Determination of Electrolyte Concentration in Saliva by Potentiometric Method

Određivanje koncentracije elektrolita u slini potenciometrijskom metodom

Vlatko Rumenjak*
Stjepan Milardović**
Ljubica Vranić***
Ivan Kruhak****
Zdravko Rajić*****

* Clinical Institute of Laboratory Diagnosis, Zagreb University School of Medicine and Clinical Hospital Centre
** Institute of General and Inorganic Chemistry, Zagreb University, Faculty of Chemical Engineering and Technology
*** Zagreb Health Centre
**** Institute of Chemistry, Zagreb University, Faculty of Food Technology and Biotechnology
***** Institute for Children's and Preventive Stomatology, Zagreb University School of Dental Medicine

Summary

A method for concurrent determination of potassium, total calcium and chloride concentrations by ion-selective electrodes is described in this paper. A sample of whole saliva from the bottom of the mouth cavity was diluted ten times with magnesium acetate solution (16 mmol/L), which lead to calcium ionization. This method corresponded well with reference methods and may be used to determine electrolyte concentration in saliva due to its simplicity. Among three groups of subjects aged 7 - 12 years, the highest calcium concentrations were found in the group of subjects affected by dental caries. Concentration variability is highest in calcium concentration, whereas it is lower in other respective electrolytes.

Key words: *saliva, electrolytes in saliva, dental caries*

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Introduction

Determination of the electrolyte composition of saliva is particularly interesting with regard to development of dental caries, a process manifesting in demineralization and proteolysis of dental tissue. Knowledge of electrolyte, particularly calcium, concentration in saliva is of primary importance in the study of interaction between hard dental tissue and saliva (1,2).

Various methods have been used to determine electrolyte concentration in saliva, and those originally used were flame photometry, atomic absorption spectrophotometry and colorimetry. However, potentiometric methods have become widely accepted methods for determination of electrolyte concentration in saliva due to their simplicity and rapidity of measurement procedure. Since generally accepted IFCC recommendations on application of this measuring principle are lacking, various instruments and techniques using this measuring principle are numerous (3).

This study describes potentiometric method used to determine electrolyte concentration. Potassium, total calcium and chloride concentrations were determined. Method of total calcium determination was based on substitution of bound calcium from protein complex, using a protein-binding ion to obtain a more stable complex. In surplus of magnesium ions, ionization of calcium occurs and magnesium binds to protein complex. Magnesium acetate was used in the study to substitute calcium from protein complex.

It has already been established that calcium may be released from protein complex using HCl solution (4,5), although it was not possible to apply this method in the study due to concurrent determination of chloride concentration.

Materials and methods

Subjects

The study included children aged 7 - 12 years from a children's home, which ensured homogeneity of subjects, and diminished effect of nutritional habits on composition of saliva. The subjects were divided into three groups as follows: group A with intact teeth (N=12), group B with carious teeth (N=9), and group C with treated teeth (N=11). Examinations were performed in accordance with the basic principles of the Helsinki Declaration (World Health Authority, 1975).

Saliva sample taking

Mixed saliva samples from the bottom of the mouth cavity were on two occasions with a one week delay. Samples were aspirated into a 2 mL plastic syringe and closed after pressing out surplus air.

Reagents

Standards. Two self-developed standards were used to calibrate ion-selective electrodes: standard 1 with potassium, calcium and chloride concentrations of 2.0 mmol/L, 0.3 mmol/L, 3.0 mmol/L, and standard 2 with potassium, calcium, and chloride concentrations of 6.0 mmol/L, 1.0 mmol/L and 12.0 mmol/L, respectively. Magnesium acetate was used to adjust ionic strength of both standards at 50 mmol/L.

Magnesium acetate solution was prepared by dissolving 3.43 g of magnesium acetate tetrahydrate in 1 L of demineralized water. Both the standard solutions and washing solutions were prepared with demineralized water from Millipore-MilliQ System. All chemicals used were analytically pure.

Measurement instruments

pH measurement was performed on ABL a 330 (Radiometer, Copenhagen, Denmark) analyzer for determination of acid-base balance. All precision and reproducibility controls performed prior to measurement were within standard deviations.

Potassium, calcium and chloride electrolyte concentrations were determined using ion-selective electrodes on a laboratory analyzer manufactured at the Institute for General and Inorganic Chemistry, Zagreb Faculty of Chemical Engineering and Technology, in collaboration with Clinical Institute of Laboratory Diagnosis, Zagreb University School of Medicine and Clinical Hospital Centre. The instrument consisted of three ion-selective electrodes and a reference electrode, two peristaltic pumps for sample taking and washing, and a computer. The self-developed program was in QBasic and instrument operated as an autonomous system. Analytical properties of the instrument, evident in work with blood samples, has already been described (6).

The method used to determine electrolyte concentrations was compared using (IL 943) flame photometer (Instrumentation Laboratory, Milano, Italy), Pye Unicam atomic absorption spectrophotometer (Oxford, England), and colorimetric procedure for potassium, calcium and chlorides, respectively.

Chlorides were measured using manual procedure (Herbos test package, Sisak, Croatia, cat. no. 12500), and results were read on SP 500 spectrophotometer (PU Oxford, Great Britain).

Method

Prior to measurement, the instrument was calibrated using undiluted standards 1 and 2, and the saliva sample was diluted by magnesium acetate solution at 1:10. After measurement the results read for potassium, total calcium and chlorides were multiplied by factor 10. After two measurements, calibration was continuously tested using standards 1 and 2.

Determination of total calcium concentration

Calcium may be found in saliva as ionized or protein-bound calcium. In case of a surplus of magnesium ions, a substitution occurs: magnesium binds to proteins instead of calcium which becomes ionized. This substitution occurs due to a difference in stability of the complex: stability constant (K_s) of magnesium compounds with aminoacids is higher than that of calcium compounds with the same aminoacids (Table 1).

Table 1. *Stability constants logKs of some calcium and magnesium compounds with some aminoacids (25 °C, immensurable dilution)*

Tablica 1. *Konstante stabilnosti logKs kalcijevih i magnezijevih spojeva s nekim aminokiselinama (25 °C, neizmjerno razrjeđenje)*

aminoacids	H^+	Ca^{2+}	Mg^{2+}
glycine	9.77	1.43	3.45
	9.78	1.38	3.44
alanine	11.0	1.24	1.96
glutamine	9.67	1.43	1.9

Precision and accuracy of method

Accuracy of the method was tested by diluting the standard 1 solution with magnesium acetate solution, and expressed as a percentage. Precision of the method was tested by repeated electrolyte concentration determinations in random samples with lower concentration (N=10), and by determining electrolyte concentration of one of the samples with higher concentration (N=10) after calibration of the instrument.

Statistical methods

Statistical processing included calculation of mean values of measured concentrations, standard deviations and variability coefficient, whereas correlation examination included calculation of slope and intercept and correlation coefficient.

Results

Accuracy and precision of method

Results of accuracy and precision tests are presented in Table 2. Good results in accuracy were achieved for all three ions. Precision of concentration determination was higher for potassium and chloride as compared to calcium.

Table 2. *Results of precision tests*

Tablica 2. *Rezultati preciznosti metode*

(N=10)

	Potassium	Calcium	Chloride
Mean value (mmol/L)	22.88	0.917	19.58
Standard deviation (mmol/L)	1.26	0.10	1.11
Variation coefficient %	5.7	10.9	5.6

(N=10)

	Potassium	Calcium	Chloride
Mean value (mmol/L)	13.1	1.22	25.46
Standard deviation (mmol/L)	0.7	0.109	1.57
Variation coefficient %	5.3	8.9	6.2

Results of accuracy tests
Rezultati točnosti metode
(standard 1, N=10)

	Theoretic	Measured	Difference
Potassium mmol/L	6.0	5.84	-2.7%
Calcium mmol/L	1.0	1.0	0.0%
Chlorides mmol/L	13.0	13.2	1.5%

Effect of magnesium ions

Magnesium interference on calcium ion-selective electrode membrane was tested by tenfold diluting of standard 1 by magnesium acetate solution. Results were the same as those obtained in precision testing (Table 2). Calcium membrane selectivity constant (log K_{CaMg}) was - 4.9, indicating that magnesium concentration may be $10^{4.9}$ fold that of calcium and yet cause to interference (7).

Measurement of saliva pH

Saliva pH ranged from 6.098 to 7.559.

Effect of ionic strength

Ionic strength of the standards and magnesium acetate solution was 50 mmol/L. Effect of changes in ionic strength of samples on results of measurement was avoided through tenfold dilution of saliva sample.

Correlation with other methods

Potassium. Results are presented in Table 3 and Figure 1. Correlation coefficient (r=0.951) and correlation slope corresponded satisfactorily to reference method.

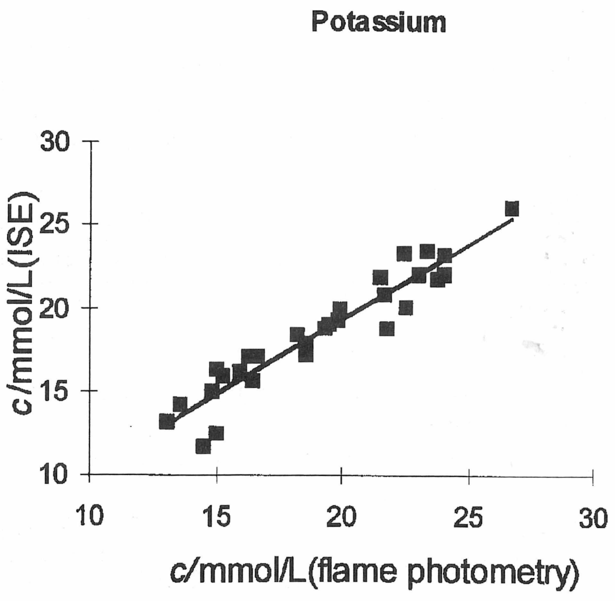

Figure 1. *Correlation slope for potassium between ion-selective electrode (ISE) and flame photometry*

Slika 1. *Pravac korelacije za kalij između ion-selektivne elektrode (ISE) i plamene fotometrije*

Table 3. *Results obtained with comparison between reference methods and ISE*

Tablica 3. *Rezultati usporedbe potenciometrijske i referentnih metoda*

a) Potassium (n=50)

	mean values mmol/L	standard dev. mmol/L	slope	intercept
ISE	18.48	3.56		
flame photomet.	19.05	3.71	0.911	1.12
correlation		0.951		

b) Total calcium (n=50)

	mean values mmol/L	standard dev. mmol/L	slope	intercept
ISE	1.02	0.337		
A.A.S.	1.00	0.322	0.868	0.117
correlation		0.909		

c) Chlorides (n=50)

	mean values mmol/L	standard dev. mmol/L	slope	intercept
ISE	18.88	3.33		
colorimetric	19.31	3.85	1.07	-0.855
correlation		0.926		

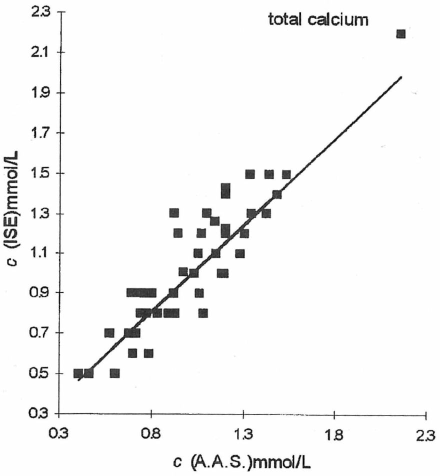

Figure 2. *Correlation slope for total calcium between ion-selective electrode (ISE) and atomic absorption spectrophotometry (A:A:S:)*

Slika 2. *Pravac korelacije za ukupni kalcij između ion-selektivne elektrode (ISE) i atomske apsorpcione spektrofotometrije (A:A:S:)*

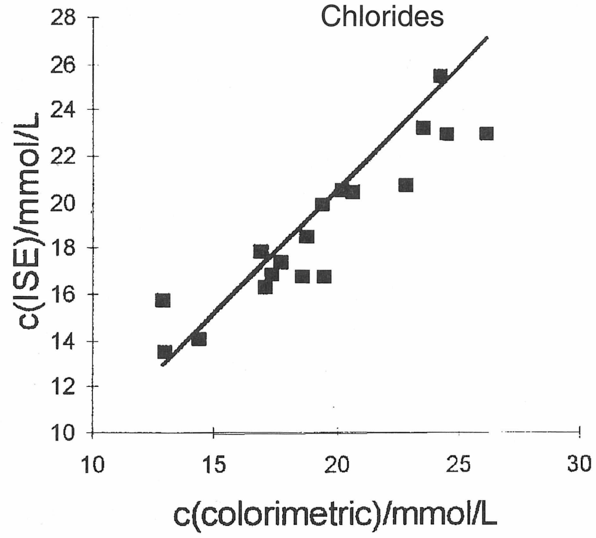

Figure 3. *Correlation slope for chlorides between ion-selective electrode (ISE) and manual colorimetric determination*

Slika 3. *Pravac korelacije za kloride između ion-selektivne elektrode (ISE) i ručnog kolorimetrijskog određivanja*

Calcium. Compared results for calcium are presented in Table 3 and Figure 2. Correlation coefficient (r=0.909) and correlation slope corresponded well to reference method.

Chlorides. Results of comparing chlorides with colorimetric method are presented in Table 3 and Figure 3. Correlation coefficient (r=0.926) and correlation slope yielded satisfactory results.

Results of electrolyte determination in saliva

Results obtained by the method described above are presented in Tables 4 and 5, with mean values and variability coefficients. High variability coefficients in calcium pointed to considerable differences in concentrations within some groups. On the basis of results it could be pointed out that among the three groups calcium concentration was highest in group B with carious teeth, but also with highest variability coefficient. Difference in calcium concentration determination between the first and the second determination was the highest. Potassium and

Table 4. *Results of the first measurement. Mean values and standard deviations are expressed in mmol/L; variability coefficient is expressed percentually*

Tablica 4. *Rezultati prvog mjerenja. Srednje vrijednosti i standardna devijacija izražene su u mmol/L, a koeficijent varijacije u postocima*

	Group A			Group B			Group C		
	Mean value	Stnd. dev.	Var. coeff.	Mean value	Stnd. dev.	Var. coeff.	Mean value	Stnd. dev.	Var. coeff.
Potassium	19.7	4.8	24.5	22.1	4.73	21.4	21.4	2.7	12.6
Calcium	0.921	0.72	78	1.14	0.831	72.9	1.08	0.297	27.5
Chlorides	15.9	1.9	12.4	19.0	4.1	21.8	15.4	3.3	21.5

Table 5. *Results of the second measurement. Potassium, total calcium and chloride concentrations and standard deviations are expressed in mmol/L; variability coefficient is expressed percentually*

Tablica 5. *Rezultati drugog mjerenja. Koncentracije i standardna devijacija kalija, ukupnog kalcija i klorida izražene su u mmol/L, a koeficijent varijacije u postocima*

	Group A			Group B			Group C		
	Mean value	Stnd. dev.	Var. coeff.	Mean value	Stnd. dev.	Var. coeff.	Mean value	Stnd. dev.	Var. coeff.
Potassium	20.6	5.2	25.1	23.6	3.0	12.7	19.1	3.0	15.7
Calcium	1.33	0.79	59.4	1.73	0.89	51.4	0.86	0.75	87.2
Chlorides	15.4	3.0	19.5	19.1	7.2	37.7	15.2	3.1	20.4

chloride concentrations were highest in group B (carious teeth), with less pronounced variability as compared to calcium. No statistically significant differences were observed in potassium and chloride concentrations between the first and the second determination in the same group ($p<0.005$).

Discussion

Considerable variability of the compositions of body fluids can be observed. It is especially true for saliva whose compositions may vary greatly (1). Accuracy of electrolyte determination using ion selective electrodes largely depends on ionic strength of the sample; substantial difference between ionic strength of the standard and the sample causes a measurement error (3). Effect of change in ionic strength on measurement result is avoided by dilution of saliva. Magnesium is only slightly present in saliva (8) and its *in vivo* effect on calcium distribution is negligible. Furthermore, the effect of pH on binding of calcium to proteins has been established (4,5): calcium is released from the complex at low pH value and becomes ionized. However, if chloride concentration is also determined, this method of calcium concentration determination cannot be used. In the method described, magnesium was found appropriate for effecting calcium release: concentration in saliva was low, whereas difference in stability of calcium and magnesium compounds caused quantitative transfer of calcium to ionized form due to the surplus of magnesium. A wide range of pH was observed in examined samples, but with no effect on results of electrolyte determination.

Despite variability coefficient for calcium of 8.9-- 10.9, the method described may be used for assessment of electrolyte concentration in saliva due to its simplicity and good correlation with reference methods. Intraindividual and interindividual variability of calcium concentration in saliva is considerably increased as compared to variability coefficient of the method (Tables 4 and 5).

Results of the study indicate a connection between the process of development of dental caries and electrolyte, particularly calcium, concentration in saliva. Calcium ionization due to addition of magnesium acetate points to possible interacting effect of dietary electrolytes on electrolyte distribution in saliva.

Conclusions

The method of electrolyte determination in saliva described above correlated well with reference methods. Results of electrolyte determination indicated increased calcium concentration in saliva in subjects with dental caries. Considerable interindividual variability was found in salivary concentrations of electrolytes, particularly calcium.

ODREĐIVANJE KONCENTRACIJE ELEKTROLITA U SLINI POTENCIOMETRIJSKOM METODOM

Sažetak

U članku je opisana metoda za istodobno određivanje koncentracije kalija, ukupnoga kalcija i klorida u slini ion selektivnim elektrodama. Uzorak pune sline s dna usne šupljine razrijeđen je 10 puta otopinom magnezijeva acetata (16 mmol/L), čime je kalcij preveden u ionizirani oblik, a istodobno je ionska jakost tako pripremljenih otopina držana stalnom (50 mmol/l). Opisana metoda pokazala je dobro slaganje s usporednim metodama i zbog svoje jednostavnosti može se rabiti za određivanje koncentracije elektrolita u slini. Rezultati koncentracije elektrolita dobiveni u trima skupinama ispitanika u dobi od 7 - -12 godina pokazuju da su najviše koncentracije kalcija u skupini s karijesom. Varijabilnost koncentracije najveća je kod kalcija, a kod ostalih je mjerenih ionskih vrsta u slini manja.

Ključne riječi: *slina, elektroliti u slini, karijes.*

Address for correspondence:
Adresa za korespondenciju:

Vlatko Rumenjak, MSc
Clinical Institute of
Laboratory Diagnosis,
Rebro Clinical Hospital
Centre, Kišpatićeva 12,
10000 Zagreb
(Emergency laboratory)
tel & fax: 01 212 079

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