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QUANTIFICATION OF ERYTHROCYTE MAGNESIUM AND POTASSIUM USING ATOMIC ABSORPTION SPECTROPHOTOMETRY

Key words: erythrocytes, magnesium, potassium, Mg^{2+}, K^+, atomic absorption spectrophotometry.

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ABSTRACT

A simple, relatively rapid and reliable atomic absorption spectrophotometric method was developed for the determination of both erythrocyte magnesium (Mg^{2+}) and potassium (K^+) concentrations in a single dilution of the lysate sample. The separated erythrocytes were washed with cold isotonic lithium nitrate solution to remove trapped plasma, white blood cells and platelets as completely as possible. The washed red cells were

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lysed by diluting with 10 mM hydrochloric acid (1:25), and analyzed for Mg^{2+} and K^{+} contents. Results obtained for Mg^{2+} was 2.33 ± 0.17 mmol/liter packed cells, n=675, and for K^{+} 90.82 ± 5.88 mmol/liter packed cells, n=675. The coefficients of variation for both were < 8 %. Values obtained by the proposed method were not significantly different from those obtained by other published methods ($P > 0.05$).

INTRODUCTION

Magnesium (Mg^{2+}) and potassium (K^{+}) are the main intracellular cations and only very small percentages of total body Mg^{2+} and K^{+} are located in the extracellular fluid. It has become increasingly evident that there is a need to monitor the levels of magnesium and potassium not only in the serum, but also inside the cells of patients' tissues with specific disease conditions and those being treated with certain drugs[1-4]. Both Mg^{2+} and K^{+} play vital roles in maintaining the normal function of living cells[5], and their depletion may lead to many abnormalities including cardiac arrhythmias which may be fatal[2].

Assessment of intracellular electrolyte content involves mainly analysis of muscle biopsy samples, which is traumatic to the patient, time consuming, expensive and not routinely done. The use of

erythrocytes, as the most accessible cells for investigation, show that the knowledge of their Mg^{+2} and K^{+} content is clinically useful in diagnosing conditions such as nonrespiratory alkalosis[6] and essential hypertension[7], and in patients treated with drugs such as thiazide diuretics[8] and digoxin[9]. Furthermore, measurements of erythrocyte Mg^{2+} and K^{+} may represent a relatively simple means of assessing tissue Mg^{2+} and K^{+} status.

Currently there is no one suitable assay method for concurrent measurement of both erythrocyte Mg^{+2} and K^{+} concentrations in the same specimen on a routine basis. The existing methods in literature are either designed for determination of erythrocyte Mg^{+2} [10-13] or K^{+} [9,14,15].

The availability of an easy, efficient and dependable method to determine the cellular concentration of Mg^{2+} and K^{+} on a routine basis seemed to be necessary for various reasons which include :

(a) The serum levels of Mg^{2+} and K^{+} may not reflect the cellular deficiencies, (b) Mg^{2+} is necessary to allow intracellular replenishment of K^{+}, (c) many patients have diseases which may lead to intracellular deficiencies of Mg^{2+} and K^{+}, and (d) drugs used in the treatment of those conditions may cause further depletion of the electrolytes.

Such a method would allow greater research capabilities, better drug monitoring, and better patient protection and care.

The present study was initiated to develop a simple, reproducible and fairly rapid atomic absorption spectrophotometric method for estimation of both erythrocyte Mg^{+2} and K^{+} concentrations in a single dilution of lysed cells by the use of appropriate wash and standard solutions.

EXPERIMENTAL

Apparatus

A double-beam atomic absorption spectrophotometer (Varian, Model AA775, USA) was used for measurement of Mg^{+2} and K^{+} concentrations. The instrumental conditions were as follow :

	For Mg^{+2}	For K^{+}
Principal line	202.5 nm	404.4 nm
Light source	Hollow cathode lamp	Hollow cathode lamp
Max.lamp current	3.5 mA	5.0 mA
Flame:		
Bandpass, nm	1.0	1.0
Flame type	Air/Acetylene	Air/Acetylene
Read-out mode	Absorbance	Absorbance

A Beckman Model J-6B Centrifuge (USA) was used.

An Eppendorf Varipette 4710 piston pipette (Eppendorf

Geratebau Netheler + Hinz GmbH, Hamburg, W. Germany) with continuously adjustable volume (100 - 1000 µL) was used. When pipetting viscous packed cells, the tip was rinsed three times in the viscous cells before pipetting; after pipetting, the outside of cells-filled tip was wiped off with lint-free tissue. For emptying into a container (e.g. volumetric flask), the tip was touched to the inside of the container and the Eppendorf button was pressed slowly downward to the first stop. After waiting about three seconds, the button was pressed on the second stop. The pipette was then slid out in the same vertical position to avoid sucking back any liqiud.

Reagents

All reagents used were of analytical grade. All solutions were prepared in de-ionized, distilled water from reagent-grade materials, unless otherwise stated.

Acid-washed glassware were used throughout. Magnesium and potassium standard preparations were stored in polythene bottles.

Concentrations of magnesium and potassium were expressed as mmol / liter of packed erythrocytes.

Lithium Nitrate (isosmotic, 155.3 mmol/liter): Accurately weighed 19.102 g of $LiNO_3.3H_2O$ (BDH, Poole-England) were dissolved in water and diluted to one liter.

Electrolyte Standard Solution (as stock): Accurately weighed 7.083 g of KCl (Merck, W. Germany), 0.3507 g of NaCl (Merck, W. Germany), and 0.418 g of $MgCl_2.6H_2O$ (Fluka, Switzerland) were dissolved in one liter of water to obtain a stock solution containing 95.0 mmol of K^+ (3715 ppm), 6.0 mmol of Na^+ (138 ppm) and 2.06 mmol of Mg^{2+} (50 ppm) per liter. This standard stock solution could be stored without alteration in polythene bottles for ten weeks at 4°C.

0.5 M Hydrochloric Acid (as stock): Approximately 5 N solution (a 50 mL bottle, BDH, England) of HCl was diluted by water to make 500 mL.

Preparation of Working Standard Solutions : A series of five standard magnesium solutions (ranging 0.5 to 5.0 μg/mL) and potassium solutions (ranging 37.0 to 372.0 μg/mL) were prepared on appropriate dilutions of the electrolyte standard stock solution with 10 mM hydrochloric acid. Each series was sprayed at the beginning and end of each set of determination. Calibration curves were obtained by plotting absorbance versus concentration (in ppm). These working standards could be stored unaltered for 2 weeks at 4°C.

Calibrators: A standard solution with Mg^{2+} and K^+ concentrations in the range of the diluted lysed samples was prepared by appropriate dilution of the electrolyte stock solution with 10 mM HCl, in the same

manner as the solutions to be analysed, and aspirated in-between the lysed samples.

Blood Sampling

Fresh venous blood samples were obtained from Blood Bank (King Khalid University Hospital, Riyadh, Saudi Arabia). The blood was collected, using citrate-phosphate-dextrose buffer as anticoagulant, from twenty eight healthy donors (males and females, aged 18 to 45 years), and stored at 4°C. These blood samples were used, without further pretreatment, within a maximum of seven days after collection.

Procedure

Four milliliters of freshly supplied venous blood were centrifuged (1800 g) for five minutes at 10°C; the plasma and buffy coat were removed. Red blood cells were washed twice by suspending in isosmotic lithium nitrate solution, to remove plasma trapped between cells. During the washing, the cells were separated from washing solution by three-minutes centrifugation at 2400 g at 10°C in a Beckman J-6B centrifuge. The washed erythrocytes were lysed by diluting (1:25) with 10 mM HCl. For accuracy and good precision the "wash out" pipetting technique, described under apparatus section, was employed. The diluted sample was then shaken well to allow for complete cells haemolysis, and the resultant lysate was filtered through sintered

funnel and used for analysis of magnesium and potassium in atomic absorption spectrophotometer. Readings of results (in ppm) were made with reference to standard curves (see figures 1 and 2). In every case, a calibrator was aspirated and readings taken. 10 mM HCl was also passed through the flame at intervals for autozeroing. The erythrocyte Mg^{2+} (or K^{+}) concentration was calculated (in mmol/ liter of packed cells) from the following formula:

$$\text{mmol electrolyte/liter} = \frac{M \times D}{\text{atomic wt. of electrolyte}}$$

where,

M = Measured value in ppm,

and D = Dilution factor.

(All estimates were made at least in duplicate)

Reproducibility

Within-day reproducibility of the proposed method was assessed by assaying twenty seven 4-ml aliquots (from one volunteer) for erythrocyte magnesium and potassium three times in one day.

Day-to-day reproducibility was assessed by assaying the twenty seven samples (kept at 4°C) once every three days for ten days.

Method Comparison

Eight samples were analyzed in triplicate for determination of erythrocyte potassium using emission

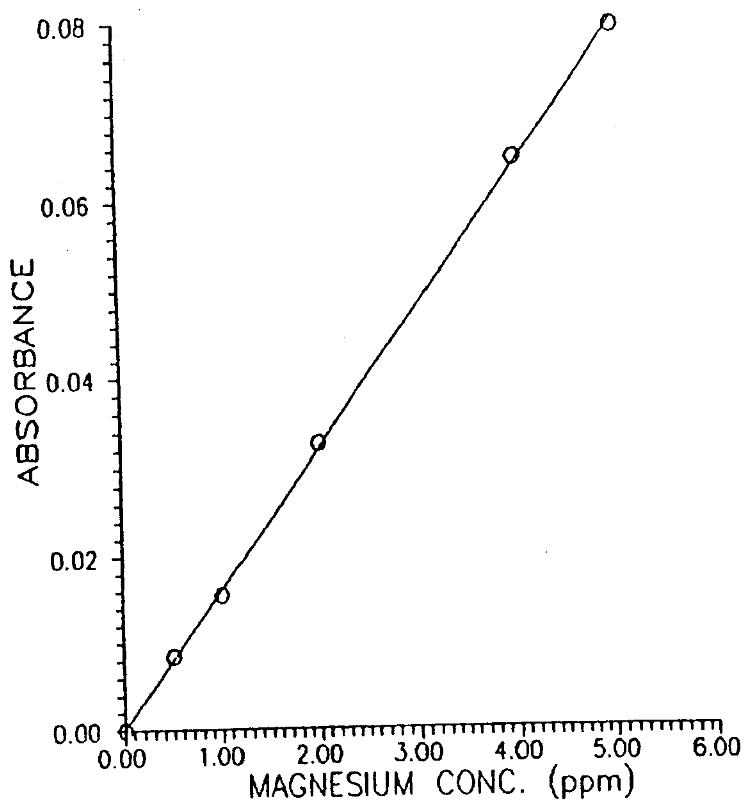

FIG. 1. Calibration curve of magnesium.

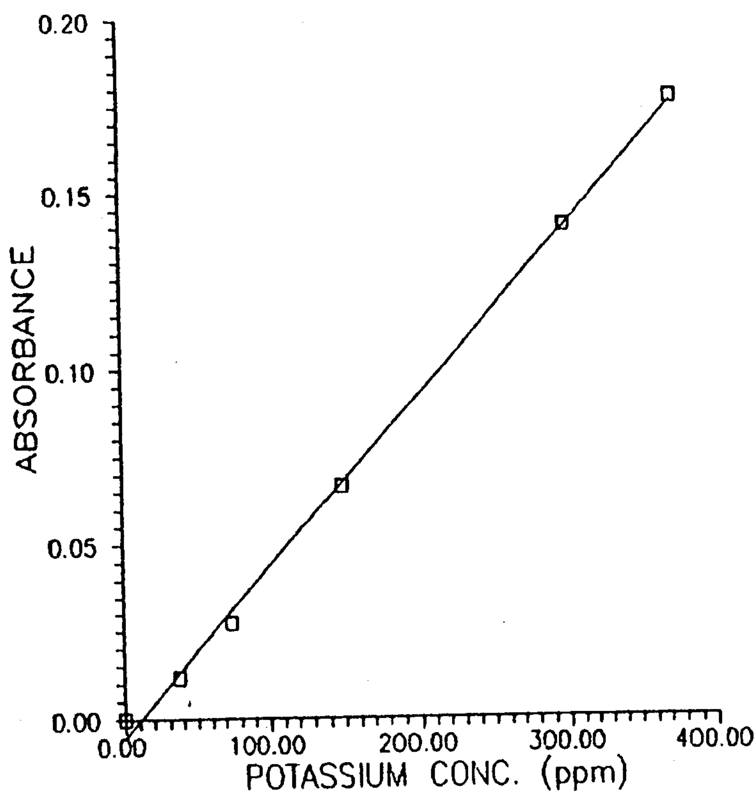

FIG. 2. Calibration curve of potassium.

photometric technique reported by Fortes etal[15], and magnesium using atomic absorption method reported by Boston etal.[10]. The results were compared with those of the proposed method, using Student's t-test for paired observations.

RESULTS

Figures 1 and 2 show typical calibration curves of Mg^{+2} and K^{+}, respectively, prepared from a standard electrolyte solution. A least-squares linear regression evaluation of the absorbance versus concentration (in ppm) relation gave absorbance = 1.58×10^{-2} (concentration) + 4.93×10^{-4} with a correlation coefficient of 0.9997 for Mg^{2+}, and absorbance = 4.89×10^{-4} (concentration) - 4.91×10^{-3} with a correlation coefficient of 0.9992 for K^{+}.

Table I summarizes the results of erythrocyte Mg^{2+} and K^{+} analysis as obtained by the proposed method. The measured range for potassium was 80.50 to 100.91 mmol/liter packed cells (n=675), and for magnesium was 2.08 to 2.69 mmol/liter packed cells (n=675).

Reproducibility

The within-day coefficient of variation was 3.03% for Mg^{+2}, and 5.01% for K^{+}. The day-to-day coefficient of variation was 3.90% for Mg^{+2} and 6.43% for K^{+}. These results were shown in Table II.

TABLE I

Summary of results of erythrocyte analysis for Mg^{2+} and K^{+} in healthy subjects

	Magnesium (mmol/L cells)	Potassium (mmol/L cells)
No. of samples (n)	675	675
Mean	2.33	90.82
SD	0.17	5.88
CV,%	7.30	6.48

TABLE II

Reproducibility Data for Erythrocyte Mg^{+} and K^{+}, n=27

	Magnesium	Potassium
Within-day:		
No. of analyses	3	3
Mean (mmol/L cells)	2.21	93.31
SD	0.06	4.68
CV, %	3.03	5.01
Day-to-day:		
No. of analyses	3	3
10 days.		
Mean	2.17	95.05
SD	0.08	6.12
CV, %	3.90	6.43

Method comparison

Table III shows results of comparing erythrocyte magnesium analysis by a published method[10] and the proposed method as run in our laboratory, using the same blood specimen.

Table IV shows results of comparing erythrocyte K^+ by a published method[15] and the proposed method, using same blood specimen.

DISCUSSION

The present study was undertaken in order to develop a simple and a reliable method for rapid detection of erythrocyte magnesium and potassium. Washing erythrocytes free from plasma by centrifugation and resuspension in iso-osmolar solution has been described previously[9,15,19]. Removal of trapped plasma by washing the cells twice with lithium nitrate was found to be quite satisfactory, as none of the elements was detected in the final supernate. Acidification with 10 mM HCl was adopted to enhance solubility of Mg^{2+} and K^+ in deionized water; furthermore, it may act as a spectroscopic buffer. It is possible that shaking of cells with the acidified water may liberate the protein-bound electrolytes. The filteration of the lysed samples helped in removing organic materials, such as cell wall fragments or other macromolecular

TABLE III

Comparison of the mean erythrocyte Mg^{+2} concentration (in mmol/liter cells) obtained by a published method[10] (A) and the proposed method (B)

Variables	(A)	(B)
Data :		
	2.03	2.22
	2.17	2.34
	2.11	2.30
	2.34	2.44
	2.43	2.57
	2.66	2.59
	2.31	2.18
	1.94	2.24
Statistical results:		
Mean	2.25	2.36
Standard Deviation	0.23	0.15
Standard Error	0.08	0.05
CV %	10.46	6.61
Calculated T value = 2.104		
At 95 % T from T-Table = 2.365		

TABLE IV

Comparison of the mean erythrocyte K^+ concentration (in mmol/liter cells) obtained by a published method[15] (A) and the proposed method (B)

Variables	(A)	(B)
Data :		
	92.04	92.00
	91.94	91.63
	95.82	94.32
	92.26	92.78
	86.77	95.46
	91.40	94.61
	94.89	100.25
	94.76	99.49
Statistical results:		
Mean	92.48	95.07
Standard Deviation	2.84	3.24
Standard Error	1.00	1.14
CV %	3.07	3.41
Calculated T value = 2.086		
At 95% T from T-Table = 2.365		

organic constituents which may block the tubings of the instrument's atomiser. Even if there is some of the organic material in the filterate it may have insignificant effect on the erythrocyte Mg^{2+} and K^{+} concentrations. This was proved by comparing electrolytes content obtained by the proposed method with values obtained after prelimenary ashing of the same specimen (unpublished observations).

Mean erythrocyte electrolytes (expressed as mmol/liter packed cells) for normal volunteers was 2.33 ±0.17 mmol for Mg^{2+} and 90.82 ± 5.88 mmol for K^{+} (Table I). These values compare favorably with the normal values obtained in literature (Table V).

The coefficient of variation of 7.30 % for erythrocyte Mg^{2+} and 6.48 % for K^{+} (Table I) may be due to errors inherent in the whole procedure and atomic absorption spectrophotometry. Furthermore, differences in the volunteers may account for the wide variation in the ranges of erythrocyte Mg^{2+} and K^{+} contents. It is important to point out that in all cases, limits of the normal range deviates from the mean by less than 10 %.

The within-day and day-to-day reproducibility of the proposed method (Table II) was acceptable for the samples studied.

As shown in Tables III and IV, the absence of any significant difference in the values of the erythrocyte

TABLE V
Reference ranges found for erythrocyte Mg^{2+} and K^{+} in healthy subjects

Electrolyte	Mean (±S.D.)	Reference
K^{+} :		
	99.33 ± 10.37 mEq/liter erythrocytes	8
	102.40 ± 7.90 mmol/liter erythrocytes	6
	70.0 to 91.0 mEq/liter erythrocytes	11,12
	99.10 ± 5.30 mmol/liter erythrocytes	13
Mg^{2+}:		
	4.41 ± 0.64 mEq/liter erythrocytes	8
	6.30 ± 0.98 mg/100 ml erythrocytes	10
	3.9 to 5.3 mEq/liter erythrocytes	11,12
	2.30 ± 0.17 mmol/liter erythrocytes	13

Mg^{2+} and K^{+} concentrations obtained by the use of the newly proposed method and other established assay procedures[10,15], indicates the reliability of our method. The procedure of the proposed method is simple and rapid. For instance, in the method of Boston et al.[10], the determination of erythrocyte Mg^{2+} concentration required more than 48 hrs, whereas in the proposed method time required for complete analysis is

less than one hour. Furthermore, the proposed method measures both erythrocyte Mg^{2+} and K^{+} levels in a single dilution of a lysate sample, whereas the previously published methods were mainly designed for the measurement of either Mg^{2+} and K^{+}.

Despite these important features however, the relatively high cost of instrumentation involved with the proposed assay method may restrict its wider applicability.

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