Investigating Escape of Low MW Siloxanes from PDMS Matrix in Aqueous Solution

by

Alicia Cochran

Submitted to the Department of Materials Science and Engineering

On May 10, 2013

In partial fulfillment of the Requirements for the Degree of Bachelor of Science

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Abstract
Knowledge of the oxygen levels in a tumor is a current goal of cancer research due to the importance of hypoxia on tumor growth and treatment. Previous work by the Cima group at MIT has shown that an oxygen sensor made from low molecular weight siloxanes (LMWS) in a PDMS matrix can be implanted during a biopsy and effectively measure oxygen levels in rat tumors with MRI. The Cima group also found that the sensors experienced signal loss over time when stored in air. The purpose of this investigation was to explore the loss of signal over time of the sensor when stored in aqueous solution. The signal reduction over time in environments similar to the body could be approximated by measuring the spin-lattice relaxation times of the sensor in various aqueous solutions. It was hypothesized that diffusion of the LMWS from the sensor to the surrounding environment was the potential cause of the signal loss over time, so the amount of LMWS that escaped into aqueous solution was investigated.

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Introduction

1.1 Oxygen, Tumors, and Treatments

It is well known in the field of cancer research that tumor hypoxia has important implications in growth and treatment. A high hypoxic fraction in tumors can promote metastasis and reduce the efficacy of treatments [1]. A hypoxic tumor is known to be resistant to ionizing radiation; in fact, J. Wang et al. demonstrated that hypoxic tumors require larger doses of radiation compared to non-hypoxic tumors to achieve a similar clinical outcome [2]. Physicians could more effectively tailor treatments to individual patients and individual tumor conditions if they had a reliable, non-invasive method of measuring the pO₂ in the tumor microenvironment.

1.2 Siloxanes as Oxygen Sensors

Siloxanes have been explored in the literature as effective oxygen sensors for magnetic resonance (MR) measurements. Polydimethylsiloxane (PDMS) is currently widely used in medical devices, as it is generally non-toxic and inert. The structure of some siloxanes makes them particularly attractive as an oxygen sensor, due to chain length and side groups. O₂ is a non-polar molecule, and is attracted to the non-polar side groups of some siloxanes, particularly those with methyl side groups. Chains of lower molecular weight have been found to be highly effective at sensing changes in pO₂ with MR measurements. Kodibagkar, Cui, Merritt, and Mason found that hexamethyldisiloxane (HMDSO), with a molecular weight of 162.38 amu, was effective as a dynamic sensor of pO₂ in vivo. Their design involved using injections of liquid HMDSO into tissue or tumors. This technique was effective for the duration of
their measurements, but H35O has a half-life of 35 hours in the body [3], so this method is not desirable for long term monitoring of oxygen levels without repeat injections. Low molecular weight siloxanes are liquids at room temperature, and as such will all run into the longevity problem if used on their own as sensors in the body.

Vassiliou, Liu, and Imaad have addressed this problem by designing a sensor that mixes the durable properties of a higher weight siloxane polymer with the oxygen sensing properties of a low weight siloxane. The sensor designed by Vassiliou et al. is made with dodecamethylpentasiloxane (DDMPS) (Figure 1), a low molecular weight siloxane, dissolved in a highly cross-linked polydimethylsiloxane (PDMS) matrix and thermally cured to form a solid. It is implanted during a routine biopsy and left in place to be read with MRI. Experiments performed by Vassiliou and coworkers have confirmed that PDMS with DDMPS acts as an effective oxygen sensor that can be read using MRI.

\[
\text{CH}_3 - \text{CH}_3 - \text{CH}_3 - \text{CH}_3 - \text{CH}_3 - \text{H}_3\text{C-Si-O-Si-O-Si-O-Si-O-Si-CH}_3
\]

\[
\text{CH}_3 - \text{CH}_3 - \text{CH}_3 - \text{CH}_3 - \text{CH}_3
\]

**Figure 1.** Dodecamethylpentasiloxane (DDMPS), a polymer with 5 siloxane groups and methyl side groups. Picture from Sigma Aldrich.

### 1.3 Partition Coefficients

The partition coefficient of a compound describes solubility of the compound in two immiscible solvents. This is expressed as a ratio of concentrations in each
solvent at equilibrium. The partition coefficient is important for this experiment because it was hypothesized that DDMPS was diffusing from the PDMS matrix into aqueous solutions; however, data on the partition coefficient of DDMPS was not readily available. Being able to extract DDMPS from water into an organic solvent was crucial for this experiment in order to measure the amounts present in solution. Examination of the structure of DDMPS (Figure 1) shows that it is a non-polar molecule, and will likely be hydrophobic, which means it will prefer the organic solvent to the aqueous solvent. The solubility of DDMPS in water and toluene was determined through extraction experiments, which will be described later.

1.4 Relaxation Time

The spin-lattice relaxation time is an important parameter in MR measurements. It is a decay constant for the recovery of the z component of the nuclear spin magnetization towards its thermal equilibrium value. This value is useful in this experiment to illustrate a change in the material over time. The $T_1$ value, when measured at the same place in a sample over time, can show that a change has occurred in the material to allow the relaxation time to increase or decrease. A decrease in $T_1$ will indicate a loss of DDMPS in this experiment all other things being equal.

1.5 Problem Statement

The problem addressed in this thesis is the loss of signal over time of the sensor. Experiments have been carried out by Vassiliou et al. previously that
indicate a reduction in signal strength over time of the sensor, and it has been hypothesized that the LMW siloxanes are escaping the PDMS matrix; however, this hypothesis has not been tested in an aqueous solution, so the effects of an aqueous environment, like that in the body, on the signal strength over time are not known.

Additionally, the effects of an aqueous environment mixed with proteins on the migration of the DDMPS are unknown. If the DDMPS prefers hydrophobic phases, which appears likely from the non-polar structure of the molecule, it might migrate more readily into an aqueous solution with some hydrophobic proteins. This is an important consideration because in the body, the sensor will be exposed to a variety of environments that are not pure water, like blood and tissue.

A variety of measurements need to be taken in order to test for signal loss and to examine the cause. The magnetic signal of H+ ions can be measured in the sample using nuclear magnetic resonance (NMR) and can be monitored over time. This process might be slow to see results, as the migration could take days or weeks to occur appreciably. Another method employed to see if DDMPS is migrating into the solution is to perform extractions of the solutions and test the compounds present.

Several methods of measuring the DDMPS in solution were considered, including HPLC-UV and GC-MS. Access to equipment presented some limitations on what could be measured. Čavić-Vlasak, Thompson, and Smith, in a review article on measuring silicones in biological matrices, found that GC-MS had the best detection limits on low molecular weight siloxanes, with detection possible in the ppm and ppb range \(^\text{[4]}\). Further literature searches revealed previous works using GC-MS to
quantify low concentrations of LMW siloxanes in rat tissue, with detection limits of 10 ppb \cite{5}. Gas chromatography- mass spectrometry was the measurement chosen because the amount of migrated DDMPS was expected to be low.

**Experimental Procedure**

**2.1 Chemicals and Equipment**

The diallyl phthalate (DAP), which was used as an internal standard for GC-MS, and the DDMPS, 97%, were purchased from Sigma Aldrich. The toluene, HPLC grade 99.7%, was purchased from Alfa Aesar. The PDMS was prepared using a Sylgard 184 kit, purchased from Dow Corning. The FBS was purchased from Life Technologies. The PBS, pH 6.5, was purchased from Teknova. The NMR measurements were performed on a NMR-MOUSE probe, using a flat coil that measured 2mm above the coil. The results of these measurements were analyzed using LabVIEW software. The GC oven used was a 6890N Network GC System from Agilent Technologies, equipped with a Rtx-1 Crossbond 100% dimethyl polysiloxane 30.0m in length, 250 in diameter, 1.00 μm pore size column from Restek. The mass spectrometer used was a 5973 Network Mass Selective Detector from Agilent.

**2.2 Sample Preparation**

Sample sensors were prepared using a multi-step process. First, PDMS was prepared using the kit, with 10 parts by weight of the base to 1 part by weight of the curing agent. The mixture was stirred vigorously for 1 minute and then put in a degassing chamber for 10 minutes to remove air bubbles. The PDMS was added to a
20 mL glass vial after degassing with DDMPS at a ratio of 30% PDMS and 70% DDMPS by weight. This mixture was stirred for 10 minutes using a vortexer. The PDMS-DDMPS mixture was then added to 20 mL glass vials in 2mL amounts, forming 2mm thick layers on the bottoms of the vials. These samples were then cured at 80°C for 2 hours. 15mL of various solutions were added to the vials after curing. The solutions added were deionized (DI) water as a control, phosphate buffered saline (PBS), and fetal bovine serum (FBS). These samples were stored in a 37°C chamber to mimic body conditions.

50% and 25% DDMPS samples were prepared in addition to the 70% DDMPS-30% PDMS samples to form a calibration curve for the NMR measurements. These samples had 15 mL of DI water added to them, as the control.

2.3 Nuclear Magnetic Resonance Measurements

The NMR measurements were carried out on a heated coil, at 40°C to keep the samples close to body temperature. The samples were placed in the incubator after they were made for 30 minutes to warm up to 37°C, then brought out one at a time to be measured to minimize heat loss. The glass vial was set on top of the flat coil and a method was run to measure 2mm above the coil, or at the top layer of the solid sample. The relaxation times were calculated using the LabVIEW software. Each sample was returned to the incubator after measurement to wait for the next measurement. The solutions were replaced with fresh solutions once per week during this investigation.
2.4 Gas Chromatography- Mass Spectrometry Measurements

All GC-MS measurements were performed in the DCIF Laboratory. DAP was used as internal standard at 25ppm. All samples were prepared in toluene. The conditions that the samples were separated at were as follows: the injector temperature was set at 280°C, the injector setting was pulsed splitless, the carrier gas was helium, the flow rate was 1mL/min, and the initial temperature of the column was set at 100°C and ramped at a rate of 20°C/min to 320°C. Injections of 1 µL were made using a 10 µL Hamilton Gastight Syringe. The mass spectrometer was operated in the scan mode.

The samples measured with GC-MS were prepared the same way as the samples that were measured using NMR. The difference in the samples is that in the NMR measurements, the solid DDMPS-PDMS is investigated, while in the GC-MS, the liquid phase is investigated for DDMPS that has partitioned into solution. The amount of DDMPS that had diffused into the various solutions was attempted to be quantified.

A calibration curve of concentrations from 0.25 ppm to 50 ppm was prepared by adding a known amount of DDMPS to toluene and performing serial dilutions, then spiking with the internal standard. This was necessary to be able to quantify the amount of DDMPS that was extracted from the samples.

2.5 Extraction

The DDMPS needed to be extracted from the aqueous solution into an organic solvent, toluene, in order to measure the concentration of DDMPS that
partitioned into the solutions. This solvent was chosen due to the GC column available, its solubility with DDMPS and DAP, and its immiscibility with water. Test samples of known concentrations of DDMPS in PBS were prepared to test the efficacy of the extraction method. Initially, samples were prepared of various concentrations of DDMPS in PBS by serial dilution. Then, samples were prepared for extraction by taking 5mL of the DDMPS-PBS solution, adding 5 mL of toluene, shaking for 20 minutes, then extracting using a separatory funnel. The extraction results for this method, which are presented in Table 3, were inconsistent and a second extraction method was attempted. Samples were prepared as previously, using a serial dilution method, then were prepared for the new method of extraction by taking 1mL of the DDMPS-PBS solution and adding 5mL of toluene, shaking for 1 hour, then extracting using a separatory funnel. The extraction results for this method, presented in table 4, were inconsistent again, and a third method was attempted. The DDMPS-PBS solutions for this method were not prepared by serial dilution, but by adding a known volume of known concentration DDMPS in toluene to a vial, evaporating the toluene, adding 1 mL of PBS to achieve the desired ppm concentration, then shaking for 30 minutes. These samples were then prepared for extraction by adding 5mL of toluene, shaking for 1 hour, then extracting using a separatory funnel. The FBS presented a challenge in extraction due to the proteins present in the solution acting as surfactants and creating foam. An effective extraction method for the FBS samples was never determined.
Results and Discussion

3.1 NMR Results

Samples to test on NMR were prepared at 25%, 50%, and 70% DDMPS with DI water as the solution in order to be able to correlate the changing relaxation times with the amount of DDMPS present in the sample. The relaxation time of each of these was measured following the procedure described previously, and these results were used to generate a calibration curve (Figure 2). This curve will help correlate the T1 to concentrations of DDMPS in the sample.

![Calibration Curve NMR](image)

\[ y = -2E-05x + 0.0021 \]
\[ R^2 = 0.99763 \]

**Figure 2.** Calibration curve of the percent of DDMPS vs relaxation rate

The relaxation times of each sample were measured on the day they were prepared, again 2 hours later, and again 2 weeks later to see the effect of time on
potential loss of signal. These data are presented in table 1 and figure 3. No sample showed considerable change during this period.

<table>
<thead>
<tr>
<th></th>
<th>DI Water</th>
<th>PBS</th>
<th>FBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>1036.9988 ms</td>
<td>1023.7899 ms</td>
<td>1037.5631 ms</td>
</tr>
<tr>
<td>After 2 Hours</td>
<td>1026.9637 ms</td>
<td>1007.7409 ms</td>
<td>1037.7632 ms</td>
</tr>
<tr>
<td>After 2 Weeks</td>
<td>1046.6810 ms</td>
<td>1076.2361 ms</td>
<td>1089.9409 ms</td>
</tr>
</tbody>
</table>

Table 1. Relaxation times of samples, all values in milliseconds

Figure 3. Relaxation rates (1/T1) for samples in various media over a two week period.

The relaxation times for each of these samples did not appear to change appreciably during the two week monitoring. The small variances in the times could be attributed to measurement noise. A longer time measurement is needed to verify that the signal changes very little over the period of this study.

The amount of DDMPS in each sample at each measurement was calculated using the calibration curve generated and is presented in Table 2. The value calculated for the control sample of DI water with 70% DDMPS solid at the initial measurement indicates that the calibration curve needs more points to more
accurately quantify the amount of DDMPS in the sample. The apparent increase in
DDMPS after two weeks is likely attributable to both the need for a more accurate
calibration curve and possibly measurement noise. More data needs to be taken to
quantify these results.

<table>
<thead>
<tr>
<th></th>
<th>DI Water</th>
<th>PBS</th>
<th>FBS</th>
</tr>
</thead>
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<tr>
<td>Initial</td>
<td>56.8</td>
<td>56.2</td>
<td>56.8</td>
</tr>
<tr>
<td>After 2 Hours</td>
<td>56.3</td>
<td>55.4</td>
<td>56.8</td>
</tr>
<tr>
<td>After 2 Weeks</td>
<td>57.2</td>
<td>58.5</td>
<td>59.1</td>
</tr>
</tbody>
</table>

Table 2. Percent DDMPS in sample, calculated with calibration curve

3.2 GC-MS Results

A calibration curve was first generated in order to be able to quantify the
amount of DDMPS found in the extracted samples. Known concentrations of DDMPS
in toluene were prepared and spiked with the internal standard, as described
previously. The responses of the DDMPS were re-calculated as a ratio of the
responses of the internal standard in each sample to account for instrument
variation. The concentrations and responses of the DDMPS are presented as a ratio
of concentrations and responses of the internal standard. The calibration data is
presented in Figure 4.
Calibration Curve GC-MS

\[ y = 2.3068x + 0.1656 \]
\[ R^2 = 0.9962 \]

Figure 4. Calibration curve for concentration of DDMPS in toluene

A sample chromatogram is shown in Figure 5. The retention times were attributed to the two molecules present in the solvent by matching the mass spectra of the peaks against literature values. The main characteristic ions of the 10.17-minute peak are 369, 281, 147, and 73, which were confirmed in the literature to be the characteristic ions of DDMPS \[ ^5 \]. The main characteristic ions of the 13.53-minute peak were 189 and 149, which were confirmed in the literature to be the characteristic ions of DAP \[ ^6 \]. The retention time for DDMPS is 10.17 minutes and the retention time for DAP is 13.53 minutes. The mass spectrum of the DDMPS peak is shown in Figure 6.
Figure 5. Chromatogram of 50ppm DDMPS and 25ppm DAP in toluene

Figure 6. Mass spectra of DDMPS
Known concentration samples were prepared in PBS and extracted into toluene, according the procedures described previously, in order to determine the extraction efficiency of the extraction methods. The data for the samples extracted with the first method is presented in Table 3, the data for the second method is presented in Table 4, and the data for the third method is presented in Table 5. The expected concentrations are reduced for the samples in Table 4 and 5 by a factor of 5 because the extraction method diluted the ppm of the samples by a factor of 5.

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Prepared Concentration (ppm)</th>
<th>Expected Concentration (ppm)</th>
<th>Actual Concentration (ppm)</th>
<th>Percent Extracted (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>Below Cal</td>
<td>n/a</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>5</td>
<td>Below Cal</td>
<td>n/a</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>5</td>
<td>0.23</td>
<td>4.6</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>10</td>
<td>0.75</td>
<td>7.5</td>
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<tr>
<td>5</td>
<td>10</td>
<td>10</td>
<td>1.79</td>
<td>17.9</td>
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<td>6</td>
<td>10</td>
<td>10</td>
<td>5.94</td>
<td>59.4</td>
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<tr>
<td>7</td>
<td>10</td>
<td>10</td>
<td>5.38</td>
<td>53.8</td>
</tr>
<tr>
<td>8</td>
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<td>25</td>
<td>25.18</td>
<td>100.72</td>
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<td>9</td>
<td>50</td>
<td>50</td>
<td>58.76</td>
<td>117.52</td>
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Table 3. Extraction data for method 1

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<tr>
<th>Sample #</th>
<th>Prepared Concentration (ppm)</th>
<th>Expected Concentration (ppm)</th>
<th>Actual Concentration (ppm)</th>
<th>Percent Extracted (%)</th>
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</thead>
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<td>40.9</td>
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<td>50</td>
<td>10</td>
<td>4.3</td>
<td>43</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>5</td>
<td>0.34</td>
<td>6.8</td>
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<td>25</td>
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<td>1</td>
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</table>

Table 4. Extraction data for method 2
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<th>Sample #</th>
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<th>Expected Concentration (ppm)</th>
<th>Actual Concentration (ppm)</th>
<th>Percent Extracted (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
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<td>10</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>5</td>
<td>Below Cal</td>
<td>n/a</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>5</td>
<td>Below Cal</td>
<td>n/a</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>5</td>
<td>Below Cal</td>
<td>n/a</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>10</td>
<td>0.67</td>
<td>6.7</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>10</td>
<td>0.61</td>
<td>6.1</td>
</tr>
</tbody>
</table>

*Table 5. Extraction data for method 3*

The data is highly variable and inconsistent in the first extraction method. The amount extracted between samples in the second method is more consistent, but the overall extraction percentage changes highly between different concentrations and is not highly effective. The third method is consistent between samples, but still overall ineffective. Using any of these methods on a sample of unknown concentration would result in data that is impossible to quantitate. The amount of DDMPS in the aqueous samples that were prepared and incubated has not been fully investigated due to the difficulties with these methods and overall time constraints.
Conclusions

The data collected in the NMR experiments provides important implications for this a sensor designed from this material. The relative stability of the relaxation times over the 2 weeks implies that the signal loss over time in various aqueous solutions is minimal, and that little to no DDMPS is diffusing from the solid DDMPS-PDMS into these solutions. This is promising for this sensor, because it implies that it will be useful in the body for long periods of time, and can provide insight into tumor pO₂ over many weeks of treatment without needed any further invasive procedures. This can be an important diagnostic and treatment tool for oncologists, letting them highly tailor treatment plans to individual patients and tumors.

The amount of GC-MS data available to analyze is lacking due to time constraints and difficulties with the procedure; however, the basis for analyzing future data is set up and can easily be applied to data once a consistent and efficient procedure is determined. Even if the extraction method had been perfected, it seems unlikely, given the conclusions drawn from the NMR data, that any large amount of DDMPS would've been found in these solutions. Because the NMR data supported the theory that the DDMPS would likely preferentially stay in the PDMS matrix, the results of the GC-MS measurements aren't necessary to be able to draw the conclusion that signal loss over time is greatly reduced once the material is stored in an aqueous media. The previous loss of signal was observed in samples stored in air, and the loss of DDMPS to the air was likely due to the low molecular weight chain's volatility.
Acknowledgements

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References


