DETERMINATION OF BISPHENOL *A* BY SYNCHRONOUS FLUORIMETRY USING PROCAINE HYDROCHLORIDE AS SELF-QUENCHING FLUORESCENCE PROBE

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A new synchronous fluorimetric method for the analysis of bisphenol A has been developed based on procaine hydrochloride as a self-quenching fluorescence probe. In acidic solution, procaine hydrochloride could be diazotized with sodium nitrite; then the diazotized product could react with bisphenol A in NH_3-NH_4Cl buffer and produce the quenching of fluorescence of diazotized procaine hydrochloride-NH_3-NH_4Cl solution. Based on this observation, an inhibitory fluorimetric method is reported for the determination of trace bisphenol A. The synchronous spectral peaks of the reaction system are at 225 and 270 nm. The spectra of the two peaks can be separated, and bisphenol A can be determined directly. The possible mechanism of the reaction has also been discussed. Under optional conditions, bisphenol A can be determined over the concentration range of 0.10 to 1.4 μg/mL with a correlation coefficient of 0.99. The detection limit is 0.04 μg/mL at a signal-to-noise ratio of 3. The relative standard deviation (RSD) for 11 repetitive determinations of 1.0 μg/mL bisphenol A is 0.32%. The utility of this method was demonstrated by determining bisphenol A in hot water in contact with commercially available table-water bottle samples.

Keywords: *bisphenol A, synchronous fluorimetry, detection, procaine hydrochloride, quenching fluorescence probe.*

ОПРЕДЕЛЕНИЕ БИСФЕНОЛА *А* МЕТОДОМ СИНХРОННОЙ ФЛУОРИМЕТРИИ С ИСПОЛЬЗОВАНИЕМ ГИДРОХЛОРИДА ПРОКАИНА В КАЧЕСТВЕ САМОЗАТУХАЮЩЕГО ФЛУОРЕСЦЕНТНОГО ЗОНДА

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Новый синхронный флуориметрический метод для анализа бисфенола А разработан на основе прокаина гидрохлорида в качестве самозатухающего флуоресцентного зонда. В кислотном растворе гидрохлорид прокаина может быть диазотирован нитритом натрия, затем диазотированный продукт взаимодействует с бисфенолом А в буфере NH_3-NH_4Cl и вызывает тушение флуоресценции диазотированного раствора прокаина гидрохлорида NH_3-NH_4Cl. Представлен основанный на этом явлении ингибирующий флуориметрический метод определения следовых количеств бисфенола А. Синхронные спектральные максимумы для реакционной системы расположены при 225 и 270 нм. Эти максимумы могут быть разделены, и бисфенол А может быть определен непосредственно. Обсуждается возможный механизм реакции. В оптимальных условиях бисфенол А может быть определен в диапазоне концентраций 0.10—1.4 мкг/мл с коэффициентом корреляции 0.99. Предел обнаружения 0.04 мкг/мл при отношении сигнал/шум 3. Относительное стандартное отклонение для 11 повторных определений 1.0 мкг/мл бисфенола А составляет 0.32%. Полезность метода продемонстрирована путем определения бисфенола А в горячей воде, в которой находились бутылки из-под минеральной воды.

Ключевые слова: *бисфенол А, синхронная флуориметрия, обнаружение, гидрохлорид прокаина, флуоресцентный зонд.*

Introduction. Bisphenol *A* (BPA, 2.2-bis(4-hydroxyphenyl)propane), a raw material (monomer) for the synthesis of epoxy resins, polystyrene resins, and polycarbonate plastics, usually exists in daily plastic products, such as water bottles, feeding bottles, food cans, and tableware. It was reported that more than ca. 2000 tonnes of BPA are annually released into the environment through domestic and industrial activities under normal conditions of use [1]. As one of the most important endocrine disrupting chemicals (EDCs), BPA can mimic the action of the hormone estrogen and disturb the estrogen–estrogen receptor binding process [2]. Further, BPA can pose a natural threat to the environment and drinking water sources as it leaches into them easily as an environmental waste. It has become the biggest man-made threat to the environment as well as human health; therefore, we need a selective, rapid and reliable method for the identification and determination of trace amounts of BPA in different type of samples. Up to now, various analytical technologies have been developed for BPA determination, including enzyme-linked immunosorbent assay [3], optical immunosensor [4], high-performance liquid chromatography (HPLC), various detectors [2, 5–8], gas chromatography-mass spectrometry (GC-MS) [9], chemiluminescence [10], and electrochemical detection [11–14]. Our previous work reported procedures for the determination of BPA in hot water in contact with commercially available table-water bottle samples by using electrochemiluminescence detection [15] and the diazotization-coupling spectrophotometry method combined with HPLC [16].

Fluorescence spectroscopy has attracted wide attention with the advantages of high sensitivity, simplicity, easy operation, and quick response [17]. It is already known to be very useful for developing environmentally friendly analytical methodologies. BPA shows native fluorescence in organic solvents but its fluorescence efficiency in aqueous medium is too low to be directly analyzed. Wang and Lin [18] reported a reversible fluorescence sensor based on insoluble β-cyclodextrin polymer for direct determination of BPA. They observed that the addition of β-cyclodextrin could remarkably enhance the fluorescence intensity of BPA in aqueous solutions. The fluorescence sensor exhibits a dynamic detection range from 6.0×10^{-6} to 1.0×10^{-3} mol/L with a detection limit of 1.0×10^{-6} mol/L.

BPA in the presence of phenol was determined using a method based on first-derivative spectrofluorimetry by Del Olmo and Vilchez [19]. This method involves a micro liquid–liquid extraction of sodium chloride saturated water samples with diethyl ether followed by direct fluorimetric analysis of extracts. The concentration range over which the method was applied was 0.5–10.0 μg/L of BPA. The detection limit was 0.07 μg/L. Sun et al. [17] developed an aptamer-based fluorescent method for the analysis of BPA based on the specific recognition of aptamers and the inner filter effect of gold nanoparticles on the fluorescence of CdTe quantum dots. The linear correlation to BPA concentration was exhibited from 10 to 80 ng/mL (R^2 = = 0.99) with the detection limit of 1.86 ng/mL. In this study, a new synchronous fluorescent method is reported utilizing procaine hydrochloride as a self-quenching fluorescence probe. The method is based on the reaction between BPA and a diazo compound of procaine hydrochloride. In acidic solution, procaine hydrochloride can be diazotized with sodium nitrite to obtain a diazo compound. It is found experimentally that the diazo compound can react with BPA in NH_3-NH_4Cl buffer, causing the fluorescence quenching of the diazo compound of procaine in NH_3-NH_4Cl solution. There is a linear relationship between the inhibitive intensity of fluorescence emission and the concentration of BPA. The method is rapid, sensitive, and has been applied to the determination of BPA in real samples.

Experimental. BPA was purchased from Shanghai Chemical Reagent Factory and Shanghai Biotechnology Co. Ltd. (Shanghai) and was recrystallized prior to use. Procaine hydrochloride was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Standard stock solution of BPA was prepared by dissolving the appropriate amounts in 0.5 mL ethanol, diluted to a final concentration of 0.10 mg/mL with water, which was kept at 4°C in the dark. Working solutions were prepared by further diluting the stock solution in water. Sodium nitrite was obtained from Kelong Huaxue Shiji (Chendu, China). Hydrochloric acid, ammonium hydroxide (25–28%) and ethanol were obtained from Jiangsu Yongfeng Chemical Reagent Co., Ltd (Danyang, Jiangsu, China). All reagents were of analytical-reagent grade unless stated otherwise. Ultrapure water (Millipore Milli-Q water, 18.2 MΩ cm) was used throughout the experiments.

Spectrofluorimetric measurements were made on a HitachiF-4600 luminescence spectrometer equipped with a xenon discharge lamp and 1.0 cm quartz cuvettes (Hitachi, Japan). Both the operation and data processing were controlled by the Fluorescence Data Software. Absorption spectra were acquired on a 1102 ultraviolet-viaible (UV) spectrophotometer (Shanghai Tian Mei Scientific instrument Co,. Ltd, Shanghai, China) equipped with 1.0 cm quartz cuvettes.

Procedures. A certain portion of 0.10 mg/mL of procaine hydrochloride solution was transferred into a 10.0 mL standard flask, and 0.2 mL of 0.10 mol/L HCl solution and 0.2 mL of 0.10 mg/mL sodium nitrite were added. The mixture was shaken and contained in ~0–5°C for 5 min. Procaine hydrochloride was first diazotized with sodium nitrite. Next a certain portion of 0.10 mg/mL of BPA solution was added to the mixture and shaken. Than 4.4 mg/mL $NH_3 \cdot H_2O$ solution was added diluted to the mark with water and mixed well.

All fluorescence measurements were made at a scan rate of 1200 nm/min using 5 nm excitation and emission windows. Recording synchronous fluorescence spectrum, $\Delta\lambda$ = 68 nm, was selected. The excitation and emission spectra and the synchronous fluorescence spectrum were recorded on the luminescence spectrometer. A blank solution containing no BPA was also prepared in this way and the output recorded as the background. The peak height of synchronous fluorescence spectrum was estimated at 270 nm on the longitudinal coordinate axis (absolute value). The inhibitive fluorescence intensity was calculated by $\Delta F = F_0 - F_S$, where F_S was the intensity of sample solution and F_0 was the signal of blank solution. The different height ΔF was used for quantification.

Results and discussion. *Spectral characteristics*. The fluorescence excitation and emission spectra were scanned using an F-4600 spectrofluorometer (Fig. 1). It can be seen that the λ_{ex}^{max} and λ_{em}^{max} of blank solution are at 272 and 340 nm (Fig. 1a,b).

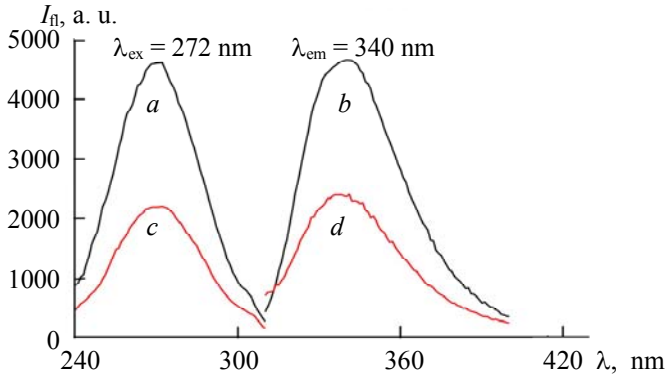


Fig. 1. Excitation (a, c) and emission (b, d) spectrum of the reaction solution (c,d) and reagent blank (a, b), λ_{em} = 340 nm (a, c) and λ_{ex} = 272 nm (b, d); BPA, 1.0 μg/mL, procaine hydrochloride, 4.0 μg/mL.

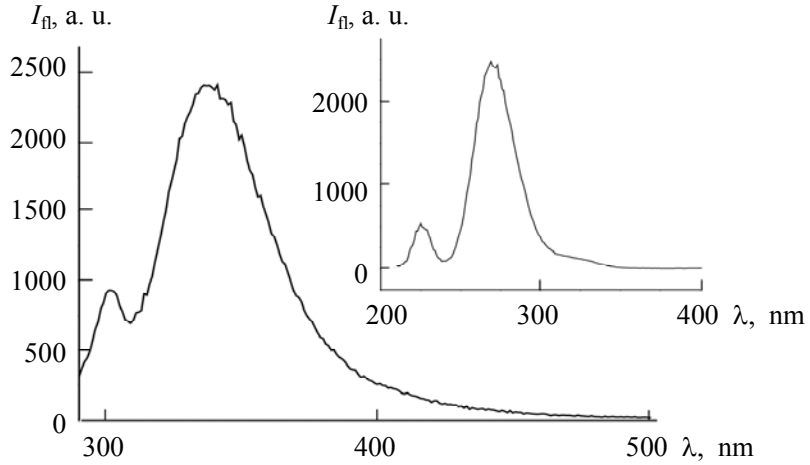


Fig. 2. Fluorescence emission signal of the reaction solution containing 1.0 μg/mL BPA. Inset: synchronous fluorescence emission signal of the reaction solution. $\Delta\lambda$ = 68 nm; procaine hydrochloride, 4.0 μg/mL.

The presence of the trace BPA has an inhibitory effect on the reaction (Fig. 1c,d). However, the spectra of the reaction solution have another peak at 306 nm (Fig. 2), which may belong to BPA. Bisphenol *A* manifests weak fluorescence property in aqueous solutions because of its low fluorescent efficiency [18]. These two peaks of the fluorescence spectra cannot be separated entirely. To resolve the problem of spectral overlap, the synchronous fluorescence method was used. Obviously, these two peaks were separated on the synchronous fluorescence spectrum (Fig. 2, inset, 225 and 270 nm). So, BPA can be more sensitively and accurately determined by basing the measurement on this synchronous fluorescence spectrum. In addition, test found that the synchronous fluorescence spectral peaks were not separated well during the $\Delta\lambda$ below or above 68 nm. Therefore, $\Delta\lambda$ = 68 nm was selected for recording the synchronous spectrum of the reaction and the blank solution. Furthermore, it was observed that there was a liner relationship between ΔF (peak at 270 nm) and the concentration of BPA.

Optimization of quench fluorescence intensity. To take full advantages of the procedure, the experiment conditions should be optimized. For this purpose, various experimental parameters have been optimized by setting all parameters constant and optimizing them one at a time. In these experiments, the concentration of BPA was kept to 1.0 μg/mL. Each experiment was replicated at least three times.

According to the procedure, the procaine hydrochloride was first reacted with sodium nitrite in acidic solution. The effect of acid contained in the solution on fluorescence emission intensity was initially examined. The following media have been prepared in the present experiments: HCl, H_2SO_4, H_3PO_4, and CH_3COOH at the same concentration. It was found that better inhibition occurred in acidic medium containing HCl. With increasing concentration of HCl, the inhibitive fluorescence intensity increased and reached a maximum value at 4.0×10^{-3} mol/L. Therefore, 4.0×10^{-3} mol/L HCl was chosen as the acidic medium for the diazotization of procaine hydrochloride.

In the present system, sodium nitrite is a diazotizing reagent. The influence of the concentration of sodium nitrite has been investigated in the range of 4.0–14.0 μg/mL. It is shown that with increase in sodium nitrite concentration, the ΔF was also increased, when the concentration of sodium nitrite reached to a certain value, the inhibitive fluorescence values tended to a maximum value. Therefore, 8.0 μg/mL sodium nitrite was used for further study. The effect of the diazotization reaction time was investigated between 1 and 20 min. A diazotization reaction time of 5 min after no ΔF increase was chosen as the optimum.

The concentration of the procaine hydrochloride also has an effect on the inhibitive fluorescence intensity. The results showed that an increase in procaine hydrochloride concentration caused an increase in the fluorescence intensity for both the blank reaction and the sample reaction, and the plot of ΔF versus procaine hydrochloride concentration shows that the ΔF increased with increase in concentration from 1.0 to 4.0 μg/mL. Therefore, 4.0 μg/mL procaine hydrochloride was used for all experiments.

The procaine hydrochloride was diazotized with sodium nitrite in acidic solution, followed by coupling with BPA in NH_3-NH_4Cl buffer. The effect of ammonium hydroxide concentration on both the sample and the blank reactions was varied in the range 88.0–306.0 μg/mL. It was found that an increase in ammonium hydroxide concentration caused an increase in the fluorescence intensity of both the blank reaction and sample reaction. The highest ΔF was obtained at the concentration of 220.0 μg/mL. Therefore, a final concentration of 220.0 μg/mL ammonium hydroxide was considered to be the best choice.

The effects of reaction temperature and time were investigated in the range of 0–60°C and 3–30 min, respectively. The mixture solutions of 1.0 μg/mL of bisphenol A were cooled or heated in a water bath of different temperature. It was found that the temperature had no significant influence on the signal in the 0–30°C range. Higher temperature resulted in a decrease in ΔF. So we selected room temperature as the reaction temperature. In addition, the results of the coupling reaction of the mixture solutions of 1.0 μg/mL of BPA occurring for different times in room temperature were investigated. When the coupling reaction time occurred for more than 15 min, the ΔF changed gently. So, 15 min was selected as optimum. The inhibitive fluorescence intensity of the sample system remained stable for at least 24 h at room temperature.

Analytical parameters of BPA. Under the optimum conditions mentioned above, the calibration curve was obtained for BPA determination by plotting the ΔF versus BPA concentration, which gave a linear range from 0.10 to 1.4 μg/mL with a correlation coefficient of 0.9949 (Fig. 3, inset). The linear regression equation of the calibration graph is $\Delta F = -42.59 + 2321.70C$ (1.0 μg/mL). The typical recording output of the sample system for measurements of BPA is shown in Fig. 3. The relative standard deviation for 11 repetitive determinations of 1.0 μg/mL BPA was 0.32%. The detection limit for BPA is 0.04 μg/mL at a signal-to-noise ratio of 3.

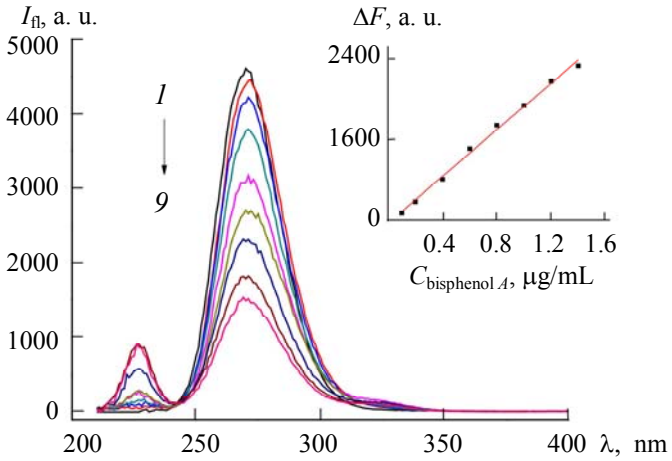


Fig. 3. Typical synchronous fluorescence emission signals of the reaction solution. Concentrations of bisphenol *A* were blank (1), 0.1 (2), 0.2 (3), 0.4 (4), 0.6 (5), 0.8 (6), 1.0 (7), 1.2 (8), and 1.4 μg/mL (9). Inset: plots of inhibitive fluorescence emission intensity vs bisphenol *A* concentration.

Selectivity. To study the selectivity of the proposed method, the influences of some species on the determination of 1.0 μg/mL BPA was investigated under the optimum conditions. Some ions commonly existing in water and organic substances that could be used in HPLC (development of an HPLC-FL method for BPA) were chosen for the selectivity test. When the effect of each foreign species on the peak height was less than 5.0%, it was thought that they do not interfere with the determination of BPA. The obtained results are summarized in Table 1. Some ions and the organic substances did not interfere with the determination of BPA. Therefore, the proposed method has good selectivity.

TABLE 1. Tolerance to Different Substances in the Determination of 1.0 μg/mL Bisphenol *A*

Species added	Maximum tolerable mole ratio[a]
Na^+, K^+, Cl^-, Ba^{2+}, EDTA, methanol, acetonitrile	500
Ca^{2+}, Zn^{2+}	200
Mg^{2+}, Al^{3+}	100
Fe^{3+}	50

[a] 500 is the greatest ratio tested.

Applications. The proposed method has been applied to the determination of BPA in table-water bottle samples. The sample solution was prepared based on [20]. Before the examination, three beverage bottles were washed with Milli-Q water, solarized at room temperature, and cut into pieces of 0.5 cm^2. An appropriate amount of each sample was taken, put in a conical flask, and 25.0 mL of water was added. The solutions were heated in a water bath (70.0 ± 0.2°C) for 2 h, and then cooled to room temperature. Finally, a portion of the prepared sample was diluted with Milli-Q water to 50.0 mL and used for the fluorescence determination of BPA. The recovery study was performed by spiking samples with BPA at different levels. The experiments results are shown in Table 2. The recoveries of 96.1–105.0% of BPA with an average of 101.4% indicate that no serious interference is observed in the samples. Therefore, BPA can be determined by the synchronous fluorimetric method.

TABLE 2. Results of Bisphenol *A* Determinations in Table-Water Bottle Samples

Sample	Added, μg/mL	Measured, μg/mL	RSD (n = 3), %	Recovery, %
1	0.00	0.52	1.9	
	0.10	0.64	4.5	103.2
	0.50	0.98	3.5	96.1
2	0.00	0.00		
	0.40	0.42	3.3	105.0
	0.70	0.71	2.5	101.4

Possible reaction mechanism. From the ultraviolet-visible absorption spectra shown in Fig. 4, we can see that procaine hydrochloride system has peaks at 220 and 290 nm (Fig. 4a), and BPA has absorption peaks at 224 and 278 nm (Fig. 4b) between 210 to 700 nm. The absorption spectrum of reaction system indicates two new peaks at 277 and 449 nm (Fig. 4c). With increase in BPA concentration, the absorbance intensity of the peak at 449 nm increased (Fig. 4, inset). So, it is concluded that a reaction takes place and new compounds could be formed.

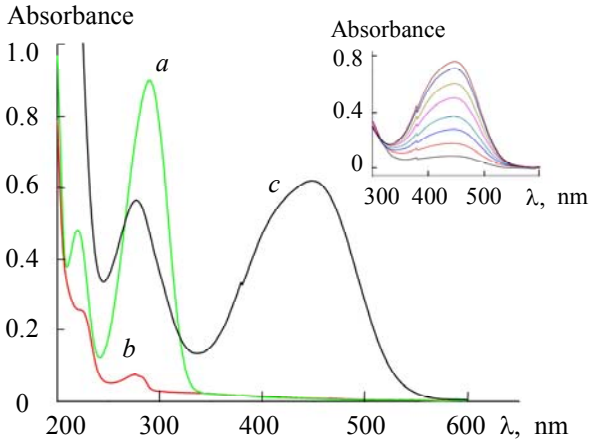
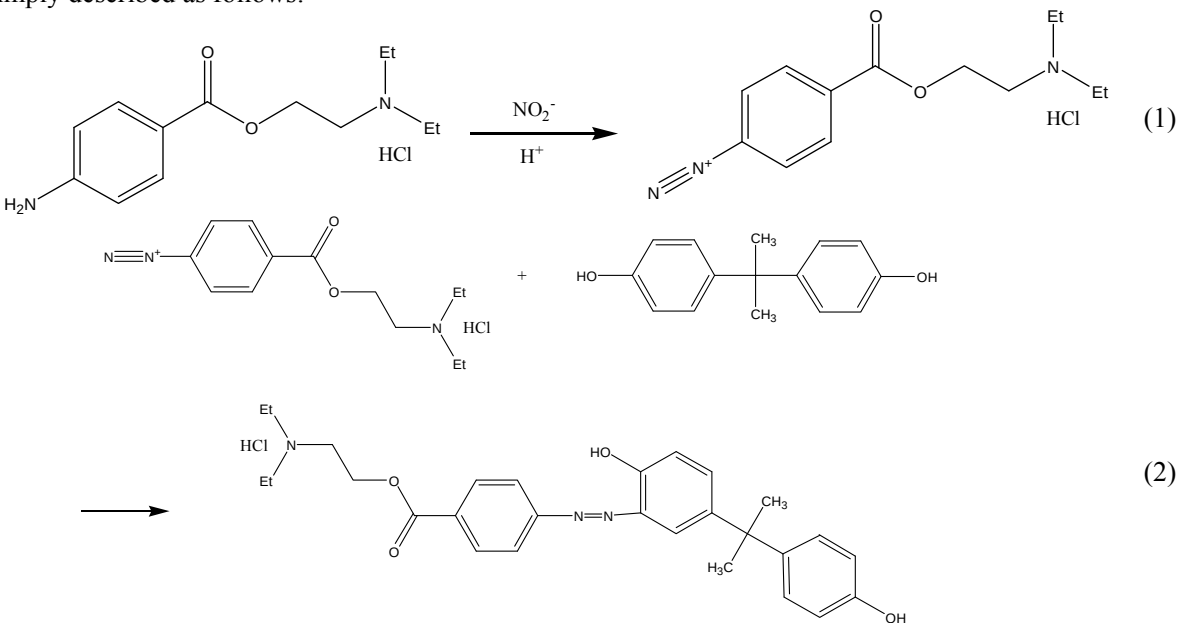


Fig. 4. UV-visible absorption spectra of 12.0 μg/mL procaine hydrochloride (a), 2.0 μg/mL bisphenol *A* (b), and 12.0 μg/mL procaine hydrochloride + 4.0×10^{-3} mol/L HCl + 20.0 μg/mL $NaNO_2$ + 2.0 μg/mL bisphenol *A* + 528.0 μg/mL $NH_3 \cdot H_2O$ (c). Inset: the typical recording output of the reaction solution. The concentration of bisphenol *A* was (low to high) 0.20, 0.40, 0.80, 1.20, 1.60, 2.00, 2.40, 2.80 μg/mL.

The Stern–Volmer curve deviates from linearity, which allows us to assume that we are dealing with a mixed type of quenching. It is generally agreed that in the case of linear 1-naphthol Stern–Volmer dependence, quenching of fluorescence occurs due to diffusive collisions [21]. In our reaction system, the Stern–Volmer dependence is nonlinear, this is caused by the interaction of BPA and diazoprocaine.

According to the above experiments and discussion, in our experiments, procaine was first diazotized with sodium nitrite to obtain a diazo compounds in acidic solution; then the diazo compounds can couple with BPA to produce an azo-compound in NH_3-NH_4Cl buffer, and the azo-compound has a maximum absorption at 449 nm (Fig. 4c). This results in quenching of fluorescence. The mechanism of the reaction can be simply described as follows:



Conclusion. A new synchronous fluorimetric method has been demonstrated for the determination of bisphenol *A* based on the diazotization-coupling reaction. The variation in synchronous fluorescence signal was proportional to bisphenol *A* concentration. Bisphenol *A* can be determined directly at μg/mL. In all the cases, recoveries of 96.1–105.0% were obtained. The proposed method is rapid, inexpensive, simple in operation, and easy to popularize.

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