Development and Applications of a Simple Spectrophotometric Method for Determination of Bisphenol A in 'New' and 'Used' Polycarbonate Baby Feeding and Water Bottles

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Abstract

Recently endocrine disorders have become a big concern. Endocrine-disrupting chemicals are contaminants interfering with hormone biosynthesis, metabolism, or change how the endocrine system works. Bisphenol A is an endocrine disruptor having weak estrogenic activity and competes with body native estrogen. Polycarbonate containers are known to be major sources of bisphenol A that leaches to the container's content through hydrolysis. This study aimed at monitoring levels of bisphenol A exposure to polycarbonate users through a novel spectrophotometric technique. The polycarbonate samples used were baby feeding bottles and portable water bottles. The Spectroscopic method employed a diazotization reaction procedure resulting in the formation of yellow azo-dye that is easily detected using UV-Vis spectroscopy. Prior to use, the sampled 'suspected' polycarbonate bottles were verified to be Polycarbonate using Fourier transform infrared spectroscopy.

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The Fourier transform infrared spectra of thin plastic solids were identified by scanning between, 500-4000 cm^{-1} for characterization. The resulting spectra were compared with those of standard polycarbonate plastics reported in the literature. Characteristic polycarbonate peaks at 2970, 1970, 1504, 1187, 1013, 1079 cm^{-1} associated with the –C-H, C=O, C=O, O-C-O, -CH$_3$ stretching vibrations respectively confirmed sampled bottles were polycarbonates. Bisphenol A levels for all the sampled polycarbonate bottles in the various environments ranged between 0.17 – 2.82 µgmL^{-1}. All the samples surpassed the tolerable daily intake for BPA of 4 µg/kgbw/day recommended by the European Food Safety Authority and this calls for serious consideration.

Keywords: Bisphenol A; Diazo-coupling Reaction; Polycarbonate Bottles; Infant Formula.

1. Introduction

Many substances resulting from anthropogenic activities interfere with physiological roles of living organisms, and later on adversely affect the status of their health. Bisphenols, which are predominantly manufactured worldwide, are the primary substances within this category of compounds. The main type of Bisphenol is known as Bisphenol A (BPA), which is a chemical found in high quantities globally and has an impact on the environment. Bisphenol A was first manufactured in 1891, but was not utilized broadly until its uses in the plastics business were distinguished during the 1950s [1]. The chemical formula for Bisphenol A is (CH3)$_2$C(C$_6$H$_4$OH)$_2$ mainly used in the production of polycarbonates PC [2-4], with plastic additives. Polycarbonates mainly find use as food contact products i.e., recyclable beverage bottles, feeding bottles for children and storage containers. Epoxy resins are usually used in beverage and food cans to form protective linings. BPA is a colorless solid, soluble in organic solvents, but not soluble in water [5]. It is used in the manufacture of infant feeding bottles and water bottles, and generally tend to leach from the bottles into their contents [6]. Approximately 95% of BPA manufactured is used to make polycarbonate and epoxy resins, [7]. The remaining 5% is utilized in a ramification of merchandise, that consist of phenoplast resins, phenolic resins, unsaturated polyester resins, can linings, antioxidants and inhibitors for PVC manufacturing, ethoxylated BPA, components used in thermal paper manufacturing, changed polyamide, compounding factor used in manufacturing of automobile tires, flame retardants, automobile system, optical media consisting of DVDs, electric gadget production, dental sealants, supermarket thermal receipts amongst others [8-10]. Bisphenol A disrupts the endocrine system mimicking estrogen and thyroid hormone, acting as metabolic and immune disruptor. This implies that the health effects of BPA are adverse and this can be attributed to its potential to cause hormonal imbalance in natural systems [11-14]. Because humans are continually exposed to BPA through various routes such as consumption, inhalation, and contact with the skin, it leads to negative health effects. Consequently, it is essential to conduct more extensive studies and make advancements in order to protect human health and ensure the environment is not compromised.

Many techniques have been employed for determining the levels of BPA in the environment, it's fate and BPA's behavior, among the techniques include, capillary electrophoresis, gas chromatography coupled with mass spectrometry (GC-MS) [15], high performance liquid chromatography mass spectrometry (HPLC-UV-Vis) [16], enzyme linked immune sorbent assay (ELISA) and electrochemical methods. Pretreatment procedures have also been done for extraction in solid-phase, liquid-liquid extraction and immuno-affinity chromatography, HPLC-

MS and ELISA for precisely determining BPA levels in complex matrices. These techniques are effective as they are rapid but also expensive [17]. We develop and use a simple diazotization method for the detection of BPA in PC bottles usually used in making baby feeding bottles and water carrying bottles.

2. Materials and Methods

2.1. Sampling

In this study, 'suspected' polycarbonate (PC) sample bottles were either sourced from a local supermarket or from the neighbouring community. Two types of bottles were used; namely baby feeding bottles (labeled as B-series) and water/juice containers (labeled in the W-series). Initially all the sampled bottles were selected on 'suspicion' that is their choice was based on characteristics like excellent toughness, clearness and good thermal stability which are known characteristics of PC polymers. Of the B series B1, B2, B3 were new (had never been used before-bought) and B4, B5 and B6 were of 'used category'. For this study, 'used' bottles were defined as those that were more than 2 months old in usage. These bottles were obtained in exchange for new ones.Similarly the water/juice bottle samples were labeled W1, W2, W3 which were of the 'new' and W4, W5, W6 which were 'used'. A total of six water/juice bottle samples were employed. All the samples were then transported to the Chemistry Department, Physical Chemistry laboratory at the University of Nairobi for analysis. At the laboratory, all the bottles were subjected to the manufacturer's first time bottle washing procedure after which the washing water was discarded.

2.2. Chemicals and Reagents

The reagents utilized in spectrophotometric technique included; 99% analytical grade BPA standard and 99.9% methanol (CH_3OH) both purchased from Sigma Aldrich. Other reagents included; 99% sodium nitrite from BDH PROLABO; 37% (w/w) hydrochloric acid and 98% (w/w) sulphuric acid from Merck; 98% analytical grade sodium hydroxide pellets; 99.5% aniline from Sigma Aldrich; 67% nitric acid; 99.8% acetonitrile, Sigma Aldrich; and 99.5+% acetone purchased from Alfa Chemicals. Other products used included baby formula (from supermarkets) and juices.

2.3. Apparatus and Instruments

The instruments used in this study were Shimadzu IRAffinity-1S - Fourier Transform Infrared Spectrophotometer and Shimadzu UV-1700 PharmaSpec UV-VIS Spectrophotometer in addition to other common laboratory equipment.

2.4. Confirmations Text for Bottle Nature

To confirm the nature of the sampled bottles whether they were PC or not, a small piece was obtained from each of the bottles and analyzed by Fourier-transform infrared FTIR spectroscopy. Samples were analysed in their solid state without any further preparation. A Shimadzu IRAffinity-1S - Fourier Transform Infrared Spectrophotometer was applied using the attenuated total reflectance mode (ATR) within the 4000-500 cm^{-1}

range. Before each measurement, ethanol was used to clean the ATR crystal, and a background spectrum was run to make sure that the solvent had completely evaporated. Polymer identification was achieved based on comparisons with reference pure BPA spectra from literature/library. The analysis confirmed that all 12 samples were made of polycarbonate nature.

2.5. Procedures for the Construction of a BPA Calibration Curve using the Diazotization Method

2.5.1. Preparation of the Aniline Monomer

The aniline monomer liquid was triply distilled to purify it and consequently stored under nitrogen away from light - aniline is hygroscopic and also tends to darken due to atmospheric oxidation when exposed to light. The distilled aniline was then used to prepare a 1000 ppm aniline stock solution by taking 0.93 mL of the distilled aniline into a 1000 mL volumetric flask containing some distilled water/methanol mixture and shaken until dissolving. The flask was then topped up to the mark using distilled water.

2.5.2. Diazotization of Aniline/ Construction of Calibration Curve

1 mL of 120 μgml^{-1} aniline was added to a mixture containing 1 mL of 0.1 mol L^{-1} HCl and 1 mL 100 μgml^{-1} $NaNO_2$. The solution was shaken thoroughly, thereafter, 1 mL of BPA 120 μgml^{-1} was added and the mixture was shaken intermittently for 2 minutes followed by addition of 1 mL 0.1 $molL^{-1}$ NaOH for complete coupling reaction. It was then filled to the mark with distilled water, after color development the absorbance was measured in 1.00 cm quartz cells at 424 nm using distilled water as a reference. This was a two-step process involving; formation of the diazonium salt and the diazo-coupling process.

2.5.3. Optimization of the Daizotization/ Diazo-coupling Reaction Conditions

To achieve the best performance of the developed method a series of experiments were performed to optimize the reagents and time of diazo-coupling reaction. The parameters optimized includes concentrations of aniline, sodium nitrite, sodium hydroxide, hydrochloric acid and diazotization reaction time. The optimization was carried out base on one factor at a time, other conditions remained constant during each optimization.

2.6. Spectrophotometric Determination of the Calibration Curves for BPA

Once the optimized conditions for the diazotization/diazo-coupling process were obtained, a calibration curve for the BPA was determined. The intensity of azo- dye hue was reflective of the concentration of BPA present. Different concentration of BPA in the range 2-12 ppm were used and process was repeated severally. During the spectrophotometric measurements a 1 cm cuvette was used to measure the absorbance of the resultant yellow dye. A 20 mL methanol solution was used as a calibration blank.

2.7. Sample Preparation for BPA Detection

Before use of the milk and infant formula for BPA leaching studies, the two baby foods were pre-prepared. To

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prepare the baby formula and milk, protein precipitation was done according to the method used by (Bashir and Audu, 2020). Briefly, 100 mL of boiling water was poured to a polycarbonate baby feeding bottle, after which 3 spoons (12.0 g) of the baby formula was added as directed by the manufacturer. The mixture was well stirred and set aside for two hours. After that, the mixture was acidified with 100 μL of concentrated hydrochloric acid. Then 5 mL of the acidified solution was combined with 10 mL of acetonitrile in 20 mL conical flask. The precipitate that formed was centrifuged for 10 minutes at 4000 rpm and filtered using Whiteman filter papers after which 10mL of ultra-millipore water was added. The resultant solution was measured for any migrated BPA using UV-Vis spectrometry, this process was repeated for milk.

For the remainder of the test samples namely distilled water and lemon water, exactly 20 mL distilled water and /or lemon water were placed in the pre-prepared PC bottles and allowed to stand for 2 hr prior to analysis for BPA. Sample contents were subject to different temperatures before been placed in the PC bottles. The temperatures employed were 25 ^{o}C, 50 ^{o}C and 95 ^{o}C.

However, where the sample bottles were scarce and unobtainable from the same source, analysis was done utilizing bottles that had been cut into small pieces for easier analysis. Sample bottle pieces of varying weights 0.5 to 1.3g were selected for analysis to represent the entire population. The experiment was then conducted in boiling tubes and the PC bottle pieces placed into the samples being analysed.

For effect of contact time experiments, the food contents were allowed to stand in the characterized PC bottles for a different lengths of time namely 1, 6 and 12 hr with regular shaking. Consequently, the amount of BPA leached were measured spectrophotometrically after diazo-coupling process.

2.8. Method Validation

Method validation was conducted in terms of recovery and sensitivity.

2.9. Recovery Experiments

The Spectrophotometric method recovery experiments were performed with distilled water which had been spiked with BPA at concentration of 2, 4 and 6 ppm and analysed like the unknown samples. The sensitivity of the method was determined in terms of the limit of detection (LOD) and the limit of quantification (LOQ) the limit of detection (LOD) and limit of quantification (LOQ) were obtained by taking several measurements of diazo-coupling reagent blank (n = 10) and calculated from the slope of the calibration curve according to ICH guidelines. LOD = 3 S/m, and LOQ= 10 S/m, where "S" is the standard deviation of several measurements of the blank and "m" is the slope of the calibration curve.

3. Results and Discussion

3.1. Confirmation of Nature of the Sampled Bottles

 Figure 1 (a) shows the FT-IR spectrum of one of the sampled 'suspected' PC bottles. A comparison with a BPA

FTIR spectra from a library sample (Fig. 1(b)) was done. Results show very similar peaks in both spectra indicating the sampled bottles were PC in nature.
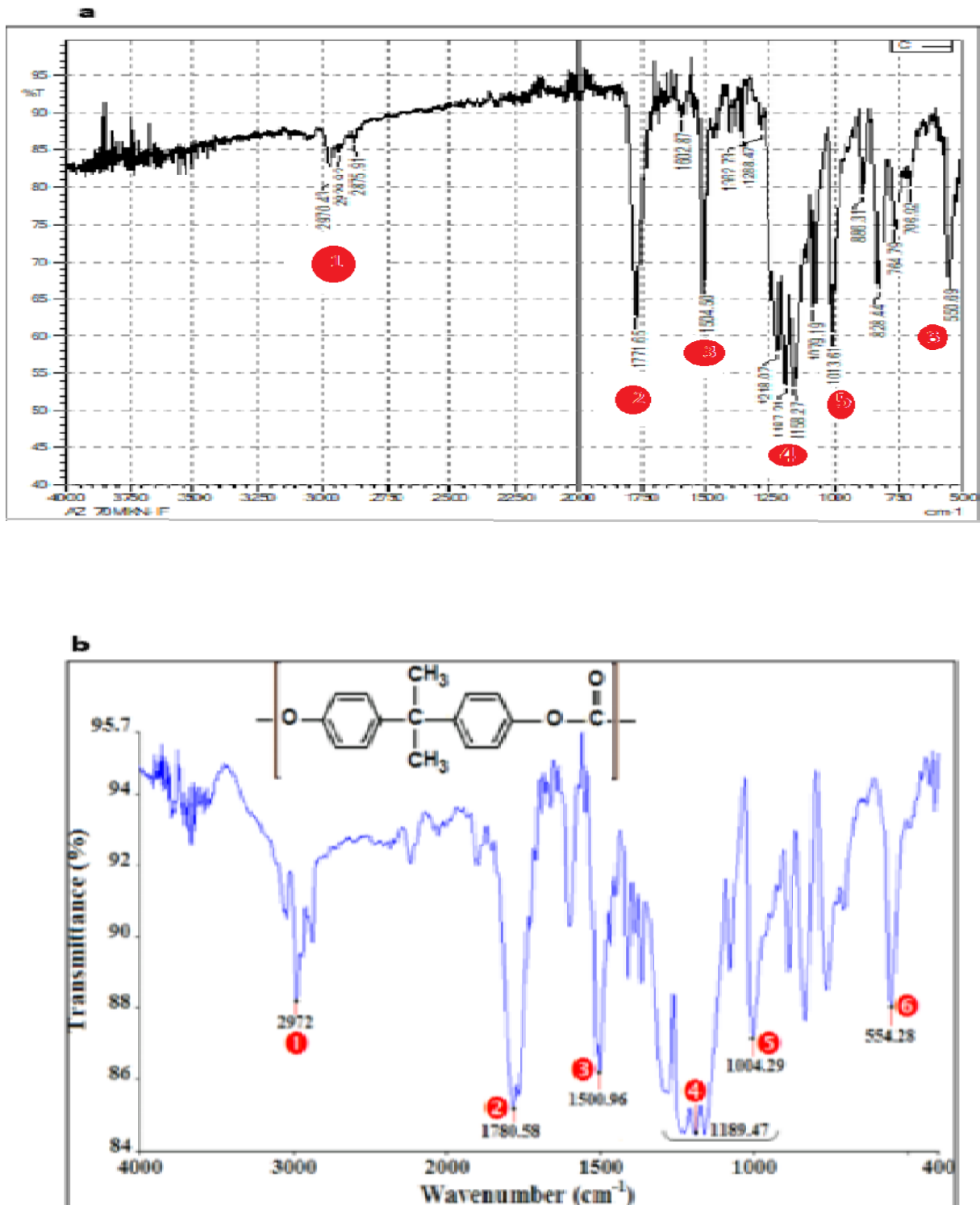

Figure 1: (a) FTIR spectra of one of the sampled PC bottles suspected to contain BPA. (b) FTIR of pure BPA obtained from library

The prominent peaks in both FTIR spectra were; characteristic vibrations at 2970 the vibrations attributed to the

stretching vibration of C-H aromatic ring; peaks at 1770 attributed to carbonate group (C=O) vibration; peaks at 1504 for the C=C vibration of the aromatic rings; peaks at 1187 and 1013 associated with vibrations from the two phenolic rings; bands corresponding to stretching deformations of asymmetric and symmetric O-C-O carbonate group and finally at 1079 can be attributed to CH_3 vibrations. These results are similar to what is found in the literature [18] for polycarbonate plastics as shown in figure 3 (b). Slight shifts in the peaks were attributed to the fact that the BPA in PC is in different chemical environment that may lead to shifts compared to the case of a pure BPA sample. However the major peaks 1-5 characteristic of BPA (figure 1 b above) were also identified in the 'suspected' PC sample (figure 1 a above).

### 3.2. Mechanism for the Formation of the Diazonium Salt

The aniline monomers in the presence of sodium nitrate and hydrochloric acid under controlled temperature conditions react to form the diazonium salt. Scheme 1 below gives the general mechanism for the formation of the diazonium salt $C_6H_5N^+$=NCl$^-$. Once the diazonium salt is formed, the salt then reacts with e.g bisphenol A to produce a yellow azo dye with an absorbance at 424nm. The reaction for the formation of this azo-dye is given below;
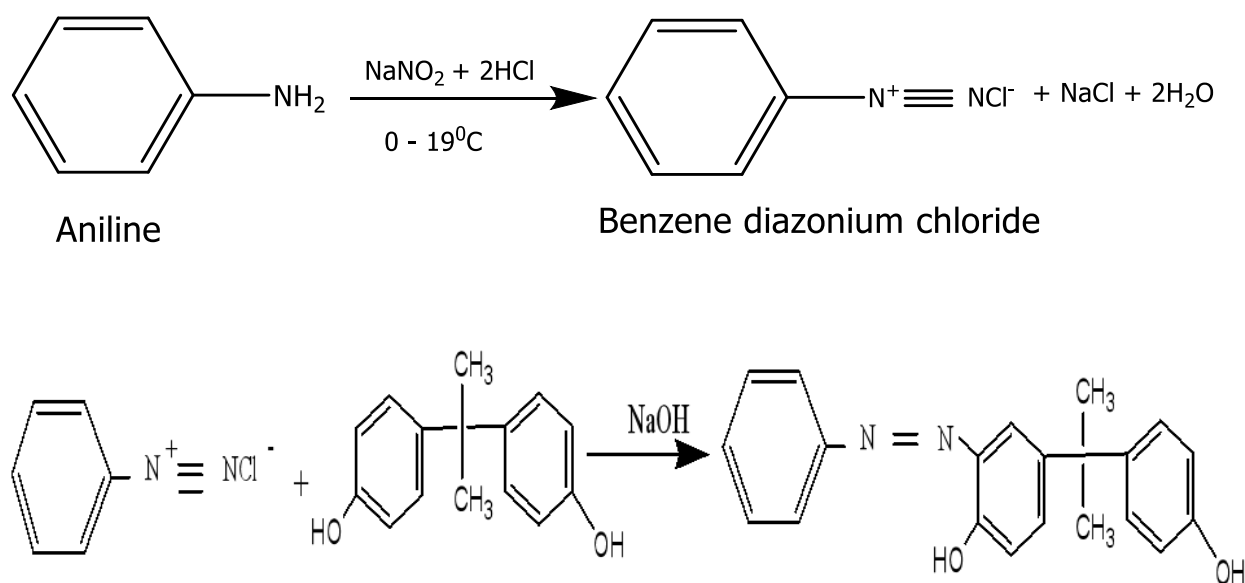
Figure 1: Mechanism for formation of a diazonium salt. Coupling reaction to form the azo dye

3.3. Optimization of Reaction Conditions for Spectrophotometric Technique

Prior to the detection of BPA, there was need to obtain the optimal condition at which the diazotization and the diazo-coupling processes were taking place. Reagents optimized were; sodium hydroxide, aniline, hydrochloric acid and sodium nitrate (figures 2 (a-d)). The realized optimized concentrations for the reagents were 120 μgmL^{-1} aniline, 0.1 $molL^{-1}$ hydrochloric acid, 100 μgmL^{-1} sodium nitrite and 0.1 $molL^{-1}$ sodium hydroxide. Though double maxima was observed for aniline, the concentration corresponding to the first maxima was taken as optimal because the consequential drop followed by a rise was thought unusual.
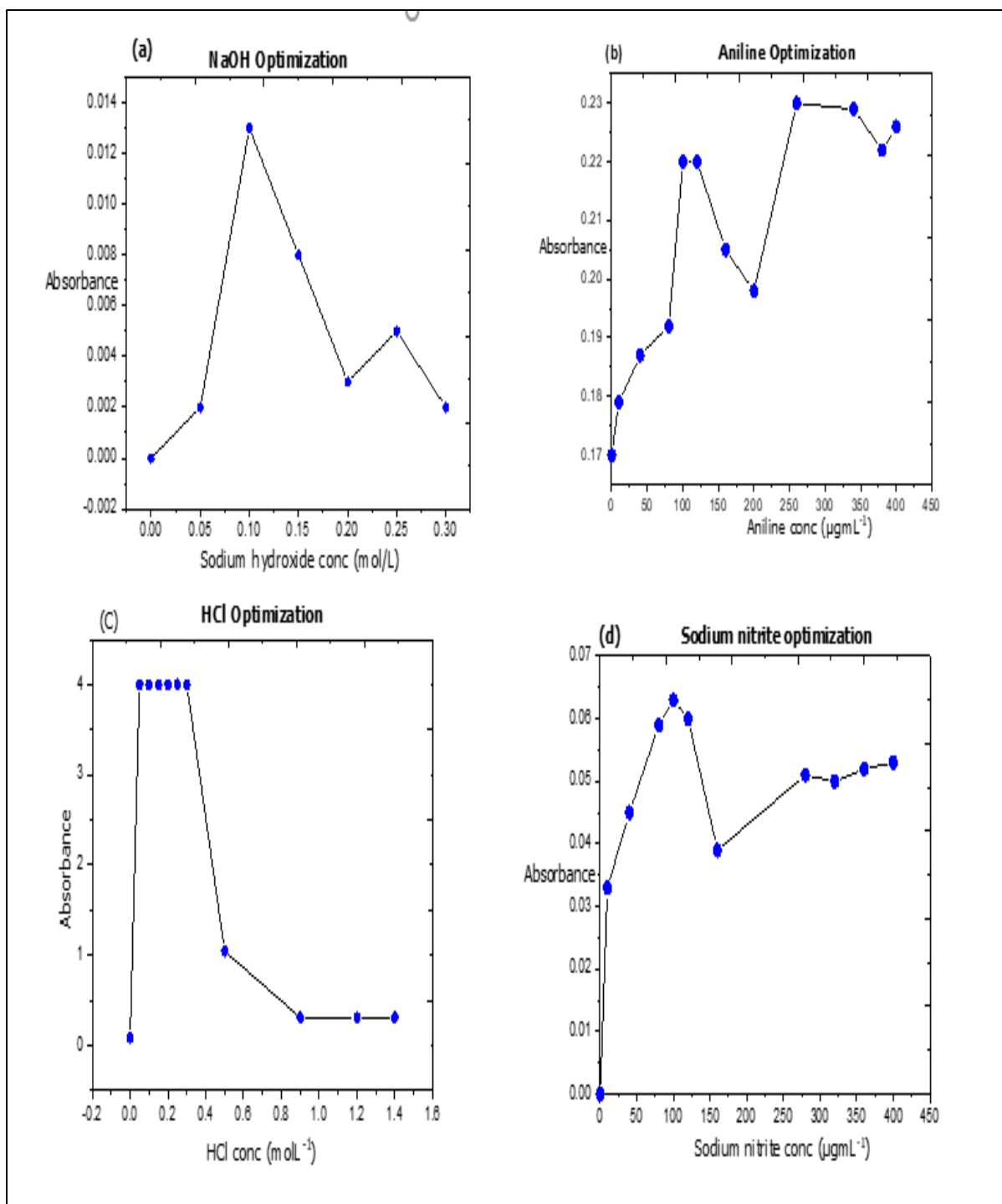
Figure 2: (a-d) parameters optimized for diazo-coupling reaction

Under the above optimized conditions, the realized absorbance data was plotted versus BPA concentration resulting into a BPA calibration curve with strict adherence to the Beer-Lamberts Law recording a linear correlation index, R^2 value of 0.999. The calibration curve was prepared by serially diluting a 100 ppm of 0.1M BPA stock solution to give final standard solutions of concentrations between 0 - 12 µg mL^{-1} concentrations. This was the expected range of BPA in the environment. The absorbance of all solutions were recorded on a UV-Vis spectrophotometer at λ_{max} = 424 nm and the calibration curve constructed is shown in figure 3 below.
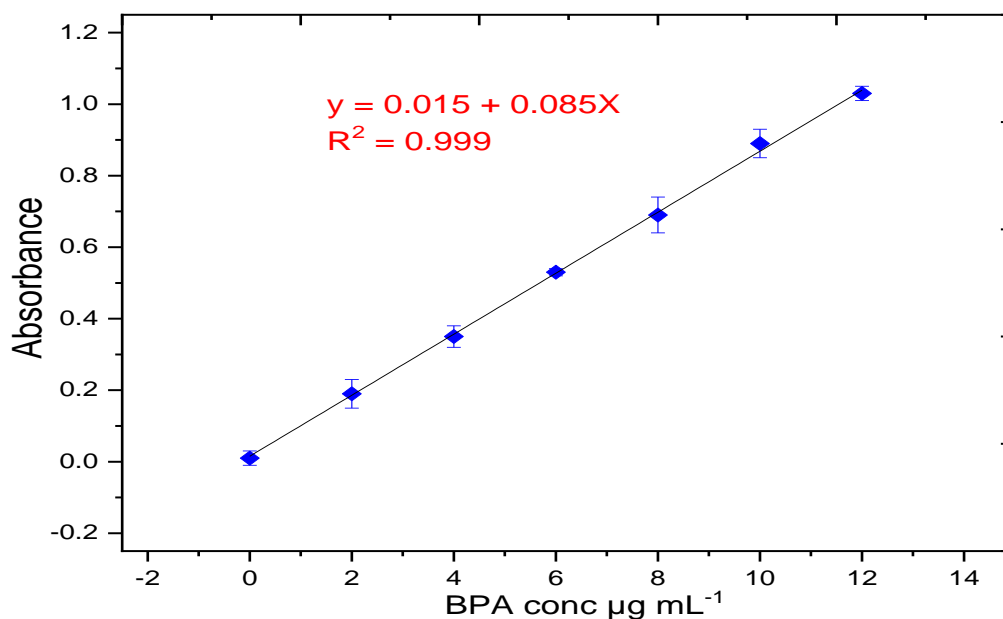
Figure 3: Calibration curve used for the BPA analysis constructed from UV-Vis data

A straight line calibration curve passing through the origin was obtained. The curve depicted adherence to Beer's law along BPA concentration range of (0 (blank) -12 µg mL^{-1}. The linear regression obtained is given by the equation; $Y = 0.0116 + 0.0859 X$ with correlation coefficient of $R^2 = 0.9991$. From the data of several such results, the LOD and LOQ were calculated using the formulae: LOD = 3.3 δ /S. Where δ is the calibration curve intercepts' standard deviation. The LOD and LOQ were estimated to be 0.8 µg mL^{-1} and LOQ 2.4 µg mL^{-1} respectively.

3.5. Recovery Test for Spectroscopic Method

The accuracy of the developed diazotization method for BPA determination was proofed through analytical recovery experiments done using 2, 4 and 6 µg/mL BPA concentrations as indicated in Table 1.

Table 1: Recovery Test for Spectroscopic Method

Analyte (BPA)	Standard added (µg mL^{-1})	Found (µgmL^{-1})	Recovery/%	RSD / %
	2.0	2.10	105.0	3.45
	4.0	4.12	103.0	2.09
	6.0	6.20	103.3	2.32

Good recovery percentages were recorded for BPA quantification method and they ranged from 103.0 ± 2.09 - 105.0 ± 3.45 %. Therefore, the method's sensitivity was analytically acceptable and being simple, quick and having the ability to determine sensitivity, this method was advantageous.

3.6. Comparison of Average BPA Concentrations Obtained using 'New' and 'Used' Bottles Containing Different Content at Various Temperatures

Table 2 below shows the levels of BPA leached from the various food substances at different temperatures.

**Table 2:** Average Detected BPA Concentrations in μgmL^{-1}

Bottle type/ Bottle content	[BPA], (μgmL^{-1})achieved at different Temperatures			[BPA], literature (μgmL^{-1})
Distilled Water	**25°C**	**50°C**	**95°C**	
Average (W1-3-New)	0.17±0.04	0.43±0.02	0.63±0.05	**0.50±0.84 [19]**
Average(W4-6-Used)	0.30±0.05	0.58±0.06	0.68±0.05	**0.80±0.11 [2]**
				0.13-0.68 [20]
Lemon Water				-
Average (W1-3-New)	0.57±0.05	0.83±0.05	1.2±0.07	
Average(W4-6-Used)	1.0±0.06	1.3±0.08	1.6±0.11	
Milk				
Average (B1-3-New)	0.86±0.04	2.36±0.05	2.52±0.07	**1.78-3.85 [2]**
Average(B4-6-Used)	1.34±0.04	2.60±0.02	2.76±0.08	**0.13±0.27 [20]**
Infant Formula				
Average (B1-3-New)	0.93±0.03	2.13±0.06	2.24±0.11	
Average(B4-6-Used)	1.35±0.06	2.33±0.05	2.53±0.09	

A glance at Table 2 above shows that the BPA levels detected compared well with those reported in literature [2, 19, 20]. The average BPA levels per category indicate increase with rising temperature. When considering the actual range of BPA for all the samples, a range of 0.17 – 0.78 μgmL^{-1} in distilled water and 0.78 – 2.82 μgmL^{-1} in milk was obtained. Zhiqun and his colleaguese, 2017 [20] using a novel spectrophotometric method for the determination of BPA based on the diazo-coupling technique reported similar values to this work. In the mentioned study, the range of values realized for BPA leachates in milk samples were 2.36-3.83 μgmL^{-1} while as those reported from water bottles had arrange of 2.66 and 4.83 μgmL^{-1}. It is also interesting to note that their LOD and LOQ values of 0.05 μgmL^{-1} and 0.15 μgmL^{-1} respectively were in close agreement with those reported in this work. Bashir and Audu, (2020) [2] on BPA migration experiments based on the diazo-coupling principle and using UV-VIS spectroscopy found BPA levels of 0.37-5.93 μgmL^{-1} with a reported LOD of 0.48 μgmL^{-1}. The authors noted that BPA migration into foods was influenced by the type of solvent used. Elsewhere in a study for the quantification of bisphenol A levels in infant PC bottles using HPLC technique, Bashir and Audu, 2021 [21] reported BPA levels of 20-27 ng/mL. Diverse BPA leachate concentrations have been reported by different authors with some being very low compared to those reported here. A study on migration of BPA from PC baby bottles using UHPLC-MS/MS recorded highest BPA level of 0.0612± 0.0015 μgmL^{-1} and an LOD of 3.5 ng/L [22]. BPA concentrations migrating from re-usable plastic drinking bottles of

levels of 0.047μg/L based on the UHPLC-MS/MS method were reported [23]. Yet another study on BPA migration from PC baby bottles and sippy cups reported a value of 31.5 ng/L in water and noted that there was a 1.4 fold increase in BPA value in 5 % ethanol [24]. Thus various studies have reported diverse BPA migration concentrations. The US Environmental Protection Agency, 2010, (Torelable Daily Intake, TDI) has stated a BPA limit of 50 μg BPA/kg body weight. The European Food Safety Authority (EFSA) revised its original BPA of 0.05 mg/kg/body weight [25]] to 4 μg/kgbw/day limit [27-29]. Clearly then it means the BPA ingested daily as a result of consuming water and/or food substances from PC bottles can have diverse values depending on experimental conditions, source of bottles amongst other parameters. The values obtained in this value are clearly above the allowed limits. In considering that BPA exposure pathways are many (not just through food/water intake) and these could easily contribute also to the daily BPA exposure levels, the implications are that the BPA exposure levels could well be beyond the estimates here. It is evident that caution should be taken in using PC containers either for adults and/or babies or in fact given the adverse effects that BPA containers should be banned altogether. Polycarbonate bottles use cannot be regarded as safe.

3.7. Factors Affecting BPA Leaching

The trends realized in this study, while comparing effects of various factors in BPA leachability are presented in figure 4 (a-d). The parameters considered were temperature, type of food/drink and bottle type ('new/'used). From the graphs it can be depicted that there was slightly higher BPA leaching at 95°C compared to either 50°C or 25°C. This is also evident from Table 2. Increased leaching with increasing temperature could be explained by the fact that at higher temperature the degree of BPA hydrolysis into free monomers increased. With elevated temperature the enhanced reaction kinetics led to a release of more BPA in to solution. Similar trends were observed for BPA leaching from lemon water and infant formula at different temperatures. Cao and his colleagues, 2008, [30] remarked that BPA migration was directly related to temperature. In the mentioned study, BPA migration was found to be directly related to temperature with BPA concentration varying from 228 to 521 μg/L as the temperature increased after being heated at 70º C for 6 days. Similar findings on which BPA migration was temperature influenced were reported by Bahare and his colleagues., 2023 [31]. In another study investigating the release of BPA under house hold conditions from baby bottles marketed in Italy, migrated BPA level were found to vary with temperature being 13.80 ng/mL at 40º C and 23.20 ng/mL at 80º C a 68% BPA migration increase [32]. Elsewhere BPA migration into water placed in a new PC baby bottle was investigated by varying the temperature between 40-100 degrees C. Levels of the BPA concentrations obtained between the two temperature limits were 0-03-0.13ppb- a whopping 333% increased [33]. The authors noted that BPA migration levels increased rapidly when the temperature of the water was over 80 ºC.
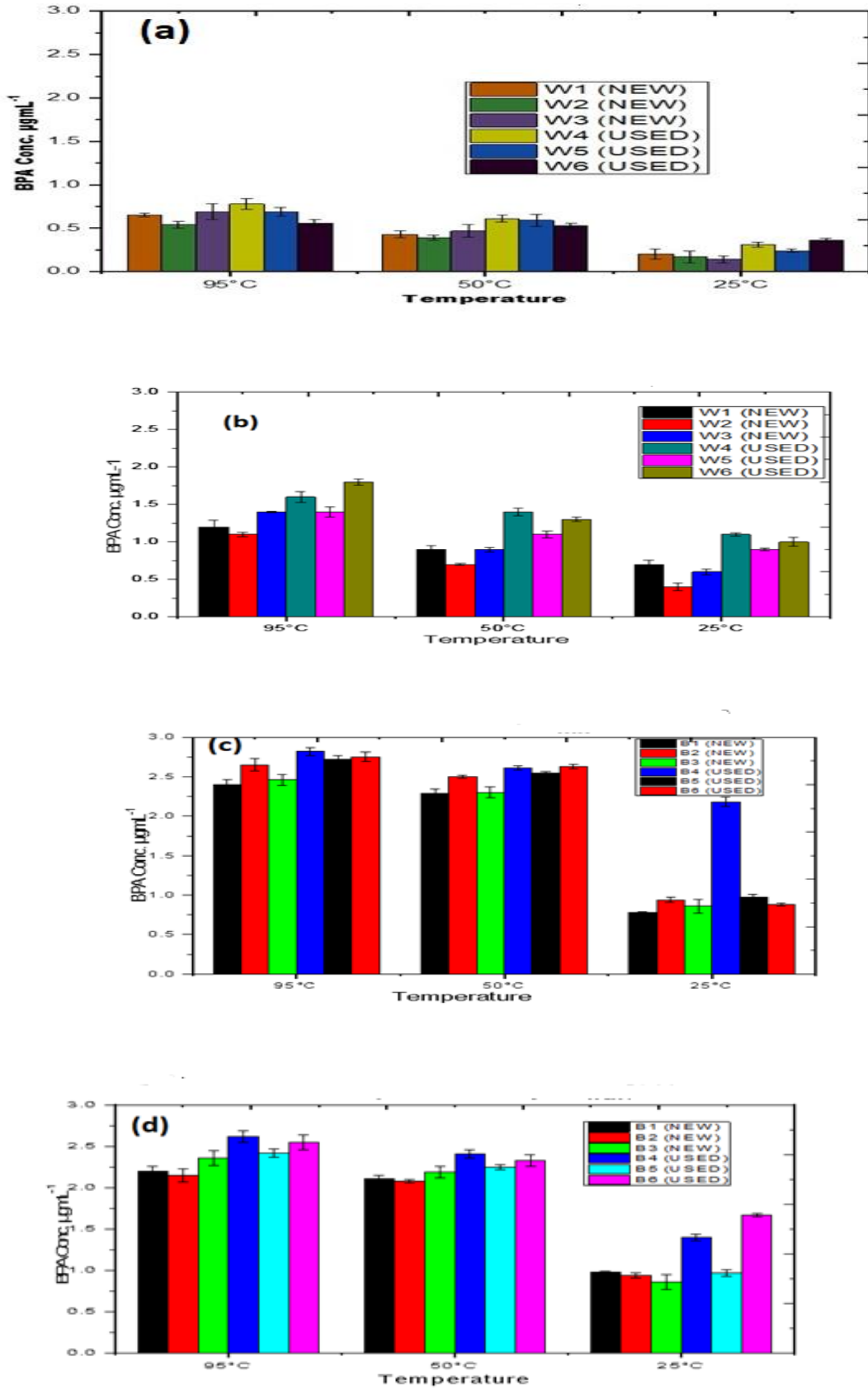
Figure 4: Effect of temperature on BPA levels leached (a) from distilled water, (b) Lemon water, (c) milk and (d) infant formula

Another phenomenon that was observed was the fact that irrespective of the temperature, BPA leachability was slightly higher in 'used' bottles. The leaching of BPA from 'used' water bottles W4, W5, and W6 series was higher (across all temperatures) may be due to the fact that these bottles had been subjected to harsh condition during washing and sterilization techniques practiced by mothers before feeding the baby. Washing, brushing and sterilizing has eventually loosened the BPA polymer bonds on the surface of the plastic material and this action has caused free BPA monomers to leach into the bottles while feeding [33, 34]. Other cleaning methods alternative that do not involve use of heat treatment or harsh chemicals should be sought. Brede and his colleagues, (2003), [35] on investigating the effect of dishwashing, boiling, brushing of PC bottles on BPA migration concluded that these practices favoured BPA migration. After washing PC baby bottles more than 50 times, the levels of migrated BPA increased form 0.23 ± 0.12 to $8.40\pm0.45\mu g/L$.

It was also found that the extent of leaching depended upon, the type of content in the bottle/constituting media. The leaching of BPA in the distilled water was lower than the values obtained in lemon water. For instance while as the amount of detected BPA levels in μgmL^{-1} in 'new' and 'used' water bottles containing lemon water were in the range of 1.10 ± 0.03 to 1.4 ± 0.08 and 1.60 ± 0.01 to 1.80 ± 0.13 μgmL^{-1}respectively, those of the same category containing only distilled water exhibited much lower values. The values obtained for the 'new' and 'used' water bottles containing only distilled water were in the ranges; 0.54 ± 0.04 to 0.69 ± 0.09 and 0.56 ± 0.03 to 0.78 ± 0.06 μgmL^{-1}respectively. It seems the acidic environment of lemon water would trigger or accelerate BPA hydrolysis into free monomer. These results were measured at $95^{o}C$. In general, the constituting solvent affected the amount of BPA migrating. In one study, the increase of ethanol concentration from 5 to 50 % over a period of 10 days at a temperature of 65° C lead to BPA leachate increase from 0.87 to 5.9 μgmL^{-1} [36]. In a study from Colombia, different BPA concentrations were reported depending on the solvent employed for constituting the samples. The BPA concentrations obtained in water, 3% ethanol, 15% Ethanol, 95 % Ethanol and isooctane were 2.6 ± 0.2; 1.4 ± 0.2; 4.8 ± 0.2; 5.4 ± 0.3; 7.1 ± 0.06 μgL^{-1} respectively clearly showing the effect of a solvent on BPA migration [20]. Elsewhere while testing solvent effect on BPA migration, different solvents were used namely; 20% ethanol, 4% acetic acid, and n-hexane. It was found that migration was highest in n-hexane with levels of BPA being 7.44-20 mg/kg in hexane, clearly indicating that the solvent/or constituting medium will after the extent of BPA migration [37]. The influence of constituting solvent on the BPA migration in the current study was not explored. Methanol was used as a constituting agent in the study especially for BPA/aniline and at the same time use of concentrated HCl in the infant milk precipitation could be explored. The effect of both milk and infant formula on the amount of BPA migrated showed that the type of food placed in the PC bottles had a direct effect on the amount leached. Value of BPA concentration of between great than 2 μgmL^{-1} at $95^{o}C$ were measured. These values were way high compared to those obtained in the case of either distilled water or lemon water. The range of BPA levels from infant formula at this temperature in 'new' and 'used' baby feeding bottles respectively were; 2.15 ± 0.06 to 2.36 ± 01 μgmL^{-1}. The values of the BPA migrated in 'used' baby feeding bottles were; 2.42 ± 0.11 to 2.62 ± 0.07 μgmL^{-1}. This observation could be due to the fact that BPA has higher solubility in lipids as compared to water since it is hydrophobic and hence it's more soluble in fatty material. The effect of lipid content on the amount of BPA absorbed could be further justified by the fact that, the BPA concentrations were higher in milk than in infant formula. The infant formula is usually made form skimmed milk which has less fat than the whole milk. The range of BPA concentrations (μgmL^{-1}) obtained

for milk in both 'new' and 'used' Baby feeding bottles were; 2.40 ± 0.06 to 2.46 ± 0.07 and 2.72 ± 0.11 to 2.82 ± 0.05 µgmL^{-1} respectively. This indicates more BPA migration in to milk as compared to infant formula.

Increased contact times leads to increased BPA migration. In this study, BPA migration concentration obtained both new and used water bottles carrying different beverages were in the range 0.55 ± 0.02 µgmL^{-1} to 3.02 ± 0.02 µgmL^{-1} respective. There was a marked increase in BPA migration as the contact time was increased and the situation was amplified with temperature increase. Wang and his colleagues, 2019, [38] on his study on determination of bisphenol analogues in food contact plastics concluded that BPA migration increased with contact time and the situation was exacerbated by rise in temperature. In a certain study, the amount of BPA at 40 degrees C that migrated ranged from 0.11 microg/L in water when incubated for 8hr to 2.30 microg/L when incubated for 240hr in 5% ethanol [39]. This indicates that consideration of both type of solvent and contact times are important in BPA migration. In another study on release of BPA from polycarbonate baby bottles based on HPLC with a fluorescence detector it was noted that the trademark was an essential factor in BPA release [40] indicating that the source of the PC bottles was important and the practice of the manufacturing body. Different PC bottles from different manufacturer will yield different BPA values.

4. Conclusion

FTIR analysis of all sampled 'suspected' PC bottle while compared with the library data showed they were all polycarbonates. The characteristic peaks associated with polycarbonates observed were; 2970 cm^{-1} due to C-H stretching of aromatic rings; C=O stretching vibrations at 1770cm^{-1}; C=C stretch vibrations at 1504 for the benzene rings, O-C-O vibrations at 1187cm^{-1} and CH$_3$ stretching vibrations at 1079 cm^{-1} amongst others. Aniline monomers through a diazotization process formed the diazonium salt that was coupled with either the BPA standard of the BPA leachate. Calibration curves made with the BPA standards based on yellow dye formation with a labda max at 424 nm were used to determine levels of BPA leaching from different environments. It was found out that the PC bottles leached BPA levels of between $0.17 - 0.78$ µgmL^{-1} in distilled water and $0.78 - 2.82$ µgmL^{-1} in milk. These levels surpassed the BPA tolerable daily intake (TDI) of 5 µg/kgbw/day advocated by EFSA. From the results obtained in this study we can conclude that polycarbonate bottles use cannot be regarded as safe. Various parameters including temperature, type of solvent, contact time, pH of food content, age of the PC bottle could have influence on the amount of BPA leachability.

5. Declaration

Limitations of the study

It is important to pinpoint that this study was subject to limitation which may/may not affect the results. Firstly, the sample size was rather small and an extensive study should be carried out to uphold or refute the results. Secondly since the bottles were purchased from the same region, it could be that they could be traced to same point of origin. Thirdly, in constituting some of the solutions such as the aniline (insoluble in water) and BPA, the role of methanol (part of the constituting solvent) to the contribution of BPA concentration migration could be investigated. It is also important to note that BPA analogue (BPF, BPS, and others) could be equally harmful

and an extensive study involving the analogues could give a more complete picture.

Recommendations

Since this was only a preliminary study, it is recommended that an extensive study of BPA migration be carried out to further verify the status. Use of different methodologies/instruments for measurements is also recommended. Analysis should also include bisphenol analogues which are also toxic. Employing a variety of instruments/techniques for comparison of results is recommended

Conflict of interest

The authors have no financial or non-financial interests to disclose relevant in the preparation of this manuscript

Data Availability

The datasets obtained in this study are available from the corresponding author upon reasonable request.

Author contributions

All authors equally contributed to this study directly in its entirety; from its conception, design, characterization and manuscript preparation. All authors read and approved the final manuscript

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