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# COMPARISON OF ION CHROMATOGRAPHY AND NEUTRON ACTIVATION ANALYSIS FOR THE MICRODETERMINATION OF $Na^+$ AND $K^+$ IN MUSCLE

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1. The simultaneous determination of $Na^+$ and $K^+$ in small tissue samples by means of neutron activation (NAA) and ion chromatography (IC) is described.

2. A 5 x $10^{11}$ n $cm^{-2}$ $s^{-1}$ neutron flux was used for $Na^+$ analysis and the induced $^{24}Na$ and $^{42}K$ activities were measured without chemical separation with a Ge(Li) detector coupled to a 4096 channel analyzer. A minicomputer on line with the analyzer was employed for the interpretation of gamma spectra.

3. When IC was used, the sample and the standard were dissolved in the solutions before injection into the ion chromatograph. The ion concentration in the effluent solution was measured by electric conductivity.

4. The two methods were compared in terms of time required, sensitivity, accuracy, precision, simplicity and operational cost of the analysis.

5. Both techniques proved to be excellent from the point of view of the reliability of the results, and can be used for the determination of $Na^+$ and $K^+$ in biological samples.

Key words: neutron activation, ion chromatography, muscle $Na^+$ and $K^+$ determination.

## Introduction

The changes in water and electrolyte metabolism were initially explained on the basis of the known concentration of the electrolyte in plasma or serum. This explanation, however, was found to be unsatisfactory because the ion concentrations in plasma and serum are not always the same as that of the body as a whole. It should also be pointed out that plasma is only a relatively small fraction of the extracellular liquid volume, which is likewise a fraction of the total water content of the body. It follows that any alteration observed in a tissue should be analyzed in the same tissue. Since muscle tissue is abundant, has a relatively constant composition and is readily available, it can easily be used to monitor both hydrosaline and energy metabolism. Thus muscle biopsies are important sources of information on alterations affecting intracellular fluid and electrolyte balance ($Na^+$, $Cl^-$, $K^+$ and P) in patients with metabolic myopathies and acid-base disorders, including renal insufficiency (Bergstrom, 1962; Batra *et al.*, 1976).

Since only a small mass is removed by a needle biopsy, a sensitive analytical method is needed for the required analyses (Bergstrom, 1962). In addition, the simultaneous determination of several elements is very important, since the results must often be obtained from small samples in a short time. On the basis of these considerations, we compared the simultaneous determination of $Na^+$ and $K^+$ by neutron activation (NA) and ion chromatography (IC).

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## Material and Methods

### *Muscle sample*

A sample from bovine gluteous muscle was dissected and only the internal parts, free of superficial contaminants, were used for the analyses. Residual connective tissue and fat material were also removed from the sample. The fragments were rapidly washed with twice-distilled water and filter paper was used to remove excess water and adhering blood. The fresh sample was dried for 24 h at 50°C and pulverized in an agate mortar. The powdered sample was stored in a glass vessel in a desiccator under vacuum (Dubois *et al.*, 1966).

### *Standard*

Powdered animal muscle (H-4) supplied by the International Atomic Energy Agency (AIEA) was used as a standard. The reported $Na^+$ and $K^+$ contents (Parr, 1980) were confirmed by IC and NA analysis using standard NaCl and KCl solutions.

### *Neutron activation*

Portions of about 25 mg of the sample or the H-4 standard were introduced into small polyethylene bags and sealed by heating. Two samples of each set were compared with the standard analyzed alternately. Irradiation was carried out using an IEA-RI reactor at $5 \times 10^{11}$ n $cm^{-2}$ $s^{-1}$ thermal flux. After 3 h of cooling, the net areas of the $^{24}Na$ and $^{42}K$ photopeaks were measured with a Ge(Li) detector coupled to a multichannel analyzer on line with a minicomputer. Figure 1 shows the gamma spectrum obtained. The high resolution of the Ge(Li) detector permits the simultaneous determination of $Na^+$ and $K^+$ in the sample.

### *Ion chromatography*

A new technique based on the classic principle of ion exchange to separate the ions to be studied in a sample, usually with two columns in series, was utilized (Small *et al.*, 1975; Anderson, 1976; Fritz *et al.*, 1984). The first column contains a low-capacity resin of the pellicular type whose function is to separate the ions in the sample solution. The solution is then passed into a second column which contains resin of high-exchange capacity (stripper column). The function of the stripper column is to remove or retard the anions of the eluent

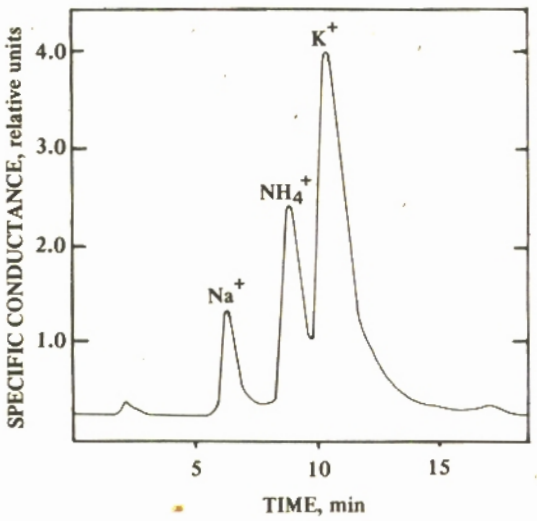


Figure 1 - Gamma ray spectra obtained after activation analysis of muscle biopsy material. Dry muscle (25 mg) was irradiated for 30 min at $5 \times 10^{11}$ n $cm^{-2}$ $s^{-1}$

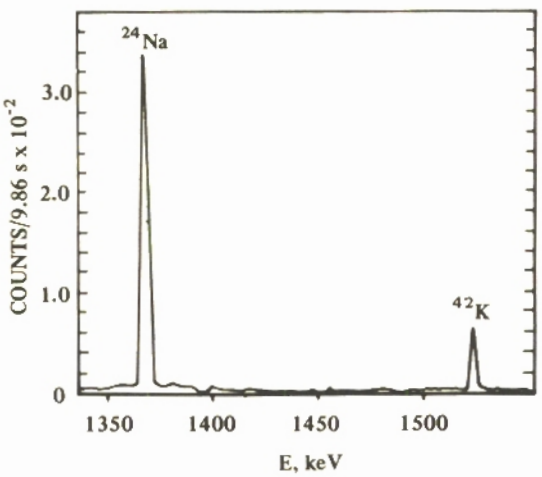


Figure 2 - Ion chromatography of $Na^+$ and $K^+$ present in muscle biopsy material. The sample solution contained 20 mg muscle and 0.2 mg was injected into the apparatus.

Table 1 - *Comparison of neutron activation analysis and ion chromatography for the determination of $Na^+$ and $K^+$ in H-4 standard muscle powder.*

The data are reported as means ± SD for 8 determinations.

| Element | Method | Expected (mg/g) | Found (mg/g) |
|---|---|---|---|
| K | NAA | 1.58 | 1.53 ± 0.08 |
| | IC | | 1.56 ± 0.08 |
| Na | NAA | 0.206 | 0.201 ± 0.012 |
| | IC | | 0.199 ± 0.009 |

used for the separation, thus transforming the species eluted into a form which does not interfere with the measurement of the electrical conductivity of the ions to be studied. The corresponding peaks are recorded and/or integrated.

A Dionex Model 10 ion chromatograph was injected in a volume of 100 $\mu$l into a 3 x 200 mm separating column eluted with 5 mN $HNO_3$ at 150 ml/h at 480 psi. The stripper column was 9 x 100 mm.

*Sample preparation*

Previous chemical digestion of sample and standard was carried out at 70°C for 30 min. A mixture of 5 ml 65% $HNO_3$, 2 ml 25% HCl and 4 ml 70% $HClO_4$ was used for this purpose.

After evaporating the acid mixture, the residue was dissolved in twice-distilled water, the solution was transferred to a volumetric flask and twice-distilled water was added again

Table 2 - *Determination of $Na^+$ in dry bovine muscle tissue by neutron activation analysis and ion chromatography.*

Each roman numeral refers to an independent analysis.

Neutron activation

| $Na^+$ (mg/g) | | | | | |
|---|---|---|---|---|---|
| I | II | III | IV | V | VI |
| 0.165 | 0.159 | 0.160 | 0.178 | 0.160 | 0.171 |
| 0.160 | 0.150 | 0.158 | 0.174 | 0.172 | 0.165 |
| 0.157 | 0.158 | 0.159 | 0.160 | 0.164 | 0.159 |
| 0.134 | 0.163 | 0.155 | 0.173 | 0.168 | 0.157 |
| $\bar{X}$ = 0.154 | $\bar{X}$ = 0.158 | $\bar{X}$ = 0.158 | $\bar{X}$ = 0.171 | $\bar{X}$ = 0.166 | $\bar{X}$ = 0.163 |

Total mean ($\bar{X}$) = 0.162 Variance ($s^2_{\bar{x}}$) = 4 x $10^{-6}$

Ion chromatography

| $Na^+$ (mg/g) | |
|---|---|
| I | II |
| 0.148 | 0.163 |
| 0.148 | 0.163 |
| 0.154 | 0.154 |
| 0.163 | 0.154 |
| 0.163 | 0.172 |
| $\bar{X}$ = 0.155 | $\bar{X}$ = 0.161 |

Total mean ($\bar{X}$) = 0.159; Variance ($s^2_{\bar{x}}$) = 9 x $10^{-6}$

up to 50 ml. Only 100 µl was introduced into the ion chromatography system and about 20 min were needed for the analysis. The sample ion peaks were compared to external standard peaks for identification by means of retention time and concentrations were determined by comparison of peak heights or areas.

## Results and Discussion

Data obtained for the determination of $Na^+$ and $K^+$ by neutron activation analysis and by ion chromatography are shown in Figures 1 and 2, respectively. The precision and accuracy of the methods were determined by statistical tests (Nalimov, 1963). When the results were compared with the expected values for the standard (Table 1), no difference was observed at the 0.95 confidence level (*t*-test). The mean values ($\bar{X}$) of the NA and IC results were also found to be statistically identical at the same confidence level.

Precision was determined by the F-test. The variances ($s^2\bar{x}$) agreed at the 0.95 confidence level, thus indicating that the methods have the same precision.

The results obtained for the muscle sample, which are presented in Tables 2 and 3, were divided into several groups. Each group is related to operations carried out on the same day. No difference was observed among the mean values of the groups at the 0.95 confidence level, showing that the results were not affected by neutron flux variations or by any instability of the equipment.

The excess mass used for the analysis

Table 3 - *Determination of $K^+$ in dry bovine muscle tissue by neutron activation analysis and ion chromatography.*

Each roman numeral refers to an independent analysis.

Neutron activation

| $K^+$ (mg/g) | | | | | |
|---|---|---|---|---|---|
| I | II | III | IV | V | VI |
| 1.66 | 1.44 | 1.54 | 1.57 | 1.63 | 1.56 |
| 1.48 | 1.61 | 1.54 | 1.59 | 1.58 | 1.51 |
| 1.45 | 1.54 | 1.62 | 1.38 | 1.54 | 1.60 |
| 1.24 | 1.60 | 1.45 | 1.58 | 1.63 | 1.47 |
| $\bar{X}$ = 1.46 | $\bar{X}$ = 1.56 | $\bar{X}$ = 1.54 | $\bar{X}$ = 1.53 | $\bar{X}$ = 1.60 | $\bar{X}$ = 1.54 |
| Total mean ($\bar{X}$) = 1.54 | | | | Variance ($s^2_{\bar{x}}$) = $4 \times 10^{-4}$ | |

Ion chromatography

| $K^+$ (mg/g) | |
|---|---|
| I | II |
| 1.66 | 1.69 |
| 1.66 | 1.69 |
| 1.52 | 1.52 |
| 1.52 | 1.52 |
| 1.50 | 1.65 |
| $\bar{X}$ = 1.57 | $\bar{X}$ = 1.61 |
| Total mean ($\bar{X}$) = 1.59 | Variance ($s^2_{\bar{x}}$) = $9 \times 10^{-4}$ |

prevented fluctuations arising from possible sample heterogeneity, even though appreciable variations in the same muscle tissue have not been observed (Nadkarni and Morrison, 1973; Batra *et al.*, 1976; Heydorn, 1984).

According to the criterion of Currie (Currie, 1968), 5 x $10^{-7}$ g Na and 4 x $10^{-5}$ g K may be quantitatively analyzed by NA analysis without chemical separation, under the conditions described here. According to the equipment manual, the sensitivity of the IC technique (Dionex Corporation, 1979) is 5 x $10^{-8}$ g for Na and $10^{-7}$ g for K. Since 8 to 30 mg of tissue are removed by needle biopsies, i.e. 2 to 7.5 mg of dry sample, it may be concluded that the sensitivity of both methods is sufficient for Na and K determinations at this level.

The time required was the same for both methods up to the preparation of the dry sample. After this point, the time required for NA was 3.5 h more than required for the IC technique. Indeed a dry sample is not needed for IC analysis, since its water content is known. This characteristic gives an advantage to the IC technique, although wet samples may be used also in NA analysis, but much more care is needed in the preparation of the sample to be irradiated.

The sensitivity of both methods is sufficient for $Na^+$ and $K^+$ determination in the biological samples. The IC technique, besides requiring less time than the NA technique, also uses less sophisticated equipment for obtaining the same results. However, the NA technique is more advantageous when several elements are to be analyzed and a nuclear reactor is available.

## References

**Anderson, C.** (1976). Ion chromatography: a new technique for clinical chemistry. *Clinical Chemistry*, 22: 1424-1426.

**Batra, G.L., Bewley, D.K., Edwards, R.T.H.** and **Jones, D.A.** (1976). Analysis of muscle biopsies for sodium, chlorine, potassium and phosphorus by neutron activation. *International Journal of Applied Radiation and Isotopes*, 27: 271-276.

**Bergstrom, J.** (1962). Muscle electrolytes in man. *Scandinavian Journal of Clinical and Laboratory Investigation*, 14: 1-110.

**Dubois, J., Colard, J.** and **Vis, H.L.** (1966). The study of hydroelectrolytic metabolism in man by muscular biopsies analysis (with special references to neutron activation). *Journal of Nuclear Biology and Medicine*, 39: 39-51.

**Fritz, J.S., Du Val, D.L.** and **Barron, R.E.** (1984). Organic acid elements for single-column ion chromatography. *Analytical Chemistry*, 56: 1177-1182.

**Heydorn, K.** (1984). *Neutron Activation Analysis for Clinical Trace Element Research*. 2 Vols. CRC Press, Boca Raton, pp. 434.

**Nadakarni, R.A.** and **Morrison, G.H.** (1973). Multielement instrumental neutron activation analysis of biological materials. *Analytical Chemistry*, 45: 1957-1960.

**Nalimov, V.V.** (1963). *The Application of Mathematical Statistics to Chemical Analysis*. Addison-Wesley, Reading, pp. 294.

**Parr, R.M.** (1980). *Intercomparison of Minor and Trace Elements in Animal Muscle (H-4)*. IAEA (IAEA-RL/69), Vienna, pp. 65.

**Small, H., Stevens, T.V.** and **Bauman, W.C.** (1975). Novel ion exchange chromatographic method using conductimetric detection. *Analytical Chemistry*, 47: 1801-1809.

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