

CHAPTER III

EXPERIMENTS

Materials.

1. Treated Seawater.

Coastal seawater as collected from the east coast of the Upper Gulf of Thailand near Sichang Island at the depth of 1 m from the sea surface. Its salinity was approximately 30 ppt. It was treated by the method which was described by Dharmvanij (105) and presented in Appendix 1, in order to reduce the metals concentrations in seawater before using it as the experimental medium.

The reason for using treated seawater, instead of the artificial seawater, is to avoid the contamination of metals from the chemical reagents used in preparing the artificial seawater and because there are no procedure for preparing the artificial seawater in such a way that its components are the same as the natural seawater.

2. Spiked Seawater.

Add 50 μ l of secondary standard metal solution (10 ppm) into 50 ml treated seawater in 250 ml-beaker. The volume is made up to 100 ml by weighing in order to make a concentration of metals (Cu, Ni, Pb and Zn) in treated seawater to 5 ppb. The weight of blank solution must be the same as the spiked seawater.

3. Reagents.

3.1 Super Q Water. This is prepared from double-distilled water which is distilled in quartz vessel and later passed through a "Millipore" Super Q deionizer. Super Q water is used throughout in all the experiments.

3.2 Nitric Acid. Purified by sub-boiling distillation of AR grade HNO_3 and stored in precleaned polyethylene bottle.

This purified nitric acid is used for preparing 4N nitric acid by diluting 250 ml concentrated nitric acid to 1000 ml of Super Q water.

The 10 % nitric acid which is used in cleaning all experimental apparatus is prepared by diluting 100 ml concentrated nitric acid to 1000 ml with Super Q water.

3.3 Stock Standard Solution. Commercially available stock standard solution (1000 ppm) of Cu, Ni, Pb and Zn are used in preparation of the primary standard solutions (100 ppm). These reagents are BDH reagent (spectrochemical grade).

3.4 Primary Standard Solution. Primary standard solution (100 ppm) is prepared by diluting 500 ul of stock standard solutions (1000 ppm) to 5 ml with 4N nitric acid. The primary standard solutions are stored in the precleaned polyethylene vials and kept in a refrigerator.

3.5 Secondary Standard Solution. Secondary standard solutions (10 ppm) are prepared by diluted 500 ul of primary standard

solution to 5 ml with 4N nitric acid and stored in the precleaned polyethylene vials and kept in the refrigerator.

3.6 Chelating Agents.

3.6.1 Ammonium Pyrrolidine Dithiocarbamate (APDC)

Solution. Preparing 1, 2, 4 and 6 % (w/v) aqueous solution of APDC by dissolving 1, 2, 4 and 6 g of APDC in 50 ml Super Q water in 100 ml-volumetric flask and the volume is made up to 100 ml. These solutions are purified by shaking with methyl isobutyl ketone (MIBK) prior to use in order to make it free of metals impurities. Purifications are made by extraction each solution 3 times with MIBK. For the first extraction, APDC solution is extracted with MIBK in a ratio 10 : 1 and 20 : 1 at the second and third extraction. Each extraction is performed by shaking for 5 min in a separatory funnel, allowing for each phase to separate and withdraw lower layer. Discard the upper layer which is MIBK. The APDC solution is freshly prepared each time prior to use for solvent extraction. Trade name of used APDC is Fluka.

3.6.2 Dithizone. Prepared 0.06 % (w/v) dithizone in chloroform by dissolving 0.06 g purified dithizone in 50 ml distilled chloroform in 100 ml-volumetric flask and make volume to 100 ml with distilled chloroform.

3.7 Organic Solvent.

3.7.1 Methyl Isobutyl Ketone (MIBK). This organic solvent, which is AR grade from Baker, is purified by redistillation at 115-116 °C.

3.7.2 Chloroform. This organic solvent is purified by redistillation at 60-65 °C.

3.8 Ammonium Hydroxide Solution. Purify by placing two beakers containing concentrated ammonium hydroxide solution (AR grade) and another containing Super Q water to about half full, in a vacuum dessicator, reduce the pressure inside the dessicator by a vacuum pump. After leaving it to stand at least 24 h the purified ammonium hydroxide solution is obtained from the beaker originally containing Super Q water. The purified solution is a dilute solution of ammonium hydroxide.

4. Instrumentation.

4.1 Atomic Absorption Spectrometry. Each of samples consisting of blanks, spiked samples and reference materials are analysed by Graphite Furnace Atomic Absorption Spectrometry (GFAAS) or by Flame Atomic Absorption Spectrometry (FAAS) depending on the metal concentrations in solution. A Perkin-Elmer Model 403 spectrophotometer equipped with a microcomputer-controlled HGA-400 is used for all atomic absorption measurements of copper, nickel and lead. Sample solutions are delivered to the furnace in 20- μ l volume using a Perkin-Elmer AS-1 autosampler. Absorbance is read from a digital recorder panel. Samples are held in precleaned polyethylene cups prior to injection. Pyrolytically coated graphite tubes are used exclusively. For the atomic absorption measurement of Zn, it is done by Flame AAS on Perkin-Elmer Model 2380. Instrumental parameters for heated graphite furnace and Flame AAS are given in Table 3.1 and 3.2 respectively.

Table 3.1 Instrumental parameters for GFAAS.

Element	Sample Volume (ul)	Dry cycle	Ashing	Atomization	Tube Clean
Cu	20	80 °C 5 s ^a 5 s ^b 120 °C 10 s ^a 10 s ^b	900 °C 15 s ^a 15 s ^b	2300 °C 0 s ^a 5 s ^b	2400 °C 2 s ^a 3 s ^b
Ni	20	80 °C 5 s ^a 5 s ^b 120 °C 10 s ^a 10 s ^b	1000 °C 15 s ^a 15 s ^b	2500 °C 0 s ^a 5 s ^b	2600 °C 2 s ^a 3 s ^b
Pb	20	80 °C 5 s ^a 5 s ^b 120 °C 10 s ^a 10 s ^b	800 °C 15 s ^a 15 s ^b	2000 °C 0 s ^a 5 s ^b	2200 °C 2 s ^a 3 s ^b

a : Ramp times, b : Hold time, Purge gas : Oxygen free nitrogen
Light sources : Hollow cathode lamp, s = Second.

Table 3.2 Instrumental parameters for flame AAS Model 2380.

Element : Zn	, Light source : Hollow cathode lamp.
Flame Type : Air-C ₂ H ₂	, Wavelength : 213.9
Spectral band width : 0.7	

4.2 Mechanical Shaker. A shaker with adjustable speeds and times knobs is used for shaking the solution in extraction procedure.

4.3 Centrifuge. This equipment is used for separation of the small solid particles of manganese dioxide from the solution.

4.4 pH Meter. For all pH measurements.

5. Apparatus.

5.1 Separatory Funnel. All 250 ml-separatory funnel are made from teflon.

5.2 Micropipette and Micropipette Tip. Micropipettes, which are 5, 1 and 0.1 ml-adjustable volumes, are used for introducing the small volume of one solution into the other solution. All micropipette tips are acid-cleaned before using.

5.3 Plastic Wares. All of them, which are used in the experiments, are made from low density polyethylene.

6. Cleaning Procedure.

All experimental apparatus are cleaned as described in Appendix 3.

Procedure.

1. General Scope of Investigation.

Experimental investigations are performed and divided into three main parts, the First Extraction, the Back Extraction and the

Validation of the achieved solvent extraction method with the Certified Reference Materials.

In the first extraction, the experiments are designed to investigate the factors affecting the extractability of the solvent extraction technique which are as follows : comparison of the differences between two processes in which the pH of sample is adjusted before and after addition of chelating agent, the appropriated volumes of organic solvent to be used, the range of pH of sample for extraction, the minimum shaking time for extraction, the minimum concentration of chelating agent and minimum time for phase separation.

For back extraction, it is designed to investigate the minimum volume of 4N nitric acid , which is used for back extraction, and the minimum shaking time for extraction. The stabilities of metals in the nitric acid extract is investigated after the improved method is obtained.

Finally, testing of the efficiency of achieved solvent extraction procedure with the reference materials, this step is performed in order to validate the achieved method.

Each experiment is done dublicately and the triplicate measurements of each extracted metal are done.

Processes outline for the investigation as mentioned above is showed in Fig. 3.1

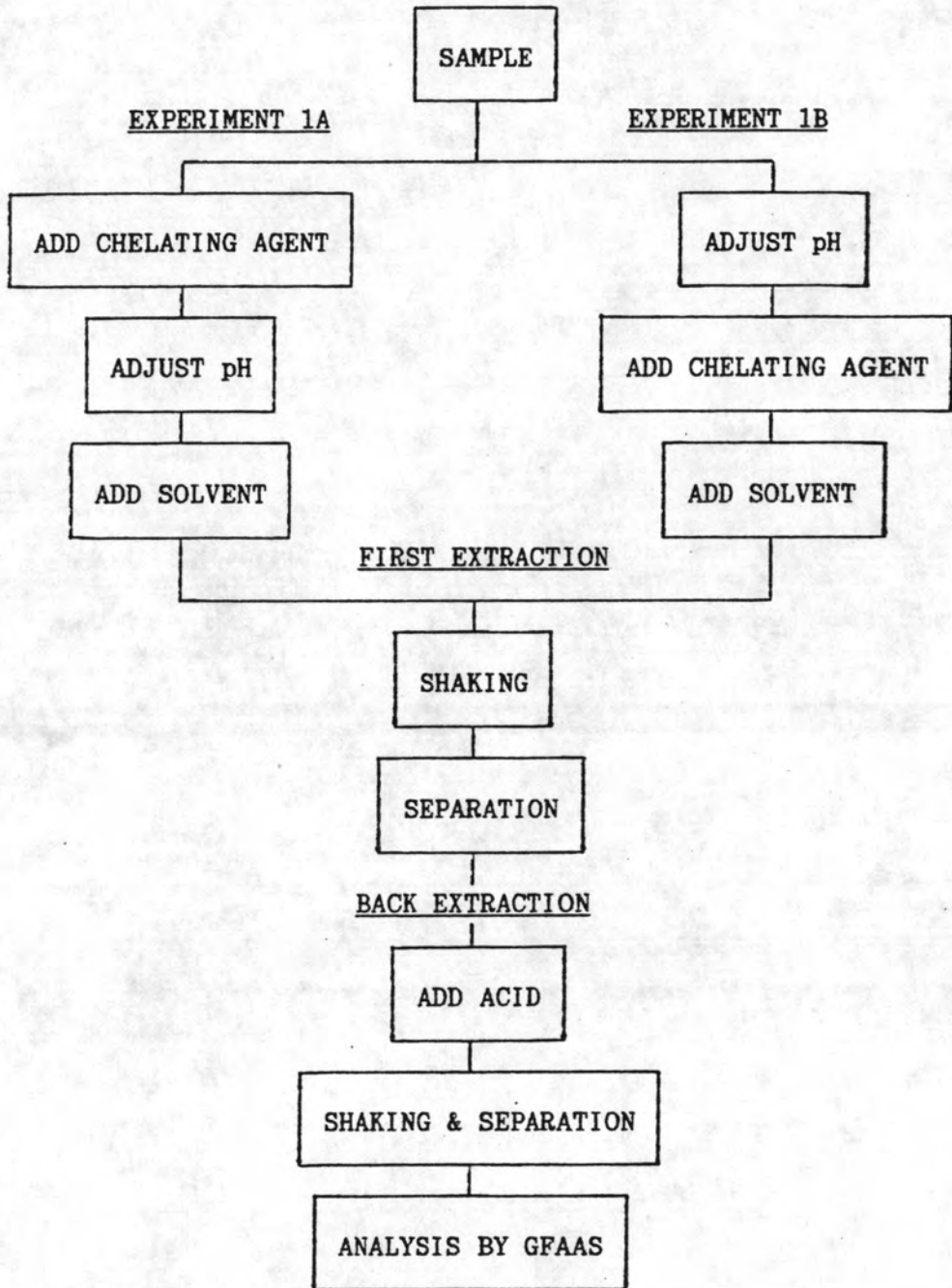


Fig. 3.1 General outline of steps involved in Solvent Extraction Method.

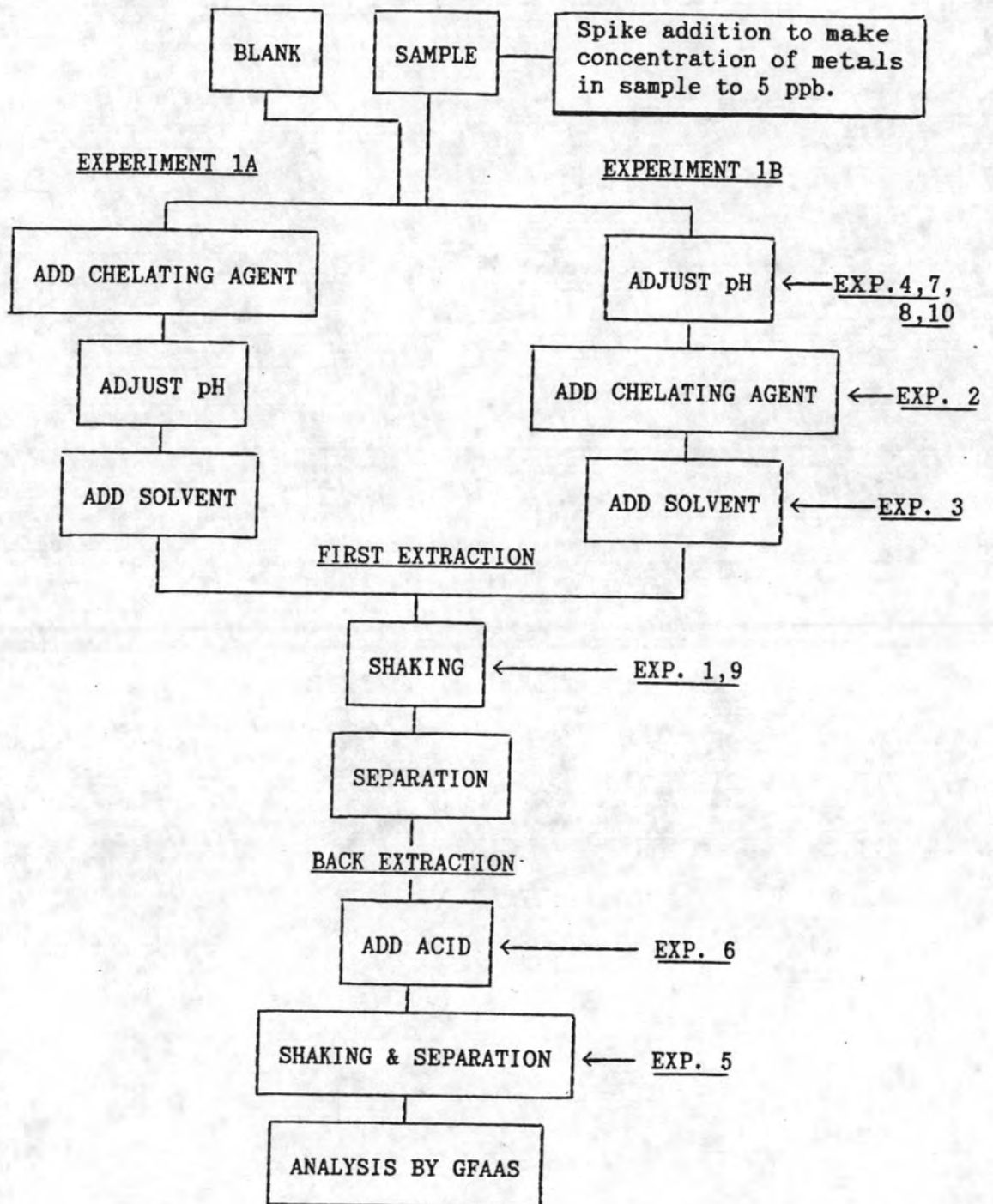


Fig. 3.2 Diagram showing the steps of Solvent Extraction Method to be investigated.

2. Investigation Procedure.

Several experimental investigations, which are designed and performed in this study, are showed in Fig. 3.2. Details and methods of such experiment are described below.

2.1 Experiment 1 : Investigation of the Appropriate Sequence and Optimum Shaking Time of the First Extraction. Since there is a difference in the first extraction about the step of adding chelating agent into the sample before and after the pH adjustment of sample (aqueous phase) and the variation of shaking time for extraction, as showed in Table 1.17, which is used for extraction of the interested metals from sample. So, this experiment is designed in order to investigate these conditions.

2.1.1 Outline of Method. Copper ion in 100 ml spiked seawater sample forms complexes with chelating agent, 2 % APDC, before (Experiment 1A) and after (Experiment 1B) adjusting pH of sample. Organic solvent, MIBK, is added into sample to extract copper from aqueous phase. Shaking times for single extraction is varied from 30 s to 20 min. Back extraction is done by adding 4N nitric acid into the organic phase in order to transfer copper from organic phase to aqueous phase. The contents of copper in nitric acid are measured indirectly three times by the atomization absorbance of copper by GFAAS. Outline of this experiment is showed in Fig. 3.3.

2.1.2 Procedure of Experiment 1A.

(a) Add 5 ml of 2% APDC aqueous solution to 100 ml spiked seawater.

(b) Adjust pH of the sample to 4 by 4N nitric

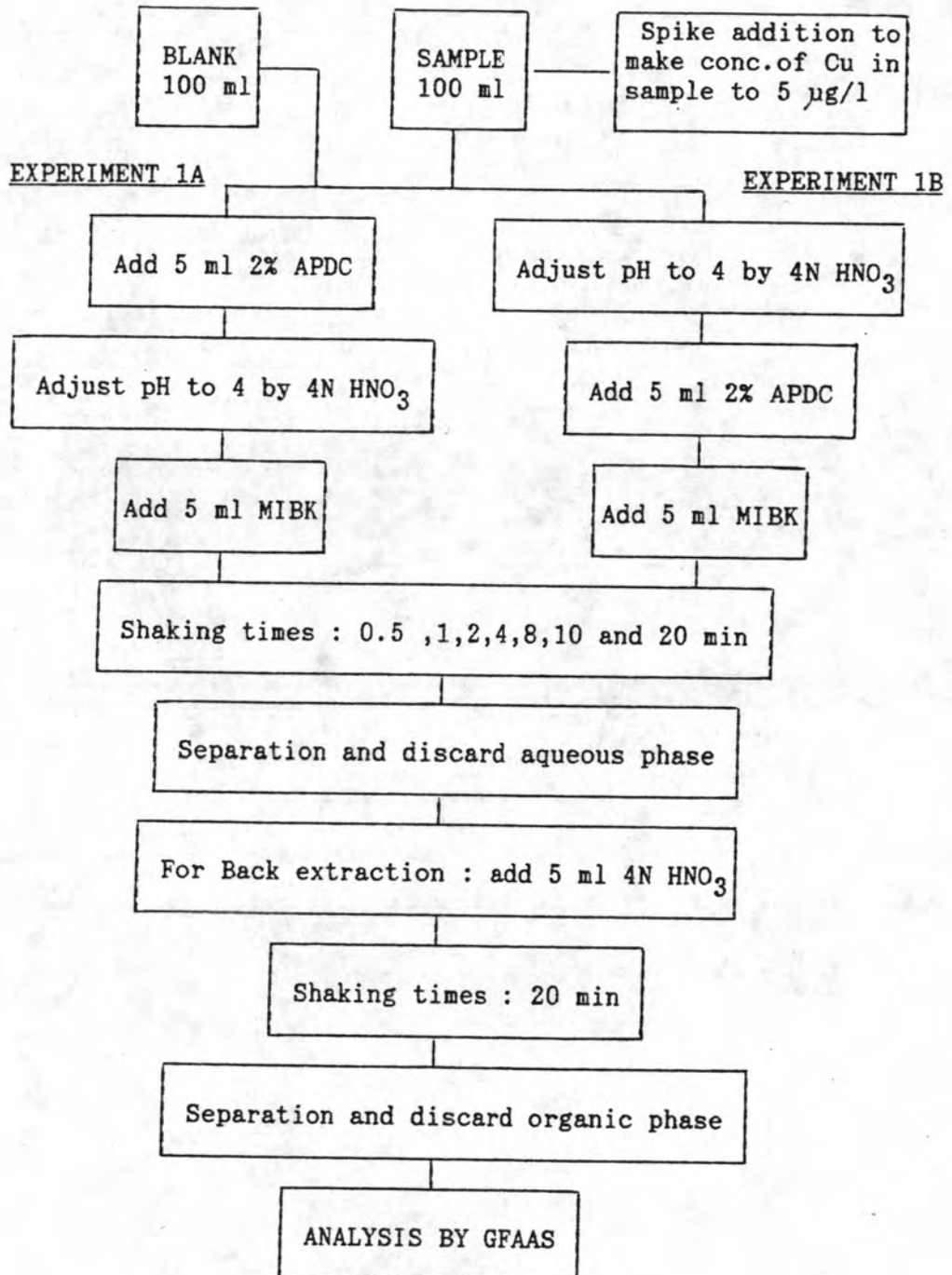


Fig. 3.3 Experimental design for investigation of the appropriate sequence and optimum shaking time of the first extraction.

acid and/or ammonium hydroxide solution, if sample is previously acidified to a pH < 4 for the purpose of preservation.

(c) Transfer the sample to a 250 ml-separatory funnel and add 5 ml MIBK by micropipette.

(d) Shaking the sample by shaker. Two sample are prepared for shaking at 30 s, 1, 2, 4, 8, 10 and 20 min respectively.

(e) Allow the solution in the separatory funnel to separate into aqueous and organic layer. After 20 min, drain off the aqueous layer (bottom layer).

(f) For back extraction , 5 ml 4N nitric acid is pipetted into the organic layer which is obtained from step (e).

(g) Shake the funnel for 20 min and let it stand for 20 min.

(h) Drain off the aqueous phase (nitric acid) into a 8 ml-precleaned polyethylene vial and store in a refrigerator before further analysis by GFAAS.

2.1.3 Procedure of Experiment 1B. Do the same as Experiment 1A except step (b) is done prior step (a).

2.1.4 Blank. All experiments will be performed together with a blank which is prepared by using 100 ml of treated seawater instead of spiked seawater.

2.1.5 Analysis of Sample.

(a) Prepare a series of standard copper solution for making a calibration curve. Each of the solution is prepared by diluting the secondary copper standard solution (10 mg/l)

with 4N nitric acid. The calibration curve is prepared by plotting the absorbances versus the concentration.

(b) If any dilution is necessary prior to measurement, 4 N nitric acid will be used instead of Super Q water.

(c) Instrumental parameters for GFAAS such as lamp current , wavelength , slit width etc. are set according to the instrumental manual. The operating conditions are showed in Table 3.1.

(d) The concentration in microgram per liter ($\mu\text{g/l}$) and percentage extraction (% E) of copper in each sample are calculated from the slope of the calibration curve which is prepared as mentioned in step (a).

2.2 Experiment 2 : Investigation of the Optimum Concentration of Chelating Agent. Because of the different concentrations of the chelating agent, APDC, employed by various workers as showed in Table 1.17, this experiment is conducted in order to investigate the appropriate quantity of APDC to be used in the first extraction.

2.2.1 Outline of Method. The concentration of Chelating agent, is used for the formation of complexes between Cu and APDC, is varied from 1, 2, 3, 4, 5 and 6 % (w/v) respectively. The results obtained from the Experiment 1 is applied to this experiment accordingly. The steps in Experiment 2 is showed in Fig. 3.4.

2.2.2 Procedure of Experiment 2.

(a) Add 50 μl of secondary standard solution (10 ppm) of Cu to a 50 ml treated seawater. Make up the volume to 100 ml by weighing. The concentration of treated seawater sample is 5

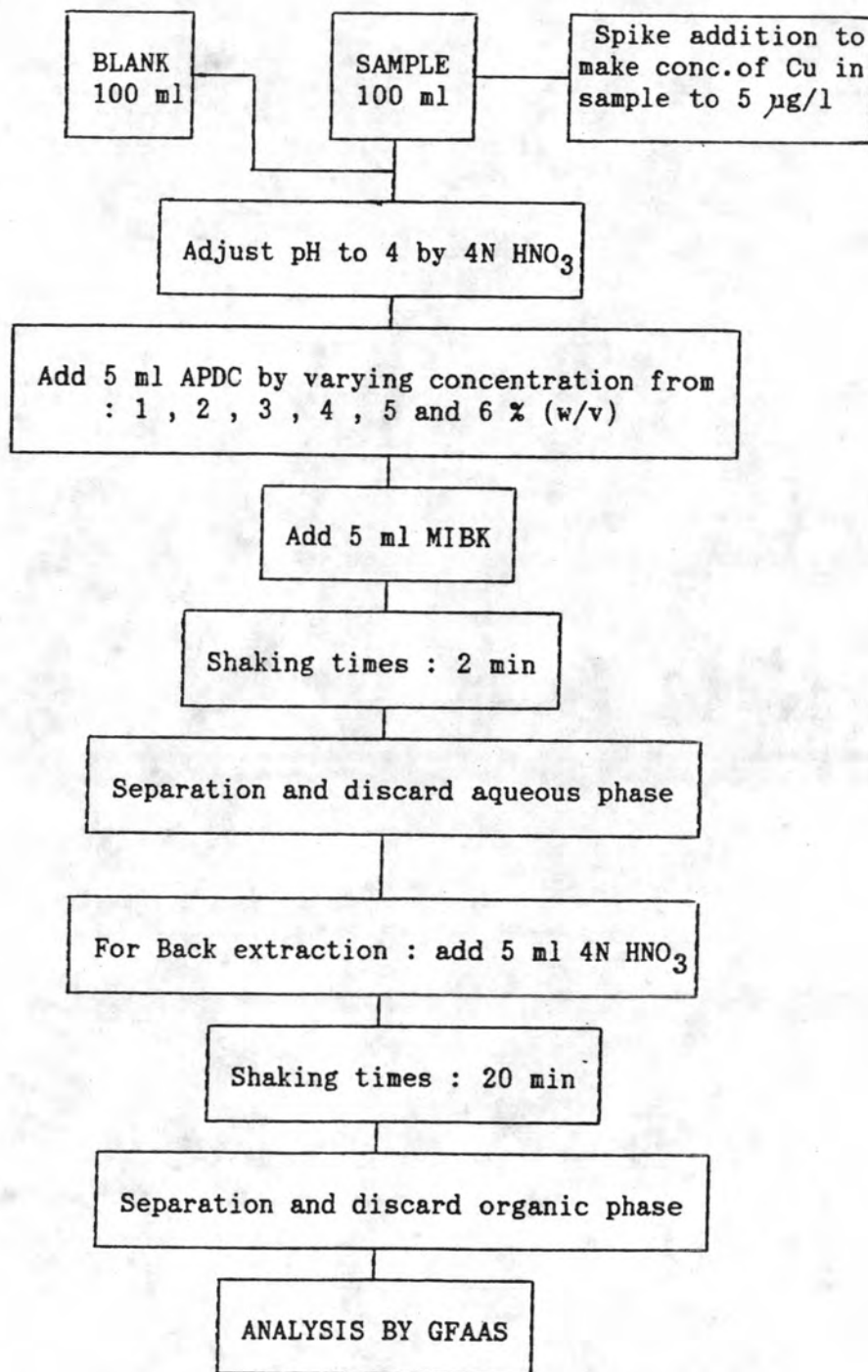


Fig. 3.4 Experimental design for investigation of the appropriate concentration of chelating agent in the first extraction.

ppb.

(b) Adjust pH of the sample to 4 by 4N nitric acid and/or ammonium hydroxide.

(c) Transfer the sample to a 250 ml-separatory funnel.

(d) Add 5 ml of APDC solution into each samples. The concentration of APDC used for chelation is varied from 1, 2, 3, 4, 5 and 6 % (w/v) respectively.

(e) Add 5 ml MIBK.

(f) Shake the sample by a shaker. Times for shaking is 2 min.

(g) Allow the solution in the separatory funnel to separate into two phases for 20 min. Drain off the aqueous layer (bottom layer) with particular care.

(h) For the back extraction, add 5 ml 4N nitric acid into the organic phase which is obtained from step (g).

(i) Shaking the funnel at least 20 min and let it stand for another 20 min.

(j) Drain off the aqueous phase (bottom layer) into a 8 ml-precleaned polyethylene vial and store in the refrigerator before further analysis by GFAAS.

2.2.3 Analysis. Do the same as described in Experiment 1.

2.3 Experiment 3 : Investigation of the Optimum Volume of the Organic Solvent.

2.3.1 Outline of the Method. After adjusting the pH

of a 100 ml treated seawater to 4, an appropriate amount chelating agent which has been verified (ca, 5 ml 2% APDC) is added to the sample. Volume of MIBK added is varied from 2.5 to 15 ml, following by back extraction with 4N nitric acid. Fig. 3.5 shows step in Experiment 3.

2.3.2 Procedure of Experiment 3.

(a) Do the same as Section 2.2.2 step (a) to (c) in Experiment 2.

(b) Add 5 ml 2 % (w/v) APDC into the sample.

(c) The volume of MIBK added into each sample varies from 2.5 , 5.0 , 10.0 and 15.0 ml respectively.

(d) From this step until the last step of this experiment do the same as step (f) to (j) in Experiment 2 , section 2.2.2 .

2.3.3 Analysis. Do the same as described in Experiment 1.

2.4 Experiment 4 : Investigation of the Optimum pH of Aqueous Phase at the First Extraction.

2.4.1 Outline of the Method. The pH of the sample , 100 ml, is adjusted from 2 to 8 by 4N nitric acid and/or ammonium hydroxide before adding chelating agent , APDC , and follows by organic solvent , MIBK, for the first extraction. Back extraction is done by adding 4N nitric acid. The procedure of this experiment is diagrammatically showed in Fig. 3.6.

2.4.2 Procedure of Experiment 4. Do the same as described in Section 2.3.2 step (a) to (d) Experiment 3 except the pH

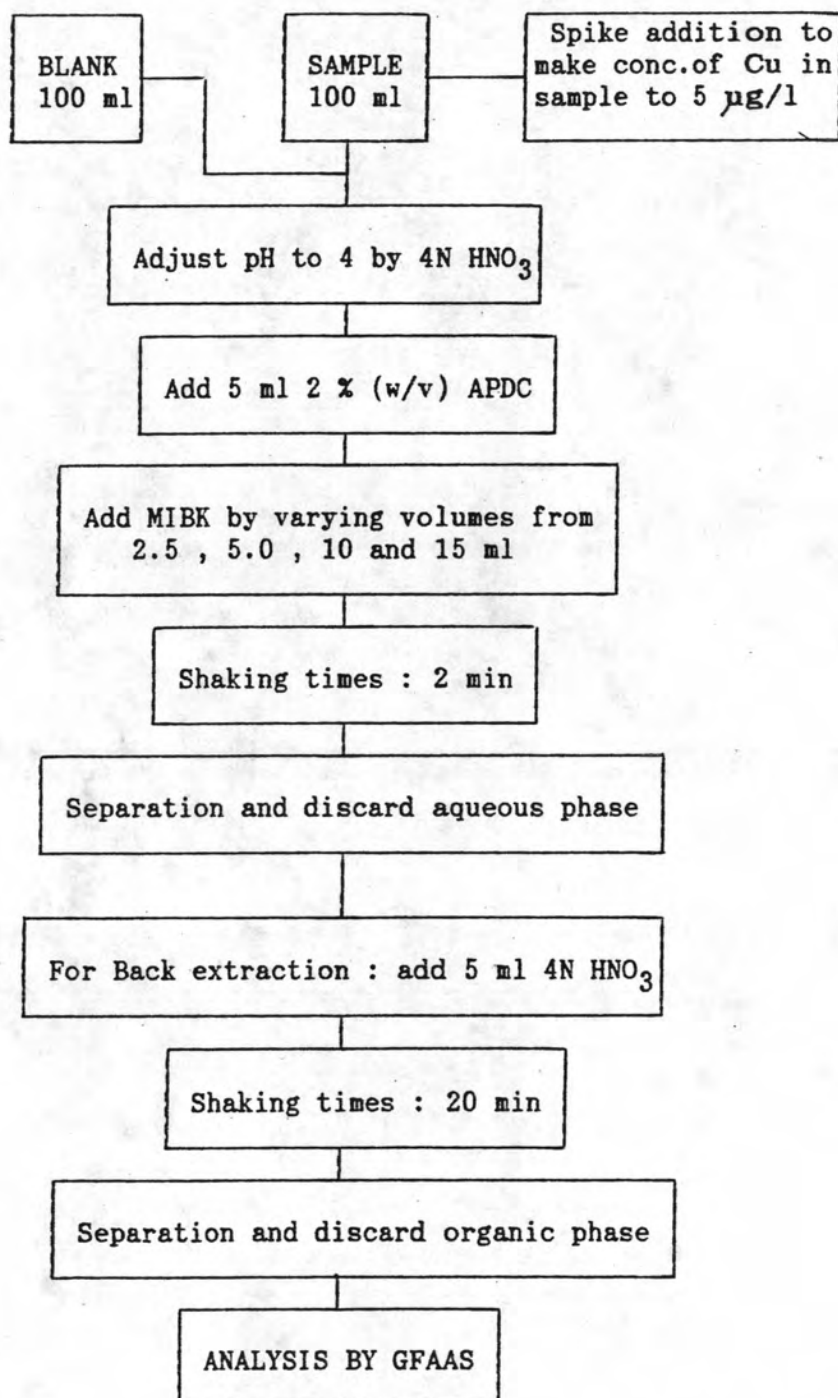


Fig. 3.5 Experimental design for investigation of the optimum volume of the organic solvent of the first extraction.

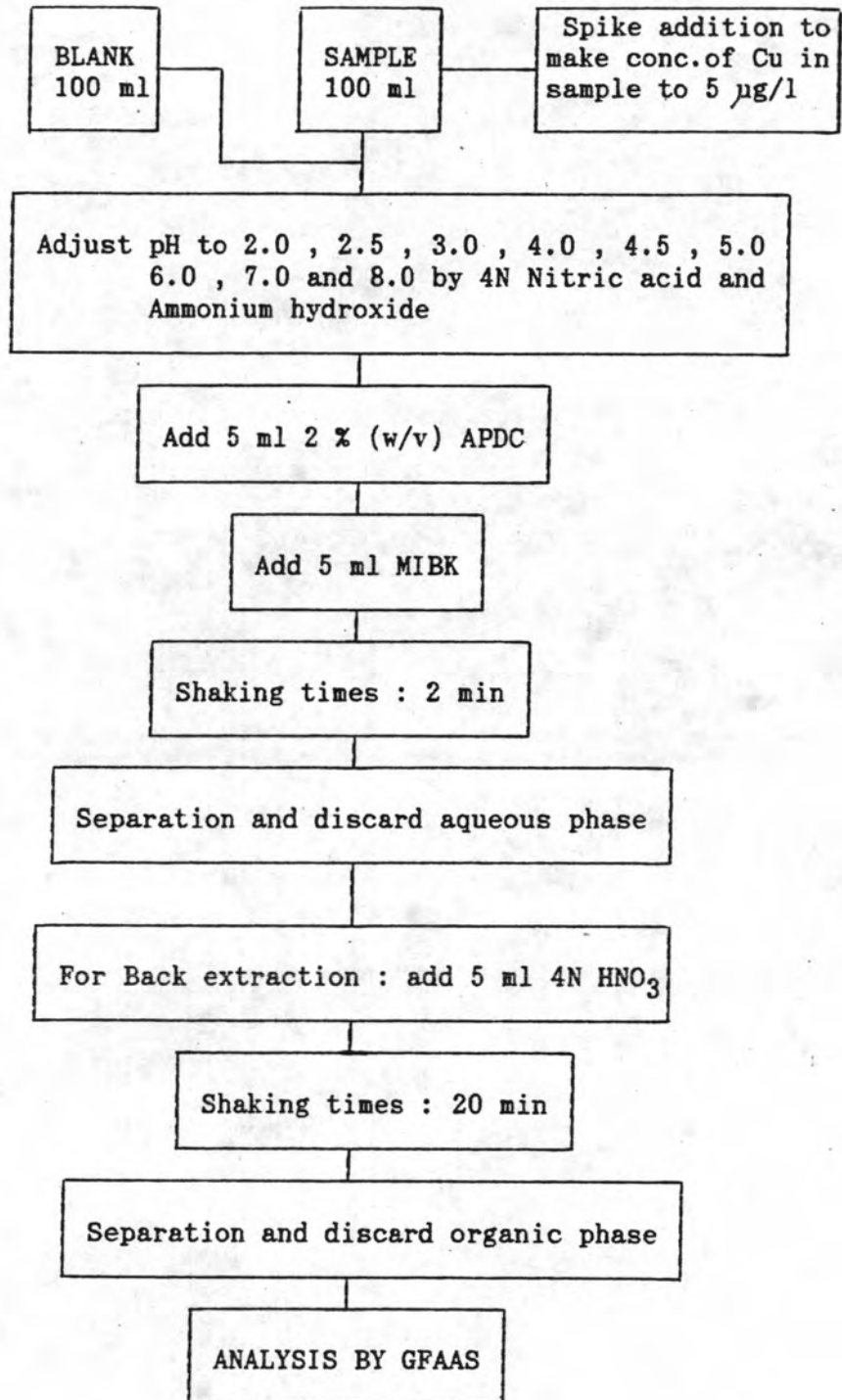


Fig. 3.6 Experimental design for investigation of the optimum pH of the aqueous phase of the first extraction.

of each sample is adjusted to 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0 and 8.0 by 4N nitric acid and/or ammonium hydroxide and the volume of MIBK is 5 ml.

2.5 Experiment 5 : Investigation of the Optimum Shaking Time of Back Extraction.

2.5.1 Outline of Method. This experiment is designed in order to determine the optimum shaking time for back extraction after finishing the first extraction. Shaking time which is used in this experiment is varied from 30 s to 20 min.

2.5.2 Procedure of Experiment 5.

(a) Do the same as described in Section 2.4.2 step (a) to (c) in Experiment 4 except the pH of aqueous is adjusted to 2.8 and the shaking time of each sample for back extraction is varied from 30 s, 1, 2, 4, 8, 10 and 20 min respectively.

(b) After shaking, allow it to stand at least 20 min for phase separation.

(c) Drain off the aqueous phase, nitric acid, with particular care and collect it in a precleaned polyethylene vial, 8 ml, and keep it in the refrigerator for further analysis by GFAAS.

2.6 Experiment 6 : Investigation of the Optimum Volume of Nitric Acid for Back Extraction.

2.6.1 Outline of Method. After the pH of sample is adjusted to pH 2.8 by nitric acid before adding 2 % APDC. MIBK is added into the sample followed by shaking. Allow for phase separation, then drain off the aqueous phase. For back extraction, add nitric

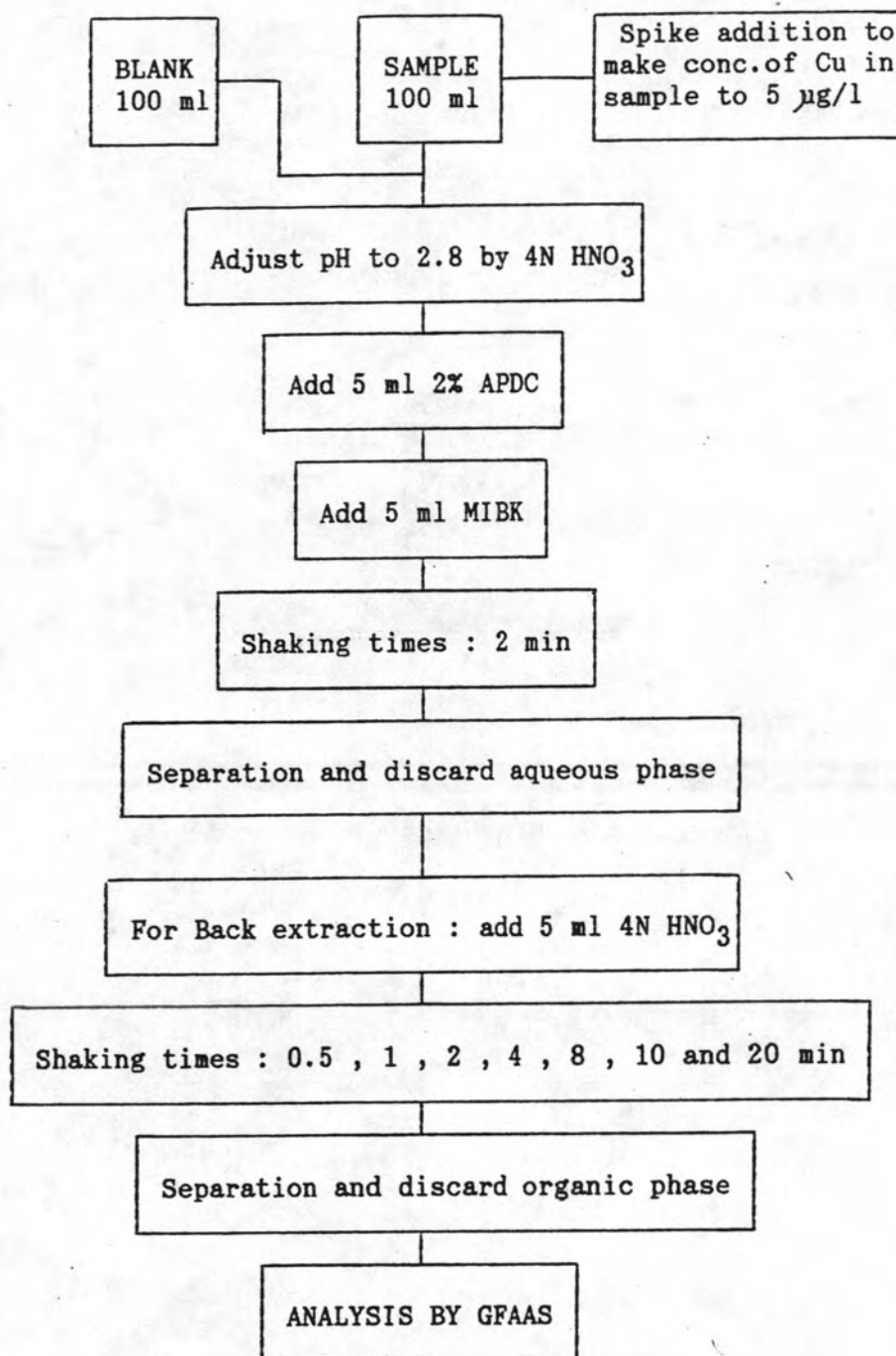


Fig. 3.7 Experimental design for investigation of the optimum shaking time of back extraction.

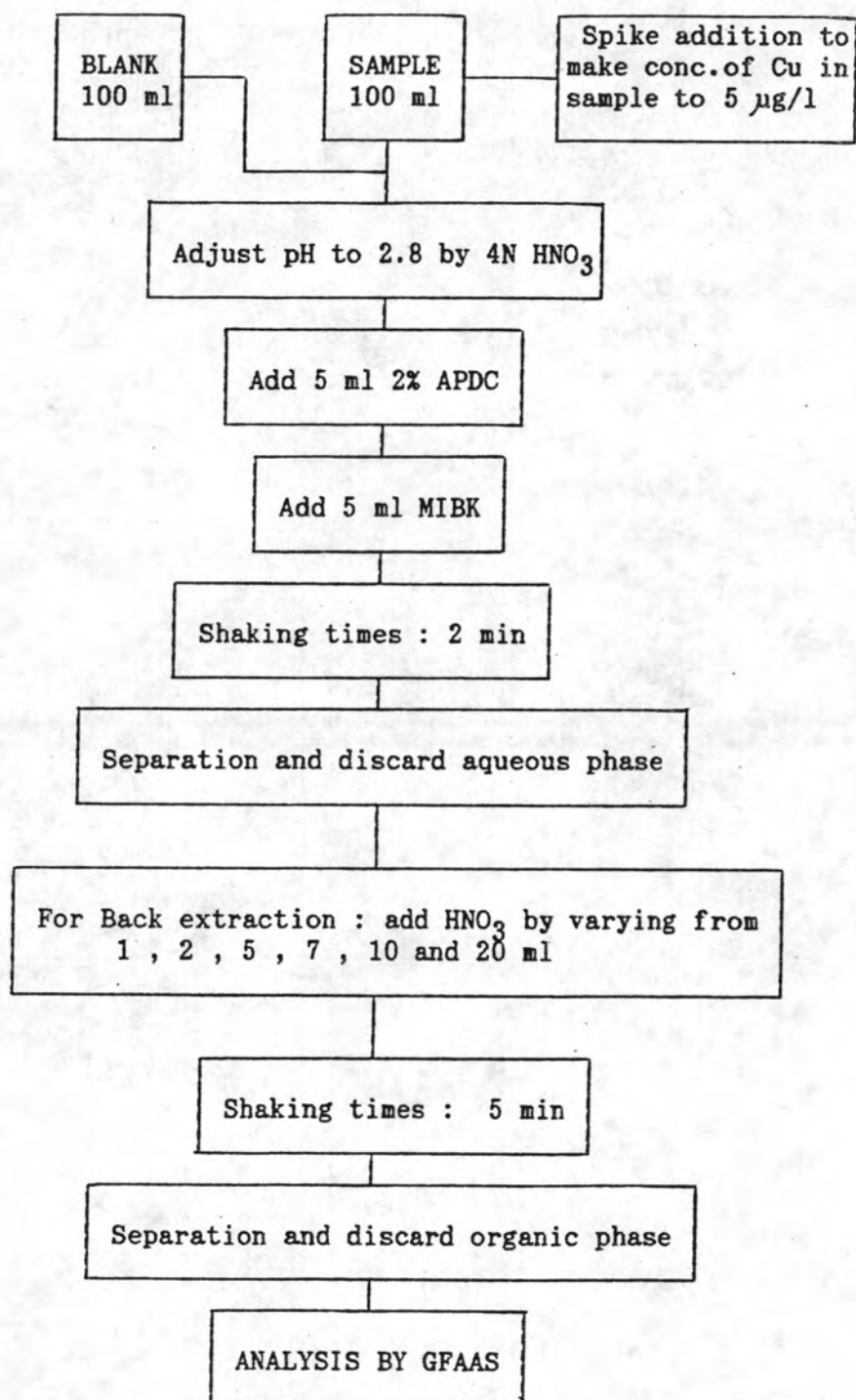


Fig. 3.8 Experimental design for investigation of the optimum volume of nitric acid for back extraction.

acid by varying its volume from 1 to 20 ml in order to investigate the appropriate volume of 4N nitric acid. Then shaking, separation and collect the aqueous phase for further analysis by GFAAS.

2.6.2 Procedure of Experiment 6. Do the same as described in Section 2.5.2 in Experiment 5 except the shaking time for back extraction is 5 min and the volume of 4N nitric acid is varied from 1, 2, 5, 7, 10 and 20 ml respectively.

2.7 Experiment 7 : Investigation of the Optimum pH for the First Extraction of Ni.

2.7.1 Outline of Method. This experiment is performed by using the optimum conditions that have been previously used for determining the Cu concentration except at the step of adjusting pH of the aqueous prior to extraction.

2.7.2 Procedure of Experiment 7. Do the same as described in Section 2.6.2 (Experiment 6) except the analyte is Ni instead of Cu and the pH of each sample is varied from 2, 3, 4, 5, 6, 7 and 8 by using 4N nitric acid and/or ammonium hydroxide solution.

2.8 Experiment 8 : Investigation of the Optimum pH for the First Extraction of Pb.

2.8.1 Outline of Method. This experiment follows the same procedure as Experiment 7 except the interested metal is Pb instead of Ni.

2.8.2 Procedure of Experiment 8 is the same as described in Section 2.7.2, Experiment 7 but the analyte is changed

from Ni to Pb and the instrumental operating conditions of GFAAS for measuring the absorbance are changed accordingly.

2.9 Experiment 9 : Investigation of the Optimum Shaking Time for the First Extraction of Zn. Because Zn formed a weakly complex with APDC, therefore, this condition for Zn is reinvestigated in order to assure the optimum shaking time for the first extraction of Zn.

2.9.1 Outline of Method. The same as described in Experiment 7 except the analyte is Zn instead of Ni, the pH of the aqueous is adjusted to 4 and the shaking time of each sample is varied from 2 to 20 min.

2.9.2 Procedure of Experiment 9.

(a) Add 50 μ l of a secondary standard solution of Zn (10 ppm) to 50 ml treated seawater. Make volume to 100 ml by weighing.

(b) Adjust pH of the sample to 4.

(c) Do the same as described in Section 2.7.2 Experiment 7 except the shaking time for the first extraction is varied from 2, 4, 8, 10 and 20 min and the instrument, which is used for metal concentration analysis, is changed from GFAAS to Flame AAS.

2.10 Experiment 10 : Investigation of the Optimum pH for the First Extraction of Zn.

2.10.1 Outline of Method. The same as described in Section 2.9.2, Experiment 9.

2.10.2 Procedure of Experiment 10. The same as described in Section 2.9.2, Experiment 9 except the pH adjustment step in which the pH is varied from 2 to 8 and shaking time for solvent extraction is 4 min.

2.11 Experiment 11 : Investigation of Multielemental Solvent Extraction and Stability of Metals in the Acid Extract. The purpose of this experiment is to extract Cu, Ni, Pb and Zn in single extraction by using the previously verified conditions in order to test the extractability of an improved solvent extraction method and to investigate the stability of the interested metals in the acid extract which are obtained from back extraction of the APDC-MIBK Extraction System.

2.11.1 Outline of Method. The Cu, Ni, Pb and Zn in treated seawater will be extracted by using APDC as chelate and MIBK as organic solvent, followed by back extraction with 4N nitric acid and analyses by GFAAS and Flame AAS. Each experimental conditions, used in this experiment is optimized and verified in previous experiments (Fig. 3.9).

2.11.2 Procedure of Experiment 11.

(a) Add 50 μ l of Cu, Ni, Pb and Zn secondary standard solutions (10 ppm) to 50 ml of treated seawater. Make volume to 100 ml with treated seawater by weighing.

(b) Adjust pH to 5 by nitric acid and/or ammonium hydroxide solution and transfer to 250 ml-separatory funnel.

(c) Add 5 ml 2 % (w/v) APDC.

(d) Add 5 ml MIBK.

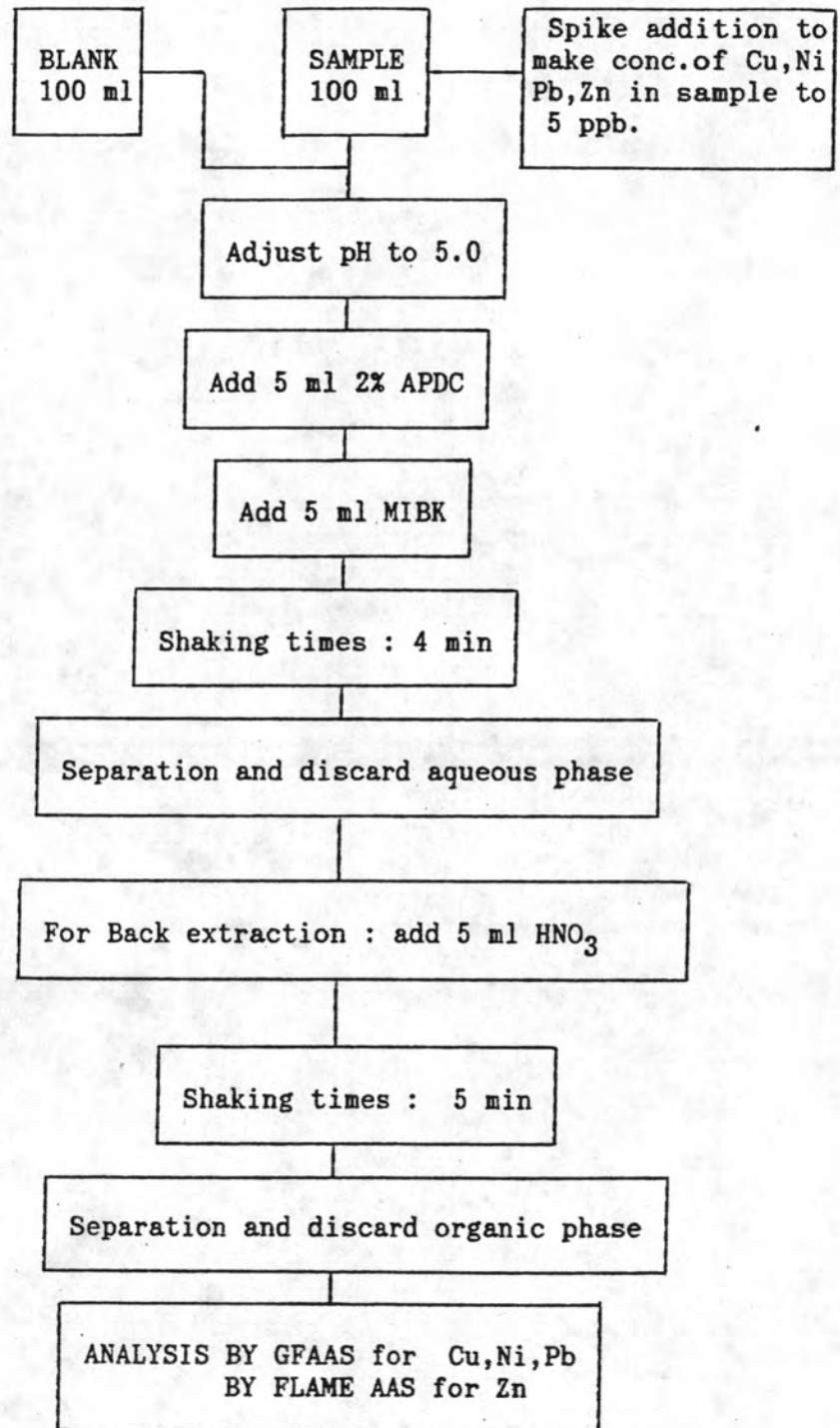


Fig. 3.9 Flow chart of Improved Solvent Extraction followed by Back Extraction with Nitric Acid.

(e) Shaking for 4 min and let the sample stand for phase separation and drain off the lower layer.

(f) Add 5 ml 4N nitric acid to the organic phase in the separatory funnel, shaking for 5 min and let it stand for phase separation.

(g) Collect the lower layer in a precleaned 8 ml-polyethylene vial and keep it in the refrigerator until further analysis.

2.12 Experiment 12 : Investigation the Appropriated pH of the Dithizone-Chloroform Solvent Extraction System. This experiment is designed in order to reinvestigated the appropriated pH for the dithizone-chloroform solvent extraction system.

2.12.1 Outline of Method. The extraction procedure follows the procedure in Section 2.11.2 Experiment 11 except in step (b) the pH adjustment vary from 1 to 8 and dithizone and chloroform are used as chelating agent and organic solvent respectively. The concentration and volume of both reagents are the same as described in Smith and Windom (76).

2.12.3 Procedure of Experiment 12. The same as described in Section 2.11.2 (Experiment 11) except chelating agent and organic solvent which use in this experiment are 0.06 % (w/v) dithizone in chloroform and purified chloroform and pH of the sample vary from 1 to 8.

2.13 Experiment 13 : Validation the Improved Solvent Extraction Method. The purpose of this experiment is to validate the improved solvent extraction technique by using the certified reference

materials from the National Research Council of Canada.

2.13.1 Procedure of Experiment 13. The same as described in Section 2.11.2, Experiment 11 but using the Certified Reference Material NASS-1 (Seawater), CASS-1 (Nearshore seawater) and SLRS-1 (Riverine water) as samples.