ISSN 2079-9780, Review Journal of Chemistry, 2015, Vol. 5, No. 1, pp. 1–20. © Pleiades Publishing, Ltd., 2015.
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Chronology of the Research on Methods for Determining the Potassium Concentration in Human Blood

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Received October 29, 2013; in final form, May 20, 2014

Abstract—Studies of human blood, performed in clinical diagnostic laboratories, are conducted on numerous indicators. Potassium concentration is one of these. This review presents the chronological development (since the mid-1950s) of techniques for determining the concentration of potassium in plasma, serum, and whole blood, which are based on different analytical methods, such as flame photometry, potentiometry, ion chromatography, mass spectrometry, X-ray fluorescence, spectrophotometry, turbidimetry, and luminescence. Brief information about these methods and the results of comparative examinations are given. It is noted that potentiometric methods for determining the concentration of potassium in plasma, serum, and whole blood are widely used in small and medium-sized laboratories, as well as in large clinical and diagnostic centers. Flame photometric methods are commonly used as the reference in comparing measurement results obtained by other techniques. Ion-chromatographic techniques also claim this status, and mass spectrometric techniques are used for certification of reference materials.

Keywords: potassium, plasma, serum, blood, measurement, concentration, procedure, analysis, clinical diagnostic laboratory.

DOI: 10.1134/S2079978014040025

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1. INTRODUCTION

At present, the studies of patient blood performed in clinical diagnostic laboratories are carried out on numerous parameters characterizing the content of a variety of chemical compounds, such as potassium, sodium, calcium, magnesium, zinc, chlorine, phosphorus, urea, uric acid, glucose, cholesterol, total protein, albumin, creatinine, total and direct bilirubin, alanine aminotransferase, aspartate aminotransferase, cholinesterase, lactate dehydrogenase, γ-glutamyl transferase, amylase, lipase, alkaline phosphatase, creatine kinase, and others.

One of these indicators is the potassium concentration. It is believed that it is from 3.5 to 5.5 mol/m^3 (mmol/L) in the blood of healthy humans. Deviation from these values is a sign of hypokalemia (potassium deficiency) or hyperkalemia (potassium excess), indicating the possible development of a number of diseases.

The concentration of potassium in human blood can be determined by several analytical methods, proposed in different periods, that have successfully operated or continue to operate at this time.

In connection with the above, the purpose of this review is an attempt to reflect the most significant events in the history of the formation, development, and application of these methods in practice.

It is seems that this purpose can be achieved through a chronological narrative of the information that was published on the above topics in the journal *Clinical Chemistry*, which was founded in 1955 and since

Table 1. Results of flame photometric determinations of potassium concentration in blood serum containing proteins and protein-free serum

Results of determination of potassium concentration, mmol/L										
Blood serum with proteins	3.8	3.9	4.2	3.9	5.0	5.5	4.1	4.9	5.0	5.4
Protein-free serum	3.7	3.7	4.2	3.7	4.8	5.2	4.0	4.8	4.9	5.2
Difference in the measurement results, %	2.7	5.4	–	5.4	4.2	5.8	2.5	2.1	2.0	3.8

then has earned recognition from specialists as one of the most authoritative editions on clinical diagnostics.

So, let us consider the studies on the development of methods for determining the potassium concentration in human blood, starting from the mid-1950s up to the present time.

2. STUDIES IN THE 1950-s AND 1960-s

In 1956 Drayer pointed out that Lundegardh developed the basis for the method of flame photometry in 1930. However, the real prerequisites for the application of this method of determining the potassium concentration in human blood were created only after the invention of a relatively simple device with high performance and satisfactory accuracy, which was made by Barnes et al. in 1945. It was noted that the dependence of the intensity of the flame emission on the potassium concentration in the solution introduced into the flame was of a complex character. Linearity was observed only at concentrations up to several dozens mmol/L. A deviation from linearity was caused both by the processes of the potassium atom's self-absorption of the resulting radiation and by the emergence of radiation of other atoms present in the solution. In this case, two analytical procedures could be applied, i.e., direct measurement and measurement using an internal standard. Direct measurement was based on the dependence of the intensity of the flame emission on the potassium concentration in the solution introduced into the flame. Measurement using an internal standard was based on the dependence of the ratio of the flame emission intensities caused by the potassium concentration itself and a fixed concentration of another component of the analyzed solution. Direct measurement provided higher sensitivity of measurements of the potassium concentration, and measurement using an internal standard made it possible to obtain a wider linear range of measurements and to compensate partially for the fluctuations caused by flame combustion and the introduction of the studied solution to it [1].

In view of these circumstances, Drayer proposed a method for determining the potassium concentration in blood serum up to 8 mmol/L. The basis for this procedure was flame photometric measurement using an internal standard. An aqueous solution of lithium nitrate at a concentration of 395 mmol/L was used as an internal standard. This solution (0.1 mL) was added to 0.1 mL of the serum under assay. The volume of the formed mixture was brought to 10 mL with distilled water, after which the mixture was sprayed into the flame of a photometer. When conducting repeated tests of 100 samples of blood serum, the mean relative error did not exceed 2.7, 0.85, 0.33, and 0.23% at a potassium concentration of 2, 4, 6, and 8 mmol/L, respectively [1].

In 1959 Natelson et al. developed a method for determining the potassium concentration in blood serum up to 12 mmol/L. The procedure was based on the X-ray fluorescence method. A serum volume of 25 μL was dosed on an area of a filter paper bounded by a circular barrier of wax. Then, the filter paper was dried at room temperature or 60°C and placed in a X-ray fluorimeter to measure the fluorescence intensity of potassium atoms at wavelength K_α 3.74 Å. The dependence of the fluorescence intensity of the potassium concentration in blood serum was linear. A comparison of the results obtained by the developed and the flame photometric methods demonstrated that both techniques had similar relative errors of measurement of potassium concentration of 4.5 mmol/L in serum, but the X-ray fluorescence technique was characterized by lower productivity [2].

In 1965 London and Marymont reported on the flame photometric determination of potassium concentration in protein-free blood serum. The removal of proteins from the serum was performed with a suspension of urease enzyme in tris-acetate buffer and methanol. The measurement results for potassium concentration in blood serum containing proteins and protein-free serum are shown in Table 1. It is easy to note that the potassium concentration in the protein-free blood serum is higher on average by ~3.1% [3].

In 1967 Dahms proposed a procedure for determining the activity of potassium ions in whole blood or serum. The procedure was based on the potentiometric method of analysis using a glass "potassium" elec-

trode. The glass composition of "potassium" electrode was as follows: 27% sodium oxide Na_2O, 4% aluminum oxide Al_2O_3, and 69% silicon oxide SiO_2. The output signal of the electrode was described by the relation [4]

$$E_K = E_{K0} + S_K \log(a_{K^+} + k_{Na} a_{Na^+}), \quad (1)$$

where E_K is the output signal of the glass "potassium" electrode, V; E_{K0} is the standard potential of the glass "potassium" electrode, V; S_K is the sensitivity constant of the glass "potassium" electrode, V; k_{Na} is the selectivity constant of the glass "potassium" electrode relative to sodium ions; a_{K^+} and a_{Na^+} are the activity of potassium and sodium ions in the studied blood (serum), respectively, mol/L.

To determine the activity of sodium ions (in order to correct the output signal of "potassium" electrode), a glass "sodium" electrode was used. The glass composition of "sodium" electrode was as follows: 11% sodium oxide Na_2O, 18% aluminum oxide Al_2O_3, and 71% silicon oxide SiO_2. The output signal of this electrode was described by the expression [4]

$$E_{Na} = E_{Na0} + S_{Na} \log a_{Na^+}, \quad (2)$$

where E_{Na} is the output signal of the glass "sodium" electrode, V; E_{Na0} is the standard potential of the glass "sodium" electrode, V; S_{Na} is the sensitivity constant of the glass "sodium" electrode, V.

Designation a_{Na^+} was given in the notation of the formula (1).

The values of E_{K0}, k_{Na}, E_{Na0}, and S_{Na} were determined at the stage of electrode calibration by two standard solutions, and the value of S_K was assumed to be 0.0617 [4].

The response time of glass "potassium" and "sodium" electrodes did not exceed 50 s. The volume of blood (serum) required for the analysis was estimated at the level of 1 mL. In contrast to the flame-photometric and X-ray fluorescence techniques, the proposed procedure made it possible to determine not the potassium concentration but another physiologically important indicator—the activity of potassium ions in blood (serum). At the same time, the potentiometric procedure was not as rapid as the flame photometric methods [4].

In 1968 Dahms examined the relationship between the potassium concentration in blood serum and the activity of potassium ions a_{K^+} in serum, which was determined by potentiometric procedure using a glass "potassium" electrode [5]

$$a_{K^+} = K_K C_K, \quad (3)$$

where K_K is the conversion factor and C_K is the potassium concentration in blood serum, mol/L.

It was experimentally shown that the conversion factor should be found by the ratio [5]

$$K_K = \gamma_K \frac{C_s}{C_w}$$

where γ_K is the activity coefficient of potassium ions in aqueous solution; C_s is the water content in solution, g/L; and C_w is the water content in blood serum, g/L.

The obtained results indicated that it is necessary to take into account the entire chemical composition, primarily the protein content, when measuring the potassium concentration in blood serum [5]. Note that this conclusion to some extent is consistent with the results of flame-photometric studies previously reported by London and Marymont.

3. STUDIES IN THE 1970-s

Because of the low accuracy of the measurements of potassium ion activity in blood serum using a glass "potassium" electrode (due to the significant influence of sodium ions in the serum), Wise et al. developed a potentiometric technique using a liquid ion-exchange electrode in 1970. The technique allowed determination of the potassium concentration from 0.5 to 500 μmol/g in protein-free blood serum. Protein removal was carried out by centrifugation of serum for 10 min at 2700 rpm. The potassium concentration was determined according to expression [6]

$$E_K^o = E_{K0}^o + 0.1184 \log(\gamma'_K C'_K), \quad (4)$$

where E_K^o is the output signal of liquid ion-exchange electrode, V; E_{K0}^o is the standard potential of activity of potassium ions; γ'_K is the average activity coefficient of potassium ions; and C'_K is the potassium concentration in blood serum, mol/g.

Table 2. Comparison of the results of potentiometric and flame-photometric measurements of potassium concentration in blood serum

Serum sample	Results of measurements by the potentiometric procedure, mmol/L	Results of measurements by the flame-photometric procedure, mmol/L
1	4.6, 4.5, 4.4, 4.4	4.4
2	4.7, 4.6, 4.6	4.5
3	5.6, 5.5, 5.5	5.4
4	4.4, 4.4, 4.3, 4.3, 4.3, 4.3, 4.3, 4.3	4.2

The average value of the activity coefficient of potassium ions γ'_K was given in the tables [6], and the potassium concentration C'_K was associated with the concentration C_K (see relationship (3)) as follows:

$$C'_K = C_K/\rho, \tag{5}$$

where ρ is the serum density, g/L.

This liquid ion-exchange electrode showed high selectivity in measuring the potassium ions relative to sodium ions. At concentrations of sodium and potassium in blood serum of 100 μmol/g, the ratio of its output signals was 93 : 1. Moreover, to eliminate the effects of undesirable serum components, a cellophane membrane was used in the electrode assembly. The response time did not exceed 1 min (when the electrode was conditioned in a solution similar to blood serum). Table 2 shows the results of comparative tests of the developed procedure and the flame-photometric technique for determining the potassium concentration in real serum samples. The data reflect the fundamental similarity of the results obtained by the above procedures, although the potentiometric slightly overestimates (by 0.1–0.2 mmol/L) the results [6].

In 1970 Neff et al. improved the method proposed by Dahms in 1967 for determining the activity of potassium ions in blood. The improved technique was also based on the potentiometric method using the glass "potassium" and "sodium" electrodes. However, during the calibration of the electrodes by two standard solutions, the procedure allowed for calculation of the values of E_{K0} and k_{Na} at $S_K = S_{Na}$ or that of E_{K0} and S_K at a given value of k_{Na} (see expressions (1) and (2)). Both variants of calculations provided satisfactory results for measurements in the analysis of whole blood or blood plasma. At the same time, because of the noted operational instability of a glass "potassium" electrode, it is advisable to carry out periodic calibration not on two but three standard solutions and then calculate the values of E_{K0}, S_K, and k_{Na} [7].

In 1970 Neff developed another procedure for potentiometric determination of the potassium concentration in plasma, serum, and whole blood using glass "potassium" and "sodium" electrodes. The procedure was based on the following approximation equations [8]

$$C_K = (g_{K1}C_{Na} + g_{K2})\frac{E_K - E_{K2}}{E_{K1} - E_{K2}} + g_{K3}C_{Na} + g_{K4},$$

$$C_{Na} = g_{Na1}\frac{E_{Na} - E_{Na2}}{E_{Na1} - E_{Na2}} + g_{Na2}$$

where C_K and C_{Na} are the concentration of potassium and sodium in plasma, serum, and whole blood; E_{K1} and E_{K2} are the output signals of the glass "potassium" electrode when contacting with the first and second standard solutions, V; E_{Na1} and E_{Na2} are the output signals of the glass "sodium" electrode when contacting with the first and second standard solutions, V; and g_{K1}, g_{K2}, g_{K3}, g_{K4}, g_{Na1}, and g_{Na2} are the calibration constants for analysis of plasma, serum, or whole blood.

To find the constants g_{K1}, g_{K2}, g_{K3}, g_{K4}, g_{Na1}, and g_{Na2}, the calibration should be made by four standard solutions. In this case, it was necessary to use the additional results of flame-photometric measurements during calibration in the analysis of whole blood [8].

In 1971 Miyada et al. reported on a potentiometric procedure for determining the potassium concentration in plasma, serum, and whole blood using an ion-selective electrode with a neutral liquid ion-exchange membrane. The output signal of the electrode was described by Nicolsky equation [9]

$$E_K^o = E_{K0}^o + \frac{2.303RT}{F}\log(a_{K^+} + 0.0023a_{Na^+} + 0.01a_{NH_4^+}), \tag{6}$$

Table 3. Results of determination of potassium concentration in the blood serum samples

Serum sample	Measurement results, mmol/L			Serum sample	Measurement results, mmol/L		
	PP	FPP1	FPP2		PP	FPP1	FPP2
1	4.0	4.0	4.0	16	5.0	5.0	5.2
2	4.7	5.1	4.8	17	4.5	4.4	4.6
3	4.6	4.5	4.7	18	4.1	4.1	4.2
4	3.8	3.8	3.9	19	4.7	4.9	4.9
5	4.3	4.4	4.4	20	6.6	6.6	6.8
6	4.8	5.0	4.7	21	4.3	4.3	4.1
7	4.5	4.8	4.8	22	4.6	4.7	4.7
8	4.2	4.1	4.9	23	4.0	3.9	3.9
9	3.4	3.4	3.4	24	3.9	3.9	3.7
10	4.0	4.1	4.1	25	4.0	4.1	4.2
11	3.6	3.6	3.6	26	4.1	4.1	4.0
12	3.7	3.8	3.8	27	5.0	5.0	5.1
13	4.1	4.2	4.1	28	4.4	4.2	4.1
14	4.7	4.9	5.0	29	4.1	4.1	4.0
15	4.1	4.0	4.1	30	5.1	5.2	5.1

PP, potentiometric procedure; FPP, flame-photometric procedure.

Table 4. Results of comparative measurements of potassium concentration in the samples of whole blood and plasma

Sample	Potassium concentration, mmol/L			Sample	Potassium concentration, mmol/L		
	blood (PP)	plasma (PP)	plasma (FPP)		blood (PP)	plasma (PP)	plasma (FPP)
1	4.7	4.6	4.6	4	4.5	4.4	4.4
2	4.6	4.6	4.5	5	4.8	4.8	4.4
3	3.9	3.9	3.7	6	4.5	4.9	4.5

PP, potentiometric procedure; FPP, flame-photometric procedure.

where R is the universal gas constant ($R = 8.31$ J/(mol · K)) [10]; T is the temperature of the analyzed plasma, serum, or whole blood, K; F is the Faraday constant ($F \approx 96486.7$ C/mol) [10]; and $a_{NH_4^+}$ is the activity of ammonium ions, mol/L.

Other designations correspond to those in formulas (1) and (4).

As compared with a glass "potassium" electrode, this electrode provided higher ($0.1/0.0023 \approx 43.5$) selectivity of measurements of potassium ions relative to sodium ions. Thus, the determination of potassium ions can be performed without the correcting amendment, since the sodium ion activity is usually in a range from 130 to 150 mmol/L and the ammonium ion activity is not greater than 0.15 mmol/L in plasma, serum, or whole blood. To perform the assay, 200 μL of plasma, serum, or whole blood was required. The response time was estimated at the level of 1 min. Table 3 presents the results of measurements of the potassium concentration in serum samples by the proposed potentiometric procedure and by techniques developed by two different manufacturers of flame-photometric equipment. The data suggest a correlation between the results of determination by flame photometry and potentiometry. The results of measurements of the potassium concentration in the samples of whole blood and plasma by potentiometric and flame-photometric procedures are shown in Table 4. The results of the potentiometric analysis of whole blood and the corresponding plasma were essentially the same. This circumstance was important from a practical point of view, because it provided an opportunity to significantly reduce the labor intensity of determinations [9].

In 1972 Kim et al. compared experimentally the characteristics of spectrophotometric and turbidimetric techniques and the procedure for direct flame-photometric measurements, as well as the procedure for

Table 5. Effect of ammonia on the measurement results of potassium concentration in blood serum using an ion-selective electrode with valinomycin-containing membrane

Sample	Results of determination of potassium concentration, mmol/L
Initial blood serum	3.9
Ammonia-added serum (pH 8)	4.5
Ammonia-added serum (pH 9)	6.0

flame-photometric measurements using an internal standard, which were intended to determine the potassium concentration in blood serum. The spectrophotometric procedure was based on the precipitation of potassium by sodium kobaltinitrite and measurement of the emerald-green color of the solution of a formed cobalt choline ferrocyanide complex. In the turbidimetric technique, the reagent consisting of ethylenediaminetetraacetate, boron, trichloroacetic acid, ethyl alcohol, and sodium hydroxide was used. When this reagent acted on serum, a suspension of white microparticles was formed, and the weakening of intensity of the light flux passing through a suspension was determined. In the procedure for direct flame-photometric measurements a gas mixture of propane–oxygen was used; in the flame-photometric procedure with the internal standard, propane–air was used. The measurement error in the spectrophotometric technique exceeded the specified limits (±8%) for the determination of low, medium, and high concentrations of potassium in blood serum. The measurement error in the turbidimetric procedure was not satisfactory for determining low potassium concentrations. The technique of direct flame-photometric measurements provided the required accuracy, but periodic calibration of the equipment was required because of reading drifts. The flame-photometric technique using the internal standard also met the requirements for the accuracy of determination of the potassium concentration in serum, but stable readings began to be registered only after the onset of the heat balance of the equipment (in 0.5–1 h after switching on) [11].

In 1973 Smith et al. developed a potentiometric procedure for determining the potassium concentration in blood serum and plasma using an ion-selective electrode with a polyvinyl chloride membrane containing natural antibiotic valinomycin. The standard deviation of the relative measurement error was less than 1.8%. To perform the analysis, less than 0.25 mL of serum (plasma) was required. The response time did not exceed 1 min. The difference between the results of determining the potassium concentration in blood serum (plasma) by the developed procedure and by the technique for flame-photometric measurements using an internal standard and a gas mixture of propane–air was regarded as insignificant [12].

In 1974 Lustgarten et al. proposed a potentiometric procedure for determining the potassium concentration in blood serum using an ion-selective electrode with a valinomycin-containing membrane and preliminary dilution of the serum with an aqueous buffer solution containing triethanolamine, hydrazine sulfate, and nitric acid. The dependence of the output signal of the electrode on the potassium concentration was approximately linear. Some deviation from linearity was observed only at a concentration of potassium less than 2.5 mmol/L. The electrode had high selectivity of measurements. Only the presence of ammonia had a significant impact on the results of determining the potassium concentration in blood serum (Table 5). Glucose, sodium heparin, lithium ions, urea, penicillin, ampicillin, tetracycline, gentamicin, erythromycin, and other chemical compounds did not virtually affect the readings of the electrode. The serum volume required for the analysis was 0.2 mL. The standard deviation of the relative error of measurement corresponded to 1.39%. The discrepancy between the results of determining the potassium concentration using the proposed procedure and the flame-photometric technique did not exceed 0.3 mmol/L [13].

In 1974 Cook et al. reported on a flame-photometric procedure for determining the potassium concentration in blood serum. In this technique, instead of using a photomultiplier registering the radiation intensity at one wavelength as a flame emission receiver, a television camera that provides information on the emission spectrum was used. As a result of the research, virtually no differences in calculating the potassium concentration based either on height or the area of the peak emission were found. However, when direct flame-photometric measurements and those using an internal standard were performed, only minor differences in the results of determinations were recorded; higher reproducibility of the potassium concentration in blood serum (standard deviation of the relative error ~1.7%) was achieved with the use of an internal standard (a solution of cesium or lithium) [14].

In 1975 Mohan and Bates considered the possibility of using only aqueous standard solutions for calibrating ion-selective electrode (particularly an electrode with a liquid ion-exchange membrane based on

a solution of 10 mg of valinomycin in 25 mL of diphenyl ether) in potentiometric procedures for determining the potassium concentration in blood serum [15].

In 1976 Bokelund estimated experimentally the error components of flame-photometric determinations of the potassium concentration in blood serum. The study was conducted according to the procedure of direct flame-photometric measurements using an acetylene-based flame and preliminary dilution of serum with 5% aqueous isopropyl alcohol. The standard deviation of the relative error of determination of the potassium concentration was estimated at 1.6%. The contribution of the serum dilution error and the error of measuring the flame emission intensity was virtually the same (1.13%) [16]

$$Z_{FP} = \sqrt{Z_{SD}^2 + Z_{FI}^2} = Z_{SD}\sqrt{2} = Z_{FI}\sqrt{2}$$

where Z_{FP} is the standard deviation of the relative error of flame-photometric determinations of potassium concentration in blood serum, %; Z_{SD} is the standard deviation of the relative error of serum dilution, %; and Z_{FI} is the standard deviation of the relative measurement error of flame emission intensity while the diluted serum is introduced in it, %.

In 1976 Anderson reported on a new analytical method developed by Small et al.—ion chromatography. Two ion-exchange columns were used in the implementation of this method. The first (separation) column was used to separate the studied ions from each other in a flow of buffer solution (eluent), and the second (suppression) column was used to reduce the background electrical conductivity of the buffer solution, in which the electrical conductivity of separated ions was measured using conductometric detection. As a result of these processes, the time-dependent change in the electrical conductivity of the buffer solution was registered at the detector output, which looked like peaks resembling the spectrogram. In this case, the time corresponding to the peak maximum identified the ion passing through conductivity detector, the area or height of the peak, and the quantitative content of this ion in the buffer solution [17].

Based on the ion chromatography method, Anderson proposed a procedure for determining the potassium concentration up to 16 mmol/L in blood serum. The technique involved a preliminary 40-fold dilution of serum, the use of a Chromex DCS-X2-55 separation column, and a Chromex DA-X10-55 (6 × 500 mm) suppression column at a flow rate of 138 mL/h. The time for ion chromatographic determination of potassium concentration did not exceed 5 min [17].

In 1977 Ladenson analyzed the technique of direct potentiometric determination (without preliminary dilution) of potassium concentrations in whole blood. It was shown that there were no discrepancies in the measurement results for potassium concentration in whole blood and plasma. Similar to the studies of Miyada et al., this circumstance was of great practical importance when performing mass analyses in clinical diagnostic laboratories because of the sharp decrease in labor costs and increased productivity [18].

In 1979 Osswald et al. developed a technique for the direct potentiometric determination of potassium concentration in whole blood during open-heart surgery. The procedure was based on the use of an electrode with a liquid ion-selective membrane having the following composition: 1% valinomycin, 32.9% polyvinyl chloride, and 66.1% di(2-ethylhexyl) sebacate. The electrode showed high selectivity in measuring potassium ions relative to sodium ions, 16000 : 1 (at concentrations of these ions of 1 mol/L). In systematic calibration, the standard deviation of the relative error was less than 0.23%. Table 6 shows the results of measurements of potassium concentration in whole blood performed by the developed potentiometric technique and those in plasma using two flame-photometric techniques. When comparing the presented data, it is easy to note the high correlation of the results [19].

In 1979 Ladenson estimated the possibilities of another technique of direct potentiometric determination of the potassium concentration in whole blood, serum, and plasma. The technique was also based on the use of an electrode with a liquid membrane based on a neutral ion exchanger valinomycin. The dependence of the electrode output signal on the potassium concentration in blood serum or plasma was linear up to 10 mmol/L. A change in the content of sodium from 119 to 171 mmol/L, calcium from 2.77 to 6.1 mmol/L, magnesium from 0.54 to 1.73 mmol/L, lithium from 1.5 to 6.6 mmol/L, creatinine from 51 to 219 mg/L, uric acid from 94 to 265 mg/L, and urea from 350 to 1810 mg/L did not affect the results of measurements of potassium concentration in serum. The insignificant effect of pH of the analyzed medium was noted (Table 7). At a phosphate concentration in aqueous solution of 40 or 80 mg/L, deviations in the electrode readings were absent, but at concentrations of 230 and 380 mg/L the results of determination of potassium concentration were lower by 1.8 and 2.8%, respectively. A change of ammonia concentration in blood by 1 mmol/L caused a decrease in the electrode readings by 0.6%; and the introduction of a standard solution with a protein concentration of 177 g/L resulted in an increase by 1.25%. The results of measurements of potassium concentration in whole blood and plasma were actually the same. In addition, a comparison of results for determining the potassium concentration in serum using

Table 6. Results of determination of potassium concentration in whole blood and plasma

Sample	Potassium concentration, mmol/L			Sample	Potassium concentration, mmol/L		
	blood (PP)	plasma (FPP1)	plasma (FPP2)		blood (PP)	plasma (FPP1)	plasma (FPP2)
1	4.23	4.5	4.5	11	5.65	5.5	5.6
2	3.97	3.9	4.0	12	4.45	4.6	4.7
3	4.60	4.6	4.7	13	4.42	4.5	4.6
4	6.36	6.1	6.2	14	4.90	5.0	5.1
5	5.48	5.5	5.5	15	5.65	5.8	5.9
6	2.79	2.9	2.8	16	5.63	5.7	5.8
7	3.38	3.4	3.4	17	5.25	5.3	5.5
8	3.57	3.7	3.7	18	5.45	5.6	5.8
9	3.33	3.4	3.5	19	4.67	4.7	4.8
10	3.38	3.4	3.5	20	4.33	4.5	4.5

PP, potentiometric procedure; FPP, flame-photometric procedure.

Table 7. Effect of the medium pH on the results of determination of potassium concentration

pH of the medium	Measurement results, mmol/L	pH of the medium	Measurement results, mmol/L
9.64	3.85	5.93	3.94
7.57	3.87	5.55	3.93
7.02	3.86	4.26	3.95
6.57	3.91	2.84	3.96

the evaluated technique with those obtained by the flame-photometric method testified to the higher precision of direct potentiometric measurements (Table 8) [20].

4. STUDIES IN 1980-s

In 1980 Flevet et al. investigated a technique for determining the potassium concentration in blood serum; the method was based on the use of ion-selective electrode with a valinomycin-containing polymer membrane. It was found that the selectivity of the measurement of potassium ions relative to sodium ions was about 5000 : 1. The presence of albumin in serum did not practically change the results of determination of potassium concentration. However, a time-dependent change in the electrode characteristics was observed, and the presence of iodide ions at a concentration of 40–50 mmol/L in serum defined the devi-

Table 8. Accuracy of the determination of the potassium concentration in blood serum by the method of direct potentiometric measurements and the flame-photometric procedure

Serum sample	Results of measurements of the potassium concentration			
	mean value, mmol/L		standard deviation of relative error, %	
	PP	FPP	PP	FPP
1	3.07	3.1	1.5	2.2
2	6.32	6.4	1.2	2.2
3	6.79	6.6	1.7	2.0

PP, potentiometric procedure; FPP, flame-photometric procedure.

ation of the electrode readings by 5–10% (presumably due to the formation of a complex compound based on iodide and potassium ions) [21].

In 1980 Czaban and Cormier tried to explain why the potassium concentration values in blood serum obtained by the procedures for direct potentiometric measurements exceeded the values obtained using the flame-photometric techniques. The explanation of this fact was based on the presence of proteins and lipids in the serum. Although they usually occupy about 7% of its volume, the possibility of chemical interactions between potassium and these organic compounds was not taken into account [22].

In 1982 Langhoff and Stelness examined experimentally the effects of proteins and lipids on the results of the determination of the potassium concentration by the method of direct potentiometric measurements with valinomycin-based ion-selective electrode and by the method of flame-photometric measurements. The presence of albumin at a concentration of 70 g/L in the analyzed medium caused a decrease in the results of potentiometric determination of potassium concentration by less than 2% and in the results of flame-photometric determinations by 5.5%. The presence of lipids in the blood plasma in fact did not change the results of potentiometric measurements but reduced the results of flame-photometric measurements by 16% [23].

In 1982 Gramlich et al. proposed a procedure for determining the potassium concentration in blood serum using the mass spectrometric method. Measurement of the ratio of $^{39}K/^{41}K$ potassium isotopes after the addition of a known amount of a solution containing the isotope ^{41}K to a pre-weighed sample of serum was the basis of the technique. To find the measurement result, the following expression was used [24]:

$$C_{KS} = C_{KA}\frac{W_A(A_A - B_A L)}{W_S(B_S L - A_S)}, \quad (7)$$

where C_{KS} and C_{KA} are the potassium concentrations in a serum sample and the added solution, g/g; W_S and W_A are the weight of a serum sample and the added solution, g; A_S and A_A are the portions of ^{39}K isotope in a serum sample and the added solution; B_S and B_A are the portions of ^{41}K isotope in a serum sample and the added solution; and L is the measured ratio of potassium isotopes $^{39}K/^{41}K$.

Finally, the potassium concentration in blood serum was determined by the formula [24]

$$C_K = C_{KS}\frac{\rho}{M}$$

where M is the atomic weight of potassium (M = 39.0983 g).

Other designations are given in designations for formulas (3), (5), and (7).

The proposed technique was characterized by a relative error in determining the potassium concentration in serum of less than 0.1%, which made it possible to use it as a reference. The data in Table 9 illustrate the accuracy of measurements of potassium concentration in seven parallel analyses of one serum sample performed by two laboratory assistants [24].

In 1982 Chariton et al. developed a procedure for determining the potassium concentration in serum up to 10 mmol/L using polyester test-strips coated with polyvinyl chloride, diphenyl phthalate, and valinomycin. The serum was diluted 9 times with a solution of erythrosine B; 140 μL of diluted serum was dosed to cover the test strips and, after standing at room temperature for 4 min, was washed with water. Then, a test-strip was placed in the measuring device, in which the absorption or reflection of its coating when irradiated with light with a wavelength of 550 nm was determined. The dependence of the reflection on the potassium concentration in blood serum was close to directly proportional [25]:

$$A_r \approx bC_K$$

where A_r is the reflection characteristic of the test-strip coating and b is the constant.

The dependence of absorption was of a more complex character [25]:

$$A_a \approx \frac{C_K}{b_0 + b_1 C_K},$$

where A_a is the spectral absorption capacity of the test-strip coating and b_0 and b_1 are the constants.

In both expressions C_K corresponds to the designation to the relation (3).

Concentrations of sodium up to 900 mmol/L and lithium up to 10 mmol/L did not affect the results of measurements of potassium concentration. In addition, the results of determining the potassium concentration in blood serum by the developed technique were correlated to the results obtained by flame-photometric method [25].

Table 9. Results of determination of the potassium concentration in blood serum by the mass spectrometry procedure

Number of parallel determination	Measurement results, mmol/L		Number of parallel determination	Measurement results, mmol/L	
	Lab assistant 1	Lab assistant 2		Lab assistant 1	Lab assistant 2
1	3.4981	3.5020	6	3.5255	3.5288
2	3.5443	3.5427	7	3.5189	3.5197
3	3.5203	3.5221	Mean value		
4	3.5135	3.5145		0.0140	0.0131
5	3.5158	3.5156	Standard deviation		
6	3.5145	3.5119			

Table 10. Results of measurements of the potassium concentration by the procedures of direct potentiometric determination with chemical field-effect transistor (PPT), direct potentiometric measurements with ion-selective electrode (PPE), and the flame-photometric procedure (FPP)

Measurement results, mmol/L		
PPT	PPE	FPP
3.8	4.05	4.1

In 1983 Sibbald et al. reported that the chemical field-effect transistors are still not widely used in clinical diagnostic laboratories, although the first such measuring device was described by Bergveld in 1970. In order to eliminate this situation, Sibbald et al. proposed a procedure for the direct potentiometric determination of potassium concentration in whole blood using the field-effect transistor, which had a shutter based on polyvinyl chloride and valinomycin. The procedure made it possible to measure the potassium concentration from 0.1 to 100 mmol/L. The output signal of the transistor obeyed the Nernst equation [26]

$$E_K^m = E_{K0}^m + \frac{2.303RT}{F}\log a_{K^+}$$

where E_K^m is the output signal of the chemical field-effect transistor, V, and E_{K0}^m is the standard potential of transistor, V.

Other designations are given in designations for formulas (1) and (6).

When performing 25 consecutive determinations of potassium concentration of 4.5 mmol/L, the standard deviation was 0.04 mmol/L. The response time of the field-effect transistor was estimated at the level of 2 s. Table 10 shows the results of measurements of potassium concentration by the proposed method, the method of direct potentiometric determination with ion-selective electrode, and the flame-photometric technique [26].

In 1984 Fogh-Andersen et al. investigated the conditions for blood storage before analysis. As a result, it was found that the optimum temperature of blood storage while determining the potassium concentration is 20°C (Table 11). Moreover, Fogh-Andersen et al. noted the correlation of the measurement results for potassium concentration obtained using the potentiometric technique with ion-selective electrodes and the flame-photometric procedures [27].

In 1985 Maas et al. stated that the activity of potassium ions in whole blood or serum determined by the techniques of direct potentiometric measurements with ion selective electrodes should be multiplied by a correction factor to get the potassium concentration, which is a result of flame-photometry determinations. This coefficient depends on the mass content of water in blood (serum), the activity coefficient of potassium ions in plasma, the extent of binding of potassium ion to the chemical compounds, and the differences between the potentials of the liquid junction of the reference electrode with plasma and the standard solutions used for calibration. When solving practical problems, it can be assumed that the potassium concentration in blood plasma determined by the procedures for direct potentiometric measurements with ion-selective electrodes coincides with the concentration obtained in flame-photometric determinations. To do this, it is sufficient to perform the calibration of ion-selective electrodes by standard solutions with constant ionic strength (and hence a constant activity coefficient of potassium ions), which

Table 11. Change of potassium concentration in whole blood at different storage temperatures

Storage time, h	Potassium concentration, mmol/kg		
	at storage temperature 4°C	at storage temperature 20°C	at storage temperature 37°C
0	4.18	4.18	4.18
2	4.38	4.17	4.19
4	4.51	4.13	4.02
8	5.19	4.18	4.55
24	7.75	4.06	14.80

would ensure compliance of the output signals of the electrodes with the Nernst equation and be consistent with the data of flame-photometric measurements [28].

In 1985 Wong et al. developed a spectrophotometric procedure for determining the potassium concentration up to 15 mmol/L in plasma, serum, and whole blood. The procedure was based on the interaction of potassium, crown ether, and picrate to form the complex compound [29]

$$\text{potassium} + \text{crown ether} + (\text{picrate}) \leftrightarrow (\text{potassium} - \text{crown ether})^{+}(\text{picrate})^{-}$$

Aqueous solution (0.9 mL) containing picric acid (8.56 mmol/L) and dimethyl sulfoxide (282 mmol/L), and 3.0 mL of diphenyl ether solution containing 18-Crown-6 (3 mmol/L) and hexachloroethane (1.28 mol/L) were added to 37.5 μL of serum (plasma) at ambient temperature. After shaking the reaction mixture, the organic phase was separated and the absorbance was measured at wavelength of 415 nm. The dependence of the absorbance on the potassium concentration was directly proportional [29]

$$A = 0.043C_K,$$

where A is the absorbance of the organic phase.

Designation C_K coincides with the designation to expression (3).

The presence of calcium up to 200 mg/L, cobalt up to 0.5 mg/L, manganese up to 25 mg/L, copper up to 40 mg/L, zinc up to 12 mg/L, magnesium up to 10 mmol/L, ammonium up to 1.2 mmol/L, triglycerides up to 5 g/L, hemoglobin up to 1.5 g/L, and bilirubin up to 0.5 g/L had no effect on the results of determining the potassium concentration in serum (plasma). The selectivity of measurements of potassium relative to sodium was 600 : 1. Before assaying the whole blood, the plasma was separated by centrifuging. The results of determining the potassium concentration by the developed technique were correlated with the results of flame-photometric measurements [29].

In 1986 Shintani proposed an ion chromatographic method for determining the potassium concentration in blood serum. At first, serum was filtered, and then 10 μL of the filtered serum was injected into the ion chromatograph equipped with a cation-exchange column. Nitric acid at a concentration of 6.3 mmol/L was used as an eluent. The flow rate was 1 mL/min. Determination of the potassium concentration was carried out at ambient temperature. The runtime of ion-chromatographic analysis did not exceed 5 min. A ten-fold repeated assay of the same sample of serum by ion chromatography gave a standard deviation of the relative measurement error of 2.13%. Table 12 shows the results of determination of the potassium concentration in 110 serum samples by the proposed procedure and the potentiometric technique using an ion-selective electrode. The discrepancy between these results was estimated to be 0.1 mmol/L [30].

In 1986 Oesch U. et al. indicated that, in general, the output signal of ion-selective electrode is described by the Nikolskii–Eisenman equation [31]

$$E = E_0 + E_D + \frac{2.303RT}{z_i F}\log\left[a_i + \sum_{j\neq i} k_{ij} a_j^{(z_i/z_j)}\right],$$

where E is the output signal of ion-selective electrode (electromotive force arising between ion-selective and reference electrodes), V; E_0 is a constant component of the potential difference between ion-selective and reference electrodes, V; E_D is the liquid-junction potential generated between reference electrode and sample solution, V; a_i, and a_j are the activity of the measured and interfering ions, mol/L; z_i and z_j are charge numbers of the measured and interfering ions; and k_{ij} is the selectivity factor of the measured ion relative to interfering ion.

Table 12. Results of determination of potassium concentration in 110 serum samples by the procedures of ion-chromatography and potentiometry with an ion-selective electrode

Mean potassium concentration in serum, mmol/L	
Ion-chromatographic procedure	Potentiometric procedure
4.0	4.1

Table 13. Required characteristics of potassium-selective electrode for determining the potassium concentration in plasma, serum, and whole blood

Measurement range, mmol/L	Change in the liquid junction potential, mV	Selectivity coefficient of potassium ion relative to				
		hydrogen ion	lithium ion	sodium ion	magnesium ion	calcium ion
more than 3.5–5.0	no more than 0.46	no more than 630	no more than 0.02	no more than 0.00025	no more than 0.0015	no more than 0.0012

Other designations correspond to those for relation (6).

Based on this expression, Oesch et al. formulated the requirements to be met by a potassium-selective electrode for determining the potassium concentration in plasma, serum, and whole blood (Table 13). Most of the potassium-selective electrodes produced at that time had a polyvinyl chloride membrane with valinomycin, and their characteristics mainly met these requirements. At the same time, research on the replacement of valinomycin with synthetic crown ethers was conducted; the results demonstrated the possibility of creating potassium-selective electrodes with a measurement selectivity similar to that of electrodes with valinomycin [31].

In 1987 Hajós et al. compared two ion chromatographic procedures for determining the potassium concentration in blood serum. In the first, a separation column (9 × 250 mm) was used in combination with a suppression column; in the second, only a separation column (3 × 100 mm) was used. Nitric acid at a concentration of 5 mmol/L was used as an eluent. The serum samples were diluted, acidified, and filtered prior to the injection in ion chromatograph. The results of measurement of potassium concentration by both methods were virtually identical (2.2 and 2.3 mg/L); however, the time for determination was 9.6 min by the first technique and 6.25 min by the second. The presence of sodium at a concentration of 30 mg/L did not affect the measurement results of potassium concentration, as the retention time of sodium in the first technique was 7.2 min and 3 min in the second technique [32].

In 1987 Thode et al. investigated experimentally the value of the Donnan relationship for potassium ions (equation (8)) by means of potentiometric measurements at different protein concentrations in serum (Table 14). These results confirmed that the activity of potassium ions in the protein-containing serum increases as a result of a decrease in plasma volume [33]

$$a_{K^+}^{(P)} \Big/ a_{K^+}^{(PF)}, \quad (8)$$

where $a_{K^+}^{(P)}$ is the activity of potassium ions in blood serum containing proteins, mol/L; and $a_{K^+}^{(PF)}$ is the activity of potassium ions in protein-free serum, mol/L.

In 1988 Kumar et al. reported that the possibility of spectrophotometric determination of potassium concentration in plasma and serum has increased many times because of the results obtained by Pedersen C. J., Lehn J. M., and Cram D. J., the 1987 Nobel Prize Laureates. These scientists designed and synthesized novel macrocyclic compounds (crown ethers, cryptands, and spherands), which, like the natural antibiotic valinomycin, can selectively form chemical complexes with potassium. The selectivity of the formation of chemical complexes was explained by the presence of the so-called cavity in the structure of these compounds, which allows interaction with the potassium ion rather than another ion [34].

Based on the principles developed by Pedersen, Lehn, and Cram, Kumar et al. proposed a spectrophotometric procedure for determining the potassium concentration in plasma and serum. A reagent under the trade name ChromoLyte (350 μL) containing buffer solution (pH 7.3), stabilizer, and macrocyclic compound was added to 3.5 μL of plasma (serum). The reaction proceeded at a temperature of 37°C for 2 min, and then the absorbance of the resulting solution was measured at wavelength of 500 nm. The dependence of absorbance on the potassium concentration was linear up to 10 mmol/L. The standard deviation of the relative measurement error did not exceed 1.5% (Table 15). Calcium (0.9 mmol/L), mag-

Table 14. Results of experimental studies of the Donnan relationship for potassium ions at different albumin concentrations

Albumin concentration, g/L	Donnan relationship	Albumin concentration, g/L	Donnan relationship
120	1.09	29	1.02
75	1.05	14	1.01

Table 15. Standard deviation of relative error in measurements of potassium concentration

Potassium concentration, mmol/L	Standard deviation of relative error, %	Potassium concentration, mmol/L	Standard deviation of relative error, %	Potassium concentration, mmol/L	Standard deviation of relative error, %
2.6	1.5	5.6	1.0	10.4	0.5

nesium (2.6 mmol/L), iron (4.3 mmol/L), pyruvate (2.3 mmol/L), lactate (2.6 mmol/L), salicylate (1.9 mmol/L), ascorbic acid (0.3 mmol/L), acetaminophen (0.3 mmol/L), ethanol (33 mmol/L), glucose (56 mmol/L), urea (8.3 mmol/L), uric acid (1.2 mmol/L), and creatinine (3.1 mmol/L) did not affect the results of the determination of the potassium concentration in plasma (serum). Sodium (from 80 to 200 mmol/L) caused changes in the potassium concentration measurement results by not more than 0.1 mmol/L. The presence of bilirubin (170 mmol/L) in plasma (serum) increased the results of potassium concentration determination by 0.4 mmol/L. Ammonium ions (because of their size, which is similar to potassium ions) had a significant effect on the measurement results. At the same time, a comparison of the results of determining the potassium concentration by the proposed method and by the potentiometric procedure using an ion-selective electrode showed no significant discrepancies [34].

In 1989 Berry et al. developed a kinetic spectrophotometric procedure for determining the potassium concentration in blood serum using pyruvate kinase enzyme. Since pyruvate kinase was activated in the presence in serum not only of potassium ions but of sodium and ammonium ions, therefore at first the following reactions were performed:

1) binding of sodium ions in a complex with cryptand

$$Na^+ + \text{cryptand} \rightarrow Na^+ - \text{cryptand}$$

2) removal of ammonium ions as a result of the reaction with glutamate dehydrogenase

$$NH_4^+ + \text{2-ketoglutarate} + NADH \xrightarrow{\text{glutamate dehydrogenase}} \text{glutamate} + NAD^+ + H_2O,$$

where *NADH* is the reduced form of nicotinamide adenine dinucleotide; NAD^+ is nicotinamide adenine dinucleotide [35].

And then:

1) pyruvate kinase activated by potassium ions stimulated the conversion of phosphoenolpyruvate to pyruvate

$$\text{Phosphoenolpyruvate} + ADP \xrightarrow{\text{pyruvate kinasse, } K^+} \text{pyruvate} + ATP$$

where *ADP* is adenosine diphospate; *ATP* is adenosine triphosphate;

2) the formed pyruvate was converted to lactate under the action of lactate dehydrogenase

$$\text{pyruvate} + NADH + H^+ \xrightarrow{\text{lactate dehydrogenase}} \text{lactate} + NAD^+$$

3) the change in the absorbance of the solution formed at the last enzymatic reaction at a wavelength of 340 nm characterized the transition of *NADH* to NAD^+ and depended on the potassium concentration in blood serum [35].

To implement the assay, 200 μL of the reagent containing cryptand (under the trade name Kryptofix 221) and glutamate dehydrogenase were added to 10 μL of serum. The mixture was kept at 37°C for 2 min. Then 12 μL of the reagent containing pyruvate kinase and lactate dehydrogenase were introduced, and a change of absorbance (at a wavelength of 340 nm) of the solution formed was registered at 5-second intervals for 2 min. The dependence of the change in absorbance with time on the potassium concentration in blood serum was linear up to 8 mmol/L. The presence of albumin (20 g/L), bilirubin (300 μmol/L), ammonium chloride (2.5 mmol/L), lithium chloride (5 mmol/L), sodium chloride (55 mmol/L), magnesium sulfate (2 mmol/L), calcium chloride (2.5 mmol/L), zinc sulfate (20 μmol/L), copper sulfate

Table 16. Results of determination of potassium concentration in blood serum

Serum sample	Mean value of potassium concentration, mmol/L	Standard deviation of relative error, %	Serum sample	Mean value of potassium concentration, mmol/L	Standard deviation of relative error, %
1	4.697	1.40	2	7.105	1.35
1	4.720	1.22	2	6.959	1.31

(20 μmol/L), iron chloride (20 μmol/L), and aluminum nitrate (20 μmol/L) in serum caused a change in the results of the determination of the potassium concentration by less than 0.1 mmol/L. The results of 20 repeated measurements of potassium concentration in two serum samples that were carried out on different days are presented in Table 16. The standard deviation of the relative error of measurement of potassium concentration did not exceed 1.4%. According to Berry et al., there was no difference in the results of potassium concentration determination by the developed technique and by the flame-photometric measurements [35].

5. STUDIES IN THE 1990-s

In 1990 Bracken et al. proposed a potentiometric procedure for determining the potassium concentration up to 10 mmol/L in plasma, serum, and whole blood using an ion selective electrode with a membrane of polyvinyl chloride and dioctyl sebacate with the addition of valinomycin and tetrakis(4-chlorophenyl)borate. The standard deviation of the relative error of measurement of potassium concentration did not exceed 2%. The results of the determinations were correlated with the results of the flame-photometric measurements. Acetaminophen (300 mg/L), warfarin (100 mg/L), acetyl salicylic acid (500 mg/L), and salicylic (400 mg/L) and ascorbic (100 mg/L) acids had an insignificant effect, and citrates (10 g/L) and oxalates (8 g/L) significantly influenced the results of determination of the potassium concentration [36].

In 1992 Kimura et al. developed a kinetic spectrophotometric procedure for determining the potassium concentration in blood serum using enzyme tryptophanase [37]. Since tryptophanase was activated in the presence of both potassium ions and ammonium ions in serum, the removal of ammonium ions was at first carried out by the glutamate dehydrogenase-catalyzed reaction:

$$NH_4^+ + 2-\text{oxoglutarate} + NADPH \xrightarrow{\text{glutamate dehydrogenase}} \text{glutamate} + NADP^+ + H_2O, \qquad (9)$$

where *NADPH* is the reduced form of nicotinamide adenine dinucleotide phosphate and $NADP^+$ is nicotinamide adenine dinucleotide phosphate [37].

After that, the enzyme tryptophanase, activated by potassium ions, catalyzed the transformation of tryptophan [37]

$$\text{tryptophan} + 5H_2O \xrightarrow{\text{tryptophanase, } K^+} \text{indole} + \text{pyruvate} + NH_4^+ + OH^-$$

and the formed ammonium ions entered the reaction (9). The change in absorbance (at a wavelength of 340 nm) of the solution formed in the course of reaction (9) reflected the transformation of *NADPH* to $NADP^+$ and was proportional to the potassium concentration in blood serum. To perform the assay, 250 μL of a reagent containing 2-oxoglutarate (18.6 mmol/L), *NADPH* (0.41 mmol/L), tryptophan (18.6 mmol/L), and glutamate dehydrogenase were added to 15 μL of serum. The mixture was kept at 37°C for 5 min. Then 200 μL of the reagent containing tryptophanase were introduced, and in 80 s the change of absorbance (at a wavelength of 340 nm) of the solution formed was registered at 20-second intervals for 220 s. The dependence of change in absorbance with time on the potassium concentration in blood serum was linear up to 7 mmol/L. Ammonium ions (up to 3 mmol/L), sodium ions (100–200 mmol/L), lithium ions (up to 5 mmol/L), NH_4^+ (from 1.29 to 6.49 mmol/L), Ca^{2+} (from 2.2 to 5.0 mmol/L), Mg^{2+} (from 0.8 to 5.0 mmol/L), Zn^{2+} (up to 40 μmol/L), Mn^{2+} (up to 40 μmol/L), Fe^{3+} (up to 40 μmol/L), hemoglobin (up to 5.2 g/L), bilirubin (from 10 to 218 mg/L), and albumin (up to 60 g/L) virtually did not affect the results of determination of potassium concentration of 4.1 mmol/L in serum. The results of 10 repeated measurements of potassium concentration in three samples of blood serum are shown in Table 17. The standard deviation of the relative error of determination of potassium concentration was less than 1.32%. There was no discrepancy between the results of measurements of potassium concentration in the developed and flame-photometric techniques [37].

Table 17. Results of determination of potassium concentration in three samples of blood serum

Measurement results, mmol/L					
Sample 1		Sample 2		Sample 3	
3.40	3.38	4.56	4.46	5.56	5.53
3.40	3.34	4.53	4.45	5.53	5.48
3.34	3.32	4.51	4.42	5.59	5.42
3.38	3.28	4.51	4.51	5.48	5.51
3.42	3.40	4.42	4.48	5.48	5.48
Mean value	3.366	Mean value	4.485	Mean value	5.506
Standard deviation of relative error, %	1.32	Standard deviation of relative error, %	1.05	Standard deviation of relative error, %	0.89

In 1992 Ng et al. analyzed the technique for determining the potassium concentration in plasma and serum by the method of reflectance photometry. In this technique, the used test-strips contain three layers: 1) the sample application layer; 2) the layer consisting of glass fiber for the sample transport; 3) the measuring layer, which contains valinomycin and a pH indicator dye, 4-[(2,6-dibromonitrophenyl)azo]-2-octadecyloxy-1-naphthol [38].

On the surface of the sample application layer, 30 μL of plasma (serum) were dosed. The specimen diffused through the transport layer to the measuring pad, where potassium ions contained in plasma (serum) bound valinomycin

$$K^+ + \text{valinomycin} \rightarrow K^+ - \text{valinomycin},$$

and an equivalent amount of protons were released into plasma (serum), which changed the color of the pH indicator. Then the test-strip was placed in the receiving chamber of a measuring instrument at 37°C, and the instrument displayed the potassium concentration in plasma (serum) in 140 s. The measurement was conducted at a wavelength of 643 nm. The dependence of the output signal on the potassium concentration was linear in the range from 2.2 to 11.5 mmol/L. The presence of bilirubin (299 μmol/L) and triglycerides (46.5 mmol/L) in plasma (serum) caused a change in the results of potassium concentration determination by less than 0.12 mmol/L. The results of measuring the potassium concentration in three certified solutions obtained using the reflectance photometry method, the potentiometric method with an ion-selective electrode, and the flame-photometric method are shown in Table 18. It is easy to notice that the deviation of the results obtained using the reflectance photometry technique does not exceed 2% of the certified value [38].

In 1992 Burritt pointed to the desirability of standardizing methods of direct potentiometric measurements with ion-selective electrodes. It was emphasized that the main reason for the observed differences in the results of determining the potassium concentration in blood by direct potentiometric methods, indirect potentiometric methods (with pre-dilution of the sample), and the flame-photometric technique was the excessively wide range of equipment and materials for calibration [39].

In 1992, Gunaratna et al. reported that, in accordance with the data for 1991, approximately 96% of clinical diagnostic laboratories applied potentiometric methods using ion-selective electrodes to determine the potassium concentration in blood; only about 4% used the flame-photometric methods. Moreover, the techniques of direct potentiometric measurements with electrodes containing a membrane of polyvinyl chloride with the addition of valinomycin constituted more than 50% of the potentiometric procedures. This was explained by the simplicity, high performance (analysis time from 10 to 60 s), and reproducibility of determinations. It was noted that direct potentiometric techniques using ion-selective electrodes made it possible to measure the activity of potassium ions and recalculate it in concentration, while the flame-photometric techniques made it possible to measure only the potassium concentration. Although the activity of potassium ions is a more capacious physiological indicator, the long-held belief about the need to determine the potassium concentration in blood determined the expediency of designing standard materials in which the potassium content would correspond to the results of the flame-photometric measurements. After testing in numerous clinical diagnostic laboratories, Gunaratna et al. submitted such materials—certified specimens based on the frozen human blood serum—and thus solved the long-debated problem [40].

In 1994 Steen et al. evaluated the applicability of the kinetic spectrophotometric technique for determining the potassium concentration in blood serum using enzyme pyruvate kinase, which was proposed

Table 18. Results of measurements of potassium concentration in certified solutions by the procedures of reflectance photometry, potentiometry with ion-selective electrode, and flame photometry

Procedure	Potassium concentration, mmol/L		
	Solution 1	Solution 2	Solution 3
Reflectance photometry	5.91	3.95	2.05
Potentiometry with ion-selective electrode	6.14	4.07	2.03
Flame photometry	5.92	3.95	1.96
Certified value of potassium concentration in the solution	6.03	4.03	2.05

by Berry et al. While performing the studies, a low selectivity of measurements of the potassium concentration in the presence of sodium was found [41].

In 1994 Van Pelt compared the standard deviations of the relative error of determining the potassium concentration in blood serum by the enzymatic spectrophotometric procedure, the flame-photometric method, and the method of indirect potentiometric measurements with ion-selective electrode (Table 19). The comparison results clearly showed a greater accuracy for the potentiometric and flame-photometric techniques [42].

In 1994 Hubl et al. compared the results of the potassium concentration determination in blood serum of patients suffering from a number of diseases. The results were obtained by the kinetic spectrophotometric technique with the use of pyruvate kinas as developed by Berry et al., the procedure for indirect potentiometric measurements with ion-selective electrode based on valinomycin, and the flame-photometric technique using a propane-based gas mixture and cesium solution as an internal standard. Although much of the observed deviations in the measurements of potassium concentration did not have medical significance, the results of determinations by potentiometric and flame-photometric methods were considered to be more accurate [43].

In 1998 Thienpont et al. organized the quality control for five procedures of direct potentiometric measurements and three procedures of indirect potentiometric measurements for determining the potassium concentration in blood serum. Verification was conducted in several clinical diagnostic laboratories. To certify the potassium concentration in the examined serum samples, the ion chromatographic technique was used (eluent, an aqueous solution of sulfuric acid at a concentration of 7 mmol/L). This method provided a systematic relative error of attestation of less than 0.65%, a standard deviation of the relative error up to 1.5%, and a total relative error of ~1.6%. The potentiometric techniques demonstrated the compliance to the requirements (systematic relative error of measurements of potassium concentration in blood serum, no more than 1.6%; and the total relative error, no more than 6.3%). In this case, the main cause of the found deviations was not the shortcomings of the potentiometric methods, but inadequate organization of their use in clinical diagnostic laboratories (installation and maintenance of equipment not in accordance with the documentation, conducting internal inspections without the recommended samples for quality control, etc.) [44].

In 1999 Hartland and Neary confirmed experimentally that the potassium concentration in serum is increased by an average of 0.4 mmol/L when sampling blood for examination because of the potassium release from erythrocytes and platelets. In this regard, Hartland and Neary indicated the importance of selecting the equipment used in blood sampling, as well as the fact that plasma is the preferred medium for the determination of potassium concentration [45].

6. STUDIES IN THE 2000-s

Since hemoglobin (one of the erythrocyte components) affects the potassium concentration in blood plasma, Hawkins presented the results of a study of the potassium / hemoglobin ratio in 2002 and 2003. It was found that this ratio can be in a rather wide range (from 0.21 to 0.345 mmol/g) and that the average value of the ratio between the potassium and hemoglobin concentrations is 0.284 mmol/g [46, 47].

In 2004 Shepherd and Baldwin found that contamination with products of vital activity of the bacteria *Pseudomonas aeroginosa* may occur with nonsystematic use of equipment for potentiometric measurements of the potassium concentration in blood. The contamination is eliminated by washing the internal parts of the equipment with gentamicin solution [48].

Table 19. Standard deviations of relative error in measuring the potassium concentration in blood serum

Procedure	Standard deviation of relative error, %
Enzymatic spectrophotometry	1.57
Indirect potentiometry with ion-selective electrode	0.75
Flame photometry	0.74

In 2006 Dimeski et al. proposed a formula to adjust the results of indirect potentiometric measurements of potassium concentration in blood containing a significant amount of lipids (cholesterol and triglycerides) [49]

$$C_K^{\mathrm{C}} = C_K^{\mathrm{M}} + 0.004C_{\mathrm{L}}, \tag{10}$$

where C_K^{C} is the corrected concentration of potassium, mol/L; C_K^{M} is the measured corrected concentration of potassium, mol/L; and C_{L} is the concentration of lipids, mol/L.

In contrast to the relation (11) put forward by Steffes and Freier in 1976, the proposed expression took into account the effect of not only triglycerides but also cholesterol [49]

$$C_K^{\mathrm{C}} = C_K^{\mathrm{M}} + \frac{(0.21C_T - 0.6)C_K^{\mathrm{M}}}{100}, \tag{11}$$

where C_T is the concentration of triglycerides, g/L.

Other designations coincide with those for formula (10).

In 2006 Carayannopoulos et al. analyzed the fluorescent method for determining the potassium concentration in blood. The method was based on "quenching" the luminescence of macrocyclic cryptand covalently bound to *o*-alkoxyaniline-based luminophor in the presence of potassium ions. The pH of the medium being analyzed and the presence of calcium, sodium, and lithium in it insignificantly affect the results of potassium concentration measurements. Ammonium chloride resulted in an increase in potassium concentration determination by ~ 1 mmol/L at a concentration of 0.5 mmol/L, by ~ 1.5 mmol/L at a concentration of 1 mmol/L, by ~ 4.5 mmol/L at a concentration of 3 mmol/L, and by ~ 6.5 mmol/L at a concentration of 5 mmol/L. This fact was explained by the chemical interaction of a macrocyclic cryptand with both potassium ions (~ 0.133 nm in size) and ammonium ions (~ 0.143 nm in size). At the same time, Carayannopoulos et al. noted that ammonium chloride at a concentration of 5 mmol/L did not influence the results of measurements of potassium concentration by the methods of direct and indirect potentiometric measurements with ion-selective electrodes [50].

In 2006 Lin and Tusa agreed with the results of studies conducted by Carayannopoulos et al. but emphasized that cases in which the concentration of ammonium ions in blood exceeds 0.5 mmol/L are extremely rare in medical practice [51].

7. CONCLUSION

The chronological development of procedures for determining the potassium concentration in plasma, serum, and whole blood is considered since the middle 1950s. In accordance with the methods applied for analysis, we can distinguish among the developed techniques the following (Fig. 1): flame-photometric, potentiometric, ion chromatographic, mass-spectrometric, X-ray fluorescence, spectrophotometric, turbidimetric, and luminescence.

In this case, the flame-photometric techniques can be further divided into procedures using an internal standard and procedures without its use. According to the type of sensor used, potentiometric techniques can be subdivided into the techniques with a glass "potassium" electrode, a liquid potassium-selective electrode, a solid-state potassium-selective electrode, and a field-effect transistor; they can also be classified as direct and indirect potentiometric procedures, depending on the presence or absence of sample preparation. Spectrophotometric techniques can be divided into enzymatic and nonenzymatic ones, as well as procedures of reflectance photometry.

Flame-photometric techniques for determining the concentration of potassium in plasma and serum, both with and without using an internal standard, have been applied in clinical diagnostic laboratories for almost 60 years. Solutions of lithium or cesium are used as an internal standard, and the gas mixture forming the flame is typically a propane–air mixture. Flame-photometric methods are considered expert methods because of the reliability of the measurement results, proven by years of research, and entrenched views regarding the need to determine the potassium concentration in blood. However, the runtime of

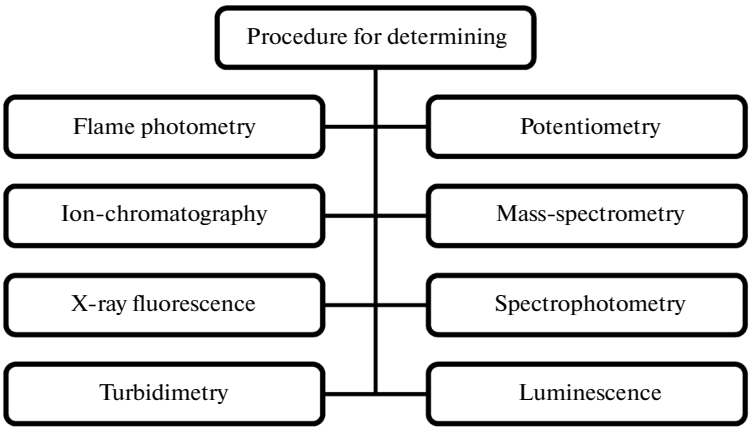

Fig. 1. Classification of the procedures for determining the potassium concentration in blood.

flame-photometric analysis often constitutes a few minutes, and the gas mixtures used can be a source of explosive and flammable situations.

Potentiometric procedures for determining the potassium concentration in plasma, serum, and whole blood have been applied in clinical diagnostic laboratories for more than 50 years. During this time they have undergone numerous changes and improvements. Thus, techniques using a glass "potassium" electrode are thing of the past, and techniques using field-effect transistors are not widely used in practice because of the difficulties that arose in their production technology. At present, potentiometric techniques with ion-selective electrodes on the basis of valinomycin or its analogs are characterized by the maximum level of use, both in small and medium-sized laboratories and in large diagnostic centers. Moreover, both direct potentiometric techniques and procedures for indirect potentiometric analysis are widely used; the results of measurements obtained using these techniques are regarded as coincident with the results of determination of potassium concentration by flame-photometric methods.

Ion-chromatographic methods for determining the potassium concentration in plasma and serum are "younger" techniques that have emerged since the middle 1970s but are claiming the title of expert techniques. Ion-chromatography techniques found preferential use in research organizations because of the rather long runtime of assay (usually for a few minutes) and the relatively high cost of the equipment.

Mass-spectrometric methods for determining the potassium concentration in blood were proposed at the turn of the 1970s and 1980s. The implementation of these techniques takes a long time and requires expensive equipment and highly skilled personnel. However, mass-spectrometric techniques are recognized as expert techniques intended for the certification of reference materials because of their very high precision (the relative error of potassium concentration determination is estimated at 0.1%).

X-ray fluorescence techniques for determining the potassium concentration in blood serum were developed in the late 1950s. These techniques had a measurement accuracy comparable to that of the flame-photometric methods but were inferior to them in performance. Along with this, the cost of X-ray fluorescence equipment was and remains quite high, and its exploitation may harm the health of the staff.

Nonenzymatic spectrophotometric procedures for determining the potassium concentration in blood serum were still known in the 1960s, but they showed a lower measurement accuracy as compared to the flame-photometric techniques. Further development of these procedures was accompanied by improvement in the accuracy and selectivity of the determination of potassium concentration; however, the nonenzymatic spectrophotometric techniques still can not compete with the flame-photometric and potentiometric methods in the above indicators. In addition, their implementation requires much more time, consumables, and labor costs.

Enzymatic spectrophotometric techniques for determining the potassium concentration in plasma and serum appeared at the turn of the 1980s and 1990s. Initially, quite high hopes were placed on these techniques, but the subsequent application in the solution of practical tasks showed a low precision and selectivity of the measurements, in contrast to the flame-photometric and potentiometric techniques. In addition, enzymatic spectrophotometric procedures are not without the drawbacks of the nonenzymatic spectrophotometric procedures (prolonged runtime of the assay, a variety of consumables, and significant laboriousness).

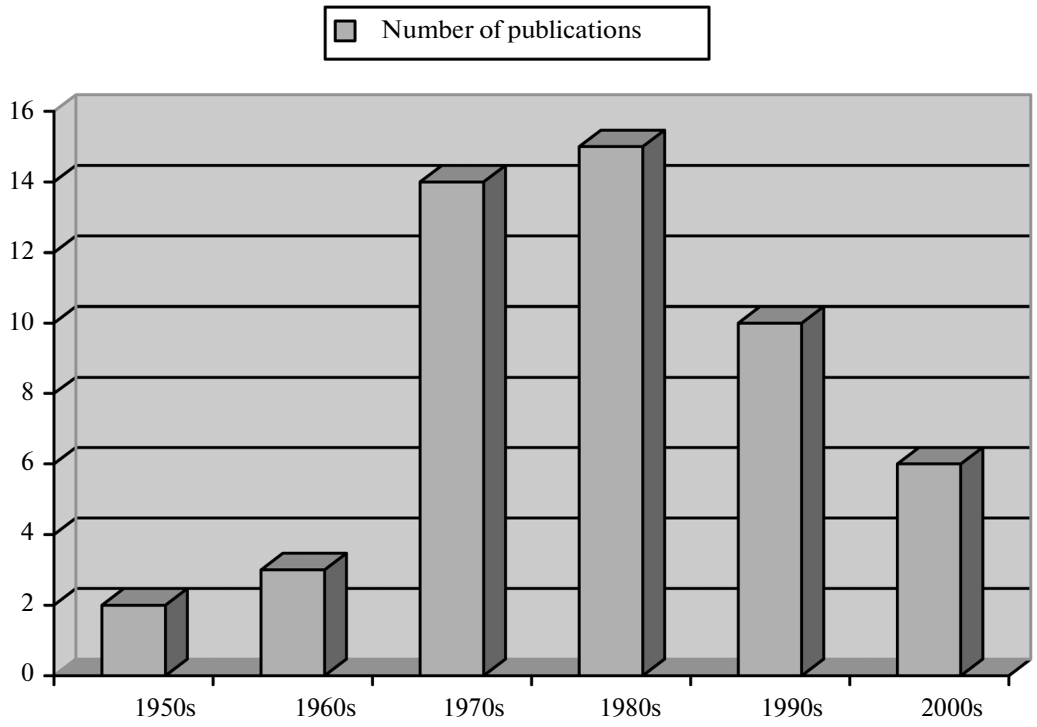

Fig. 2. Number of landmark publications on the procedures for determining the potassium concentration in blood in the journal *Clinical Chemistry*.

The procedures for reflectance photometry satisfy the requirements of rapid analysis and are successfully used in small clinical diagnostic laboratories. Turbidimetric methods for determining the potassium concentration in blood is practically not applied, and luminescence techniques are exploited in a limited number of organizations.

In conclusion, we note that the highest activity in the development of methods for determining the potassium concentration in plasma, serum, and whole blood was observed in the 1970s, 1980s, and 1990s (Fig. 2).

In the 2000s, this activity was significantly decreased, as the main methodological, science and technical problems were solved. The developed methods passed the stage of improvements and enhancements and firmly took up positions in the conducting of routine analyses. At present, if there are any questions, they are mainly related to the organization of the functioning (management) of clinical diagnostic laboratories and not to the quality of the methods for determining the potassium concentration in plasma, serum, or whole blood.

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Translated by G. Levit