DETERMINATION OF Bisphenol A RELEASED FROM POLYCARBONATE INFANT FEEDING BOTTLES BY UV-VIS SPECTROPHOTOMETRY

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Received 24 September 2020; accepted 08 October 2020, published online 30 October 2020

Abstract
Bisphenol A (BPA) is a synthetic organic compound used as a raw material for the production of polycarbonate plastics, which are widely used in infant feeding bottles, kitchen utensils and other food packaging materials. The migration of bisphenol A from polycarbonate feeding bottles into water and milk samples was investigated using UV-Vis spectrophotometry based on diazotization-coupling reaction. The proposed method involves diazotization of 4-aminobenzenesulphonic acid under acidic condition in the presence of sodium nitrite at low temperature, followed by its coupling with Bisphenol A, in alkaline medium to produce yellow color azo-dye. The diazo-coupling reagents and time were optimized, the azo-dye formed has absorption maximum at 439nm. BPA was detected within the range of 0.37 – 5.93 μg/mL in the samples using standard addition method. The validation parameters were LOD: 0.48 μg/mL, LOQ: 1.62 μg/mL, while recovery for spiked samples were averagely 97.87 % and %RSD ranged between 0.12- 4.9%. Milk samples have exceeded the tolerable daily intake (TDI) for BPA of 50μg/kgbw/day recommended by EFSA.
Key words: Bisphenol A, Migration, polycarbonate, Azo-dye, Standard Addition.

1.0 Introduction
Bisphenol A, 2, 2 - Bis-(4-hydroxyphenyl)-propane (BPA), is one of the most important synthetic materials largely used in plastic industry, with the chemical formula \((\text{CH}_3)_2\text{C}(\text{C}_6\text{H}_5\text{OH})_2\) belonging to the group of diphenylmethane derivatives. BPA is used as a raw material for the production of polycarbonates and a key monomer for the production of epoxy resins and as non-polymeric additives in other products. Polycarbonates are polymers containing carbonate groups (-O-(C=O)-O-), produced from bisphenol A and phosgene \((\text{COCl}_2)\) or bisphenol A and diphenyl carbonate \((\text{C}_6\text{H}_5\text{O})_2\text{CO}\). Polycarbonate plastics are typically hard and clear and are marked with the resin identification code number ‘7’. They are used in food and drink packaging, infant bottles, toddler sipping cups, tableware, and food containers (1). Epoxy resins are used to line metal products such as cans for food, bottle tops, and water supply pipes. The use of polycarbonate containers for the purpose of storage is controversial, because of their hydrolysis (degradation by water) often referred to as leaching occurring at high temperature, releases bisphenol A (1). BPA can inevitably migrate into foodstuffs and beverages from packaging material, resulting in trace amount of BPA being ingested (2).
BPA can leach from polycarbonate into liquid foods by two different processes: diffusion of residual BPA present in bottle after the manufacturing process, and hydrolysis of the ester bond that binds them with polycarbonate or resin depending on the temperature, acidity and basicity conditions (3). For dried foods, diffusion is the most common process. Release of BPA into food depends on the contact time, temperature, and type of food. Thus the majority of human exposures to BPA occur mainly via diet, through packaged food and beverages (2). The highest estimated intake of bisphenol A occur in infants and children, averaging 0.375 ± 0.02 ppb per body weight per day for infants till 3 years of age (4). Their intake is high because BPA is suspected to leach from the plastic baby bottles as well as the linings of cans used in storing powdered and liquid formula. Bisphenol A has been characterized as potential endocrine disrupting compounds which can mimic or block the normal hormone function in animals and human, causing adverse effect resulting in incidence of diseases/dysfunction.
There is evidence of reproductive toxicity in laboratory animals and possible health effects in humans (2). Pregnant mothers (their embryos) and children are the most vulnerable populations to endocrine disruptor exposure. Bisphenol A has been detected using traditional analytical technique such as: liquid chromatography (LC), gas chromatography coupled with mass spectrometry (GC-MS) and enzyme-linked immunosorbent assay (5). In recent years, spectrophotometric method has attracted attention due to its simple operation, rapid response time, low cost, high sensitivity, instrument availability and capability of real sample analysis (6).

The spectrophotometric method based on diazo-coupling reaction has been used for the estimation of drugs and other organic compounds. It is a reaction of aromatic amino compounds with sodium nitrite to form diazonium salt, which undergoes substitution or coupling reaction to form azo-dyes. The coupling of diazotized sulphanilic acid has been used to determine Resagline Hemitartrate in drug formulation (7). Similarly the coupling of diazotized clenbuterol and sulphamethoxazole for the estimation of bisphenol A has been reported (8, 9).

To the best of our knowledge, the coupling reaction of sulphanilic acid with bisphenol A has not been reported, thus the study aimed to investigate the possible coupling reaction between BPA and sulphanilic acid with subsequent determination of BPA in polycarbonate infant feeding bottles.

The development of diazo-coupling reactions occur in two steps; firstly the sulphanilic acid reacts with sodium nitrite in acidic medium (HCl) at low temperature to produce the diazonium salt. Thereafter, the diazotized sulphanilic acid is coupled with BPA in alkaline medium to produce a yellow azo-dye that can be monitored spectrophotometrically.

2.0 Materials and Methods
2.1 Reagents and Standard: The chemicals used were BPA D_{16} standard (> 99%, Sigma-Aldrich, USA) sulphanilic acid, sodium nitrite, hydrochloric acid, methanol, sodium hydroxide and acetonitrile. Water used was purified by Synergy UV Milli-Q water purification system from Millipore. Glass ware were soaked in 0.2M nitric acid overnight then washed thoroughly, rinsed with distilled water and dried in an oven at 105°C. The use of plastic containers were avoided throughout the analysis.

Standard stock solution of BPA 1mg/mL was prepared by dissolving in methanol and kept at 4°C in an amber bottle, working solutions were prepared by further diluting the stock with distilled water. Oven dried sodium nitrite was used to prepare a stock of 1mg/mL which was stored in an amber bottle in refrigerator.

2.2 Selection of polycarbonate bottles
Based on physical properties of polycarbonate plastics, hard and clear feeding bottles with resindentification code ‘7’ were selected during sampling. The samples were further characterized by Fourier-transform Infrared Spectrometry (FTIR) and compared with polycarbonate reference material (Fig. 1).

2.3 Optimization of Reaction Conditions
A series of experiments were performed to optimize the reagents and time of diazo-coupling reaction. The parameters optimized includes concentrations of sulphanilic acid, sodium nitrite, sodium hydroxide, hydrochloric acid and diazotization reaction time. The optimization were carried out base on one factor at a time, other conditions remained constant during each optimization.

2.4 Procedure
1mL of 120 μgml⁻¹ Sulphanilic acid was added to a mixture of cold solution containing 1mL 0.1mol L⁻¹ HCl and 1mL 140 μgml⁻¹ NaNO₂. The solution was shaken thoroughly and allowed to stand for 5 minutes in an ice bath (0°C-5°C). Thereafter, 1mL of 0.12 mg mL⁻¹ BPA was added and the mixture was shaken intermittently for 5 minutes, this was followed by addition of 1mL 0.1 mol L⁻¹ NaOH (pH= 13) for complete coupling reaction. It was then filled to the mark with distilled water and allowed to stand for 10 minutes, after color development the absorbance was measured in 1.00 cm quartz cells at 439 nm. Below is the scheme showing the two step reactions.
2.5 Samples preparation

2.5.1 Polycarbonate bottle fragments: In order to extract BPA, the polycarbonate bottle was first washed thoroughly with double distilled water, dried and then cut into small fragments of about 5 × 5 mm in size. 1.5 g of the fragment was weighed and soaked in 10% ethanol for 24 hours. The solution containing the fragments were ultrasonically extracted for 2 hours in a water bath (95 ± 0.5 °C). The leachate solutions were cooled to room temperature, filtered with 0.45 µm-filter membrane, and analyzed by standard addition method.

2.5.2 Extraction of BPA using hot water: Three different brands of polycarbonate infant feeding bottles of 60 mL capacity were washed according to manufacturer’s instruction for the first time use and the water was discarded. The bottles were then filled with boiling HPLC grade water similar to actual use by nursing mothers (10), and allow to stand for two hours. Thereafter, the leachate solution was used to determine the amount of BPA released by standard addition method.

2.5.3 Extraction of BPA using reconstituted infant formula: Two different powdered infant formulae were used and prepared for protein precipitation as follows, 100 ml of boiling water was transferred to polycarbonate baby feeding bottle, followed by addition of 3 spoons (12.0 g) of powdered milk as instructed by manufacturers. The mixture was shaken thoroughly and allowed to stand for two hours. The mixture was then acidified with 100 µL concentrated hydrochloric acid. Then 5 mL of the acidified solution was transferred into a 20 mL conical flask and mixed with 10 mL acetonitrile, placed on a rotatory shaker and was shaken for 5 minutes. The resulting precipitate was centrifuged for ten minutes at 4000 rpm and filtered with 0.45 µm-filter membrane (10). The clear solution was evaporated to dryness at 40 °C, 20 ml HPLC grade water was added to the residue, which was analyzed for BPA by standard addition method.

2.6 Recovery Experiments: The Spectrophotometric method recovery was carried out with HPLC grade water which has been spiked with BPA at concentration of 2, 4 and 6 µg/ml and analyzed like the unknown samples.

2.7 Limit of detection and Limit of quantification: 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 = 3S/m, and LOQ = 10S/m, where “S” is the standard deviation of several measurements of the blank and “m” is the slope of the calibration curve.

2.8 Standard Addition Method

5 ml of the leachate solutions were added to four labelled 10 ml volumetric flasks, followed by addition of BPA standards of 0, 2, 4 and 6 µg mL⁻¹ concentrations. The solutions were subsequently made up to 10 ml volume with distilled water. BPA was analyzed by coupling 1 ml of the prepared solution with diazotized sulphanilic acid at each concentration in triplicates to produce a yellow azo-dye. Standard addition curve was constructed by plotting mean absorbance obtained for the 4 sets.
of samples against concentration of added standards.

3.0 Results and discussion

Figure 2 (a-e) presents the results for optimization of diazo-coupling reagents and conditions, the chosen conditions were selected based on their ability to give maximum absorbance values and were maintained throughout the studies. These concentrations were 120 µg/mL sulphanilic acid, 0.1 molL⁻¹ hydrochloric acid, 140 µg/mL sodium nitrite and 0.1molL⁻¹ sodium hydroxide (pH=13). Others were 5 minutes diazotization reaction time and 10 minutes time for maximum color development for measuring the absorbance of the azo-dye. The standard addition plots for some samples were presented in figure 3, constructed using mean absorbance against concentration of added standards. The concentration of BPA in the original sample was obtained by backward extrapolation to the x-axis when y = 0. The analysis were first carried out using five bottle fragments samples of varying weight (1.5 to 15g), the BPA leaching was detected in all the fragments samples and found to increase with increase in weight (0.373-5.93 µg/mL⁻¹). The results of BPA migration in hot water and milk stored in the bottles were 0.40 ± 0.05, 0.80 ± 0.11 and 0.52 ± 0.10 µg/mL⁻¹ for the three water samples, 3.85 ± 0.20 and 1.78 ± 0.07 µg/mL⁻¹ for the milk samples respectively. The detection of BPA in all the samples above could be due to hydrolysis of the polymer, because according to published studies by some authors (11) there is evidence that when polycarbonate is in contact with water, a chemical reaction known as hydrolysis occurs. Milk samples have much higher BPA than the boiled water, which could be attributed to the presence of BPA in powdered milk due to diffusion of residual BPA from epoxy resin lining in the can container. Following the initial analysis of BPA in the two powdered infant milk identified as N1 and P1 gave a result of 0.74 ± 0.10 and 1.24 ± 0.10 µg/mL⁻¹ respectively, indicating the presence of BPA before the migration test.

(a)                                                                                  (b)

Figure 1: FTIR of (a)Polycarbonate feeding bottle compared with (b)polycarbonate reference material from the library
Figure 2 (a-e): Optimization of diazo-coupling reagents and conditions. (a) Sulphanilic acid (b) HCl (c) Sodium nitrite (d) Sodium hydroxide and (e) Effect of diazotization reaction time.
The results of this study is very similar to what was reported by Peijin et al. (8), BPA migration of 0.50 – 0.84 µg/mL in four different types of polycarbonate containers. Makinwa and Uadia (12) from south-western Nigeria also reported average BPA concentration in old and new feeding bottles as 0.217±0.05 and 0.885±0.11 µg/mL respectively, similar to the result of hot
water in new feeding bottles. Similarly Zhiqun et al, (9) reported BPA in water bottles and milk between the range of 0.56 – 0.68 µgmL\(^{-1}\) and 0.13 – 0.27 µgmL\(^{-1}\) respectively, bisphenol A in milk is much lower than the present study probably due to different sources of the milk powder as well as the PC containers in which the milk was stored. The results were equally lower than those obtained by (13) which reported a spectrophotometric analysis for BPA migration in various infant materials (bottles, bowls, Sippy cups, spoons etc.) within the range of 2.54 – 95 µgmL\(^{-1}\), probably due to strong simulant (methanol) used in their analysis. 

The recovery of the spectrophotometric method given as the mean percentage recovery values were found to be 99.4 ± 0.12, 97.87 ± 4.88 and 99.63 ± 3.78 for three different concentrations added. Low values of standard deviation and relative standard deviation (0.12 - 4.9%) confirm a high level of accuracy for the proposed method. Ruggedness data of BPA on different days showed no significant difference as indicated by the t-tests at 5% probability level. The results of 2.0±0.12, 2.05±0.08 and 3.92±0.07, 4.00±0.13 with low %RSD which ranged between 1.78 – 5.03% for BPA concentrations of 2 and 4 µgmL\(^{-1}\) respectively. This showed that the method is reliable with high reproducibility as well as accuracy. The LOD and LOQ were found to be 0.48 µgmL\(^{-1}\) and 1.62 µgmL\(^{-1}\) respectively, which were similar to 0.42 and 1.28 µgmL\(^{-1}\) reported in (14) and 0.48 and 1.60 µgmL\(^{-1}\) reported by Eudes (13).

The estimated safe level known as the Tolerable Daily Intake (TDI) of bisphenol A set up by European Food Safety Authority (4) was given as 50µg/kgbw/day. The average migration value of BPA in water and milk samples obtained in this study were 0.57 µgmL\(^{-1}\) and 2.82 µgmL\(^{-1}\) respectively. The estimated daily intake from these results assuming an infant of 5kg consuming at least 300mL of water and milk in a day, would be 34.20 µg/kgbw/day and 169.20 µg/kgbw/day respectively. This indicates that the average daily intake of BPA in water in the present study is less than the TDI of 50 µg/kgbw/day. But the average daily intake in milk has exceeded the TDI by 3 folds. The high value in the milk samples can be attributed to already diffused BPA into the milk powder from the lining of the can container.

**Conclusion**

The use of spectrophotometric method base on diazo-coupling reaction for the determination of BPA has been achieved and confirmed by HPLC. The diazo-coupling reagents and conditions which includes concentrations of sulphanilic acid, sodium nitrite, hydrochloric acid, sodium hydroxide and diazotization reaction time has been optimized. All the PC feeding bottles tested were found to have leached BPA at detectable amount, the level of released BPA differs significantly between the samples, BPA was also found in the milk samples prior to storage in the PC bottles attributed to diffusion of residual BPA from the coating in the metal containers.

BPA did migrate into the surrounding food in varying amounts implying that children have been exposed to this monomer from early age. Milk samples have exceeded the tolerable daily intake (TDI) for BPA of 50µg/kgbw/day recommended by EFSA. From the findings of this research the use of polycarbonate feeding bottles cannot be regarded as safe for the level of BPA consumed at every feeding of the infant.

**Acknowledgements**

The authors wish to thank Mr. Musa Beli from central Laboratories Complex, Mr. Muhammad Salihu, Mr. Umar Na‘i of Biochemistry laboratories Bayero University Kano, for their technical assistance. We also wish to thank the Kano State College of Education and Preliminary Studies, for the study leave given to one of us (A.B.Bashir).

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