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Bis-phenol A: origin, fate, dilemma and the effects of increased background organics on its adsorption by powdered activated carbon.

Final Thesis

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ABSTRACT

This study was conducted to simply observe whether or not background organics had an effect on the adsorption of Bisphenol A (BPA) by powdered activated carbon (PAC), and to what degree. Bisphenol A is an endocrine disruptor used in many applications, for which sanitary issues are still in controversial debate. In this experiment we observed that under natural levels of organic matter (River Han of Seoul with a DOC of 2.38 ppm), double amount of PAC is required to adsorb BPA to the levels it would have been adsorbed without competing background organics. A contact time of 240 minutes was proven to be a minimum in reaching adsorption equilibrium. We can determine from this experiment that BPA is very well adsorbed by activated carbon, but in presence of heavy organic competition (Double DOC with Humic acid), the adsorption sees its efficiency considerably reduced (ca. 20% for a 200% increase in background organics), and shall then be taken into account in water treatment facilities by using an adequate amount of PAC and reasonable contact time. The values expressed in this report should not be used in practice unless a thorough study is done. This report has answered whether or not background organics had an effect on BPA's adsorption, as a basis for conducting further studies such as dynamic column studies, to determine the requirements of a water treatment system (Activated carbon) of a specific size, in contact time and PAC concentration. Thus it falls in the qualitative category of report and should not be used as if it were a quantitative work. In addition to genuine experiments, a selection of scientific reports belabors the concern of BPA's ubiquity in our environment, its toxicity in low concentrations and difficulty of its monitoring in the environment as well as in closed systems (detection limits).

Key words — Bisphenol A, BPA, Powdered activated carbon, PAC, Background organics, Water treatment, Endocrine disruptor, EDC, Adsorption, Humic acid, HPLC, Isotherm, Freundlich isotherm fit, surface water, river, Han, Seoul.

Foreword

This work was conducted in Soongsil University (Seoul – South Korea), in addition to the duties I had to perform during my internship in professor Hong Song Ho's postgraduate water treatment research laboratory.

The topicality and controversy of Bisphenol A, fueled my curiosity and eagerness to uncover information about a subject I was not aware of. I have learned a tremendous amount of skills and knowledge on conducting an experiment from start to end, and yet understand how superficial the work I did actually is.

The more one knows about a subject, the more one understands how vast is the unknown. In the realm of environmentalism, the former statement extends to knowing how grim and dire the state of the Earth is, under current impetuous and unethical corporatocratic management.

I would like to thank my colleagues and friends of the laboratory, as well as all who supported me during my stay in Korea, for their help, scientific knowledge, and true kindness. The help they offered me is simply unmeasurable, without which I would still be working on the many problems I encountered.

More precisely I would like to thank Professor Hong Song Ho for kindly welcoming me in his department, as well as Soul, Yoon and Sun for their infinite kindness.

Kevin Thien - Tampere, 2009.

Table of Contents

Terms and symbols

BPA: Bisphenol A
HCl: Hydrochloric acid
CO_2: Carbon dioxide
US or USA: United States of America
UV: Ultraviolet
PC: Polycarbonate
PTFE: Polytetrafluoroethylene, also known as Teflon®
PVC: Polyvinyl chloride
DOC: Dissolved organic carbon
DW: Deionized water
RW: River water
HA: Humic acid
EDC: Endocrine Disrupting Chemical
VTG: Vitellogenin
PAC: Powdered activated carbon
HPLC: High Precision Liquid Chromatograph
v/v% : Volume per volume percentage
Endocrine: Relative to endocrine system; release of hormones controlling growth, sex, mood,...
RT: Retention time (time at which a certain molecule crosses the UV detector in HPLC)

Units
ml = 10^{-3} liter; μl = 10^{-6} liter; nl = 10^{-9} liter
ppm = part per million, usually 1 ppm = 1 mg/l (depending on specific gravity)
ppb = part per billion; 1 ppm = 1000 ppb
psi: pounds per square inch; 1 psi = 6.894757 kPa

1. Introduction

Bisphenol A a major compound (monomer) in the fabrication of canned food resin liners, polycarbonate and PVC, has recently been of particular concern as it has proven to leach from its polymerous form under high temperature or caustic environment. Known to be an endocrine disruptor since the 1930s, Bisphenol A had also been used as a pesticide.

A few years ago, growing concerns about the endocrine disruption function of BPA arose, as baby bottles made of PC could leach the estrogen-like chemical into infant's diet, having tremendous effects on their growth especially their reproductive system. In 2008, Canada declared a ban on BPA use in baby bottles and infant products. However, the ubiquity of the toxic compound in our environment and in the market (2 million tons produced in 2003), creates room for information and disinformation.

The toxicity of BPA as an endocrine disruptor is said to be maximal at very low dosage which is ironically very difficult to detect on an industrial scale (food analysis, waste water analysis,...).
BPA does not have a long half-life (1 to 10 days under aerated conditions), however its ubiquity in our environment is of rising concern especially for industrial waste waters containing BPA. Aquatic fauna (e.g. fish) could well be affected by the presence of BPA and thus affect a long ecological chain.

Also, BPA has been proved to disrupt the roots of leguminous plants in their intake of nutrients. Although easily decomposed in nature, BPA has been found to remain for years in river sediments, a typical oxygen deprived environment. The presence of BPA in industrial effluents is of growing concern, especially in the fabrication of thermal papers, thus infecting the wastewater of pulp and paper factories.

This study focuses on natural waters and the possibility of BPA being present in surface waters used for drinking purposes. Thus, the primary question of concern is whether background organics offer “resistance” to the correct adsorption and efficiency of activated carbon filtration, and to what extent. Such study could also be of concern to industries rejecting organic wastes containing BPA, such as chemical plants, paper plants, etc...

This study will try to answer the following question: “Is carbon filtration efficient in removing BPA, and how an increased background organic concentration affects its adsorption.”

2. Theoretical Background

2.1. *Polymerization using BPA*

a) Bisphenol A

Bisphenol A, $C_{15}H_{16}O_2$ is obtained by condensation of acetone and phenol, as illustrated in *illustration 1*, and according to the equation: $(CH_3)_2CO + 2\ C_6H_5OH \rightarrow (CH_3)_2C(C_6H_4OH)_2 + H_2O$

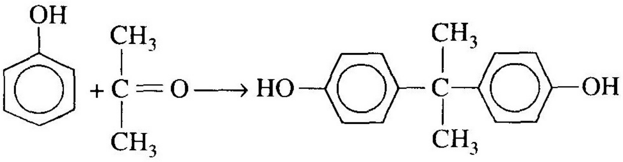


Illustration 1: Synthesis of BPA [SINHA (26)]

The particle “A” originates from “Acetone”. One part of acetone reacts with 2 parts of phenol. Hence the name Bis-phenol A. The reaction is catalyzed by an acid such as HCl. Usually an excess of phenol is used in the condensation to assure complete reaction. The only by-product of the condensation is water, and excess phenol that hasn't reacted. [WIKIPEDIA (2)]
The main polymers using Bisphenol A are epoxy resins and polycarbonates.

b) Epoxy resin

The raw materials for the epoxy resins are mostly epichlorohydrin C_3H_5ClO and bisphenol A . Qualities of epoxy resins are: toughness, low shrinkage, high adhesion to many substrates and good alkali resistance. Epoxy resins may be prepared by reacting epichlorohydrin and a dihydric phenol. But these raw materials are expensive. Therefore the most common type of phenol used for the industrial production is bisphenol A.[SINHA (26)]

In a typical process, 1 mole of bisphenol A is taken with 4 moles of epichlorohydrin in a batch reactor and heated to a temperature of about 100°C under nitrogen atmosphere. This process called condensation has an organic layer separated from the rest upon completion, which is then dried with sodium sulphate, and vacuum distilled to remove the unconverted monomers. [SINHA (26)]

The general formula for epoxy resins is shown in *illustration 2*.

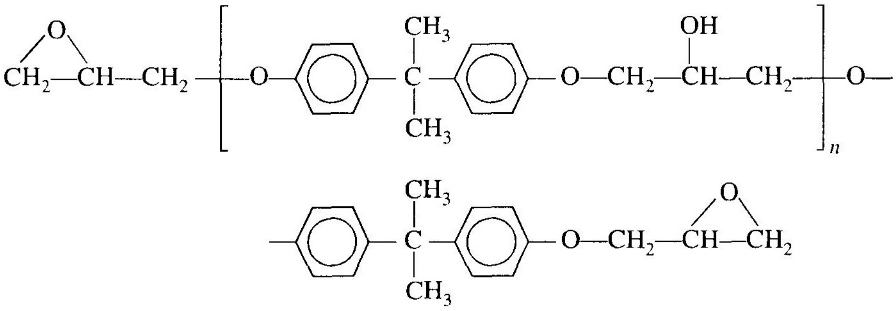


Illustration 2: synthesis of epoxy [SINHA (26)]

Epoxy resins have a variety of uses such as surface coating (water tubes, canned food,...) adhesives, potting and encapsulation, lamination and tooling. There exist non-bisphenol A epoxies, but these have the disadvantage of a higher cost.[SINHA (26)]

c) Polycarbonate

Polycarbonates of bisphenol A may be prepared by varying methods. The most common processes are phosgenation and ester-exchange. *Illustration 3* depicts an ester-exchange process of making polycarbonate.

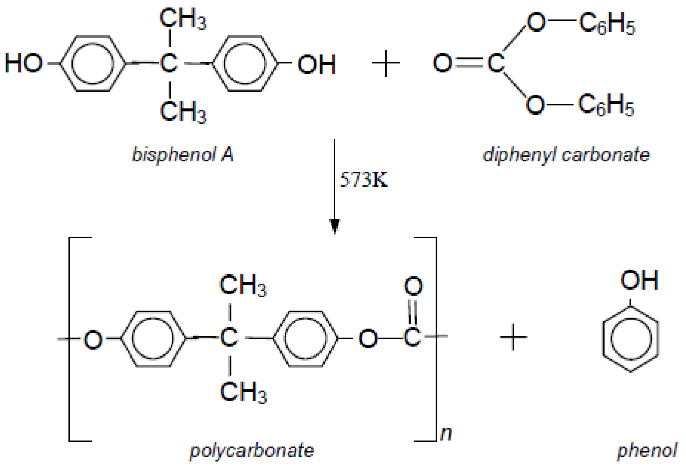


Illustration 3: Polymerization of polycarbonate(4)

The ester-exchange process uses high temperature in which Bisphenol A is unstable. Thus an excess of diphenyl carbonate is used to assure a complete reaction of BPA. Polycarbonates have a crystalline structure as shown in *illustration 4*. This explains the transparent property of polycarbonates.

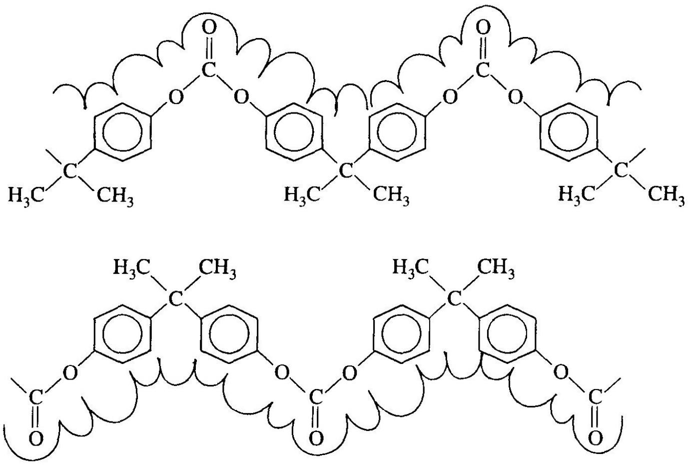


Illustration 4: Bisphenol A polycarbonate structure [SINHA (26)]

The crystalline structure of polycarbonate is somehow limited, which explains its toughness. Properties of PC include its transparency, rigidity and toughness up to 140°C. It also is self extinguishing, and has a fair resistance to weak aqueous acids. However it has limited resistance to chemicals, UV light and alkali substances. [SINHA (26)]

Polycarbonate is known to leach BPA under strong heat, harsh detergents and acid food/beverages. Therefore polycarbonate products should not be microwaved, cleaned in dishwasher, cleaned with harsh detergents, nor contain hot beverage/food.

d) Other sources of BPA

Toilet paper has been identified as being an important source of endocrine disruptor to wastewater, as recycled papers often include thermal papers [GEHRING et al. (20)]. Thermal paper such as credit card receipts, etc.. contain BPA which is used as a color developer molecule [TAKAHASHI et al. (19)] They are thought to be the major contributor of BPA, contaminating recycled paper products. Up to 46 mg/kg (dry mass) of BPA has been found in recycled-paper-made toilet paper [GEHRING et al. (20)(25)].

2.2. Environmental Toxicity

a) In aquatic ecosystems

A study [MANDICH et al. (5)] analyzed the effect of BPA exposure to 1-year-old common carp, during a period of up to 14 days exposure, in an in vivo experiment. It has been observed that male carps, had severe alteration of their reproductive organs at 1 µg/l (1 ppb) and 10 µg/l (10 ppb) of BPA. Vitellogenin (VTG) is a glycolipo-protein that occurs in female fish. It acts as a bio-marker for endocrine disrupting chemicals such as BPA, as VTG will be produced in a dose-dependent manner to BPA. Male fish end up producing VTG under estrogen-like endocrine disruptors, like BPA. [WIKIPEDIA (6)].

Under laboratory or effluent exposure, different fish species have shown increased levels of VTG caused by BPA. Many specimens are able to respond to low BPA concentrations by synthesizing VTG [MANDICH et al. (5)]. The study on carp showed that 9.1% of males were vitellogenic (showing VTG concentrations >11,000 ng/ml) after 14 day of exposure to 1 ppb, and 42.9% of them with 10 ppb.[MANDICH et al. (5)] This stresses the fact that small doses, do have an effect on the environment.

On the other hand, Dutch field research [BELFROID et al. (7)] rejected the risk of BPA at levels that would trigger VTG (i.e. an estrogenic response), in natural areas such as coastal sea or surface water. BPA has been detected in fish liver and muscle, but concentration was apparently 100 times less than that which triggers a clear VTG response in a laboratory experiment.

However, the study showed that levels of BPA were changing over time and thus the effect on the environment was variable. Also, as fish and shellfish migrate from point to point, it is difficult to underline a clear dose-response to pollution of BPA at a certain time.

Nevertheless, in opposition to a concentration of BPA in solution (i.e. water) that fluctuates as it naturally degrades, BPA has been shown to accumulate and remain unoxidized in sediments [ZENG et al. (11)]. Benthic fauna that lives and feeds on these sediments could accumulate BPA from it; thus species of shellfish that feed on this benthic fauna, could be exposed to BPA on a chronic basis via such a secondary pollution channel.[BELFROID et al. (7)]

Apart from being accumulated by living tissues, BPA can be either photo-oxidized, bio-degraded or adsorbed to sediments [SUN et al. (12)]. However, a Japanese study [NAKAJIMA et al.(10)] shows that freshwater micro-algae can metabolize BPA to their glycosides, in a process called glycosylation. Glycosides are molecules in which a chemical part is bound to a sugar part. [WIKIPEDIA, (8)]. The concentration of BPA falsely diminishes, as it is not only oxidized or degraded, but could be stored as glycosides. That study reveals that BPA can accumulate in plants and algae in a neutral form, but can then be digested back to BPA, when plants or algae containing BPA glycosides, are consumed. [NAKAJIMA et al.(10)]

b) In terrestrial ecosystems

Along with other remaining chemicals, BPA can be found in waste water sludges. It has been observed that BPA disrupts the nitrogen fixing capacity of leguminous plants such as Alfalfa, and then may considerably delay the harvest times of fields using contaminated wastewater sludge. [FOX et al. (13)].

The study of Dresden university of technology on recycled papers being contaminated with BPA from thermal paper waste, warns of the mixing of such paper with biological waste intended to be used as organic fertilizer, for the reasons stated above. [GEHRING et al. (25)].

2.3. BPA's safety: a real dilemma

Information, disinformation or incomplete mentioning of the experimental settings in which testings take place, are omnipresent. [MYERS et al.(14)]. Many groups and associations financed by plastic making corporations are often set to refute the frightening scientific discoveries or psychosis that settle in public opinion, as the following excerpt illustrates:

"BPA is one of the most extensively tested of all substances and has been used safely for over 50 years. BPA safety has been widely studied. Based on the weight of the scientific evidence, health authorities throughout the world conclude that BPA-based materials are safe in their intended uses, both in consumer and industrial applications."
[PlasticsEurope (22)]

The scientific debate over BPA is constant. The European Union and the U.S. Food and Drug Administration say the chemical is safe, as a recent European commission risk assessment report of 392 pages affirms [IHCP (23)]. BPA production is of tremendous commercial importance. The main producers of BPA are USA, Japan, Brazil, Russia, Germany, Belgium, Spain, the Netherlands and Thailand. With 1.1 million tons of BPA produced in USA alone [PALMER et al. (21)].

Interestingly Canada, a country that recently banned BPA-containing baby products, does not belong to the list of countries producing the most BPA. Hence it seems that economical reasons are motives for softening or impeding the discrimination of BPA products and their potential risk on the Environment.

2.4. Activated carbon filtration

Activated carbon filtration is in simple words: a small amount of carbon that offers a great surface to which molecules will adhere, and therefore be removed from a liquid. Coal (lignite and sub bituminous), wood (sawdust) and coconut shells but also organic waste products such as coffee bean shells, banana skins, bark from tree felling, straw, fruit stones, which all on carbonization produce porous carbons, which can be activated to an appropriate porosity. [MARSH et al. (28)]

The porosity of a carbon as it is, is not sufficient. Therefore we speak of activating carbon in an effort to increase porosity. First, takes place a physical and thermal activation by gasification of the carbon with water vapor or CO_2 at 800 to 900°C , which creates more porosity. Then, comes a chemical activation. Three chemical processes purify the activated carbon: zinc chloride promotes extraction of water, phosphoric acid combines chemically and lastly, potassium hydroxide provokes a kind of internal explosion of the structure, similar to a grain of corn popping to a popcorn. [MARSH et al. (27)]

Important characteristics of an adsorbent are parameters such as pore size distribution, surface chemistry (functionality) and mineral matter content. Adsorption capacity depends on accessibility of the organic molecules to microporosity which is dependent on their size. Thus, under appropriate experimental conditions, small molecules such as phenol can access micropores; natural organic matter (NOM) can access mesopores and lastly bacteria, have access to macropores. [MARSH et al. (29)]

The terminology in adsorption is rather confusing, therefore it is important to recall that:

- an *adsorptive* is a molecule in a solution that is to be adsorbed (e.g. BPA in solution).
- an *adsorbate* is a molecule that has been adsorbed by activated carbon (e.g. BPA on PAC)

- an *adsorbent* is what activated carbon is, a highly porous matter that adsorbs molecules (e.g. PAC) [MARSH et al. (28)]

It is said that "*an increase in activated carbon's surface acidity after an oxidation, causes a decrease in phenol adsorption from dilute aqueous solution*." [MARSH et al. (29)]. Therefore in this study, we will try to answer whether carbon filtration is efficient in removing BPA, and how an increased background organic concentration affects its adsorption.

3. Experimental section

3.1. Reagents and materials

a) In table form

All reagents were of analytical-reagent grade and de-ionized water was used throughout the experiments. Before analysis with HPLC or DOC, all samples were filtered with a 0.45 µm filter.An overall look of all specificities is seen in *Table 1* .

Table 1: Summary of all configurations

Name	Characteristics	Origin
Bisphenol A	100 ppm stock solution of BPA, 10 mg of BPA granule dissolved in 100 ml of distilled water containing 5 v/v % of ethanol. Stored at 5°C	Industrial sample. Pure BPA granules.
Activated carbon	325-mesh PAC	Calgon™ , type F400 . 1st generation
Natural water	Filtered to 0.45 µm, stored at 5°C	Water from river Han, Seoul.
Humic acid	14.54 mg/l in natural water.	Humic acid. Batch #21520BB Aldrich (H16752 – 2006)
DOC : Multi N/C 3000	NPOC, Potassium Hydrogen Phtalate standard. NDIR detection. All sample filtered to 0.45 µm before analysis.	Analytik Jena AG, Germany
HPLC	Column:Novapak C18 4µ 4,6x250 mm cartridge Methanol concentration for cleaning: 100% pumps: 70% Acetonitrile CH_3CN: HPLC grade. Autosampler: Midas Software: Autochro 3000	Younglin Instruments (2 pumps and UV detection) CH_3CN, FW 41.05 from Duksan pure chemical co.

b) Detailed explanation

- ***Bisphenol A***

Bisphenol A was provided as solid granules of pure BPA, as used in industrial polymerization.

BPA has a relatively low solubility in water, that ranges from 120 to 300 ppm at 21,5°C [WIKIPEDIA (2)]. A method of dissolving BPA with a certain amount of ethanol was found from a study made by Akira Motoyama [MOTOYAMA et al. (1)]. In order to make a 100 ppm stock solution of BPA, 10 mg of BPA granules are dissolved in 100 ml of distilled water containing 5 v/v % of ethanol (100 mg/ml).

Thus, with the use of a micro-pipette we inject 100 µl of ethanol into 100 ml of stock solution containing 10 mg of BPA into a volumetric flask. The flask is stirred with a magnetic rod and kept at a constant mild temperature (30°C), for a day. The ethanol's density makes its input negligible in the calculation of concentrations: thus the specific gravity is unchanged. 100 µl of ethanol (100 mg/l) is equivalent to a concentration of 100 ppm.

These stock solutions were stored at 5°C upon complete dissolution of BPA. When necessary, the stock solutions were further diluted with distilled water to give final concentrations such as 10 mg/ml (10 ppb), or else as desired. It is important to note that the presence of ethanol would disturb the results given by DOC tests, as they will not directly correspond to the concentration of BPA alone, but that of 24 g/mol of carbon from ethanol (C_2H_6O : 2 molecules of carbon at 12 g/mol each) and 180 g/mol of carbon from BPA ($C_{15}H_{16}O_2$: 15 molecules of carbon at 12 g/mol each) in their respective concentration. Fortunately, ethanol is not adsorbed by activated carbon and will thus not impede on the results.

- ***Activated Carbon***

In order to increase the adsorption surface, hence efficiency, activated carbon needs to be converted to Powdered Activated Carbon: PAC. Activated carbon was provided by Calgon™ (Calgon carbon corporatum (Pittsburgh, PA. USA.)) and was of type F400 (Filtrasorb 400), first generation. Reducing the Activated carbon to powder (PAC) requires crushing it with a mortar at slow speed and mortar pressure. It is then meshed down with sieves of different mesh size. In order to qualify for PAC quality, the mesh should be greater than 80-mesh sieve (0,177 mm) [WIKIPEDIA (24)].

In this experiment we used a 325-mesh sieve, which translates as having 325 lines in a square inch. PAC is then refined through a succession of washing and purification processes. Fine sieved powder is washed at least 3 times with DW; particles are left to settle down on the bottom and the remaining “dust” on top or suspended in the liquid, is thrown away. This process is repeated until only clear black matter remains on the bottom in clear water. Dissolved air is expelled out under vacuum. The solution is stirred and the vacuum is repeated, until no air remains trapped in the PAC or dissolved in the DW. PAC is then drained and dried in an oven for at least 24 hours at 120°C.

- ***Natural water***

The natural water used for our experimentation was that of the river Han, Seoul. A total of 80 liters (2 times 40) was collected in May. This water was filtered to up to 0.45 µm, in several filtering steps to remove coarse particles first and finer last. Filters of 0.45 µm were made of glass microfiber from Whatman® Schleicher & Schuell, GF/C category number 1822110. The filtered water was then stored at 5°C prior to usage. When referring to this natural water we will use the letters “RW”, standing for “river water”.

- ***Humic acid***

In order to assess the efficiency of BPA removal by PAC in an organic-rich environment, Humic acid was used to increase the organic concentration. The Humic acid used was the following: Humic acid. Batch #21520BB Aldrich (H16752 – 2006) “Humic acid, sodium salt, tech”

We need to assess how much dissolved organics are present in our sample river water (RW). Then we need to determine the effect of added humic acid on the final DOC. The required increased background organics that we wished to analyze is at least double that of the natural DOC of our river water.

As illustrated in *table 2*, 15 mg of Humic acid gives approximately 5 ppm of DOC. As the river water has a natural DOC of 2.21 ppm we would like the increased-background-organic sample to be roughly double that of the river water, hence c.a. 4.4 ppm.

Table 2: Determination of Humic acid (HA) effect on DOC.

Sample	**Filtered to**	**DOC in mg/l (ppm)**	**Standard Deviation of DOC in mg/l**	**Analyzed volume in µl**	**Dilution**
RW	0.45 µm	2.55	0.046	1	1 in 1
RW + 10 mg of HA	0.45 µm	4.46	0.076	1	1 in 1
RW	0.45 µm	2.21	0.053	1	1 in 1
RW + 15 mg of HA	0.45 µm	5.12	0.076	1	1 in 1
RW + 20 mg of HA	0.45 µm	6.16	0.063	1	1 in 1
RW + 30 mg of HA	0.45 µm	7.71	0.029	1	1 in 1
Standard (1 ppm)		1.06	0.020	1	1 in 1
DW		0.07			

Note: DW water was inserted before and after each test to clean the system.

5 mg/l of HA seemed to increase the DOC by 1 ppm when mixed with river water. Thus the final HA concentration for our experiment was set to 14.54 mg/l, to give an estimated 2.9 ppm contribution to DOC.

3.2. *Instruments*

a) DOC analyzer

In order to qualify for dissolved organic compounds, all samples were filtered with a 0.45 µm membrane (HA type). DOC measurements were done with a Multi N/C 3000 from Analytik Jena AG, Germany. The analyzer used NPOC (non purgeable organic carbon) method. The standard solution of DOC to calibrate the machine was Potassium Hydrogen Phthalate ($C_6H_4(COOK)(COOH)$) from Nacalai Tesoue, Japan. The oxidation method was combustion. Detection is NDIR: Non dispersive infrared detector.

b) HPLC method and calibration

HPLC characteristics: Younglin Instruments (2 pump and UV detection)

Column:Novapak C18 4µ 4,6x250 mm cartridge

Methanol concentration for cleaning: 100%

pumps: 70%

Acetonitrile CH_3CN: HPLC grade. FW 41.05 from Duksan pure chemical co.

Autosampler: Midas

Software: Autochro 3000

After researching studies that involved the use of a HPLC for detection of BPA using similar elements such as the same column or solvent, 5 different methods were found, as illustrated in *table 3.*

Table 3: Compilation of HPLC methods for BPA analysis

Acetonitrile mix	**Flow rate in ml/s**	**UV wavelength in nm**	**Column type**	**Characteristics**		**Retention time**
7 : 1	0.8	278	C18	5 µm	250x4.6 mm	27.98 min
1 : 1	0.8	276	C18			8 min
1 : 1	1	220	ODS3			
1 : 1		254	C18	5 µm	250x4.6 mm	17.76 min
1 : 1	1	230	C18			

For reasons of economy and prevalence of its use in former studies, the mixing of Acetonitrile to deionized water was set to 1:1. Also, a relatively fast retention time would allow faster analysis of multiple time-consuming tests, and a slow flow rate would allow to keep the critical pressure of the HPLC at its lowest possible. The HPLC had problems with its second pump and after the intervention of a qualified engineer, the pressure rose dramatically above safety limits if the flow rate was pushed above 1 ml/s. Thus our calibration-optimization-process started with a flow rate of 0.6 ml/s giving a reasonable pressure of ca. 2500 psi and a UV wavelength of 222 nm.

The tests illustrated in *table 4*, were conducted with a 20 µl injection of 10 ppm BPA. A minimum of 3 run was used for calculating the average result.

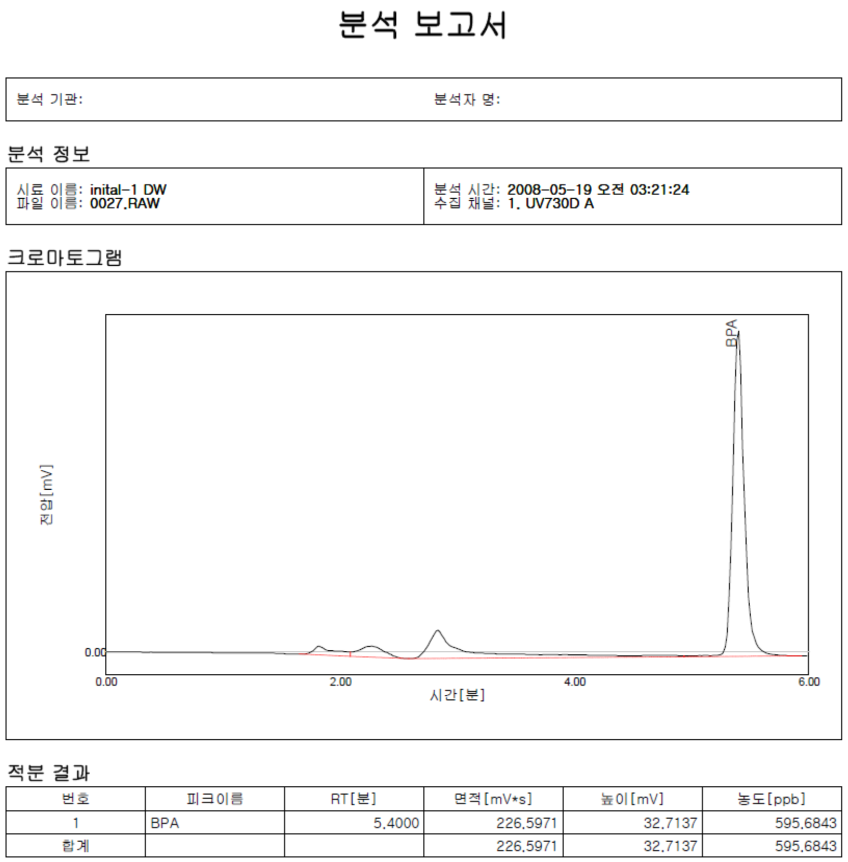
Table 4: Finding the optimal wavelength

Wavelength in nm	Resolution: average peak area in mV/s
217	1254
220	1234
222	1232
223	1305
224	1320
225	1349
226	1270
230	1277
250	50

Non exhaustive tests with flow rates such as 0.8 ml/s resulted in poor results. 0,6 ml/s proved to be an optimal tuning as it gave good clear peaks, a fast running time compared to slower flow rates (retention time around 5 min), a lower internal pressure (whence safety for the machine), and an economical value as less solvents were used.

The final tuning was thus set to:
Flow rate of 0.6 ml/s,
UV wavelength of 225 nm
Mixing of 1:1 Acetonitrile/DW
Retention time (RT): ca. 5 min

The injection micro pipette was previously of 20 µl, but was changed in the meantime to 100 µl, which fortunately gave better peaks. Before each injection, the micro pipette was cleaned 3 times with methanol (MeOH), and the injector 2 times (MeOH). The micro pipette was then washed twice with the sample, and we injected the sample once in the injector (ca. 100 µl). An example of peak quality obtained can be seen on *illustration 5*.



분석 보고서

분석 기관:
분석자 명:

분석 정보

시료 이름: inital-1 DW
파일 이름: 0027.RAW
분석 시간: 2008-05-19 오전 03:21:24
수집 채널: 1. UV730D A

크로마토그램

적분 결과

번호	피크이름	RT[분]	면적[mV*s]	높이[mV]	농도[ppb]
1	BPA	5.4000	226.5971	32.7137	595.6843
합계			226.5971	32.7137	595.6843

Illustration 5: BPA peak at RT: 5.4 min with an area of 226.6 mV/s corresponding to ca. 595 ppb

In *illustration 5*, the data beneath the graph relates to the concentration of BPA. The retention time RT is of 5.4 min, the area of BPA's response peak is 226.59 mV/s and its peak height 32.7 mV, this translates as 595.68 ppb. The ppb conversion of BPA has been realized thanks to an internal standardization.

- ***Internal Standardization***

Once the parameters of the HPLC are tuned to give the best performance, we need to create an internal standardization to translate the findings (area in mV/s) into understandable concentrations (ppm, ppb, ...). Several dilutions of BPA are prepared: 10 ppm, 1 ppm, 500 ppb, 200 ppb, 100 ppb, 50 ppb, 20 ppb, 10 ppb, 5 ppb, 0 ppb and tested with the HPLC.

The result is then plotted on a graph that will give us the coefficient of the slope of the correlation between concentrations and BPA response (area in mV/s). We can then estimate the concentration of BPA in any unknown sample, as seen in *illustration 5*.

Illustration 6 shows the processing of internal standardization done manually. Response peak areas on the left are averages of 3 tests of the corresponding dilution. The main graph illustrates the total plot of the data, whereas the upper right-hand graph is a zoom from 0 to 200 ppb and the lower right-hand graph, a zoom from 0 to 50 ppb. As one can see, the values change when getting to lower concentrations, exposing limits of detection. Thus, a certain range of error should be taken into consideration.

ppb	***mV/s***
10000	3685
10000	3670
10000	3722
1000	383.75
1000	383.23
1000	377.58
500	193
500	195
500	195.3
200	81.26
200	85.25
200	80.65
100	45.56
100	42.13
100	43.04
50	23.74
50	23.12
50	23.66
20	15.2
20	10.62
20	10.02
10	6.45
10	6.15
10	7.07
0	3.83
0	3.93
0	4.28

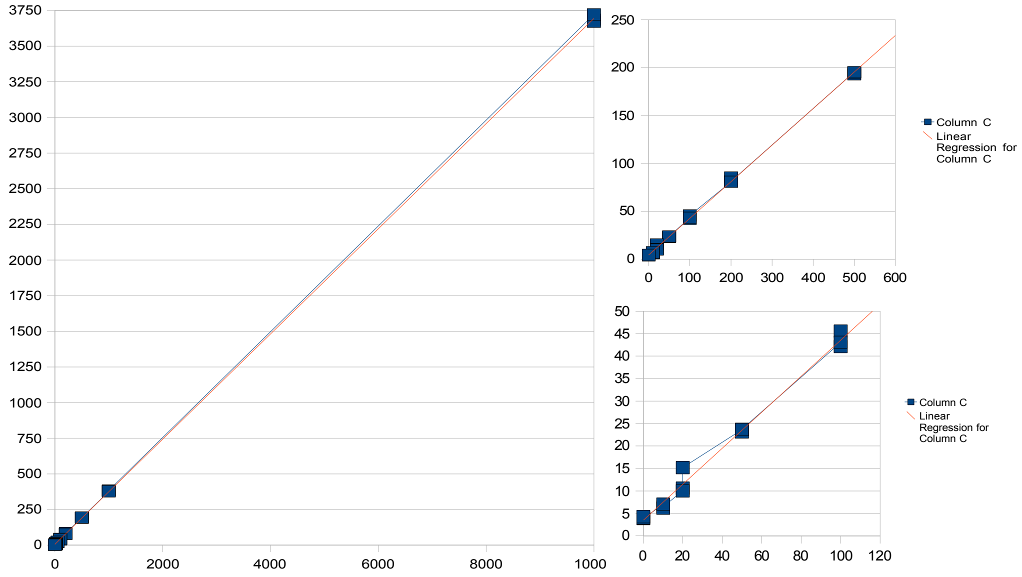


Illustration 6: Manual internal standardization

All data and ppb conversions in our experiment have been calculated with the integrated HPLC internal standardization, as seen in *Annex A*.

- ***Detection limits***

At first, we operated with the original injection tube of 20 µl and managed to get reasonable values for 20 ppb. The detection threshold proved to be 10 ppb. On receiving a new injection tube of 100 µl (5 times bigger), results were considerably improved in their resolution and peak height, from 3 to 7 times better.

Conducting non exhaustive experiments on low concentrations, the new detection limit proved to be 2.5 ppb. However, the authenticity of the dilution remains questionable at such a low concentration, and it is therefore safer to say that the HPLC could give credible results for up to 20 ppb. Since our resolution was not optimal, the design of the experiment took the former statement into consideration. High concentrations of BPA exposed to low amounts of PAC, would not ensure complete removal of all detectable BPA, but reach equilibrium. Equilibrium is reached when the given activated carbon concentration has adsorbed the maximum amount of molecules it can adsorb, and therefore all remaining molecules in solution will not be removed.

According to studies on the limits of detection of BPA with HPLC, a noticeably similar configuration could obtain detection limits of up to 0.2 µg/ml, which corresponds to 0.2 mg/l (200 ppb) without sample preparation [RYKOWSKA et al. (4)]. This underlines the difficulty of monitoring low concentration of BPA as a British study points out by saying that : "*Occurrence and low-level treatability of EDCs would require more advanced analytical instrumentation and preconcentration strategies.*" [YEOMIN et al. (8)]

3.3. Method

Before conducting time-consuming tests, we needed to determine in what "time-frame" the complete adsorption of BPA by PAC occurs. The first tests performed were non-exhaustive tests that took place on a long time-scale, in order to determine the maximum time required for equilibrium (maximum adsorption possible for PAC of a given concentration of BPA). Then knowing the time required for process, an experimentation on the adsorption kinetics versus contact time for a set concentration of PAC will be conducted, followed by an isothermal test.

a) Preliminary test

A preliminary test will help us determine a time frame of complete adsorption / saturation of PAC adsorptive property. Therefore we base our target on a 5 day experiment in which every day consist of an analysis. Bottles of 330 ml are used for the test. These have been thoroughly cleaned in a solution containing sulfuric acid (H_2SO_4), and dried in an oven for at least a full day at 120℃.

Deionized water (DW) containing 500 ppb of BPA solution is analyzed. Five different concentrations of PAC are used: 20, 50, 100, 200, 400 mg/l. As the experiment is set to last for 5 days with an analysis every 24 hours, this makes 5 analyses per day, hence 25 bottles. One extra bottle containing no PAC but DW+BPA will be used as a control bottle to attest that the concentration is not affected by other parameters.

The preliminary test revealed that the complete adsorption by PAC of BPA up to the capacity of PAC (i.e. equilibrium), occurred within 24 hours. It is essential to know this time frame in order to conduct the two following experiments: “kinetic of adsorption vs contact time” and “isotherm”.

b) Contact time and adsorption

We would like to observe the evolution of adsorption of BPA by PAC, when exposed to different amounts of PAC as well as different environments such as natural background organics (RW). In this experiment, we arbitrarily chose to take 3 different amounts of PAC: 15 mg/l, 24 mg/l and 45 mg/l. We also chose to lead the experiment with only two waters: RW and DW, all containing 600 ppb of BPA.

We chose to analyze the samples at 6 different times: 0 min, 15 min, 30 min, 60 min, 120 min and 240 min. The maximum time 240 min (4 hours), is often encountered in scientific articles dealing with the adsorption of BPA, as being the equilibrium time. Zero minutes corresponds to the analysis of the sample without contact to PAC, thus having 600 ppb BPA. A graphic summary of the different samples and their samplings is illustrated in *table 5*.

Table 5: Sampling plan of the "time to time" test

PAC in mg/l	**Time in minutes**				
	15	**30**	**60**	**120**	**240**
15	RW	RW	RW	RW	RW
15	DW	DW	DW	DW	DW
24	RW	RW	RW	RW	RW
24	DW	DW	DW	DW	DW
45	RW	RW	RW	RW	RW
45	DW	DW	DW	DW	DW

At first, all amounts of PAC are weighted and put in their respective bottles of 330 ml, labeled with the following information: sampling time, amount of PAC and type of water. 30 bottles of 330 ml are prepared. Bottles of 330 ml have been thoroughly cleaned in a solution of H_2SO_4 and dried in an oven prior to use.

River water (RW) and DW receive a certain amount of BPA at 100 ppm in order to have a final concentration of 600 ppb. Five liters of RW + BPA and 5 liters of DW + BPA are prepared in 5 liter Teflon containers. As the real amount required is slightly less than 5 liters, the rest will be used to determine the 0 min BPA concentration.

When ready, all 30 bottles are entirely filled with the BPA solution, to avoid air bubbles and possible effects of oxidation. A pastille of Teflon is placed between the water meniscus and the cap of the bottle to create an air proof / waterproof seal. The timing then starts when bottles are full. Bottles requiring long time frames are placed in a tumbler that will slowly shake them evenly. For shorter time frames such as 15 min and 30 min, the mixing is done by hand.

At the planned time, a portion of each of the 330 ml bottle is filtered with a 0.45 µm membrane (PTFE 0.45 µm filters from Target®), to remove any PAC. Enough is taken for HPLC and possibly DOC test control, and then stored at 5°C. All samples are analyzed by HPLC to find their remaining BPA concentration. Results are plotted in the *Result and discussion* chapter.

c) Isotherm tests

Adsorption is often described with isotherms. Adsorption isotherms are normally developed to evaluate the capacity of Activated Carbon for adsorption of a particular molecule.[MARSH et al. (29)]. Because adsorption is relative to temperature, in order to assess the adsorption of a certain molecule, experiments are done at an identical, constant temperature: hence "isotherm". In an effort of normalization and ease of comparison between different types of adsorbent, quantities adsorbed are usually expressed in the unit mass of the adsorbent. [WIKIPEDIA (3)]

Isothermal adsorption tests will demonstrate how much adsorbate (BPA, organic matter,...) on the adsorbent (PAC) has accumulated at equilibrium (time where no more adsorption takes place). In this experiment the contact time is set to 24 hours for all samples, in order to make sure that the equilibrium is reached. Thus, only the concentration of PAC will be the variable here.

Five types of PAC for 3 types of water are taken as illustrated on *table 6*. HA stands for humic acid and is added to the river water (RW) to act as an increased background organics sample. A concentration of 14.54 mg/l of humic acid was added to the river water. (cf. r*eagents and materials / humic acid*)

Table 6: PAC amount per water

Type of water	PAC in mg/l				
DW	2	4	6	8	10
RW	4	8	12	20	30
RW + HA	4	8	12	20	30

Note that DW has smaller amounts of PAC (cf. *Table 6*). This has been set to avoid DW being totally deprived of its BPA upon equilibrium, as no other organic matter than BPA would impede the adsorption. If all BPA had been adsorbed it would have been difficult to get credible results with the HPLC, as our detection limit was restricted at low concentration.

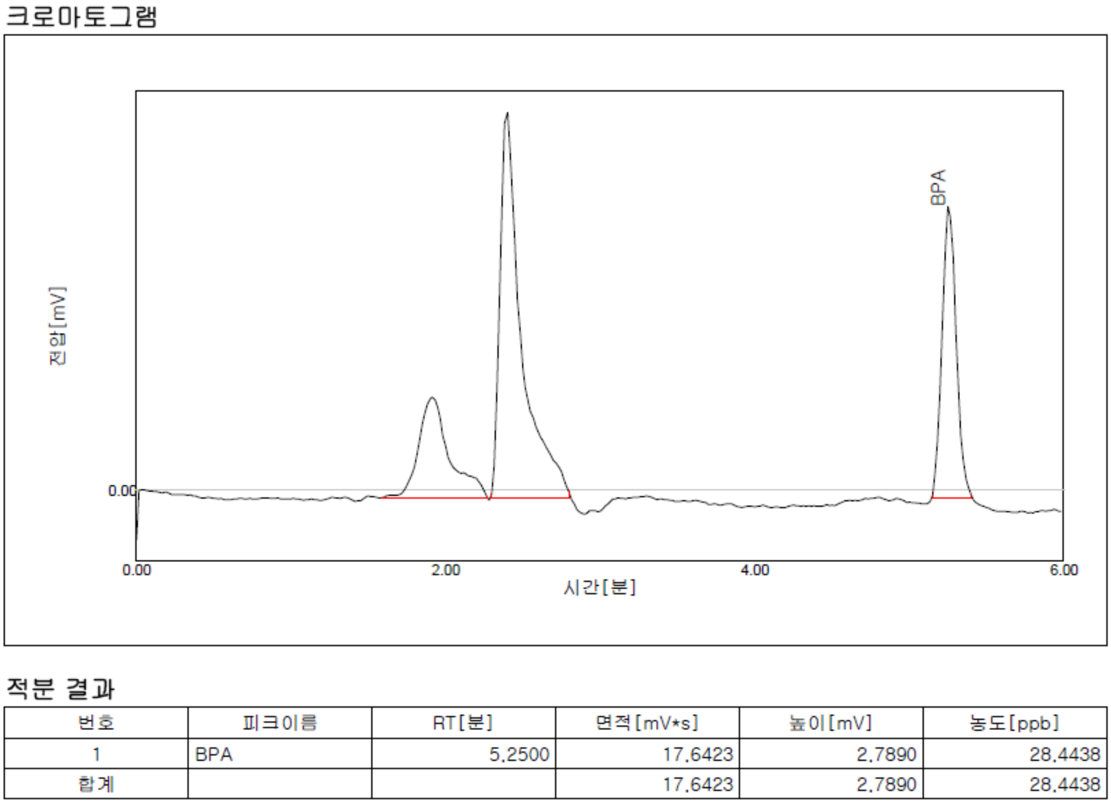
The experimental process is similar to the one of the "*contact time and adsorption test*", but this time 1 liter bottles are used in an effort to reduce the error, as smaller amounts of PAC are required. Bottles have also been thoroughly cleaned in a solution of H_2SO_4 and dried in an oven prior to usage.

All 15 bottles + 1 control bottle containing BPA and DW but no PAC, are put in a mixing tumbler at constant temperature and left for 24 hours. All samples are then filtered at 0.45 µm, controlled for DOC and stored at 5°C until HPLC analysis. All samples are analyzed by HPLC to find their remaining BPA concentration. Results are plotted in the *Result and discussion* chapter.

4. Results and discussion

Examples of HPLC results of samples after adsorption: *illustration 7 and 8*

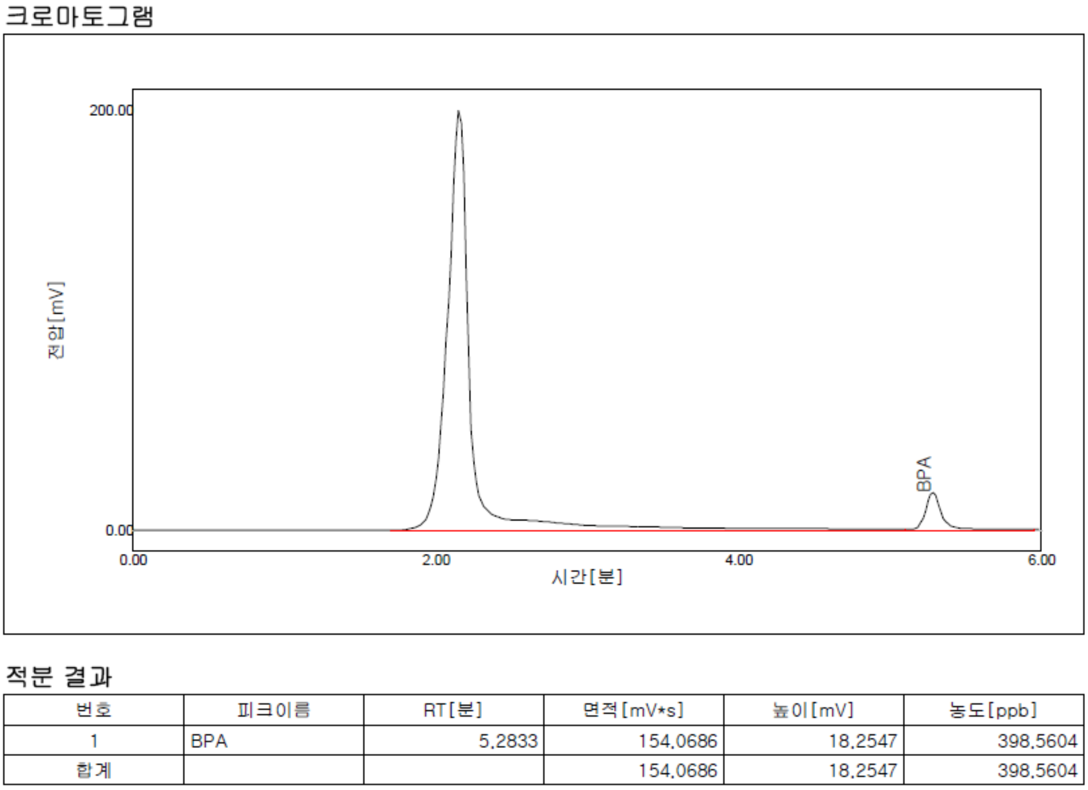
Note that they have different y-axis scale. Thus *illustration 7,* even though of a bigger concentration than *illustration 8,* appears smaller.



적분 결과

번호	피크이름	RT[분]	면적[mV*s]	높이[mV]	농도[ppb]
1	BPA	5,2500	17,6423	2,7890	28,4438
합계			17,6423	2,7890	28,4438

Illustration 7: Small concentration of BPA detected on HPLC after adsorption.



적분 결과

번호	피크이름	RT[분]	면적[mV*s]	높이[mV]	농도[ppb]
1	BPA	5,2833	154,0686	18,2547	398,5604
합계			154,0686	18,2547	398,5604

Illustration 8: Big concentration of BPA detected on HPLC after adsorption.

4.1. Contact time vs concentration of adsorptive

Illustration 9 shows the plot data of BPA concentration remaining in solution after a certain contact time. This graph has been built simply by converting the results as shown in *illustration 7 and 8* to concentrations in µg/l (ppb), using the internal standardization in *annex A*. As initial concentration of BPA was intended to be close to 600 ppb but was not exactly that due to large volumes prepared, the y-axis in *illustration 9* is simply the remaining concentration at t time (C), divided by the initial concentration (Co) of that particular type of water, hence C/Co.

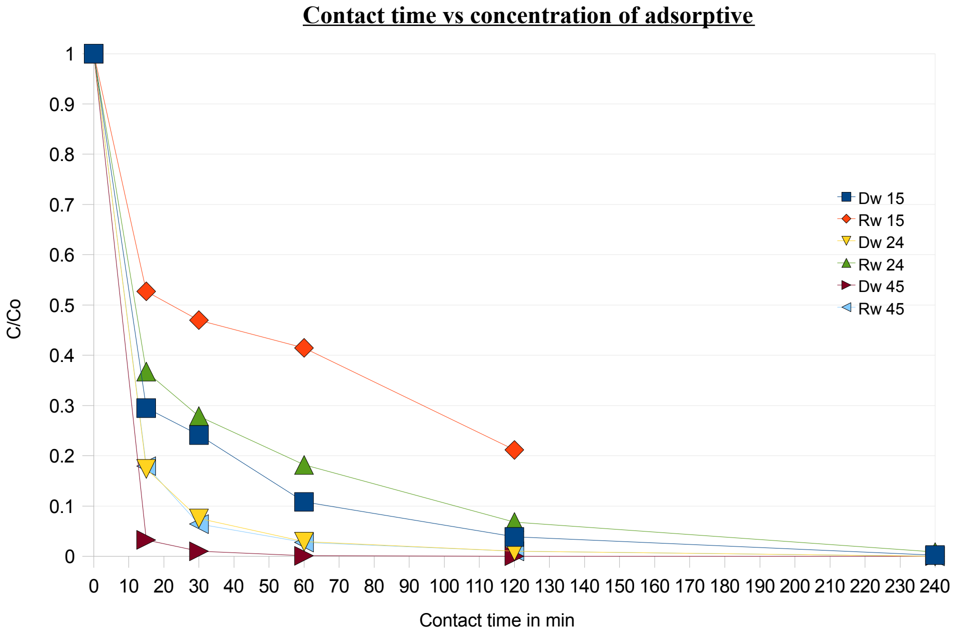


Illustration 9: Contact time versus concentration of adsorptive

Results for RW15 (River water and 15 mg/l of PAC) is lacking data, for the unfortunate reason that the sample broke in the tumbler. However, we can easily see that after 240 min of contact time, the PAC would not have adsorbed all of the BPA. In comparison with that, DW 15 did much better but complete adsorption is still questionable. RW 45 is behaving roughly similarly to DW 24, we can thus understand that under natural levels of organic matter, double amount of PAC is required to adsorb BPA to the levels it would have been adsorbed without competing background organics. It is important to recall from *table 2* that RW had an average DOC of 2.38 ppm. Roughly speaking, 240 minutes seemed to be justified as an ideal contact time for reaching equilibrium.

4.2. Isotherm test

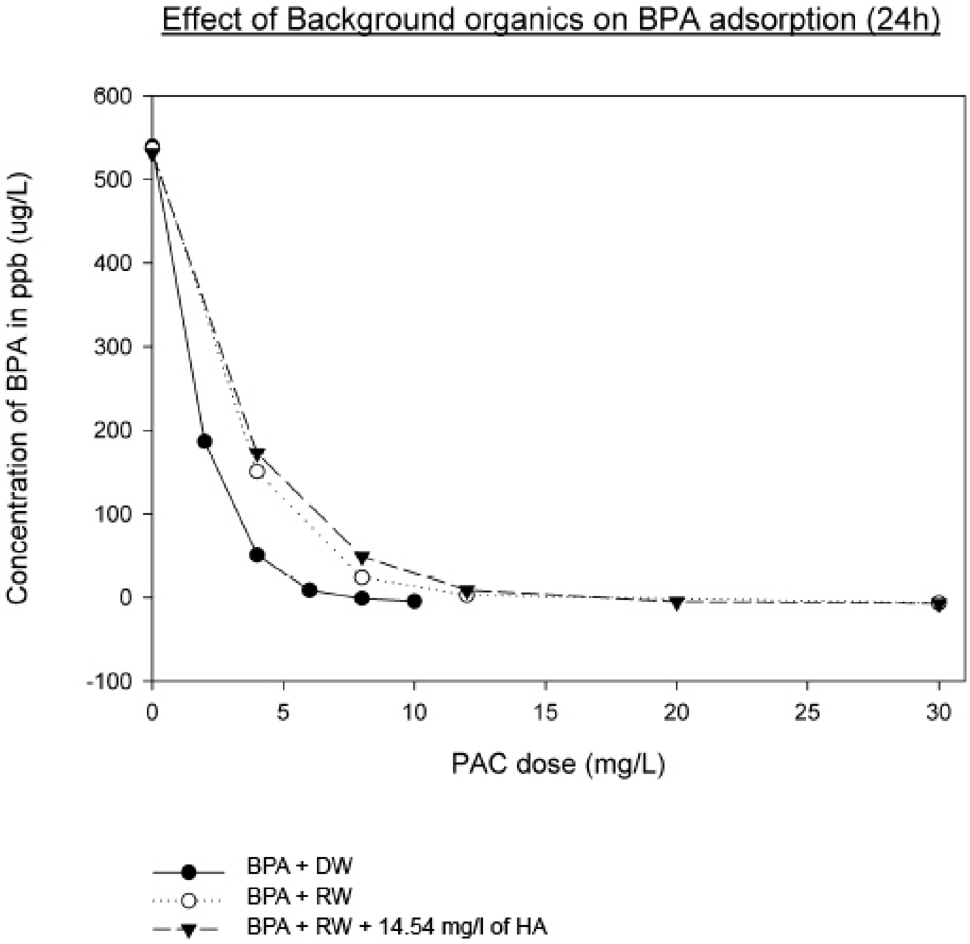


Illustration 10: Effects of Background organics on BPA adsorption

Illustration 10 shows a plot of the isothermal test. Similar to the contact time vs concentrations experiment, we can see that background organics have an effect on the adsorption. However, the degree of that effect is difficult to quantify, hence the need to convert the values with the Freundlich equation. We can nevertheless confirm that the change of PAC dosage for DW was justified, as it reached complete removal with ca. 9 mg/l of PAC. If the PAC dosage had not been changed, we would have got only 2 computable points. Also as seen on the graph, one point is missing for BPA + RW, at 20 mg/l of PAC. This was also due to a broken sample in the tumbler. This could not be corrected later due to a lack of time, and the fact that doing this particular dosage anew would have had a different initial concentration and temperature, hence different results. We used the following formula inspired by the Freundlich coefficient on adsorption:

$$Q=\frac{mg/l\ of\ adsorbate}{mg/l\ of\ PAC} \quad \text{with}$$

Adsorbate = initial adsorptive concentration - (minus) adsorptive concentration at equilibrium.

The result Q is a unit-less coefficient that is however expressed as $\frac{g}{g}$. Grams of adsorbate over grams of adsorbent.

The data similar to the one plot in *illustration 10*, is then recalculated using the above equation, and put in order as shown in *table 7.*

Table 7: Concentration at equilibrium of BPA vs Q of adsorption for each water

Concentration of BPA at equilibrium	**Q value**		
mg/l (ppm)	DW	RW	RW + HA
0.186579162	0.17639		
0.172418894			0.08968
0.150554572		0.09659	
0.050609846	0.12219		
0.048937018			0.06027
0.023790335		0.06414	
0.008843386			0.04352
0.008427439	0.08849		
0.002604187		0.04452	
0.000999005	0.06754		
0.000995370	0.05440		
0.000994710			0.02682
0.000993399		0.01812	
0.000992754			0.01795

Values in *table 7* are then plot into a graph, as seen in *illustration 11*.

The x-axis value was converted to g/l and all axis' scales were converted to logarithm, in order to represent the data as a Freundlich isotherm fit. In this graph we can understand that, when only adsorbing BPA (BPA + DW), the amount of adsorbate is higher than the amount of adsorptive left in solution ($C_{adsorbate} > C_{adsorptive}$). However, the data of RW (BPA + RW) and HA (BPA + RW + 14.54 mg/l of HA), shows us that at first BPA is well adsorbed, (trend similar to BPA + DW as $C_{adsorbate} > C_{adsorptive}$) but then the adsorption of BPA starts being slowed down by background organics competing on the adsorbent (The ratio $C_{adsorbate}/C_{adsorptive}$ diminishes) and the trend is even reversed at 1.8 g/g for RW and 1.75 g/g for HA ($C_{adsorbate} < C_{adsorptive}$), as background organics obstruct the way.

Deciphering the Freundlich isotherm fit visually is easy, but quantitatively it is more difficult. It should be understood that values of a same kind are not connected to each other. Thus for the DW fit, concluding that “the more BPA is adsorbed, the more BPA is left in solution”, does not make any sense. The points 1.8 g/g with 0 g/l left and 1.9 g/g with 1 g/l left are isolated values that have each reached equilibrium. These are ratios.

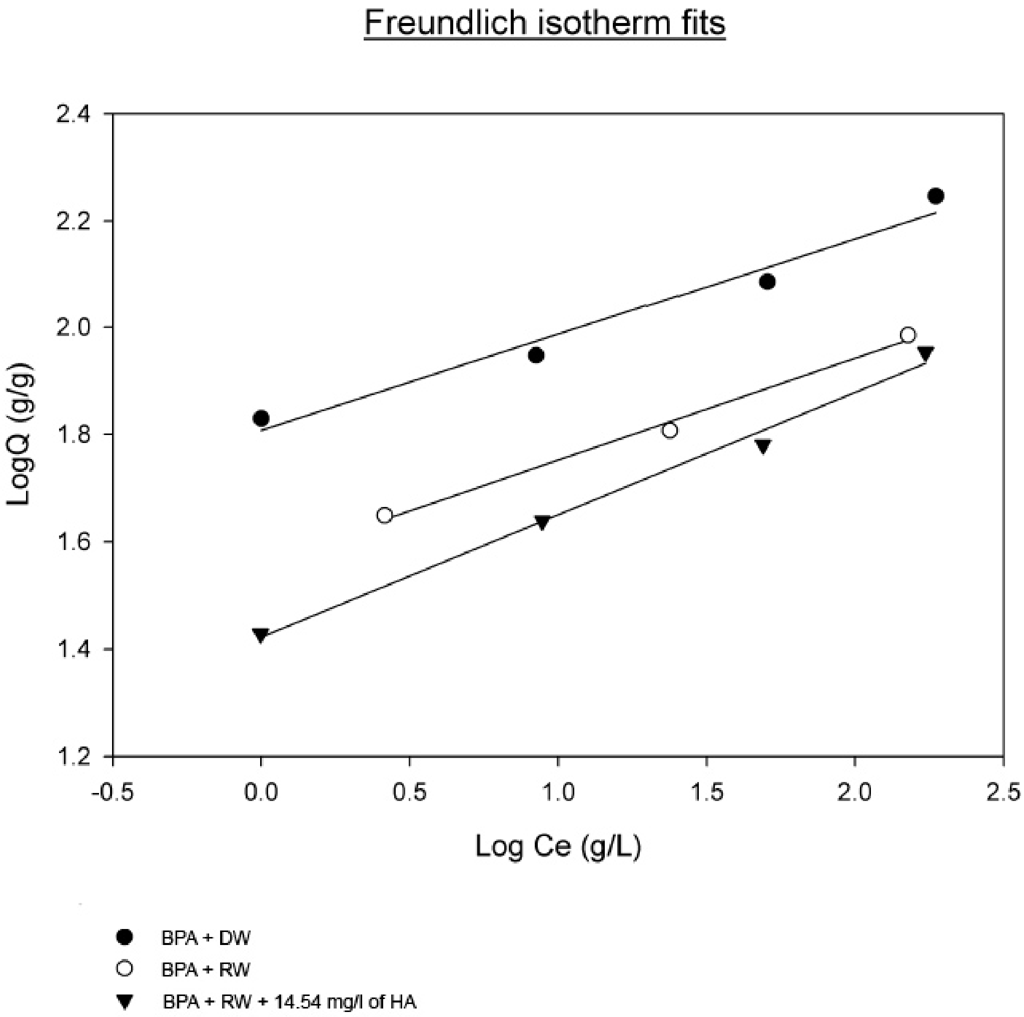


Illustration 11: Freundlich isotherm fit

At equal amounts of BPA on adsorbent (e.g. 1.8 g/g), the DW fit has no BPA left in solution (ca. 0 g/l) but RW has ca. 1.45 g/l and HA ca. 1.75 g/l. We can thus deduce that with an increase of 200% in background organics (HA's DOC is double that of RW's DOC), the efficiency of the adsorption is reduced by 20%.

Further analysis would have been required to clearly determine the amount required for practical usage. A breakthrough curve, is often used to determine water treatment (Activated carbon) system size requirements, contact time and carbon usage rates. This is usually done with dynamic column studies. However, the results of this experiment were conclusive to answer whether or not background organics had an effect on the adsorption of BPA, and to which degree it did impede it.

4. Conclusion

With this study we have observed that under natural levels of organic matter (RW's DOC is ca. 2.38 ppm), double amount of PAC is required to adsorb BPA to the levels it would have been adsorbed without competing background organics. Also, 240 minutes seemed to be justified as being the minimum contact time to reaching equilibrium.

We can determine from this experiment that BPA is very well adsorbed by activated carbon, but in presence of heavy organic competition, the adsorption sees its efficiency considerably reduced (ca. 20% for a 200% increase in background organics), and shall then be taken into account both by the amount of PAC used, and the contact time chosen (as seen thanks to the “*contact time vs concentration*” experiment).

The values expressed in this report should not be used in practice unless a thorough study is done. This report has answered whether or not background organics had an effect on BPA's adsorption, as a basis for conducting further studies such as dynamic column studies, to determine a water treatment system's (Activated carbon) size requirements, contact time and PAC concentration. Thus it falls in the qualitative kind of report and should not be used as if it were a quantitative work.

This study has modestly demonstrated the extent to which background organics are impeding the adsorption and removal of BPA. However, studies enumerated earlier in this study, demonstrated the importance and alarming effect of BPA at very low concentrations. The limit of detection in our experiment was essentially handicapped by a combined lack of knowledge, material and time.

Nevertheless, this demonstrates the concern where routine checking of the efficiency of carbon filtration in the removal of EDCs, can never be certain of absolute removal, or of its removal to certain standards, as BPA's capacity to be filtrated will fluctuate with background organics present in effluents, not to mention the difficulty of detecting low concentrations.

Low dosages that are easily encountered in the environment, the toxicity of which many refuted, do indeed have effects such as:

- the possibility of developing prostate cancer when exposed during development (infancy) [HO et al. (15)]
- oversized growth of female snails [OEHLMANN et al. (16)]
- increase in obesity potential [MASUNO et al. (17)]
- altered behavior and brain structure [KUBO et al. (18)]

It is thus important to stress that despite the many ways in which BPA and EDCs can be removed, and activated carbon being one efficient method, it is impossible to claim certainty of its complete removal and claim that safe dosage has been reached, as the toxicity of these compounds are not simply reduced to a dose-response relationship. Indeed, research has yet to confirm the role of lower dosages in overall toxicity, compared to bigger amounts. BPA is technically non persistent, and degrades easily, but its ubiquity is of major concern for the perennation of species and the entire ecosystem.

Finally, I would like to share the following quote from Rykowska's study, as it illustrates well the dilemma which we are facing : "*It must also be kept in mind that humans are exposed not only to this one compound but to many potentially risky materials in the environment. Therefore, even if the concentration of each hazardous compound is below a safe limit, their sum can exceed the level tolerated by an organism and trigger negative effects.* " [RYKOWSKA et al. (4)]

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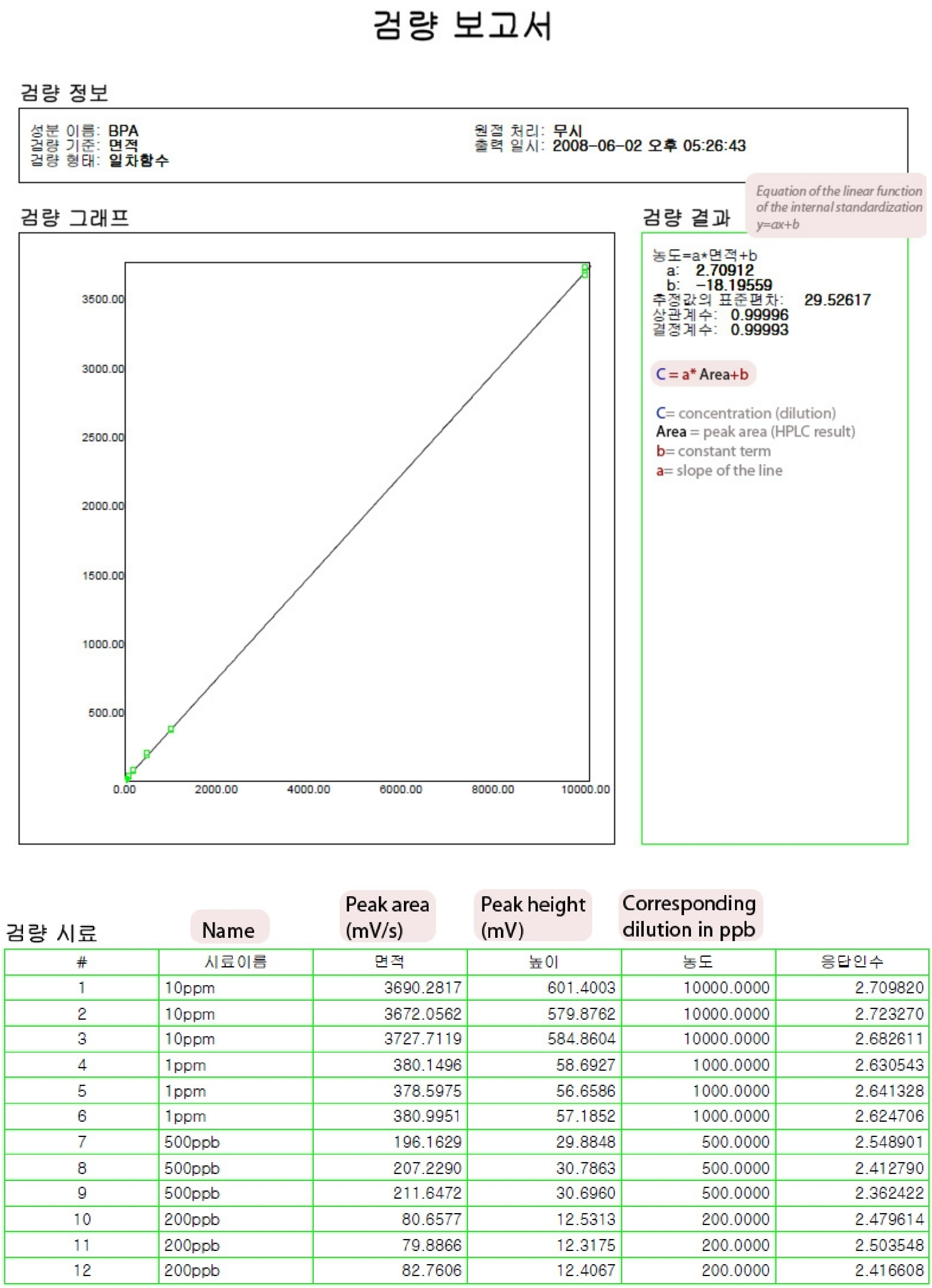
6. Appendices

APPENDIX A:

HPLC automatic internal standardization



검량 보고서

검량 정보

성분 이름: **BPA**
검량 기준: **면적**
검량 형태: **일차함수**
원점 처리: **무시**
출력 일시: **2008-06-02 오후 05:26:43**

검량 그래프

검량 결과

Equation of the linear function of the internal standardization y=ax+b

농도=a*면적+b
a: **2.70912**
b: **−18.19559**
추정값의 표준편차: **29.52617**
상관계수: **0.99996**
결정계수: **0.99993**

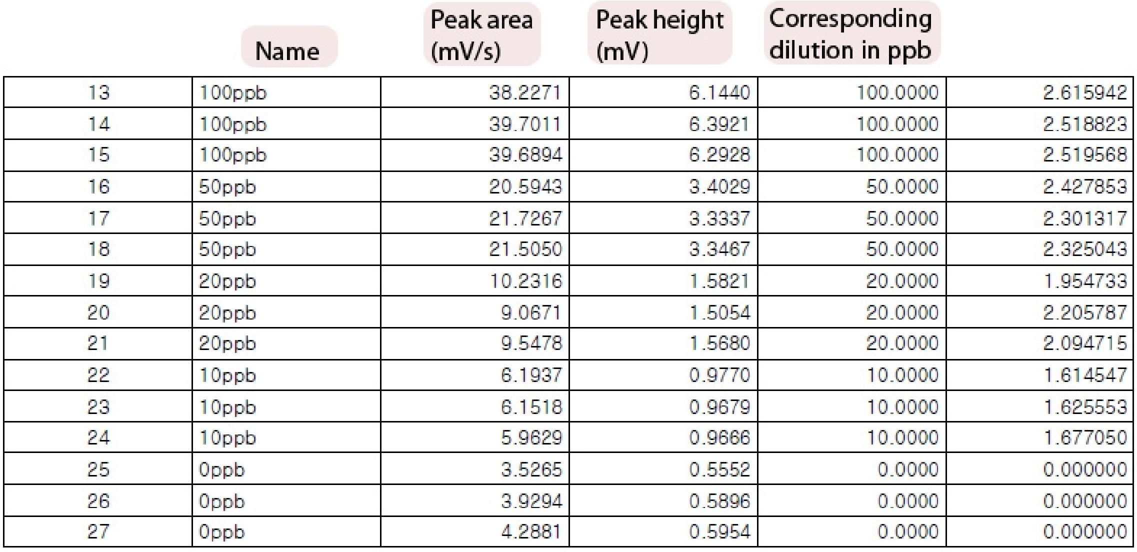
C = a* Area+b

C= concentration (dilution)
Area = peak area (HPLC result)
b= constant term
a= slope of the line

검량 시료

Name — Peak area (mV/s) — Peak height (mV) — Corresponding dilution in ppb

#	시료이름	면적	높이	농도	응답인수
1	10ppm	3690.2817	601.4003	10000.0000	2.709820
2	10ppm	3672.0562	579.8762	10000.0000	2.723270
3	10ppm	3727.7119	584.8604	10000.0000	2.682611
4	1ppm	380.1496	58.6927	1000.0000	2.630543
5	1ppm	378.5975	56.6586	1000.0000	2.641328
6	1ppm	380.9951	57.1852	1000.0000	2.624706
7	500ppb	196.1629	29.8848	500.0000	2.548901
8	500ppb	207.2290	30.7863	500.0000	2.412790
9	500ppb	211.6472	30.6960	500.0000	2.362422
10	200ppb	80.6577	12.5313	200.0000	2.479614
11	200ppb	79.8866	12.3175	200.0000	2.503548
12	200ppb	82.7606	12.4067	200.0000	2.416608



	Name	Peak area (mV/s)	Peak height (mV)	Corresponding dilution in ppb	
13	100ppb	38.2271	6.1440	100.0000	2.615942
14	100ppb	39.7011	6.3921	100.0000	2.518823
15	100ppb	39.6894	6.2928	100.0000	2.519568
16	50ppb	20.5943	3.4029	50.0000	2.427853
17	50ppb	21.7267	3.3337	50.0000	2.301317
18	50ppb	21.5050	3.3467	50.0000	2.325043
19	20ppb	10.2316	1.5821	20.0000	1.954733
20	20ppb	9.0671	1.5054	20.0000	2.205787
21	20ppb	9.5478	1.5680	20.0000	2.094715
22	10ppb	6.1937	0.9770	10.0000	1.614547
23	10ppb	6.1518	0.9679	10.0000	1.625553
24	10ppb	5.9629	0.9666	10.0000	1.677050
25	0ppb	3.5265	0.5552	0.0000	0.000000
26	0ppb	3.9294	0.5896	0.0000	0.000000
27	0ppb	4.2881	0.5954	0.0000	0.000000