

# Coated Copper Wire Calcium Selective Microelectrode for Applications in Dental Medicine

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*A coated wire calcium selective microelectrode for biological use was developed, comprising a PVC selective matrix containing calcium ionophore IV coated on copper wire, previously covered with a solid-state contact mixture. The obtained calcium microsensor presented a Nernstian answer in a concentration range of  $10^{-1}$  to  $10^{-6}$  mol/L. The selectivity coefficients over the main interfering ions of biological interest proved that the calcium microelectrode is highly selective. Also, the response time (6s) and repeatability have been determined. The pH variation did not significantly modify the calcium microelectrode answer, being stable over the pH range (6.7-7.3) of interest. The obtained calcium microelectrode is simple, inexpensive and able to give reliable electrochemical response, recommending itself as a solution for assessing the level of inorganic ions of the gingival crevicular fluid and saliva.*

**Keywords:** calcium microsensor, coated wire microsensor, solid-state contact mixture, PVC selective mixture, gingival crevicular fluid

During last years, important progress has been obtained in the area of biological and clinical analysis using ion selective electrodes with membranes based on macrocyclic transporters [1-3]. In potentiometric methodology, a significant development was represented by the ion selective electrodes. These selective devices allowed the application of the potentiometric method for complex samples analysis, including for clinical environment. One of the well-known application of the microelectrodes with membranes based on ionophores is the usage of flux injection analysis systems for determining  $\text{Na}^+$  and  $\text{K}^+$  ions in blood [4]. A successful class of selective microelectrodes is the microelectrodes realized by covering conducting wires with selective membranes, being simple and without requiring internal reference electrode. Although in such system a thermodynamic equilibrium between selective membrane and its solid support is not established, the covered wire microelectrodes are easy to be prepared and the function is satisfactory [5], with a Nernstian behavior and a good selectivity. Lot of efforts have been directed towards the development of sensitive microdevices for applications in pharmaceutical formulations analysis. Depending on their dimension, form and configuration, the microsensors could be also used as portable tools for medical diagnosis.

In the context of our keen interest to develop and introduce new sensitive microdevices able to determine various inorganic species into saliva and gingival crevicular fluid, we developed a coated wire calcium selective microelectrode as an alternative to an ISFET structure based sensor [6]. Because both saliva and the gingival crevicular fluid (GCF) are considered as possible markers for periodontal disease evolution [7-10] we consider that the development of various types of microsensors able to

assess the level of different ions in GCF could be of great importance for early diagnosis and evaluation of periodontal disease.

The aim of the present paper is to introduce a coated wire calcium selective microsensor for applications in dental medicine.

## Experimental part

In the performed experiments, the following chemicals have been used: tetrahydrofuran (THF) (Fluka) as solvent, high molecular weight polyvinylchloride (PVC) (Fluka) for the polymeric matrix, o-nitro phenyl octyl ether (NPOE) (Fluka) as plasticizer, [12, (4-ethylphenyl) dodecyl] (Fluka), potassium tetrakis (p-chloro) phenyl borate (KTpClPB) (Fluka) as lipophilic agent and graphite (Fluka). In addition, there has been used epoxy resin (Ultrapure SU 2227, Victor, Italy), hardener (Desmodur RFE) (Bayer AG). As electroactive agent it was the calcium ionophore IV (N, N-Dicyclohexyl-N', N'-dioctadecyl-3-oxapentamide (Merck). The electrolyte solutions have been prepared using highly pure salts provided by Merck and ultrapure water ( $18.2 \text{ M}\Omega$ , ELGA System). Standard solution of calcium have been obtained from calcium chloride (Merck) and the interfering solutions from the corresponding chloride salt for: magnesium, sodium, lithium, potassium, barium, strontium and ammonium (Merck). The analyte solutions have been prepared by subsequent dilution from an initial stock solution,  $10^{-1} \text{ mol/L CaCl}_2$ . The studies dedicated to the pH influence on the potentiometric answer of the calcium microelectrode have been conducted in phosphate buffered solutions (Merck).

For microelectrode manufacturing, copper wires of approximately 0.4-0.5 mm diameter and 5-10 cm length

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have been used. Electrochemical measurements have been performed on a multichannel potentiometer with associated software (ISEMS-4, Medisen). As well known, in all electrochemical measurements, the indicating electrode, the calcium selective electrode to be developed, and the reference electrode need to be present simultaneously. Therefore, in order to comply with the specificity of the indicating electrode, a homemade micro-sized solid-state Ag/AgCl reference electrode [11] was used.

#### Calcium selective membrane

The calcium selective membrane was prepared using a PVC cocktail [12]. In 5 mL of THF solvent were thoroughly mixed: PVC 29%, plasticizer NPOE 68%, calcium ionophore IV 2% and KpTCIPB 1%, all added in mass percentage (w/w). This membrane was further used to obtain the microelectrode.

#### Solid-state calcium selective microelectrode

The solid-state contact mixture containing: graphite, 50% (wt), epoxy resin, 35% (wt) and hardener, 15% (wt) was dissolved in THF solvent. While mixing, the appropriate viscosity was reached and the copper wires were dipped in the mixture in order to be covered. This procedure was repeated 6 to 8 times to assure a correct and uniform coverage of the copper wires. The wires were left overnight in opened air, at room temperature.

The previously prepared calcium selective membrane was used as dipping media for the conditioned copper wires. As consequence, the membrane matrix was coextruded being deposited over the solid-state contact material. The calcium selective electrode obtained was conditioned for 24h in  $10^{-2}$  mol/L  $\text{CaCl}_2$  solution before its usage. In figure 1 some of the obtained calcium selective microelectrodes are presented.

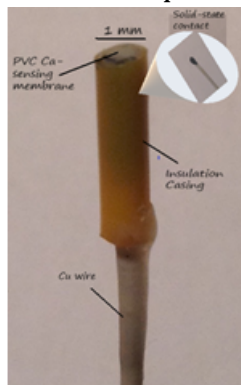


Fig. 1. Calcium selective microelectrode

#### Electrochemical characterization

The calcium microelectrodes were electrochemically characterized. The specific potentiometric behavior has been evaluated, as well as the selectivity against main interfering ions. These interfering ions were chosen taking into account the final application of the microelectrodes - for the clinical area. In addition, the response time, detection limit and working pH range of the prepared  $\text{Ca}^{2+}$ -selective microelectrode have been investigated.

The separate solution method was applied for the determination of selectivity coefficients. Considering that our newly prepared calcium selective microelectrode is answering to a solution containing only the ion  $\text{Ca}^{2+}$ , then the electrochemical potential varies according to:

$$E_{\text{Ca}} = E^{\circ'} + \frac{RT}{2 \cdot F} \ln a_{\text{Ca}} \quad (1)$$

where:  $E_{\text{Ca}}$  - electrochemical potential of the calcium selective microelectrode in solution containing only

calcium (mV),  $E^{\circ'}$  - standard electrochemical potential of the calcium-selective microelectrode (mV),  $R$  - the universal gases constant,  $8.314 \text{ J K}^{-1} \text{ mol}^{-1}$ ,  $T$  - absolute temperature, (K),  $F$  - the Faraday constant (coulombs/mole of electrons),  $96,485.34 \text{ C/mol}$ , and  $a_{\text{Ca}}$  - chemical activity of calcium.

If, by contrary, the solution would contain only the interfering ion,  $i$ , calcium - selective microelectrode's potentiometric answer becomes:

$$E_{\text{Ca},i} = E^{\circ'} + \frac{RT}{2 \cdot F} \ln a_i + \frac{RT}{2 \cdot F} \ln K_{\text{Ca},i}^{\text{pot}} \quad (2)$$

where:  $E_{\text{Ca},i}$  - electrochemical potential of the calcium selective microelectrode in solution containing only interfering ion,  $i$  (mV),  $a_i$  - chemical activity of interfering ion,  $i$ , and  $K_{\text{Ca},i}^{\text{pot}}$  stands for selectivity coefficient of calcium against interfering ion,  $i$ .

When working at equal activities for calcium and interfering ion,  $i$ , then from the previous relationships one obtains:

$$E_{\text{Ca},i} - E_{\text{Ca}} = \frac{RT}{2 \cdot F} \ln K_{\text{Ca},i}^{\text{pot}} = \frac{2.303 \cdot RT}{2 \cdot F} \lg K_{\text{Ca},i}^{\text{pot}} \quad (3)$$

$$\lg K_{\text{Ca},i}^{\text{pot}} = \frac{2 \cdot F \cdot (E_{\text{Ca},i} - E_{\text{Ca}})}{2.303 \cdot RT} = \frac{2(E_{\text{Ca},i} - E_{\text{Ca}})}{59.16} = \frac{(E_{\text{Ca},i} - E_{\text{Ca}})}{29.58} \quad (4)$$

We could also approach this method using the potentiometric determinations of the two solutions (calcium and interfering ion) when the activities  $a_{\text{Ca}}$  and  $a_i$  are varied and the potentials defined by equations (1) and (2) are graphically represented. Such diagram should contain two linear graphs from where it would be determined the activities values,  $a_{\text{Ca}}$  and  $a_i$ , for which the two electrochemical potentials would become equal,  $E_{\text{Ca},i} = E_{\text{Ca}}$ . In this situation, the selectivity coefficient is calculated according to:

$$K_{\text{Ca},i}^{\text{pot}} = \frac{a_{\text{Ca}}}{a_i} \quad (5)$$

Saliva content in calcium ions is  $1.2 \times 10^{-3}$  -  $2.80 \times 10^{-3}$  mol/L, which is similar to the calcium content in plasma. Calcium is one of the inorganic ions, which has been intensely studied as a potential indicator for periodontal disease in saliva. Saliva with an increased calcium concentration proved to be characteristic for patients with periodontitis [7]. The gingival crevicular fluid could contain  $10^{-2}$  mol/L calcium in healthy patients and up to  $1.59 \times 10^{-2}$  mol/L calcium for in moderate periodontitis [10]. Following our intentions to develop a solid-state contact calcium-selective microsensor for assessing the calcium level in the gingival crevicular fluid (GCL) and saliva, the calcium micro-device should be tailored in such way to be highly sensitive for calcium within the specific concentration range  $10^{-2}$  mol/L -  $1.2 \times 10^{-3}$  mol/L.

#### Results and discussions

The Nernstian behavior of the obtained  $\text{Ca}^{2+}$ -selective microelectrode was tested for  $\text{Ca}^{2+}$  solutions with concentration ranging between  $10^{-6}$  and  $10^{-1}$  mol/L. The specific behavior could be followed in figure 2 where the potentiometric response of two identical  $\text{Ca}^{2+}$ -selective microelectrodes is presented. The specific potentiometric performance of the prepared  $\text{Ca}^{2+}$ -selective microelectrode was studied against the following cations:  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Li}^{+}$ ,  $\text{Na}^{+}$ ,  $\text{K}^{+}$ ,  $\text{NH}_4^{+}$ ,  $\text{Sr}^{2+}$ ,  $\text{Ba}^{2+}$  with concentrations ranging between  $10^{-6}$  and  $10^{-1}$  mol/L. It was noticed that the prepared microelectrode exhibited fast, selective and

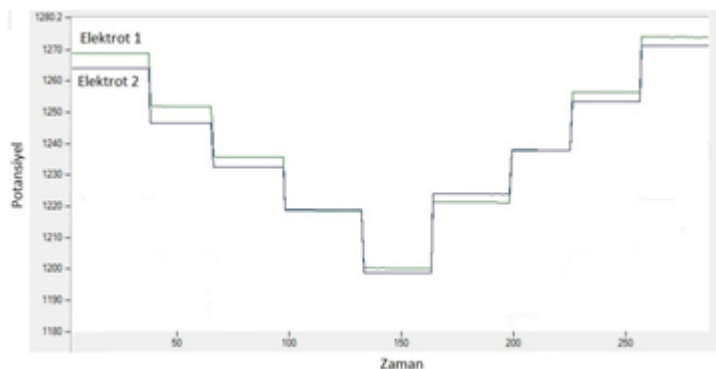


Fig.2 Potential-time plots for two identical PVC matrix based  $\text{Ca}^{2+}$ -selective microelectrodes

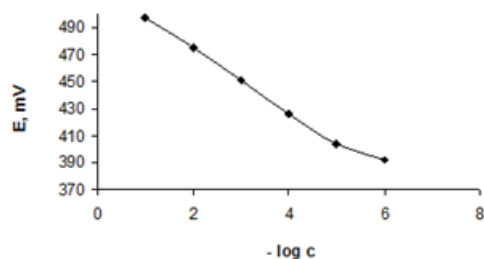


Fig.3 Calibration curve for  $\text{Ca}^{2+}$  - selective microelectrode

reproducible response against  $\text{Ca}^{2+}$  ion in the presence of interfering ions.

One of the most important dynamic characteristics is the detection limit of ion selective electrodes. For the obtained calcium-selective microelectrode the detection limit was calculated using the calibration curve, which is presented in figure 3. For the  $\text{Ca}^{2+}$  - selective microelectrode the determined detection limit was  $3.26 \cdot 10^{-6}$  mol/L, which is satisfactory for the performances that we are seeking for.

In order to establish the selectivity constants, potentiometric measurements of the calcium solution and interfering ion solutions with the PVC matrix based  $\text{Ca}^{2+}$  - selective microelectrode were done. The potentiometric behavior of  $\text{Ca}^{2+}$  - selective microelectrode against different interfering ions is presented in figure 4.

During this study, the selectivity coefficients have been calculated applying the separate solution method [13, 14] using the electrochemical potentials equalization. Therefore, the concentrations of the calcium ion solution that gives equal potential measured in the  $10^{-2}$  mol/L solution of the interfering ion have been determined. The calculated values are presented in table 1.

The experimental results recorded showed that the prepared  $\text{Ca}^{2+}$ -selective microelectrode is highly selective against the various interfering ions as presented in figure 4.

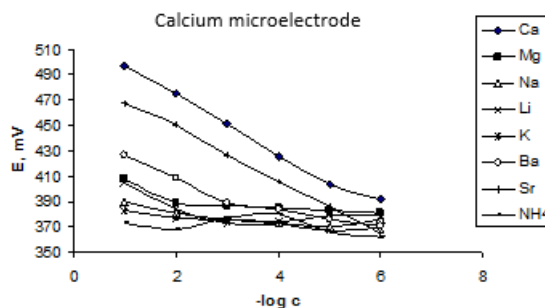


Fig. 4 Potentiometric behavior of PVC-matrix based  $\text{Ca}^{2+}$  selective microelectrode against different interfering ions

**Table 1**  
SELECTIVITY CONSTANTS CALCULATED FOR PVC-MATRIX  $\text{Ca}^{2+}$  - SELECTIVE MICROELECTRODE

Interfering ion, $i$	$K_{\text{Ca},i}$	$\log K_{\text{Ca},i}$
$\text{Mg}^{2+}$	$2.39 \times 10^{-4}$	-3.62
$\text{Na}^{+}$	$1.09 \times 10^{-4}$	-3.96
$\text{K}^{+}$	$7.38 \times 10^{-5}$	-4.13
$\text{Li}^{+}$	$1.47 \times 10^{-4}$	-3.83
$\text{NH}_4^{+}$	$3.06 \times 10^{-5}$	-4.51
$\text{Sr}^{2+}$	$9.43 \times 10^{-2}$	-1.02
$\text{Ba}^{2+}$	$1.70 \times 10^{-3}$	-2.77

The calcium microelectrode described in the present paper has been developed for further clinical applications. For such applications, the response time is very important, as we are seeking to obtain a quick and accurate potentiometric answer. In figure, 5 the variation of the response time for the calcium microelectrode could be followed. The microelectrode has been immersed in standard calcium chloride solutions applying two subsequent concentration sequences: from  $10^{-1}$  to  $10^{-6}$  mol/L and from  $10^{-6}$  to  $10^{-1}$  mol/L, respectively. In the transition from  $10^{-4}$  mol/L  $\text{Ca}^{2+}$  to  $10^{-3}$  mol/L  $\text{Ca}^{2+}$  solution, the time corresponding to 95% of the equilibrium time of the microelectrode potential has been calculated according to IUPAC [13]. The response time ( $t_{95}$ ) of the PVC-matrix  $\text{Ca}^{2+}$ -selective microelectrode was lower than 6 s. We could conclude that the obtained selective device presents a very short and satisfactory response time.

Another important dynamic characteristic of the calcium microelectrode is the repeatability. It is known that for getting reliable results for repeatability, we must perform the same electrochemical procedure multiple times. In order to avoid supplementary error, during this experiment, the following working conditions were secured: the same researcher applied the same procedure using the same materials and equipment keeping unchanged the environmental conditions and all determinations were done

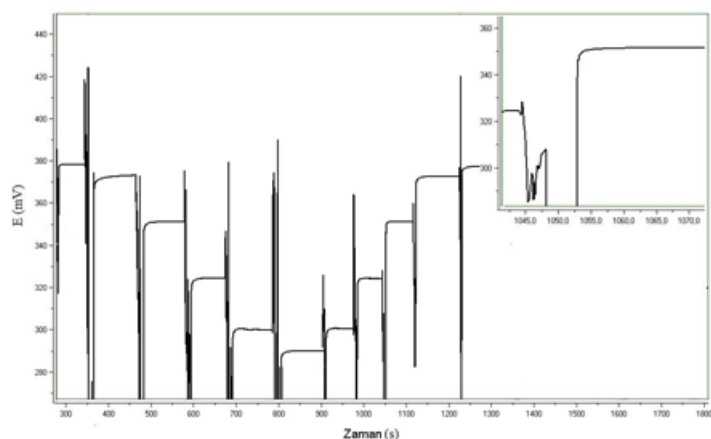


Fig. 5 Response time of the  $\text{Ca}^{2+}$ -selective microelectrode



$[Ca^{2+}]$ , mol/L	Mean Potential (mV)	Standard deviation	Standard deviation of the mean
$10^{-2}$	268.02	0.98	0.20
$10^{-3}$	246.06	0.43	0.09
$10^{-4}$	222.86	0.38	0.08

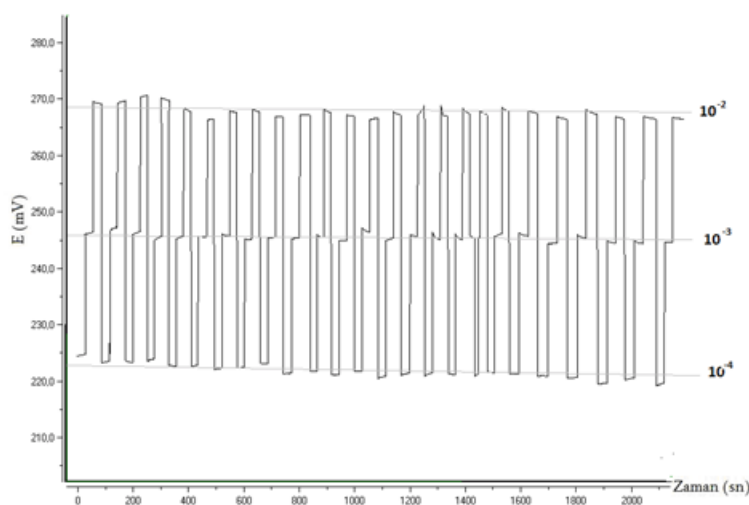


Fig.6. Repeatability of the obtained  $Ca^{2+}$ -selective microelectrode

in a short period of time. For determining the repeatability parameter, the microelectrode was immersed in different calcium chloride solutions, namely  $10^{-4}$ ,  $10^{-3}$ , and  $10^{-2}$  mol/L, respectively. The immersion procedure was performed/repeated 25 times in a row, when approximately the same potential values were recorded after each measurement. This could be considered as an indication regarding a very good repeatability of the calcium microelectrode. The specific behavior during the applied procedure to determine the repeatability is presented in figure 6. The test run for  $10^{-2}$  mol/L  $Ca^{2+}$  solution gave for the standard deviation of the mean 0.20 compared with that ones for  $10^{-3}$  mol/L  $Ca^{2+}$  solution which was 0.09 and for  $10^{-4}$  mol/L  $Ca^{2+}$  solution when 0.08 was recorded. In table 2 the statistical parameters based on the measurements shown in figure 5 are presented.

The lower value of the standard deviation of the mean (table 2) indicates a higher reliability of the results.

The environment of the clinical samples to be analyzed using the obtained calcium microelectrode is dependent upon the pH variation. As consequence, it is important to establish the pH working range for the microelectrode.

After potentiometric calibration of the microelectrode for standard calcium solutions with concentrations ranging between  $10^{-1}$  mol/L to  $10^{-6}$  mol/L, measurements to follow the influence of pH on the electrochemical answer of the microelectrode were performed. Phosphate buffer solutions ( $5 \cdot 10^{-3}$  mol/L) were used to obtain a variation of pH from 4 to 9. In these buffered solutions, the calcium ion concentration was maintained constant to  $10^{-3}$  mol/L or to  $10^{-4}$  mol/L. In figure 7 the microelectrode electrochemical behavior in solutions with varied pH could be observed.

The prepared  $Ca^{2+}$ -selective microelectrode does not show a significant potential change between pH = 4 and pH = 9. Therefore, we could conclude that the microelectrode is functioning without being significantly affected by the pH of the medium ranging between 4 and 9. It is known that, in oral cavity, saliva's pH is maintained within the neutral range between 6.7 to 7.3 and the resting pH is not lower than 6.3 [15]. It has to be noted that for the physiological pH range 6.3 - 7.3, the recorded

Table 2  
STATISTICAL PARAMETERS

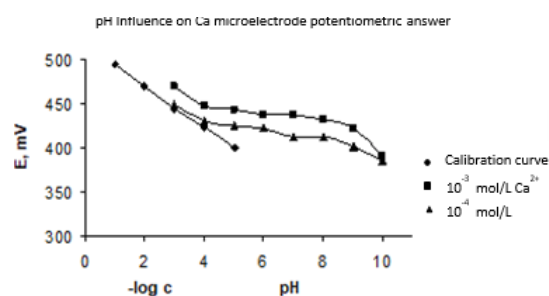


Fig.7 pH working range of the PVC-matrix based  $Ca^{2+}$ -selective microelectrode

electrochemical potentials do not vary with more than 5 mV in answer.

## Conclusions

Due to its characteristics and miniaturization, the obtained calcium microelectrode could be easily used in biomedical environment, clinical studies and particularly in dental medicine for gingival crevicular fluid assessment in periodontal disease. Our previous presented CHEMFET [6] could be considered as a natural extension of the actual presented coated-wire calcium microsensor. The obtained solid-state contact coated wire calcium microsensor proved high selectivity for calcium against main interfering ions present in GCF or saliva. The electrochemical dynamic characteristics of the calcium microsensor: nernstian response, 6s for response time, detection limit of  $3.26 \cdot 10^{-6}$  mol/L allow its usage for assessing the calcium level from complex biological matrices. Much more, the stability of its electrochemical answer within the physiological pH range (6.3-7.3) transforms the developed solid-state calcium microelectrode into a useful tool for investigations over the evolution of the calcium level in GCF or saliva.

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