CHAPTER 2

EXPERIMENTAL

2.1 Instrumentations

The chromatographic system consisted of a Waters (Milford, MA, USA) Model 600 pump, a Rheodyne (Cotati, CA, USA) model 7725 stainless steel injector (20 μl loop), a UV-Vis spectrophotometric detector (Jasco, Tokyo, Japan) operated at 585 nm. A Purospher® STAR C_{18} reversed-phase column (75 mm x 4 mm I.D, particle size 3 μm, Merck) was used as an analytical column, and was fitted with a NovaPak C_{18} guard column (Merck). Flow rate of the mobile phase was maintained at 1.0 ml min^{-1} and column temperature was kept at 30 °C. Detection was accomplished by absorbance measurements at 585 nm.

2.2 Chemicals

Throughout this study all water used was distilled and then deionised using a UHQ system (Elga, Bucks, England). HPLC grade methanol $\geq$ 99.9% purity was obtained from Merck (Darmstadt, Germany).

ICP/DCP standard solution of Zr(IV) (9,908 ppm in 4.6 wt% HCl) and hafnium(IV) sulfate 99.9% purity were obtained from Aldrich (Steinheim, Germany). Solution of {2-(5-bromo-2-pyridylazo)-5-[N-propyl-N-(3-sulfopropyl) amino] phenol, disodiumsalt, dihydrate}(PAPS) (Dojindo Laboratories, Kumamoto, Japan) were prepared freshly in water before used. AR grade of sodium fluoride (NaF) 99% purity (Ajax Finechem, Auckland, New zealand), tetrabutylammoniumbromide (TBABr) 99% purity (Aldrich, Steinheim, Germany) and sodium hydroxide (NaOH) $\geq$ 99% purity (Merck, Darmstadt, Germany) were also used. Reagents used in the preparation of geological samples were as follows: AR grade of nitric acid 65% by wt. (HNO_3) and hydrochloric acid 37% by wt. (HCl) were obtained from Merck, Darmstadt, Germany.

Granite rock samples (KP112) were obtained from Dr. Parinya Puthapiban, Department of Geology, Mahidol University, Kanchanaburi.

2.3 Preparation of solutions

2.3.1 Preparation of hafnium (IV) solution

A stock solution of hafnium 1,000 mg L^{-1} was prepared by dissolving 0.0519 g of hafnium (IV) sulfate in 5% HCl and sonicated for about 20 min before transferring the solution to a 25 ml volumetric flask and filling up to the mark with 5% HCl.

2.3.2 Preparation of fluoride solution

A stock solution of fluoride 60 mM was prepared by dissolving 0.0630 g of sodium fluoride in deionized water and make volume to 25 ml in volumetric flask.

2.3.3 Preparation of Br-PAPS solution

A solution of Br-PAPS 5 mM was freshly prepared by dissolving 0.0134 g of Br-PAPS in deionized water and make volume to 5 ml in volumetric flask.

2.3.4 Preparation of mobile phases

Mobile phases were prepared by dissolving TBABr, fluoride and acetic acid in water, adding the required volume to the desired amount of methanol, adjusting the pH with sodium hydroxide and then diluting with water in order to adjust the final methanol concentration to the desired level. The optimal mobile phase composition was methanol-water (65-35 v/v) containing 5 mM TBABr, 0.05 mM fluoride and 10 mM acetic acid at pH 5.

2.4 Sample preparation

Triplicate 0.0500 g powdered rock samples were prepared in Teflon vials. A few drops of water were added to wet the powdered rock. After carefully adding 3 ml of concentrated HNO_3 and 1 ml of concentrated HCl into the each vial, the lids were screwed on and the samples were digested on a hotplate at 70 ^{o}C. The lids were opened carefully sometimes in order to release the bubble gas and waited until the solutions were clear and sample solutions were then evaporated to incipient dryness. Then 2 ml water was added to the residues and continued heating about 30 minutes. Leave the solution cool down, then transferred the solution to 25 ml a volumetric flask and filled to the mark with water. Finally, the sample solutions were filtered through a 0.45 µm membrane before HPLC analysis.

2.5 Pre-column complex formation

The following techniques were used for pre-column formation of the ternary complex for standard solutions and for sample solutions:

2.5.1 Pre-column formation for standard solutions

In the case of standard solutions, fluoride solution (0.2 ml, 1 mM) was placed into a plastic container and then 0.2 ml of freshly prepared PAPS solution (5 mM) was added, followed by a standard solution of Zr(IV) and Hf(IV). The pH of the solution was adjusted to pH 3.7 with sodium hydroxide and the solution was transferred to a 10 ml volumetric flask and filled to the mark with water.

2.5.2 Pre-column formation for sample solutions

The rock samples contained many other metals that were capable of forming complexes with PAPS. Therefore, a higher concentration of PAPS was required to used in real sample. The preparation was as following: the digested rock sample (0.25 ml) was added to 0.2 ml of fluoride solution (1 mM), followed by 0.5 ml of freshly prepared PAPS solution (5 mM) in a plastic container. The solution was adjusted to pH 3.7 with sodium hydroxide and transferred to a 10 ml volumetric flask and filled to the mark with water.