An Analysis of Bisphenol A Leaching from Polycarbonate Plastic by Solid Phase Microextraction

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An Analysis of Bisphenol A Leaching from Polycarbonate Plastic by Solid Phase Microextraction

Honors Thesis

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April 19, 2009
This thesis for honors recognition has been approved for the
Department of **Natural Sciences**.

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Abstract

This experiment encompassed analyzing water samples for Bisphenol A (BPA) from polycarbonate containers with Solid Phase Microextraction (SPME). Standards were used, ranging in concentration from 0ppb-100ppm. Analyses were completed on the gas chromatograph coupled to a mass spectrometer detector using the selected ion monitoring (SIM) mode. SPME was effective as a technique for screening for BPA in water samples stored in polycarbonate containers. Concentrations of standards as low as 1 ppb were detected by GCMS SIM mode. BPA in water bottle samples ranged from 0-.76ppb as determined by a calibration curve generated from standards. The calibration curves showed a consistent logarithmic response with respect to concentration when samples were analyzed in SIM mode. It was concluded that SPME provided a good method for detecting BPA. Other techniques that should be explored are use of standard addition and spiking samples.
INTRODUCTION

HISTORY

In 1891 Bisphenol A (BPA) was first synthesized and since 1936 has been known to be an estrogen derivative. Its link to plastics lies in the fact that it has also been used as a monomer in polycarbonate (PC) plastics and as a plasticizer in Poly Vinyl Chloride (PVC). BPA has also been used as a key ingredient in epoxy resins.

STRUCTURE

The structure of BPA can be seen below as two phenol rings attached to a central carbon also containing two methyl groups. BPA is also known as BPA, 4,4’-(propan-2-ylidene) diphenol, p’-isopropylidenebisphenol and 4,4’-isopropylidenediphenol.

![Figure 1: Above the structure of Bisphenol can be seen.](image)

USE IN PLASTICS

BPA has been used in the manufacturing of plastics since before the 1960s. Worldwide the production of BPA is currently more than 6 billion pounds per year, and since 1980, U.S. production of BPA has increased by nearly five times.
LEACHING

Levels Observed:

The panel at HCRA attempted to investigate levels of BPA at least one order of magnitude below the current Lowest Observed Adverse Effect Level which was seen to be 50 mg/kg-day\(^4\). The reference dose of BPA was 0.05mg/kg-day which is defined as the maximum acceptable oral dose of any toxic substance. The daily intake of BPA in adults within the general population ranges from 0.008-1.5 µg/kg per day\(^5\). This is clearly below the reference dose declared by the USEPA which was verified by vom Saal and Hughes in 2005\(^7\).

Levels Detected:

There has been a wider range BPA levels detected in other parts of the world. In 2005, a paper was published from the National Chung-Hsing University on the leaching of BPA from polycarbonate plastic\(^6\). The range of BPA levels reported was 0.6-3.8 µg/L from a total of five polycarbonate plastic samples\(^6\).

USE OF POLYCARBONATE PLASTIC

In today’s society, it is difficult to go anywhere and not see some sort of polycarbonate plastic. The brightly colored water bottles that are ubiquitous on college campuses and in hiker’s backpacks are generally made from polycarbonate plastic. Polycarbonate has also been used in the manufacturing of baby bottles as well as other consumer products.
HEALTH CONCERNS

As recently as June 11, 2008, the National Toxicology Program released a brief to help provide the public with its conclusions regarding BPA’s possible effects on reproductive health as well as child development. In 2004 a panel was formed by the HCRA to help determine mainly, but not limited to, the effects on reproduction of BPA. This study looked at the hormonal effects of BPA on a variety of animals, including humans, but specifically males. The males were analyzed to better detect variations in estrogen levels. According to The Scientific Committee on Food in 2002 the estimated exposure of humans to BPA was $4.8 \times 10^{-4}$ mg/kg-day whereas the European Union’s assessment in 2003 was that the human exposure is, on average, $9.0 \times 10^{-3}$ mg/kg-day. The panel examined studies as recent as April 2002, and in the end it concluded that more information was needed to determine just what the low dose exposure could mean in the long term.

In 2008 the NTP concluded that there was some concern for the fetal/infant/children population when exposed to high levels of BPA, though the levels of concern were not specified, but there was negligible concern for pregnant women or others not exposed consistently to BPA. The NTP report also included studies on rats which indicated there is clear evidence of adverse developmental effects at high doses, which constitutes exposures above 5mg/kg of body weight per day, of BPA in the form of fetal death, decreased litter size, decreased number of live pups per litter, reduced growth, and delayed puberty in male and female rats.
A study conducted using data from a 2003-2004 National Health and Nutrition Examination Survey examined 1455 adults BPA urinary concentrations. Of those examined higher concentrations of BPA were associated with; cardiovascular disease, diabetes, and clinically abnormal concentrations of the liver enzymes GGT, alkaline phosphatase and lactate dehydrogenase\(^7\). Cell-signaling disruption was also seen from BPA at ingestion exposures as low as 0.23ppt\(^8\). In September, 2008 the Yale School of Medicine reported decreased brain function due to daily BPA exposure equal to the lowest observed adverse effect level (LOAEL) in primates\(^9\). In April 18, 2008 Nalgene announced it was phasing out the production of PC bottles\(^10\).

**TRADITIONAL METHODS OF ANALYSIS**

It is possible to detect BPA using other methods of analysis. HPLC as well as solid phase extraction have both been used in numerous studies to detect BPA. Direct injection GCMS analyses were attempted before changing the current method to solid phase microextraction. The issue when directly injecting samples involved BPA showing up in trace amounts when there was an abundance of BPA present in the standards prepared. Concentrations of BPA could also be analyzed at very low levels via liquid/liquid extraction.

**SPME BACKGROUND**

Solid phase microextraction (SPME) is a highly versatile technique that has been in use since the early 90s\(^11\). It was developed as a method for which solvents were not necessary. It has also been observed that analyses with SPME can yield concentrations in
the parts per trillion range. Since the research at hand was designed to merely detect how much BPA would be present in water bottle samples, having a low detection limit would be an admirable attribute.

**HYPOTHESIS**

The SPME technique can be applied to the detection and quantification of BPA in water samples stored in polycarbonate containers.
EXPERIMENTAL

My method for analysis was based on Chia-Min Chang et al. 2005 but with some modification. A method outlined in *The Journal of Chromatography* was also considered in formulating my method of analysis\(^{12}\). After running the first samples, it became apparent that modification would be necessary in order to best detect the BPA. The following sections illustrate the initial attempts made and the final parameters set for all analysis.

MATERIALS

**SPME:**

Solid phase microextraction was used as a method of analysis. A manual injector with a fiber of 85μm thickness made of polyacrylate (PA) was used for all analyses. Direct injection of samples in preliminary tests yielded a trace response when the concentrations were substantial to warrant a response. SPME was chosen to concentrate what was being injected in order to produce a response in which a calibration curve could be generated.

**GCMS:**

The GCMS that all analyses were run on was an Agilent 6890 GC series and 5973 MSD series. The column with which all samples were run through was a HP-5MS (Crosslinked 5% PHME Siloxane) column with 30mx0.25mmx0.25μm film thickness. A blank run was conducted after each conditioning to determine how much BPA remained on the fiber. It was at this point that the conditioning parameters were altered in order to remove a greater amount of BPA that remained on the fiber which allowed for more accurate sampling. The sample desorption time was set at 3 minutes. Before the GC inlet
was purged the fiber stayed in the inlet for a total of 5 minutes to increase the amount of BPA coming off. The GC inlet was kept at 280°F, and the oven was ramped from 80-300°F at a rate of 20°F per minute which resulted in a total run time of 14 minutes. The observed retention time was 12.32 min, but the peak was located near the end of the run and was broad as well as part of the tailing off of the column. After a response was seen, the run time was increased by adding a hold time of four minutes once 300°F was reached to see if the chromatogram peak could be sharpened. This was successful. Initially, all chromatograms were run in SCAN mode which detects a range of ions. Because of other substances present in the water, a response due to BPA was more difficult to see. So it was determined the select ion monitoring (SIM) mode might provide a better way to see the BPA. Ions 213 and 228 were chosen because they were used in the Chang experiment and are characteristic to BPA fragmenting in the GCMS. A representative chromatogram and mass spectrum are shown for a standard solution containing 10 ppb BPA in DI water in Figures 2 and 3.

![Figure 2: SIM chromatogram including m/z 213 and m/z 228 for a 10 ppb sample of BPA in deionized water.](image-url)
STANDARDS

All glassware was washed and dried before use. Standards were made to generate a calibration curve ranging from 100ppm to 1ppb in 1:10 dilutions. The standard preparation was made with acetone for the 100ppm standard and then diluted with DI water to observe the solubility characteristics of BPA. Standards were made two times. The first of the two standards was made to explore analysis techniques involving the SPME fiber. This set was also made to fine tune the parameters within the Agilent software. The second set of standards was made in order to generate the calibration curve that would be used to determine sample concentration. There was concern surrounding the standards sitting too long. This was solved by a method outlined in the *Journal of Chromatography* which involved re-making the standards on a weekly basis\(^\text{11}\). Rather than remake the standards each week, all standards were run within a week of being made. To try to solve a problem of stagnancy in the standards, the second set of standards was remade, stored at 4°C, and analyzed within three weeks.
SAMPLES

Each bottle was filled with DI water and allowed to sit at room temperature unexposed to any UV light which could have affected the leaching of the BPA. All attempts were made to keep the experiment practical but contained. Because so much of the process involved the development of the method, it seemed necessary to minimize all parameters that could be controlled. Samples were handled in a manner identical to that of the standards to emphasize continuity. Each sample was collected in a 40ml glass vial capped with a Teflon septum and refrigerated until ready for analysis, a method identical to that outlined in the Chang experiments\(^6\).

WATERBOTTLES

The bottles sampled can be seen in Table 1.

<table>
<thead>
<tr>
<th>Brand</th>
<th>Color</th>
<th>Volume</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nalgene</td>
<td>green</td>
<td>500ml</td>
<td>2.5yrs</td>
</tr>
<tr>
<td>Nalgene</td>
<td>light</td>
<td>1000ml</td>
<td>6yrs</td>
</tr>
<tr>
<td>Camelback</td>
<td>black</td>
<td>750ml</td>
<td>3yrs</td>
</tr>
</tbody>
</table>

Table 1: Shown here are the three bottles which samples were taken from throughout this experiment.

Bottles of different ages and colors were used in order to make sample extraction as applicable to everyday life as possible. The colors were not chosen on purpose, nor were the volumes. But as it happened, the differing volumes, colors, and ages provided a wider range of parameters to test than just the type of plastic.
METHOD DEVELOPMENT

PROOF OF PRINCIPLE

BPA was extractable onto the polyacrylate fiber and produced a detectible response in the GCMS as can be seen in the prior figures 2 and 3. After standards were analyzed, a trend was seen where the abundance peaks were increasingly larger as the concentration increased from 0ppb to 100ppm. Some variables that needed to be taken into account were other substances possibly present in water which could hide the BPA peaks. Any variation in method from the time the fiber was exposed to a sample to the time it was ready to be exposed to another sample could also skew results. It was also necessary to analyze the deionized water to see what the SIM response for the BPA ions would be. There was an undetectable amount of BPA present in the deionized water which was assumed to be zero for all future analyses.

SIM MODE

SIM mode was chosen because the response of the GCMS needed to be geared towards the analyte of interest. Since that analyte was BPA, masses 213 and 228 were chosen.

THE FIBER

The fiber also had to be conditioned in order to cleanse it of any remaining BPA. Residual BPA present on the fiber would skew all future analyses. The polyacrylate (PA) fiber conditioning parameters set forth by Supelco in its product insert (2006) were modified from one hour to one hour and fifteen minutes. It was noticed that less residual
BPA remained on the fiber when the time was increased. Of the three polyacrylate fibers used, the first was for testing out the sampling techniques and the GC techniques. The second and third were used for final standard analysis and sample analysis, respectively. The second and third fibers were subject to the same conditioning method as well as storage when not in use. The only difference between the first and last two was that the first had different conditioning and sampling techniques used in order to determine the optimum method.

**ANALYSIS**

**The Standards:**

The SPME apparatus was subjected to de-ionized water to determine if BPA was present in the solvent water; the BPA signal intensity was not statistically different from the noise in the chromatogram, and therefore it was assumed that any BPA in the water would have a minimal effect.
RESULTS AND DISCUSSION

In order to determine relative concentrations of samples, standards were needed to generate a calibration curve. Preliminary analysis of the water bottles showed BPA concentrations would be less than 100ppb. The calibration curve for the 1ppb to 100ppb can be seen in Figure 4 below.

A logarithmic trend line was used because after further research, SPME showed a tendency to produce nonlinear calibration curves. Within the standards, BPA was present at such concentrations that it could be detected. It became apparent after the samples were run that a calibration curve ranging from 0-10ppb would be sufficient to develop an equation with which to quantify the samples. The standards made to generate this calibration curve can be seen in Figure 5.

\[ y = 876817 \ln(x) + 1 \times 10^6 \]
\[ R^2 = 0.9883 \]

Figure 4: Standard concentrations ranging from 1ppb to 100 ppb with a logarithmic trend line to approximate further experimental samples.
Figure 5: Logarithmic calibration curve for standards of DI water (assumed 0 ppb), 1 nob BPA and 10 nob BPA.

After the samples were run, the logarithmic equation from Figure 5 was used to calculate the concentrations as shown in Table 2. The logarithmic trend line was generated using Excel’s Regression Data Analysis tool pack. It should be noted that the oldest bottle does show the greatest amount of BPA. This particular bottle was subject to temperatures in the range of 300° six months prior to analysis as part of another experiment. Needless to say, however, the bottle still shows the highest concentration of BPA.

<table>
<thead>
<tr>
<th>Area</th>
<th>Concentration (ppb)</th>
<th>Approx age (yrs)</th>
<th>volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cb</td>
<td>239158</td>
<td>.02</td>
<td>750</td>
</tr>
<tr>
<td>climate</td>
<td>146929</td>
<td>ND</td>
<td>500</td>
</tr>
<tr>
<td>green</td>
<td>898833</td>
<td>.76</td>
<td>1000</td>
</tr>
</tbody>
</table>

*Table 2:* Results from water analyses of three Polycarbonate bottles held at T=20°C for 24 hours. The climate sample was not distinguishable from the BPA already present in the water and was said to be not detected (ND).
The dependence of the GCMS response (peak area) on concentration is nonlinear. Although there are examples of acceptable calibration curves in Mass Spectrometry that are nonlinear, these are usually associated with high concentrations and near saturation of the electron impact source and the electron multiplier\textsuperscript{14}. As a result it was necessary to eliminate as many variables as possible that could have been responsible for the nonlinearity of the calibration curve.

**POSSIBLE EXPLANATION FOR NON-LINEAR RESPONSE**

**Saturation:**

There was concern that the fiber would become saturated and thus BPA molecules would be unable to adsorb onto the surface. After working with standard concentrations ranging from 1ppb to 100ppm, the abundances did not seem to reach a plateau, so for the purpose of analyzing the less concentrated samples, saturation was assumed a non-issue.

**Carryover:**

Carryover was an issue since the affinity of the BPA for the fiber was so strong; while being exposed to the inlet of the GC, the fiber was never completely blank when the next sample was taken. While this would have a minimal effect on standard analysis for increasing concentrations, it could potentially have a large effect on the samples which had unknown concentrations. This problem was managed by increasing the conditioning time. There was still a little bit of residual BPA left, but the fiber was continually conditioned until the response was minimal.
Stirring:

To account for the slow diffusion of BPA through water, stirring was used on one set of standards to determine if a detectable difference in the GCMS response peak areas could be detected. The thought was that the mass being transferred to the fiber via diffusion would deplete the BPA in the region adjacent to the fiber if stirring was not present. A different response was not present, so the effects of diffusion were ignored for the remainder of the analyses.

Oxidation:

This was a concern since the polyacrylate fiber would be repeatedly exposed to high temperatures, and after the first round of samples was run, the fiber began to significantly darken. Since two other fibers remained, one was used for the second set of standards and one for the water bottle samples. The fiber for the standards was only exposed to 10 samples and was therefore only exposed to the inlet 20 times. The fiber only slightly darkened, which was to be expected and then remained unchanged. Since the method was exactly the same for the second set of standards as it was for the samples, any oxidation would have occurred in both cases and skewed results in the same way. Oxidation of the fiber was thus considered negligible.

Samples:

The samples needed to be treated in the exact same manner as the standards in order to have repeatable results. For this reason the samples were stored with the standards and timed while being exposed to the fiber. The exposure conditions were also the same since each sample analyzed was done so in the fume hood. There was concern
surrounding the reproducibility of results. The amount of carryover of BPA and the
inability to obtain a consistent GCMS response of zero post conditioning the fiber were
both cause for changing the method of analysis. This was further evidence of a different
method needing to be used to better determine the concentration of BPA leaching from
the polycarbonate bottles.

STANDARDS

At first different exposure times were used for the different standards as I refined
my methods. However, in order to eliminate all variables related to the GCMS response, I
eventually settled on exposure and desorption times of 50 min and 3 min respectively. In
the Chang method, 50 min exposure times were used and the amount of BPA desorbed
increased with desorption time until it reached a maximum at 3 minutes. In order to
optimize the time used, lesser times were initially tested and then increased on
subsequent analyses to see if a difference could be detected. When the response reached a
plateau, greater times were not tested. The exposure and desorption times used for the
standards as well as the water bottle samples were equivalent to those used in the Chang
method. For all of my standards and samples, I had to use a uniform exposure and
desorption time.

THE FIBER

The polyacrylate fiber was originally conditioned for one hour. However, the
BPA had too much affinity for the fiber, and there was concern regarding the BPA from
previous analysis remaining on the fiber and increasing the amount of BPA seen in the
proceeding sample runs. This was managed by conditioning for fifteen minutes longer
than the suggested time to allow more BPA to desorbe from the fiber. The longer condition did decrease how much BPA was left over. A run was made following each condition in an attempt to quantify how much BPA remained on the fiber.

By increasing the exposure time, the chemical integrity of the fiber coating was assumed at risk. Because it was recommended by the manufacturer that 100 runs per fiber be completed before the fiber would be quantitatively unreliable, the 15 runs, even with a longer conditioning time, were considered to be sufficient. There were also concerns that the fiber might become saturated with each analysis. After analyzing the ppm standard, the peak area was in the $10^8$ range. When the ppb standards and samples showed peak areas in the range of $10^5$ and $10^6$, saturation was assumed not to have happened.

Oxidation of the fiber became a concern when, in the preliminary analyses, the fiber coloration was darkened. Close attention was paid while working with the standard fiber as well as the sample fiber. Both were used for a small number of analyses and the method was such that discoloration was not seen.

**SAMPLES**

In order to maintain consistency, the bottles (from which the samples came) were filled with DI water at the same time, stored at the same temperature for the same period of time before samples were taken. Since there were so many parameters involved, consistency was practiced as much as possible. For this reason, it was assumed that any variables that the samples encountered would have been consistent throughout the sampling process.
Variation was seen between the first and the second trial conducted on each sample. Since the major variables had been contained, the variation was thought to be the result of residual BPA remaining on the fiber. The magnitude of residual BPA was decreased when the conditioning time between GCMS runs was increased. Multiple analyses of each sample were also run to insure consistency. When values seemed improbable or too high, samples taken after a longer period of time were analyzed. Since the second set of samples had been exposed to the bottle longer, the concentrations of BPA should have been higher when compared to the samples taken after 24 hours. If the value of the 24 hour sample exceeded the 1 week sample, the 24 hour sample data were thrown out on the basis of residual BPA skewing the results.
CONCLUSION

By using SPME coupled to GCMS in SIM mode, BPA was detected in experimentally prepared standards as well as water bottle samples. However, due to the need to make results reproducible, it was concluded that SPME is not the best method for quantifying the amount of BPA in water samples. Perhaps work with fibers of other stationary phases would be useful. With an increase in polarity the BPA may desorb better in the inlet and help eliminate the problem of carryover. SPME provides a very simple and efficient way of rapidly screening samples but in some cases produces nonlinear calibration curves as well as inconsistencies in sample analyses\(^\text{12}\). This makes SPME a difficult technique for obtaining quantitative results for some analysis. In further research, SPME could be used for detecting BPA. Also, perhaps further experimentation with types of fibers and conditioning times could be conducted. The use of internal standards or standard addition would also provide other options for BPA research that may be more reproducible.
LITERATURE CITED


