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Automatic amperometric titration method for quantitative determination of zinc oxide in ointments

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Abstract

Background: Zinc is an important trace mineral in human body, therefore development of new methods for zinc determination in drugs is an actual task.
Material and methods: Assay of standard solutions of zinc ions and solutions of ointments to be analyzed was performed automatically using the titrator TITRION, which recorded, then displayed on the screen each amperometric titration curve. The titration curves had a sudden break in the current intensity at the equivalence point, caused by anodic oxidation of the titrant and cathodic reduction of H^+ at pH 3 – 4. The stoichiometry of Zn^{2+} ion sedimentation reaction with $[Fe(CN)_6]^{4-}$ ions was studied and confirmed in the presence of the background electrolyte and the ZnO calculation formulas in the analyzed ointments were deduced.
Results: Two different ointment masses were weighed for the Zoxitin ointment and the separate calculation results were 0.4172±0.0051 g/g and 0.4212±0.0051 g/g of ointment, and in the zinc oxide ointment ω(ZnO) was 10.208±0.078 %.These results were compared with the results of ZnO analysis in ointment by the classical complexometric titration method. For the Zoxitin ointment m(ZnO) was 0.4223±0.007 g/g of ointment, and for the ointment with ZnO ω(ZnO) was 10.18±0.11%.
Conclusions: An automatic amperometric titration method was developed to quantify ZnO in ointments. The method is based on the sedimentation reaction of zinc ions with potassium ferrocyanide solution in presence of acetate buffer solution as background electrolyte.
Key words: automatic amperometric titration, zinc oxide, quantitative determination of Zn.

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Introduction

Zinc is a vital element for a healthy immune system, having an important role for cell growth, cellular metabolism promoting healthy growth in childhood, increasing resistance to infections and wound healing. Medicines with zinc content are usually for internal or external use. Zinc can also be found in preparations for ophthalmic use or in solutions for parenteral use in total parenteral nutrition.

Topically used, zinc-containing preparations exhibit emollient, anti-inflammatory, antiseptic and protective action. It contributes to the reduction and elimination of local manifestations of exudation, inflammation and irritation.

In this context, the elaboration of the methods of quantitative analysis of zinc in order to ensure the quality of pharmaceutical forms remains current.

Material and methods

The Zoxitin ointment (manufacturer Farmaprim, the Republic of Moldova) containing ZnO as the active substance of 400 mg/g of ointment and the ZnO ointment prepared by the pharmacy of *Nicolae Testemitsanu* State University of Medicine and Pharmacy, the Republic of Moldova with the ZnO mass fraction of 10 %.

Laboratory vessels: volumetric flasks with different capacities, conical flasks and beakers with capacities of 50 and 100 ml, two DAPpette automatic pipettes with capacities of 100 – 1000 µl and 1000 – 5000 µl. The masses of the ointments to be analyzed, the masses of ZnO and $K_4[Fe(CN)_6]\cdot 3H_2O$ samples used for the preparation of the analyte and standard solutions were weighed into conical flasks or glass vials using the RADWAG AS 110.RI balance.

For the determination of ZnO in the nominated ointments, the automated amperometric titration kit named titrator TITRION (manufacturer ECONIS EXPERT, Russia) was used in the titration curve recording mode. This titrator is designed to perform automated volumetric assay of solutions, in which oxido-reduction processes take place, using amperometric method based on the measurement of the current intensity in the circuit of a system of two Pt

electrodes to the application of an external voltage. The general aspect of this automated amperometric titration kit is shown in fig. 1.

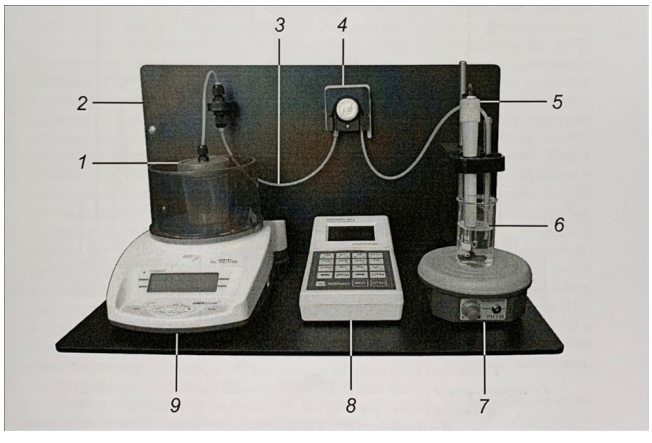

Fig. 1. External appearance of the kit for automatic amperometric titration – titrator TITRION.

1 – beaker with the titrant solution, 2 – the case of titrator TITRION, 3 – pump tube, 4 – peristaltic pump, 5 – electrode system, 6 – beaker with solution to be analyzed, 7 – magnetic stirrer, 8 – liquid analyzer EXPERT-001, 9 – balance.

The "brain" of this titrator is the liquid analyzer EXPERT-001, which contains programs for assay, measuring the current intensity (μA) and the solution fractions (ml) of the titrant added to the analyte solution over certain time intervals (s), announces the end of the titration, displays the value of the current intensity and the total volume of the titrant consumed for quantitative determination, records and displays on the screen the titration curve, which is processed by the operator to determine the titrant equivalence volume [1].

Preparation of solutions

All the solutions used in the study were prepared from the reagents, classified as "chemically pure", but the potassium ferrocyanide was recrystallized from the saturated aqueous solution upon addition of ethanol [2].

Standard and auxiliary solutions

ZnO standard solutions with molar concentrations equal to 0.005 mol/l and 0.01 mol/l were prepared in the study as follows. ZnO masses equal to 0.1017 g and 0.1627 g, respectively, were quantitatively passed into 250 ml and 200 ml volumetric flasks, and 4.0 ml of solution with c(HCl)=2 mol/l were added. The ZnO masses were dissolved, the solutions were diluted with distilled water up to the mark and homogenized. The solution with c(HCl)=2 mol/l was prepared from fixanal (c(HCl)=0,1 mol/dm^3).

In the automatic amperometric titration method as the titrant in the studying of the stoichiometry of the Zn^{2+} ion sedimentation reaction as well as to determine ZnO in the analyzed ointments, potassium ferrocyanide solutions with molar concentrations equal to 0.01 mol/l and 0.04 mol/l were used. These solutions were prepared by calculating, accurately weighing the masses of these samples of complex salt of $K_4[Fe(CN)_6]\cdot 3H_2O$, which had been quantitatively passed into a 50 ml volumetric flask, dissolved, diluted with distilled water to the mark and homogenized. The titrant solutions were used on the day of their preparation.

Zinc oxide in the solutions for the analysis of the ointments was quantitatively determined by the complexometric titration method. In this method, the disodium salt of EDTA, which is a crystalohydrate, used in general formula $Na_2H_2Y\cdot 2H_2O$ [3-5], is used as the primary standard and is also called disodium edetate [5]. Standard solutions with c(1/2 Na_2H_2Y)=0.05 mol/l and c(1/2 Na_2H_2Y)=0.1 mol/l were used in the analysis. The first solution was prepared by accurately weighing the calculated sample of $Na_2H_2Y\cdot 2H_2O$ crystalohydrate with a mass of 0.9306 g, which was quantitatively passed into a 100 ml volumetric flask, dissolved, diluted to the mark with distilled water and homogenized. The second solution was prepared from fixanal (c(1/2 $Na_2H_2Y\cdot 2H_2O$)=0.1 mol/l).

Auxiliary solutions were also used in the study. All quantitative determinations in the automatic amperometric method were performed in the presence of acetate buffer with different pH values, which was used as background electrolyte. These solutions were prepared by mixing the sodium acetate solution containing a constant mass of $Na(CH_3COO)\cdot 3H_2O$, with different volumes of concentrated CH_3COOH acid solution.

In the complexometric titrations to maintain pH=10, the ammonia buffer solution was used. This solution was prepared as follows. Ammonium chloride with mass of 5.4 g was dissolved in a minimum volume of distilled water, 35 ml concentrated NH_3 solution was added thereto and diluted with distilled water to 100 ml of buffer solution. The volume of the titrant in this method was measured using a 2.00 ml microburette.

The pH of the analyzed solutions was measured using the I-160M ionometer, connected with a glass indicator electrode and a silver–silver chloride reference electrode.

Preparation of Zoxitin ointment solution to be analyzed

In a 100 ml conical flask, about 0.2 – 0.3 g (exact mass of ointment), 4.0 ml of solution with c(HCl)=2 mol/l and 25 ml distilled water were added. The conical flask was installed on a hot asbestos plate, and the solution was heated to boiling temperature, kept near this temperature, and shaken until the entire ointment base together with ZnO dissolved. The solution was cooled then under the tap, filtered, the filtrate was taken up in a 200 ml volumetric flask. The conical flask and the filter were washed 2 – 3 times with 15 – 20 ml of distilled water, then the solution in the volumetric flask was diluted with distilled water to the mark and homogenized.

The solution to be analyzed of ZnO ointment prepared in the pharmacy of the *Nicolae Testemitsanu* State University of Medicine and Pharmacy was prepared analogously to the Zoxitin ointment solution except that about 1 g (exact mass) of the ointment was taken for the assay.

Results and discussions

The previous publications [6-10] have been devoted to the elaboration, completion and masking of interfering ions in the quantitative determination of Zn in different electrolytes by the amperometric titration method with $K_4[Fe(CN)_6]$ solution. For this, anodic oxidation of titrant excess and background electrolytes with different composition and concentrations were used, as well as the composition of the sediment, formed during assay, was all different [6].

The information about an amperometric titrator developed by ECONIS EXPERT for didactic purposes was published in the indication [11]. The titrator is equipped with two Pt indicator electrodes. Zn assay was performed with $K_4[Fe(CN)_6]$ solution in the presence of acetate buffer solution (pH 3 – 4) as background electrolyte and the sediment composition was complex salt $Zn_2[Fe(CN)_6]$.

Method of preparation and titration of the solutions to be analyzed using the titrator TITRION

Using an automated pipette, a certain volume of standard solution of Zn^{2+} ions or solution to be analyzed of the ointment was measured and added to a 50 ml titration beaker. In beaker, 10.0 ml of distilled acetate solution and distilled water were added with a pipette, so that the final volume of the solution taken for titration was 20.0 ml. The solution was then automatically titrated with standard $K_4[Fe(CN)_6]$ solution using the titrator as indicated in the operating compendium [1].

All amperometric determinations with standard $K_4[Fe(CN)_6]$ solution were performed in the titration curve recording mode and the voltage applied to the indicator electrodes was 0.2 V. The curves, which were obtained as a result of assay, had a very pronounced turning of the current intensity at the equivalence point. They were processed using the liquid analyzer EXPERT to determine the equivalence volume of the titrant [1]. As an example in fig. 2 are shown the appearance of an amperometric titration curve of a solution to be analyzed with $K_4[Fe(CN)_6]$ solution and the determination of equivalence volume of titrant.

However, at amperometric dosage with this titrant solutions, where the concentration of Zn^{2+} ions was in the order

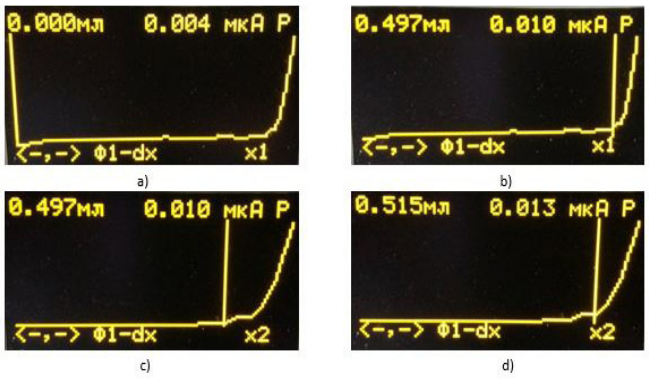

Fig. 2. Appearance of the titration curve of a solution to be analyzed with ZnO (a), its processing to determine the titrant volume in the near of the equivalence point (b and c) and the equivalence point (d).

Fig. 3. The appearance of a Zn^{2+} ion solution titration curve with $K_4[Fe(CN)_6]$ standard solution ($c(Zn^{2+}) = 5 \cdot 10^{-3}$ mol/l; $c(K_4[Fe(CN)_6]) = 0.04$ mol/l; pH 3.5).

of $n \cdot 10^{-3}$ mol/l, the appearance of the titration curves was similar to that shown in fig. 3. This aspect of the titration curve up to the equivalence point of the titrant can probably be elucidated on the basis of the equilibrium that results in the solution at the formation of the sediment [6, 12].

In studying the stoichiometry of the Zn^{2+} ion sedimentation reaction with the $K_4[Fe(CN)_6]$ standard solution by the amperometric titration method, the preparation of the solution for titration and its assay was carried out according to the procedure outlined above. The data obtained are shown in tab. 1.

Table 1

Study of stoichiometry of Zn^{2+} ion sedimentation reaction with $K_4[Fe(CN)_6]$ standard solution by the amperometric titration method with two Pt indicator electrodes

№	V (Zn^{2+}) ml	n (Zn^{2+})·10^2 mmol	V ($K_4[Fe(CN)_6]$) ml	n ($K_4[Fe(CN)_6]$)·10^2 mmol	$\frac{n(Zn^{2+})}{n(K_4[Fe(CN)_6])}$
1	0.50	0.25	0.125	0.125	2.000
2	1.00	0.50	0.252	0.252	1.984
3	1.50	0.75	0.372	0.372	2.016
4	2.00	1.00	0.498	0.498	2.008
5	2.50	1.25	0.619	0.619	2.019
6	3.00	1.50	0.748	0.748	2.005
7	4.00	2.00	1.004	1.004	1.992
8	5.00	2.50	1.243	1.243	2.011

($c(Zn^{2+})$=0.005 mol/l; $c(K_4[Fe(CN)_6)$=0.01 mol/l; $\Delta\varphi$=0.2 V; pH = 3.7)

Table 2

Study of the influence of pH of the solution on the stoichiometry of Zn^{2+} ion sedimentation reaction with $K_4[Fe(CN)_6]$ standard solution by the amperometric titration method with two Pt indicator electrodes

№	pH	V (Zn^{2+}),ml	n (Zn^{2+})·10^2 mmol	V ($K_4[Fe(CN)_6]$) ml	n ($K_4[Fe(CN)_6]$)·10^2 mmol	$\frac{n(Zn^{2+})}{n(K_4[Fe(CN)_6])}$
1	3.17	2.00	1.00	0.497	0.125	2.012
2	3.33	2.00	1.00	0.502	0.252	1.992
3	3.55	2.00	1.00	0.498	0.372	2.008
4	3.87	2.00	1.00	0.499	0.498	2.004
5	4.02	2.00	1.00	0.502	0.619	1.992
6	4.21	2.00	1.00	0.512	0.748	1.953

($c(Zn^{2+})$=0.005 mol/l; $c(K_4[Fe(CN)_6]$=0.01 mol/l; Δφ =0.2 V)

It is confirmed that in the presence of the acetate buffer solution as a background electrolyte the ratio $n(Zn^{2+})$: $n(K_4[Fe(CN)_6])$ = 2 : 1 and the sedimentation reaction takes place in the solution after the ionic equation: $2\ Zn^{2+} + [Fe(CN)_6]^{4-} = Zn_2[Fe(CN)_6]$.

It does not influence this ratio nor the pH of the solution in the range of values 3.17 – 4.02 (tab. 2), which was investigated in analogy with the stoichiometry of the Zn^{2+} ion sedimentation reaction with $K_4[Fe(CN)_6]$.

The mass of zinc oxide (m(ZnO), g/g) in one gram of Zoxitin ointment, based on the stoichiometry of the sedimentation reaction, can be calculated according to the formula:

$$m(ZnO) = 2 \times c(K_4[Fe(CN)_6] \times V(K_4[Fe(CN)_6] \times M(ZnO) \times \frac{V_0 \times 10^{-3}}{V_1 \times m_u}, \quad (1)$$

in which: $c(K_4[Fe(CN)_6])$ – the molar concentration of the titrant solution, mol/l;

$V(K_4[Fe(CN)_6])$ – the titrant's volume of equivalence, ml;

M(ZnO) – molar mass of zinc oxide, g/mol;

V_0 – the capacity of the volumetric flask with Zoxitin ointment solution, ml;

V_1 – the analyte fraction of this ointment, taken for analysis, ml;

m_u – ointment mass, taken for analysis, g.

However, the equation of the sedimentation reaction of Zn^{2+} ions with $K_4[Fe(CN)_6]$ standard solution, shows that the equivalence factor of the titrant is equal to ½. In this case, the product $2 \times c(K_4[Fe(CN)_6]$ is equal to $c(\frac{1}{2}K_4[Fe(CN)_6]$ and equation (1) simplifies and turns into the relation:

$$m(ZnO) = T_{K_4[Fe(CN)_6]/ZnO} \times V(K_4[Fe(CN)_6] \times \frac{V_0}{V_1 \times m_u}, \quad (2)$$

in which: $T_{K_4[Fe(CN)_6]/ZnO}$ – the theoretical titre of the - potassium ferrocyanide solution relative to ZnO, g/ml. The other notes are shown above.

Table 3

Determination of the mass of zinc oxide in one gram of Zoxitin ointment by automatic amperometric titration method with two Pt indicator electrodes

a – m_u=0.2871 g

№	V_1, ml	V ($K_4[Fe(CN)_6]$), ml	m (ZnO), g/g
1	0.50	0.179	0.4057
2	0.75	0.181	0.4103
3	1.00	0.277	0.4186
4	1.25	0.370	0.4193
5	1.50	0.461	0.4180
6	2.00	0.562	0.4246
7	2.50	0.741	0.4199
8	3.00	1.113	0.4204

($T_{K_4[Fe(CN)_6]/ZnO}$ = 0.0016274 g/ml; V_0=200 ml; Δφ =0.2 V; pH 3.5)

b – m_u=0.3556 g

№	V_1, ml	V ($K_4[Fe(CN)_6]$) ml	m (ZnO), g/g
1	1.00	0.116	0.4247
2	1.50	0.175	0.4272
3	2.00	0.235	0.4302
4	2.50	0.276	0.4042
5	3.00	0.348	0.4247
6	3.50	0.401	0.4195
7	4.00	0.461	0.4220
8	4.50	0.518	0.4215
9	5.00	0.568	0.4159
10	6.00	0.692	0.4223

($T_{K_4[Fe(CN)_6]/ZnO}$ = 0.0065096 g/ml; V_0=200 ml; Δφ =0.2 V; pH 3.5)

Equation (2) was used to calculate the results of ZnO analysis in Zoxitin ointment analytical solutions by the automatic amperometric titration method with two Pt-

indicator electrodes. The results are presented in tab. 3a and tab. 3b. For quantitative determination of solutions to be analyzed of this ointment, as a titrant, standard solution of $K_4[Fe(CN)_6]$ with molar concentration of the equivalent equal to 0.02 mol/l and 0.08mol/l were used, by means of which the theoretical titre $T_{K_4[Fe(CN)_6]/ZnO}$ from equation (2) was calculated.

Results of ZnO analysis in the solutions to be analyzed for the two Zoxitin ointment masses in tab. 3a and tab. 3b have been processed statistically separately and the mean mass of ZnO in one gram of ointment consisted of (0.4172±0.0051) g/g (tab. 3a) and (0.4212±0.0051) g/g (tab. 3b), with a confidence interval of 95 %.

Using the mentioned titrator, the assay of the ZnO ointment solution, prepared in the pharmacy of Nicolae Testemitsanu State University of Medicine and Pharmacy, was also made. The results obtained are presented in tab. 4.

Table 4

Determination of zinc oxide mass in ZnO ointment solution, prepared in the pharmacy of *Nicolae Testemitsanu* State University of Medicine and Pharmacy, by automatic amperometric titration method with two Pt indicator electrodes

№	V_1, ml	V ($K_4[Fe(CN)_6]$), ml	ω (ZnO), %
1	0.50	0.173	10.247
2	1.00	0.349	10.336
3	1.25	0.435	10.306
4	1.50	0.515	10.168
5	2.00	0.696	10.306
6	2.50	0.862	10.212
7	3.00	1.024	10.109
8	4.00	1.371	10.151
9	5.00	1.694	10.034

($T_{K_4[Fe(CN)_6]/ZnO}$ = 0.0016274 g/ml; V_0=200 ml; m_u=1.099 g; Δφ =0.2 V; pH 3.5)

The mass fraction of ZnO (ω(ZnO), %) in this ointment was calculated according to the equation:

$$\omega(ZnO) = T_{K_4[Fe(CN)_6]/ZnO} \times V(K_4[Fe(CN)_6] \times \frac{V_0 \times 100}{V_1 \times m_u}, \quad (3)$$

The results of the analysis ω(ZnO) in tab. 4 were statistically processed and for this ointment the mean mass fraction of ZnO was (10.208±0.078) % for the 95 % confidence interval.

To evaluate and confirm the results obtained by the automatic amperometric titration method, the analytical solutions of these two ointments were also analyzed by the complexometric method. In a 100 ml titration flask a certain volume of the ointment solution was added, measured by means of an automatic pipette, 5 – 6 ml of ammonia buffer solution with pH=10, 20 ml distilled water, 0.05 g mixture eriochrome black T indicator and the obtained solution was titrated with standard solution of $Na_2H_2Y{\cdot}2H_2O$ with c($1/2Na_2H_2Y$) equal to 0.05 mol/l or 0.1 mol/l until the change from red to violet in blue [5]. The results obtained are presented in tab. 5 and tab. 6.

Table 5

Determination of the mass of zinc oxide in one gram of Zoxitin ointment by the complexometric titration method

№	V_1, ml	V (Na_2H_2Y), ml	m (ZnO), g/g
1	3.00	0.37	0.4194
2	3.50	0.43	0.4178
3	4.00	0.50	0.4251
4	4.00	0.51	0.4336
5	4.50	0.56	0.4232
6	5.00	0.61	0.4149

($T_{Na_2H_2Y/ZnO}$ = 0.004069 g/ml; V_0=200 ml; m_u=0.2393 g; pH 10)

Table 6

Determination of zinc oxide mass fraction in the ZnO ointment, prepared in *Nicolae Testemitsanu* State University of Medicine and Pharmacy, by the complexometric titration method

№	V_1, ml	V (Na_2H_2Y), ml	ω (ZnO), %
1	2.00	0.55	10.18
2	2.50	0.70	10.36
3	2.75	0.74	9.96
4	3.00	0.83	10.24
5	3.50	0.98	10.36
6	4.00	1.09	10.09
7	4.50	1.23	10.12
8	5.00	1.37	10.14

($T_{Na_2H_2Y/ZnO}$ = 0.002034 g/ml; V_0=200 ml; m_u=1.099 g; pH 10)

Calculations of the results of the complexometric analysis of ZnO mass in one gram of ointment (m(ZnO), g/g) Zoxitin was performed according to the relationship:

$$m(ZnO) = T_{Na_2H_2Y/ZnO} \times V(Na_2H_2Y) \times \frac{V_0}{V_1 \times m_u}, \quad (4)$$

in which: $T_{Na_2H_2Y/ZnO}$ – the theoretical titre of the titrant solution relative to ZnO, g/ml;

$V(Na_2H_2Y)$ – the titrant's volume of equivalence, ml.

The other marks are shown above.

All the notes in equation (4) remained the same for equation (5), after which the mass fraction of ZnO (ω (ZnO),%) was calculated in the solution of ZnO ointment, prepared in the SUMP «Nicolae Testemitsanu» pharmacy, using the complexometric titration method:

$$\omega(ZnO) = T_{Na_2H_2Y/ZnO} \times V(Na_2H_2Y) \times \frac{V_0 \times 100}{V_1 \times m_u}, \quad (5)$$

The results in tab. 5 and tab. 6, obtained by the complexometric titration method, were statistically processed. For the Zoxitin ointment the mean ZnO mass was (0.4223±0.007) g/g of ointment, and for the ZnO ointment prepared in the pharmacy of *Nicolae Testemitsanu* State University of Medicine and Pharmacy, the mean mass fraction of ZnO constituted (10.18±0.11) %. In both calculations, the confidence interval was 95%.

These results compared to the automatic amperometric titration method with two Pt-indicator electrodes are satisfactory. However, for one and the same 95% confidence level, the uncertainty of measurement of both m(ZnO) and ω (ZnO) from their mean values in ointment (see above) is lower in the automatic amperometric titration method compared to the classical complexometric titration method. This can be explained by the fact that in the electrochemical automated assay method of solutions of ZnO in ointments by means of the mentioned titrator, the equivalence volume of the titrant was determined more accurately than visually in the complexometric titration method.

Conclusions

1. A new method of automatic amperometric dosage of ZnO in ointments was developed using the titrator TITRYRON. The developed method can be recommended for the quantitative analysis of ZnO in ointments.

2. Quantitative analysis of ZnO in ointments was performed by two methods: automatic amperometric dosing with two Pt indicator electrodes by titrator TITRION and classical complexometric. Both methods yielded satisfactory results, but the accuracy limits from the mean value of ZnO in ointments were lower in the developed method compared to the complexometric titration method.

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Authors' contributions
VO designed the trial and interpreted the data. VV acquired and interpreted the data. CC interpreted the data. MN conducted/performed the laboratory work, drafted the first manuscript. SO described and processed the physics data. LM conducted/performed the laboratory work, revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

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Ethics approval and consent to participate
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Conflict of Interests
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