Migration of Acetaldehyde and Formaldehyde from PET into Plain Water Bottles under Environmental Stress

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ABSTRACT

Bottled water consumption is an increasing world-wide phenomenom. Most bottled water is bottled in polyethyl teraphthalete (PET) packages that can leak formaldehyde and acetaldehyde generated during synthesis. The presence of these toxic compounds was analyzed with high-performance liquid chromatography (HPLC) and derivatization with 2,4-dinitrophenylhydrazine (DNPH). No significant results were found but many future implications for bottled water packaging were discovered.

INTRODUCTION

The current growth rate of bottled water industry is 25%. The main reason for this rapid growth is the taste of chemicals, particularly chlorine, used for purification of tap water (Mutsuga et al., 2005). In year 2009, bottled water sales accounted for 44% of the market volume for non-alcoholic drinks in Europe, with an average individual consumption of 105 L per year. Polyethylene teraphthalete (PET) is polyester used in a wide number of applications. PET has good properties such as transparency, light weight, strength, permeability, and high resistance to chemicals recyclability, which makes it suitable in food packaging (Bach et al., 2012).

PET is obtained by gradual polymerisation of teraphthalic acid and ethylene glycol. The first level includes dimethylteraphthalete transesterification with ethylene glycol or esterification of teraphthalic acid with ethylene glycol accompanied with the formation of bis (2-hydroxy-ethyl) terephthalate. The second level is the polycondensation of esters, bis [2-hydroxy-ethyle] terephthalate and formation of polyethylene teraphthalate (PET).

Acetaldehyde and formaldehyde are formed during the polymerization of PET bottles. After cooling, acetaldehyde and formaldehyde remain trapped in the walls of PET bottle and may migrate into the water after filling and storage (muhamad et al., 2011). Due to the high volatility of acetaldehyde and formaldehyde, there is a great chance of migration from PET bottle to the packed contents. High temperature and UV radiations may accelerate the possible migration of substance; consequently this could lead to change the taste and odor of the bottled drinking water (Azra et al., 2012; Muhamad et al., 2011).

MATERIALS AND METHODS

Some bottled water samples were drawn from the markets where they still in the validity period for human consumption.

Storage Study:

The outline of the storage study is in Figure 1. The equipment used were:

- Incubator
- UVB bulb (295-315 nm)
- Aluminum foil

One incubator was calibrated at 25°C and other one at 45°C.

UVB bulbs (295-315 nm) were installed inside the incubator and stored the sample at 25°C and 45°C. The samples at the time zero and after 15 days of storage at refrigerator temperature were taken for different analysis.

Measuring UV Intensity

Ultraviolet light intensity was measured using an EIT UV icure Plus 2 (Sterling, VA, USA). The machine was placed in one incubator directly under the UVB light bulb for one hour. The intensity was then recorded from the machine and used to calculate dosage for the samples in the storage study.

Chemical Analysis:

Acetaldehyde 2,4-DNPH and Formaldehyde 2,4-DNPH standards were purchased from Sigma-Aldrich (St. Louis,MO, USA). Acetonitrile (HPLC grade). Sodium Citrate, Citric Acid, Sodium Chloride and Hydrochloric Acid (all reagent grade) was purchased from JT Baker/Avantor (Center Valley, PA, USA). The derivatizing reagent, 70% 2,4- dinitrophenylhydrazine (DNPH, reagent grade), was also purchased from Sigma-Aldrich (St. Louis, MO, USA). Other equipment used included 500mL black screw cap bottles, plastic transfer pipettes and pH paper (Nasco, Salida, CA, USA), and sonicator (55°C, Branson 2510, Processing Equipment and Supply, Cleveland, OH, USA).

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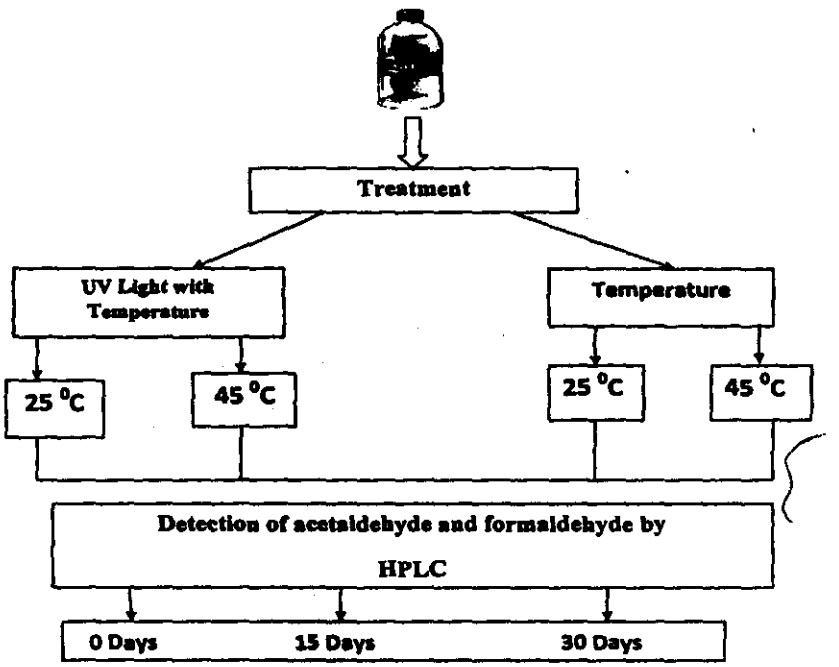

Figure1. diagram of Experimental design

SPE Sep-Pak C18 cartridges were purchased from Waters (Millipore, Milford, MA, USA) and the SPE vacuum manifold (Supelco VisiPrep™) used was purchased from Sigma-Aldrich (St. Louis, MO, USA) as well. After preparation, samples were filtered through 0.45 µm nylon syringe filters (Gelman acrodisk, 13mm, Sigma-Aldrich, St. Louis, MO, USA), placed in amber HPLC vials with PTFE/Silicon lids (National Scientific, Rockwood, TN, USA) and stored at 4°C until analysis. The High Performance Liquid Chromatograph (HPLC) used was from Agilent (Model 1200, Santa Clara, CA, USA) with an Agilent ZORBA Eclipse Plus C18 column (4.6x 150 mm x 5 µm, Agilent, Santa Clara, CA, USA) with a mobile phase of acetonitrile:water 55:45 v/v (1 mL/min flow rate), injection volume of 20µL and a UV detector set to 360nm. Nanopure water used for all reagents was supplied by a Milli-Q system (18.2MΩ/cm @ 25°C, TOC <10 ppb, Millipore, Billerica, MA, USA).

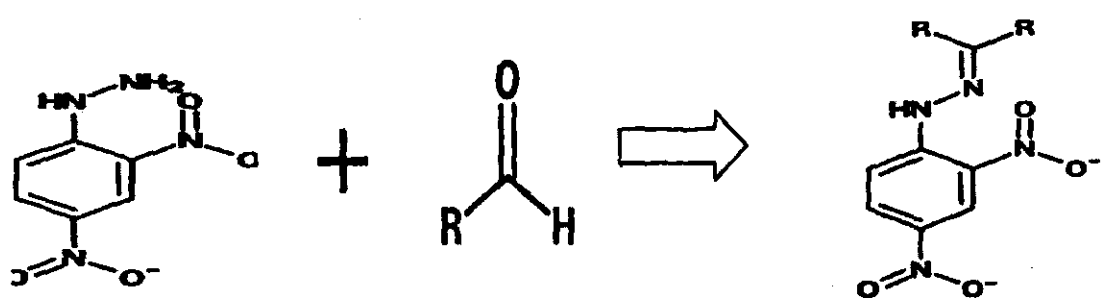

Figure 2: DNPH derivation reaction of organic aldehydes

Preparation of Calibration Curve

Calibration standards were made from a stock solution of 10mg standard in 1 mL acetonitrile. This was then diluted ten- -fold to produce a working solution of 1mg/mL. This was then serially diluted with acetonitrile (using ten-fold and two-fold series) to produce standards in the concentrations of: 1µg/mL, 5µg/mL, 10µg/mL, 25µg/mL, 50µg/mL, 100µg/mL, and 250µg/mL. Each standard (Acetaldehyde-2,4-DNPH and Frmaldehyde-2,4-DNPH) was diluted separately then joined after dilution (500µL Acetaldehyde-2,4- DNPH with 500µL Formaldehyde-2,4-DNPH at each calibration concentration). Calibration standards were placed in amber HPLC vials and also held at -4 °C until time of analysis.

Preparation of Samples for Analysis

The water samples from the storage study were removed from the refrigerator and 50mL aliquots were taken from each sample, and placed in chemical-free screw cap bottles and sealed.Each sample was then adjusted to an approximate pH of 3 using 4M HCl and pH paper. The derivatization reaction (depicted in Figure 2) was then performed by adding 1mL of the 5mg/mL DNPH stock solution (0.5g in 100mL) to each bottle. The bottles were then sealed again and sonicated at 55°C for 60 minutes. After sonication and derivation, 10mL of a saturated NaCl solution was added to precipitate the aqueous phase. The samples were allowed to sit for 10 minutes while the SPE cartridges were being prepared. To prepare the SPE cartridges, 10 mL of citrate buffer (20mL of 1M sodium citrate in 80mL of 1M citric acid) at pH 3 was used to condition the cartridges.

After the samples had separated and the cartridges were conditioned, 10mL of each supernatant was quantitatively transferred to the SPE cartridges and pulled through with a vacuum of 15 inches of Hg. The cartridges were then evacuated for 5 minutes and the waste discarded.

The analytes, acetaldehyde 2, 4 DNPH and formaldehyde 2,4 DNPH, were then eluted from the cartridge using 5mL of acetonitrile. The samples were then filtered through 0.45µm syringe filters and placed into amber HPLC vials under 4°C until analysis (kim et al., 1990).

RESULTS AND DISCUSSIONS

The calibration standards were analyzed using HPLC to generate a calibration curve. This was done by plotting the peak area of each analyte against the concentration in the standard. A linear regression analysis was then performed to determine the linear range and concentration equation for the unknown samples.

The results of this calibration curve are shown in Table 1. From the standards, the retention time of Formaldehyde (FA) was determined to be 3.943 minutes and the retention time of Acetaldehyde (AA) was 5.090 minutes.

The concentration equations were found by performing a linear regression of the calibration curve.

HPLC analysis was also performed on each of the unknown and treated samples.

After analysis, the chromatogram of each sample was visually inspected and then compared to standards and blanks to determine the presence of the analytes (FA and AA). In the samples in which either one or both of the analytes was detected, concentration was determined using the equations in Table 1. The results of these calculations, (the approximate concentrations), are shown in Table 2. For the 3 samples in which results were found, the chromatograms are included in Figure 3.

The validity of the calculations was also evaluated and it was found that for both FA and AA, the relative standard deviation was below 20% validation criterion. An attempt was also made to measure the light intensity of the UVB bulbs used in the experiment. The UV radiometer used only measured UVC intensity, so no measurement was recorded. The control samples were exposed to no UVB light.

Table 1. Linear dynamic ranges, concentration equations, and correlation coefficients for the two standards used in this study

Standard	Range (µg/mL)	Equation	Correlation Coefficient
Formaldehyde	1-50	C= Area/50.725	0.99817
	100-250	C= (Area – 351.6) / 20.782	1
Acetaldehyde	1-50	C= (Area + 13.279) / 45.806	0.99593
	100-250	C= (Area – 212.8) / 19.803	1

Table 2. Calculated concentrations of Formaldehyde and Acetaldehyde in each sample. The samples are coded with the # of storage days— temperature

Sample	Formaldehyde Concentration (µg/mL)	Acetaldehyde Concentration (µg/mL)
Time 0	ND	ND
15-25 CA	ND	ND
15-25 CB	ND	ND
15-25 LA	ND	[0.551]
15-25 LB	ND	[0.526]
15-25 LC	ND	[0.469]
24-45 CA	ND	[0.567]
24-45 CB	ND	[0.627]
24-45 LA	[0.282]	[0.611]
24-45 LB	[0.274]	[0.583]
24-45 LC	[0.236]	[0.575]

(C)ontrol or (L)ight, exposed and then replicate.

Note: ND means that the analyte in question was not detected in that sample. Concentrations with brackets around them indicate that the concentration was outside of the linear range and my not be accurate.

DISCUSSION

All of the samples from the first time point of the storage study (15 days) showed results that were either not detected, or below the limit of quantitation. This could be due to a number of reasons. The first is that the storage is not long enough. Fifteen days of exposure to UVB light and temperature may not be enough to generate a significant amount of FA and AA in such a small container. Also, when comparing the chromatograms of the samples against the blanks, the concentrations, that were found, were barely larger than the time zero control and the blank. Another possibility as to why the results were outside the limit of quantitation, This could be due to the injection volume. The injection volume for each of the calibration standards (except for 100µg/mL and 250µg/mL) was 20µL. This could have been a way too much to inject for the standards.

The vertical axis is mAU and the horizontal axis is time in min. (A) 15 day, 25 °C sample exposed to light, (B) 15 day, 45 °C sample held as a control, (C) 15 day, 45 °C sample exposed to light, reduced the peak area, possibly bringing the samples closer to the linear dynamic range. It also would have improved the resolution of the peaks and reduced the broadening seen. The last possibility was that not enough of the sample was concentrated on the SPE cartridge to produce significant results. We did, however. Find some results in this study. We found about 0.5ppm in the 25 °C samples and about 0.6ppm in the 45 °C samples. Bear in mind that these results are still well below 1ppm limit of quantitation. We also found little difference between the control and light exposed samples for 45 °C. This may mean that heat plays a larger role in the PET degradation and subsequent FA and AA formation than UVB light does. Further studies may clarify this implication. It may also be worth nothing that AA appeared to be formed in the water before FA. This may have a use in future studies or industrial applications

Table 3. Average concentrations, standard deviations, and relative standard deviations (RSD) to determine validity for each of the samples with detected concentrations of FA and AA

	Formaldehyde			Acetaldehyde		
Sample	Average Concentration (µg/mL)	Standard Deviation	% RSD	Average Concentration (µg/mL)	Standard Deviation	% RSD
15 – 25, Light	ND	N/A	N/A	0.515	0.042	8.155
15 – 45, Control	ND	N/A	N/A	0.597	0.042	7.107
15 – 45, Light	0.264	0.025	9.309	0.590	0.019	3.206

Note: ND means that analyte was not detected and N/A means that analysis was not applicable to the sample.

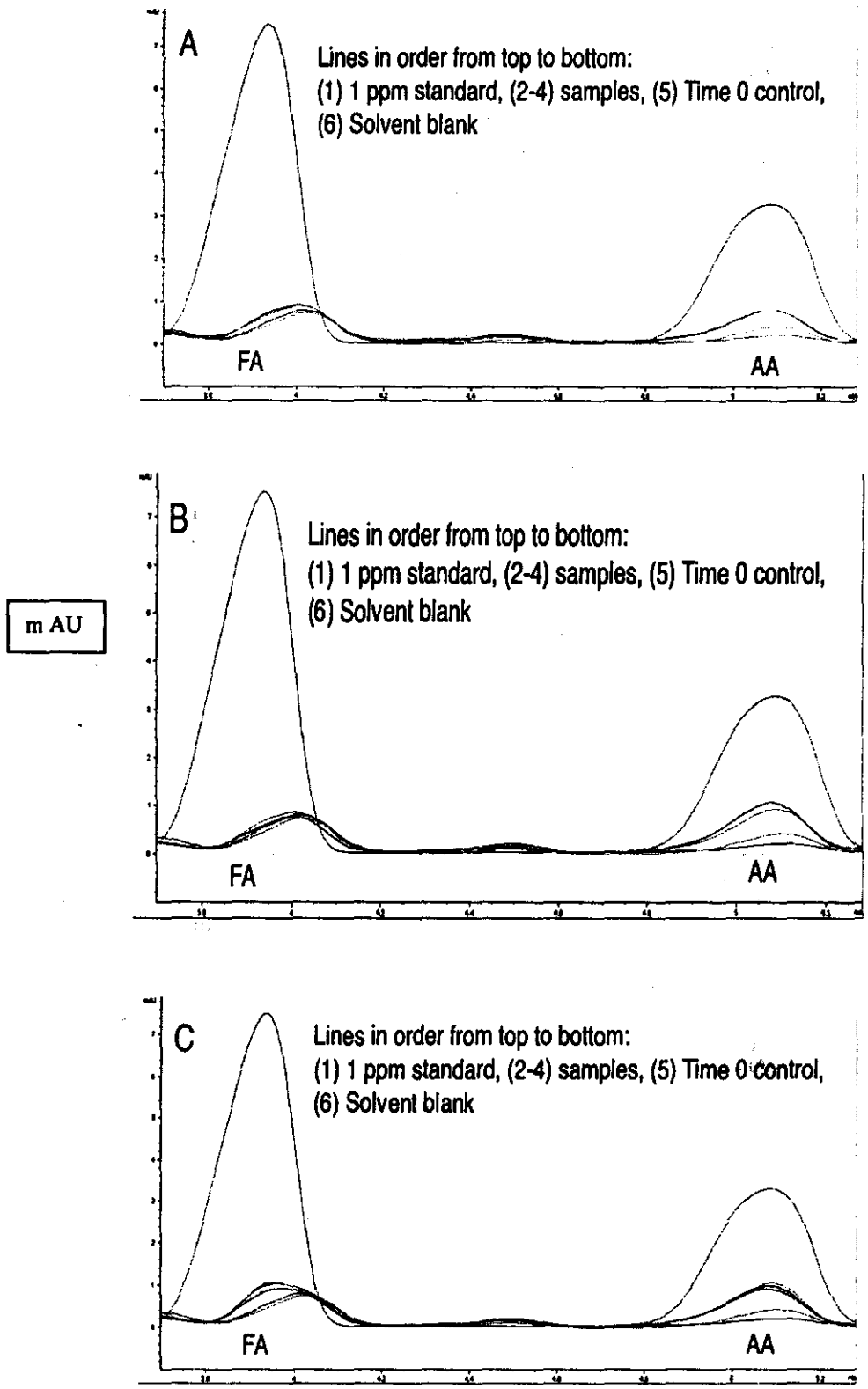

Figure 3: Chromatograms of samples with detectable FA and AA concentrations

CONCLUSION

Commercially available PET bottles were analyzed for the presence of formaldehyde and acetaldehyde due to degradation of the PET polymer during storage. Analysis was performed using an Agilent 1200 HPLC with a C18 column and pre- column derivatization with 2,4□ dinitrophenylhydrazine. Concentrations of the analytes were detected in the samples held for 15 days at 25 °C with exposure to light, and both samples held for 15 days at 45 °C (exposed to light and control).

While the detected concentrations did fall below the linear range of the calibration curve, they were still calculated for preliminary results. It was found that the 25 °C samples had 0.5ppm of acetaldehyde while the 45 °C samples contained around 0.6ppm. This may show that heat plays a larger role in the PET breakdown reactions than light. It may also show that the light effects seen in other studies are actually due to the excess heat generated by the UV light in the sample. Research on this topic needs to be continued in future storage studies.

This study also has implications for the food industry. The future of PET based packages may be in jeopardy if highly significant evidence of degradation is found.

This study can then be used to generate new ideas on the thermostability of PET based packages. The data from this study can ultimately also be used to redesign a PET package that is more thermostable, by altering the chemistry of PET to limit the migration of these harmful chemicals (Safa, 1999). There is also one last implication of this study. As the globalization of the food industry grows and the food web becomes a world□wide chain, local demands for food product functionality cannot be forgotten. Nothing is truly a one□size□fits□all situation, and that same approach cannot be used for food products and their packages. Based on the preliminary results of this study, food packages need to be considered and designed for their local region of intended use. The ambient temperatures of major regions of the world need to be looked at and taken into account when designing a package.

A PET package will not behave identically in the Artic Circle as it will in the Sahara Desert (Schmid et al., 2008), so why should we continue to assume that it will?. Let this study be an indicator to the global food market that to ensure the functionality of food products in this growing global network. Weather conditions of the climate of intended use must be a principle consideration in the design of new food packages.

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الملخص العربي

هجرة الأسيتالديهايدِ والفورمالدهيد من الفنيل اثيل تيرافثليت إلى زجاجات المياه تحت ظروف الإجهادِ البيئي

خيري محمد العماري ومحمد مصباح الزياني

ان إستهلاك الماءِ المعبّأ فى القناني ظاهرة عالمية متزايدة. وأكثر المياه المعبّأ فى القناني مكونه من الفنيل اثيل تيرافثليت والذي من الممْكِنُ أنْ يُسرّبَ الفورمالدهيد وألأسيتالديهايد المنطلق اثناء التخليق.

هذه المركّباتِ السامّةِ تم تحليلها بواسطةجهاز الكروماتوجرافى السائل (HPLC)، حيث لم يكون هناك اثر معنوي فى هذه الدراسة، ولكن العديد مِنْ الدراسات المستقبليةِ للماءِ المعبّأ سوف يتم الكشف عنها.