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Short Communication

IMPROVEMENT OF STAINING / DESTAINING STEPS OF PROTEINS SDS - PAGE

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ABSTRACT

The polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate is one of the most used method for separation and identification of proteins in biochemistry laboratories. The time of the total analysis and the sensitivity of this technique are important parameters for optimal activities in this field. The purpose of this paper is to improve the sensitivity, i.e. to obtain a stronger signal of the bands of separated proteins and to reduce as much as possible the total run of the method. For this, the composition of the running buffer and the potential applied to the electrodes was studied. The intensity of the color of the bands of separated proteins, transformed by GelAnalyser software in areas of peaks, was optimized by changing some parameters of the staining and destaining procedures.

Keywords: Polyacrylamide gel electrophoresis, SDS, SDS-PAGE, Coomassie, protein electrophoresis.

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1. INTRODUCTION

One of the most used technique to study the separation and to identify proteins is polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS). This method is rapid, simple and have a good sensitivity. The principle of separation of proteins is that in the presence of SDS, that bind to the hydrophobic part of the protein molecules, the proteins become negatively charged due to SO_3^- groups of SDS molecules. In an electric field, the proteins covered with SDS will migrate towards positive electrode, irrespective of their initial charge. The velocity of the migration of proteins covered with SDS will depend mostly on the molecular mass of the polypeptide[1,2].

The proteins separated on the gel (SDS-PAGE) in order to be detected have to be colored. There are several methods of staining the proteins in the SDS-PAGE gels: coloration with Amidoblack[3], Coomassie Brilliant Blue[4,5], Silver stain[6,7], fluorescent dyes[8], negative stains[9] with organic dyes[10] or with copper chloride[11], zinc chloride[12], potassium acetate[13], and many others.

Most of the methods used to color the proteins stain also the gel matrix. A destaining procedure is required to clear the gel and to reveal with high contrast the proteins bands. Most of the destaining methods use hazardous solvent like methanol and / or acetic acid[4].

In this study we focus on the optimization of the staining / destaining procedures, aiming to reduce the number of solutions used for these steps and the total time for coloration / discoloration of proteins bands.

2. MATERIALS AND METHODS

All chemicals used in this study were of analytical grade or purer. Most of them were bought from Sigma (Redox Lab Supplies Com S.R.L,Bucharest, Romania): Tris (#77-86-1), Glycine (#56-40-6), Sodium docecyl sulfate (#151-21-3), Tris-HCl (#1185-53-1), Glycerol (#56-81-5), 2-Mercaptoethanol (#60-24-2), Bromophenol Blue (#115-39-9), Bovine serum albumin (#9048-46-8), Acrylamide (#79-06-1), N,N′-Methylenebis(acrylamide) (#110-26-9), N,N,N′,N′-Tetramethylethylenediamine (#110-18-9), Ammonium persulfate (#7727-54-0), Coomassie Brilliant Blue (#6104-58-1), Acetic acid (#6104-58-1), Ispropanol (#67-63-0)

The electrophoresis was run in a Mini-protean III cell (Bio-Rad) at a constant voltage of 200V per slab gel using a Power PAC 300. Other items used were: casing gel mold, 8x10 cm glass plates.

Buffers preparation. Running buffers = 250 mM Tris, 1.92 M Glycine, 1% SDS, pH 8-8.5, made as 10 times concentrated (10X) stock solution, comparing with the working solution and sample buffer = 250 mM Tris-HCl, 25% Glycerol 1M 2-Mercaptoethanol, 0.05%

Bromophenol Blue, pH 6.8. Before electrophoresis, the running buffer was diluted to desired concentration.

Samples preparation. For comparison reasons, all the analyzed samples have contained bovine serum albumin (BSA) at 3 mg/mL in phosphate-buffered saline (PBS) and before running the solutions were diluted 1:2 with sample buffer.

Polyacrylamide gels preparation. In all the experiments from this study, 10% polyacrylamide with an acrylamide:BIS ratio of 30:0.8 separation gels (100 mm×80 mm×0.75 mm) and 4,5% stacking gels, prepared after Laemmli [14] with small modifications, were used. For 10% separation gels there were mixed 2.78 mL dH_2O, 3.75 mL 1 M Tris HCl (pH 8.8), 100 µL 10% SDS, 3.33 mL acrylamide/bis-acrylamide (30%/8%), 100 µL TEMED and 30 µL 20% APS. To prepare the stacking gels the following solutions were mixed: 3.645 mL dH_2O, 625 µL 1 M Tris HCl (pH6.8), 50 µL 10% SDS, 830µL acrylamide/bis-acrylamide (30%/8%), 5 µL TEMED and 15 µL 20% APS.

Staining / destaining the gels. The gels were immersed in solution A (0.05% CBB, 25% isopropanol, 10% acetic acid), heated for 30 seconds in microwave oven, shacked for 15 minutes at room temperature. Then the gels were immersed in solution B (0.05% CBB, 10% isopropanol), heated 30 seconds in microwave oven and heated again for 30 seconds but in solution C (0.002% CBB, 10% acetic acid). For destaining it was used a solution of 10% acetic acid (solution D) in which the gels were shaken for 15 minutes.

Gels documentation. After migration, gels were stained, photographed and the images were analyzed with GelAnalyzer software (http://www.gelanalyzer.com/). This software was used to transform electrophoretic bands into peaks and to calculate theirs areas. It was also used to measure the distance of migration of various peaks and to calculate Rf or to estimate the molecular weight of proteins.

3. RESULTS AND DISCUSSIONS

In Figure 1 a typical analysis of an image of SDS-PAGE gel is presented. This software transform the bands of colored proteins into peaks and the quantity of dye associated with one band into area of that peak, considered as conventional units (pixels).

This software is useful to create calibration curves and, based on them, to estimate the quantity of the protein from a band. For example, in Figure 2 there is presented the calibration curve that make a correspondence between the quantity of the protein loaded on each lane and the areas of the peaks of each lane (the software name the area of the peak as raw volume and the conventional units are in pixels). The correlation coefficients of such calibration curves were better than 0.99 for proteins with molecular masses ranging from 12 until 116 kDa.

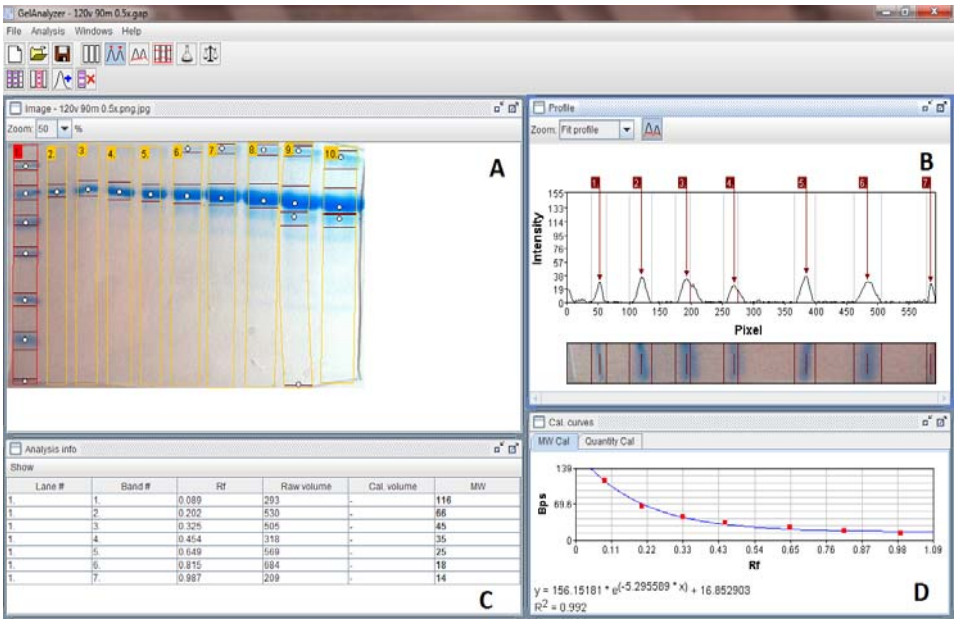

Figure 1. The main panels of GelAnalyser software. Panel A - the image of the entire electrophoresis gel. Panel B – the bands from the selected lane from panel A are transformed in peaks and their area are computed in panel C, as raw volume (conventional units). Based on the molecular weights of the markers, in panel D, the correspondence between the molecular mass and the migration (as Rf) of the protein is presented.

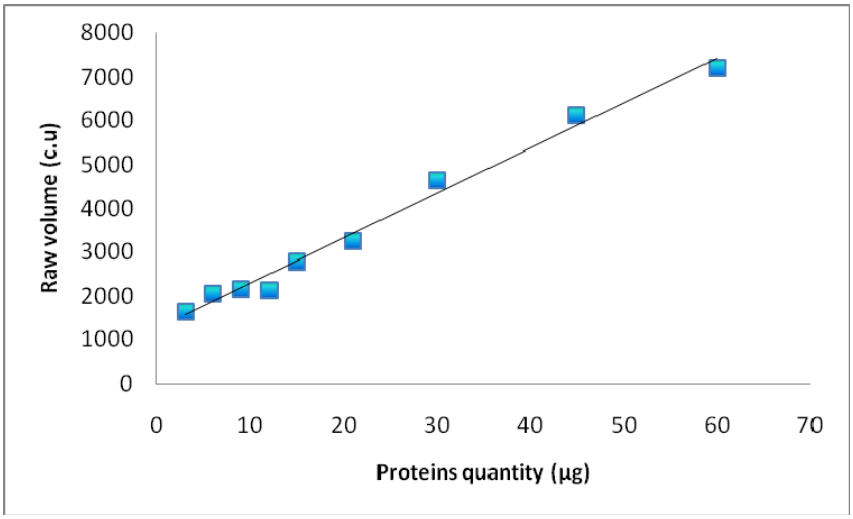

Figure 2. Variation of peak areas (color intensity of bands) on quantity of protein loaded per lane.

In order to reduce as much as possible the total running time of electrophoresis, there were studied the influence of the composition and concentration of the running buffer. As Figure 3 shows, at buffer concentration higher than 1X the migration of protein is higher than when weaker buffers are used. Considering this, the following experiments were realized with running buffer having the concentration 1X (normal dilution).

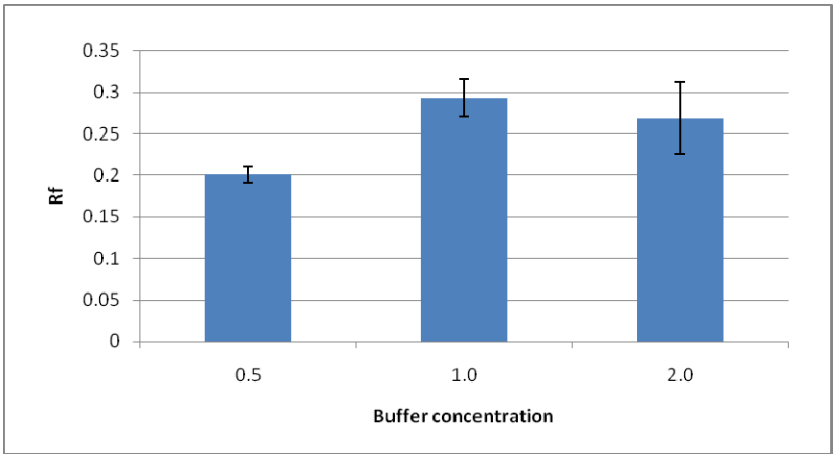

Figure 3. The dependence of Rf of protein on the running buffer concentration

The main objective of this study was to improve or to optimize the steps of the staining procedure of the gels. The most used protocol to stain the proteins separated by SDS-PAGE is staining with Coomassie Brilliant Blue R-250 (CBB) dye[15]. Currently, there are two type of procedures to stain the SDS-PAGE gels for proteins using Coomassie Brilliant Blue, the so call "classic" method, when the gels are stained overnight[5] and the "rapid" technique[4], when the gels are stained only several minutes. There are authors that have concentrated their efforts to reduce the time of the staining step and other to reduce the destaining phase[16].

For staining stage, there are necessary three solutions: solution A (0.05% CBB, 25% isopropanol, 10% acetic acid), solution B (0.05% CBB, 10% isopropanol), solution C (0.002% CBB, 10% acetic acid). For destaining, usually, it is used only a solution of acetic acid (solution D is10% acetic acid).

In the "rapid" method the gel is immersed in solution A, heated in microwave oven for 30 seconds and kept at room temperature 15 minutes, under constant shake. After 15 minutes, the gel is immersed in solution B and for 30 seconds is heated in microwave oven. The same operations are performed with C. The gel colored in this way is destained with solution D, heating it again in microwave oven for 30 seconds and that kept at room temperature for 15 minutes, on shaker, with a paper napkin placed on gel. In total, this "rapid" procedure takes around 40 minutes.

In order to speed up the staining / destaining procedures, we have applied the strategy to omit one-step at the time. From the standard "rapid" protocol, we have omitted one step and recorded the results, in this case the area of the peaks. Based on the results presented in Figure 4, we have concluded that the solution A cannot be omitted from the staining protocol, but the other two (i.e. solution B and C) may be absent, without decreasing very much the areas of the peaks.

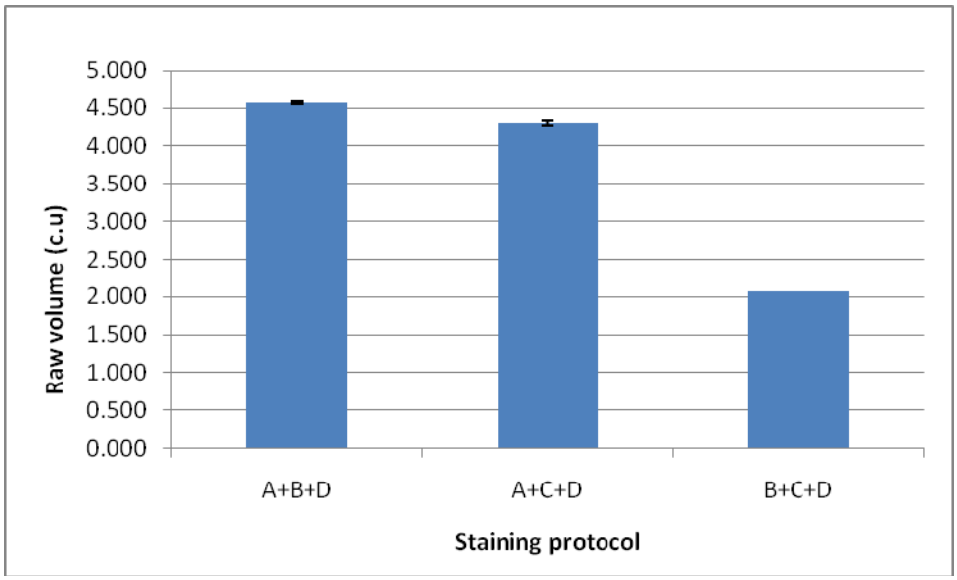

Figure 4. The dependence of the areas of the peaks on the use of the staining solutions (for details see the text)

Renouncing to use the solutions B and C, we have monitored the influence of the time of coloration of the gels with solution A. From Figure 5 one can see that a staining time of 15 minute will be enough to properly stain the gels. Larger time intervals will not increase the intensities of the bands and for the rest of the experiments the staining procedure was to heat the gels for 30 seconds in microwave oven and to color the gel for 15 minutes, under constant shaking.

Due to the fact that SDS inhibits the staining[17], we have introduced before the staining procedure a step in which the gel was simply washed with water. First the gel immersed in water was heated in microwave oven for 30 seconds, and then the gel was shaken for 1 to 10 minutes. As it can be seen from Figure 6, the stage when the gel was shaken in water can last only 3 minutes to obtain the best results.

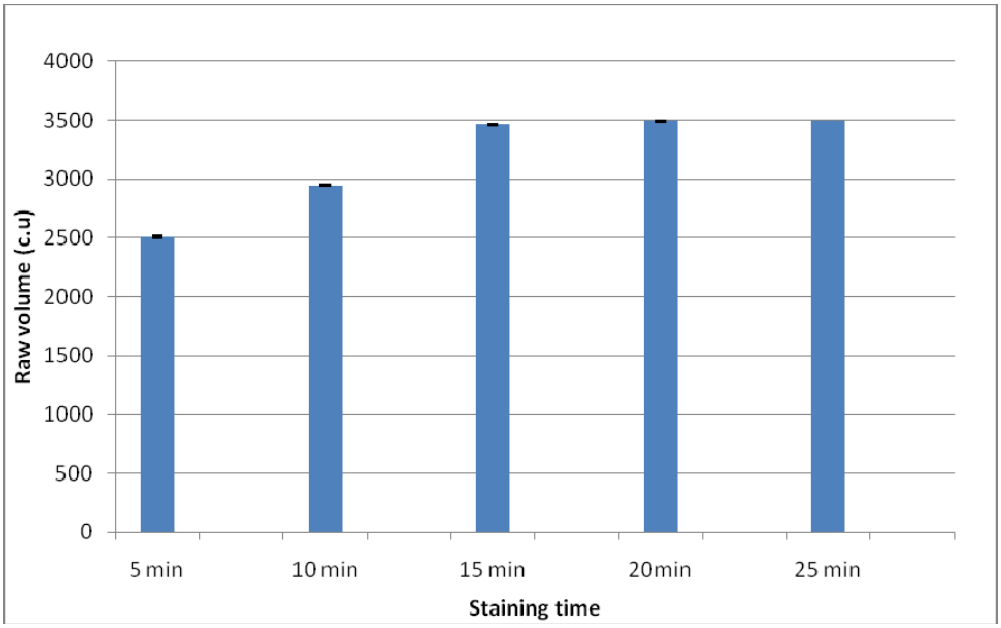

Figure 5. The dependence of the areas of the peaks on the time used for staining procedure, when only solution A was used for coloration of the gel.

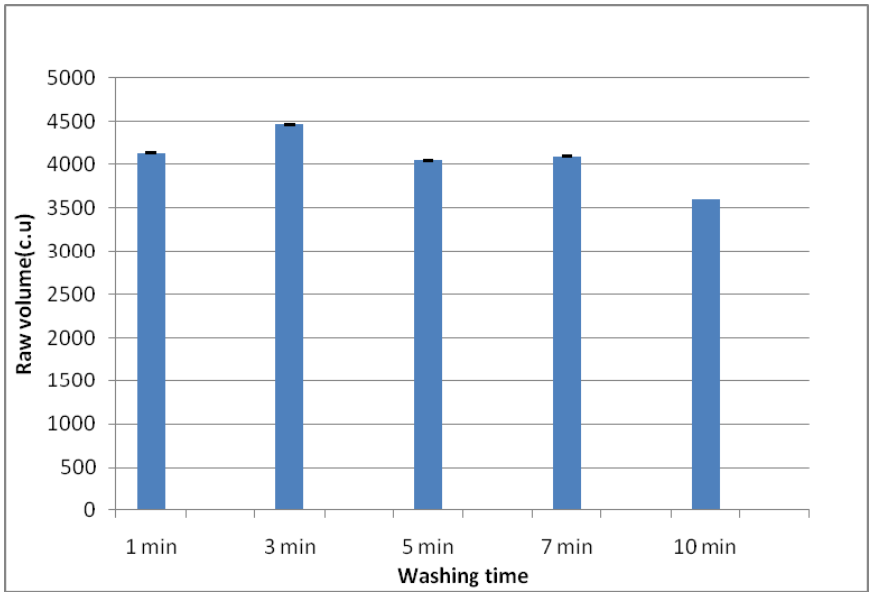

Figure 6 The influence of the time of washing step applied to the gel before staining procedure, to washout the SDS

The attempts to optimize or reduce the time of destaining procedure were addressed to observe the influence of various combination of destaining solutions, made from acetic acid, methanol, water or combination of them. From the results presented in Figure 7 one may see that comparing with the published procedure[18] when the destaining was carried out with mixture methanol / acetic acid (50% / 10%), better results, i.e. higher areas of the peaks, are obtain when the concentrations of these solvents are lower. These results conduct us to the idea to use only water for destaining and, indeed, the best results are obtained in this case. For this, we have used in the remaining experiments, water as solvent for destaining step. The gel was immersed in water, heated in microwave oven for 30 seconds and shaken for 15 minutes at room temperature.

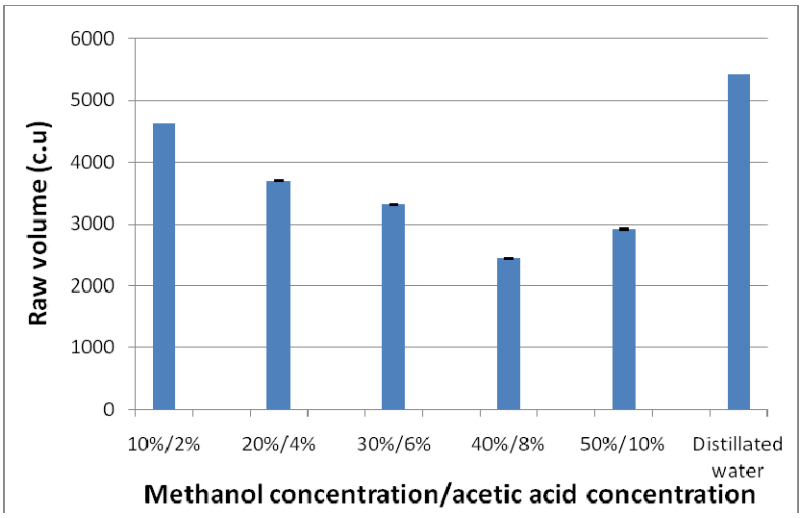

Figure 7. The influence of composition of destaining solution on raw volume (peak area) of protein bands.

This last step was further improved by changing the water repeatedly. In fact, the importance of repeating the washing procedure for destaining is presented in Figure 8, when the first figure represent the total time of the destaining procedure and the second figure represent the time of each step when the gel was shaken at room temperature. For example, the best results are obtained when the gel was maintain in water, after heating in microwave oven for 30 second 5 minutes and this procedure was repeated for 3 times (5x3).

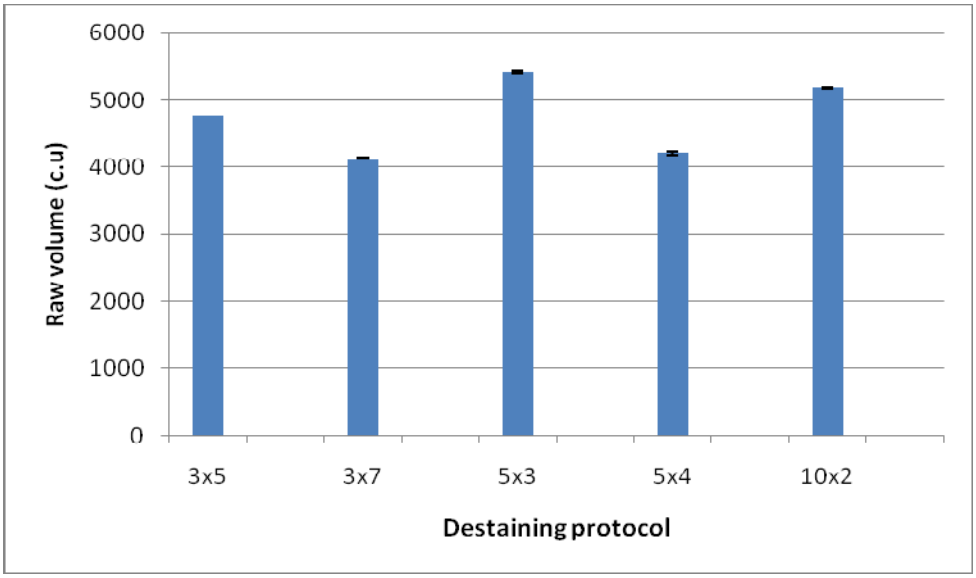

Figure 8 The influence of washing / destaining step on the areas of the peaks (bands): first figure represent the time when the gel was shaken in water, after a 30 seconds heating in microwave oven and the second figure is the number of repeated steps.

The SDS-PAGE is an analytical technique used very often for protein separation. The time of the total run and the sensitivity of this technique are important parameters for the productivity in biochemistry laboratories. In this study, we have optimized the staining / destaining procedures.

4. CONCLUSIONS

The steps of staining procedure of the gel obtained in polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate were studied and optimized. The staining procedure was reduced from 3 steps to only 1 and the time from 40 to 30 minutes. Beside this some reagents and solutions were also eliminated that conducts to reduction of the price of analysis.

The influence of the concentration of the running buffer and of the voltage applied to the electrodes was also studied. By increasing the voltage to 170 V the running time of the electrophoretic separation was reduced from 90 to 60 minutes.

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