CHAPTER 3

RESULTS AND DISCUSSION

3.1 Formation of ternary complexes and their absorption spectra

PAPS can form colored complexes with many metal ions, including Zr(IV) and Hf(IV). In this work, blue ternary complexes of Zr(IV) and Hf(IV) were formed with PAPS and fluoride throughout the pH range of 3.5-5. Absorbance of Zr(IV) and Hf(IV) complexes measured at various pH values were similar with both complexes showing strong absorbance at pH 3.7. The wavelength of maximum absorbance was at 585 nm for both Zr(IV) and Hf(IV) complexes. Figure 3.1 shows the spectra of the Zr(IV) and Hf(IV) complexes at pH 3.7.

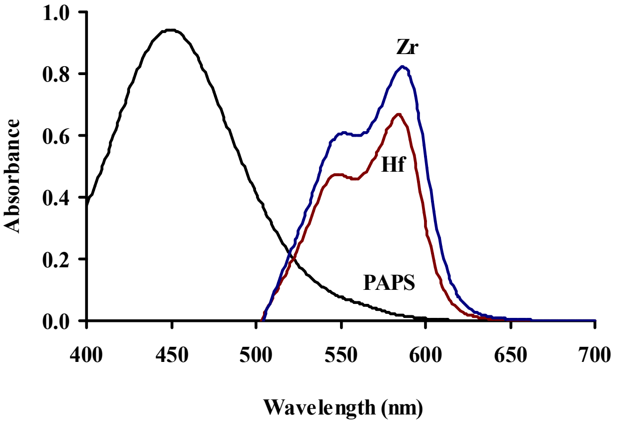

Figure 3.1 Absorption spectra of PAPS, Zr-PAPS-fluoride (0.5 mg L^{-1}) and Hf-PAPS-fluoride (2 mg L^{-1}) at pH 3.7. Conditions [PAPS] = 0.1 mM, [fluoride] = 0.02 mM.

3.2 Optimization of the pre-column complex formation

The parameters used for the pre-column formation of the ternary complex were investigated in order to obtain the highest sensitivity and reproducibility. A pH of 3.7 had been selected previously on the basis of pH effects on the absorbance of the complex. The optimal concentration of PAPS was investigated over the range of 0.01-0.15 mM for a standard of Zr(IV) (0.1 mg L^{-1}) and Hf(IV) (0.4 mg L^{-1}) in 10 mM acetic acid. The highest of peak height for both Zr(IV) and Hf(IV) were obtained with a final concentration of 0.1 mM PAPS (Figure 3.2). Since some of the metal ions present in the rock sample can also form complexes with PAPS, the optimum concentration of PAPS was also studied for rock samples. To compare both peak height and peak area of Zr(IV) and Hf(IV) complexes in rock samples, over the range of 0.1-0.3 mM PAPS, the results showed that 0.25 mM PAPS gave the best performance for rock sample (KP112). The optimum concentration of fluoride was also investigated over the range of 0.01-0.04 mM, 0.02 mM fluoride offered the best results for Zr(V) and Hf(IV) complexes (Figure 3.3) in both standard and rock sample solution.

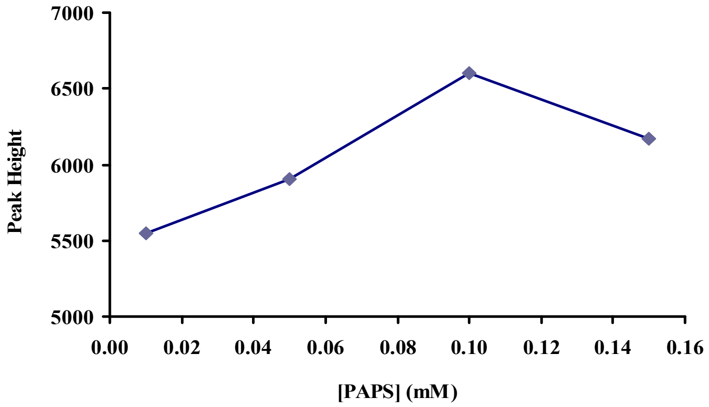

Figure 3.2 Effect of PAPS concentration on peak height of Zr(IV) (0.1 mg L^{-1})

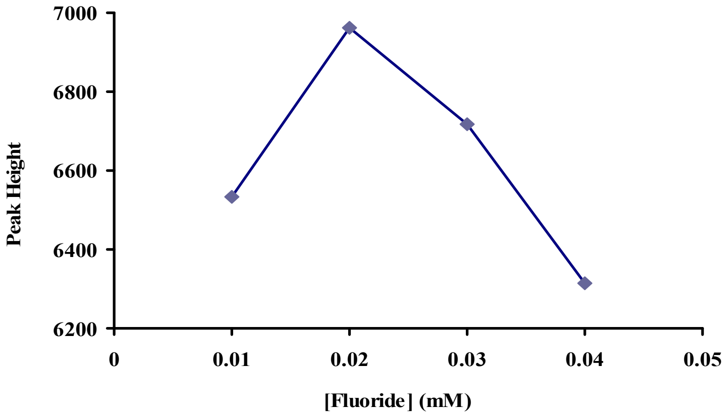

Figure 3.3 Effect of fluoride concentration on peak height of Zr(IV) (0.1 mg L^{-1})

3.3 Separation parameters

The amount of methanol in the mobile phase was found to exert a very strong effect on retention time, and the effect of methanol was observed over the range 60-80% v/v methanol. The retention time being excessively long in mobile phase that contained less than 65% methanol. However, peak area increased markedly at higher methanol concentrations. Therefore, the optimum concentration of methanol was selected to be 65%.

When using a mobile phase containing only an acetate buffer in a methanol-water (65:35 %v/v), the Zr(IV) and Hf(IV) complexes were unstable and co-eluted. After adding fluoride into mobile phase Zr(IV) and Hf(IV) complexes were stable. To increase the sensitivity of both complexes, the concentration of fluoride over the range of 0.01-0.2 mM were investigated. The results showed 0.05 mM fluoride offered the highest peak height for Zr(IV) and Hf(IV) ternary complexes. However, the composition of mobile phase containing methanol (65:35 %v/v) and 0.05 mM fluoride in 10 mM acetate buffer provided co-eluted peaks of Zr(IV) and Hf(IV) ternary complexes (Figure 3.4).

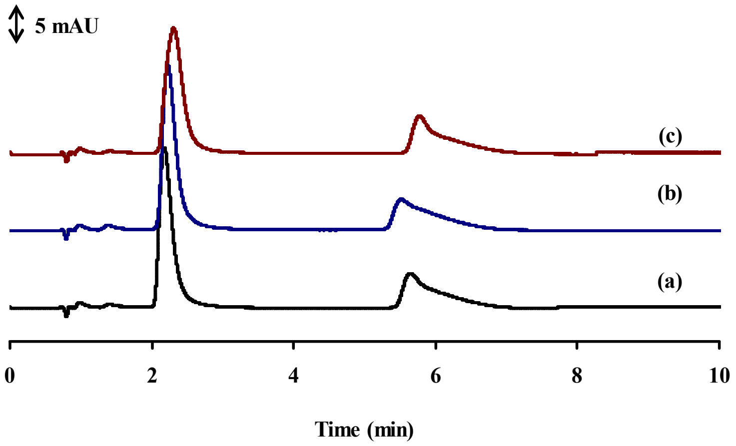

Figure 3.4 Chromatogram of (a) Hf(IV)-PAPS-fluoride (0.4 mg L^{-1}), (b) Zr(IV)-PAPS-fluoride (0.1 mg L^{-1}) and (c) standard mixture of Zr(IV) (0.05 mg L^{-1}) and Hf(IV) (0.2 mg L^{-1}). Mobile phase was methanol-water 65% v/v containing 0.05 mM fluoride and 10 mM acetate buffer at pH 5, analytical column 75 mm x 4 mm I.D., 3 µm, Purospher C_{18}; flow rate 1 ml/min; temperature 30 °C; detection at 585 nm.

To solve this problem, tetrabutylammonium bromide (TBABr) was added into mobile phase. The addition of cationic tetrabutylammonium ions (TBA^+) into the mobile phase caused an increase in retention time of both complexes, which indicated that the complexes were anionic. TBA^+ can coat on the surface of C_{18} stationary phase and also form ion-pair with Zr(IV) and Hf(IV) complexes. To find optimal concentration of TBA^+ in mobile phase, the TBA^+ over the range 0-8 mM was investigated. The graph plot between capacity factor (k′) and concentration of TBABr was shown in Figure 3.5.

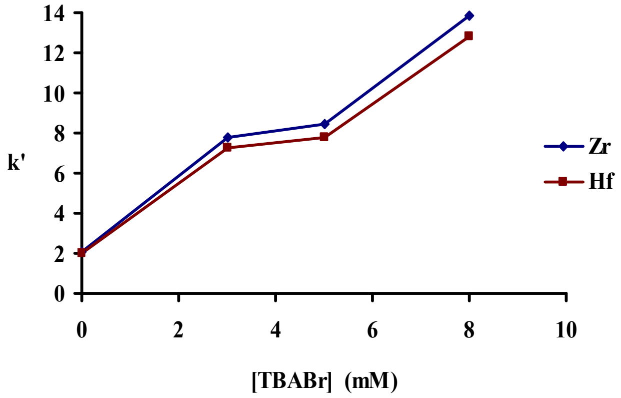

Figure 3.5 Effect of TBABr concentration on k′ of Zr-PAPS-fluoride and Hf-PAPS-fluoride. Other conditions are the same as in Figure 3.4.

Figure 3.5 showed the concentration of 5 mM TBA^+ provided good separation of Zr(IV) and Hf(IV) complexes, but both complexes still showed co-eluted peak about 40%. Figure 3.6 showed the chromatogram of standard of Zr(IV) and Hf(IV) complexes under the optimal conditions.

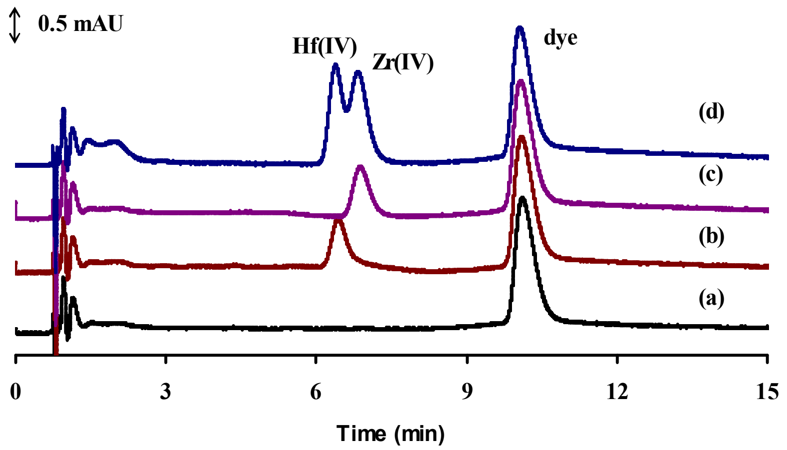

Figure 3.6 Chromatogram of (a) blank, (b) Hf(IV)-PAPS-fluoride (20 µg L^{-1}), (c) Zr(IV)-PAPS-fluoride (5 µg L^{-1}) and (d) standard mixture of Zr(IV) (10 µg L^{-1}) and Hf(IV) (40 µg L^{-1}). Mobile phase was methanol-water 65% v/v containing 0.05 mM fluoride, 5 mM TBABr and 10 mM acetate buffer at pH 5. Other conditions are the same as in Figure 3.4.

The pH of the mobile phase had a significant effect on the peak area and peak height of both complexes. Therefore, pH over the range 4-5.5 was investigated. The result showed with increased pH giving increased peak area and peak height. The optimum pH for both Zr(IV) and Hf(IV) was selected at pH 5 because of giving peak symmetry and good baseline (Figure 3.7).

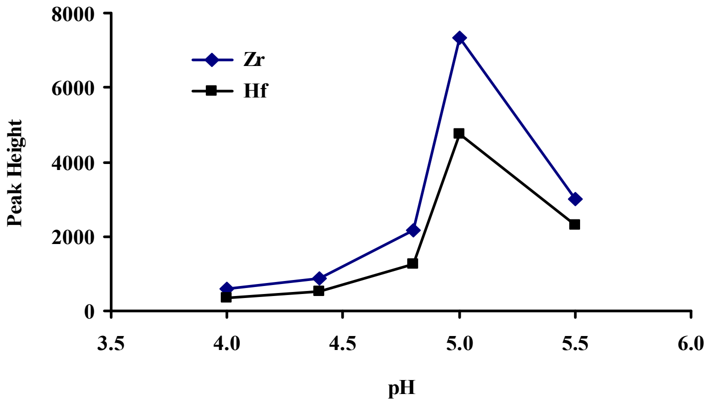

Figure 3.7 Effect of pH on peak height of Zr(IV)-PAPS-fluoride (50 µg L^{-1}) and Hf(IV)-PAPS-fluoride (200 µg L^{-1}). Other conditions are the same as in Figure 3.4.

3.4 Analytical performance parameters

Analytical performance parameters were determined under the optimized conditions: analytical column RP-C_{18} (75 mm x 4 mm I.D., 3 µm), mobile phase was methanol-water 65% v/v containing 0.05 mM fluoride, 5 mM TBABr and 10 mM acetate buffer at pH 5.

Linearity was observed for Zr(IV) and Hf(IV) standard solution using peak area. External standard calibration curves exhibited good linearity. Table 3.1 summarizes limits of detection (LODs) (determined by a signal to noise ratio of 3) and correlation coefficients for the above metals. The % relative standard deviation (%RSD) was studied to obtain the method precision. The %RSD was established by four replicate injection of each standard solution of Zr(IV) (20 µg L^{-1}) and Hf(IV) (50 µg L^{-1}) (Table 3.2).

Table 3.1

Detection limits and calibration graphs for determination of Zr(IV) and Hf(IV)

Metal	LOD (µg L^{-1})	Linearity range (µg L^{-1})	Regression equation	Correlation coefficient
Zr(IV)	0.38	0.5 - 200	y = 3181.2x-1238	0.9996
Hf(IV)	1.53	2 - 200	y = 570.63x+2646.9	0.9993

Table 3.2

% RSD for retention time, peak area and peak height of Zr(IV) and Hf(IV)

Metal	%RSD (n = 4)		
	Retention time	Peak Area	Peak Height
Zr(IV)	0.03	0.75	1.37
Hf(IV)	0.06	1.01	1.70

3.5 Determination of Zr(IV) and Hf(IV) in rock sample

Three replicates of a granite rock sample (KP112) was used for determination of Zr(IV) and Hf(IV). The analysis of this sample under the optimized conditions showed that simultaneous determination of Zr(IV) and Hf(IV) was possible. Chromatograms obtained with and without the standard addition of Zr(IV) and Hf(IV) are shown in Figure 3.8.

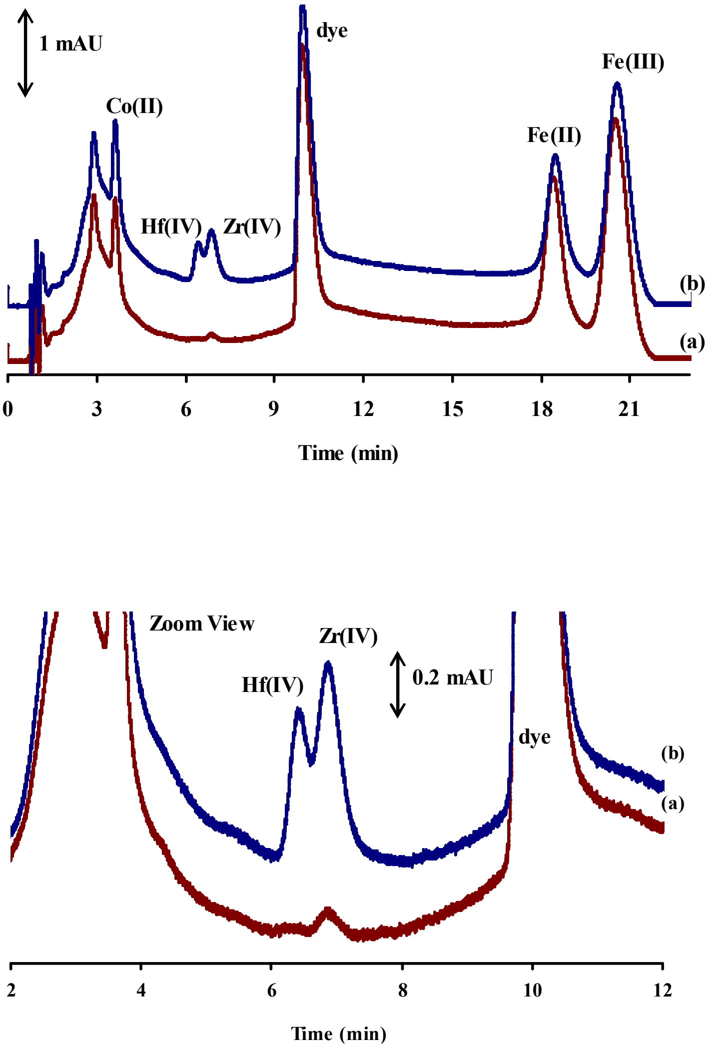

Figure 3.8 Chromatogram of (a) granite rock sample KP112 and (b) granite rock sample KP112 spiked with 5 µg L^{-1} Zr(IV) and 20 µg L^{-1} Hf(IV). Top: complete view. Bottom: zoom view. The sample contained [PAPS] = 0.25 mM, [fluoride] = 0.02 mM. Other conditions are the same as Figure 3.6.

It can be seen that clear peaks were evident for both Zr(IV) and Hf(IV). To confirm Zr(IV) and Hf(IV) ternary complexes peak, the spike of standard Zr(IV) and Hf(IV) were added into rock sample (Figure 3.4b). To determine the amount of Zr(IV) and Hf(IV) in rock sample (KP112), an external standard method was used. The result for Zr(IV) content was 14.88±0.93 μg/g in solid sample. For Hf(IV), the concentration in KP112 was below the detection limit. This illustrates the limitations of the developed method.