Development of a New Potentiometric Sensor based on home made Iodide ISE Enriched with ZnO Nanoparticles and its Application for Determination of Penicillamine

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This paper describes the preparation and application of "home-made" iodide ISE enriched with ZnO nanoparticles. Iodide ISE membrane was made of $AgI:Ag_2S:PTFE = 1:1:2$ and enriched with ZnO nanoparticles in ratio 1-5.0 wt.%. Prepared membranes were used for penicillamine (Pen) determination in acetic buffer (pH = 4-4.75) and in perchloric acid (pH = 1-2). Due to the low price method, simplicity and relative speed, a possibility for Pen determination was found. Pen determination showed better results in an acetic buffer, pH = 4 than in perchloric acid. All experiments for Pen measurements were done without pretreatment of pharmaceuticals. The determination is based on the reaction between Pen with both Ag^+ and Zn^{2+} from the electrode membrane. A newly described method has linear response range for Pen 2.45×10^{-6}-1×10^{-2} mol L^{-1} and a detection limit of 2.24×10^{-6} mol L^{-1}. The found concentrations of Pen are in a very good agreement with the declared ones with the standard deviation of 2.70 %.

1. INTRODUCTION

Penicillamine (Pen) is an amino acid often used for chelating copper ions in human bodies. Pen could be taken as a pharmaceutical (e.g. Metalcaptase®). Pharmaceuticals containing Pen are used for the treatment of Wilson's disease. Wilson's disease is characterized by an accumulation of copper in a tissue of liver, brain and eyes (edge of iris) as well as potential neuropsychiatric symptoms (tremor, ataxia, dystonia etc.) [1].

By searching the literature of the last decade, only a limited number of papers describing direct potentiometric determinations of thiols/pen [1-9] can be found. Other proposed methods are based on different techniques, such as voltammetry [10-13], spectrophotometry [14-17] and chromatographic techniques, e.g. [18].

This paper is a continuation of our efforts in the development of both new suitable potentiometric sensors [19] and methods [5, 9] useful for wide application. In testing and developing methods, our aim was to make it as simple for everyday use, low-cost and reasonable speed. On the other hand, we made a step in a new direction by adding ZnO nanoparticles. We hoped that adding ZnO nanoparticles would increase the selectivity of our membranes as well as make a possible determination of lower concentrations.

Herein we have proposed a method appropriate for the determination of Pen in extensive concentration range without a requirement for pharmaceuticals pretreatment. The newly developed method has been established as a very low-cost, simple, reasonably fast method and a very robust one. In case of applying newly developed membranes in previously described electrode body [4], this gives a decent potentiometric sensor for determination of Pen in pharmaceuticals. Because the preparation of the membrane is done in laboratory and because of the use of the potentiometric sensor, this method is very simple. It can be even more inexpensive if for the precipitation of silver iodide and silver sulfide commercially available silver nitrate, potassium iodide and sodium sulfide are used. In our studies, we used commercially available ZnO nanoparticles and PTFE.

2. EXPERIMENTAL

2.1. Reagents and chemicals

We also used the following chemicals: Sodium nitrate, $NaNO_3$, p.a., Potassium iodide, KI, p.a., Silver nitrate, $AgNO_3$, p.a., Sodium sulphide nonahydrate, $Na_2S \times 9H_2O$, p.a., Sodium acetate, CH_3COONa, p.a., Acetic acid, CH_3COOH, p.a. and perchloric acid, Kemika (Croatia) and Penicillamine, $C_5H_{11}NO_2S$, p.a., Alfa Aesar (Germany).

All solutions needed ($AgNO_3$, $Na_2S \times 9H_2O$ and KI) for precipitation of sparingly soluble salts were prepared by solving of a certain quantity of solid chemicals in suprapure water, if not stated otherwise. Suprapure water (declared conductivity of 0.04 μS cm^{-1}) was prepared by Millipore Simplicity (Millipore, USA).

Solutions used for laboratory testing were prepared by dissolving a required mass of substance in perchloric acid (pH = 1-2) and acetic buffer (pH = 4 and pH = 4.76). An acetic buffer was 0.1 M. Ionic strength of solutions was adjusted at 0.5 M by adding $NaNO_3$.

2.2. Apparatus

The previously described multi-purpose solid-state electrode body was used [5]. After the precipitation of silver iodide and silver sulfide, proper masses were weighted and mixed with PTFE and ZnO nanoparticles in powder homogenizer as shown in Table 1. Membranes had a mass of (0.5000±0.0005) g. Prepared powders for membranes were put in a press for 1 h at 740 MPa [5]. Membranes were pressed in a form of a pellet (2.0 mm i.d. and 1.2 mm long) and finally incorporated in the electrode body. Membranes' compositions are given in Table 1.

Table 1. Mass of added ZnO nanoparticles per membrane

Membrane label	Mass of ZnO nanoparticles, mg
M1	5.2
M2	10.0
M3	15.1
M4	20.2
M5	25.0
M6	0.0

Transfer of electrical charge from the membrane to millivoltmeter was described earlier [5].

The indicator electrode was an Orion 90-02 double junction reference electrode (Orion, USA), as in our previous works [1-5]. Potentiometric data during laboratory work were recorded at 25 °C with a millivoltmeter (SevenExcellence, Mettler-Toledo, Switzerland-USA) coupled to a personal computer by USB cable, while the data were recorded by the use of LabX direct pH 3.3 (Mettler-Toledo, Switzerland-USA), as in our previous works [1-5]. The uncertainty in potential measurements was ±0.0001 V.

3. RESULTS AND DISCUSSION

"Classic" ("batch") potentiometric determinations of Pen have been done by using mentioned iodide ISE enriched with ZnO nanoparticles. Due to the new design, the "home-made" iodide ISE has been tested for response to both silver ions and iodide concentration. Change of concentrations was performed by the standard dilution method. During measurement, the solution was stirred and kept at a constant temperature of 25 °C. The results for iodide are shown in Figures 1-4. We have deliberately decided not to show results for silver ions because the slope was very low, like for divalent ions, around 29 mV per decade. On the other hand, it is very interesting that the membrane response to

silver ions is fast losing at a concentration below 0.1 mM. A possible explanation for this phenomenon is making membrane surface positively charged by Zn ions in acid media from ZnO nanoparticles. Such positively charged surface attracts anions but, at the same time, retracts cations.
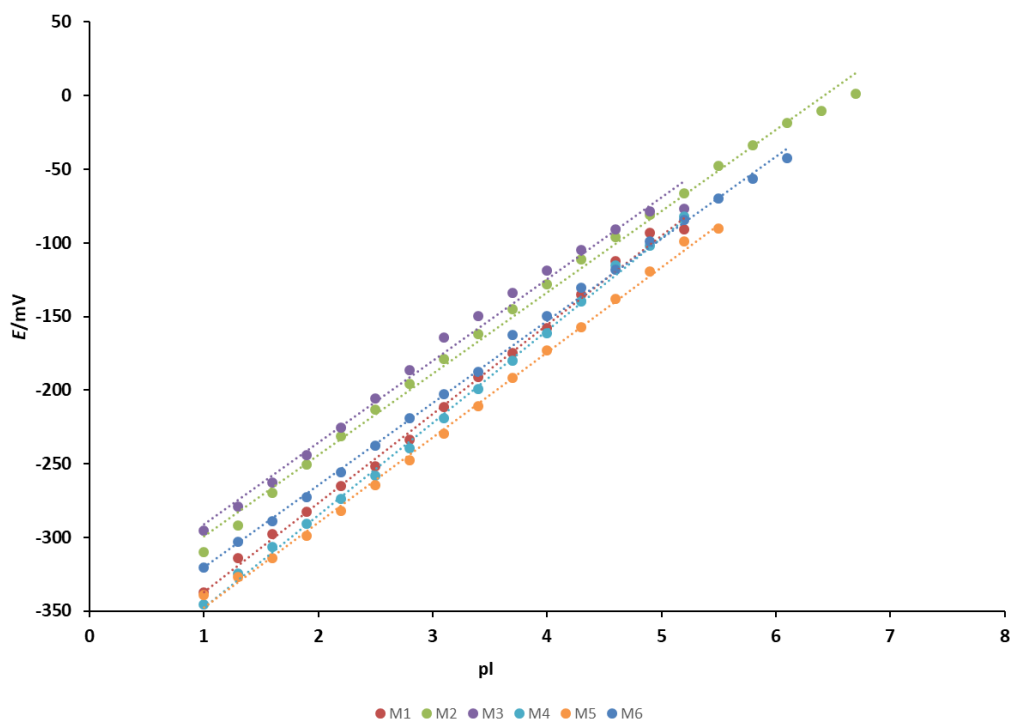

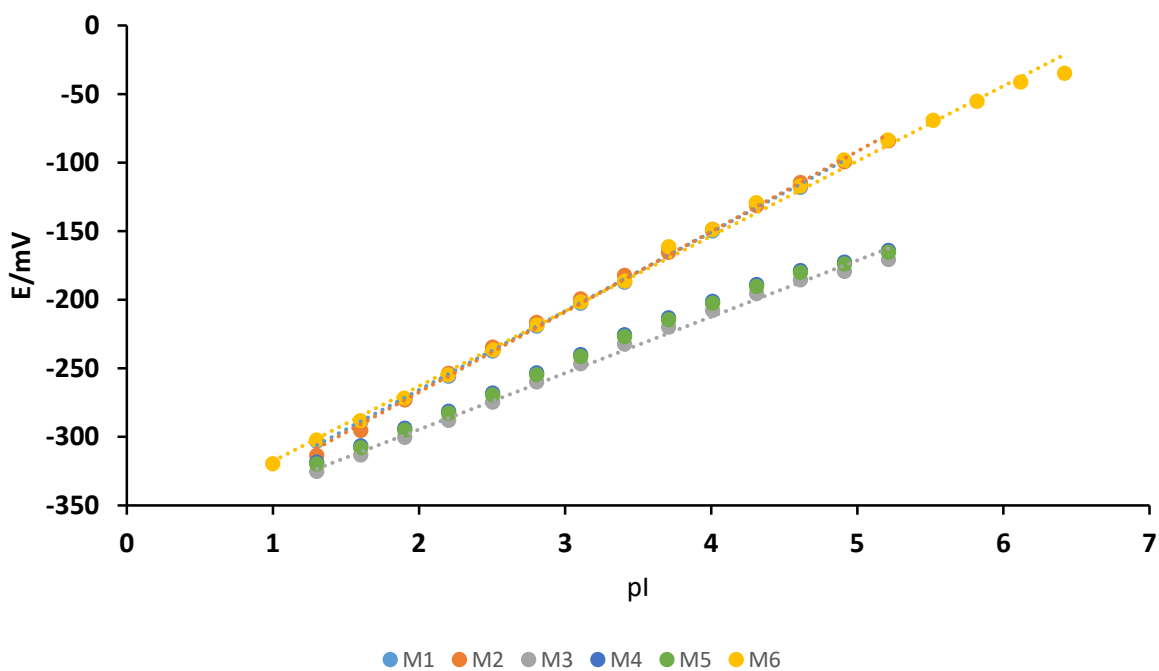**Figure 1.** Response of "home-made" iodide ISE to iodide ions, pH = 1

Figure 2. Response of "home-made" iodide ISE to iodide ions, pH = 2
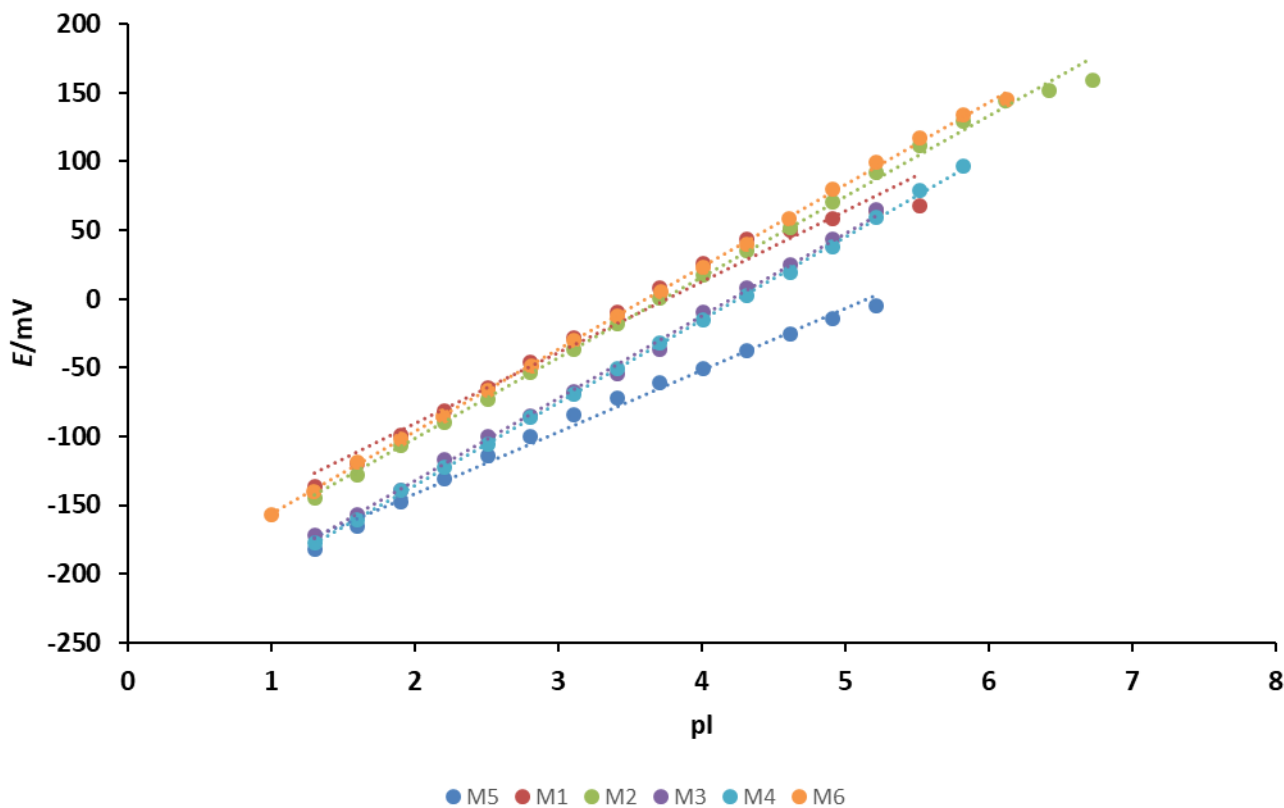
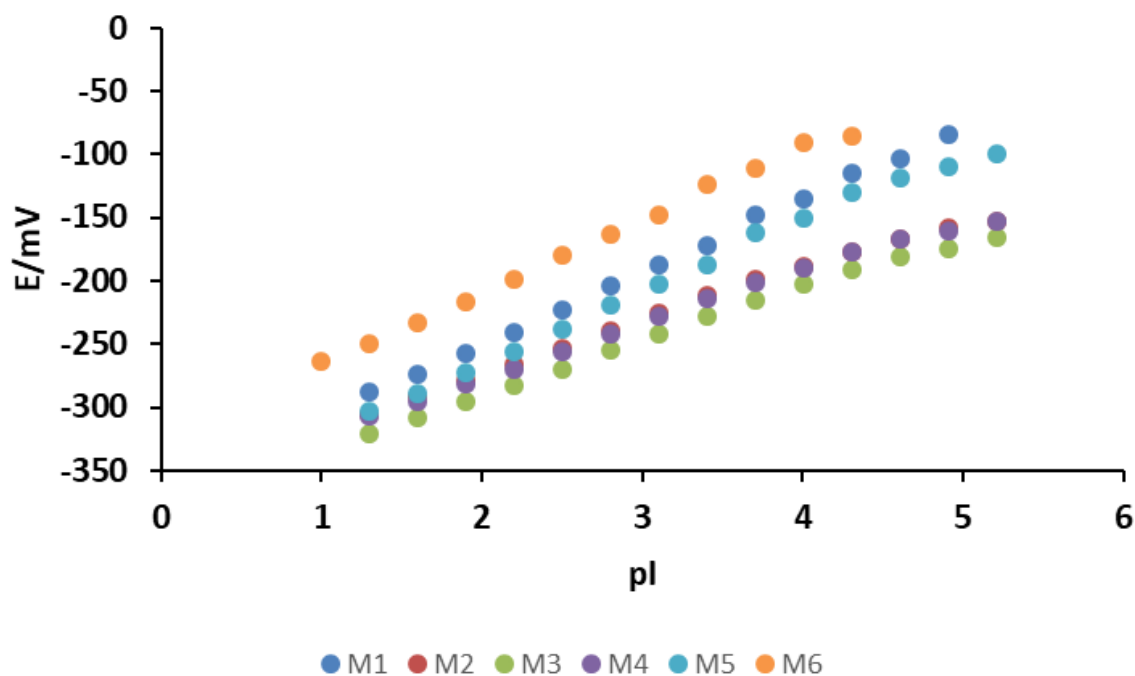**Figure 3.** Response of "home-made" iodide ISE to iodide ions, pH = 4

Figure 4. Response of "home-made" iodide ISE to iodide ions, pH = 4.75

Points in the Figures 1-4 represent experimental data while the straight line was calculated by using the method of linear regression using Microsoft Excel®. As it can be seen, the newly prepared membranes linearly follow the changing of I⁻ concentration in a wide concentration range, at least four magnitudes of order. The stable potential was reached in 30 seconds. The potential change of 55-60

mV per decade of iodide concentration change was recorded, with the correlation coefficient of 0.9900-0.9986, which is in good agreement with the theoretical Nernstian slope for monovalent cations. "Home-made" iodide ISE linearly follows I^- concentration changing with a linear response in the concentration range of $I^- = 7.6 \times 10^{-7} \text{-} 10^{-1}$ mol L^{-1} in the perchloric buffer, pH = 1-2. On the other hand, membranes had a decent response in the concentration range of $I^- = 1 \times 10^{-5} \text{-} 10^{-1}$ mol L^{-1} in an acetic buffer, pH = 4-4.75.

The response of "home-made" iodide ISE to Pen was based in the reaction of sulphur in thiol's group of Pen with Ag^+ with silver iodide from electrode membrane, by forming a complex at membrane surface described in the literature [20-23]. On the other hand, our new membranes contain ZnO nanoparticles and Zn from nanoparticles that react with sulfur in Pen, what synergistically improves both their linear response range and limit of detection. The same method for testing response of "home-made" electrode to Pen as to iodide ions was performed but shown only at pH = 4 and 4.75. Since Pen acts as a weak acid, by decreasing pH it becomes an itself conjugated acid by protonation of the thiol group. Due to the mentioned reasons, membranes showed poor response to Pen in high acidic media. The results are shown in Figures 5-6.
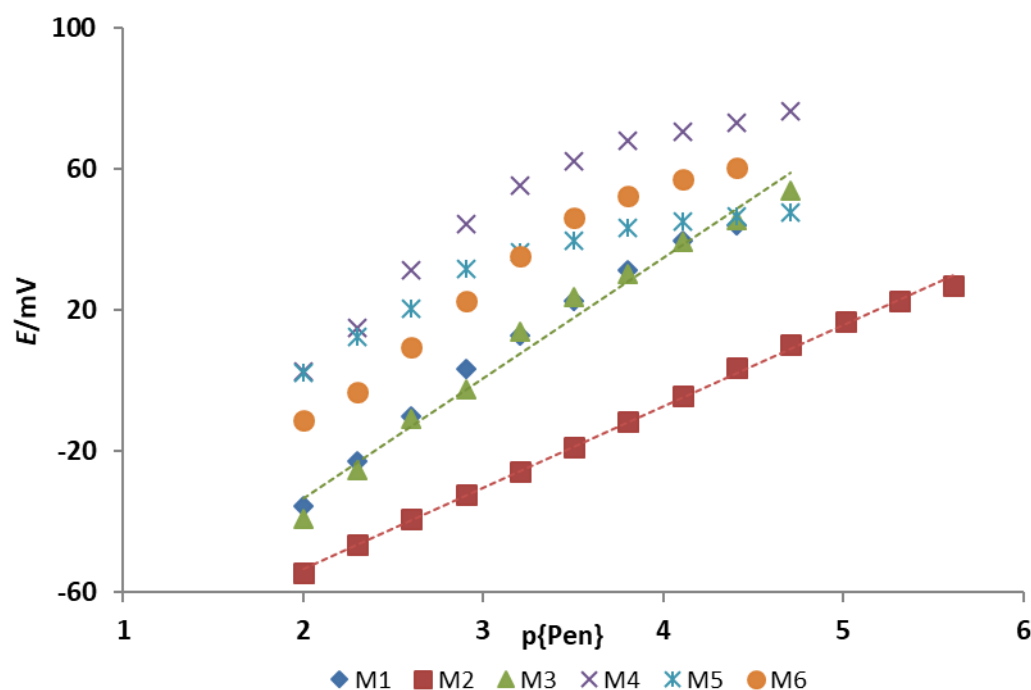

Figure 5. Response of "home-made" iodide ISE to Pen, pH = 4

In case of membranes response to Pen, we made a linear regression only for acceptable data sets. It can be seen that only membranes designate as M2, M3 and M6 showed good linear dependence by the change of Pen concentration. The best membrane has been proven to be M2, at pH = 4, with a linear response in the concentration range $2.45 \times 10^{-6} \text{-} 1 \times 10^{-2}$ mol L^{-1}, and a detection limit 2.24×10^{-6} mol L^{-1}. The stable potential was reached in about 45 seconds. The potential change of 23.00 mV, for the decade concentration change of Pen, with the correlation coefficient of 0.9983 was recorded in an

acetic buffer, pH = 4. In earlier works [1-3, 5, 9, 24] were given methods with a linear response range of, around, two concentration decades. In comparison with results obtained by Bralic & Radic [24] and Vukušić et al. [1] with commercially available iodide ISE and copper ISE, respectively, and proposed method with "home-made" iodide ISE enriched with ZnO nanoparticles, now the extending linear response range for Pen can be seen, as well as decreasing limit of detection for one and two concentration decades, respectively. Due to the use of non-toxic solvents and its simplicity, a proposed novel method is suitable for determination of the Pen's wide concentration range.

When our proposed sensor is compared to other electroanalytical sensors described in last two years, but used in completely different electroanalytical techniques, it is quite easy to see its simplicity in terms of preparation and use. Descriptions for sensors listed in Table 2 can be found in the relevant literature. Limit of detection (LOD), linear response range and technique where the described sensor is used are presented in Table 2:

Table 2 Comparison of similar sensor with one proposed in this work for penicillamine determination

Reference	LOD/mol L^{-1}	Linear range/mol L^{-1}	Technique/method type
This work	2.24×10^{-6}	2.45×10^{-6}-1×10^{-2}	Potentiometry
[25]	0.015×10^{-6}	0.03×10^{-6}–2.50×10^{-4}	Cyclic voltammetry
[26]	0.50×10^{-6}	0.50×10^{-6}–5.00×10^{-4}	Linear sweep voltammetry
[27]	0.10×10^{-6}	0.10×10^{-6}–2.50×10^{-4}	Square wave voltammetry
[28]	0.10×10^{-6}	0.50×10^{-6}–3.0×10^{-4}	Square wave voltammetry
[29]	0.10×10^{-6}	1.0×10^{-6}–9.0×10^{-4}	Differential pulse voltammetry

Although, at first glance, characteristics (LOD and linear range) given in Table 2 do not go in favor of our sensor, this is not quite the case. The main advantages of our sensor are its robustness, simplicity to use, cheap, simple and relatively fast preparation. On the other hand, it should be noted that the method described here does not require pharmaceutical pretreatment.

The proposed method can be easily implemented in pharmaceutical plants for quality control in various possibilities, e.g. on-line.

Membrane showing best response (M2) to Pen were analyzed on SEM with EDS, Figures 7-8.

Our newly proposed direct potentiometric method of Pen determination was used for measuring the concentration of Pen in tested pharmaceuticals (Table 3). Since we have experience in pharmaceuticals' testing, we used our earlier procedure [1]. The results are given in Table 2. The best results were obtained with membrane M2. They show a good agreement between measured and expected values with recorded recovery 99.88-100.57%.
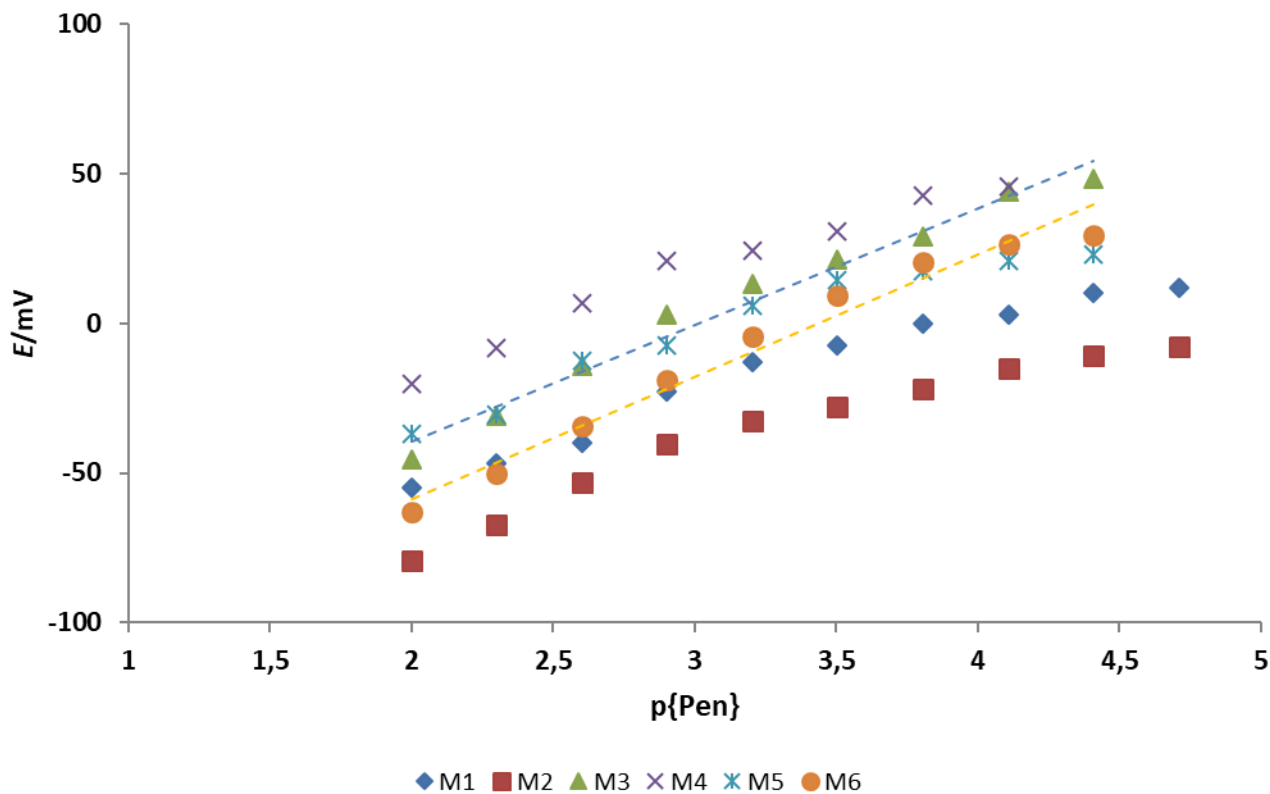
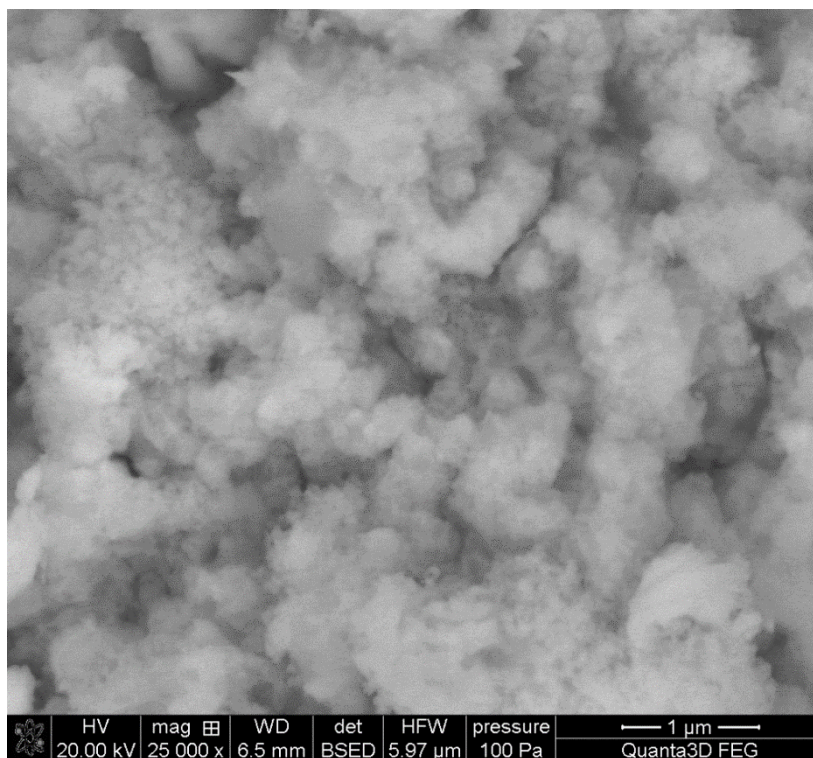**Figure 6.** Response of "home-made" iodide ISE to Pen, pH = 4.75

Figure 7. SEM image of M2

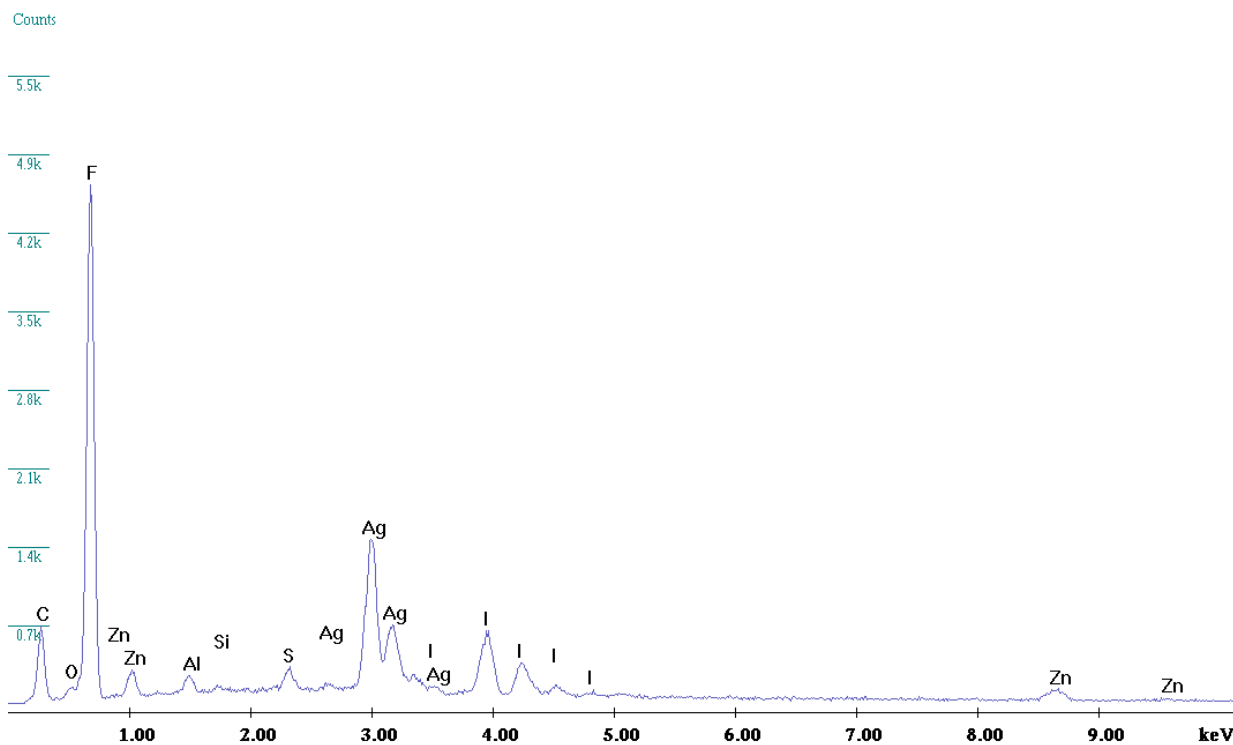Label A: Chlorite (Nrm.%= 38.86, 20.96, 34.83, 1.14, 3.84, 0.28)

Figure 8. EDS spectra of M2

Table 3. Determination of Pen in real samples for membrane M2

	Labelled, mg	**Found ±SD (%) (*n*=5)**	**Recovery (%)**
Metalcaptase®	150 (one pile)	149.82±2.65	99.88
	565 (five piles – smashed, value has been calculated)	568.22±2.78	100.57

4. CONCLUSIONS

"Home-made" iodide ion-selective electrode enriched with ZnO nanoparticles (ISE) is an adequate sensor in the direct potentiometric method for Pen determination in the acetic buffer, pH = 4. The proposed method does not require pharmaceuticals pretreatment. Linear response range is c(Pen) $= 2.45 \times 10^{-6}$ -1×10^{-2} mol L^{-1}, with the potential change of 23.00 mV per decade of Pen concentration. By earlier findings, it was supposed that Pen forms both very stable complex/sparingly soluble compound in reaction with Ag$^+$ and Zn^{2+} from the surface of the membrane and causes potential change. The found values of tested pharmaceuticals are in a very good agreement with the declared values having standard deviation of about 2.70 %.

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