longitudinal relaxivity</td>
<td>1/mM•sec</td>
</tr>
<tr>
<td>r₂</td>
<td>Transverse relaxivity</td>
<td>1/mM•sec</td>
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**Introduction**

Approximately half of the mammary tumors in women, that are 2 cm in diameter, have already metastasized. Furthermore, current mammographic standards miss 10% of palpable tumors, and of those breast lesions detected, only 25% are malignant, leading to unnecessary biopsies. Clearly a need exists for improving the detection limits (sensitivity) and the ability to differentiate benign from malignant tumors (specificity). Extraction of an agent by a tumor from the blood depends on (a) capillary surface area, S, (b) capillary permeability, P, (c) capillary blood flow, F, (d) transit time of the agent through the tumor interstitium, and (e) the plasma half-life, $T_{1/2}$ (see Figure 1). Tumors secrete angiogenic factors which both increase the capillary density, surface area, and permeability. Weidner showed that specific regions of interest (ROI) with high capillary densities act as excellent prognostic indicators for breast tumors\(^1\)\(^-\)\(^3\). In this project we are testing the hypothesis that regional differences in the permeability-surface area product acts as a prognostic indicator for breast cancer.

In order to calculate the permeability-surface area product the pharmacokinetic behavior of magnetic resonance contrast agents must be understood. Relating these pharmacokinetic models to dynamic magnetic resonance imaging with contrast agents, was first done in brain lesions by Larsson et al., Tofts and Kermode, and Brix et al.\(^4\)\(^-\)\(^6\). Their models and techniques are consistent with each other\(^7\),\(^8\) and are widely used in analyzing the time-evolution of magnetic resonance imaging contrast agents in the plasma, extravascular extracellular space (EES), and the tumor. Based on the analysis of these models, we can obtain information about an agent’s plasma half-life, distribution behavior, and a specific tumor’s permeability-surface area product; the very same extraction factors mentioned above. The original pharmacokinetic models have been used with Gd-DTPA to analyze different types of tumors: brain\(^4\)\(^-\)\(^6\), muscle\(^9\) and mammary\(^10\),\(^11\). All these analyses
are based on time-dependent measured or calculated contrast agent concentrations determined from magnetic resonance imaging experiments.

Figure 1: Contrast agent tumor extraction factors

The specific aim of this project is to identify how contrast agent size affects the permeability-surface area products of mammary tumors, identifying any intratumor or regional heterogeneity for use in characterizing tumor specificity, and any detectable differences that are characteristic of tumor type. To calculate the permeability-surface area products, we use a two compartment model and FLASH (Fast Low-Angle SHot) image signal intensities that have been converted to contrast agent concentration data by a calibrated standard curve to examine the differences in tumor uptake of a low molecular weight agent, Gd-DTPA (MW = 938.014), and a macromolecular dendrimer based agent, PAMAM-TU-DTPA G = 4 (MW ≈ 35,000).

In this paper we will cover some general background, including: the physical basis of nuclear magnetic resonance, the theory of magnetic resonance imaging, the principles
behind contrast agents, and the biological fundamentals of cancer. We will then delineate the theory behind the concentration calibration curve and the two compartment model with its attendant equations. Additionally, basic methods and a discussion of results will also be covered.

Physical Basis of Magnetic Resonance

The phenomenon of (nuclear) magnetic resonance (MR) is the electromagnetic wave emitted by certain nuclei when they attempt to re-align with a static magnetic field after being perturbed. The first magnetic resonance experiments were performed, independently, by Bloch and Purcell in 1946\(^{12}\). Magnetic resonance makes use of the nuclear spin, \( I \), exhibited by all nuclei. This nuclear spin is quantized into three states, zero (\( I = 0 \); nuclides with even atomic number and mass number), integral (\( I = 1, 2, 3... \); nuclides with odd atomic number and even mass number), and half-integral (\( I = 1/2, 3/2, 5/2... \); nuclides with odd mass number). Specific nuclides with integral and half-integral spin exhibit magnetic resonance, that is, they are magnetic resonance active\(^{13}\).

![Figure 2: Zeeman splitting of nuclear spins](image-url)

\[ H(t) = a \left(T_{1}c + b \right) \]

\[ \begin{align*}
  \Delta E &= \hbar \gamma B_0 \\
  \Delta E &= \hbar \gamma B_0
\end{align*} \]
In the most common case for magnetic resonance applications, \( I = \pm 1/2 \) (e.g. \(^1\text{H}, ^{13}\text{C}\) and \(^{19}\text{F}\)), and the waveform of the nuclear spins is\(^{14}\):

\[
\Psi(t) = a \left| \frac{1}{2} \right\rangle + b \left| -\frac{1}{2} \right\rangle
\]

(1.)

This means that the nuclides exists in some linear combination of the spin states, but we can only observe them in either the \( \left| \frac{1}{2} \right\rangle \) or \( \left| -\frac{1}{2} \right\rangle \) state. When an MR active nuclide's atoms or molecules containing its atoms, are placed in a strong, static magnetic field, \( B_0 \), the nuclear spins undergo Zeeman splitting\(^{15}\) (see Figure 2). Simple resonance theory tells us the Zeeman splitting energy is quantized\(^{16}\):

\[
E = -\gamma h B_0 m \quad \text{where} \quad m = I, I - 1, \ldots, -I
\]

(2.)

Where \( E \) is the energy of the spin state, \( \gamma \) is the gyromagnetic ratio (which is nuclide specific), and \( h \) is Planck's constant. Classically, this means that spins either align with (for \( I = 1/2 \)) or against (for \( I = -1/2 \)) the static magnetic field, \( B_0 \).\(^{17}\) From the Boltzman energy distribution, it can be shown that it is energetically more favorable (more stable) to align with the field. Therefore, a couple more spins in every million spins aligns with the field than against it\(^{18}\). Conservation of energy gives us\(^{16}\):

\[
\Delta E = \hbar \omega_0 = \gamma h B_0
\]

(3.)

Where \( \omega_0 \) is the angular frequency of the spins about \( B_0 \). This difference in energy, \( \Delta E \), produces a small excess of nuclear spins, leading to a bulk magnetization, \( M_0 \).
precessing around the large static magnetic field, \( B_0 \) (see Figure 3). In essence, a normally non-magnetic substance becomes, in an extremely short period of time, magnetized\(^7\).

From the conservation of energy above, we can write the Larmor relationship\(^6\),

\[
\omega_0 = \gamma B_0
\]

(4.)

This relationship tells us that the angular frequency of the electromagnetic radiation required to cause a transition of the nuclear spins between energy states is linearly proportional to the magnetic field it is in\(^9\). Since the Larmor relationship is no longer dependent on Planck’s constant, the angular frequency is much like the classical picture\(^6\).

\[\text{Figure 3: Bulk magnetization, } M_0, \text{ due to excess spins aligning with } B_0\]

**Excitation and Relaxation**

Once the sample is magnetized, a detectable signal must be induced. In order to produce this signal, the equilibrated energy states are perturbed. To perturb the system a radiofrequency (RF) excitation, \( B_1 \), induces a change in the nuclei from one quantum to
another\textsuperscript{19}. In order for the excitation to cause the transition it must obey the resonance condition; the excitation frequency must equal the Larmor frequency (i.e. $\omega_{\text{RF}} = \omega_0$). The RF excitation, $B_1$, is smaller than $B_0$, time-dependent, and perpendicular to $B_0$, and can be expressed (if $B_0$ is a vector in the z-direction)\textsuperscript{18}:

$$
\mathbf{B}_1(t) = B_1^*(t) \left[ \cos(\omega_{\text{RF}}t + \varphi) \mathbf{i} - \sin(\omega_{\text{RF}}t + \varphi) \mathbf{j} \right]
$$

(5.)

Where $B_1^*(t)$ is the pulse shape of the excitation, $t$ is the time, and $\varphi$ is the initial phase angle. In other words, $B_1(t)$ is a wave in the xy-plane. To better understand the behavior of the radiofrequency excitation of the bulk magnetization and its subsequent realignment with $B_0$ we convert to a rotating frame of reference with angular frequency, $\omega_{\text{RF}}$.

Conversion between the frames of reference is given by a linear transformation\textsuperscript{18} (see Figure 4):

$$
\begin{bmatrix}
M_x' \\
M_y' \\
M_z'
\end{bmatrix} =
\begin{bmatrix}
\cos \omega_{\text{RF}} t & -\sin \omega_{\text{RF}} t & 0 \\
\sin \omega_{\text{RF}} t & \cos \omega_{\text{RF}} t & 0 \\
0 & 0 & 1
\end{bmatrix}
\begin{bmatrix}
M_x \\
M_y \\
M_z
\end{bmatrix}
$$

(6.)

Which means in the rotating frame of reference, the $B_1$ excitation looks like (for zero phase, $\varphi = 0$):

$$
\begin{bmatrix}
B_x^* \\
B_y^* \\
B_z^*
\end{bmatrix} =
\begin{bmatrix}
\cos \omega_{\text{RF}} t & -\sin \omega_{\text{RF}} t & 0 \\
\sin \omega_{\text{RF}} t & \cos \omega_{\text{RF}} t & 0 \\
0 & 0 & 1
\end{bmatrix}
\begin{bmatrix}
B_x^*(t) \cos \omega_{\text{RF}} t \\
B_y^*(t) \cos \omega_{\text{RF}} t \\
B_z^*(t)
\end{bmatrix}
$$

(7.)
If the pulse shape, $B_1^e(t)$, is defined as $B_1$ for time $0 < t < t_p$ (a rectangular pulse), when we apply the RF pulse, we will flip or move the magnetization, $M_0$, away from $B_0$ and down toward the xy-plane. In other words, the application of the excitation, $B_1$, alters the quantum mechanical spin state of the nuclei, making their bulk magnetization point in a new direction. Furthermore, the longer the pulse width, $t_p$, and the stronger the radiofrequency pulse, $B_1$, the greater the flip angle, $\Theta$ (radians). The flip angle is given by $^{17}$ (see Figure 6):

$$\Theta = \gamma B_1 t_p$$  \hspace{1cm} (8.)

Figure 4: The rotating (primed) and laboratory (unprimed) frames of reference

Following the flip of the magnetization, the $B_1$ excitation is switched off and the perturbed bulk magnetization relaxes back to realign with the static field, $B_0$. This relaxation behavior is governed by the Bloch equations$^{16}$:
\[
\begin{align*}
\frac{dM_z}{dt} &= \gamma (\textbf{M} \times \textbf{B})_z + \frac{M_0 - M_z}{T_1} \\
\frac{dM_{xy}}{dt} &= -\frac{M_{xy}}{T_2} + i\gamma (B_0 M_0 - b_0 M_{xy}) 
\end{align*}
\] (9.)

Where \( M_z \) is the magnetization in the z'-direction, \( M_{xy} \) is the magnetization in the x'y'-plane, \( b_0 \) is the inhomogeneity of the \( B_0 \)-field, \( T_1 \) is the longitudinal or spin-lattice relaxation time, and \( T_2 \) is the transverse or spin-spin relaxation time.

Where \( T_{2e} \) is the transverse relaxation time in the presence of small \( B_0 \)-field inhomogeneity, \( b_0 \). That is, \( T_{2e} \) is the "effective" or "observed" transverse relaxation time and is given by:

\[
\frac{1}{T_{2e}} = \frac{1}{T_2} + \gamma b_0 
\] (11)

Figure 5: \( T_1 \) and \( T_2 \) behavior

The longitudinal relaxation time is the time it takes 63% of the magnetization to return in the z'-direction, that is for the nuclides to re-align with the static magnetic field following a perturbation. In other words, the time it takes for the spins to equilibrate with their surroundings (i.e. the lattice) is proportional to the longitudinal relaxation time. The transverse relaxation time is the time it takes for 63% of the magnetization in the x'y'-plane to dissipate, or allow the nuclides to de-phase. That is, the transverse relaxation time is related to how long it takes the spins to energetically equilibrate with each other\(^\text{17}\). Both \( T_1 \) and \( T_2 \) are dependent on a number of things, including the medium of the sample, the
molecular structure that the magnetic resonance active nuclides are part of, the presence of paramagnetic ions, the temperature, pressure, pH and strength of $B_0^{19}$.

The Bloch equations then are the rate of change of the magnetization in $z$-direction (the longitudinal component) and the $xy$-plane (the transverse component). Solving the Bloch equations for the relaxation after a $90^\circ$ pulse (where all the bulk magnetization, $M_0$, is flipped down into the $x'y'$-plane) yields$^{16}$ (see Figure 5):

$$M_x(t) = M_0(1 - e^{-\frac{t}{T_2^*}})$$
$$M_{xy}(t) = M_0e^{-\frac{t}{T_2}}$$

(10.)

Where $T_2^*$ is the transverse relaxation time in the presence of small $B_0$-field inhomogeneity, $b_0$. That is, $T_2^*$ is the "effective" or "observed" transverse relaxation time$^{14}$ and is given by$^{17}$:

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \gamma b_0$$

(11.)

This is the basic relaxation behavior of the magnetic resonance active nuclides. Specific expressions can be derived from the Bloch equations for specific pulse sequences. It should be noted that two independent processes occur in relaxation: (a) the longitudinal process as the magnetization exponentially grows and levels off in the $z'$-direction and (b) the transverse process that exponentially decays as the magnetization dephases in the $x'y'$-plane. The spinning, decaying electromagnetic wave in the $x'y'$-plane is the detected magnetic resonance signal, and is referred to as a free induction decay (FID) (see Figure 6).
RF ON
Proton spins are perturbed from their energy state; the coil applies $B_1$ for time $t_p$; the bulk magnetization, $M_0$, is flipped away from the $z'$-axis by flip angle, $\Theta$

RF OFF
Proton spins realign with or against $B_0$; the flipped magnetization relaxes due to spin lattice and spin-spin interactions; signal is induced in the coil due to relaxation.

Figure 6: Signal generation and detection

Image Formation

With what we now know about magnetic resonance, we can build a system that would cause Zeeman splitting and then be able to perturb the spins by a radiofrequency excitation, followed by a detection of the relaxation. Modern systems use a superconducting magnet to produce a large, static magnetic field, $B_0$. Typically this field is 1.5 to 2 Tesla for a clinical imaging system, 4.7 Tesla for a research imaging system, and upwards of 12 Tesla for a standard NMR spectrometer. New 3 to 4 Tesla systems are being introduced for clinical use and a new 12 Tesla system is being used on animals with a human version currently under construction. It is interesting to note that a 1.5 Tesla field is...
roughly \(3 \times 10^4\) times the magnetic field strength of the earth and is powerful enough to pick up a car\(^1\). 

To induce the radiofrequency excitation, \(B_1\), a small coil is placed around the area or sample to be imaged. This coil can be a simple loop of wire (a surface coil), a solenoid, a dual coil, or any of a number of designs\(^2\). This same coil would be used to detect the relaxation, the nonionizing electromagnetic radiation released as the spins re-equilibrated\(^3\) (see Figure 6). However, the signal detected by the coil is the blended electromagnetic waves emitted by all the relaxing spins, meaning that the signal is averaged and does not distinguish position\(^4\).

In order to distinguish position, defined inhomogeneities are added in the magnetic field of \(B_0\), such that the magnetic resonance signals can be spatially encode\(^5\). This idea of defined inhomogeneities created by linear gradients was first applied by Lauterbur in 1973\(^6,7\). On the most basic level, the gradients isolate a set number of nuclear spins in each voxel ("volume element") by selecting a slice, a "row" within the slice, and an intersecting "column" within each "row". A slice is defined by applying the slice selective gradient to the homogenous static magnetic field, \(B_0\)\(^8\). A stronger slice selective gradient leads to a thinner slice. The thinner the slice, the more accurate the image, since less tissue spins are averaged together to form each voxel. Mathematically, the slice selection profile for a slice in the z-direction is given by\(^9\):

\[
p(z) = \Pi \left( \frac{z-z_0}{\Delta z} \right) e^{i \gamma G dz (z-z_0)/2} \tag{12.}\]

Where \(\Pi\) represents a rectangular window function centered around \(z_0\). Now that the slice is determined, the plane (the "rows" and "columns") of the slice must be encoded.
To do this, two techniques, known as frequency encoding and phase encoding, are used\textsuperscript{12}. Frequency encoding works by using the Larmor relationship to give a "row" of voxels (full of nuclear spins) a slightly different frequency from the other rows. While phase encoding works by adding and subtracting increasing phase angles from the recorded signal\textsuperscript{14} (see Figure 7). Each voxel produces a specific waveform with the frequency encoding setting a specific frequency for the voxel’s signal and the phase encoding setting a specific phase for the voxel’s signal. These waves are arrayed and the encoded information in the array is processed by a number of algorithms, including projection reconstruction and discrete 2D Fourier transform techniques, to compose an image\textsuperscript{18}. 

\textbf{Figure 7: Image encoding}
Place an orange in the imager and use the gradients to encode the magnetic resonance signal, induced by the radiofrequency pulse, to form an image.

Each voxel defined by the slice selection, frequency and phase encode gradients generates a specific FID for proton spins located within the voxel. The FID has a specified frequency and phase due to the encoding.

The FID’s are demodulated and arrayed. This array is in k-space and is a Fourier space representation of the slice through the object.

The k-space array is processed into an image by a 2D Fourier transform. In this case, the y-axis is the phase encode and the x-axis is the frequency encode. The image is greyscale, based on the absolute magnitude of the magnetic resonance signal from each voxel. Typically, the greater the water (i.e. proton) content the brighter the voxel.

Figure 7: Image encoding
Proton Signal and Contrast

In most magnetic resonance imaging (MRI) applications, the hydrogen nucleus provides us with our signal. Hydrogen atoms bonded in water form an extremely concentrated solution, approximately 110 M. Additionally, 75% of the human body is composed of water which is spread throughout our entire system. Because magnetic resonance is an inherently insensitive technique, the abundant and highly concentrated solution of hydrogen protons in water is necessary for generating an image, most other magnetic resonance active nuclides are simply not as detectable. This makes hydrogen the nuclide of choice in magnetic resonance imaging of living tissue. In general, MRI does not include the signal from hydrogen in lipids, proteins or carbohydrates found in living tissue, although signals from lipids do sometimes show up as imaging artifacts (a so-called chemical shift artifact).

Although our signal comes from only one nuclide in a specific electron configuration (that of a hydrogen atom bonded to a hydroxide group) this signal differs from voxel to voxel in an imaging slice. This signal difference, called contrast, is expressed:

\[
\text{Contrast} = R_{I_a} - R_{I_b}
\]  

(13.)

Where the relative intensities of regions a and b, \( R_{I_a} \) and \( R_{I_b} \), are given by normalizing the signal intensity:

\[
R_{I_i} = \frac{S_i}{S_{\text{REF}}}
\]  

(14.)

\( S_i \) is the signal intensity of the region of interest, \( i \), and \( S_{\text{REF}} \) is the signal intensity of a reference (e.g. a vial of dilute contrast agent, a Vitamin E capsule, a known internal
standard, or even just the noise of the image). Simply stated, contrast is just signal intensity differences due to spatially dependent variations in longitudinal and transverse relaxation times, spin densities, and other physical factors, in the excited slice\textsuperscript{23}. Relaxation times are dependent on both external, which will be the same for the entire image, and internal factors, which will vary within each image. The internal factors that effect relaxation times are due to localized changes in the magnetic field experienced by the protons\textsuperscript{24}. These localized magnetic field changes are due to such things as: temperature, pH, atomic interactions between the water molecule and the surrounding molecules (proteins, lipids, carbohydrates, minerals and other constituents of living systems), diffusive and perfusive motion of the water molecule, and location of the water (e.g. intraversus extracellular water)\textsuperscript{12, 19}. The localized magnetic field variations due to the presence of another chemical species causes changes in the total magnetic field, \( B_0 \), experienced by the magnetic resonance active hydrogen nuclei. Thus\textsuperscript{17},

\[
\vec{B}_t = \vec{B}_0 + \vec{B}_{\text{loc}}
\]

(15.)

Where the localized magnetic field is given by\textsuperscript{17}:

\[
\vec{B}_{\text{loc}} = \frac{\mu_S (3 \cos^2 \theta - 1)}{r_{IS}^3} \hat{r}
\]

(16.)

Where \( \mu_S \) is the magnetic moment of nuclei S (i.e. the other molecular species, M), \( r_{IS} \) is the internuclear vector or the distance between nuclei I (the water hydrogen, H, whose oxygen is electronically interacting with the other species, M) and nuclei S, and \( \theta \) is the angle between \( r_{IS} \) and \( B_0 \). Essentially all contrast effects are due to some form of
molecular motion; changes in rotation (change of $\theta$), stretching vibrations of the bonds (change of $r_{is}$), and translation of the magnetic moments (change of $r_{is}$)$^{24}$.

Figure 8: The effects of a molecule on local magnetic fields

The effects of molecular motion are proportional to the longitudinal and transverse relaxation times$^{17, 19}$ (see Figure 8):

$$\frac{1}{T_1} \propto \langle B_{loc}^2 \rangle \left( J(\omega) + J(2\omega) \right)$$  \hspace{1cm} (17.)

$$\frac{1}{T_2} \propto \langle B_{loc}^2 \rangle \left( J(0) + J(\omega) + J(2\omega) \right)$$  \hspace{1cm} (18.)
\( \omega_c \) is the Larmor frequency of nuclei, I (the hydrogen nucleus), and the spectral density function, \( J(\omega) \), given by

\[
J(\omega) = \int_{-\infty}^{\infty} g(\tau_c) e^{-i\omega \tau_c} d\tau_c
\]

(19.)

Where \( g(\tau_c) \) is a correlation function and \( \tau_c \) is the correlation time characterizing the frequency of molecular motion (modulation). Expressions relating the relaxation times of water protons due to local field changes have been derived\(^{19}\):

\[
\frac{1}{T_1} = \frac{3}{10} \frac{\gamma_i^2 n_i}{r_{ls}^2} \left( \frac{\tau_c}{1 + \omega_c^2 \tau_c} + \frac{4\tau_c}{1 + 4\omega_c^2 \tau_c} \right)
\]

(20.)

\[
\frac{1}{T_2} = \frac{3}{20} \frac{\gamma_i^2 n_i}{r_{ls}^2} \left( 3 \tau_c + \frac{5\tau_c}{1 + \omega_c^2 \tau_c} + \frac{2\tau_c}{1 + 4\omega_c^2 \tau_c} \right)
\]

(21.)

Where \( \gamma_i \) is the gyromagnetic ratio of nuclei, I (i.e. the gyromagnetic ratio of hydrogen).

**Macromolecular and Dendrimer Contrast Agents**

To improve contrast in magnetic resonance imaging, a contrast agent is used to selectively alter the proton relaxation times of tissues containing the contrast agent\(^{25}\). The simplest way to change relaxation times is by introducing a paramagnetic species, such as Gadolinium (Gd), Cobalt (Co), Manganese (Mn), Nickel (Ni) or Iron (Fe)\(^{26}\).
Unfortunately, such paramagnetic species are inherently toxic\(^2^7\), so in order to use their relaxation altering properties they are chelated to protect the patient from the metallic ion\(^2^8\).

The presence of a paramagnetic ion causes local magnetic field changes due to dipole-dipole interactions\(^1^7\). Theory has been derived governing the paramagnetic ion's effect on relaxation\(^1^9\),\(^2^7\):

\[
\frac{1}{T_i} = \frac{1}{T_{i0}} + \frac{1}{T_{i,p}} \left( \frac{3\pi}{T^2} + \frac{1}{1 + \omega_M^2} \right) + \frac{1}{2} \frac{gS(S+1)A^2}{9} \left( \frac{3\pi}{T^2} + \frac{1}{1 + \omega_M^2} \right)
\]

(22.)

Where \( T_{i0} \) (\( i = 1,2 \)) is the relaxation time in the absence of paramagnetic ion (the water molecules which do not get electronically bound by the paramagnetic ion) while \( T_{i,p} \) (\( i = 1,2 \)) is the paramagnetic contribution to the total relaxation time (the water molecules which do get electronically bound by the paramagnetic ion). The paramagnetic component has both inner and outer sphere components:

\[
\frac{1}{T_{i,p}} = \frac{1}{T_{i,\text{inner}}} + \frac{1}{T_{i,\text{outer}}}
\]

(23.)

The inner sphere component, \( T_{i,\text{inner}} \), is given by\(^1^9\)

\[
\frac{1}{T_{i,\text{inner}}} = \frac{P_M q}{T_{i,\text{M}} + \tau_M}
\]

(24.)

\[
\frac{1}{T_{i,\text{inner}}} = \frac{P_M q}{\tau_M} \left[ \frac{1}{T_{2,M}^2} \left( \frac{1}{T_{2,M}} + \frac{1}{\tau_M} \right) + \Delta \omega_M^2 \right]
\]

(25.)
Where $P_M$ is the mole fraction of paramagnetic ion, q is the number of bound water molecules per paramagnetic ion, $\tau_M$ is the residence lifetime of the bound water, $\Delta \omega_M$ is the chemical shift due to the paramagnetic ion, and $T_{iM}$ ($i = 1, 2$) is the relaxation time of the hydrogen nuclei that are part of the bound water. Theoretical expressions for the bound relaxation times, $T_{iM}$, are given by Solomon-Bloembergen-Morgan (SBM) theory:\(^{19}\):

\[
\frac{1}{T_{1M}} = \frac{2}{15} \frac{\gamma_0^2 g^2 S(S + 1)}{r_{IS}^2} \frac{\beta^2}{1 + \omega_0^2 \tau_c^2 + \frac{7 \tau_c}{1 + \omega_S^2 \tau_c^2}} + \frac{2}{3} S(S + 1) \left( \frac{A}{\hbar} \right)^2 \left( \frac{3 \tau_c}{1 + \omega_S^2 \tau_c^2} \right)
\]

(26.)

\[
\frac{1}{T_{2M}} = \frac{1}{15} \frac{\gamma_0^2 g^2 S(S + 1)}{r_{IS}^2} \frac{\beta^2}{1 + \omega_0^2 \tau_c^2 + \frac{13 \tau_c}{1 + \omega_S^2 \tau_c^2}} + \frac{1}{3} S(S + 1) \left( \frac{A}{\hbar} \right)^2 \left( \frac{\tau_c}{1 + \omega_S^2 \tau_c^2} \right)
\]

(27.)

Where $g$ is the Landé g-factor, $S$ is the electron spin of the paramagnetic species, $\beta$ is the Bohr magneton, $\omega_S$ and $\omega_I$ are the electronic (S) and nuclear (I) Larmor precession frequencies, respectively, and $A/\hbar$ is the electron-nuclear hyperfine coupling constant. The correlation times in these expressions; the dipole-dipole correlation time, $\tau_c$, and the isotropic nuclear-electron correlation time, $\tau_e$, are expressed as,

\[
\frac{1}{\tau_c} = \frac{1}{\tau_S} + \frac{1}{\tau_M} + \frac{1}{\tau_R}
\]

(28.)
Changes in the residence lifetime of the bound water, $\tau_{m}$, the rotational correlation time, $\tau_{r}$, or the electron-spin relaxation time, $\tau_{s}$, adjust the bound relaxation times, causing changes in contrast. Recall that local magnetic field changes are dependent on changes of $\theta$, $r_{is}$ and $r_{is'}$. These changes are characterized by the times $\tau_{r}$, $\tau_{m}$, and $\tau_{s}$.

New chemical structures are constantly being proposed, synthesized and tested for setting $\tau_{r}$, $\tau_{m}$, and $\tau_{s}$ and inducing known relaxivity behavior changes to maximize contrast. Dendrimer contrast agents are novel polymeric agents with unusually high ion and molecular relaxivities causing great contrast enhancement$^{29}$. These new contrast agents are composed of a paramagnetic ion linked to the dendrimer polymer by a chelate$^{30}$. The dendrimer is a three-dimensional polymeric structure assembled by iterative chemical reactions of smaller substitutive molecules$^{28,31}$. Being relatively new agents, little has been investigated about their permeability properties compared to clinically available macromolecular agents or how the generation (i.e. size) of the dendrimer affects its extraction by the tumor. Additionally these dendrimer agents can be used for targeted contrast enhancement approaches$^{32}$.

Cancer

Finally, it is necessary to briefly review the basis of cancer biology and the language of oncology. Cancer is any malignant neoplasm, an uncontrolled new growth of cells exhibiting invasiveness and remote spread (metastasis)$^{33}$. Initiation of cancer is called carcinogenesis, which results from mutations in the DNA (the genetic instructions of the cell) that do not get repaired by cellular mechanisms. These oncogenic cells have corrupted
instructions, rather than undergoing programmed cell death (apoptosis) the cell (and its mutated progeny) continues to replicate. The cancerous mutation can be caused by a mutagen; ionizing radiation, a chemical carcinogen, or viruses\textsuperscript{34}. This mutagen either interacts directly or indirectly with the DNA. Direct mechanisms occur when the mutating agent directly effects the DNA, such as a chemical carcinogen reacting with the gene or a viral gene strand becoming inserted in the host genome. Indirect mechanisms are caused by the mutating agent forming new molecules and excited atoms within the cell, that later interact with the DNA. An indirect DNA mutation would be damage caused by free radicals of oxygen created within the cell by ionizing radiation\textsuperscript{35} (see Figure 9).

![Figure 9: Carcinogenesis](image)

The uncontrolled growth of cancer cells causes a swelling or tumefaction known as a tumor or neoplasm\textsuperscript{36}. Benign and malignant are terms for characterizing a neoplasm’s specificity\textsuperscript{35} (see Figure 10). A benign tumor is noninvasive and does not metastasize. It can still be a problem as its growth puts extra demands on the patient and its size causes it to interfere with normal function. Benign tumors may also become malignant. A malignant tumor (i.e. cancer) is invasive and spreads through the body. It is typically a metastasized cancer that is fatal. Metastasized cancer forces the body to cope with multiple
or key systems being taxed by metastasized tumors, cell growths that have dissimilar functions with the host tissue.

Growth of tumors is exponential, that is, it will grow without bound until it kills the patient or is treated, by surgery, radiation therapy, or chemotherapy. To give some idea of scale, a tumor of 1 cm³ is considered detectable by palpitation or imaging, a lesion roughly the size of a grape can be seen by an oncologist. Of patients expressing a tumor of 11.5 cm³, an apricot size lesion, approximately half of these have metastasized tumors elsewhere. In general, the upper or lethal size limit of a lesion is 1000 cm³, a tumor the size of an apple is fatal.

![Tumorigenesis Diagram](image)

**Figure 10: Tumorigenesis**

Although tumors are growths due to a single cell clonally overreplicating, tumors are heterogeneous. Tumors are composed of oxic, hypoxic and necrotic regions. The oxic regions are composed of the tumor cells which receive enough oxygen to thrive. This region is closest to the capillary bed that feeds the tumor and associated with angiogenesis (formation of new vasculature). Further from the tumor’s capillary blood supply lies the hypoxic region, which is composed of the tumor cells that are oxygen starved. Finally, the
necrotic region is composed of tumor cells that are dead or dying, that is, the cells that are not receiving oxygen and are furthest removed from the capillaries feeding the tumor. In order to fit the pharmacokinetic model presented, specific regions of the magnetic resonance images must be converted into concentrations of contrast agent for a series of time points. The data collected are magnetic resonance signal intensities of regions of interest that must related to the concentration of paramagnetic ion in those regions. Thus, the purpose of this section, is to show the theory relating the signal intensity to the contrast agent concentration. Let us begin with the theoretical signal intensity of a FLASH imaging sequence:

\[ S_{\text{FLASH}} = kN \frac{\sin \Theta (1 - E_1)}{1 - (1 - E_1) \cos \Theta} \]  

(30.)

Where \( \Theta \) is the flip angle and

\[ E_1 = e^{-TR/T_1} \]  

(31.)

Where, \( TR \) (sec) is the repetition time and \( T_1 \) (sec) is the longitudinal relaxation time.

Therefore the normalized signal intensity, \( S/S_0 \), where flip angles are identical:

\[ \frac{S}{S_0} = \frac{1 - (1 - E_1) \cos \Theta}{1 - E_{10}^2} \frac{1 - E_1}{1 - (1 - E_1) \cos \Theta} \]  

(32.)

Where,

\[ E_{10} = e^{-TR/T_1} \]  

(33.)
Theory

Relating Normalized Signal Intensities to Contrast Agent Concentrations

In order to fit the pharmacokinetic model presented, specific regions of the magnetic resonance images must be converted into concentrations of contrast agent for a series of time points. The data collected are magnetic resonance signal intensities of regions of interest that must related to the concentration of paramagnetic ion in those regions. Thus, the purpose of this section, is to show the theory relating the signal intensity to the contrast agent concentration. Let us begin with the theoretical signal intensity of a FLASH imaging sequence:\(^{37}\):

\[
S_{\text{FLASH}} = kN_{T} \frac{\sin \Theta (1 - E_{j})}{1 - (1 - E_{j}) \cos \Theta}
\]  
(30.)

Where \( \Theta \) is the flip angle and

\[
E_{j} = e^{-TR/T_{1}}
\]  
(31.)

Where \( TR \) (sec) is the repetition time and \( T_{1} \) (sec) is the longitudinal relaxation time.

Therefore the normalized signal intensity, \( S/S_{0} \), where flip angles are identical:

\[
\frac{S}{S_{0}} = \frac{1 - (1 - E_{10}) \cos \Theta}{1 - E_{10}} \frac{1 - E_{1}}{1 - (1 - E_{j}) \cos \Theta}
\]  
(32.)

Where,

\[
E_{10} = e^{-TR/T_{10}}
\]  
(33.)
Where $T_{10}$ (sec) is the longitudinal relaxation time in the absence of paramagnetic ion (i.e. in the absence of contrast agent), since $S_0$ is the signal in the absence of paramagnetic ion (i.e. $T_1 = T_{10}$), or the signal pre-injection of contrast agent (i.e. at time, $t = 0$). Thus,

$$\frac{S}{S_0} = \frac{\left(\frac{1}{1 - E_{10}} - \cos \Theta\right)}{\left(\frac{1}{1 - E_1} - \cos \Theta\right)}$$

(34.)

To relate this to contrast agent concentration, use the longitudinal relaxivity equation:

$$\frac{1}{T_1} = \frac{1}{T_{10}} + r_1 [C]$$

(35.)

Where $r_1$ (1/mM·sec) is the longitudinal relaxivity and [C] (1/mM) is the contrast agent's paramagnetic ion concentration. This tells us what the longitudinal relaxation time is as a function of contrast agent concentration. Thus,

$$\frac{S}{S_0} = \frac{\left(\frac{1}{1 - e^{-TR/T_{10}}} - \cos \Theta\right)}{\left(\frac{1}{1 - e^{-TR(T_{10} + r_1[C])}} - \cos \Theta\right)}$$

(36.)

From this expression, we can now write an equation for [C] as a function of normalized signal intensity, $S/S_0$: 

25
\[
[C] = -\frac{1}{T_{10}r_1} - \frac{\ln \left( \frac{A - 1}{A \cos \Theta - 1} \right)}{TRr_1}
\]  (37.)

The factor, \(A\) is given by:

\[
A = \left( \frac{S}{S_0} \right) \left( \frac{1 - E_{10}}{1 - E_{10} \cos \Theta} \right)
\]  (38.)

Let us calculate \(E_1\) and \(E_{10}\). From our experiment, \(TR = 0.080\) sec, \(T_{10} \approx 2.4\) sec, and \(0 \leq [C] < \infty\). Thus \(E_1\) and \(E_{10}\) are given by:

\[
1 \leq \frac{1}{1 - E_{10}} < 34.5
\]

\[
\frac{1}{1 - E_{10}} \approx 34.5
\]  (39.)

These values are much greater than the cosine of the flip angle, \(\Theta \approx 80^\circ\). That is, \(\cos \Theta = 0.1763\) and it is safe to assume that Equation (36) simplifies to:

\[
\frac{S}{S_0} = \frac{1 - e^{-TR(T_{10}^{-1} + r_1[C])}}{1 - e^{-TR/T_{10}}}
\]  (40.)

This can be re-written as,
\[
\frac{S}{S_0} = \frac{1 - C_1 e^{-C_2[C]}}{1 - C_1} \tag{41.}
\]

This is the expression for fitting all the data of the contrast agent calibration curve (except for high concentrations, \([C]\), where a T2 effect occurs). And,

\[
C_1 = e^{-\text{TR}/T10}
\]
\[
C_2 = \text{TR} \ r_1 \tag{42.}
\]

To make sure that the boundary condition, that is \(S/S_0 = 1\) when \([C] = 0\), is obeyed in the expansion, we apply the condition:

\[
\frac{S}{S_0} = \frac{1 - C_1}{1 - C_1} = 1
\]

From Equation (41), the expression for the concentration, \([C]\), is then

\[
[C] \approx -\frac{\ln \left( \frac{1}{C_1} + \frac{S}{S_0} \left( 1 - \frac{1}{C_1} \right) \right)}{C_2} \tag{43.}
\]

In order to further simplify the relationship between signal intensity and contrast agent concentration, we can use an expansion for the exponential for small \(x\) (i.e. small contrast agent concentrations, \([C]\)):

\[
e^x = 1 + x + \frac{x^2}{2!} + \frac{x^3}{3!} + ... \tag{44.}
\]
Then, to first order, we must optimize the relationship:

\[ e^{-C_2|C|} = 1 - C_2|C| \]  \hspace{1cm} (45.)

This is done by graphical analysis on a computer. Using our reported fitted \( C_2 \) for the concentration curve (see Results) we find for Gd-DTPA (\( C_2 = 0.66 \) 1/mM) that \([C] \leq 0.4 \) mM and for PAMAM-TU-DTPA \( G = 4 \) (\( C_2 = 4 \) 1/mM) that \([C] \leq 0.06 \) mM.

\[
S = \frac{1 - C_1(1 - C_2|C|)}{1 - C_1} = \frac{1 - C_1 + C_1C_2|C|}{1 - C_1} = \frac{C_1C_2}{1 - C_1} [C] + 1
\]  \hspace{1cm} (46.)

Thus\(^{38},\)

\[
\frac{S}{S_0} = m |C| + 1
\]  \hspace{1cm} (47.)

Where,

\[
m = \frac{C_1C_2}{1 - C_1} = \frac{TR r_1}{e^{\frac{TR}{T10}} - 1}
\]  \hspace{1cm} (48.)

This is the expression used for small concentrations (i.e. the linear region of the concentration curve). The boundary condition is that \( S/S_0 = 1 \) when \([C] = 0 \), let us check if the expansion holds at this condition:

\[ 1 = 1 + 0 \]
From Equation (47), we know that:

\[ [C] = \frac{1}{m} \left( \frac{S}{S_0} - 1 \right) \]

(49.)

From the theory above, two separate methods were employed for converting signal intensities to contrast agent concentrations. In both methods, the concentration calibration curve, Equation (41), was used. However different techniques were used for determining the parameters of the calibration curve, \( C_{1,2} \). For conversion of the signal intensity of the blood to contrast agent concentration in the blood we used the known pulse sequence repetition time, the longitudinal relaxation time of blood, and the relaxivity of the agent investigated to calculate \( C_{1,2} \), using Equation (42). These calculated concentrations were scaled by using: (1) the initial condition of the plasma compartment, that at time, \( t = 0 \), the concentration in the plasma volume is equal to the dose, \( D \), over the plasma volume, \( V_p \) (i.e. \( C_p(t = 0) = \frac{D}{V_p} \)), and (2) assuming that all the agent was injected at time, \( t = 0 \), that is, at \( t = 0 \) the concentration in the plasma compartment is 100% of the initial bolus injection and as time passes this percentage decreases due to distribution and excretion. Thus,

\[ C_p(t) = \left( \frac{D}{V_p} \right) \left( \frac{C_{p,\text{calc}}(t)}{C_{p,\text{calc}}(t = 0)} \right) \]

(50.)

We also used a contrast agent calibration curve to convert the normalized signal intensity of a tumor to the contrast agent concentration in that tumor. In this case, the parameters \( C_{1,2} \) of Equation (41) are fitted for from signal intensities measured at known reference concentrations\(^9\).\(^{39}\).
In order to correct our concentration calibration curve for temperature and contrast agent differences, we must be able to relate the longitudinal relaxation times of one set of conditions to another. Recall the relaxivity expression for two different agents in the same medium:

\[
\frac{1}{T_{1a}} = \frac{1}{T_{10}} + r_{1a}[C_a] \\
\frac{1}{T_{1b}} = \frac{1}{T_{10}} + r_{1b}[C_b]
\]  

(51.)

Where \(a\) and \(b\) represent the two different agents. Now, for the concentration calibration curve the longitudinal relaxation time in the absence of the agent, \(T_{10}\), is the same in both cases. At some arbitrary concentration the longitudinal relaxation rates, \(1/T_{1a}\) and \(1/T_{1b}\), will be the same. That is:

\[
\frac{1}{T_{10}} + r_{1a}[C_a] = \frac{1}{T_{10}} + r_{1b}[C_b]
\]

(52.)

The concentrations can then be related by:

\[
[C_b] = \frac{r_{1a}}{r_{1b}}[C_a]
\]

(53.)

From this expression we can adjust the concentration calibration curve for Gd-DTPA to one with any other Gadolinium-based agent by taking the ratio of the relaxivities and multiplying it by the concentration of Gd-DTPA.
Pharmacokinetic Behavior of Contrast Agents

Once we have determined the contrast agent concentration from our magnetic resonance image data, we can apply a pharmacokinetic model to the time evolution of the contrast agent. The pharmacokinetic behavior of the contrast agent concentration is described by a two compartment model as used by Tofts and Kermode 5, 40 (see Figure 11). This model is composed of a plasma and an extravascular extracellular space (EES) compartment. According to the two compartment model, following a bolus injection into the blood (i.e. the plasma compartment), the contrast agent is distributed into the EES compartment or is excreted through the kidneys.

The plasma compartment governs the time dependent concentration of contrast agent in the vasculature and connects the pieces of the model together: the kidneys and the EES. This compartment is exchanging contrast agent with the EES and losing contrast agent by excretion due to the kidneys.

The second half of the model consists of the EES of the normal tissue. This is the physiologically large amount of space outside of the vasculature between the cells 8. Essentially this can be thought of as the body, not including the blood, kidneys or cells 9. In other words, the EES is the leakage space of the body. That is, the contrast agent spreads to the EES of the normal and abnormal tissue of the body from the plasma compartment.

Linked to the plasma compartment, but separate from the EES compartment, the kidneys are a sink or excretion term for the plasma compartment. Eventually all the contrast agent will be removed by the kidneys. Although the liver is also used in excretion, it is not considered as an excretory mechanism for low molecular weight contrast agents, but may remove macromolecular agents from the blood stream. In this paper, the effects of the liver are assumed to be negligible.

The experimental region of interest (ROI) is the tumor compartment, essentially the EES of the abnormal (tumor) tissue. This small sub-compartment has a negligible effect on
The rest of the model, since the contrast agent entering and exiting of the tumor compartment is a small change to the total concentration in the remainder of the body. Therefore, the tumor's flow rates are assumed only to affect calculations dealing directly with the tumor compartment's contrast agent concentration.

Now that all of the pieces of the two compartment model have been described, let us examine how the pieces transfer contrast agent between one another. Influx and efflux of a contrast agent is different in each ROI in each compartment, so the concentration and time it takes for the agent to fill each area is different. This means that there are different flow rates, or transfer of an amount of contrast agent per unit time, for the same agent in different regions of the body. However, as we are only investigating the flow rates of the tumor, these rates will be measured for each tumor ROI while the flow rates of the rest of the body (i.e. the rest of the two compartment model) will be averaged. To calculate the flow rates, the rate of concentration change in a compartment must be found. The rate of
concentration change in a compartment is essentially the difference of influx and efflux to the compartment. Specifically, the rate of change of the concentration in a compartment is proportional to gains due to agent coming from other compartments and losses due to agent leaving the compartment. In general, for a compartment, \( i \):

\[
V_i \frac{dC_i(t)}{dt} = \sum_n K_{n \rightarrow i} C_n(t) - \sum_n K_{i \rightarrow n} C_i(t)
\]  

(54.)

Where \( V_i \, (L/kg) \) is the volume of the compartment per unit mass (for normalization between animals), \( C_i \, (mM) \) is the concentration of contrast agent in compartment, \( i \), being measured, \( C_n \, (mM) \) is the concentration of contrast agent in compartment, \( n \), \( K_{n \rightarrow i} \) \((L/kg*min)\) is the flow rate per unit concentration difference from compartment \( n \) to \( i \), and \( K_{i \rightarrow n} \, (L/kg*min) \) is the flow rate per unit concentration difference from compartment \( i \) to \( n \).

It is important to note that the volume of the compartment per unit mass, \( V_i \), is more accurately described as the volume of the compartment permeated by the contrast agent per unit mass. This means that contrast agents of differing size will express different compartmental volumes in the same animal. The flow rate constants are defined as the permeability-surface area product per unit mass of tissue:

\[
K_{i \rightarrow j} = P_{i \rightarrow j} S_{ij}
\]

\[
K_{j \rightarrow i} = P_{j \rightarrow i} S_{ji} = P_{j \rightarrow i} S_{ij}
\]  

(55.)

\[
K_{i \rightarrow j} = P_{i \rightarrow j} S_{ij}
\]

Where \( P_{i \rightarrow j} \, (m/min) \) is the permeability constant between compartments \( i \) and \( j \). \( S_{ij} \, (m^2/kg) \) is the surface area per unit mass of tissue, that is, the area between the compartments (NOTE: \( S_{ij} = S_{ji} \))\(^8\). The symbolism \( i \rightarrow j \) indicates the transfer from
compartment $i$ to compartment $j$. This transfer is not necessarily the same as $j \rightarrow i$ since active transport mechanisms and diffusion, viscosity or pressure differences will drive each side differently. Alternatively, if only simple diffusive transport is used, the permeability between the compartments is isodirectional, the same in both directions. This case is represented by the symbolism $i \leftrightarrow j$.

From the description of the two compartment model and the nomenclature given previously, an ordinary differential equation (ODE) can be written relating the rate of change of contrast agent concentration in the plasma compartment due to the loss by excretion into the kidneys and to the flow into and out of the EES compartment:

$$
V_p \frac{dC_p(t)}{dt} = K_{e \rightarrow p} C_e(t) - K_{p \rightarrow e} C_p(t) - K_{p \rightarrow k} C_p(t)
$$

(56.)

Where $C_p(t)$ (mM) is the contrast agent concentration in the plasma compartment, $C_e(t)$ (mM) is the contrast agent concentration in the EES compartment, $V_p$ (L/kg) is the plasma volume (per unit mass), $K_{e \rightarrow p}$ (L/kg*min) is the flow rate per unit volume from the EES to the plasma compartment, $K_{p \rightarrow e}$ (L/kg*min) is the flow rate per unit volume from the plasma to the EES compartment, and $K_{p \rightarrow k}$ (L/kg*min) is the excretion flow rate per unit volume due to the unidirectional transit of the contrast agent from the plasma compartment to the kidneys. Remember that these flow rates are averages for the entire body and that the agent is distributed heterogeneously throughout the body. Again, from the model, an ODE can be written for the rate of contrast agent change in the EES compartment due to concentration gains from and losses to the plasma compartment:

(61.)
\[ V_e \frac{dC_e(t)}{dt} = K_{p \rightarrow e} C_p(t) - K_{e \rightarrow p} C_e(t) \]  

(57.)

Where \( V_e \) (L/kg) is the EES volume (per unit mass). Rewriting Equation (56), yields:

\[ C_e(t) = \frac{V_p}{K_{e \rightarrow p}} \frac{dC_p(t)}{dt} + \frac{K_{p \rightarrow e} + K_{p \rightarrow k}}{K_{e \rightarrow p}} C_p(t) \]  

(58.)

Now substituting this expression into Equation (57) we have a second order ODE for \( C_p(t) \):

\[ V_p V_e \frac{d^2C_p(t)}{dt^2} + \left( K_{p \rightarrow e} V_p + K_{e \rightarrow p} V_e + K_{p \rightarrow k} V_e \right) \frac{dC_p(t)}{dt} + K_{e \rightarrow p} K_{p \rightarrow k} C_p(t) = 0 \]  

(59.)

Finding the roots of Equation (59) gives us the general solution for the plasma compartment contrast agent concentration, \( C_p(t) \) (see Appendix III):

\[ C_p(t) = A_1 e^{m_1 t} + A_2 e^{m_2 t} \]  

(60.)

Where the roots \( m_{1,2} \) are given by:

\[ m_{1,2} = -\left( K_{p \rightarrow e} V_p + K_{e \rightarrow p} V_e + K_{p \rightarrow k} V_e \right) \pm \sqrt{\left( K_{p \rightarrow e} V_p + K_{e \rightarrow p} V_e + K_{p \rightarrow k} V_e \right)^2 - 4 K_{e \rightarrow p} K_{p \rightarrow k} V_p V_e} \]  

(61.)
Using the assumption that $K_{p\rightarrow e} \gg K_{p\rightarrow k}$ and $K_{e\rightarrow p} \gg K_{p\rightarrow k}$ since the EES has a much greater interfacing surface area than the kidneys:\(^5\):

\[
-m_{1,2} = \frac{-\left(K_{p\rightarrow e} V_p + K_{e\rightarrow p} V_e\right) \pm \left(K_{p\rightarrow e} V_p + K_{e\rightarrow p} V_e\right) \sqrt{1 - \frac{4 K_{e\rightarrow p} K_{p\rightarrow k} V_p V_e}{\left(K_{p\rightarrow e} V_p + K_{e\rightarrow p} V_e\right)^2}}}{2 V_p V_e}
\]

(62.)

Now, using a binomial series expansion (for $x \ll 1$):

\[
\sqrt{1 + x} \approx 1 + \frac{x}{2}
\]

(63.)

Thus,

\[
-m_{1,2} = \frac{-\left(K_{p\rightarrow e} V_p + K_{e\rightarrow p} V_e\right) \pm \left(K_{p\rightarrow e} V_p + K_{e\rightarrow p} V_e\right) \left(1 - \frac{2 K_{e\rightarrow p} K_{p\rightarrow k} V_p V_e}{\left(K_{p\rightarrow e} V_p + K_{e\rightarrow p} V_e\right)^2}\right)}{2 V_p V_e}
\]

(64.)

Therefore the rate constants can be approximated as linearly proportional to the flow rates per unit volume. The roots, $m_{1,2}$, can be related to the distribution, $\alpha$ (1/min), and excretion rate constants, $\beta$ (1/min), respectively. These rate constants can be related to the distribution and excretion half-lives:
Where \( T_{1/2\,\text{DIST}} = \frac{\ln 2}{\alpha} \approx \frac{0.693}{\alpha} \) and \( T_{1/2\,\text{EXCRE}} = \frac{\ln 2}{\beta} \approx \frac{0.693}{\beta} \) (65.)

Where,

\[
\alpha \approx \frac{K_{p \rightarrow e} V_p + K_{e \rightarrow p} V_e}{V_p V_e}
\]

\[
\beta \approx \frac{K_{e \rightarrow p} K_{p \rightarrow k}}{(K_{p \rightarrow e} V_p + K_{e \rightarrow p} V_e)}
\]

(66.)

In the case of simple diffusive transport, where the influx and efflux between the plasma and EES compartments is the same (i.e. \( K_{e \rightarrow p} = K_{p \rightarrow e} = K_{p \rightarrow e} \)), the rate constants become:

\[
\alpha \approx \frac{K_{p \rightarrow e} (V_p + V_e)}{V_p V_e}
\]

\[
\beta \approx \frac{K_{p \rightarrow k}}{V_p + V_e}
\]

(67.)

The contrast agent concentration in the plasma, \( C_p(t) \), can now be given physiological meaning and can be written as:

\[
C_p(t) = D \left( a_1 e^{-\alpha t} + a_2 e^{-\beta t} \right)
\]

(68.)
Where \( D \) (mmole/kg) is the dose and \( a_{1,2} \) (kg/L) are the normalized concentration amplitudes for unit dose. This expression means that the contrast agent concentration in the plasma should start at a peak concentration and then biexponentially decay due to distribution and excretion of the contrast agent. In other words, the initial condition on the plasma compartment is that at time, \( t = 0 \), the concentration is a maximum equal to the dose over the plasma volume (i.e \( C_p(t = 0) = D/V_p \)). We will use this initial condition to solve for the amplitudes, \( a_{1,2} \) of Equation (68). NOTE: From Equation (60), \( A_1 = D \times a_1 \) and \( A_2 = D \times a_2 \).

Half of the two compartment model has been solved, now let us solve the second half, the EES compartment's contrast agent concentration, using Equation (57). The initial condition is that the EES compartment's contrast agent concentration is zero at time, \( t = 0 \) (i.e \( C_e(t = 0) = 0 \)) (see Appendix III). This yields:

\[
C_e(t) = \frac{K_{p \rightarrow e}}{V_e} D \left[ \frac{a_1}{K_{e \rightarrow p} - \alpha} e^{-\alpha t} + \frac{a_2}{K_{e \rightarrow p} - \beta} e^{-\beta t} - \left( \frac{a_1}{K_{e \rightarrow p} - \alpha} + \frac{a_2}{K_{e \rightarrow p} - \beta} \right) e^{-\frac{K_{e \rightarrow p}}{V_e} t} \right]
\]

(69.)

For the simple diffusive transport case \( (K_{t \rightarrow p} = K_{p \rightarrow t} = K_{p \rightarrow e}) \), this expression becomes:

\[
C_e(t) = D \left[ \frac{a_1}{1 - \frac{V_e \alpha}{K_{p \rightarrow e}}} e^{-\alpha t} + \frac{a_2}{1 - \frac{V_e \beta}{K_{p \rightarrow e}}} e^{-\beta t} - \left( \frac{a_1}{1 - \frac{V_e \alpha}{K_{p \rightarrow e}}} + \frac{a_2}{1 - \frac{V_e \beta}{K_{p \rightarrow e}}} \right) e^{-\frac{K_{p \rightarrow e}}{V_e} t} \right]
\]

(70.)
The concentration amplitudes, $a_{1,2}$, must be determined for both the plasma and EES compartments. To determine the amplitudes, use the plasma compartment’s initial boundary condition to get:

$$a_1 + a_2 = \frac{1}{V_p}$$

(71.)

This condition is also the pharmacological definition of plasma volume$^{42}$. Using this initial condition, plugging Equations (66), (68) and (69), into Equation (56), and using the assumption that $K_{p\rightarrow e} \gg K_{p\rightarrow k}$ and $K_{e\rightarrow p} \gg K_{p\rightarrow k}$, we find that:

$$a_1 = \frac{K_{e\rightarrow p} V_e + (K_{p\rightarrow e} - K_{e\rightarrow p}) V_p}{V_p (K_{p\rightarrow e} V_p + K_{e\rightarrow p} V_e)}$$

$$a_2 = \frac{K_{e\rightarrow p}}{(K_{p\rightarrow e} V_p + K_{e\rightarrow p} V_e)}$$

(72.)

For the case of simple diffusive transport ($K_{e\rightarrow p} = K_{p\rightarrow e} = K_{p\rightarrow e}$), the amplitudes reduce to:

$$a_1 = \frac{V_e}{V_p (V_p + V_e)}$$

$$a_2 = \frac{1}{V_p + V_e}$$

(73.)

Now that the plasma and EES compartments’ equations have been determined, an equation can be set up for contrast agent concentration in the tumor. The tumor compartment contrast agent concentration can be written as$^{8,9}$:
\[ C_t(t) = v_p C_p(t) + v_e C_e(t) \]  \hspace{1cm} (74.)

Where \( C_t \) (mM) is the contrast agent concentration in the tumor compartment, \( v_e \) is the tumor's EES volume fraction permeated by the agent, and \( v_p \) is the tumor's plasma volume fraction. Essentially, the tumor compartment is composed of fractions of the plasma and EES compartments. Substituting the derived contrast agent concentration expressions for the plasma and EES compartments, that is Equations (67) (68) (70) and (73), into Equation (74) and noting that the flow rates per unit concentration of the EES of the tumor are different from those averaged for the whole body\(^8,9\). That is, the flow rates incorporated in \( a_{1,2}, \alpha \) and \( \beta \) are different from those in the tumor compartment expression (this is signified by replacing the subscript \( e \) with a \( t \)), we find:

\[
C_t(t) = D \left[ a_1 \left( v_p + \frac{v_e K_{p \to t}}{V_t} - \alpha \right) e^{-\alpha t} + a_2 \left( v_p + \frac{K_{p \to t}}{V_t} - \beta \right) e^{-\beta t} - \left( a_1 v_e \frac{K_{p \to t}}{V_t} - \alpha \right) e^{-\frac{K_{t \to p}}{V_t}} - \left( a_2 v_e \frac{K_{p \to t}}{V_t} - \beta \right) e^{-\frac{K_{t \to p}}{V_t}} \right]
\]  \hspace{1cm} (75.)

Where \( V_t \) (L/kg) is the EES volume permeated by the agent of the tumor per unit mass of the tumor, \( K_{t \to p} \) (L/kg·min) is the flow rate per unit volume from the tumor to the plasma compartment, \( K_{p \to t} \) (L/kg·min) is the flow rate per unit volume from the plasma to the tumor compartment. Furthermore, using the given definition of \( v_e \), it must be true that,

\[
v_e = \frac{\text{volume of the tumor's EES}}{\text{total volume of the tumor}} = \frac{V_t}{V_T}
\]  \hspace{1cm} (76.)
$V_T$ (L/kg) is the tumor volume per unit mass of tumor (i.e. the inverse density of the tumor). For the simple diffusive transport case ($K_{t-p} = K_{p-t} = K_{p-e}$), we can simplify Equation (75) into:

$$C_i(t) = D a_i \left( v_p + \frac{v_c}{1 - \frac{v_c}{K_{p-e}}} \right) e^{\alpha t} + a_2 \left( v_p + \frac{v_c}{1 - \frac{v_c}{K_{p-t}}} \right) e^{\beta t} - \left( \frac{a_1 v_c}{1 - \frac{v_c}{K_{p-e}}} + \frac{a_2 v_c}{1 - \frac{v_c}{K_{p-t}}} \right) e^{-\frac{k_{p-e}}{v_c}}$$

(77.)

In some tumors $v_p$ is very small ($v_c \gg v_p$), thus we can approximate

$$C_i(t) = D a_i \left( v_c \frac{\alpha}{1 - \frac{v_c}{K_{p-e}}} \right) e^{-\beta t} + a_2 \left( v_c \frac{\alpha}{1 - \frac{v_c}{K_{p-t}}} \right) e^{\beta t} - \left( \frac{a_1 v_c}{1 - \frac{v_c}{K_{p-e}}} + \frac{a_2 v_c}{1 - \frac{v_c}{K_{p-t}}} \right) e^{-\frac{k_{p-e}}{v_c}}$$

(78.)

Although it is simplest to find the concentration in the tumor compartment from Equation (74) and simplifying with special cases, an ODE can also be written for the tumor compartment:

$$v_c V_T \frac{dC_c(t)}{dt} = K_{p-t} C_p(t) - K_{t-p} C_c(t)$$

(79.)

We can plug Equation (74) into this expression to get:
\[ \frac{dC_{h}(t)}{dt} - v_p \frac{dC_{p}(t)}{dt} = \frac{K_{p \rightarrow a}}{V_T} C_{p}(t) - \frac{K_{a \rightarrow p}}{v_e V_T} \left( C_{h}(t) - v_p C_{p}(t) \right) \] (80.)

Solve this ODE (see Appendix III) by plugging in Equation (68) and applying the initial condition that agent concentration in the tumor at time, \( t = 0 \), is the tumor plasma volume fraction, \( v_p \), of the initial dose diluted over the plasma volume (i.e. \( C_{h}(t = 0) = v_p D/V_p \)) gives us Equation (75). Another method, for small \( v_p \) and the simple diffusive case, expresses the tumor compartment concentration as a simplified ODE\(^5, 8\):

\[ V_T \frac{dC_{h}(t)}{dt} = K_{p \rightarrow a} \left( C_{p}(t) - \frac{C_{h}(t)}{v_e} \right) \] (81.)

Solve this ODE (see Appendix III) by plugging in Equation (68) and applying the initial condition that no agent is the tumor at time, \( t = 0 \) (i.e. \( C_{h}(t = 0) = 0 \)) gives us Equation (78). All methods and notation are consistent with one another.

Our pharmacokinetic analysis, like all others, makes assumptions about physiology of the test animal and the tumors they express in order to simplify the applied model. What follows is a summary of the assumptions made in our analysis (these assumptions will be discussed in the Discussion and Conclusions):

1. The initial contrast agent concentration, post-injection in the animal’s plasma was equal to the total prepared dose over the average plasma volume of a rat (i.e. the initial condition of the plasma compartment). All concentrations were some percentage of this dose.
3. A short transit time through the tumor EES.

4. Fast exchange of water within each compartment\(^8, 43\).

5. Simple diffusive transport was assumed making flow rates between two compartments isodirectional or the same in both directions (i.e. influx and efflux are the same).

6. The liver is not an excretory mechanism for removing the agents from the body.

7. Although the kidneys, spleen and liver are the most permeable and highly vascularized areas of the body, the permeability and surface area of the capillaries in the EES of the body is much greater than that of the kidneys (i.e. \( K_{p+e} \gg K_{p+k} \)).

8. The initial contrast agent concentration, post-injection in the tumor ROI was zero.

9. The plasma volume fraction of the tumor is much less than the EES volume fraction of the tumor (i.e. \( v_p \ll v_e \)).

10. The permeability-surface area product and EES volume fraction across a selected tumor ROI were assumed to be uniform. That is, the selected ROI was a homogeneous area of the tumor.

11. Time invariance of parameters. All measured and fitted parameters in the signal intensity to contrast agent concentration as well as the pharmacokinetic model remained unchanged during the time span of the experiment. Only the concentration in the compartments changed over time\(^8, 43\).
Materials and Methods

MRI: Animals

Female Sprague Dawley rats with N-ethyl-N-nitrosoourea (ENU) induced mammary tumors were imaged by a multislice FLASH sequence ($\Theta = 80^\circ$, TR = 70 ms, TE = 6 ms, ACQ = 0.85 min, FOV = 300 mm, slice thickness = 3.0 mm) on a 1.5 Tesla Siemens MR system at the St. Francis Medical Center, Peoria, IL. Each rat was initially imaged under anesthesia (1 mL/kg Ketamine injection), pre- and post-injection of a bolus of Gd-DTPA (0.3 mmoles/kg dose) followed, 24 hours later, by PAMAM-TU-DTPA G = 4 (polyamidoamine-thiourea-diethylene-triamine-pentaacetic acid generation 4) dendrimer (0.0576 mmoles/kg dose). Following the image collection, regions of interest were selected from the tumors and the signal intensity was measured as function of time. The signal intensity of the heart, liver and a fiduciary (a vial of Gd-DOTA) were also measured for all time points. This image data gives signal intensities of specific regions of interest as a function of time. From this data we found the normalized signal intensity as a function of time, $S/S_0(t)$, that we would convert to concentration data and analyze with the two compartment model.

MRI: Contrast Agent Concentration Curve

The data for the concentration standard curve, Equation (41), used to convert normalized signal intensity into contrast agent concentration of the tumor, was generated by imaging serial dilutions of Gd-DTPA in Sephadex gel beads (G-50-150, Pharmacia, Sweden). The concentrations used were 7.4, 4.0, 2.0, 0.99, 0.49, 0.24, 0.12, and 0.06 mM of Gadolinium as measured by inductively coupled plasma (ICP) mass spectrometry at the Hazardous Waste Research Center (HWRC) at the University of Illinois. Vials of the serial dilutions were arranged in different geometries around a rat in
the magnet and imaged under the same conditions as above. From this data we would fit the coefficients of the concentration calibration curve, Equation (41).

$T_1$-Studies

Additional measurements for correcting the concentration calibration curve were necessary in the study. In order to scale the Gd-DTPA signal intensity data, the longitudinal relaxation time at bore temperature and 37 °C was measured on the 1.5 Tesla Tec Mag system at the Beckman Institute, University of Illinois. First, the samples were heated to 37 °C in a water bath. They were quickly moved from the bath to the coil and a fast (less than 2 minutes) T$_1$ inversion recovery (IR) sequence was used to determine the longitudinal relaxation times of the serial dilutions (same concentrations as above) of Gd-DTPA in Sephadex gel beads. The longitudinal relaxation rate versus concentration was plotted and from the slope the relaxivity, r$_1$, and longitudinal relaxation rate in the absence of paramagnetic ion, 1/T$_{10}$, at 1.5 T was determined. The tissue model was done at both bore temperature and 37 °C to scale the signal intensity data gathered for the concentration calibration curve.

In order to scale concentration data for the dendrimer agent investigated, field cycling nuclear magnetic relaxation dispersion (NMRD) profiles (on the Biomedical Magnetic Resonance Laboratory’s IBM Field Cycling Relaxometer, UIUC) were obtained of the dendrimer agent in HEPES at 37 °C. This data was used for correcting the concentration calibration curve data of Gd-DTPA to that of the dendrimer agent.

Applying the Pharmacokinetic Model

Corrected contrast agent concentrations of the magnetic resonance image data of the animals were analyzed by a program (see Appendix I) written in MATLAB (The MathWorks Inc., Natick, MA). First, the normalized signal intensities of slices through the heart were converted to blood plasma contrast agent concentrations by Equation (43).
The coefficients, $C_{1,2}$, were calculated from the repetition time, the measured relaxivity of the agent in question, and a $T_{10 \text{BLOOD}} = 1.2 \text{ sec}^{44}$. This calculated plasma concentration was scaled using Equation (50), where the total dose loaded in the syringe and a theoretical plasma volume of $0.0350 \text{ L/kg}$ were assumed$^{42}$. This concentration data was fit by the plasma curve, Equation (68), for the parameters $a_{1,2}$, $\alpha$ and $\beta$ by nonlinear least-squares data fitting by the Gauss-Newton method$^{45}$. From these parameters the pharmacokinetic coefficients $K_{p \rightarrow e}$, $K_{p \rightarrow k}$, $V_e$, and $V_p$ were calculated by Equations (67) and (73).

From selected slices, signal intensities were temperature scaled using Equation (53). The temperature corrected signal intensities of the vials were plotted versus the concentration of the contrast agent. From this plot, the signal intensity of the reference samples were compared to the pre-injection signal intensity of the tumor being investigated. A relative concentration was determined by taking the signal intensity of the tumor and from the plot above converting into a concentration of contrast agent in the reference curve. This was the relative concentration that was used to scale the calibration curve, by subtracting the relative concentration from the vial concentrations and discarding negative values. That is, the pre-injection signal intensity of the tumor was correlated to a concentration of zero. The temperature scaled, normalized signal intensity was plotted versus the scaled concentrations. This data was fitted according to Equation (41) by nonlinear least-squares data fitting by the Gauss-Newton method$^{45}$. For conversion, the averaged fitted coefficients, $C_{1,2}$, of the selected slices from all the geometries were used. Since the pre-injection signal intensity was specific for each tumor the coefficients, $C_{1,2}$, of Equation (41) was specific to each tumor. Using the $C_{1,2}$ determined above, the concentration of the tumor was determined by Equation (43). The initial condition was forced by setting $C_t(t = 0) = 0$. This was fit with Equation (76) and (78) for $v_e$ and $K_{p \rightarrow e}/V_t$ by nonlinear least-squares data fitting by the Gauss-Newton method$^{45}$.
Results

$T_1$ Studies

The results of the $T_1$ measurements are necessary for the conversion of the contrast agent calibration curve due to differences in temperature or agent type. These values are summarized in Table 1. As expected, $T_{10}$ changes little due to temperature variations. Notice, that the relaxivity of the PAMAM-TU-DTPA $G = 4$ is roughly 5.5 times that of the Gd-DTPA at the same temperature.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>$r_1$ (1/mM·sec)</th>
<th>$T_{10}$ (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gd-DTPA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5 T (60 MHz)</td>
<td>7.92</td>
<td>2.39</td>
</tr>
<tr>
<td>bore temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gd-DTPA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5 T (60 MHz)</td>
<td>4.92</td>
<td>2.05</td>
</tr>
<tr>
<td>37 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAMAM-TU-DTPA $G = 4$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5 T (60 MHz) 37 °C</td>
<td>27.1</td>
<td>2.05</td>
</tr>
</tbody>
</table>

Table 1: Summary of experimentally determined $r_1$ and $T_{10}$

Contrast Agent Concentration Curve

The conversion of the signal intensities of the tumor ROI were done separately for each tumor region. The mean fitted parameters are summarized in Table 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Gd-DTPA</th>
<th>PAMAM $G = 4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_1$</td>
<td></td>
<td>0.88 ± 0.01</td>
<td>0.86 ± 0.02</td>
</tr>
<tr>
<td>$C_2$</td>
<td>1/mM</td>
<td>0.66 ± 0.07</td>
<td>4 ± 1</td>
</tr>
</tbody>
</table>

Table 2: Concentration calibration curve fitted parameters
From the fitted parameters (see Table 2), observe that $C_1$ is roughly the same for the conversions of both agents, since $C_1$ is fundamentally based on pulse sequence parameters which were not changed between agent administration. On the other hand, the parameter $C_2$ is linearly proportional to the relaxivity of the agent. This means that the fitted $C_2$ of the PAMAM-TU-DTPA $G = 4$ is $6 \pm 2$ times that of the $C_2$ for Gd-DTPA. Since the extrapolated relaxivity at 1.5 T and 37 °C from nuclear magnetic resonance dispersion (NMRD) of the PAMAM-TU-DTPA $G = 4$ is 27.1 $1/mM \cdot sec$; the measured relaxivity under similar conditions of the Gd-DTPA is 4.92 $1/mM \cdot sec$. That is, a 5.5 fold difference between the relaxivities. The signal intensity data as function of the contrast agent concentration of the Sephadex tissue models is fitted by Equation (41) to find $C_{1,2}$ shown in Figures 12 and 13.
These parameters only quantify the curve and tell us little about the actual pharmacokinetic behavior of the agents in the body. The F-test of the fit yielded an $p < 0.001$. To physiologically and pharmacokinetically quantify the parameters, Equations (65), (67), and (73) are used for both agents, as shown in Table 4.

![Graph](image)

**Figure 13:** Concentration calibration curve for PAMAM-TU-DTPA $G = 4$

**Plasma Compartment Model**

The fitted parameters for the biexponential plasma concentration behavior given by Equation (68) are shown in Table 3.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Gd-DTPA</th>
<th>PAMAM $G = 4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_1 (= D \times a_1)$</td>
<td>mM</td>
<td>4.96</td>
<td>0.707</td>
</tr>
<tr>
<td>$A_2 (= D \times a_2)$</td>
<td>mM</td>
<td>3.38</td>
<td>0.967</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>1/min</td>
<td>0.384</td>
<td>0.0929</td>
</tr>
<tr>
<td>$\beta$</td>
<td>1/min</td>
<td>0.0275</td>
<td>0.0074</td>
</tr>
</tbody>
</table>

**Table 3:** Plasma compartment fitted parameters ($p < 0.001$)
These parameters only quantitate the curve and tell us little about the actual pharmacokinetic behavior of the agents in the body. The F-test of the fit yielded an \( p < 0.001 \). To physiologically and pharmacokinetically quantitate the parameters, Equations (65), (67) and (73) are used for both agents, as shown in Table 4.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Gd-DTPA</th>
<th>PAMAM G = 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_e )</td>
<td>L/kg</td>
<td>0.0528</td>
<td>0.0251</td>
</tr>
<tr>
<td>( V_p )</td>
<td>L/kg</td>
<td>0.0360</td>
<td>0.0344</td>
</tr>
<tr>
<td>( K_{p-ve} )</td>
<td>L/kg*min</td>
<td>0.0082</td>
<td>0.0013</td>
</tr>
<tr>
<td>( K_{p-k} )</td>
<td>L/kg*min</td>
<td>0.0024</td>
<td>0.00044</td>
</tr>
<tr>
<td>( T_{1/2\text{ DIST}} )</td>
<td>min</td>
<td>1.81</td>
<td>7.46</td>
</tr>
<tr>
<td>( T_{1/2\text{ EXCRE}} )</td>
<td>min</td>
<td>25.2</td>
<td>93.7</td>
</tr>
</tbody>
</table>

Table 4: Calculated pharmacokinetic parameters of contrast agents in rats

Figure 14: Plasma curve for Gd-DTPA
First, let us explore the physiologically meaningful values. Recall, that the average rat has a plasma volume, $V_p = 0.0350 \text{ L/kg}^{42}$. Both agents will permeate the entire vasculature, since they are much smaller than even the smallest capillary. The calculated plasma volumes are close to the reported average value. As expected the EES volume, $V_e$, of the PAMAM-TU-DTPA $G = 4$ is smaller than the plasma volume, since it is a high molecular weight agent, that is a vascular or blood pool agent. Conversely, the EES volume of the Gd-DTPA is larger than the plasma volume, since Gd-DTPA is smaller and more permeable allowing it to spread or permeate more of the EES.

![Figure 15: Plasma curve for PAMAM-TU-DTPA $G = 4$](image)

Now, let us examine the pharmacokinetic values of the plasma compartment equation. The excretion half-life, $T_{1/2 \text{ EXCRE}}$, of Gd-DTPA has been measured by other groups$^{46, 47}$. The fitted value obtained for our Gd-DTPA study was 25.2 min, falling
within the range of these other measurements. Furthermore, the shorter distribution half-life, $T_{1/2 \text{dist}}$, of the Gd-DTPA makes physiological sense, since the agent has time to be distributed through the organism and detected by MRI, before being removed by the kidneys. The longer half-lives of the PAMAM-TU-DTPA $G = 4$ also seem reasonable, since a larger agent takes longer to both be distributed through and removed from the body. The plasma compartment contrast agent concentration calculated from Equation (43) and the fit, by Equation (78), are shown in Figures 14 and 15 for Gd-DTPA and PAMAM-TU-DTPA $G = 4$, respectively.

**Tumor Compartment Model**

The tumor compartment parameters are summarized in Table 5. The F-test of the fit yielded a $p < 0.001$.

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Parameter</th>
<th>Units</th>
<th>Gd-DTPA</th>
<th>PAMAM $G = 4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$K_{p,e}/V_T$</td>
<td>1/min</td>
<td>0.014</td>
<td>0.0086</td>
</tr>
<tr>
<td></td>
<td>$v_e$</td>
<td></td>
<td>0.12</td>
<td>0.013</td>
</tr>
<tr>
<td>1 Upper</td>
<td>$K_{p,e}/V_T$</td>
<td>1/min</td>
<td>0.018</td>
<td>0.0052</td>
</tr>
<tr>
<td></td>
<td>$v_e$</td>
<td></td>
<td>0.13</td>
<td>0.015</td>
</tr>
<tr>
<td>2</td>
<td>$K_{p,e}/V_T$</td>
<td>1/min</td>
<td>0.058</td>
<td>0.0060</td>
</tr>
<tr>
<td></td>
<td>$v_e$</td>
<td></td>
<td>0.094</td>
<td>0.023</td>
</tr>
<tr>
<td>3</td>
<td>$K_{p,e}/V_T$</td>
<td>1/min</td>
<td>0.036</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>$v_e$</td>
<td></td>
<td>0.092</td>
<td>0.0095</td>
</tr>
<tr>
<td>3 ROI</td>
<td>$K_{p,e}/V_T$</td>
<td>1/min</td>
<td>0.043</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>$v_e$</td>
<td></td>
<td>0.098</td>
<td>0.044</td>
</tr>
<tr>
<td>6</td>
<td>$K_{p,e}/V_T$</td>
<td>1/min</td>
<td>0.027</td>
<td>0.0046</td>
</tr>
<tr>
<td></td>
<td>$v_e$</td>
<td></td>
<td>0.092</td>
<td>0.0077</td>
</tr>
</tbody>
</table>

**Table 5:** Tumor compartment fitted parameters ($p < 0.001$)
Using the fitted parameters, $C_{1,2}$, for the concentration calibration curve and the plasma compartment parameters, $a_{1,2}$, $\alpha$, and $\beta$, the parameters for the tumor compartment expression, Equations (76) and (78), can be fitted for. The results show that a more permeable tumor will have a higher flow rate between the plasma and tumor EES, $K_{p^\text{ext}}$, (i.e. the permeability-surface area of the tumor), shown by a short contrast agent concentration rise time in the tumor compartment curve. That is, the greater the absolute value of the slope of the peak of the concentration in the tumor, the greater the permeability-surface area product.

![Graph showing concentration over time for tumor #1 with Gd-DTPA for the whole tumor (■) and its ROI (○)](image)

**Figure 16**: Fit of tumor #1 with Gd-DTPA for the whole tumor (■) and its ROI (○)

For the low molecular weight agent, a low permeable tumor has a tumor volume normalized flow rate between plasma and tumor EES, $K_{p^\text{ext}}/V_T$, of between 0.01 and 0.02
min^{−1} (e.g. tumors 1 and 6), a highly permeable tumor has a $K_{p}\cdot V_T = 0.04 - 0.06$ min^{−1} (e.g. tumors 2 and 3). In the case of the high molecular weight agent, low permeable tumors have a $K_{p}\cdot V_T = 0.005 - 0.008$ min^{−1} (e.g. tumors 1 and 6) while a highly permeable tumor (e.g. tumor 3) had a $K_{p}\cdot V_T = 0.01 - 0.04$ min^{−1}. The range of fitted $K_{p}\cdot V_T$ values for Gd-DTPA lies within other reported studies for mammary tumors\textsuperscript{48, 49}. This means that the dendrimer agent can be used to distinguish greater differences in permeability. The flow rate between plasma and tumor normalized to total tumor volume, $K_{p}\cdot V_T$, is related to the permeability-surface area product and can be thought of as a distribution rate constant of the agent in the tumor EES. Since the surface area and the total tumor volume are the same for both agents, the differences in $K_{p}\cdot V_T$ are exclusively due to permeability differences of the agents in the specific tumors. The fitted $K_{p}\cdot V_T$ values for PAMAM-TU-DTPA G = 4 are less than those of Gd-DTPA, in the same tumor; due to the dendrimer agent’s larger size it is less permeable. So for Gd-DTPA, since the permeability is the same within the tumor, differences in the permeability-surface area product must result from surface area differences. Small permeability-surface area product differences, with Gd-DTPA, between whole and ROI are due to small differences in surface area.
A tumor is made up cells and interstitial space, which includes the EES and plasma volume, connective tissues, and necrotic spaces, etc. We have used the assumption that the plasma volume fraction is negligible, since the fitted plasma volume fraction was found to be $10^{-4}$ to $10^{-3}$ times less than the EES volume fraction. Additionally, necrotic regions do not get permeated by contrast agents. Therefore, if necrotic areas are not averaged in the selected ROI, the general assumption in MR cellularity measurements is that $v_e \approx 1 - v_{\text{cellular}}$

This assumption neglects the necrotic regions, hypoxic regions, and regions of zero flow (high interstitial pressure). Additionally, as shown in our results, the size of the contrast agent determines the EES fraction of the tumor measured. The Gd-DTPA measured EES volume fraction is 2 to 10 times as great as that measured by PAMAM-TU-DTPA ($G = 4$), so the actual EES volume fraction of the tumor is greater than the value measured. Tumor
cellularity, $\nu_{\text{cellular}}$, the fraction of the tumor volume that is made up of cellular volume, has been measured by several techniques, both histological\cite{50, 51, 52} and imaging\cite{53, 54}. Histological experiments on mammary tumor cellularity have measured cellularities from 10% to 80\%\cite{55}. Magnetic resonance experiments report 69% ± 15% cellularity\cite{56}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure18.png}
\caption{Fit of tumor #2 with Gd-DTPA}
\end{figure}

From our data, the EES volume fraction with Gd-DTPA was found to be between 9-13\%. This is within the rather large range of values found by both histological and imaging techniques. The EES volume fraction of the PAMAM-TU-DTPA (G = 4) is smaller than that of Gd-DTPA, because the dendrimer agent is much larger and cannot reach or permeate as much of the tumor EES volume space.
Figure 19: Fit of tumor #2 with PAMAM-TU-DTPA G = 4

The uptake of the two different sized agents, yield different concentrations in separate tumor. But what is more interesting is in the case of tumor #3, where the whole tumor and an ROI of the tumor were investigated. For the two regions the small agent, Gd-DTPA, could distinguish no differences in the tumor heterogeneity, both regions had the same permeability and EES volume fractions. This same tumor and ROI with the larger dendrimer agent showed that the PAMAM-TU-DTPA G = 4 differentiated between the areas of tumor heterogeneity. With the dendrimer agent, the whole tumor and ROI had very different permeability-surface area products and EES volume fractions. This indicates that the dendrimer agents will be useful in in vivo determination of tumor heterogeneity. Indeed, with the low molecular weight agent, the permeability in the ROI was only 16% more than that observed for the whole tumor. However the dendrimer agent’s permeability in the ROI was 3 times that averaged over the entire tumor.
Figure 20: Fit of tumor #3 with Gd-DTPA for the whole tumor (■) and its ROI (○)

The uptake of the two different sized agents, yield different concentrations in separate tumor. But what is more interesting is in the case of tumor #3, where the whole tumor and an ROI of the tumor were investigated. For the two regions the small agent, Gd-DTPA, could distinguish no differences in the tumor heterogeneity, both regions had the same permeability and EES volume fractions. This same tumor and ROI with the larger dendrimer agent showed that the PAMAM-TU-DTPA G = 4 differentiated between the areas of tumor heterogeneity. With the dendrimer agent, the whole tumor and ROI had very different permeability-surface area products and EES volume fractions. This indicates that the dendrimer agents will be useful in in vivo determination of tumor heterogeneity. Indeed, with the low molecular weight agent, the permeability in the ROI was only 16% more than that observed for the whole tumor. However the dendrimer agent’s permeability in the ROI was 3 times that averaged over the entire tumor.
Figure 21: Fit of tumor #3 with PAMAM-TU-DTPA $G = 4$
for the whole tumor (■) and its ROI (○)
Figure 22: Fit of tumor #6 with Gd-DTPA

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Parameter</th>
<th>Gd-DTPA</th>
<th>1/min</th>
<th>PAMAM-TU-DTPA</th>
<th>1/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.056</td>
<td>&lt; 0.001</td>
<td>0.0060</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.094</td>
<td>&lt; 0.001</td>
<td>0.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.058</td>
<td>&lt; 0.001</td>
<td>0.0060</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>0.095</td>
<td>&lt; 0.001</td>
<td>0.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.057</td>
<td>&lt; 0.001</td>
<td>0.0061</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>0.095</td>
<td>&lt; 0.001</td>
<td>0.022</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>0.057</td>
<td>&lt; 0.001</td>
<td>0.0061</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Fitted parameters for tumor #6 for increasing number of time points for both Gd-DTPA and PAMAM-TU-DTPA G = 4

The two-compartment model's behavior was also investigated over longer periods of time, as shown in Table 4 and Figure 24.
Figure 23: Fit of tumor #6 with PAMAM-TU-DTPA G = 4

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Parameter</th>
<th>Units</th>
<th>Gd-DTPA</th>
<th>p</th>
<th>PAMAM G = 4</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 30</td>
<td>$K_{p,s}/V_T$</td>
<td>1/min</td>
<td>0.058</td>
<td>&lt; 0.001</td>
<td>0.0060</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>$v_e$</td>
<td></td>
<td>0.094</td>
<td></td>
<td>0.023</td>
<td></td>
</tr>
<tr>
<td>0 - 60</td>
<td>$K_{p,s}/V_T$</td>
<td>1/min</td>
<td>0.058</td>
<td>&lt; 0.001</td>
<td>0.0060</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>$v_e$</td>
<td></td>
<td>0.095</td>
<td></td>
<td>0.023</td>
<td></td>
</tr>
<tr>
<td>0 - 90</td>
<td>$K_{p,s}/V_T$</td>
<td>1/min</td>
<td>0.057</td>
<td>&lt; 0.001</td>
<td>0.0061</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>$v_e$</td>
<td></td>
<td>0.095</td>
<td></td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>0 - 120</td>
<td>$K_{p,s}/V_T$</td>
<td>1/min</td>
<td>0.057</td>
<td>&lt; 0.001</td>
<td>0.0061</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>$v_e$</td>
<td></td>
<td>0.095</td>
<td></td>
<td>0.022</td>
<td></td>
</tr>
</tbody>
</table>

Table 6: Fitted parameters of tumor #2 for increasing number of time points for both Gd-DTPA and PAMAM-TU-DTPA G = 4

The two compartment model’s behavior was also investigated over longer periods of time, as shown in Table 6 and Figure 24.
Time (min)  Gd-DTPA  PAMAM G = 4

0 - 30

0 - 60

0 - 90

0 - 120

Figure 24: Fits of tumor #2 for increasing number of time points for both Gd-DTPA and PAMAM-TU-DTPA G = 4
Previous studies have investigated the short term agent distribution patterns without looking at long term effects. In both cases the two compartment model's predictions break down past the thirty minute mark. For Gd-DTPA the breakdown is shown as the model undershoots the actual data, there is more contrast agent in the tumor than the model suggests. This is most likely due to a neglected refluxing term. Conversely, for PAMAM-TU-DTPA G = 4 the model overshoots the actual contrast agent concentration in the tumor, which is later followed by undershoot. The initial overshoot is most likely due to neglected agent removal by the liver (a possibility with the high molecular weight dendrimer agent) and the later undershoot due to neglected refluxing.
Discusson

We have adapted and developed several methods of converting signal intensities to contrast agent concentrations. The calculated concentrations have been applied to a two compartment model and produce results that are consistent with the pharmacokinetic behavior based on the animal model and contrast agent size. Many of these techniques have been delineated by other groups. However, we have coupled approaches and made necessary adjustments. For the contrast agent calibration curve we derived the fitting equation from the theoretical signal intensity of a FLASH sequence, showing both the curve before the $T_2$ effect and the linear region for small concentrations. The two compartment model was reviewed; a fundamental nomenclature and general form of the compartments' rates of change were shown. The equations for the plasma and EES compartment were derived, followed by the application of the assumptions to simplify the expressions. With these expressions, we found fitted values which agreed with values reported for physiological measurements on rats. For Gd-DTPA, $V_e = 0.0528$ L/kg, $V_p = 0.0360$ L/kg, and $T_{1/2\text{EXCRE}} = 25.2$ min and for PAMAM-TU-DTPA G = 4, $V_e = 0.0251$ L/kg, $V_p = 0.0344$ L/kg, and $T_{1/2\text{EXCRE}} = 93.7$ min. The same nomenclature and assumptions were applied to the tumor compartment using three approaches to get similar equations for the tumor concentration. The volume fractions of the tumors were also defined, since contrast agents permeate the volume spaces dependent on agent size. This was reapplied in the tumor compartment equation to get physiologically real EES volumes.

The results for the tumors we investigated are for two separate agents. The first agent, Gd-DTPA, is a known standard used in nearly all magnetic resonance imaging contrast agent experiments to compare with literature reported values. We can use these results to look at how our modeling and assumptions agree or disagree with other groups. For Gd-DTPA, $v_e = 9 - 13\%$ and $K_{p-eeq}/V_T = 0.01 - 0.06$ min$^{-1}$. The PAMAM-TU-DTPA G = 4 results are for a new agent that has not previously been investigated. For PAMAM-
TU-DTPA $G = 4$, $v_e = 0.8 - 1\%$ and $K_{p_{\text{eq}}}/V_T = 0.008 - 0.04$ min$^{-1}$. Since both agents were used on the same tumors, measured differences should be based on agent size and charge. Size differences will effect permeability surface area products and tumor EES volume fractions measurements in a predictable fashion; the larger agent will have smaller values, for the same tumor.

The goals of magnetic resonance mammography are to improve sensitivity and characterize tumor specificity. Sensitivity improvements would increase the number of mammary tumors detectable by magnetic resonance, currently 10% of palpable tumors are missed by standard x-ray mammography. Characterizing tumor specificity, that is differentiating benign from malignant neoplasms would be a valuable in vivo tool, currently only surgical biopsy is used clinically. Contrast agents are the answer for both increasing sensitivity and characterizing tumor specificity.

From the results of tumor #3 we can see how the dendrimer agent, PAMAM-TU-DTPA $G = 4$ can differentiate between two regions of tumor heterogeneity that were undetectable by Gd-DTPA. In tumor #3, $K_{p_{\text{eq}}}/V_T$ of the Gd-DTPA differed by 16% between the ROI and the whole tumor, but $K_{p_{\text{eq}}}/V_T$ of the PAMAM-TU-DTPA $G = 4$ for the same two regions differed by 200%. The dendrimer agent is much better suited for distinguishing areas of tumor permeability heterogeneity. The permeability-surface area products of the dendrimers agree with Folkman, who has shown that tumors are heterogeneous in their capillary density and surface area, since only a subset of tumor cells acquire angiogenic activity$^{34, 57}$. The high molecular weight dendrimer agent is a blood pool agent and will have the greatest contrast enhancement in the areas of greatest capillary density and the greatest permeability in regions of neovascularization. This is consistent with Brasch’s hypothesis$^{58, 59}$. These same regions are associated with angiogenesis or capillary formation and growth. Angiogenesis is driven by vascular endothelial growth factor (VEGF) also known as vascular permeability factor (VPF). Areas abundant with
VEGF are the regions of greatest angiogenesis and increased incidence of metastasis. In this way dendrimer agents may be used as an indicator of metastasis or metastatic potential. Future magnetic resonance imaging studies with histological comparisons will be necessary.

In this paper, we have shown that we are theoretically able to relate measured signal intensities to concentration using predetermined longitudinal relaxivities and relaxation rates from known samples. However, in practice, there are differences in relaxation behavior between a prepared contrast agent sample and a specific tissue containing said contrast agent. That is, the longitudinal relaxation time, $T_{10}$, of the tissue will be slightly different between tissues (e.g. plasma, EES, liver, kidney, or specific tumor, etc.) and the prepared, buffered, Sephadex samples. Similarly, the relaxivity, $r_1$, of the contrast agent in tissue will be close to but not exactly the same as the relaxivity measured in our tissue emulating vials. These small variations will be sources of error in our scaling using Equation (53). Furthermore, signal intensity differences will also persist due to these variations between the in vitro relaxation behavior of the prepared contrast agent samples and the in vivo relaxation behavior of the contrast agent in the tissues of the rat. That is, for the same amount of contrast agent in different regions of the body and the prepared contrast agent sample, the signal intensity will be different. Hence, this effect will be a source of error when we calculate the contrast agent concentration from our magnetic resonance image data.

Recall the factors for the extraction of a contrast agent by a tumor from the blood: (a) capillary surface area, $S$, (b) capillary permeability, $P$, (c) capillary blood flow, $F$, (d) transit time of the agent through the tumor interstitium, and (e) the plasma half-life, $T_{1/2\text{DIST}}$. In this study, we applied a pharmacokinetic model that assumes fast uniform blood flow, $F$, a short tumor transit time, and a time invariant $T_{1/2\text{DIST}}$, from which we fitted the time invariant permeability-surface area product, $PS$, of the tumor. These assumptions remove all mass transport effects on the agent. In an environment as heterogeneous as a tumor,
such assumptions are not necessarily valid. Furthermore, the permeability-surface area product and EES volume fraction across a selected tumor ROI were assumed to be uniform. This means that in a heterogeneous tumor, each selected ROI was considered to be homogeneous. Again, not the most valid of assumptions, but such assumptions are usually made in pharmacokinetic analyses with various imaging modalities.

More assumptions were made in order to ease the complexity of the analysis. In our study, the following assumptions were made: (a) the initial contrast agent concentration, post-injection in the animal’s plasma was equal to the total prepared dose over the average plasma volume of a rat, (b) isodirectional agent transport due to simple diffusive transport, (c) the liver is not an excretory mechanism, (d) the EES of the rest of the body is more permeable and vascularized than the combination of the kidneys, spleen and liver, (e) the initial contrast agent concentration, post-injection in the tumor ROI was zero, and (f) the plasma volume fraction of the tumor is much less than the EES volume fraction of the tumor. Of these assumptions, (b), (c), (d), (e) and (f) are typically made in modeling mammary tumors and greatly simplify the complicated mathematics that tries to mimic biological behavior. In this study, assumption (a) was chosen from a number of techniques for quantitating the blood since it gave the most physiologically real values. Essentially, we must ask the question: although the simplified pharmacokinetic equations fit the obtained data, is there perhaps hidden or overlooked information due to all of the assumptions? The answer is most likely yes. However, by applying mass transport theory to contrast agent uptake along with novel magnetic resonance imaging methods, such as FAIR (Flow-sensitive Alternating Inversion Recovery) for determining flow as well as techniques for estimating capillary surface area, we can stop making the assumptions and actually start to quantitate what is happening when an agent is extracted by a lesion.
Conclusions

In this paper, we have shown the theory behind the application of the two compartment model we have used. We have developed methods for relating the time evolution of the signal intensity to the time dependent behavior of the contrast agent concentration in the body. The method used, a concentration calibration curve, was derived from the equation for the FLASH pulse sequence. The concentration calibration curve obeyed the boundary condition of the system and a special case, for low contrast agent concentrations, was shown.

Using the calculated concentrations, we have applied previously derived expressions for the agent behavior in the plasma, EES, and the tumor, and calculated physiologically real values. Recall, for the plasma compartment with Gd-DTPA, $V_e = 0.0528$ L/kg, $V_p = 0.0360$ L/kg, and $T_{1/2 \text{ EXCRE}} = 25.2$ min and with PAMAM-TU-DTPA G = 4, $V_e = 0.0251$ L/kg, $V_p = 0.0344$ L/kg, and $T_{1/2 \text{ EXCRE}} = 93.7$ min. These values are comparable with results presented by other groups and in line with the behavior of agents of differing size.

From the plasma compartment fit, the permeability-surface area products of the tumors and ROI within the tumors were found. Recall, for Gd-DTPA, $v_e = 9 - 13\%$ and $K_{p\text{-eq}}/V_T = 0.01 - 0.06 \text{ min}^{-1}$ and for PAMAM-TU-DTPA G = 4, $v_e = 0.8 - 1\%$ and $K_{p\text{-eq}}/V_T = 0.008 - 0.04 \text{ min}^{-1}$. There is good agreement with our fitted permeability-surface area products and tumor EES volume fractions for Gd-DTPA and those reported by other groups for mammary tumors. The corresponding measurements with the PAMAM-TU-DTPA G = 4 agree well with estimates due to agent size differences. The results show that the macromolecular agent, PAMAM-TU-DTPA G = 4, is superior to Gd-DTPA for determining permeability differences within tumors. This means that macromolecular agents show the potential as a diagnostic standard for determining tumor specificity using magnetic resonance mammography.
Future Work

Tumors are inhomogeneous, that is, they have non-uniform physical characteristics, including oxic, hypoxic, and necrotic regions, with pH, temperature, capillary density, capillary permeability, and capillary blood flow differences. Our current treatment of tumors and tumor ROI assumes that selected areas are uniform. Since we know that this not the case, additional work and analysis is required.

Our study’s approximations greatly simplified the mass transport theory of an agent in two compartments. Mass transport theory governs the temporal and spatial rates of change of the concentration of a species (i.e. a contrast agent) in a system (i.e a living organism or a tumor). This mathematical treatment can be analyzed with finite element analysis, breaking the differential equation of mass transport theory into interconnected pieces of a matrix. A magnetic resonance image is essentially a matrix, with each pixel being an element of that matrix representing a signal intensity, longitudinal relaxation time, transverse relaxation time, flow, concentration of contrast agent (using our calibration curve), or heating, etc. Using the matrix formed by an image, the finite element analysis treatment of mass transport theory, flow information obtained by FAIR, and different macromolecular agents, we can accurately map permeability values. Correlating these maps with the pathology of the tumor, that is, taking a histological slice from the image “slice” and determining the specificity of the tumor, we can show that permeability of macromolecular agents is a diagnostic indicator of metastatic potential.
Appendix I: Code

CAPPharmmodel.m

function pharmaco = CAPPharmmodel(inanimaldata, inconcdata, ...
outanimaldata, outconcdatan, outplasmadata, ...
TR, dose, theoVp, lagtime, droppts, fitpts, ...
heartcol, T10Blood, r1AgentBlood, ...
guessPlasma, ...
tumorcot, T10unk, r1unk, T10kwn, r1kwn, ...
guessTumor, guessConcal)

roidata = dlmread(inanimaldata,'t'); % Read in data
Szero = roidata(1,:); % Find the pre-injection SI's
endpt = length(Szero); % Find # of cols of data
Szero = Szero(3:endpt); % Chop off time and image #

S1endpt = length(roidata); % Find tot # of SI's
SI = roidata(2:S1endpt,3:endpt); % Extract all post-injection SI's

time = roidata(:,1); % Extract time data

time = time(2:S1endpt); % Start from first time point

% Go through and normalize (calc. S/S0)

[yendpt,xendpt] = size(SI);
for xctr = 1:xendpt
    for yctr = 1:yendpt
        scaledS(yctr,xctr) = SI(yctr,xctr)/Szero(xctr);
    end
end
end
end

% Calculate plasma concentration curve from theoretical signal intensity
% of FLASH

C1 = exp(-TR/T10Blood);
C2 = TR*r1AgentBlood;

concPlasma = concCalc(scaledS(:,heartcol),C1,C2);
concPlasma = (dose/theoVp)*(concpPlasma/concpPlasma(1));

% Fit using Gauss-Newton method

[plasma,plasmares,plasmaJac] = ...
nlinfit(time,concpPlasma,'plasmafull.m',guessPlasma);

A1 = plasma(1)
A2 = plasma(2)
alpha = plasma(3)
beta = plasma(4)
Fvaluecalc(concpPlasma,plasmares,4);

a1 = A1/dose;
a2 = A2/dose;

Ve = a1/(a2*(a1+a2))
\[ V_p = \frac{1}{a_1 + a_2} \]
\[ K_{pe} = \alpha \cdot a_1 / (a_1 + a_2)^2 \]
\[ K_{pk} = \beta / a_2 \]

dlmwrite('CAPharmparm', [A1 A2 alpha beta], '\n');

% Plot the plasma models, the raw data and the fits
endind = length(time);
ptimes = [0:1/128:time(endind)];

figure(1)
plot(time,concPlasma,'ko', ... 
ptimes,plasmafull(plasma,ptimes),'k-');
title('Plasma Compartment Curve');
xlabel('Time (min)');
ylabel('Concentration of Gd in the plasma (mM)');
legend('Data','Fit');

% Reorganize the data such that the normalized signal, \( S/S_0 = 0 @ t = 0 \)
% Use the reported lagtime from the data

time = time(1:fitpts) + lagtime;
newtime = zeros(fitpts+1,1);
newtime(2:fitpts+1) = time;
time = newtime;
% Read in the concentration calibration curve data

concalldata = hdfread(inconcdatav);
concepts = concalldata(:,1); % Extract conc and signal data
sigpts = concalldata(:,2:length(concalldata));
tots = size(sigpts);
concepts = concepts(1:tots(1)-droppts,:);
concepts = r1kvn*concepts/r1unk;
sigpts = sigpts(1:tots(1)-droppts,:);
tots = size(sigpts);

tumorfits = length(tumorcol);

% Fit each column of tumor data separately

for numfits = 1:tumorfits
    fprintf('Fitting tumor concentration set %d\n',numfits);
end

% Reset the total concentration calibration curve matrices

beg = 1;
totconcdatav = [0];
totscaled = [0];

% For each concentration calibration curve data set

for ctrcc = 1:tots(2)
    S0 = Szero(tumorcol(numfits));
concdat = [0];  \% Reset the matrices holding
scaled = [0];  \% the correct pieces
tempadjpts = sigpts(:,ctrcc);

\% Find the concentration that agrees with the S0 for this
\% tumor.
\% Build the total concentration calibration curve data

for dcctr = 2:length(tempadjpts)
    S1 = tempadjpts(dctr-1);
    S2 = tempadjpts(dctr);
    C1 = concts(dctr-1);
    C2 = concts(dctr);
    if S1 <= S0 & S2 >= S0
        m = (S2 - S1)/(C2 - C1);
        b = (C2*S1 - C1*S2)/(C2 - C1);
        deltaconc = (S0 - b)/m;
    end
end

newctr = 1;
\% For each concentration...

for ctsri = 1:tots(1)
\% Make sure the adjusted concentration is still REAL
\% if it is, add it to the concdata and scaled matrices
    if concpts(ctsri)-deltaconc >= 0
        concdata(newctr) = ...
        concpts(ctsri)-deltaconc;
    end
end
scaled(newctr) = ...
  tempadjpts(ctrsi)/S0;
  newctr = newctr + 1;
end
end

% Build the total concentration calibration curve data
% from approved data sets
% Add the values found from this iteration to the end of
% the list.
  dun = beg + length(concdata)-1;
  totconcdatum(beg:dun) = concdata;
  totscaled(beg:dun) = scaled;
  beg = length(totconcdatum)+1;

% Fit using Gauss-Newton method on:
%  S/S0 = C1*[C] + 1
% From BC: S/S0 = 1 @ [C] = 0

  scalecoeff = nlinfit(concdatum,log(scaled'), ...

  'fullconcal.m',guessConcal);
  concal(ctrcc,1) = scalecoeff(1);
  concal(ctrcc,2) = scalecoeff(2);
end
C1 = concal(:,1);
C2 = concal(:,2);
avgcoeff(1) = mean(C1);

75
avgcoeff(2) = mean(C2);
stddev(1) = std(C1);
stddev(2) = std(C2);

 avalia
gcoeff

stddev

te

es = [0:1/256:max(jitter)];

% Plot the calibration curve and raw data.
figure(numfits+1)
pconc = [0:1/256:max(totconcdat
plot(totconcdat,:totscaled,'ko',...
pconc, exp(fullconcal(avgcoeff,pconc)),',k-');
title('Contrast Agent Calibration Curve')
xlabel('Concentration (mM)');
ylabel('S/S_0');
legend('Measured points','Fitted calibration curve',0);

dlmwrite(outconcdat,[totconcdat,totscaled],',n');

function conc = concCalc(normS1,C1,C2)
concTumor = concCalc(scaledS(1:fitpts,tumorcol(numfits)), ...
    avgcoeff(1),avgcoeff(2));
newconcTumor = zeros(fitpts+1,1);
newconcTumor(2:fitpts+1) = concTumor;
concTumor = newconcTumor;

[tumor,tumorres,tumorJac] = ...
    nlinfit(time, concTumor, ...
            'tumormodel.m', guessTumor);
KptOverVT = tumor(1)
ve = tumor(2)
Fvaluecalc(concTumor,tumorres,2);

ptimes = [0:1/256:max(time)];
figure(numfits+tumorfits+1)
plot(time,concTumor,'ko', ... 
   ptimes,tumormodel(tumor,ptimes),'k--');
xlabel('Time (min)');
ylabel('Concentration of Gd in the tumor (mM)');
legend('Data','Fit',0);
dlmwrite(outplasmadata, [time' concPlasma'], '
');
dlmwrite(outanimaldata, [time' concTumor'], '
');
end

function conc = concCalc(normSI,C1,C2)
conc = -log(1/C1 + (1 - 1/C1)*normSI)/C2;

plasmafull.m
function func = plasmafull(param,time)
   A1 = param(1);
   A2 = param(2);
   alpha = param(3);
   beta = param(4);
   func = A1*exp(-alpha*time) + A2*exp(-beta*time);
fullconcal.m

function normSI = fullconcal(params,conc)

    C1 = params(1);
    C2 = params(2);
    normSI = log(1 - C1*exp(-C2*conc)) - log(1 - C1);


tumormodel.m

function func = tumormodel(param,time)

given = dlmread('CAPharmparam',"n");

    A1 = given(1);
    A2 = given(2);
    alpha = given(3);
    beta = given(4);
    KptOverVT = param(1);

    ve = param(2);
    B1 = A1*ve/(1 - alpha/( KptOverVT /ve));
    B2 = A2*ve/(1 - beta/( KptOverVT /ve));
    B3 = -(B1 + B2);

    func = B1*exp(-alpha*time) + B2*exp(-beta*time) + ...
           B3*exp(-KptOverVT*time /ve);

    Figure 28: Tumor signal intensity data file structure.
Appendix II: Fitting Protocol

Configuration

The first thing the user of this code should check out is the availability of MATLAB on the system they are using. Check with the system administrator about properly setting paths and accessing the software. Then, the user should create a directory in their home directory called "matlab". Within this directory they should place the .m files: CAPharmmodel.m, plasmafull.m, fullconcal.m, and tumormodel.m. Do not place them anywhere else and do not rename them.

Data Preparation

The simplest way to prepare the ROI signal intensity data for analysis is to type it into a spreadsheet program and then export as tab delimited text. Different spreadsheet programs will have different methods for doing this, but exporting data is typically under the File or Edit menus. For the signal intensity behavior of the regions of interest the format chosen shown in Figure 25.

<table>
<thead>
<tr>
<th>Pre-injection</th>
<th>Time</th>
<th>Reference Number</th>
<th>Tumor 1</th>
<th>Tumor 2</th>
<th>...</th>
<th>Tumor n</th>
<th>Heart Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>S₀₁</td>
<td>S₀₂</td>
<td>...</td>
<td>S₀ₙ</td>
<td>S₀H</td>
</tr>
<tr>
<td>Post-injection</td>
<td>t₁</td>
<td>x₁</td>
<td>S₁₁</td>
<td>S₁₂</td>
<td>...</td>
<td>S₁ₙ</td>
<td>S₁H</td>
</tr>
<tr>
<td>...</td>
<td></td>
<td></td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Figure 25: Tumor signal intensity data file structure

Where the time column is the time in minutes since the injection (i.e. the first post-injection point is t₁). The time since injection should increase as we go down the column. The reference number is a user defined number such as an image number, study number or other user defined code. The following columns are the tumor ROI investigated, typically the final column is used for heart ROI signal intensity data. Be sure that each signal intensity corresponds to the time which it occurred. The concentration calibration curve data is prepared much the same way, see Figure 26.
The first column is the measured concentration in each vial. The following columns are measured signal intensities from the vials in different image geometries. Be sure to correspond the correct signal intensity with its concentration. Concentrations should be arranged in ascending order going down the column.

In both these examples the bold grey background is not typed, these are the names of the columns and rows. Transfer these files to the computer where MATLAB is available, using a file transfer protocol (FTP) program such as Fetch. Convert the concentration calibration curve to hyperdensity format (HDF) by using matlab. At the matlab prompt, type:

```matlab
> data = dlmread('filename.TEX', 't')
> hdfwrite('filename.HDF', data)
```

**Executing the Analysis**

Now at the matlab prompt type “CPharmmodel” with all its parameters or write a script that invokes the CPharmmodel.m function. The pieces that you the user must define are:

- `inanimaldata`...the file name of the ROI signal intensity data you created above. This file name must be within quotes (e.g. ‘rattus1.TEX’).
- `inconcddata`...the file name of the concentration calibration curve data that was converted to HDF. This file name must be within quotes (e.g. ‘conccaldata.HDF’).
outanimaldata. the file name that the you wish the time and tumor concentration data points to be exported to. This file name must be within quotes (e.g. ‘outrattus1.TEX’). It is a return delimited text file.

outconcdataline the file name that the you wish the scaled concentration calibration curve signal intensity and concentration points exported to. This file name must be within quotes (e.g. ‘outconc.DE’). It is a return delimited text file.

outplasmadata. the file name that you wish the time and calculated plasma concentration data exported to. This file name must be within quotes (e.g. ‘outplasma1.TEX’). It is a return delimited text file.

TR............. the repetition time of the FLASH sequence in seconds (e.g. 0.080).

dose............. the dose of contrast agent injected in mmole/kg (e.g. 0.3).

theoVp........ the average plasma volume of the animal model injected in L/kg (e.g. 0.0350).

lagtime........ the time between each image in minutes (e.g. 0.85).

droppts........ the number of points to drop, starting from the high end, of the concentration calibration curve, eliminates curvature back toward the x-axis due to T<sub>2</sub> effect (e.g. 0).

fitpts........... the number of points to fit in the tumor model (e.g. 33).

heartcol........ the column number of the heart data, counting the first column of data (i.e. Tumor 1) as column 1 (e.g. 7).

guessPlasma... the initial guesses for the plasma compartment, Equation (68), fit for A<sub>1</sub> (mM), A<sub>2</sub> (mM), α (min) and β (min) (e.g. [4 2 1 log(2)/23]).

tumorcol....... the list of the tumor data sets that you wish to be fitted, counting the first column of data (i.e. Tumor 1) as column 1 (e.g. [1 2]).

T10unk......... the T<sub>10</sub> of the agent you injected in the animal in seconds (e.g. 2.39).
the $r_i$ of the agent you injected in the animal in $1/mM\cdot sec$ (e.g. 7.92).

The $T_{10}$ of the agent used in the concentration calibration in seconds (e.g. 2.05).

The $r_i$ of the agent used in the concentration calibration in $1/mM\cdot sec$ (e.g. 4.92).

guessTumor... the list of initial guesses for the tumor compartment, Equation (78),
fit for $K_{p*}/V_T$ (in 1/min) and $v_e$ (e.g. [0.04 0.09]).

guessConcal... the list of initial guesses for the concentration calibration curve,
Equation (41), fit for $C_1$ and $C_2$ (e.g. $[\exp(-TR/T10unk)TR*r1unk]$).

Output

After running CAPharmmodel.m, matlab should give following results. It will give
the fitted parameters $A_1$ (A1), $A_2$ (A2), $\alpha$ (alpha) and $\beta$ (beta), as well as an F-value with
degrees of freedom for the fit. From these fitted parameters it calculates $V_e$ (Ve), $V_p$ (Vp),
$K_{p*}$ (Kpe), and $K_{p->k}$ (Kpk). The program also displays a plot of the converted
concentration of the plasma compartment and the fitted equation. Next, the program
performs the concentration calibration for the tumors. It displays the average fitted
parameters $C_1$ and $C_2$ (avgcoeff), with their standard deviations (stdev). All the
concentration calibration points are displayed on a second plot, with the curve from the
averaged coefficients. Finally, it fits for $v_e$ (ve) and $K_{p*}/V_T$ (KptOverVT) and displays the
values along with an F-value and degrees of freedom for the fit. A third plot of the
converted concentration data of the tumor ROI and the fitted curve is also displayed.
Appendix III: Mathematics

Given an ordinary differential equation (ODE) of the form:

\[ a' \frac{d^2X(x)}{dx^2} + b' \frac{dX(x)}{dx} + c'X(x) = 0 \]  \hspace{1cm} (82.)

Where \( a' \), \( b' \) and \( c' \) are arbitrary constants and \( X(x) \) is a function of \( x \). An example would be the plasma compartment ODE, Equation (59). The solution is found by assuming a general form:

\[ X(x) = A_1 e^{m_1 x} + A_2 e^{m_2 x} \]  \hspace{1cm} (83.)

This is the same as Equation (60). Substituting Equation (83) into (82):

\[ a' \left( A_1 m_1^2 e^{m_1 x} + A_2 m_2^2 e^{m_2 x} \right) + b' \left( A_1 m_1 e^{m_1 x} + A_2 m_2 e^{m_2 x} \right) + c' \left( A_1 e^{m_1 x} + A_2 e^{m_2 x} \right) = 0 \]  \hspace{1cm} (84.)

Which simplifies to:

\[ A_1 \left( a'm_1^2 + b' + c' \right) e^{-m_1 x} + A_2 \left( a'm_2^2 + b'm_2 + c' \right) e^{-m_2 x} = 0 \]  \hspace{1cm} (85.)

Since the exponential functions are linearly independent, an equivalent expression is:

\[ a'm_{1,2}^2 + b'm_{1,2} + c' = 0 \]  \hspace{1cm} (86.)
The roots, $m_{1,2}$, are found from the quadratic equation:

$$m_{1,2} = \frac{-b' \pm \sqrt{b'^2 - 4a'c'}}{2a'}$$ (87.)

While the coefficients, $A_{1,2}$, are found from the initial or boundary conditions of the problem. Another ODE encountered in pharmacokinetic modeling is of the form:

$$c' \frac{df(x)}{dx} = a' f(x) + b' g(x)$$ (88.)

Where $C$ is a constant of integration. For the particular solution we assume that $f(x)$ is of the same form as $g(x)$. That is,

Once again $a'$, $b'$ and $c'$ are arbitrary constants, while $f(x)$ and $g(x)$ are functions of $x$. First, divide through by $c'$ and define $a = a'/c'$ and $b = b'/c'$:

$$f(x) = C_0 g(x) + C_1 \frac{dg(x)}{dx} + C_2 \frac{d^2 g(x)}{dx^2} + \ldots + C_n \frac{d^n g(x)}{dx^n}$$ (94.)

Where $C_i$ ($i = 0, 1, 2, \ldots, n$) are arbitrary constants. Derivatives of $g(x)$ are taken to the $n$th order.

As a solution for $f(x)$ assume a homogenous, $f_h(x)$, and a particular, $f_p(x)$, solution. That is:

$$f(x) = f_h(x) + f_p(x)$$ (90.)

From this we can form two ODEs:

$$\frac{df_h(x)}{dx} = a f_h(x)$$ (91.)
and

\[ f(x) = C_e(t) \]

\[ g(x) = C_f(t) \]

\[ \frac{df_p(x)}{dx} = a f_p(x) + b g(x) \]  \hspace{1cm} (92.)

The solution to Equation (91) is:

\[ f_h(x) = Ce^{ax} \]  \hspace{1cm} (93.)

Where \( C \) is a constant of integration. For the particular solution we assume that \( f_p(x) \) is of the same form as \( g(x) \). That is:

\[ f_p(x) = C_0 g(x) + C_1 \frac{dg(x)}{dx} + C_2 \frac{d^2g(x)}{dx^2} + \ldots + C_n \frac{d^n g(x)}{dx^n} \]  \hspace{1cm} (94.)

Where \( C_i \) (\( i = 0, 1, 2, \ldots, n \)) are arbitrary constants. Derivatives of \( g(x) \) are taken until the function repeats itself or reduces to a constant. Plugging this assumed solution into Equation (92) allows us to solve for the particular solution. Adding the homogeneous and particular solutions gives us the complete solution, where the coefficient, \( C \), is determined by initial or boundary conditions. This is best illustrated by an example. Let's take the example where we are analyzing the EES compartment, Equation (57):

\[ V_e \frac{dC_e(t)}{dt} = K_p \rightarrow e C_p(t) - K_e \rightarrow p C_e(t) \]  \hspace{1cm} (97.)

Thus,
Due to linear independence of the exponential terms, this breaks down into two simple solutions:

\[ x = t \]
\[ f(x) = C_a(t) \]
\[ g(x) = C_p(t) \]
\[ a = -\frac{K_{e\rightarrow p}}{V_e} e^{-\alpha t} + Da_1 \frac{K_{p\rightarrow e}}{V_e} e^{-\beta t} \]
\[ b = \frac{K_{p\rightarrow e}}{V_e} e^{-\beta t} + Da_2 \frac{K_{e\rightarrow p}}{V_e} e^{-\alpha t} \]

Thus, the homogeneous solution, \( C_{eh}(t) \), from Equation (93), is

\[ C_{eh}(t) = C e^{-\frac{K_{e\rightarrow p}}{V_e}} \] \hspace{1cm} (95.)

Using the solution to the plasma compartment, Equation (68), we know that

\[ C_p(t) = D \left( a_1 e^{-\alpha t} + a_2 e^{-\beta t} \right) \] \hspace{1cm} (99.)

Thus from Equation (94) we know the particular solution, \( C_{ep}(t) \), is

\[ C_{ep}(t) = C_1 e^{-\alpha t} + C_2 e^{-\beta t} \] \hspace{1cm} (96.)

Plugging this into Equation (92) we get:

\[ -C_1 \alpha e^{-\alpha t} - C_2 \beta e^{-\beta t} = -C_1 \frac{K_{e\rightarrow p}}{V_e} e^{-\alpha t} - C_2 \frac{K_{e\rightarrow p}}{V_e} e^{-\beta t} + Da_1 \frac{K_{p\rightarrow e}}{V_e} e^{-\alpha t} + Da_2 \frac{K_{e\rightarrow p}}{V_e} e^{-\beta t} \]

\[ (97.) \]
Due to linear independence of the exponential terms, this breaks down into two simple equations

\[
C_1 \left( \frac{K_{e \rightarrow p}}{V_e} - \alpha \right) e^{-\alpha t} = Da_1 \frac{K_{p \rightarrow e}}{V_e} e^{-\alpha t}
\]

\[
C_2 \left( \frac{K_{e \rightarrow p}}{V_e} - \beta \right) e^{-\beta t} = Da_2 \frac{K_{p \rightarrow e}}{V_e} e^{-\beta t}
\]

(98.)

In other words:

\[
C_1 = \frac{Da_1 \frac{K_{p \rightarrow e}}{V_e}}{\frac{K_{e \rightarrow p}}{V_e} - \alpha}
\]

\[
C_2 = \frac{Da_2 \frac{K_{p \rightarrow e}}{V_e}}{\frac{K_{e \rightarrow p}}{V_e} - \beta}
\]

(99.)

Thus,

\[
C_e(t) = \frac{K_{p \rightarrow e}}{V_e} \left[ e^{-\alpha t} + \frac{a_2}{K_{e \rightarrow p} - \beta} e^{-\beta t} \right] + Ce^{-\frac{K_{e \rightarrow p} t}{V_e}}
\]

(100.)

Now the EES compartments initial condition is that there is no contrast agent in this compartment immediately post-injection (i.e. \(C_e(t = 0) = 0\), thus;
\[
\frac{K_{\text{p} \rightarrow \text{e}}}{V_e} D \left[ \frac{a_1}{K_{\text{e} \rightarrow \text{p}} - \alpha} + \frac{a_2}{K_{\text{e} \rightarrow \text{p}} - \beta} \right] + C = 0
\]

(101.)

Solving for \(C\) and plugging back into Equation (100) gives us Equation (69):

\[
C_e(t) = \frac{K_{\text{p} \rightarrow \text{e}}}{V_e} D \left[ \frac{a_1}{K_{\text{e} \rightarrow \text{p}} - \alpha} e^{-\alpha t} + \frac{a_2}{K_{\text{e} \rightarrow \text{p}} - \beta} e^{-\beta t} - \left( \frac{a_1}{K_{\text{e} \rightarrow \text{p}} - \alpha} + \frac{a_2}{K_{\text{e} \rightarrow \text{p}} - \beta} \right) e^{-\frac{K_{\text{e} \rightarrow \text{p}}}{V_e} t} \right]
\]
References


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