ELSEVIER

Journal of Chromatography B, 783 (2003) 367–375

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Simple and accurate determination of bisphenol A in red blood cells prepared with basic glycine buffer using liquid chromatography-electrochemical detection

Junko Sajiki*

Public Health Laboratory of Chiba Prefecture, 666-2 Nitona-cho, Chuo-ku, Chiba 260-8715, Japan

Received 19 March 2002; received in revised form 27 August 2002; accepted 27 August 2002

Abstract

For an accurate determination of bisphenol A (BPA) in red blood cells (RBC), the effect of pH on the concentration of BPA was investigated. Also, BPA recovery using ferric heme, methemoglobin (metHb) and hematin, were investigated to confirm whether BPA binds to ferric heme. BPA recovery in hemolysate was high at alkaline pH and was very low at acidic pH where oxyHb changed to metHb. BPA recovery decreased dose-dependently in metHb and hematin, but inorganic iron ions did not influence the recovery. These results suggested that BPA could be bound to ferric heme in RBC. The use of glycine–NaOH buffer (pH 11) as well as plasma had the highest recovery (97%). BPA was not detected in red blood cells of healthy adult volunteers ($n=6$). In sheep blood contaminated with BPA, BPA was detected in both plasma and RBC (10 times lower than in plasma), indicating that BPA could have migrated from plasma into RBC.

Keywords: Bisphenol A; Glycine buffer, basic

1. Introduction

Bisphenol A (BPA), a compound widely used as a monomer for the production of polycarbonate plastics and as a major component of epoxy resin for lining of food cans and dental sealants, is well-known as a weak estrogenic endocrine-disrupting chemical [1,2] and is detected in industrial wastewater and soil [3], water [4], baby food bottles [5] and plastic waste [6].

The practically ubiquitous occurrence of BPA has become a controversial issue because it is easily leached in biological samples such as serum and saliva from polycarbonate plastic bottles [7] and resin-based dental composites [8] and is likely to have an adverse effect on animals including humans [9–12]. Therefore, the determination of BPA in biological samples such as blood and tissues is essential for monitoring BPA in vivo. However, an accurate method for the determination of BPA in blood samples has not yet been established. Sajiki [13] reported that the recovery of BPA was high (above 93%) in serum, but was low (below 50%) in both hemolyzed red blood cells (RBC) and whole blood due to the existence of methemoglobin (metHb), and proposed a binding mechanism of BPA to metHb. Moreover, it was considered that the

*Tel.: +81-43-266-7989; fax: +81-43-265-5544.
E-mail address: j.sjk@ma.pref.chiba.jp (J. Sajiki).

1570-0232/02/$ – see front matter
PII: S1570-0232(02)00639-6

determination of BPA in whole blood was made possible by separating RBC from plasma.

In this study, for an accurate determination of BPA in RBC, the effect of pH on the concentration of BPA was investigated and adjustment of proper metHb concentration was carried out. Furthermore, to confirm the assumption of BPA binding to ferric heme, BPA recovery using metHb and hematin was also studied.

2. Materials and methods

2.1. Materials

Fresh sheep blood was collected from the leg vein of three sheep which were raised domestically in cages but sometimes allowed on pasture, and was preserved at 5 °C. Fresh human blood was collected from healthy volunteers. All experiments were done within 2 weeks after blood collection. Defibrinized sheep blood was purchased from Japanese pharmaceutical companies (Tokyo, Japan). BPA, iron (II) chloride tetrahydrate (minimum 99%) and iron (III) chloride hexahydrate (minimum 98%) were purchased from Wako (Tokyo, Japan). Crystallized and lyophilized bovine metHb, and porcine hematin were purchased from Sigma (St. Louis, MO, USA). Throughout the experiment, the procedures used both for preparing the BPA-free water and for the extraction were ODS-silica Sep-Pak Plus (long body, Waters, MA, USA) and Oasis HLB (60 mg/3 ml, Waters) washed with 3.5 ml special grade ethanol (EtOH, 95 v/v%) and 3.5 ml water before use, respectively. Other chemicals were of special grade (Wako). Acetate buffer (pH 2–5), phosphate buffer (pH 6–8.5) and glycine–NaOH buffer (pH 7–11) were used in this study.

2.2. Preparation of samples

2.2.1. Experiment 1. Changes of metHb and concentrations of BPA in contaminated whole sheep blood as a function of pH

Fresh whole sheep blood and commercial whole sheep blood contaminated with BPA (599 ng/ml in plasma) were diluted with the same volume of water (H-WB). One fifth of 1 ml H-WB was added to each 4.8 ml of various buffer solutions (pH 3–5, acetate buffer, pH 6–8, phosphate and pH 9–10, glycine–NaOH buffer) and allowed to stand for 30 min at room temperature. After the determination of metHb, the whole volume of each sample was applied to an Oasis-HLB column for the extraction of BPA.

2.2.2. Experiment 2. Changes of BPA recoveries in human H-RBC fortified with BPA as a function of pH

Plasma was separated from RBC by centrifuging at 1000 *g* for 10 min immediately after blood collection. After washing RBC five times with 10 volumes of phosphate buffered saline (PBS, pH 7.4), RBC were hemolyzed by addition of water (H-RBC). Cell walls were removed from H-RBC by centrifuging at 1000 *g* for 10 min. The heme concentration of H-RBC was represented by dilution with water in the present study. H-RBC solutions diluted 2–32-fold with water were fortified with BPA to yield a final concentration of 100–300 ng/ml. BPA stock solution was prepared in 25% MeOH at a concentration of 1 μg/ml. One tenth of 1 ml of H-RBC containing BPA was added to 0.3 ml phosphate- and glycine-buffer solutions (pH 6–11) and allowed to stand for 1 h at room temperature (20–25 °C).

2.2.3. Experiment 3. Effect of human plasma on the BPA concentration in the metHb and hematin solution

Authentic bovine metHb or porcine hematin was dissolved in water or 0.2 N NaOH at a concentration of 5×10^{-2}–5 mg/ml, respectively. Each 0.5 ml metHb or hematine solution was added to 0.1 ml of human serum and/or 25 ng/ml BPA. The samples were allowed to stand for 30 min at room temperature and were diluted with water to a final volume of 5 ml and the whole volume of sample was applied to an Oasis-HLB column for the extraction of BPA.

2.3. Extraction of BPA from samples

Extraction of BPA was carried out according to the method of Sajiki et al. [7]. All samples were applied to Oasis HLB, and polar lipids were removed from the column with 3.5 ml 15% EtOH and 3.5 ml

petroleum ether were used to remove nonpolar lipids after washing with 3.5 ml water. Finally, BPA was eluted with 3.5 ml ethyl acetate. The solvent was evaporated to dryness under N_2. The residue was dissolved in 1 ml acetonitrile–water (40:60, v/v). When 60 ng/ml BPA was added to the water, BPA recoveries in three fractions, 15% EtOH, petroleum ether and ethyl acetate, were 0, 0 and 100%, respectively. When 5 and 50 ng/ml BPA were added to human plasma, BPA recoveries using this method were 95 and 104%, respectively. BPA standards were prepared with water using the 1 μg/ml stock solution which was dissolved with 25% MeOH. A blank test was carried out using water, metHb, hematin, human plasma and H-RBC.

2.4. HPLC conditions

HPLC, Model LC-10 AD (Shimadzu, Kyoto, Japan) with Shim-Pack VP-ODS column (150 mm× 4.6 mm I.D., Shimadzu), and electrochemical detector (ECD, Coulochem II 5200A, ESA, MA, USA) were used for BPA analyses. The solvent system was acetonitrile–water–phosphoric acid (40:60:0.2, v/v). The flow-rate and column temperature were 1.0 ml/min and 40 °C, respectively. The injection volume was 50 μl. The conditions of the EC detector were: guard cell potential, E 600 mV; analytical cell potentials, E_1 300 mV and E_2 550 mV; sensitivity, 1 μA, respectively. Identification of BPA was made by comparing the HPLC retention times with those of the authentic standards. Further, co-chromatography using authentic standards was employed for a complete identification. For a complete identification of BPA, a HPLC system, Alliance 2690 model (Waters) with a Symmetry C_{18} column (3.5 μm, 150 mm×2.1 mm I.D.) equipped with a Waters 996 photodiode array UV–Vis detector and a ZMD Z-spray mass spectrometer using an ESI interface system (Waters), was used. The solvent system was acetonitrile–50 m*M* phosphate buffer (pH 3) (40:60, v/v). Flow-rate and column temperature were 0.25 ml/min and 40 °C, respectively. Injection volume was 10 μl. The analytical conditions in the negative ion scanning modes of ESI were as follows: capillary voltage, 3.1 kV; cone voltage, 33 V; source block temperature, 90 °C; desolvation temperature, 175 °C.

2.5. Determination of metHb

MetHb concentration in H-RBC was measured by taking the difference between optical densities at 630 nm (a peak of metHb) and at 700 nm (baseline) using a multi-purpose recording spectrophotometer (MPS-2000, Shimadzu) [14].

2.6. Statistical analysis

All reported values are the mean of duplicates or triplicates. Data were analyzed by one-way analysis of variance, and the difference among treatments was tested by the least significant difference. Test of significance between two groups was done by Student's *t*-test.

3. Results

Most of the commercial defibrinized sheep blood did not contain BPA. However, an isolated case where blood in a glass bottle contained an abundant amount of BPA was found where BPA concentration in plasma was 599.4 ng/ml as reported in a previous paper [7]. This is a contamination that occurred after sampling, e.g. due to a contaminated container or leaching from the plastic container in the course of production. BPA concentrations in H-WB with pH adjusted from 2 to 10 vary from 38 to 278 ng/ml as shown in Fig. 1. At acidic pH, H-WB shows low BPA concentrations, but high metHb concentrations. There was a significant negative correlation ($P<$ 0.01) between BPA and metHb between pH 3 and 8. BPA concentration increased with a rise in pH. When water was fortified with BPA at a final concentration of 250 ng/ml at pH 2 and 8, BPA recoveries were 88% and 95.4%, or for the above H-WB at pH 2 and 8 were 15.5% and 71.2%, respectively (Table 1).

Change of BPA recovery in fresh sheep H-WB (no BPA contamination) spiked with 28–110 ng/ml BPA as a function of storage time is shown in Table 2. MetHb concentration of whole blood was 3.7 mg/ml. BPA recovery in water fortified with 28 to 100 ng/ml BPA and incubated for 4 days, was above 90%. In all samples fortified with various concentrations of BPA, BPA recovery was the lowest at day 0 (assay was done 10 min after BPA addition) and it

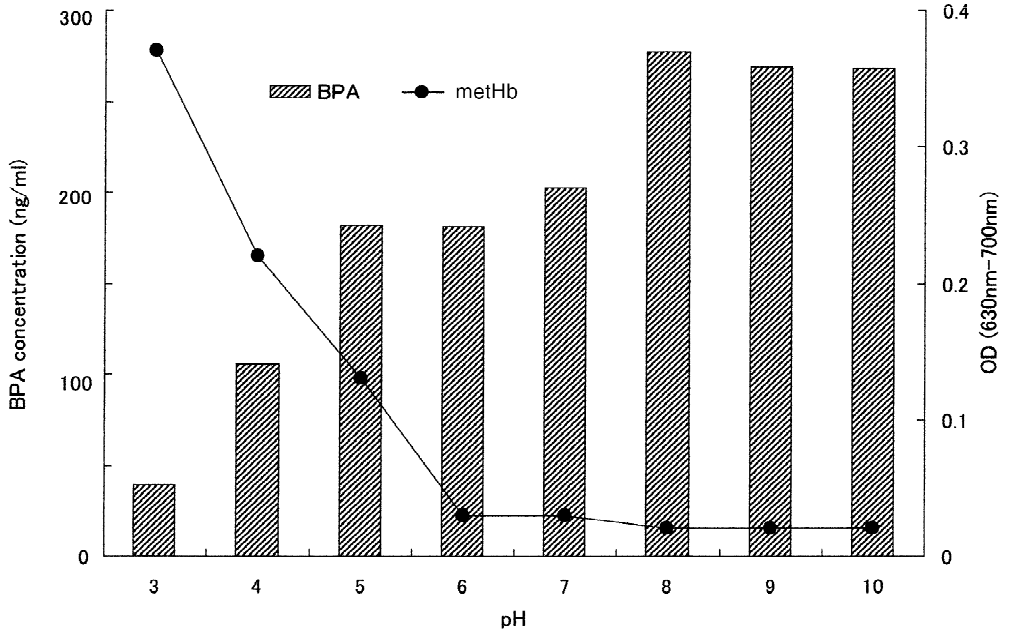

Fig. 1. Changes of BPA concentration and metHb content in BPA-contaminated sheep H-WB as a function of pH. Data represent the mean values of duplicates. Samples were allowed to stand at room temperature for 30 min. MetHb is represented as OD (630–700 nm) of metHb peak in H-WB diluted 50 times with water. pH 3–5 (50 m*M* acetate buffer), pH 6–7 (50 m*M* phosphate buffer) and pH 8–10 (50 m*M* glycine buffer).

increased significantly ($P<0.01$) at day 1 and was kept on the same level until day 4. On all experimental days, BPA recovery tended to be higher as the amount of BPA fortified increased.

To investigate if heme iron could be related to the BPA recovery in H-RBC, BPA recovery tests were carried out using metHb (stable ferric type of Hb in aerobic condition) and hematin (ferric heme) solutions. As shown in Fig. 2, the higher the concentration of metHb and hematin added, the lower the recovery of BPA. As reported [13], when 4.2 mg/ml metHb solution was fortified with BPA, BPA recovery was 32% 10 min after the addition, but it was restored to 48% after 30 min. After that, the level was held to around 55% until 60 min but gradually decreased again to 38% at 120 min.

Table 1
Changes of BPA and metHb concentration in sheep H-WB fortified with BPA at pH 2 and 8

	pH 2			pH 8		
	BPA (ng/ml)	BPA recovery (%)	metHb (ΔOD)	BPA (ng/ml)	BPA recovery (%)	metHb (ΔOD)
Water	0					
Water+BPA[a]	220.1	88.0		238.4	95.4	0.02[c]
H-WB[b]	5.4		0.37[c]	154.3		
H-WB[b]+BPA[a]	44.2	15.5		332.3	71.2	

Data represents the mean values of duplicates. ΔOD, the difference of optical density between 630 nm and 700 nm.
[a] 250 ng/ml.
[b] Diluted blood two-fold with water.
[c] Data show the value using hemolysate diluted 25-fold with buffer solution.

Table 2
Changes of BPA recovery in H-WB[a] after fortifying with various concentrations of BPA

BPA concentration spiked (ng/ml)	BPA recovery (%) day after BPA addition			
	0	1	2	4
28	$22.8^{a\alpha}$	$62.1^{b\alpha}$	$60.0^{b\alpha}$	$54.5^{b\alpha}$
55	$24.6^{a\alpha}$	$73.2^{b\alpha}$	$70.5^{b\alpha}$	$67.0^{b\beta}$
83	$43.5^{a\beta}$	$72.6^{b\alpha}$	$70.0^{b\alpha}$	$67.3^{b\beta}$
110	$41.5^{a\beta}$	$76.7^{b\beta}$	$78.0^{b\beta}$	$79.5^{b\gamma}$

Data represent the mean of duplicates. Values with different superscript alphabetical letters in the same row are significantly different ($P<0.01$). Values with different superscript greek letters in the same column are significantly different ($P<0.01$).

[a] Fresh sheep whole blood diluted twofold with water.

Similar tendency was also observed in 0.42 mg/ml metHb solution fortified with BPA. In the 0.42 mg/ml hematin, however, BPA recovery was very low 10 min after the fortification with BPA, and the level continued to drop to around zero until 120 min. In particular, hematin drastically lowered BPA recovery even at the concentration of 0.04 mg/ml (Fig. 3). The concentration showing 50% BPA recovery in hematin was 275 times higher than in metHb (BPA decreased by half at 0.012 mg/ml for hematin and 3.3 mg/ml for metHb). However, addition of 17% human plasma enhanced BPA concentration almost completely at the concentration of 0.42 ng/ml metHb (Fig. 3). The order of addition of plasma and BPA did not affect the BPA recovery. On the other hand, addition of 0.5 μg/ml Fe^{2+} or Fe^{3+} did not change the BPA recovery.

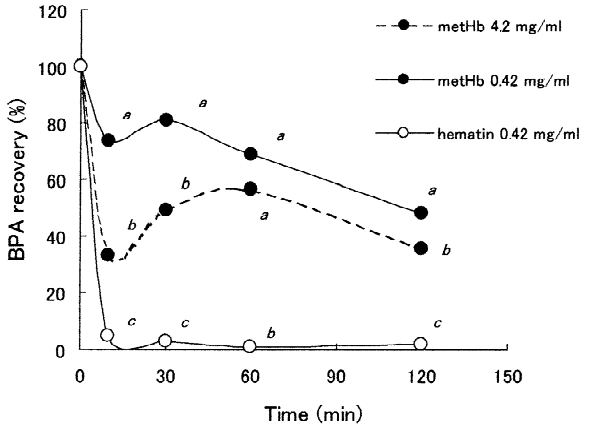

Fig. 2. BPA recovery as a function of time after fortification with BPA in metHb or hematin solution. Data represent the mean values of triplicates. Fortified with 25 ng/ml BPA. Samples were allowed to stand at room temperature. BPA recovery with different superscript letters at the same experimental time are significantly different ($P<0.01$).

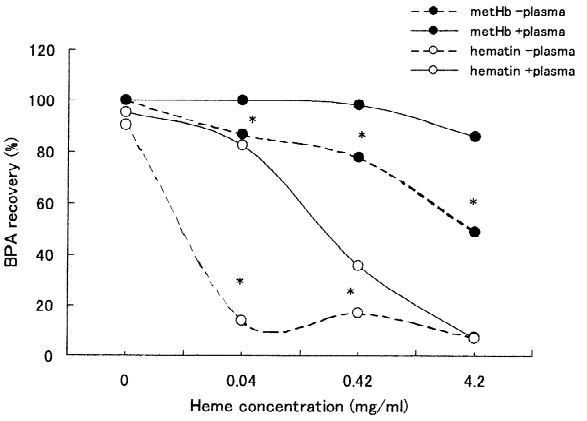

Fig. 3. BPA recovery as a function of the metHb or hematin concentration in a water fortified with BPA with or without human plasma. Data represent the mean values of triplicates. Fortified with 25 ng/ml BPA. Samples were allowed to stand at room temperature for 30 min. *Significant difference ($P<0.01$) in the same sample with and without plasma (17% at a final concentration).

BPA recovery in H-RBC depended on the heme concentration as shown in Fig. 4. BPA recoveries decreased drastically within 10 min and the recovery level continued for 60 min in all three samples and the BPA recoveries at 24 h after the fortification with BPA were not significantly different from the value at 60 min. The higher the dilution of H-RBC, the higher the BPA recovery. When H-RBC diluted 12-times with water was fortified with 300 ng/ml BPA together with 18–360 m*M* glycine–NaOH buffer solutions (pH 10), BPA recovery was above 90% (Fig. 5), but below this buffer concentration, the recovery was low. Changes of BPA recovery in H-RBC diluted 12-times with water using 50 m*M* phosphate and glycine–NaOH buffers (37.5 m*M* final concentration in the reaction mixture) as a function of pH are shown in Fig. 6. BPA recoveries in H-RBC with both phosphate and glycine–NaOH buffers were higher than that with water (pH 7.5). In the phosphate buffer, BPA recovery was significantly

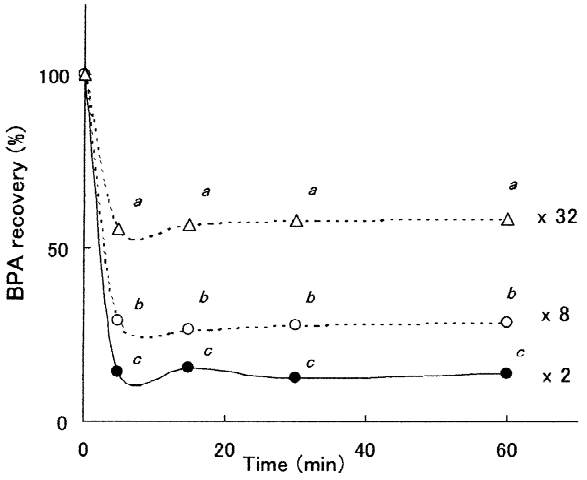

Fig. 4. Changes of BPA recovery in human H-RBC with various concentration of heme as a function of time after the fortification with BPA. The numbers in the figure represent the dilution times of RBC with water. Data represent the mean values of duplicates. Samples were allowed to stand at room temperature. Fortified with 100 ng/ml BPA. BPA recovery with different superscript letters at the same experimental time are significantly different ($P<0.01$).

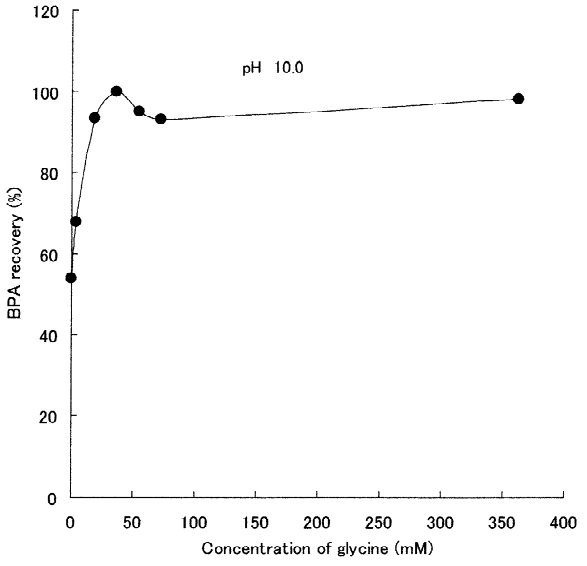

Fig. 5. Changes of BPA recovery in H-RBC as a function of the concentration of glycine at pH 10. Data represent the mean values of duplicates. Samples were allowed to stand at room temperature. Fortified with 300 ng/ml BPA. H-RBC was finally diluted with RBC 48 times.

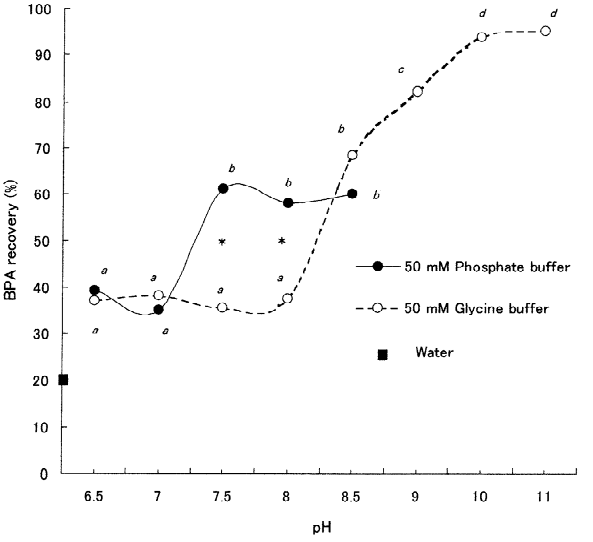

Fig. 6. Changes of BPA recovery in H-RBC as a function of pH using phosphate and glycine–NaOH buffers Data represent the mean values of duplicates. Samples were allowed to stand at room temperature for 60 min. Fortified with 300 ng/ml BPA. BPA recovery with different superscript letters in the same kinds of buffer are significantly different ($P<0.01$). *BPA recovery between two kinds of buffers in the same pH are significantly different ($P<0.01$). Solid square represents the BPA recovery of control.

higher ($P<0.01$) from pH 7.5 to 8.5 compared to that below pH 7.5. In the glycine–NaOH buffer, BPA recovery was significantly higher ($P<0.01$) from pH 8.5 to 11 compared to that below pH 8.5, especially at pH 10 and 11 where recovery was above 95%. At pH 7.5 to 8, phosphate buffer showed higher recovery than glycine buffer. Table 3 shows the BPA recovery in H-RBC reacted with various kinds of solvents. BPA recovery was the lowest in the H-RBC where water was the reaction solvent. BPA recovery in the H-RBC with Na_2HPO_4 was significantly higher than that in the H-RBC with KH_2PO_4 ($P<0.01$). BPA recovery in the H-RBC with 50 m*M* glycine buffer at pH 10 and 11 and human plasma was above 90%.

BPA in RBC of six healthy volunteer human bloods was not detected using the method as shown in Fig. 7. BPA contamination in RBC of commercial sheep blood whose plasma has a BPA contamination of 599 ng/ml [7] was 57 ng/ml.

Table 3
BPA recovery in H-RBC[a] added with various kinds of solvents[b] or human plasma

Solvents	pH	BPA recovery (%)[c]
H_2O	7.8	53.6
50 m*M* KH_2PO_4	4.7	65.5
50 m*M* Na_2HPO_4	9.0	84.0
50 m*M* Phosphate buffer	8.0	74.6
50 m*M* Glycine	6.0	62.5
50 m*M* Glycine buffer	8.0	63.6
	9.0	83.3
	10.0	90.0
	11.0	97.6
50 m*M* NaOH	11.0	86.3
Human plasma	7.2	97.0

[a] Twelve-fold diluted human RBC with water containing 1.4 μg/ml BPA at 37 °C for 2 h.
[b] Reacted H-RBC with three times volume of solvents at 25 °C for 1 h.
[c] Means of duplicates.

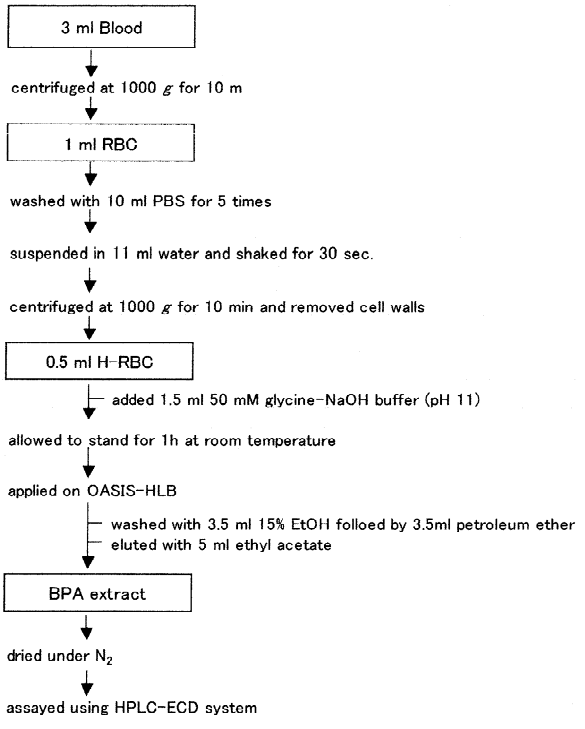

Fig. 7. Flow diagram for the determination of BPA in RBC.

4. Discussion

Researchers sometimes encountered commercial blood or plasma containing surprisingly high concentrations of BPA as we previously reported [7]. BPA contamination might occur through manufacturing products after blood collection but not due to uptake from their environment since BPA is leached in biological fluid such as saliva and plasma. Recently, a new problem in which BPA is eluted from polycarbonate and polysulfone hemodialyzer in hemodialysis patients has occurred [15]. Although BPA concentrations in plasma of healthy animals including humans are very low, those in patients exposed to plastic medical devices might be elevated. Thus the monitoring of BPA in human blood is important to know the prevalence of BPA, one of the endocrine-disrupting chemicals, in the circulation systems of humans. To date, an accurate method for the determination of BPA in plasma has not been devised. However, the confirmation of an accurate method for BPA determination in RBC-containing samples is desired because of the low recovery of BPA due to the presence of hemes.

Although BPA concentration in plasma was not influenced by the change of pH, that in whole blood of commercial sheep blood contaminated with a high amount of BPA varied depending on pH. The facts that BPA concentration was lower at acidic pH and higher at alkaline pH, and negative correlation between metHb value and BPA concentrations, might suggest that metHb has an important role in a significantly low BPA recovery in whole blood. When the hemolysate was fortified with BPA in acidic conditions (pH 2) where the colour could change from red to brown by changing oxyHb to metHb, a large amount of BPA (84.5%) disappeared. The phenomenon also shows that metHb has an impact on BPA recovery.

Sajiki [13] reported that the BPA recovery was high (above 93%) in plasma, but was low in hemolyzed blood. Moreover, BPA concentration in authentic metHb which corresponds to the physiological concentration of human blood decreased to a low level which was inhibited by addition of plasma, implying that BPA might be bound to metHb in RBC of blood. In the present study, BPA recovery decreased drastically in hematin solution at a lower

concentration than in metHb solution and the decrease in BPA recovery depended on the metHb concentration. As hematin is a ferriporphyrin which has lost globin from metHb, these results suggest that the decrease in BPA recovery might be caused by the binding of BPA to hemes. The difference in the BPA recovery between metHb and hematin might be due to stereochemical properties. BPA recovery did not change in the presence of inorganic iron ions, which indicated that BPA binded to heme not to inorganic ions.

The fact that very low BPA concentration was observed within 10 min after the fortification of either H-RBC or metHb solution with BPA, and an inhibitory effect of plasma on the decrease of BPA concentration in metHb solution did not change regardless of the order of addition of serum and BPA to metHb or vice versa, imply that BPA binds to heme proteins immediately after the spike of BPA, and plasma acts as some kind of hindrance to the binding of BPA. Miyakoda et al. [16] reported that BPA recovery in the fetus whole body homogenate (87%) was lower than that in the maternal plasma (94%). Lower BPA recovery in the liver (49%) than in the muscle (79%) of rainbow trout also reported by Pederson and Lindholst [17] is considered to have arisen from the existence of ferric heme in situ.

A similar negative correlation was observed between concentration of hemes in H-RBC and BPA recovery at every assay time after the fortification with BPA, and BPA recovery did not change for 24 h after the fortification at three levels of heme prepared by the dilution of H-RBC with water, which suggest that BPA could bind to hemes in saturation quickly (within 10 min). On the other hand, BPA recovery was lowest at 10 min after the fortification with BPA and increased significantly with the passage of time when H-WB was fortified with BPA. The finding suggests that BPA could bind to hemes immediately after the fortification with BPA and release gradually from heme due to the presence of plasma. In the H-WB, BPA recovery was significantly higher at high concentration of BPA fortified (83–110 ng/ml) than low concentration of BPA (28–55 ng/ml) immediately after the BPA fortification, which indicates that a definite volume of BPA could bind to heme in H-WB.

Although the mechanism of BPA released from heme by addition of plasma has never been clarified in the present study, some kinds of plasma proteins (albumin, IgG and transferrin) which enhanced BPA recovery [18] indicate an involvement of plasma protein in the BPA recovery in H-RBC. The addition of phosphate and glycine to H-RBC which was treated with BPA for 2 h enhanced BPA recovery even at pH lower than water, implying that physiological materials in plasma might give an impact on the release of BPA from heme in RBC.

Moreover, other elements such as anions in the solution might be associated with the BPA recovery besides pH. Significant difference ($P<0.01$) was observed in the BPA recoveries between two kinds of buffers (phosphate and glycine) at pH 7.5 and 8.0 when H-RBC was fortified with BPA in the buffer solutions, indicating that the kind of buffer could influence the BPA recovery at the specified pH. Also, the fact that glycine buffer above pH 10 showed high BPA recovery in H-RBC compared with 50 m*M* NaOH adjusted to pH 11 with HCl also supports an involvement of amino acids in BPA recovery. From the fact that the glycine pK_a is 9.78 and a similar result was obtained in the experiment using methionine (data not shown), the anion type of the amino acid might be related to the enhancement of BPA recovery.

It was reported that BPA recovery was enhanced when over 17% plasma was added to H-RBC where 0.4 ml sheep RBC was diluted to 6 ml with water [13], though the maximum BPA recovery was still low at 79%. In both H-RBC and authentic ferric heme solutions, the heme concentration seemed very important in the binding or releasing of BPA to or from ferric heme. The low BPA recovery in a solution with plasma (29%) and high concentration of ferric heme (0.42 mg/ml hematin) could mean that either high concentration of ferric heme or low concentration of plasma could influence the release of BPA from heme. Addition of 25% plasma showed high BPA recovery (97%) in H-RBC with a final dilution of 48 times. However, there are some demerits to using plasma in enhancing BPA recovery. One is BPA contamination in plasma and the other is the existence of unknown substances interfering with the HPLC-ECD assay. EC detection has high sensitivity for phenol compounds in biological samples [19,20] although the HPLC-ECD method is

easy to handle. To avoid these problems, employing glycine–NaOH buffer (pH 11) instead of plasma was devised as a simple solution for the BPA determination in H-RBC as shown in Fig. 7. Glycine buffer (pH 11) was preferred to other buffers for preventing BPA from binding and releasing to ferric heme.

The presence of BPA in the plasma of blood from healthy volunteers even in a very low quantity (0–1.6 ng/ml) [7] suggests that contamination from the environment might have occurred, however, no BPA was detected in the same RBC indicating that BPA in plasma did not migrate to RBC. On the other hand, the fact that 54 ng/ml BPA were detected in the BPA-contaminated commercial sheep red blood cells might suggest the possibility of BPA penetration into red blood cells through cell walls when blood was contaminated with a high concentration of BPA. The low BPA recovery in H-RBC immediately after the fortification with BPA, which increased significantly from day 1 until day 4, suggests that BPA might be released from heme as a function of time.

5. Conclusion

Adjusting the pH of H-RBC samples with glycine–NaOH buffer (pH 11) resulted in higher recovery (97%) than with other buffers. Since BPA could be bound to ferric hemes such as metHb and hematin which are easily formed in acidic conditions, acidic treatment should be avoided for the determination of BPA in blood and blood tissues.

References

[1] A.V. Krishnan, P. Starhis, S.F. Permuth, L. Tokes, D. Feldman, Endocrinology 132 (1993) 2279.
[2] J.A. Brotons, M.F. Olea-Serrano, M. Villalobos, V. Pedraza, N. Olea, Environ. Health Perspect. 103 (1995) 608.
[3] C.A. Staples, P.B. Dom, G.M. Klecka, S.T. O'Block, L.R. Harris, Chemosphere 36 (1998) 2149.
[4] A. Gonzalez-Casado, N. Navas, M. del Olmo, J.L. Vilchez, J. Chromatogr. Sci. 36 (1998) 565.
[5] K.A. Mountfort, J. Kelly, S.M. Jickells, L. Castle, Food Addit. Contam. 14 (1997) 737.
[6] T. Yamamoto, A. Yasuhara, Chemosphere 38 (1998) 2569.
[7] J. Sajiki, K. Takahashi, J. Yonekubo, J. Chromatogr. B 736 (1999) 255.
[8] N. Olea, R. Pulgar, P. Perez, F. Olea-Serrano, A. Rivas, A. Novillo-Fertrell, V. Pedraza, A.M. Soto, C. Sonnenschein, Environ. Health Perspect. 104 (1996) 298.
[9] S.C. Nagel, F.S. Vom Saal, K.A. Thayer, M.G. Dhar, M. Boechler, W.V. Welshons, Environ. Health Perspect. 105 (1997) 70.
[10] F.S. Vom Saal, P.S. Cooke, D. L Buchanan, P. Palanza, K.A. Thayer, S.C. Nagel, S. Parmigiani, W.V. Welshons, Toxicol. Ind. Health 14 (1998) 239.
[11] K.L. Howdeshell, A.K. Hotchkiss, K.A. Thayer, J.G. Vandenbergh, F.S. Vom Saal, Nature 401 (1999) 763.
[12] A. Takeshita, N. Koibuchi, J. Oka, M. Taguchi, Y. Shishiba, Y. Ozawa, Eur. J. Endocrinol. 145 (2001) 513.
[13] J. Sajiki, J. Chromatogr. B 755 (2001) 9.
[14] J. Sajiki, K. Takahashi, Eisei Kagaku 35 (1989) 414.
[15] H. Yamasaki, Y. Nagake, H. Makino, Nephron 88 (2001) 376.
[16] H. Miyakoda, M. Tabata, S. Onodera, K. Takeda, J. Health Sci. 45 (1999) 318.
[17] S.N. Pederson, C. Lindholst, J. Chromatogr. A 86 (1999) 17.
[18] J. Sajiki, J. Yonekubo, Environ. Sci. 8 (2001) 162.
[19] M.E. Murphy, J.P. Kehrer, J. Chromatogr. 421 (1987) 71.
[20] G. Achilli, G.P. Cellerino, G. Melzi d'Eril, S. Bird, J. Chromatogr. A 697 (1995) 357.