CHAPTER III

EXPERIMENTAL

3.1 Apparatus

UV-Visible spectrophotometer

The absorption spectra of BF_2-curcumin were recorded by a UV-visible spectrophotometer, HP Hewlett Packard 8435 (Agilent Technologies), in the wavelength range of 350 to 800 nm.

Fourier Transform Infrared Spectrometer (FT-IR)

The BF_2-curcumin coated resin was characterized by a Fourier Transform Infrared spectrometer (FT-IR), model Nicolet 6700 (Thermo Scientific). The IR spectra were recorded from 400 to 4000 cm^{-1} in attenuated total reflectance (ATR) mode.

Fourier Transform Raman Spectrometer (FT-Raman)

The BF_2-curcumin coated resin was characterized by a Fourier Transform Raman spectrometer (FT-Raman), model Spectrum GX (Perkin Elmer). The Raman spectra were recorded from 400 to 3600 cm^{-1} with a 4 cm^{-1} resolution.

Diffuse reflectance ultraviolet visible spectrophotometer

The reflectance spectra of BF_2-curcumin coated resin were recorded by a diffuse reflectance ultraviolet visible spectrophotometer (DR-UV-Vis), model UV-2500PC (Shimadzu), using barium sulfate as background substrate.

Inductively coupled plasma optical emission spectrometer (ICP-OES)

The concentration of As(III) in solution was measured by an inductively coupled plasma optical emission spectrometer (ICP-OES), model iCAP 6500 Duo (Thermo Scientific). The operating conditions are listed in Table 3.1

Table 3.1 The ICP-OES operating conditions for As determination

Operating condition	
RF power	1150 W
Auxilary gas flow	0.5 L/min
Nebulizer gas flow	0.6 L/min
Coolant gas flow	12 L/min
Analysis pump rate	50 rpm
As emission wavelength	189.042 nm

pH meter

The pH of all solutions was measured using a pH/mv meter, model UltraBASIC-10 (Denver).

Camera

The photographs were taken with Lumix FZ 150 digital camera (Panasonic).

3.2 Chemicals

All chemicals used in this work were of analytical grade and listed in Table 3.2.

Table 3.2 Chemicals list

Chemicals	Supplier
Acetone	Merck
Amberlite XAD-2	Supelco Analytical
Ethanol	Merck
Hydrochloric acid 37%	Merck
Nitric acid 65%	Merck
Sodium arsenate	BDH Chemicals
Sodium arsenite	BDH Chemicals
Sodium carbonate	Fluka
Sodium hydroxide	Merck
Sodium nitrate	Fluka
Sodium phosphate	Merck
Sodium sulphate	Merck
Sodium thiosulphate	Merck

3.3 Synthesis of difluoroboron-curcumin (BF_2-curcumin)

BF_2-curcumin was synthesized by following the procedure of Chaicham et al. [25]. Briefly, 1.02 mL (8 mmol) borontrifluoride diethyletherate ($(C_2H_5)_2OBF_3$) was added into a 250 mL two-neck round bottom flask containing curcumin 2.947 g dissolved in methanol (200 mL). The reaction mixture was refluxed at 60°C for 2 h under nitrogen atmosphere and then cooled down to room temperature. The BF_2-curcumin was obtained as a red solid.

3.4 Preparation of solutions

As(III) solution

As(III) solution (1000 mg/L, 50 mL) was prepared by weighing exactly 86.7 mg of sodium arsenite and dissolving in deionized water.

As(V) solution

As(V) solution (1000 mg/L, 50 mL) was prepared by weighing exactly 138.7 mg of sodium arsenate and dissolving in deionized water.

BF_2-curcumin solution

BF_2-curcumin solution (1 mM, 5 mL) was freshly prepared by dissolving 2.08 mg of BF_2-curcumin solid in 60% (v/v) ethanol and sonicated for 3 minutes before used.

Sodium hydroxide solution

Sodium hydroxide solution (1 M) was prepared by dissolving the appropriate amount of sodium hydroxide in deionized water.

Hydrochloric acid

Hydrochloric acid solution (1 M) was prepared by diluting the concentrated Hydrochloric acid (37%) in deionized water.

3.5 Colorimetric and naked eye detections of arsenic in solution system

3.5.1 Preliminary study for naked eye detection of arsenic

In this experiment, 30 µL of BF_2-curcumin in 60% (v/v) ethanol was added into a cuvette followed by a designated volume of 100 mg/L and 1000 mg/L

As(III) stock solution. The solution was made up to 3 mL with 60% (v/v) ethanol. After the addition of As(III), the mixture was shaken and immediately observed for the solution color by naked eye. Its absorbance was measured by UV-visible spectrophotometer in the wavelength range of 350-800 nm. The resulting color of the BF_2-curcumin solution with As(III) was compared with the BF_2-curcumin solution with As(V) treated in the same manner. The parameters including concentration of BF_2-curcuminin, pH, and sampling time were optimized.

3.5.2 Effect of concentration of BF_2-curcumin solution

1 mM BF_2-curcumin solution was diluted with deionized water to 5, 10, 20, and 30 μM. These BF_2-curcumin solutions were tested with As(III) solution in different concentrations from 0-200 μM. The colors of solutions were visually observed and their absorbance were measured by UV-Vis spectrophotometer at 632 nm.

3.5.3 Effect of pH

As(III) solution (50 μM) was used as a sample to investigate the effect of pH in the range of 3 to 10. The pH of this solution was adjusted by the addition of 1 M HCl and/or 1 M NaOH solution. The effect of pH of As(III) solution was compared with a control solution, which was performed by using deionized water without As(III), in the same pH range. The concentration of BF_2-curcumin was maintained at 10 μM. The color of solution and its absorbance was detected by naked eye and UV-Vis spectrophotometer at a 632 nm.

3.5.4 Sampling time

Ten μM BF_2-curcumin solution containing 10, 50 and 80 **μM** of As(III) solution were used as samples to study the stability of resulting color on different sampling time from 0 to 30 minutes. After the addition of As(III) solution, the mixture was shaken for 30 seconds and examined for the stability of resulting color by UV-Vis spectrophotometer at 632 nm at the designated sampling time. The pH of As(III) solution was fixed at pH 8.

3.5.5 Interferences study

The effect of interferences from common co-existing anions was studied by adding 1 mM of NO_3^-, I^-, CO_3^{2-}, $S_2O_3^{2-}$, PO_4^{3-} and SO_4^{2-} to 10 μM BF_2-curcumin solutions and compared to those containing 100 μM As(III) standard solution. The solutions of As(III) and interfering anions were adjusted to pH 8 before use. After that, all solutions were added into cuvettes and then shaken for 30 seconds. The colors of solutions were observed by naked eyes and their absorbance were measured by UV-Vis spectrophotometer at 632 nm within 3 minutes.

3.5.6 Method validation

In terms of naked-eye detection, 200 μL of As(III) solution was added into a cuvette containing 30 μL of 1 mM BF_2-curcumin in 60% (v/v) ethanol and made up with 60% (v/v) ethanol to 3 mL. The solution was shaken for 30 seconds and visually observed its color within 3 minutes. Under the optimal conditions, the color of BF_2-curcumin solutions with As(III) standard solutions in the concentration of 0, 25, 50, 70, 90 and 100 μM were used as a color calibration chart for naked eye detection of As(III) in water samples. Subsequently, the limit of detection (LOD) was estimated by the lowest concentration producing minimum change of color that can be visually differentiated from that of the blank solution.

In terms of UV-Vis spectrometry, As(III) standard solution in the concentration range of 0-100 μM were used as samples to evaluate the method performance. 200 μL of As(III) standard solution was added into a cuvette containing 30 μL of 2 mM BF_2-curcumin in 60% (v/v) ethanol and made up to 3 mL with 60% (v/v) ethanol. Then, this solution was shaken for 30 seconds and measured by UV-Vis spectrophotometer at 632 nm. The analysis was repeated 6 times for each concentration under the optimal conditions. The calibration curve was plotted with absorbance (Abs.) of each concentration of As(III) standard solutions that was calculated from Equation 3.1;

$$\text{Abs.} = \text{Abs}_{\text{sample}} - \text{Abs}_{\text{blk}} \qquad (3.1)$$

Where $\text{Abs}_{\text{sample}}$ and Abs_{blk} represented the absorbance of As(III) standard solution in each concentration and blank solution that were obtained from a UV-Vis spectrophotometer at 632 nm. The signal of the concentration that is the limit of detection (S_{LOD}) was calculated from Equation 3.2 [52];

$$S_{LOD} = S_{blk} + 3SD_{blk} \qquad (3.2)$$

Where S_{blk} and SD_{blk} represented the signal and standard deviation of the blank solution, respectively. The limit of detection (LOD) was calculated by comparing S_{LOD} to the linear equation from the calibration curve. In addition, a mixture of As(III) and As(V) (1:1) was used as a model for total As determination and its result was compared with As(III) determination in the same manner.

3.6 Naked eye detections of arsenic in solid system

3.6.1 Pretreatment of Amberlite XAD-2 resin

The Amberlite XAD-2 resin was stirred with deionized water for 24 hours, and then filtered and washed with deionized water. The resin was dried at 100°C for 2-3 days in an oven. It was sieved (~500 μm) and kept into a desiccator before use.

3.6.2 Preparation and charaterization of BF_2-curcumin coated resin

Two grams of Amberlite XAD-2 resin beads were stirred with 25 mL of BF_2-curcumin solution for 24 hours. The BF_2-curcumin coated resin was filtered and washed with deionized water. The color of coated resin clearly changed from white to orange indicating the incorporation of BF_2-curcumin onto the beads. The coating of this resin was later confirmed by Fourier transform infrared spectrometer (FT-IR), Fourier transform Raman spectrometer (FT-Raman) and diffuse reflectance ultraviolet visible spectrophotometer (DR-UV-Vis).

For characterization by the DR-UV-Vis spectrophotometer, the spectra of Amberlite XAD-2 resin and BF_2-curcumin coated resin were plotted between Kubelka-Munk function or $f(R)$ and wavelength. The $f(R)$ was calculated from Equation 3.3 [53];

$$f(R) = \frac{(1-R)^2}{2R} \qquad (3.3)$$

when $f(R)$ = Kubelka-Munk function

R = reflectance of sample

3.6.3 Effect of pH

Twenty milligrams of BF_2-curcumin (70 µM) coated resin and 1×10^{-3} M As(III) solution were used as a sample to investigate the effect of pH in the range of 3 to 10. The pH of this As(III) solution was adjusted by the addition of 1 M HCl and/or 1 M NaOH solution. After the loading of 1 mL As(III) solution into a test tube containing BF_2-curcumin coated resin, the mixture was shaken for approximately 1 minute and observed for the changes in resin color by naked eyes. The effect of pH on the color of resin in As(III) solution was compared with a resin color in the control solution, which was performed by using deionized water without As(III), in the same pH range.

3.6.4 Effect of concentration of BF_2-curcumin for resin coating

In order to study the effect of BF_2-curcumin concentration on resin colors, various concentrations of BF_2-curcumin in the range of 10, 20, 30, 50 and 70 μM were used for resin coating before testing with As(III) solution. This As(III) solution was varied in the range of 0-1×10-3 M and adjusted to pH 10 before use. After that, 1 mL of As(III) solution was loaded into a test tube containing 20 mg of the prepared resins. The colors of the BF_2-curcumin coated resin were observed by naked eyes.

3.6.5 Method validation

One millilitre of As(III) standard solution in the concentration range of 0-5×10^{-3} M, that was adjusted to pH 10 with 1 M NaOH solution, was brought into a microcentrifuge tube containing 20 mg of BF_2-curcumin coated resin. The mixture was shaken for approximately 1 minute and observed the color change of coated resin by naked eyes. Under the optimal conditions, the changes of resin color by As(III) standard solutions in the concentration 0, 3×10^{-5}, 1×10^{-4}, 5×10^{-4}, 1×10^{-3} and 5×10^{-3} M were used as a color calibration chart for naked eye detection of As(III) in water samples. The residual concentration of As(III) in solution was measured using an inductively coupled plasma optical emission spectrometer (ICP-OES), in order to evaluate the reaction mechanism of BF_2-curcumin and As(III). The limit of detection (LOD) was determined by the minimum change of color that can be visually differentiated for the naked-eye detection.

For total As determination, a mixture of As(III) and As(V) (1:1) was used as a model sample for total As determination with this method. Under the optimal conditions, i.e., 50 μM BF_2-curcumin coated resin, pH 10 of As solution, the changes of resin color in total As standard solutions in the concentration 0, 3×10^{-5}, 1×10^{-4}, 5×10^{-4}, 1×10^{-3} and 5×10^{-3} M were used as a color calibration chart for naked eye detection of total As in water samples.

3.6.6 Regeneration of BF_2-curcumin coated resin

The BF_2-curcumin coated resin with 1x10-3 M As(III) solution was used as a sample to study the regeneration of BF_2-curcumin coated resin. 1 mL of 1 M HCl was added into a microcentrifuge tube containing 20 mg of used resin. The solution was shaken and instantly observed for the change of resin color by naked eyes. In addition, the regenerated resin was used as a sample to investigate the repeatability for As(III) determination. The regenerated resin was washed with deionized water and then tested with 1x10-3 M As(III) solution. The resin color was visually observed.

3.7 Application in real samples

3.7.1 Determination of As(III) in water samples

3.7.1.1 Solution system

The real water samples used in this work were a bottled drinking water and Chulalongkorn University's pond water. The water samples were filtered and adjusted to pH 8 with 1 M HCl and/or 1 M NaOH solution.

In terms of naked eye detection, 200 μL of a water sample, previously spiked with As(III) in the concentration range of 30, 50, 70 and 130 μM, was added into a cuvette containing 30 μL of 1 mM BF_2-curcumin in 60% (v/v) ethanol and made up to 3 mL with 60% (v/v) ethanol. Then this solution was shaken for 30 seconds and visually observed for the change of solution color within 3 minutes. The concentration of As(III) in this sample was estimated by comparing with a color calibration chart of the As(III) standard solutions in section 3.5.6.

In terms of UV-Vis spectrophotometry, a bottled drinking water was used as a sample. 200 μL of a bottled drinking water sample, spiked with As(III) in the concentration range of 10, 25, 50 and 75 μM, was added into a cuvette containing 30 μL of 2 mM BF_2-curcumin in 60% (v/v) ethanol and made up with 60% (v/v) ethanol to 3 mL. Then this solution was shaken for 30 seconds and its absorbance was measured by UV-visible spectrophotometry at 632 nm within 3 minutes. The amount of As(III) in spiked sample was calculated from the linear equation of the calibration curve plotted between the absorbance calculated from Equation 3.1 and the concentration of As(III) in the range of 2 to 100 μM. The percentages of recovery of As(III) in these samples were calculated from Equation 3.4.

$$\%\text{recovery} = \frac{Cf}{Cs} \times 100 \qquad (3.4)$$

when Cf = the concentration of As(III) found in the spiked sample (μM)

Cs = the concentration of As(III) spiked in the sample (μM)

3.7.1.2 Solid system

In the solid system, this proposed method was applied to detect As(III) in Chulalongkorn University's pond water. Before use, the pond water was filtered and adjusted to pH 10 with 1 M NaOH solution. After that, 1 mL of the pond water sample, spiked with As(III) in the concentration range of 3×10-5, 5×10-4, 1×10-3 M (pH 10), was loaded into a microcentrifuge tube containing 20 mg of 50 μM BF_2-curcumin coated resin. The mixture was shaken for approximately 1 min and observed for the color change of coated resin by naked eyes. The concentration of As(III) in this sample was evaluated by comparing with a color calibration chart of the As(III) standard solutions in section 3.6.5.

3.7.2 Determination of total As in water samples

3.7.2.1 Solution system

Bottled drinking water was used as a sample to study the application of this method for determination of total As. 200 μL of a bottled drinking water sample, spiked with total As (As(III) and As(V) in 1:1 ratio) in the concentration range of 10, 30 and 50 μM (pH 8), was added into a cuvette containing 30 μL of 2 mM BF_2-curcumin in 60% (v/v) ethanol and made up to 3 mL with 60% (v/v) ethanol. Then, this solution was shaken for 30 seconds and its absorbance was measured by UV-visible spectrophotometer at 632 nm within 3 minutes. The spiked recovery of As in these samples were determined by Equation 3.4.

3.7.2.2 Solid system

In terms of solid system, 2 types of waste water (TK 80 and TK 81) from the PTT Public Company Limited were used as samples. The waste waters were filtered and adjusted to pH 10 with 1 M NaOH before test. 1 mL of each waste water sample was loaded into a microcentrifuge tube containing 20 mg of BF_2-curcumin coated resin. Then, the mixture was shaken approximately 1 minute and observed the color change of resin by naked eye. The concentration of total As in this sample was evaluated by comparing with a color calibration chart of the total As standard solution in section 3.6.5 and the results were compared with those obtained by ICP-OES method.