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THE INFLUENCE OF ACID PROTEIN PRECIPITANTS ON THE SPECIFICITY OF THE REACTION OF NINHYDRIN WITH AMINO ACIDS

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Aim. The purpose of the work was to determine the effect of trichloroacetic (TCA) and perchloric ($HClO_4$) acids on the result of ninhydrin reaction with various amino acids.

Methods. A standard method of amino acid detection using a ninhydrin reagent was applied. Optical spectra and density of reaction products were determined spectrophotometrically.

Results and conclusions. As a result, it was found that the studied acids change the spectral characteristics of the products of the ninhydrin reaction with amino acids. TCA significantly reduced the optical density of chromophores, and $HClO_4$ also led to a significant shift of the spectra of the reaction products into the short-wavelength region. An exception was the reaction with proline, as a result of which a well-defined maximum appeared in the product spectrum: $\lambda = 620$ nm in the presence of TCA and $\lambda = 515$ nm with $HClO_4$. At the same time, in the presence of $HClO_4$, the reaction became highly specific for proline.

The conditions for the ninhydrin reaction with proline upon addition of $HClO_4$ were analyzed in detail. As a result, a new method of highly specific determination of proline in the presence of other amino acids was proposed.

Key words: ninhydrin reaction, amino acids, proline, trichloroacetic acid, perchloric acid.

For practical purposes, proteinases are widely used in various industrial biotechnological processes [1–4] and medicine [5–7]. The reaction of amino acids with ninhydrin, which is a well-known reagent for proteins, peptides, and amino acids, is most often used to study proteolytic activity [8]. Moore and Stein made a significant contribution to the widespread use of the ninhydrin reaction [9]. These scientists analyzed in detail the main indicators and parameters of the reaction (properties of chromogenic products, duration of heat treatment and color preservation time, pH value, intensity of color and spectra of the products based on interaction with individual amino acids), proposed a method of color stabilization, etc. Thanks to the work of Moore and Stein, the method of determining amino acids using the ninhydrin reaction has become routine and is widely used in the chromatographic analysis of the amino acid composition of proteins, as well as in studying the activity of proteinases, collagenases, etc. [10].

Despite the ancient history of ninhydrin reaction, all its features have not yet been ascertained. Thus, we did not find detailed information in the literature about how protein precipitants — perchlorate ($HClO_4$) and trichloroacetic (TCA) acids — will affect the result of ninhydrin reaction with amino acids. These acids are usually used to separate the high-polymer molecules of substrate and enzyme from hydrolysis products [9], which is important for a more accurate analysis of proteolytic activity. Therewith, it should be emphasized that the ninhydrin reaction is not specific and the vast majority of amino acids form a chromogenic product with approximately the same optical density [8].

Thereby, the aim of the work was to determine how TCA and $HClO_4$ will affect the specificity and peculiarities of ninhydrin reaction with various amino acids (optical spectrum, duration of chromogenic products preservation, time of heat treatment, etc.).

Materials and Methods

L-amino acids (Serva), TCA and $HClO_4$, dimethylsulfoxide (DMSO), methyl cellosolve (MC), isopropanol of the "ag" or "rg"x qualification were used in the work. Ninhydrin was purified by double recrystallization from water.

The effect of TCA was studied at a final concentration of 4.5% and 10%, and the effect of perchloric acid was in the range of final concentration from 4.1% to 10.4%. It is known that the minimum indicated doses of these acids are sufficient for proteins separation and low-molecular products of their hydrolysis [11]. Therefore, lower concentrations of acids were not studied.

In the experiments, a scheme for detecting amino acids using a ninhydrin reagent was used as a standard, as described in [10]. As a control, a volume of water equivalent to the volume of acid precipitant used in the experiment was added to 0.5 ml of a 1 mM aqueous solution of amino acids. After 15–20 min ninhydrin solution was poured in and the mixture was incubated in a boiling water bath (95±3 °C). The volume of the ninhydrin reagent and the duration of heat treatment were varied depending on the experiment tasks. After cooling the mixture to room temperature (10 min), a solvent (ethanol, isopropanol, methylcellosolve, dimethylsulfoxide or a mixture of the last two) was added. The optical spectra and optical density of the reaction products were determined on a Cary 60 UV-Vis spectrophotometer in quartz cuvettes with an absorbing layer thickness of 1 cm.

The ninhydrin reaction result (chromogenic product yield) was expressed in units of optical density. Experiments were performed in 3–5 independent repetitions. Data processing was carried out using standard Microsoft Excel package: average arithmetic values and their standard errors were calculated, as well as graphs were constructed and approximation equations and their probability level were obtained.

Results and Discussion

It was found that the ninhydrin reaction result depended both on the applied precipitants and on the investigated amino acids. In the presence of TCA, for all amino acids (except imino acids), the optical absorption spectrum maximum of the reaction products remained unchanged and amounted to 570 nm, as in the standard method [10]. However, this acid in most cases significantly reduced the optical density (Table 1).

As it can be seen from the presented data (Table 1), it is difficult to find a connection between the structure of the amino acid and the effect of TCA on chromogenic products formation of the ninhydrin reaction. The formation of the colored product is significantly inhibited when studying amino acids of completely different groups (noted in Table 1 with a gray marker). Oxyproline in the studied conditions did not have a pronounced peak in the spectrum at all. At the same time, the minimum studied doses of TCA practically did not affect the analysis of glycine and alanine, and the effect of high acid concentration was relatively moderate.

Table 1. **The effect of TCA on the ninhydrin reaction result**

Reaction conditions	Researched amino acids *, **									
	Gly	Ala	Leu	Glu	Gln	Arg	Trp	His	Lis	Cys
Control, without TCA	1.50/ 100%	2.09/ 100%	2.10/ 100%	2.01/ 100%	1.73/ 100%	1.02/ 100%	1.20/ 100%	1.20/ 100%	1.55/ 100%	1.42/ 100%
4.5% TCA	1.48/ *99%*	2.00/ *96%*	0.41/ *20%*	0.42/ *20%*	1.16/ *66%*	0.20/ *20%*	0.16/ *20%*	0.73/ *60%*	0.66/ *43%*	1.25/ *88%*
10.0% TCA	1.20/ *80%*	1.44/ *67%*	0.00/ *0%*	0.00/ *0%*	0.16/ *9%*	0.00/ *0%*	0.00/ *0%*	0.08/ *7%*	0.00/ *0%*	0.24/ *17%*

Note: * — the value of the optical density in the maximum zone of the optical spectrum (λ = 570 nm) and ** — its level in the experiment compared to the control in % are indicated; experimental conditions — 1.5 cm^3 of ninhydrin reagent was added to 0.5 cm^3 of a 1 mM amino acid solution, the duration of heat treatment was 20 min, the final solvent of the reaction products: 3 cm^3 of a mixture of isopropanol and water in a 1:1 ratio.

A special picture was observed when determining proline. In the presence of TCA in the reaction mixture, a clearly defined wavelength maximum of $\lambda = 620$ nm appeard in the optical spectrum of the reaction products (Fig. 1). Furthermore, in the case of proline, an increase in TCA concentration led to an increase in the optical density of the resulting solution unlike the options with other amino acids.

Compared with TCA, the influence of $HClO_4$ on the ninhydrin reaction result was more significant (Fig. 2). For all investigated amino acids, the maximum characteristic of the reaction under standard conditions (λ = 570 nm) completely disappeared in the optical absorption spectra of the chromogenic reaction product. At the same time, the total optical density also decreased significantly. The reaction with lysine and tryptophan revealed an increase in optical density in a highly stretched region in the range of λ = 460–500 nm. Only with proline, the reaction product spectrum had a well-defined maximum at $\lambda = 515$ nm, absent for other amino acids, including oxyproline.

Thus, the addition both perchloric acid and TCA ensured the specificity of the reaction to proline.

The ninhydrin reagent used as a standard [10] had a pH value of 5.5. After adding TCA or $HClO_4$ to the amino acid solution, the acidity of the amino acid mixture with the ninhydrin reagent increased significantly and was ≤ 2. The peculiar behavior of proline in the ninhydrin reaction in an acidic medium was noted long ago [12]. Various methods of determining proline using an acidic ninhydrin reagent were proposed, of which the Bates' et al. one became the most popular [13].

Without special analytical studies, it is difficult to estimate the chemical mechanism of action concerning the investigated precipitating acids on the ninhydrin reaction result with amino acids. Perhaps, being strong oxidants and reagents capable of nucleophilic substitution, they affect the key stages of the reaction such as oxidation of amino acids and condensation of the resulting products. The role of $HClO_4$ as a cyclization catalyst is also known [14]. Friedman and Sigel [15] showed that the reaction of amino acids with ninhydrin depends both on the basicity of amino groups and steric features of amino components.

The high specificity of proline detection with a ninhydrin reagent in the presence of $HClO_4$ prompts detailed analysis and optimization of the main conditions for the procedure. First of all, it is necessary to analyze the following points:

1) to choose the optimal concentration of perchloric acid,

2) to specify the dependence of the reaction results on the duration of heat treatment with a ninhydrin reagent,

3) to determine the shelf life of the chromogenic product,

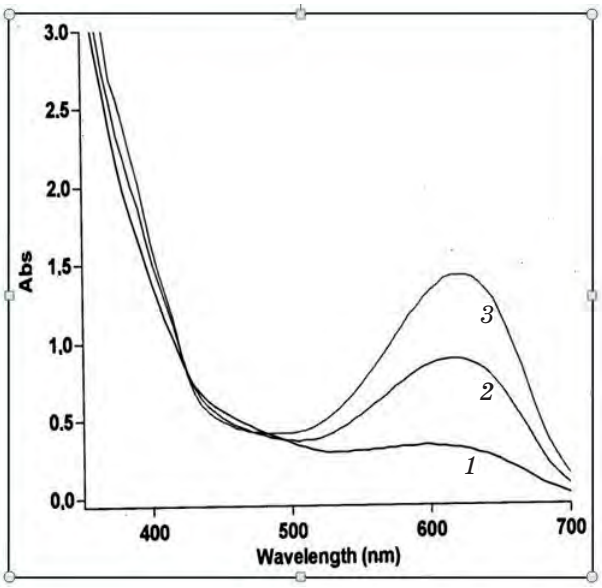

Fig. 1. **Spectrum of proline reaction products with ninhydrin in the presence of TCA:**
1 — proline without TCA, *2* — proline with 4.5 % TCA, *3* — proline with 10 % TCA, experimental conditions: 0.5 cm^3 of 1 mM imino acid solution, 1.5 cm^3 of ninhydrin solution, heat treatment duration was 20 min, after cooling 3 cm^3 of isopropanol mixture and water (1:1) were added.

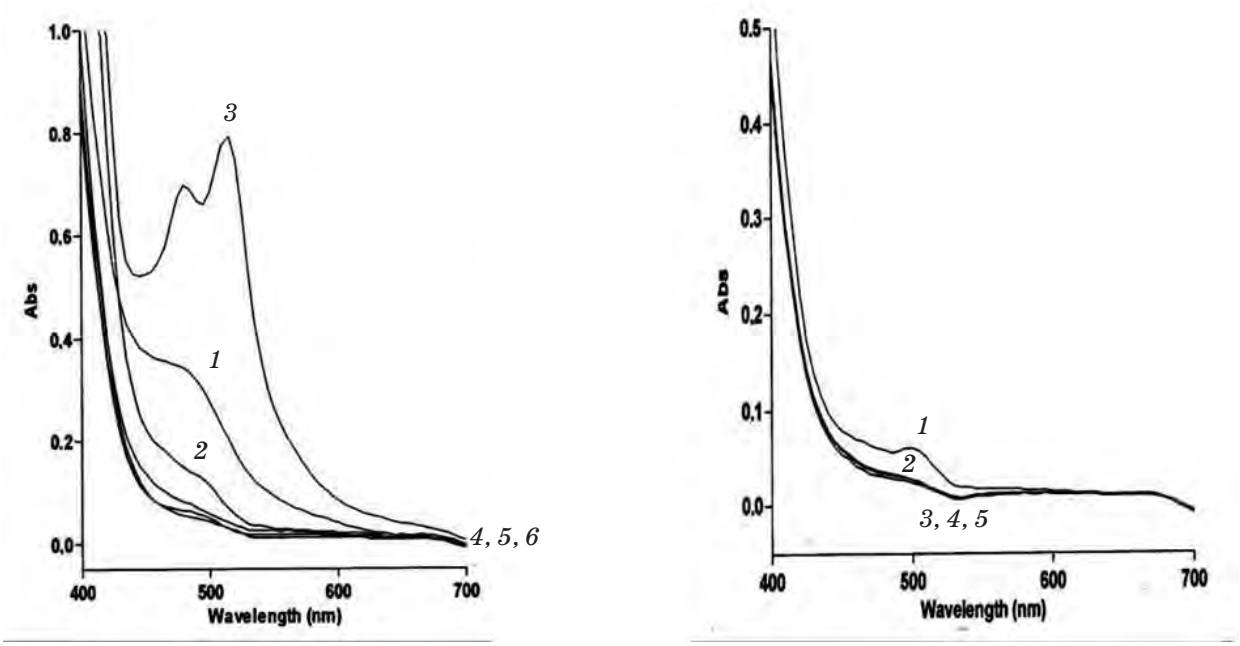

Fig. 2. **Reaction products spectra of the studied amino acids with ninhydrin in the presence of 4.1% $HClO_4$:**
a) *1* — lysine, *2* — tryptophan, *3* — proline, *4* — histidine, *5* — cysteine, *6* — glutamine;
b) *1* — oxyproline, *2* — arginine, *3* — glycine, *4* — alanine, *5* — leucine;
experimental conditions: 0.5 cm^3 of 1 mM amino acid solution, 1 ml of ninhydrin solution, heat treatment duration was 40 min, after cooling 2 cm^3 of DMSO and MC (1:1) mixture were added.

4) to find out the optimal ratio of the main components of the reaction mixture (non-hydric reagent and solvent),

5) to deafine the effect of other amino acids on the detection of proline (interference of proline with other components of proteins),

6) to find the linear dependence region of the optical density on proline concentration.

Elucidation of the above will contribute to a method development for proline specific determination. This method will primarily help to study the importance of this aminoacid in the protective reactions of organisms, and will also be useful for studying the functioning of proteinases, especially collagenases. The results of the search for the optimal concentration of $HClO_4$ are shown in Fig. 3. As it can be seen, increasing the acid dose is responsible for a negative effect on the detection of the colored product. Perhaps this is related to the increased destruction of the aminoacid by high concentrations of perchloric acid.

The influence of heat treatment duration on the final product optical density is given in Fig. 4, from which it can be seen that the reaction reaches a maximum in an hour. According to various protocols, the recommended processing time was from 20 to 60 min [16]. Thus, the obtained results fit into the specified terms.

The weak point of the ninhydrin reaction was the relative instability of the colored products. Different ways of increasing their stability are known: addition of complexing cations (Co^{2+}, Cu^{2+}, Ca^{2+}, etc.), use of non-aqueous solvents, pH value choice [8, 9, 17, 18].

The results of different solvents using after heat treatment were compared, as well as pH value changes (Table 2). It can be seen that ethanol and isopropanol, as the most frequently used solvents for ninhydrin reaction products [8, 9, 16–18] did not provide color stability under our conditions. Thus, when using isopropanol, a finely dispersed chromophore precipitate was noticed in the reaction mixture already after an hour, which caused a decrease in optical density indicators. It is known that one of the colored products of the ninhydrin reaction, hydrindanthin, dissolves very poorly in aqueous media and is unstable. Reducing the acidity of the environment by adding alkali (options No. 3 and 4) did not give a positive result. The most effective solvents were DMSO, MC and their mixture. With their use, the chromophores remained stable under these conditions for an hour. Perhaps this was primarily due to the high solubility of these substances. The introduction of reducing agents (dithiothreitol or ascorbic acid) did not protect the reaction products, but on the contrary, worsened the result.

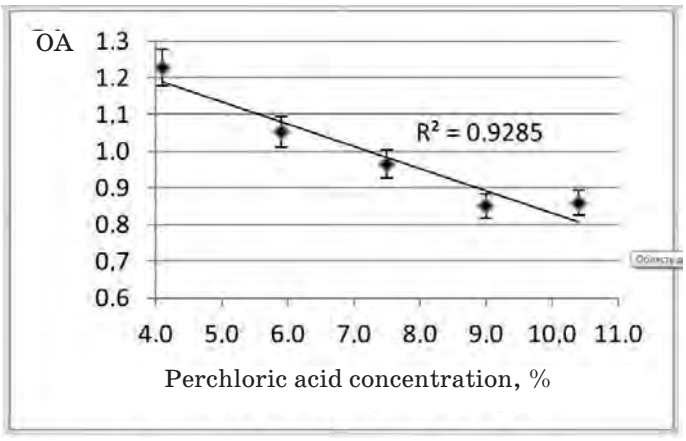

Fig. 3. **The influence of different concentrations of $HClO_4$ on the chromogenic product yield in the ninhydrin reaction**

Optical absorption (OA) at a wavelength of $\lambda = 515$ nm.
Experimental conditions: 0.050, 0.075, 0.100, 0.125, 0.150 cm^3 of 45% $HClO_4$, 1 cm^3 of ninhydrin reagent were added to 0.5 cm^3 of 1 mM proline solution; after heat treatment (45 min) and cooling (10 min), 2 cm^3 of a mixture of DMSO and MC (1:1) was added. R^2 is the reliability value of the linear approximation.

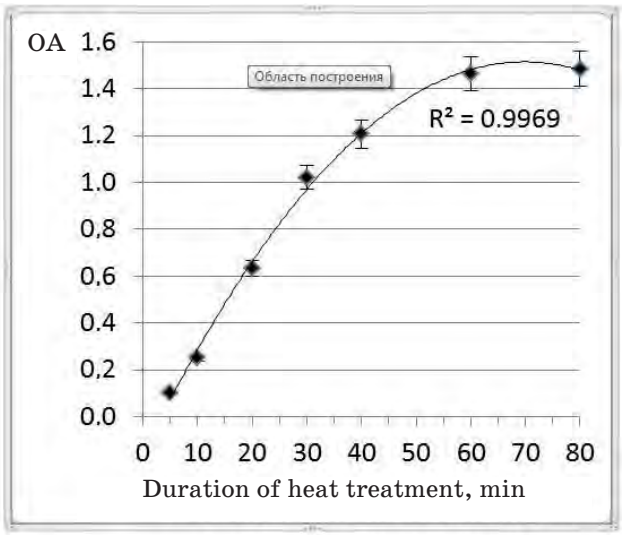

Fig. 4. **Quantity dependence of the obtained colored product (optical absorption, 515 nm) on the duration of treatment in a boiling water bath (min)**

Experimental conditions: 0.5 cm^3 of 1 mM proline solution with 0.05 cm^3 of 45% $HClO_4$, 1 cm^3 of ninhydrin reagent; after the end of heat treatment and cooling (10 min), 2 cm^3 of a mixture of DMSO and MC (1:1) was added. R^2 is the reliability value of the polynomial approximation.

It also was found that the stability of the ninhydrin reaction products depended not only on the solvents used, but on the duration of heat treatment as well (Table 3). As it can be seen from the presented data, stability was achieved when the reaction mixture was heated for 30 min and above. At the same time, high values of optical density, convenient for analysis, were provided.

The ratio of its components is important for the reaction optimization. Naturally, an increase in the relative dose of the ninhydrin reagent and a decrease in the amount of solvent (Tables 4, 5) leads to an increase in the value of the optical density of the reaction mixture. From the data presented, a tendency to stabilize the color could be observed with an increase in the relative proportion of the solvent. However, excessive dilution of the mixture with a solvent significantly impaired the analytical significance of the measurement.

From the given data (Tables 3–5), it can also be assumed that the ninhydrin reaction requires a certain time to stabilize the chromogenic products. This requires about 30 min after heat treatment. Thus

Table 2. **Stability dependence of ninhydrin products with proline on the solvents composition in perchloric acid presence**

No. of option	Reaction conditions: the solvent composition of the reaction products; the final pH value in the mixture	Optical density change (%) in time after the start of measurements (min)						
		0	30	60	90	120	150	180
1	Ethanol + water (2:1); pH ≤ 2	100*	87**	80**	«–»	«–»	«–»	«–»
2	Isopropanol+water (1:1); pH ≤ 2	100*	53**	36**	«–»	24**	«–»	«–»
3	Isopropanol+water (1:1); pH 5.5–6.5	100*	55**	39**	«–»	28**	«–»	«–»
4	Isopropanol+water (1:1)+9 мМ NaOH; pH 5.5–6.5	100*	49**	41**	«–»	29**	«–»	«–»
5	DMSO; pH ≤ 2	100*	93**	90**	87**	81**	77**	73**
6	MC; pH ≤ 2	100*	89**	80**	76**	67**	65**	63**
7	DMSO + MC (1:1); pH ≤ 2	100*	97**	90**	89**	67**	67**	62**
8	DMSO + 10 мМ dithiothreitol; pH ≤ *2*	100*	77**	70**	66**	63**	61**	57**
9	DMSO + 10 мМ ascorbic ac; pH ≤ 2	100*	54**	37**	29**	22**	20**	«–»

Note: * the initial measurement of the optical density of the reaction products was carried out after 30 min after completion of the reaction, the value of which was taken as 100 %;

** — the final value of the optical density in comparison with the initial value;

«–» — measurements were not performed.

Experimental conditions: 0.5 cm^3 of a 1 mM solution of proline with 0.05 cm^3 of 45% $HClO_4$ (in option №. 3, another 0.05 cm^3 of 0.1 N NaOH was added), 1 cm^3 of ninhydrin reagent; after heat treatment (30 min) and cooling (10 min), 2 cm^3 of a certain solvent was added (in option №. 4, 0.2 cm^3 of 0.1 N NaOH was added to the solvent).

Table 3. **Color preservation of the ninhydrin products with proline in the presence of perchloric acid at different times of heat treatment**

Duration of heat treatment (min)	Initial optical density	Optical density change (%) in time after completion of the reaction (min)				
		10	30	40	60	120
5	0.120± 0.004	100*	79**	74**	73**	70**
10	0.250± 0.008	100*	89**	87**	85**	79**
20	0.700± 0.022	100*	94**	93**	90**	83**
30	1.100± 0.034	100*	95**	94**	91**	83**
40	1.260± 0.038	100*	95**	94**	91**	83**
60	1.440± 0.044	100*	96**	93**	91**	83**
120	1.510± 0.045	100*	95**	93**	91**	84

Note: * — the initial measurement of the optical density of the products was carried out after 10 min after completion of the reaction, the value of which was taken as 100%;

** — the final value of the optical density in comparison with the initial one.

Experimental conditions: 0.5 cm^3 of 1 mM proline solution with 0.05 cm^3 of 45% $HClO_4$, 1 cm^3 of ninhydrin reagent; after the end of heat treatment and cooling (10 min), 2 cm^3 of DMSO+MC (1:1) mixture was added.

Table 4. **Products stability dependence of the ninhydrin reaction with proline in the presence of perchloric acid on the ratio of proline solution volumes and ninhydrin reagent volume and the volume ratio to the volume of reaction solvent mixture**

Ninhydrin reagent volume, cm^3	Volume ratio of the proline solution to the volume of the ninhydrin reagent	Solvent volume, cm^3	Solvent volume ratio to the total volume of the mixture	Initial optical density	Changes of optical density (%) over time, min	
					30	60
0.25	1.0 : 0.5	3.75	0.83 : 1.00	0.42± 0.01	100	107*
0.50	1.0 : 1.0	3.50	0.78 : 1.00	0.61± 0.02	100	102*
1.00	1.0 : 2.0	3.00	0.67 : 1.00	0.97± 0.02	100	98*
1.50	1.0 : 3.0	2.50	0.56 : 1.00	1.11± 0.04	100	96*
2.00	1.0 : 4.0	2.00	0.44 : 1.00	1.38± 0.04	100	93*

Note: the initial measurement was made after 30 min after completion of the reaction and cooling (10 min), which value was taken as 100%;

* — optical density value compared to the initial one.

Experimental conditions: 0.5 cm^3 of 1 mM proline solution + 0.05 cm^3 of 45% $HClO_4$; the volume of the reaction mixture in all versions is constant (4.50 cm^3); duration of heat treatment — 45 min; solvent was a mixture of DMSO+MC (1:1).

Table 5. **Ninhydrin reaction optimization with proline in the presence of perchloric acid according to the solvent volume ratio to the reaction mixture volume**

Solvent volume, cm^3, (total volume of the reaction mixture, cm^3)	Solvent volume ratio to the volume of the reaction mixture	Initial optical density	Changes of optical density value (%) of the reaction products in relation to the initial measurement during observation (min)		
			30 min	60 min	90 min
1.5 (3.05)	1:1.0	1.09± 0.04	100	98*	94*
2.0 (3.55)	1:1.3	1.04± 0.03	100	98*	93*
2.5 (4.05)	1:1.7	1.00± 0.03	100	99*	95*
3.0 (4.55)	1:2.0	0.98± 0.03	100	99*	97*
4.0 (5.55)	1:2.7	0.83± 0.03	100	102*	100*
4.5 (6.05)	1:3.0	0.83± 0.03	100	105*	105*

Note: the initial measurement was made after 30 min after completion of the reaction and cooling (10 min), the value of which was taken as 100%; * — optical density value compared to the initial one. Experimental conditions: 0.5 cm^3 of 1 mM proline solution + 0.05 cm^3 of 45% $HClO_4$; volume of ninhydrin reagent — 1 cm^3; the total volume of the reaction mixture in different versions is not the same; duration of heat treatment was 45 min; the solvent was a mixture of DMSO+MC (1:1).

(Tables 4, 5), it is exactly after this time the optical density of products slowly decreases (no more than 1–3% per hour.

Based on the experiments, we can recommend the most optimal conditions for conducting the ninhydrin reaction with proline in the presence of perchloric acid: to 0.5 cm^3 of the proline solution it need to add $HClO_4$ solution to a final concentration of 4–4.1%, to add 1 cm^3 of the ninhydrin reagent (according to the prescription work 10), to hold in a boiling water bath for up to 60 min, to cool to room temperature and at the end to add 3 cm^3 of the DMSO + MC mixture. To make the measurement at a wavelength of 515 nm 30 min later.

The proposed method is also convenient from the point of view that the same ninhydrin reagent can be used both for the specific determination of proline and for the determination of other amino acids by conventional methods.

Despite the fact that separately all the studied amino acids did not show a noticeable reaction with ninhydrin in the presence of perchloric acid, it is necessary to check their

effect on the detection of proline by analyzing mixtures of amino acids (Table 6). To assess the interference of amino acids with proline, the deviation degree of the reference values obtained for proline from the optical density indicators of the mixtures was calculated. As can be seen from the table, a significant part of the investigated amino acids in equimolar ratios with proline (glycine, alanine, oxyproline, arginine, glutamic and aspartic acids, etc.) have a weak influence on the determination of proline.

The SH group is a nucleophile, therefore it can participate in nucleophilic addition or substitution reactions and compete with NH_2 groups of amino acids for the reaction with ninhydrin [15, 17]. As a result of such competition, the amount of chromogen in the ninhydrin reaction of proline in the presence of methionine and cysteine was decreased. The increase in color with lysine is associated with the interaction of ninhydrin with the ε-amino group of the amino acid [8].

For comparison, a similar analysis was performed according to the widely used method of Bates et al. [13], which is considered a reference. In the most cases, the proposed method provided greater specificity for proline determination in the presence of other amino acids and was superior to the reference method.

A number of successive dilutions of proline were prepared to construct a calibration graph. Proline solutions with a concentration from 0.01 mmol/dm^3 (0.576 μg in the analyzed sample of 0.5 cm^3) to 20.00 mmol/dm^3 (1151.300 μg in the analyzed sample) were analyzed.

The range of application of the proposed method, that is, the preservation of the linear relationship between the optical density and the amount of amino acid,

Table 6. **Proline interference with other amino acids in reaction with ninhydrin in the presence of perchloric acid**

Amino acids	The degree of deviation of the optical density (%) of the mixture of amino acids in relation to the values for proline	
	The original method	The method of Bates et al., 1973
Proline 1 мМ	0.0	0.0
Proline 1 мМ + glycine 1 мМ	+0.5	+7.2
Proline 1 мМ + alanine 1 мМ	+1.6	+18.2
Proline 1 мМ + valine 1 мМ	−4.8	+14.5
Proline 1 мМ + oxyproline 1 мМ	+0.2	+3.3
Proline 1 мМ + threonine 1 мМ	−5.0	+5.8
Proline 1 мМ + methionine 1 мМ	−8.3	+19.6
Proline 1 мМ + arginine 1 мМ	−1.2	−4.5
Proline 1 мМ + asparagine 1 мМ	−1.6	+11.9
Proline 1 мМ + glutamine 1 мМ	−1.8	−8.6
Proline 1 мМ + lysine 1 мМ	+5.8	+4.9
Proline 1 мМ + leucine 1 мМ	−2.0	+3.2
Proline 1 мМ + isoleucine 1 мМ	+0.2	+9.4
Proline 1 мМ + cysteine 1 мМ	−3.3	−3.2
Proline 1 мМ + histidine 1 мМ	−6.0	+19.7
Proline 1 мМ + phenylalanine 1 мМ	−5.9	−1.2
Proline 1 мМ + tryptophan 1 мМ	+1.6	−18.6
Proline 1 мМ + tyrosine 1 мМ	+1.5	+8.3
Proline 1 мМ + glutamic acid 1 мМ	−1.2	+5.3
Proline 1 мМ + aspartic acid 1 мМ	+1.3	−12.9

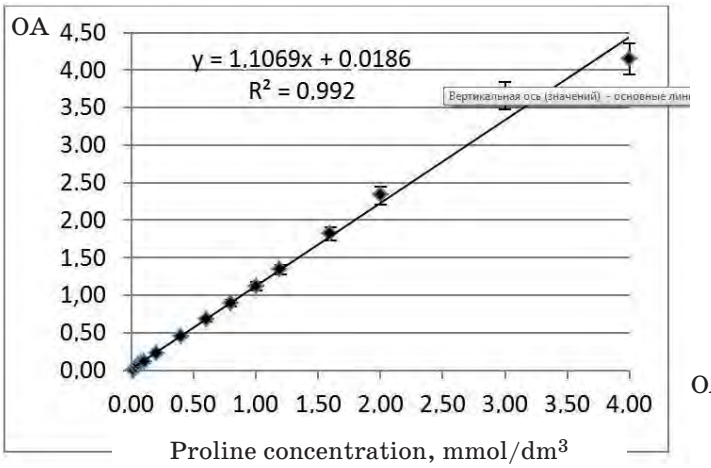

Fig. 5. **Calibration graph for proline:** OA — optical density; the specified approximation equations and its reliability level (R^2).

is maintained up to a concentration of 4.00 mmol/dm^3 (230.26 μg in the analyzed sample) with an approximation coefficient of $R^2 = 0.992$ (Fig. 5). Proline detection limit: 0.01 mmol/dm^3 (0.576 μg in the analyzed sample); the limit of quantitative determination is 0.02 mmol/dm^3 (1.151 μg in the analyzed sample). The average standard error (SE) for the entire range of measurements is 3.32% of the optical density of the samples.

Thus, the study of the effect of acid precipitants of proteins made it possible to propose a method for the specific determination of proline using a standard ninhydrin reagent. The main results of the work can be formulated as follows.

Trichloroacetic (TCA) and perchloric ($HClO_4$) acids change the spectral characteristics of the products of the ninhydrin reaction with amino acids.

In the presence of TCA, the optical density of reaction products decreases for most amino acids without TCA shifting the maximum of the optical spectrum. In the presence of $HClO_4$, for most amino acids, the maximum characteristic of the ninhydrin reaction under standard conditions ($\lambda = 570$ nm) completely disappears and the optical spectrum of the chromophore shifts to the short-wavelength zone. At the same time, the total optical density of the chromophore also decreases significantly.

The exception is the reaction with proline, as a result of which a well-defined maximum appears in the product spectrum: $\lambda = 620$ nm in the presence of TCA and $\lambda = 515$ nm with $HClO_4$. Moreover, in the presence of perchloric acid, the reaction becomes highly specific for proline.

On the basis of the obtained results, a method of highly specific determination of proline was developed [19].

In our opinion, the use of the proposed specific method for the determination of proline in combination with ways specific to other amino acids (oxyproline and glycine) and in combination with standard methods for the determination of amino acids can contribute to the elucidation of the features of recognition sites in collagen molecules during their hydrolysis by collagenases. This approach can also be useful for studying the mechanism of action of other proteinases.

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ВПЛИВ КИСЛОТНИХ ОСАДЖУВАЧІВ ПРОТЕЇНІВ НА СПЕЦИФІЧНІСТЬ РЕАКЦІЇ НІНГІДРИНУ З АМІНОКИСЛОТАМИ

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Мета. Визначити вплив трихлороцтової (ТСА) та перхлоратної ($HClO_4$) кислот на результат реакції нінгідрину з різними амінокислотами.

Методи. Застосовували стандартний метод виявлення амінокислот з використанням нінгідринного реагента. Оптичні спектри та густину продуктів реакції визначали спектрофотометрично.

Результати та висновки. Виявлено, що досліджувані кислоти змінюють спектральні характеристики продуктів нінгідринної реакції з амінокислотами. ТСА суттєво знижує оптичну густину хромофорів, а $HClO_4$ до того ж призводить до значного зміщення спектрів продуктів реакції у короткохвильову область. Винятком є реакція з проліном, у результаті якої у спектрі продуктів з'являється чітко виражений максимум: $\lambda = 620$ нм за присутності ТСА та $\lambda = 515$ нм з $HClO_4$. При цьому за присутності $HClO_4$ реакція стає високоспецифічною щодо проліну.

Аналізовано умови проведення нінгідринної реакції з проліном при додаванні $HClO_4$. Запропоновано спосіб високоспецифічного визначення проліну за присутності інших амінокислот.

Ключові слова: нінгідринна реакція, амінокислоти, пролін, трихлороцтова кислота, перхлоратна кислота.