

The object of research is a Stadier-type apparatus for analytical electrophoresis of proteins. In the milk proteins research, in addition, there is a need to carry out serial express analyses of their various groups, as well as the isolation of individual homogeneous fractions. The dimensions of working chambers for analytical, express, and micro preparative electrophoresis of caseins and milk whey proteins were proposed to solve this task. For each type of electrophoresis, different chambers and formers are used without changing the design of the apparatus. The apparatus is suitable for electrophoretic systems used for the analysis of milk proteins. Analysis of casein in the anodic system of a homogeneous polyacrylamide gel in the presence of urea allows identification of the main fractions:  $\alpha_{S1}$ -CN-8P,  $\alpha_{S1}$ -CN-9P,  $\alpha_{S2}$ -CN-10P,  $\alpha_{S2}$ -CN-11P,  $\alpha_{S2}$ -CN-12P,  $\alpha_{S2}$ -CN-13P,  $\beta$ -CN-5P,  $\kappa$ -CN-1P and three  $\beta$ -casein fragments  $f(29-209)$ ,  $f(106-209)$  and  $f(108-209)$ . Express electrophoresis in the presence of urea reveals four fractions of caseins:  $\alpha_{S1}$ -CN,  $\alpha_{S2}$ -CN,  $\beta$ -CN, and  $\kappa$ -CN. The analysis of whey proteins in the Davis native disc electrophoresis system allows identification of  $\beta$ -Lg A,  $\beta$ -Lg B,  $\alpha$ -La, BSA fractions, and a group of immunoglobulin fractions. The express electrophoregram differs by a common band A and B variants of  $\beta$ -Lg. Due to an adequate selection of electrophoretic systems, it is possible to identify semi-quantitatively all the main fractions of milk proteins under analytical or express mode. The adapted apparatus also makes it possible to conduct micro preparative electrophoresis and obtain the main fractions of milk proteins. In this case, the yield of electrophoretically pure proteins is:  $\beta$ -CN-5P (23±5 %),  $\beta$ -Lg (A+B) (27±6 %),  $\alpha$ -La (11±3 %), and purified groups of  $\alpha_{S1}$ -CN-8P+ $\alpha_{S1}$ -CN-9P (25±6 %),  $\alpha_{S2}$ -CN-(10-13P) (6±1.5 %) and  $\kappa$ -CN-1P (7±2 %). The apparatus could be used at enterprises producing dairy protein products

**Keywords:** apparatus for electrophoresis, casein fractions, milk whey proteins, electrophoretic systems

# ADAPTATION OF STADIER'S APPARATUS FOR ELECTROPHORESIS OF MAIN MILK PROTEINS

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## 1. Introduction

Interest in the protein fractions of cow's milk primarily concerns the fractions that exhibit biological activity or are enzymes [1]. These include  $\alpha$ -lactalbumin, immunoglobulins, lactoferrin, lactoperoxidase, individual proteins of fat globules, etc. [2]. It is also important that fractional specificity is characteristic of numerous biologically active peptides that can be formed from the main proteins of milk during the biochemical processes of production of fermented milk products [3, 4]. The fractional composition of proteins is related to the quality indicators of milk [5] and cheese [6]. There is growing interest in the protein fractions of buttermilk, which is a byproduct of butter production [7]. Therefore, it is obvious that affordable and effective methods of qualitative and quantitative analysis of the fractional composition of milk proteins are necessary during its processing. Such methods include electrophoresis, which is widely used in the study and control of cow's milk proteins [8] and their genetic variants [9]. Electrophoresis allows identification of milk proteins of different species of mammals [10]. Also, electrophoresis is a convenient method for serial express analyses of individual groups of milk proteins [11]. However, existing universal procedures, as well as devices for electrophoresis, do not take into account significant differences in the structure, composition, and properties of individual

groups of milk proteins. These groups include caseins (~80 %), major whey proteins (~18 %), a multifractional group of minor proteins, and a very specific group of fat globule proteins [1, 2]. Also, different devices are often used for different types of electrophoresis (analytical, express electrophoresis, preparative). All this complicates the application of such an effective method as electrophoresis at dairy enterprises.

Therefore, it is a relevant task to adapt parameters of the apparatus for analytical and preparative electrophoresis of various groups of milk proteins under the conditions of production laboratories at dairy enterprises.

## 2. Literature review and problem statement

Different electrophoretic methods were used to analyse milk proteins: frontal electrophoresis, isoelectric focusing, capillary electrophoresis, many variants of zonal electrophoresis [8, 12]. For the most part, these methods are relevant for scientific research. Zone electrophoresis in polyacrylamide gel (PAG) is the most promising for serial analyses of milk proteins under production conditions. It is various systems of electrophoresis in PAG that are most often used for the analysis of milk proteins.

The Committee on Nomenclature, Classification, and Methodology of Milk Proteins of the American Dairy

Science Association (ADSA) recommended the use of electrophoretic systems in PAG for the identification of protein fractions of milk [13]. In reality, two groups of milk proteins are of practical interest under production conditions: caseins and whey proteins. These groups differ significantly in their properties, degree of heterogeneity, amino acid composition, spatial structure, and molecular weights [1]. Considering this, ADSA suggests using two different electrophoretic systems for the analysis of caseins and whey proteins [13].

Both systems belong to anodic electrophoretic systems and differ in the conditions of conducting electrophoresis. For proteins of the casein complex, a system of homogeneous PAG in the presence of urea is recommended. Under such conditions, casein fractions do not form aggregates and are effectively separated according to their charges. Based on the results obtained by this type of electrophoresis, a modern classification of caseins was created. Various variants of this method have been used in many casein studies and analyses. In particular, the most common is analytical electrophoresis and its express variant [14]. In these cases, devices with different working chambers are used. To separate purified fractions, a system for preparative electrophoresis was developed with the appropriate modification of the apparatus [15]. The disadvantage of the system of homogeneous PAG with urea is the impossibility of simultaneous analysis of caseins and whey proteins, which form blurred bands on electrophoregrams [11].

For the analysis of milk whey proteins, ADSA recommends the very common Laemmli disc electrophoretic system [13]. In this case, electrophoresis takes place in the presence of sodium dodecyl sulfate (SDS), and protein fractionation occurs based on the difference in their molecular weights [16, 17]. The disadvantages of such a system are the mandatory denaturation of all whey proteins and, accordingly, their loss of native structure and biological activity, so it cannot be used for the preparative isolation of individual fractions of this group of milk proteins. For successful separation and identification of the main protein fractions of milk whey, the disc electrophoresis system for neutral and weakly acidic proteins can be used [18, 19]. Such an electrophoresis system is useful if the preservation of the native protein structure is important. Express [11] and preparative [20] versions of whey protein electrophoresis have also been developed on its basis. However, using this type of electrophoresis, it is difficult to analyse products that contain denatured whey proteins.

Therefore, various devices and electrophoretic systems are used for the analysis of milk proteins. In particular, these are devices for horizontal and vertical electrophoresis, electrophoresis in PAG tubes and plates. Currently, various modifications of devices for vertical electrophoresis in PAG plates are most often used [21]. There are many varieties of such devices, which are designed for the analytical version of various electrophoretic systems. This also applies to systems used to analyse the main groups of milk proteins. Such devices are not suitable for express electrophoresis variants, which is important in serial analyses of protein dairy products and are not at all suitable for preparative electrophoresis of milk proteins.

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### 3. The aim and objectives of the study

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The aim of our work was to adapt the apparatus for electrophoresis in vertical PAG plates for analytical separation

and preparative separation of proteins of the casein complex and milk whey, which could allow more detailed and more efficient control over production processes of milk protein products.

To achieve the goal, the following tasks were formed:

- to select the basic model of the apparatus for electrophoresis in vertical PAG plates and adapt the parameters of the electrophoretic chamber and formers for analytical and preparative electrophoresis of milk proteins;

- to acquire electrophoregrams of proteins of the casein complex on an adapted device in electrophoretic systems for analytical, preparative, and express electrophoresis in the presence of urea;

- to acquire electrophoregrams of milk whey proteins on an adapted device in electrophoretic systems for analytical, preparative, and express electrophoresis under native conditions.

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### 4. The study materials and methods

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The object of our study is an apparatus for electrophoretic analysis and preparative separation of milk proteins in vertical plates of polyacrylamide gel using appropriate electrophoretic systems for different groups of milk proteins.

The main hypothesis assumes the possibility of constructing a universal apparatus for different types of electrophoresis (analytical, express, and preparative) in different electrophoretic systems for the analysis, fractionation, and isolation of milk proteins. At the same time, the fractional composition, the difference in molecular weights, and the differences in the physical and chemical properties of the main groups and fractions of milk proteins should be taken into account.

The apparatus and formers for electrophoresis were made from organic glass in the biochemistry laboratory of the Department of Food Biotechnology and Chemistry at Ternopil Ivan Puluj National Technical University. The parts were glued using dichloroethane. Platinum electrodes were used in the device. Parts of the electrophoretic chambers were made of inorganic glass. For the preparation of PAG and buffer solutions (for the gel, for samples, and electrodes), reagents from the company “Reanal” (Hungary) and reagents of a high degree of purification, produced in Ukraine, were used.

Fresh milk from PJSC “Ternopil Dairy Factory” was used to obtain samples of proteins of the casein complex and whey. For defatting, the milk was centrifuged twice at 4000 rpm for 10 minutes on an OPN-8 centrifuge. The total preparation of casein was obtained by isoelectric precipitation. The pH value was adjusted to the isoelectric point using 1 M HCl. Casein was precipitated by centrifugation (5000 rpm, 10 min). The precipitate was dissolved in distilled water with the addition of 1 M NaOH. The pH value did not exceed 7.5. The procedure was repeated twice. After that, the casein was lyophilized. The whey preparation was obtained after isoelectric precipitation of casein from skimmed milk. To purify whey proteins from low-molecular-weight compounds, gel filtration was performed on Sephadex G-25 columns from the Pharmacia company. For gel filtration, chromatographic columns from a set for liquid chromatography of the company “Reanal” (Hungary) were used. Whey albumin (BSA) and  $\alpha$ -casein from the company “Sigma” were used as markers for the identification of protein fractions of milk during electrophoretic studies. Homogeneous  $\beta$ -lactoglobu-

lin ( $\beta$ -Lg) and  $\alpha$ -lactalbumin ( $\alpha$ -La) were isolated by preparative electrophoresis [20]. Homogeneous  $\alpha_{S1}$ -CN and  $\beta$ -CN casein fractions were obtained by differential precipitation in the presence of urea followed by purification by ion exchange chromatography on DEAE cellulose [22].

For analytical and preparative electrophoresis of casein complex proteins, an electrophoretic system was used in homogeneous PAG in the presence of urea [15, 18]. Express analysis of the fractional composition of caseins was performed according to the method described previously in [14]. For analytical and preparative electrophoresis of milk whey proteins, a modified Davis disc electrophoresis method was used under native conditions for neutral and weakly acidic proteins [18, 20]. The procedure of express electrophoresis of whey proteins is described in [11]. Quantitative processing of PAG plates was performed using the *imread* graphic image reading function.

Spectrophotometry in the ultraviolet region of the spectrum was used to determine the concentration of milk proteins. The research was carried out on a SF-46 spectrophotometer. The following absorption coefficients ( $D_{dm}^{1\%}$ ): were used to calculate the concentration: 8.2 – for total casein; 10.1 –  $\alpha_{S1}$ -CN; 4.6 –  $\beta$ -CN; 9.6 –  $\kappa$ -CN; 12.3 – for proteins of milk whey; 9.6 – for  $\beta$ -Lg; 20.1 – for  $\alpha$ -La, 6.7 – for BSA; 13.6 – for Ig.

Mathematical and statistical treatment of the results was carried out using Microsoft Office Excel 2007 package.

## 5. Results of electrophoretic studies of milk proteins on an adapted apparatus in various electrophoretic systems

### 5.1. Selection of the basic model and adaptation of the apparatus for electrophoresis of milk proteins in vertical plates of polyacrylamide gel

A Stadier-type apparatus for electrophoresis in vertical PAG plates was chosen as the basic model for adaptation to the conditions of milk protein analysis. The advantages of such an apparatus are the ability to easily change the number of samples for analysis and their volume. At the same time, various electrophoretic chambers and formers for starting cells are used without changing the design of the apparatus itself [23]. It is also important that on such devices it is convenient to compare the electrophoretic properties of several different samples under exactly the same conditions. This is especially important for the analysis of complex multifractional protein compositions such as milk whey proteins [1, 2]. Also, the Stadier-type apparatus can be adapted for both analytical and preparative electrophoresis of milk proteins.

When choosing sizes of the electrophoretic chambers, the location of the bands of different groups of milk proteins in the PAG plates relative to the starting line in the corresponding electrophoresis systems was taken into account. The optimal length of the gel plate is 90 mm with a gel thickness of 3 mm. This length allows one to effectively separate all the protein fractions of the casein complex and whey proteins. Also, this length is sufficient for separation of the main fractions of caseins and whey proteins during preparative electrophoresis.

A critical procedure for acquiring high-quality electrophoregrams during electrophoresis is the introduction of the sample into the starting cell. To simplify the procedure, the thickness of the PAG plate was increased to 3 mm. This allows the formation of cells for samples where all four side walls of the cell are formed by PAG. Moreover, the width of the cell at the top is greater and is equal to the thickness of the gel plate. Near the starting line, the width of the cell is 1.5 times smaller. This facilitates the introduction of the sample and prevents the passage of proteins between the PAG plate and the chamber wall.

The proposed version of the device is intended for simultaneous analysis of 3 to 7 protein samples during analytical and express electrophoresis. In express electrophoresis, in order to save reagents, the volume of the electrophoretic chamber can be reduced by shortening its length. In the case of preparative electrophoresis, one large starting cell is used for samples [15, 20]. At the same time, in order to regulate the volume of the protein sample, PAG plates with a thickness of both 3 and 5 mm, as well as different chamber widths, are provided. The dimensions of the chambers for different types of electrophoresis of milk proteins and different numbers of samples are given in Table 1.

All variants of electrophoretic chambers consist entirely of glass elements. This improves heat transfer during electrophoresis. At the same time, PAG plates do not overheat and there is no need to use a special cooling system. Also, the use of glass elements of the chambers ensures a perfectly even surface of PAG plates.

Table 1

The size of chambers for different types of electrophoresis of milk proteins

Number of cells for samples*	Analytical electrophoresis (mm)	Express electrophoresis (mm)	Preparative electrophoresis			
			Variant I		Variant II	
			Chamber size, mm	Sample volume, $\mu$ l	Chamber size, mm	Sample volume, $\mu$ l
3	3×39×90	3×39×55	–	–	–	–
5	3×81×90	3×81×55	3×81×90	500	5×81×90	1000
7	3×111×90	3×111×55	3×111×90	700	5×111×90	1500

Note: in all PAG plates for preparative electrophoresis, one sample cell is formed

The volume of milk protein samples for analytical and express electrophoresis can be from 5 to 50  $\mu$ l. In the case of preparative electrophoresis, the optimal sample volume for variant I of the electrophoretic chamber is within 500–700  $\mu$ l, and for variant II – 1000–1500  $\mu$ l.

### 5.2. Electrophoresis of casein complex proteins in an adapted Stadier-type apparatus

Analytical electrophoresis of casein complex proteins was performed in homogeneous PAG in the presence of 4.5 M urea according to the method described in [18]. An electrophoretic chamber with dimensions of 3×81×90 mm and a former for five cells were used for the analysis. 10  $\mu$ l of the same sample of casein isolated from one batch of milk was introduced into the cells. Thus, all differences in the qualitative composition and quantitative indicators can be associated only with the process of electrophoresis and densitometry.

Fig. 1 shows a typical electrophoregram of a casein sample and its densitogram. With the use for homogeneous casein markers, the placement of casein fractions, which is characteristic of this electrophoretic system, was estab-

lished. The qualitative composition of the casein fractions on all five strips of the PAG plate was identical.

The content of four groups of caseins ( $\alpha_{S1}$ -CN,  $\alpha_{S2}$ -CN,  $\beta$ -CN, and  $\kappa$ -CN) was quantified by the area of densitogram peaks of each sample, as shown in Fig. 1b. Based on our densitograms, the content of each protein fraction from all proteins of the sample was calculated, and their average content and standard error were determined (Table 2).

Chambers with the same dimensions as for analytical electrophoresis can be used for casein express electrophoresis. However, in order to save reagents, shorter chambers can be chosen (Table 1). At the same time, it is necessary to raise the level of the buffer in the lower electrode chamber by placing a bar of organic glass of the appropriate volume in it. Express electrophoresis of the same casein sample was performed in five strips of a PAG plate according to the method described previously in [14]. This method makes it possible to identify the main casein fractions in just 90 minutes.

A typical result of casein express electrophoresis is shown in Fig. 2. The qualitative composition of casein fractions on all five lanes is identical. It should be noted that the  $\alpha_{S2}$ - and  $\kappa$ -casein fractions are not clear and blurred on the electrophoregrams.

Quantitative processing of the densitogram of such electrophoregrams is complicated and can concern only two fractions:  $\alpha_{S1}$ -CN and  $\beta$ -CN. In this case, the error can be more than 10 %.

For preparative electrophoresis of casein complex proteins, a chamber with dimensions of 3×81×90 mm and a suitable former were used. Preparative electrophoresis was performed according to the method described in [15]. Fig. 3 shows a stained PAG plate after preparative electrophoresis of 500  $\mu$ l of a 1 % casein solution.

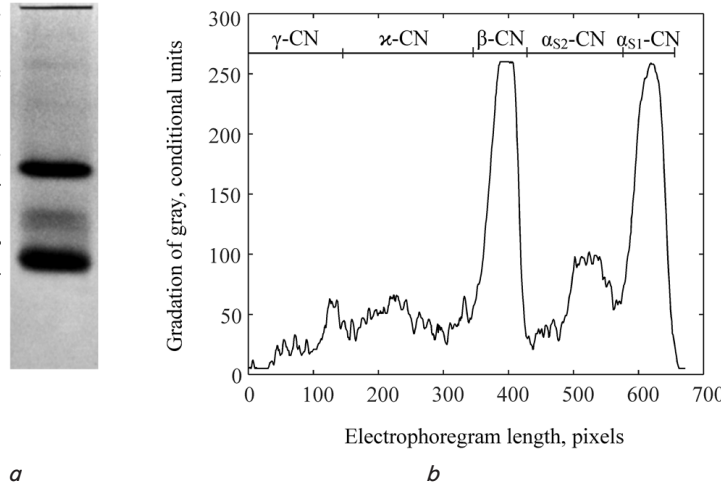


Fig. 1. Electrophoresis of proteins of the casein complex in the system of homogeneous PAG in the presence of 4.5 M urea: a – electrophoregram; b – densitogram

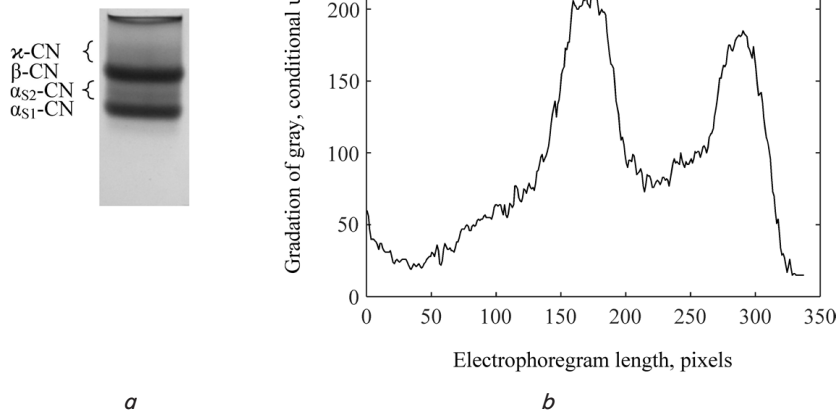


Fig. 2. Express electrophoresis of casein complex proteins: a – electrophoregram; b – densitogram

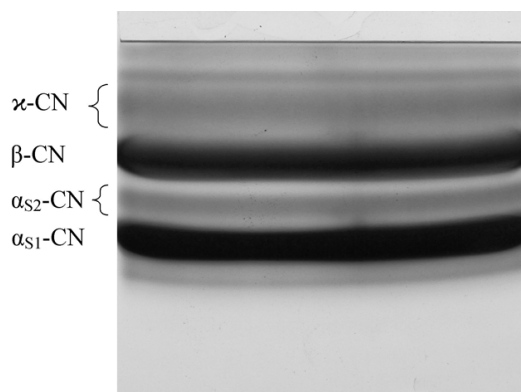


Fig. 3. Stained polyacrylamide gel plate after preparative electrophoresis

Table 2

Content of casein fractions in five casein samples

Casein sample	Fractions of proteins in casein complex			
	$\alpha_{S1}$ -CN, %	$\alpha_{S2}$ -CN, %	$\beta$ -CN, %	$\kappa$ -CN, %
1	44	10	38	11
2	41	11	36	12
3	38	11	37	13
4	42	10	35	13
5	42	9	33	15
Average value ( $M \pm n, n=5$ )	41.4±2.0	10.2±0.8	35.8±1.7	12.8±1.3

According to the results of five separations, the yield of the electrophoretically homogeneous  $\beta$ -CN fraction was 23±5 %, and the  $\alpha_{S1}$ -CN,  $\alpha_{S2}$ -CN, and  $\kappa$ -CN fraction groups were 25±6 %, 6±1.5 %, and 7±2 %, respectively % of all proteins of the casein sample.

### 5. 3. Electrophoresis in the adapted apparatus of milk whey proteins

Analytical electrophoresis of milk whey proteins was performed using a native disc electrophoresis system for neutral and acidic proteins [18]. A PAG plate with five cells was polymerized in a chamber with dimensions of

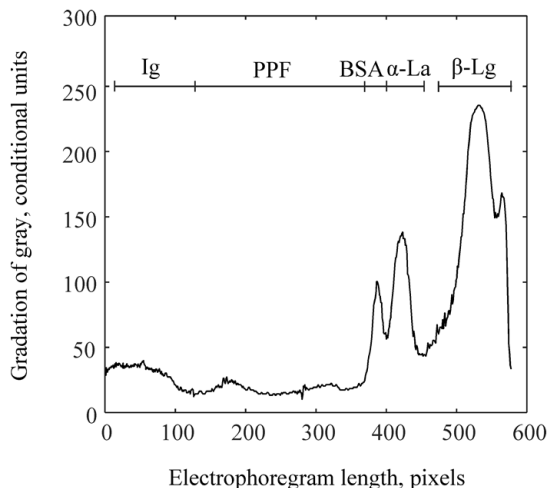
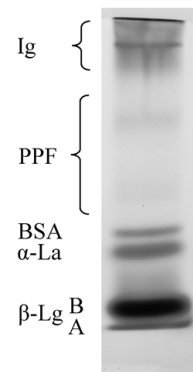
3×81×90 mm. To objectively assess the reproducibility of the results, the same sample of milk whey proteins (10 μl) was introduced into all cells. A typical electrophoregram and densitogram of whey proteins takes the form shown in Fig. 4. The qualitative composition of fractions in all five samples is identical.

Quantitative analysis of the fractional composition was performed on the basis of densitometry of five samples, which were separated on one PAG plate. At the same time, the peak areas of four fractions: β-Lg, α-La, BSA, and Ig were determined, as shown in Fig. 4, b. The results of calculating the content of each fraction from all proteins of the sample are given in Table 3.

For express electrophoresis of milk whey proteins, a native electrophoretic system was used in homogeneous PAG with preservation of the concentration effect [11]. Such a system allows identifying the main fractions of whey proteins in less than one hour. Fig. 5 shows the electrophoregram and densitogram of a whey sample. According to the fractional composition, this electrophoregram differs from the analytical one by the composition of some minor fractions, as well as the combined band A and B of β-Lg variants.

The quantitative analysis was carried out based on the determination of peak area of four fractions (β-Lg, α-La, BSA, and Ig) on the densitograms of five samples, as shown in Fig. 5, b. The results are given in Table 4.

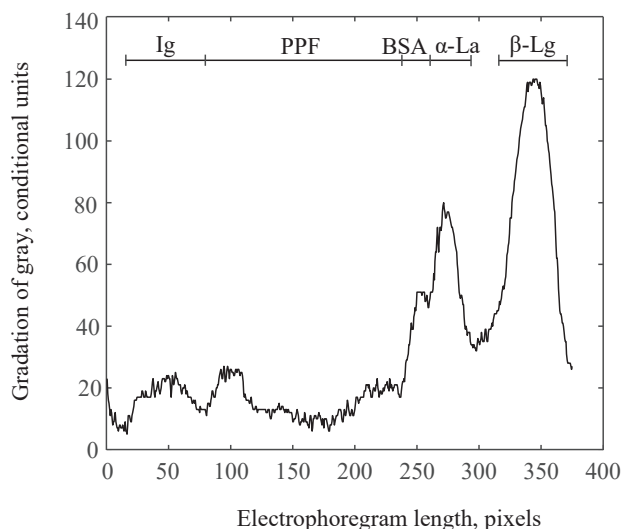
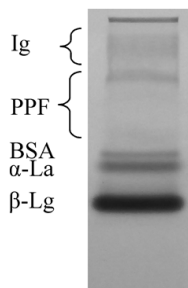
The apparatus proposed in the work also allows for preparative electrophoresis of milk whey proteins. To isolate two electrophoretically pure fractions, the disc electrophoresis technique was used under native conditions [20]. Preparative electrophoresis was performed in a chamber with dimensions of 3×81×90 mm. The stained PAG plate after preparative fractionation of 500 μl of milk whey takes the following form (Fig. 6).



a

b

Fig. 4. Disc electrophoresis of milk whey proteins: a – electrophoregram; b – densitogram



a

b

Fig. 5. Express electrophoresis of milk whey proteins: a – electrophoregram; b – densitogram

The average yield of two electrophoretically homogeneous fractions (β-Lg and α-La) based on the quantitative processing of the results of five preparative electrophoresis was 27±6% and 11±3%, respectively, from all the proteins of the sample.

Table 3

Content of protein fractions in five samples of milk whey

Whey sample	Whey protein fractions			
	β-Lg, %	α-La, %	BSA, %	Ig, %
1	37	17	13	19
2	32	18	12	19
3	35	14	10	17
4	41	15	9	14
5	36	17	8	20
Average value (M±n, n=5)	36.2±2.9	16.2±1.5	10.4±1.9	17.8±2.1

Table 4

Content of protein fractions of five samples of milk whey according to the results of express electrophoresis

Whey sample	Whey protein fractions			
	β-Lg, %	α-La, %	BSA, %	Ig, %
1	34	16	9	18
2	37	13	10	16
3	34	18	11	18
4	44	17	8	15
5	41	17	9	19
Average value (M±n, n=5)	38.0±4.0	16.2±1.7	9.4±1.0	17.2±1.5

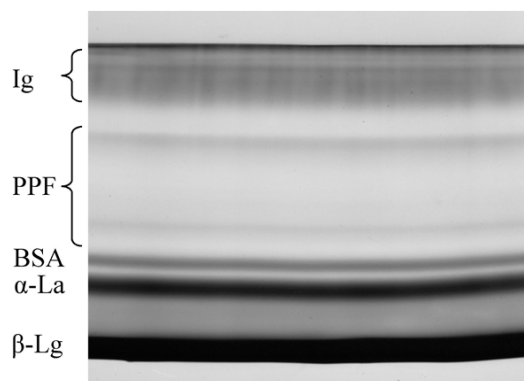


Fig. 6. Preparative electrophoresis of milk whey proteins

## 6. Discussion of results of electrophoresis of milk proteins in an adapted Stadler-type apparatus

The design of the proposed apparatus for electrophoresis of milk proteins is quite simple. It can be manufactured in laboratories from available materials: organic and inorganic glass, dichloroethane, platinum wire for electrodes. The special structure of the formers allows to form cells that ensure reliable introduction of samples. It is this procedure that is critical for obtaining high-quality electrophoregrams and requires high qualification. To carry out electrophoresis, the device can be connected to a typical direct current source with current stabilization up to 250 mA and voltage up to 500 V.

The dimensions of the chambers for the formation of PAG plates make it possible to carry out all types of electrophoresis, which are recommended for the analysis of milk proteins. Depending on the type of electrophoresis, the number of preparations and the necessary repetitions, from one to seven samples can be analyzed simultaneously. At the same time, different chambers are used without changing the design of the device.

The proposed electrophoresis apparatus can identify the main fractions of caseins ( $\alpha_{S1}$ -CN B-8P,  $\alpha_{S1}$ -CN B-9P,  $\alpha_{S2}$ -CN A-10P,  $\alpha_{S2}$ -CN A-11P,  $\alpha_{S2}$ -CN A-12P,  $\alpha_{S2}$ -CN A-13P,  $\beta$ -CN A<sup>2</sup>-5P,  $\kappa$ -CN A-1P) and three fragments of  $\beta$ -casein.  $\beta$ -Lg A,  $\beta$ -Lg B,  $\alpha$ -La, BSA and group IG can be determined from milk whey proteins. To identify minor whey proteins, it is necessary to use highly purified marker preparations of the corresponding proteins. In the case of determining the degree of homogeneity of a certain milk protein preparation, there is no need to identify it since it is the main part of the preparation. In this case, analytical electrophoresis can be used for any milk protein fractions.

When using the proposed device, as in the case of other devices, the simultaneous analysis of all milk proteins is complicated. This is due to a large number of fractions that have similar properties and can be superimposed on electrophoregrams [1, 2]. Also, overlapping of bands of different proteins can occur due to a large difference in the content of different fractions. Therefore, the common variant of electrophoresis with SDS in the Laemmli system does not always provide an opportunity to effectively separate and identify all fractions [8], especially in the absence of appropriate marker proteins. If possible, we recommend doing this separately when using analytical electrophoresis for caseins in the presence of urea, and native disc electrophoresis for whey proteins [11]. The placement of protein fractions on the

electrophoregrams was determined using the appropriate marker proteins. However, the results indicate the possibility of identifying all the main fractions of caseins and whey proteins by the characteristic patterns of electrophoregrams even without marker proteins (Fig. 1, 4).

Express electrophoresis can be useful if rapid analysis of milk protein samples is required. They allow identification of four casein fractions ( $\alpha_{S1}$ -CN,  $\alpha_{S2}$ -CN,  $\beta$ -CN, and  $\kappa$ -CN). All these fractions, except for  $\beta$ -CN, include groups of proteins with the same primary structure. Separation into variants formed due to the difference in the number of phosphoserine residues ( $\alpha_{S1}$ - and  $\alpha_{S2}$ -casein), as well as oligosaccharides ( $\kappa$ -CN), does not occur. However, this may be sufficient to control the fractionation or proteolysis of casein [14]. In the case of express electrophoresis of whey proteins, all major fractions can be identified. Only the genetic variants of  $\beta$ -Lg A and B cannot be separated (Fig. 5).

The capabilities of the proposed device also include preparative electrophoresis. Considering the relatively small number of proteins in the samples, it is more correct to call it micro preparative. At the same time, the same electrophoretic systems as for analytical electrophoresis are used. This ensures high efficiency of fractionation and allows the isolation of electrophoretically homogeneous proteins from casein ( $\alpha_{S1}$ -CN,  $\alpha_{S2}$ -CN,  $\beta$ -CN, and  $\kappa$ -CN) and whey ( $\beta$ -Lg and  $\alpha$ -La). In this case, it is important to use the native electrophoresis system for whey proteins. In the case of SDS, denatured fractions can be isolated [20]. The use of such fractions is limited. They lose their native structure and biological activity during sample preparation.

The deviation in the quantitative assessment of the content of protein fractions in PAG plates according to literature data can be from 5 to 10 % [8]. This is related to the conditions of conducting electrophoresis, the processes of staining, scanning, or photographing and densitometry of gels. When comparing the data obtained in the same PAG plate, the influence of these factors decreases. Taking this into account, the method of electrophoresis of milk proteins can be classified as semi-quantitative [8]. Due to the high content of the main protein fractions of casein and milk whey, one can use 10V amido black for staining gels. At the same time, staining occurs much faster [11, 14, 24]. This is especially important when performing express electrophoresis. If it is necessary to detect and identify minor fractions or when analysing the homogeneity of milk protein preparations, it is advisable to use the more sensitive Coomassie dye [25]. It is also worth noting that when comparing the content of caseins (especially  $\alpha_{S1}$ -CN,  $\alpha_{S2}$ -CN, and  $\beta$ -CN) with other proteins, it is necessary to take into account the peculiarities of their binding of acidic dyes [25].

The given results of electrophoresis indicate the possibility of effective use of the adapted apparatus for the analysis of the main groups of milk proteins in vertical plates of polyacrylamide gel. Without changing the design of the device, two variants of analytical electrophoresis (properly analytical and express electrophoresis), as well as micro preparative electrophoresis of casein complex proteins and milk whey proteins can be performed using different working chambers. Our results of electrophoresis in terms of qualitative and quantitative indicators do not differ from similar electrophoregrams described in the literature and allow semi-quantitative identification, as well as the isolation of electrophoretically pure main fractions of milk proteins.

It should be noted that preparative electrophoresis is limited by the volume and concentration of proteins in the samples. Chambers for micro preparative electrophoresis allow the fractionation of samples in the amount of less than one gram. The minimum number of detected proteins is limited by the sensitivity of the used dye. In this case, one can use the more sensitive Coomassie dye, which allows one to detect a strip containing 10 µg of protein.

During the analysis, the qualitative composition of milk proteins is completely reproduced. Quantitative indicators are reproduced within 5–9 %, which is typical for electrophoretic methods. In the future, more accurate quantitative indicators can be obtained when using standard fractions of milk with a given concentration. It should also be noted that for an accurate quantitative analysis of the content of protein fractions, it is necessary to additionally apply other methods. Electrophoresis can be used for preliminary quantification.

The selection of operating chamber parameters is designed for the analysis of the main groups of milk proteins and may not be suitable for more complex protein compositions. Certain complications of using the device are associated with the lack of disposable chambers with ready-made gel plates. Perhaps the production of the most popular chamber options could be adjusted in the future.

In connection with the growing interest in the proteins of fat globules, it would be advisable to work out the methodology of their analysis and identification on the adapted device.

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## 7. Conclusions

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1. The proposed apparatus for electrophoresis is adapted for the analysis of the main groups of milk proteins (caseins, whey proteins) in vertical PAG plates. The device can be manufactured in the conditions of scientific and factory laboratories from available materials (organic and inorganic glass). The optimal dimensions of working chambers for various types of electrophoresis have been established. The original structure of the formers allows analysing from 1 to 7 samples. The trapezoidal shape on the cross-section of the cells for samples allows obtaining high-quality electrophoregrams. The device is suitable for the main types of electrophoretic systems used for the analysis of milk proteins.

2. Analytical version of casein complex protein electrophoresis in the adapted apparatus in the presence of 4.5 M urea allows identification of all casein fractions according to the modern classification of cow's milk caseins. These include  $\alpha_{S1}$ -CN-9P,  $\alpha_{S1}$ -CN-8P,  $\alpha_{S2}$ -CN-13P,  $\alpha_{S2}$ -CN-12P,  $\alpha_{S2}$ -CN-11P,  $\alpha_{S2}$ -CN-10P,  $\beta$ -CN-5P,  $\kappa$ -CN-1P and three  $\beta$ -casein fragments f(29-209), f(106-209), and f(108-209). Using the express version of electrophoresis in the presence of urea, four casein fractions can be identified:  $\alpha_{S1}$ -CN,  $\alpha_{S2}$ -CN,  $\beta$ -CN, and  $\kappa$ -CN. The average value and standard

deviations of the relative content of casein fractions were calculated on the basis of densitometry of analytical electrophoregrams of five samples. They are for  $\alpha_{S1}$ -CN – 41.4±2.0 %,  $\alpha_{S2}$ -CN – 10.2±0.8 %,  $\beta$ -CN – 35.8±1.7 %, and  $\kappa$ -CN – 12.8±1.3 %, which is acceptable for electrophoretic methods, which are semi-quantitative. Micro preparative electrophoresis of total casein was also performed using the adapted apparatus. At the same time, an electrophoretically pure  $\beta$ -CN-5P fraction was obtained with a yield of 23±5 % and purified groups  $\alpha_{S1}$ -CN,  $\alpha_{S2}$ -CN, and  $\kappa$ -CN with a yield of 25±6 %, 6±1.5 %, and 7±2 %, respectively. The calculation was performed on the basis of the results of five micro preparative electrophoresis.

3. Analytical native disc electrophoresis of milk whey proteins allows identification of all their main fractions:  $\beta$ -Lg A,  $\beta$ -Lg B,  $\alpha$ -La, BSA, and Ig fractions. To identify minor fractions, it is necessary to use marker proteins. The variant of express electrophoresis of whey proteins under native conditions of a homogeneous PAG differs only in a common band for  $\beta$ -Lg A and  $\beta$ -Lg B. The average value and standard deviations of the relative content of whey protein fractions were calculated on the basis of densitometry of the electrophoregrams of five samples. Their values are 36.2±2.9 % for  $\beta$ -Lg (A+B), 16.2±1.5 % for  $\alpha$ -La, 10.4±1.9 % for BSA, and 17.8±2.1 % for Ig, which corresponds to their relative content in milk whey. Standard deviations are acceptable for electrophoretic methods. Micro preparative electrophoresis of milk whey proteins made it possible to isolate two electrophoretically homogeneous fractions –  $\beta$ -Lg and  $\alpha$ -La with a yield based on five micro preparative electrophoresis – 27±6 % and 11±3 %, respectively.

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## Conflicts of interest

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The authors declare that they have no conflicts of interest in relation to the current study, including financial, personal, authorship, or any other, that could affect the study and the results reported in this paper.

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The study was conducted without financial support.

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## Data availability

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The data will be provided upon reasonable request.

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## Use of artificial intelligence

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The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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