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A Study of the Determination of Rubidium in Human Erythrocytes by Atomic Absorption Spectrophotometry

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ABSTRACT

In the determination of Rb by atomic absorption spectrophotometry (AAS), potassium or cesium are recommended to be added to interfere with the ionization of Rb but the amount of the ion to be added is something vague. And as far as the reports concerning the determination of Rb in biological fluids especially in human erythrocytes by AAS were surveyed, only a few papers were found to be mentioned it but the method were different from each other as follows; one report recommended to add only sodium to increase the sensitivity, another suggested to add potassium only, but the other recommended to add both cations for the same purpose.

The purpose of the present study is to elucidate a rapid and accurate method to determine Rb in human erythrocytes.

When erythrocytes were diluted 1:50 with potassium at the final concentration of 10,000 ppm, following results were obtained.

1) Sensitivity of absorbance increased about a threefold compared to that with no addition.
2) Concentrations could be determined within the range from 0 to 60 ppm.
3) Influences of coexisting cations in erythrocytes such as potassium, sodium and so on within the physiological range could be negligible.
4) Influences of acids, such as nitric acid and hydrochloric acid, used during preparation or determination could be also negligible.
5) Preparations of erythrocytes such as wet-ashing with nitric acid or deproteinization with trichloroacetic acid (TCA) were appeared to be needless.

Recently Rb, one of the alkali metals, has been reported to have neurochemically, neurophysiologically and behaviourally opposite properties to those of lithium salts[1,3,5,7,9,16,19,20], and thus rubidium salts have been suggested to be a potential antidepressant[6,11,12]. But in the application of Rb to the affective disorders the necessity of caution has been suggested[2] because of its long biological half life. To avoid the intoxication of Rb, blood monitoring is required for clinical application. From the ethical point of view the author used erythrocytes as a model of nerve cells. Rb in erythrocytes was determined by atomic absorption spectrophotometry (AAS).

In general, at the determination of Rb by AAS, other alkali metals such as potassium or cesium are recommended to be added to interfere with the ionization of Rb. The reports concerning the determination of Rb in erythrocytes were surveyed, but only a few papers were found to have mentioned details. Wood[18] suggested that samples such as plasma, erythro-

cytes and urine should be diluted in saline to give 10,000 ppm Na, but Del Vecchio[2] recommended to add potassium at the final concentration of 1,000 ppm on the determination of Rb in blood and plasma. On the other hand, Lieberman[9] suggested to dilute samples with the solution containing 25 meq/liter of sodium and 50 meq/liter of potassium, and Sutter[17] reported that the marked enhancement effect of ions is necessitated the preparation of standard which contained equivalent amount of sodium and potassium. Thus the methods mentioned above were different from each other and the method of determination of Rb in erythrocytes has not been standardized yet.

The aim of the present study is to elucidate a rapid and accurate method to measure the concentration of Rb in human erythrocytes by means of AAS.

MATERIALS AND METHOD

Adequate volume of venous blood was drawn into a plastic syringe containing heparin, and centrifuged at 4°C, 3,000 rpm for 5 min, then the plasma and buffy coat were removed and discarded. Then the sample was washed three times with 100 mM $MgCl_2$ by centrifugation under the same condition mentioned above. In principle, as the preparation of erythrocytes for the determination of Rb, 1 ml of packed erythrocytes was wet-ashed with 5 ml of concentrated nitric acid and 1 ml of sulfuric acid in a Kjeldahl flask of a wet-ashing equipment model J-2320 (Top Co., Ltd. Japan).

Then the ashed solution was diluted 1:50 in the solution examined below for the determination. A Nippon-Jarrel-Ash Model AA-780 atomic absorption spectrophotometry (AAS) with a laminar flow burner was used. Samples were atmized in an air-acetylene flow (Air: 3.0 kg/cm^2, 8 L/min. Acetylene: 1.0 kg/cm^2, 2 L/min). The Rb-neon hollow cathode lamp (WL-22824A; Westinghouse Co., Ltd. USA) was operated at 20 mA using the 7800.2 Å Rb resonance line. The lamp was warmed up for at least 30 min before the determination. The concentration switch was set at the high concentration position. The contents were determined with standard curve method. Rb standard solution was made from Rb standard stock solution (1,000 ppm in 0.1 N HCl, Kanto Chemical Ltd. Japan). Acids used in the present study were all at super special grade.

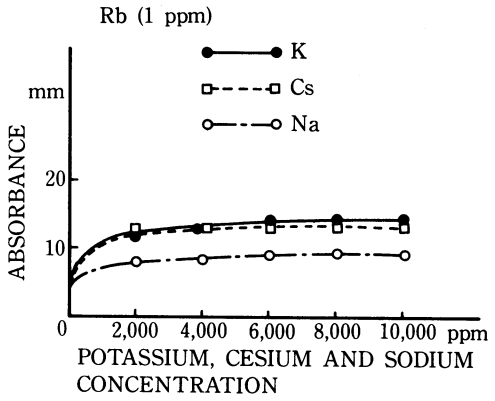

Fig. 1. Influence of potassium, cesium and sodium on rubidium (1 ppm) absorbance

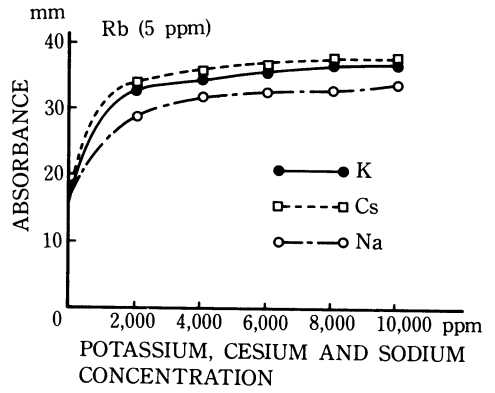

Fig. 2. Influence of potassium, cesium and sodium on rubidium (5 ppm) absorbance

RESULTS

Influences with the Ionization

In the method of determination of Rb by AAS, it is recommended that other alkali metals such as potassium, sodium or cesium are added to interfere with the ionization of Rb in order to gain increased sensitivity. Figs. 1—3 showed the results of comparisons of the gain in sensitivity obtained by adding potassium, sodium or cesium. At a Rb concentration of either 1,5 or 10

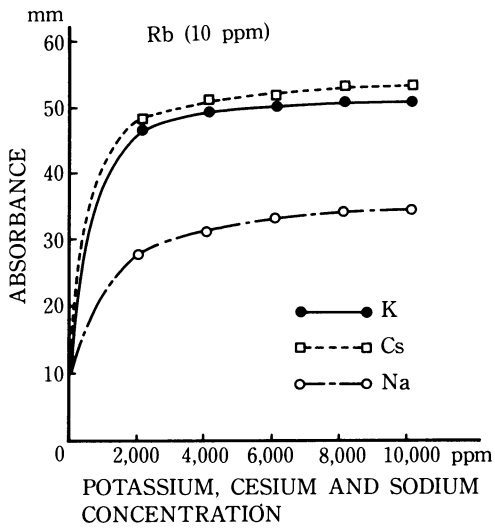

Fig. 3. Influence of potassium, cesium and potassium on rubidium (10 ppm) absorbance

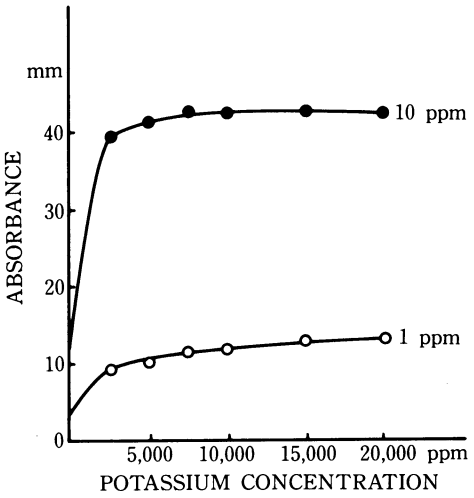

Fig. 4. Influence of potassium on rubidium (1, 10 ppm) absorbance

ppm, potassium and cesium showed approximately the same gain, although sodium showed about a 60% gain compared with the former two cations. As potassium chloride was more easily obtainable than cesium chloride, potassium was used in the following studies to obtain the necessary gain in sensitivity.

The next step was to determine the optimum concentration of potassium to add for the stable gain in sensitivity. As shown in Fig. 4, at a Rb concentration of 1 ppm, the gain increased in parallel with potassium concentration. However, at a Rb concentration of 10 ppm, the gain reached a plateau at a final potassium concentration of 7,500 ppm. In accordance with these results, the final potassium concentration to be added was fixed at 10,000 ppm, thus allowing a safety margin for the gain in sensitivity.

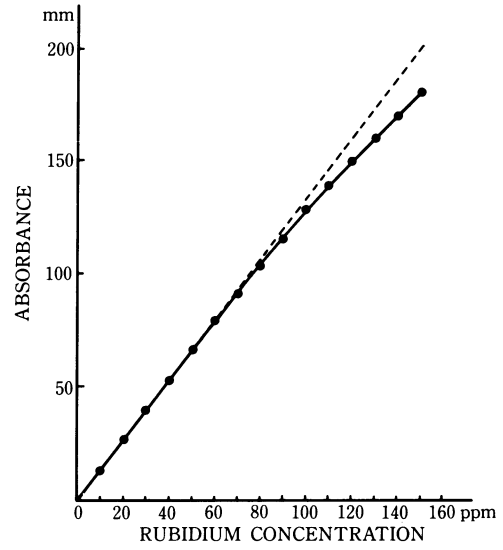

Fig. 5. Linearity of standard curve by addition of potassium (10,000 ppm)

Linearity of the standard curve

Fig. 5 showed the linearity of the standard curve under the condition mentioned above, and the linearity was maintained up to a Rb concentration of 60 ppm.

Influences of the coexisting cations in erythrocytes

Influences of the coexisting cations in erythrocytes such as potassium, sodium, calcium, magnesium and iron were examined. Potassium in erythrocytes became negligible in the determination when a final potassium concentration of 10,000 ppm was added, because potassium concentration in erythrocytes within the physiological range became about 70 ~ 80 ppm when erythrocytes were diluted 1:50. Iron coexists in erythrocytes in the form of hemoglobin, however

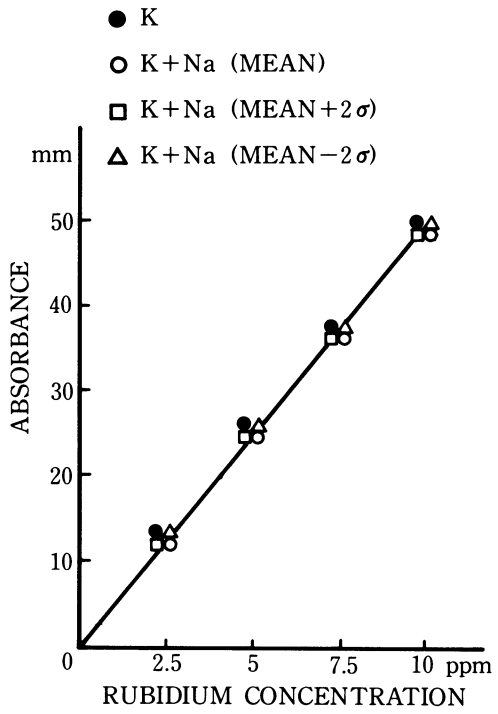

Fig. 6. Influence of sodium of physiological concentration in erythrocytes on rubidium absorbance

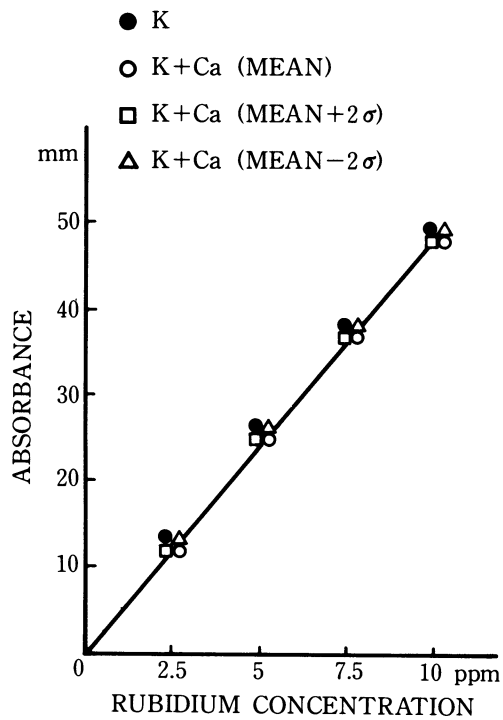

Fig. 7. Influence of calcium of physiological concentration in erythrocytes on rubidium absorbance

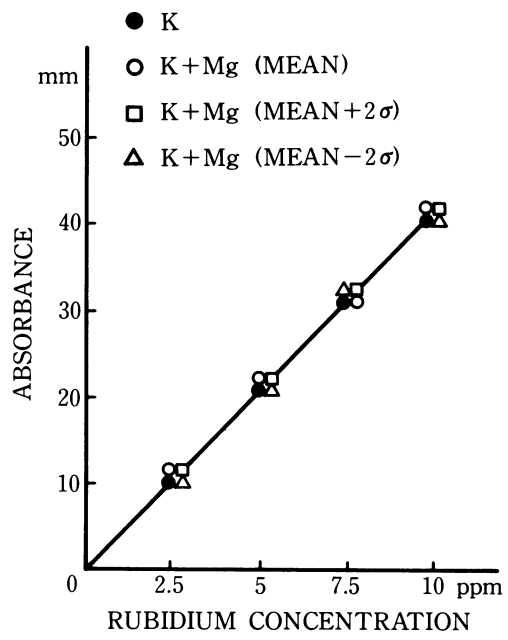

Fig. 8. Influence of magnesium of physiological concentration in erythrocytes on rubidium absorbance

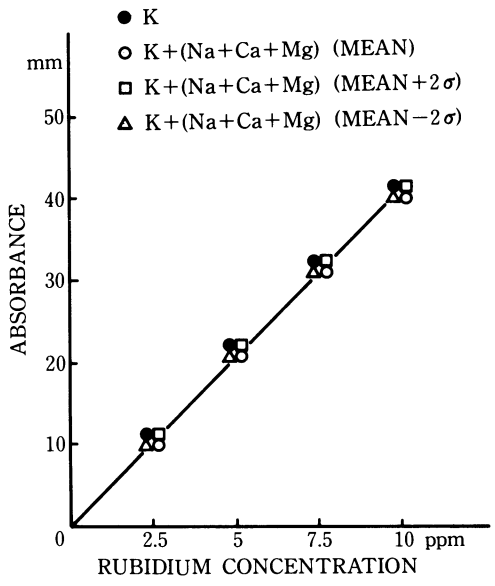

Fig. 9. Combined influence of sodium, calcium and magnesium of physiological concentration in erythrocytes on rubidium absorbance

during the wet-ashing with HNO_3 and H_2SO_4, iron sulfate (III) is formed and precipitated, thus eliminating the iron content. The influences of

sodium, calcium and magnesium within the physiological range of concentrations existing in erythrocytes were examined. However, no influence of cations on Rb absorbance was observed as shown in Figs. 6—8. The combined influence of sodium, calcium and magnesium within the physiological range of concentrations in erythrocytes was examined, because they might act together to the absorbance of Rb. Again, however, no influence of cations on Rb absorbance was observed as shown in Fig. 9.

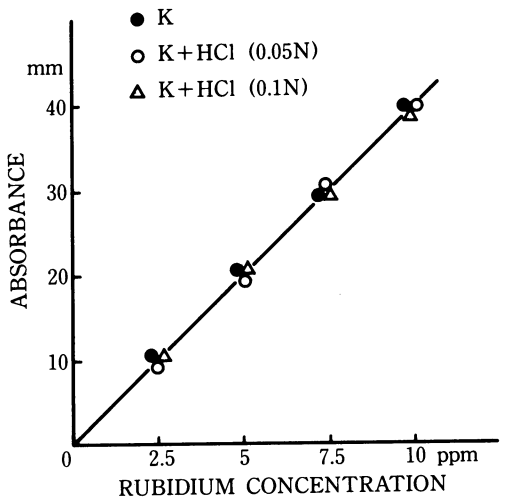

Fig. 10. Influence of hydrochloric acid on rubidium absorbance

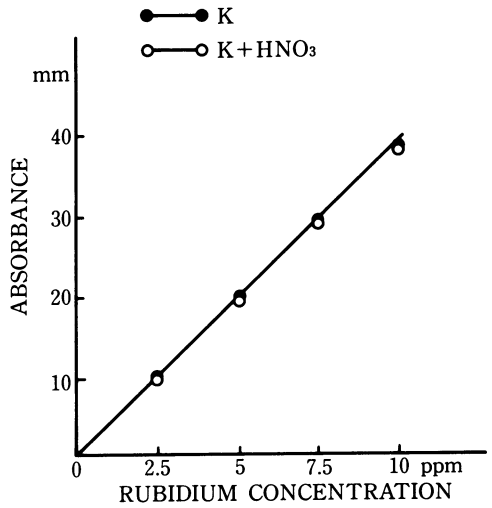

Fig. 11. Influence of nitric acid (0.28N) on rubidium absorbance

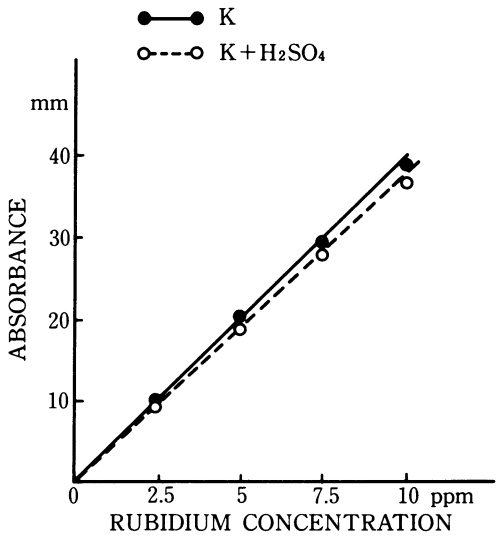

Fig. 12. Influence of sulfulic acid (0.73N) on rubidium absorbance

Influences of acids

Acids have also been reported to interfere with atomic absorption spectrophotometric determination and so the effects of HCl, HNO_3 and H_2SO_4 were examined. The standard solutions of metals for AAS were usually acidified with 0.1N NCl, HCl might be assumed to have no influence on the determination by AAS. In order to confirm the influence of HCl, we carried out tests to examine the effects of 0.05N and 0.1N HCl on the absorbance of Rb from 0 to 10 ppm. No influence of HCl on the absorbance was observed as shown in Fig. 10. The effect of HNO_3 on the absorbance seemed to be negligible, since it is heated so intensively that it is driven off in vapor during the ashing procedure. Fig. 11 showed the effect of HNO_3, assuming that 1 ml of HNO_3 (final concentration of 0.28N) remained in the wet-ashed solution, while the absorbance of Rb was formed to be independent of HNO_3. Fig. 12 showed the influence of H_2SO_4 on Rb absorbance. H_2SO_4 at a final concentration of 0.73N decreased the gain in sensitivity, and it was not driven off in vapor during the ashing procedure. Clearly, therefore, erythrocytes should be wet-ashed without H_2SO_4, or H_2SO_4 should be added to the standards at the same concentration as that of the ashed solution.

Comparisons of absorbances of samples with different preparations

We prepared erythrocytes, in principle, by ashing them with HNO_3. In general, materials such as erythrocytes used to be initially deproteinized with trichloroacetic acid (TCA). We therefore compared the absorbances of samples containing the same Rb concentration but prepared by three different methods. The first preparation was ashed with HNO_3, the second, deproteinized with TCA and the third, diluted with only deionized water, and potassium at the final concentration of 10,000 ppm was added to each sample.

On the assumption that the mean absorbance value of samples ashed with HNO_3 was 100 mm, that of the samples deproteinized with TCA also showed 100 mm, however that of samples diluted with water gave an absorbance of 98 mm. TCA at the concentration used in the deproteinization of erythrocytes decreased the gain in sensitivity and during the procedure of deproteinization, fine precipitates tended to become easily intermixed in samples for determination, so that they often clogged the suction tube. For this reason, deproteinization with TCA could not be recommended for determinations using AAS.

DISCUSSION

Although there have been several studies on the properties of Rb in comparison with lithium, there are few reports concerning the determination of Rb in biological materials especially in human erythrocytes[2,9,17,18]. Among them, Wood[18] examined the method of determination of Rb in human erythrocytes, plasma and urine, and suggested to add sodium at the final concentration of 10,000 ppm. On the other hand, Del Vecchio[2] reported the method of determination of Rb in blood and plasma, and suggested to add potassium at the final concentration of 1,000 ppm. Moreover Lieberman[9] reported that they found added potassium to be superior to added sodium in enhancing Rb, and sodium acts to slightly depress the enhancing effect of potassium. Nevertheless they used the solutions containing both sodium and potassium to add to the standards and samples, and reported that their method was applicable to the analysis of all biological materials. Sutter[17] also suggested that the marked enhancement effect of ions necessitated the preparations of standard solutions which contained equivalent amounts of sodium and potassium.

Thus each author reported the different method from each other. The reasons of differences might be considered that each method was aimed to determine Rb in different materials containing the different composition of coexisting cations, and that the determination of Rb in biological materials is not so necessitated as lithium in clinical use at present.

In regard to the method of determination of Rb in human erythrocytes, because potassium is the main component of the cations in erythrocytes and the effects on Rb absorbance increased in the sequence of $Na < K < Cs$ as Sanui[15] and Sutter[17] reported and as we examined, potassium in excess (final concentration of 10,000 rpm) is recommended to be added to the standards and materials. Therefore, the determination of Rb in human erythrocytes by AAS could be proceeded without any special preparation of erythrocytes.

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