Rapid Quantitative Turbidimetric Spot Test Analysis of Potassium In Blood Serum

Matthieu Tubino*a, Rafael L. de Souzaa and Nelci F. Hoehrb

a Instituto de Química, Universidade Estadual de Campinas, CP 6154, 13083-970 Campinas- SP, Brazil
b Departamento de Patologia Clínica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, CP 6111, 13083-970 Campinas - SP, Brazil

A spot test quantitative turbidimetric method was developed for the determination of potassium in blood serum. The precipitation of potassium is done in basic medium with the classical analytical reagent sodium tetraphenylborate. The measurement of the turbidity is directly performed in this solution without further dilution using a micro-turbidimetric cell developed in this work. Blood serum was mixed with the buffer and the analytical reagent solution. The turbidity is measured in a spectrophotometer at 700 nm. The calibration curve is a straight line in the working concentration range, presenting a correlation coefficient equal to 0.9998. The analytical results obtained with this method were compared with those obtained through the use of a potassium ion selective electrode. Complete agreement was achieved between the two methods at the 99% confidence level. The observed mean RSD was 3.8%.

Keywords: potassium, serum, tetraphenylborate, turbidimetric, micro analysis, spot test

Introduction

Potassium is the major intracellular cation. Ninety percent of the total body potassium is free and therefore exchangeable, while the remainder is bound in red blood cells, bone and brain tissues. However, only approximately two percent (50-60 mmol) of the total is located in the extracellular fluid, where is readily accessible for measurement. The plasma potassium concentration is controlled mainly by the kidneys. The K+ filtered at the glomerulus is reabsorbed in the proximal tubule. The distal tubules regulate its amount excreted in the urine. Potassium excretion is stimulated by aldosterone, but it also depends upon the glomerular filtration rate, extracellular hydrogen ion concentration, sodium and water excretion.1

Hypo- and hyperkalemia are most commonly encountered electrolyte abnormalities in hospitalized patients.2,3 Potassium depletion occurs when output exceeds intake. Increased loss of potassium is a frequent occurrence and such loss can be from the gut, through vomit or through the kidneys.4 Low potassium levels are dangerous as the lack of this ion increase muscular irritability that as final consequence can stop cardiac action. Drug therapy is often implicated in the pathogenesis of potassium depletion. Hypokalemia may be due to potassium depletion but can also be a result of redistribution of potassium from the extra- to the intracellular compartment. Hypokalemia is also a common complication of acute myeloid leukemia and has been ascribed to renal tubular loss of potassium, increased uptake of potassium by the active cell mass, and treatment with some antibiotics.5

Potassium excess can be due to excessive intake or decreased excretion. A normal intake may be excessive if
Excretion is reduced (e.g. renal failure). Excessive intake is otherwise virtually always iatrogenic and parenteral. The danger of hyperkalemia is that it can cause cardiac arrest and can kill without warning. This may occur in the absence of any warning clinical signs. It lowers the resting membrane potential, shortens the cardiac action potential and increases the velocity of repolarization. Cardiac arrest with ventricular fibrillation may be the first sign of hyperkalemia.

Variations of the extracellular potassium concentration have been implicated in the regulation of the cerebral blood flow and it is well established that the concentration of extracellular potassium ions is regulated by glial cells.

Spot test analysis, is an analytical method more frequently used for qualitative purposes. The material used is very often of very low cost and the procedure is very simple. This method is particularly useful in situations where the sample size is small or when it is desirable to decrease the quantity of the sample as in clinical analysis. Nowadays, with the advanced development of the electronics, the miniaturization of equipments and with the decrease of the cost of such material, it is possible to develop quantitative spot test analytical methods that can be used for the determination of a variety of analytes in different matrices.

The use of sodium tetraphenylborate (TPB) as analytical reagent for potassium was recognized by Wittig et al. about fifty years ago. Surprisingly, the turbidimetric method for the determination of this ion using this reagent was adequately developed only forty years later. This method was adapted for flow injection systems and used for the determination of potassium and ammonium in various matrices.

Potassium can be also determined by an ion-selective-electrode. Direct potentiometry of potassium is increasingly used in the clinical laboratories. However some accuracy problems can occur, because measurements with ion selective electrodes depend on the ionic activity, whereas the flame photometer measures the stoichiometric concentration.

In the present work sodium tetraphenylborate was used for the determination of potassium in blood serum. The use of a turbidimetric cell, with small optical path of approximately 1 mm, avoids the necessity of further dilution, contrasting with the methods that use an ion selective electrode or flame photometry.

**Experimental**

**Apparatus**

Spectrophotometer: A Femto® visible single beam spectrophotometer, model 600, fixed at 700 nm, was used for the turbidity measurements (Turbidity = - log Transmittance; $T_b = -\log T$). An Ultrospec 2000 Pharmacia Biotech®, single beam spectrophotometer, with 1.0 cm optical path quartz cells, was used to obtain the visible spectra. In the Laboratory of Clinical Pathology the analyses of potassium in the serum samples, used in this work, were performed in a Hitachi 917 Analyzer using a potassium ion selective electrode.

Turbidimetric cell: In order to avoid dilution of the matrix, a turbidimetric cell with small optical path was developed. It consists of a glass cylinder 60 mm high, 6 mm of external diameter and 1 mm of internal diameter (optical path). The present work used a glass capillary bar from which pieces of 60 mm in length were cut. The used glass here was not of optical quality. It was necessary to verify the transparency of each cell at the working wavelength, 700 nm, in order to select the optically equal ones. Certainly this selection will not be necessary if pieces of optical glass or a transparent rigid plastic are used instead of ones. This cell can be easily filled by capillary action.

Support for the turbidimetric cell: In order to support the turbidimetric cell in the spectrophotometer, the support depicted in Figure 1 was constructed. Due to its size, it can be used with any spectrophotometer. Two pieces were constructed, one of aluminum painted with black ink and the other of black P.T.F.E. The two presented equal behavior.

**Chemicals and solutions**

All the chemicals used in this work were of analytical grade. The water used in all experiments was distilled in a glass distiller and then deionized in a Milli QPlus deionizer. Sodium tetraphenylborate (TPB) 3% m/v: 3.0 g of the...
chemical were dissolved in 100 mL of water. This solution was prepared daily, as TPB decomposes in solution with time. Sodium tetraborate (TB) buffer solution (pH 9.1): 0.38 g of the chemical were dissolved in 100 mL of water. Glycerin 10% m/v: 10 g of glycerin were dissolved in 100 mL of water. Standard stock potassium solution: a stock solution containing 300.0 µg/mL (7.5 mmol L⁻¹) of potassium was prepared by dissolving 0.2855 g of dried KCl in 500.0 mL of water. Serum samples: Blood was collected in a tube without anticoagulant. After natural coagulation the sample was centrifuged during 10 min at 3000 rpm. The serum was separated and kept in small tubes closed with rubber caps.

Analytical procedure

100.0 µL of serum samples were mixed with 100.0 µL of the TB buffer solution, 100.0 µL of the glycerin solution and 400.0 µL of the 3% m/v TPB in a spot test porcelain plaque and stirred for 30 seconds with a tiny glass rod. One minute latter the turbidimetric cell was filled with the solution by capillary action. The turbidity was measured in the spectrophotometer at 700 nm. The result was compared with the calibration curve.

Calibration curve: The calibration curve was constructed using the standard stock solution of potassium. Adequate dilutions were performed in order to obtain a standard range from 2.5 to 7.5 mmol L⁻¹. These solutions were treated in the same way as above described in the analytical procedure. Daily, two standard solutions (3.75 mmol L⁻¹ and 6.25 mmol L⁻¹) were used to control the validity of the calibration curve. The blank was obtained with water, as it was not observed significant signal difference between water and the solution containing all the reagents excepting potassium.

Results and Discussion

Sodium tetrphenylborate is a highly specific reagent for potassium, mainly in alkaline medium. Therefore, no interferences of other ions are expected in potassium determination in blood serum, using this reagent.

In Figure 2 can be seen the visible spectra, from 400 to 800 nm, of a solution of serum (400 µL) with tetraborate buffer (400 µL), glycerin 10% solution (1.6 mL), water (400 µL) (spectrum a) or sodium tetrphenylborate 3% (400 µL) (spectrum b). A quartz cell with an optical path of 1.00 cm was used to obtain the spectra. Observing the spectra, where the total "absorbance" must be understood as the sum of the absorbance plus the turbidity, it is easy to conclude that the wavelength where there is the smallest interference of the serum solution (curve a), associated with the lowest turbidity (curve b) is at about 700 to 800 nm. The use of higher wavelengths, compared to the commonly used 420 nm, decreases the turbidity effect and this is desirable in the proposed method as the decreased turbidity in addition to the small optical path permits us to avoid further dilution of the sample. The wavelength of 700 nm was chosen for the analytical procedure.

The absorbance, at 700 nm, of all the solutions used in the procedure, showed that they do not significantly contribute for the final turbidimetric value. Therefore, for simplicity, water was used as the blank.

The study of the influence of the TPB on the obtained final turbidity was performed varying its concentration from 0.5% to 3.0% m/v. It was observed that the precipitation of the potassium tetrphenylborate, with the time, strongly depends on the TBP concentration. Using the solution containing 3% m/v of the reagent, the reaction is completed in less than 1 minute. Therefore this concentration was used for the analytical procedure.

Figure 3 shows that, in the working range, the obtained calibration curve was a straight line that is described by the equation Tₜ = 0.0321 + 0.0594 C, where Tₜ is the turbidity and C the potassium concentration in mmol L⁻¹. The correlation coefficient is 0.9998. Small differences of the linear coefficient of the calibration curve are observed from one day to another and can be attributed to small instrumental effects, one of which probably results from the fact that the turbidimetric glass cells used in this work are not optically equivalent. The use of optical quality glass or optically identical transparent plastic capillaries
will certainly diminish these differences and enhance the analytical results.

One hundred blood serum samples already analyzed in the Laboratory of Clinical Pathology of the Clinic Hospital of the University were submitted to the analytical procedure proposed in this work. Three aliquots of each sample were treated according to the proposed procedure leading to three turbidity values. The samples from 1 to 50 and those from 51 to 100 were analyzed in two subsequent days, respectively. The calibration curve was checked in the second day and showed a small difference only in the linear coefficient, easily corrected.

In the Laboratory of Pathology, the analysis was performed in a Hitachi 917 Analyser that uses a potassium ion selective electrode. The specified precision of this instrument is 1.4%. Experiments were performed to check this RSD value and the specification of the instrument was confirmed. As three aliquots of the hundred samples analyzed by this instrument were subjected to three independent analyses, using the proposed turbidimetric method, for statistical purposes these hundred samples were considered as being three hundred.

Figure 4 shows the linear regression that correlates the analytical results ($y$) of the turbidimetric method with those ($x$) of the ion selective electrode method. This line is described by the equation $y = -0.0631 + 1.024 \times x$. It is easy to note that the calculated slope and intercept are very close to the “ideal” 1 and 0 respectively. Also, for the 99% confidence level the Student’s $t$ value is 2.58 and the confidence limits ($t \times SD$) for the intercept and for the slope are ±0.189 mmol and ±0.0452 respectively. The dashed lines in the figure represent the confidence limits. Considering the slope and the intercept values and the confidence limits there is no evidence for systematic differences between the two sets of results at a 99% confidence level. The mean RSD of the proposed turbidimetric method was found to be 3.8%.

Considering the quality of the analytical results, the specificity character, the small sample volume, the operational and the instrumental simplicity and the low cost aspect of the proposed method, we conclude that it can be recommended for the analysis of potassium in blood serum. It must be remembered that the use of optical quality glass or identical transparent plastic capillaries will certainly enhance the analytical results. In the case of plastic capillaries an additional characteristic can be considered as they will be disposable, leading to more operational simplicity.

Acknowledgments

The authors thank Fundação de Amparo à Pesquisa, FAPESP, and Conselho Nacional de Desenvolvimento Cientifico e Tecnológico, CNPq, for financial support.

References


Received: November 4, 2003
Published on the web: August 9, 2004

FAPESP helped in meeting the publication costs of this article.