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ANALYSIS OF CHITOSAN MOLECULAR WEIGHT PROFILE BY ELECTROPHORESIS IN A POROSITY STEP GRADIENT POLYACRYLAMIDE GEL

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Chitosan is biocompatible and biodegradable natural biopolymer widely applied in various fields of biology, medicine, and pharmacy, however, its effects significantly depend on the degree of polymerization (DP) and the degree of deacetylation (DDA) of polymer chains. Evaluation of the chitosan chain diversity by DP requires the use of a highly expensive method of high-performance size exclusion chromatography. The aim of our study was to determine the molecular weight profile of chitosan specimens by the use of electrophoresis in a porosity step gradient polyacrylamide gel and to evaluate the efficacy of this method in monitoring the purification of chitosan fragments and its derivatives. Two types of step gradient porosity gels were used: 1) gels of layers with acrylamide concentration 2.5, 3.5, 5.0, 10.0, 15.0, 20.0 % w/v for native chitosan or its high molecular fragments; 2) gels of layers with acrylamide concentration 2.5, 5.0, 10.0, 15.0, 20.0, 25.0 % w/v for low molecular chitosan fragments. The main amount of molecules from the chitosan pool was localized in the type 1 gel in the region of 550-40 kDa and distributed among three bands, which in different samples differed significantly in percentage. Electrophoresis of chitosan fragments fractionated by gel permeation chromatography provided a clear separation of medium molecular weight fragments (50–400 kDa) in type 1 gel and of low molecular weight fragments (3–40 kDa) in type 2 gel. Thus the method of chitosan electrophoresis in a step-gradient porosity of polyacrylamide gel was developed which permits to characterize the molecular weight profile of chitosan specimens polymer chains and is effective in monitoring the isolation of chitosan fragments by gel penetration chromatography of molecular weights from 3 to 400 kDa.

K e y w o r d s: chitosan, determination of molecular weight, electrophoresis, step gradient porosity, polyacrylamide gel.

Chitosan is a natural biopolymer which is widely applied in different fields of biology, medicine and pharmacy due to its biocompatibility and biodegradability [1-3]. However, its biological effects significantly depend on its physicochemical characteristics, the most important among which are considered the molecular weight, resp., degree of polymerization (DP) and degree of deacetylation (DDA) of polymer chains [4,5]. These parameters of commercial chitosan specimens are indicated in catalogs as average values, which, however, do not reflect the real degree of heterogeneity of chitosan chains. It is considered that the molecular weight of chitosan polymer chains may be extended from 10 kDa to 1.000 kDa. The heterogeneity of chitosan chains by DP is characterized by polydispersity index (PDI), which is defined as the relation M_n/M_m, where Mn is an average molecular weight relative to the number of particles, M_m is an average molecular weight dependent on the molecular weight of particles. PDI can be calculated on basis of the molecular weight profile of chitosan chains. The fractionation of chitosan chains according to DP was firstly performed by means of gel-penetration chromatography (GPC) [6-8]. Thereafter the technology of high performance-size exclusion chromatography (HP-SEC) was applied and after a series of consecutive improvements, the modern method of molecular weight profile estimation of chitosan chains in specimens was developed [9-12]. Yet the highly expensive

equipment and special requirements for materials and condition of the analyt limit the wide application of this method.

Another approach to fractionation of chitosan and its oligomers is gel electrophoresis which was initiated by P. Audy and A. Asselin [13]. Authors revealed that the optimal for electrophoresis of chitosan is polyacrylamide gel in acidic buffer systems. However, the method had some disadvantages. The main amount of chitosan migrated as an even extended strip without separation into bands. Only low molecular chitosan oligomers obtained by enzymatic cleavage were resolved into distinct bands. The absence of molecular weight markers applicable in acidic electrophoresis systems made it difficult to separate the electrophoregram into fractions according to distinct molecular weights. Besides, some part of material was retained in the start position and not entered the gel due to a very high molecular weight (over 1.000 kDa). Despite these circumstances, we suggest that analysis of molecular weight profile of chitosan specimens by use of gel electrophoresis is perspective as a convenient alternative to HP-SEC.

It was revealed in our studies on chitosan that electrophoresis in polyacrylamide gel with a stepwise gradient of porosity significantly improved the image of electrophoregram by separation of chitosan into several bands in accordance with molecular weight due to the retardation on pore-size gradient steps. This makes it possible to characterize different chitosan specimens by molecular weight profile which may be useful for their comparison and identification.

The aim of our study was to determine the molecular weight profile of chitosan chains in specimens by use of electrophoresis in the step-gradient porosity of polyacrylamide gel as well as to evaluate the efficacy of this method in monitoring the process of purification of chitosan fragments and its derivatives.

Two types of step-gradient porosity gels were developed: 1) gels of layers with acrylamide concentration 2.5, 3.5, 5.0, 10.0, 15.0, 20.0% w/v for analysis of native chitosan or its high molecular fragments; 2) gels of layers with acrylamide concentration 2.5, 5.0, 10.0, 15.0, 20.0, 25.0% w/v for analysis of low molecular chitosan fragments. The proposed method was proved by investigations of chitosan specimens from crabs, shrimps, honeybee corpses as well as in the monitoring of fractionation and purification of chitosan fragments by GPC [14, 15]. In the present paper the optimized version of preparation of gels with a step-gradient porosity, performance of electrophoresis, quantitative evaluation of electrophoregrams and their interpretation are described. Besides, we developed the kit of molecular weight markers applicable in acidic electrophoresis systems which includes alkaline proteins clupein, cytochrome c and its oligomers, human immunoglobulins. It permitted to determine with sufficient accuracy the molecular weight ranges of chitosan bands.

Materials and Methods

The Kit of reagents for preparation of polyacrylamide gel, buffer solutions and Amido black 10 B for staining of electrophoregrams were purchased from Reanal (Hungary). Ammonium persulfate was a product of the Angarsk chemical plant (Rf). All other chemicals were of the analytical reagent grade quality.

The following chitosan specimens were investigated: chitosan (Aldrich, USA), “practical grade” and “low molecular weight grade”; shrimps chitosan Celox (Medtrade products Ltd., UK) and also from ChitoPharm (Norway), types L (large), M (medium) and S (small) (a generous gift from ChitoPharm company); chitosan Organika (Health products Inc., Canada); chitosan “Bioprogress 300” (Rf); chitosan Tjan-Shi (China). Specimens of chitosan from crab shells, honeybee corpses and their fragments after limited hydrolysis were obtained in our laboratory as described earlier [14, 15]. Fragmentation of chitosan by limited acid hydrolysis was performed in mild (0.5 M HCl in 0.5 M acetic acid at 80°C for 1 h) or in strong (heating of chitosan powder embedded with HCl for 1 h in a boiling water bath) conditions. Subsequent fractionation of fragments was performed by GPC using Bio-Gel P-300 (Bio-Rad Laboratories, USA) or Acrylex P-30 (Reanal, Hungary).

The following stock solutions were prepared for the construction of step-gradient porosity polyacrylamide gels.

1. Buffer A (Buf A): 1N KOH 4.8 ml, acetic acid 1.72 ml, TEMED 0.4 ml, adjusted with distilled water to 10 mL, pH 4.5.

2. Buffer B (Buf B): 1N KOH 4.8 mL, acetic acid 0.75 ml, TEMED 0.4 ml, adjusted with distilled water to 10 ml, pH 5.0.

3. Acrylamide solution 10% (w/v) (Acr10): acrylamide 0.9 g, bis-acrylamide 0.10 g, adjusted with water to 10 ml. Proportion Acr/bisAcr = 9:1.

4. Acrylamide solution 30% (w/v) (Acr30): acrylamide 8.82 g, bis-acrylamide 0.18 g, adjusted with water to 30 ml. Proportion Acr/bisAcr = 49:1.

5. Acrylamide solution 35% (w/v) (Acr35): acrylamide 6.72 g, bis-acrylamide 0.28 g, adjusted with water to 20 ml. Proportion Acr/bisAcr = 24:1.

6. Ammonium persulfate (APS): 1% (w/v) aqueous solution, prepared ex tempore.

7. Buffer for electrode vessels: β-alanine 3.12 g, acetic acid 0.8 ml, distilled water to 800 ml, pH 4.5.

Solutions with acrylamide concentrations 2.5, 5.0 and 20.0 % (w/v) were prepared for the construction of gel type 1 as it is shown in Table 1.

Solutions of layers with distinct concentrations of acrylamide for resolving gel were prepared by mixing solutions Acr 20% and Acr 5% in proportions indicated in Table 2. Solution Acr 2.5% was used for obtaining 3.5% and 2.5% resolving gels. Volumes of indicated solutions were calculated for gel dimensions 10.5(w) × 11.5(h) × 0.15 cm, total volume 18.11 ml.

Resolving gel was prepared by consequent manual overlaying of acrylamide solutions of distinct concentration in vertically installed casting gel mold and left for 8-12 hrs at temperature 18-22°C for polymerization. Thereafter solution of stacking 2.5% gel was applied and a comb for sample wells formation was introduced and left for 8-12 hrs for polymerization. Volumes of solutions were calculated just for construction of gel with the abovementioned dimensions.

In our experiments, we used casting gel mold made in laboratory using the photographic glass plates, as spacers were used glass strips of 0.15 cm thick. The leak of applied solutions was prevented by clamps and sealing up the camera with a melted wax composed of three parts of paraffin and one part of dentistry technical wax. The bottom spacer was eliminated after polymerization of gel at preparing the unit for electrophoresis. If possible, commercial electrophoresis units may be used. The most suitable by dimensions are Sigma single gel vertical electrophoresis units (Cat. No. Z375039, gel dimensions 16.5×14.5×0.15 cm; and TV100 mini vertical electrophoresis unit, Cat.No Z649767-1EA). The proportions of constituents in solutions for preparation of gel layers ought to be recalculated and adapted to the volume of the acquired electrophoresis unit.

Table 1. Composition of acrylamide solutions for preparation of type 1 gel with a step-gradient of acrylamide concentration from 2.5 to 20%

20% Acr solution, ml		5% Acr solution, ml		2.5% Acr solution**, ml	
Buf A	0.90	Buf A	1.00	Buf B	0.30
Acr30	4.80	Acr30	1.35	Acr10	0.60
Water	1.10	Water	5.10	Water	1.20
APS*	0.40	APS	0.55	APS	0.30
Volume	7.20	Volume	8.00	Volume	2.40

Notes. APS* solution is prepared just before gel construction. **2.5% Acr solution is prepared two times, firstly, 1.2 ml is used for obtaining of 3.5% acrylamide layer and the rest (about 1 ml) for application of 2.5% acrylamide as resolving gel. After gel polymerization (for 6-12 h) 2.5% Acr solution was prepared again and used for formation of stacking gel with sample slots. Abbreviations used in Tables 1-4 correspond to those in the list of stock solutions

Solutions for preparation of gel type 2 are shown in Table 3. There was introduced an additional 25% w/v acrylamide gel for achievement of low molecular chitosan fragments resolution. Loose 2.5% and 3.5% acrylamide gels were omitted.

Mixing of solutions for preparation of gel with gradient of acrylamide concentration from 5 to 25%

Table 2. Mixing of solutions for the construction of the type 1 gradient porosity gel

Layer #	20% Acr, ml	5% Acr, ml	2.5% Acr, ml	Volume of layer, ml	Conc. of Acr (%)	ProportionAcr/ bisAcr
1	3.6	0	0	3.6	20.0	50/1
2	2.4	1.2	0	3.6	15.0	50/1
3	1.2	2.4	0	3.6	10.0	50/1
4	0	3.6	0	3.6	5.0	50/1
5	0	0.8	1.2	2.0	3.5	35/1
6	0	0	1.2	1.2	2.5	10/1

Table 3. Composition of acrylamide solutions for preparation of type 2 gel with a step-gradient of acrylamide concentration from 5 to 25%

25% Acr solution, ml		20% Acr solution,ml		5% Acr solution, ml		2.5% Acr solution, ml	
Buf A	0.60	Buf A	1.1	Buf A	1.1	Buf B	0,4
Acr35	3.60	Acr30	4.8	Acr30	1.2	Acr10	0.8
Water	0.65	Water	1.0	Water	4.4	Water	1.6
APS	0.20	APS	0.3	APS	0.5	APS	0.4
Volume	5.05	Volume	7.2	Volume	7.2	Volume	3.2

is shown in Table 4. They were used for construction of gel type 2 (a step-gradient of acrylamide concentration from 5 to 25%) by overlaying solutions as it was described above for gel type 1.

Markers of molecular weight (M_r) for electrophoresis in acidic buffer system were composed of the following cationic proteins: human immunoglobulins, isolated by chromatography on T-gel and containing IgG (150 kDa), IgA (380 kDa) and IgM (900 kDa) [16, 17], clupein sulfate, M_r 4.40 kDa (Fluka), cytochrome *c* from bovine heart, M_r 12.3 kDa, (Sigma, USA), and its oligomers, which were prepared in the laboratory as described below. To 180 µl of aqueous solution of cytochrome *c* (concentration 1.7-2.0% w/v) was added 20 µl of 1.0 M sodium acetate buffer, pH 5.0, followed by 25 µl of 2.5% v/v solution of glutaric aldehyde (final concentration 0.28%). The mixture was left for 90 min at 20-22°C. The reaction was stopped by passing the mixture through "Sephadex" G-25 column (volume 3.5-4.0 ml, height 10-12 cm), equilibrated with 0.05 M ammonium acetate buffer, pH 6.0. Concentration of cytochrome *c* in the combined eluate was determined by spectrophotometry taking into account that 0.01% solution of cytochrome *c* has OD 0.58 at 400 nm (optical pass 1.0 cm). The solution was lyophilized or preserved with 0.04% sodium azide and stored at 4°C for about 5-6 months. The calibration graph for determination of molecular weight using the abovementioned marker proteins is shown in Fig 1. It was based on the interrelation between the logarithm of molecular weight and the mobility of marker protein relative to the reference protein, in our case cytochrome c, its mobility was accepted as 1.0.

Probes of chitosan for electrophoresis were prepared from chitosan gel which was obtained by adjustment of chitosan solution (5-10 mg/ml in 0.5 M acetic acid) to pH 8.5 with 5% ammonium hydroxide. Sediment was collected by centrifugation and washed with distilled water. Concentration of solid residue in chitosan gel was determined gravimetrically by the weighing of gel aliquot before and after drying at 105°C. Usually, it was in the range of 2-3%. Working probes were prepared by suspending 20 mg of wet gel in the defined volume of water for obtaining chitosan concentration 2 mg/ml. Thereafter 5-7 µl of 1M ammonium acetate buffer, pH 4.5, or 0.5 M acetic acid was introduced for dissolution of chitosan. Sucrose was added to a concentration 5-7% (w/v) and thereafter, a small volume of 1% aqueous solution of methylene blue for monitoring the running of electrophoresis.

Electrophoresis was conducted in vertically installed gel. Into wells were introduced 15-20 µl

Table 4. Mixing of solutions for obtaining type 2 gradient porosity gel

Layer #	25% Acr	20% Acr	5% Acr	Volume of layer, ml	Conc. of Acr, %	Proportion Acr/bisAcr
1	5.0	0	0	5.0	25.0	25/1
2	0	3.6	0	3.6	20.0	50/1
3	0	2.4	1.2	3.6	15.0	50/1
4	0	1.2	2.4	3.6	10.0	50/1
5	0	0	3.6	3.6	5.0	50/1

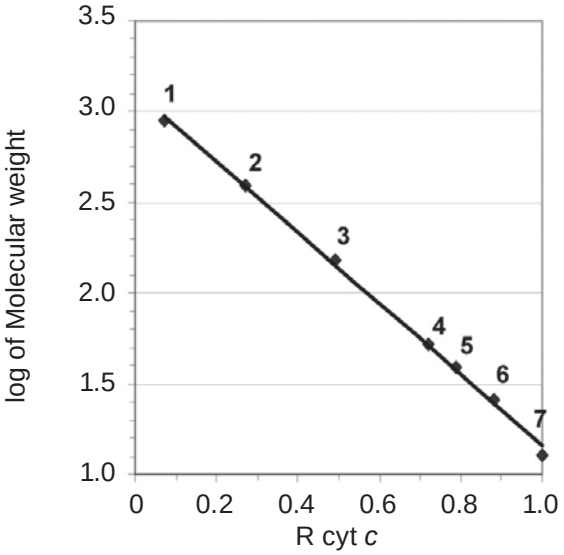

Fig. 1. Calibration graph for determination of the molecular weight marker proteins in acidic electrophoresis system. Enumeration of marker proteins: 1 – IgM (900 kDa), 2 – secretory IgA (385 kDa), 3 – IgG (150 kDa), 4 – cytochrome c tetramer (52 kDa), 5 – cytochrome c trimer (39 kDa), 6 – cytochrome c dimer (26 kDa), 7 – cytochrome c monomer (13 kDa). Points of mean values were calculated at n = 8, SD's are not visible as being too small

of probes containing 5-20 μg of chitosan. Electrophoresis was conducted at 7-9 mA for 12-14 h using β-alanine–acetic acid electrode buffer, pH 4.5 (cathode – bottom electrode). Running was stopped when methylene blue band reached about 15 mm to the lower edge of gel. Gels were stained with 0.01% Amido black 10 B in methanol-water-acetic acid (40:50:10) for 10-12 h, destained during 48 h in 2 changes of the same solvent to a clear background, the image was taken with a digital camera Canon A590 IS. Quantitative evaluation of electrophoregrams and determination of the percentage ratio of electrophoretic bands were performed with the aid of the program "Image J".

Statistics. Quantitative results are presented as means ± SD. The significance of difference between compared data was analyzed with GraphPad Prism (version 6; GraphPad Software, La Jolla, Ca, USA) using Student's *t*-test (unpaired *t*-test with Welch's correction). Two levels of significance were used: $*P < 0.05$ and $**P < 0.01$.

Results and Discussion

An example of electrophoregram of selected chitosan specimens in type 1 gel is shown in Fig. 2. As it can be seen, all chitosan specimens possess some quantity of material retained at the start line and did not migrate even in a staking 2.5% gel. It corresponds to single or aggregated chitosan chains of very high molecular weight (over 1.000 kDa), on electrophoregrams it was signed as peak 1. Chitosan material which migrated in gel was usually separated into six fractions which were formed due to the retardation of chitosan with subsequent molecular weight in six borders between gel layers with stepwise decrease in size of pores.

The main quantity of chitosan was localized in 3 fractions (peaks 3, 4 and 5) in the molecular weight region from 40 to 550 kDa which differed significantly by percentage ratio in different samples (Fig. 1, *A*). Some chitosan specimens, like Chitopharm L (large), M (medium) and S (small), missed low molecular weight chains and showed less number of fractions. These specimens were subjected to a more detailed analysis of their electrophoretic plots (Fig. 3).

Statistical analysis revealed that in sample M as compared with sample L it was noticed a moderately significant increase of fractions 4 and 5, and in sample S was highly significant increase of fractions 4 and 5 with a lower molecular weight (ranges 90-240 and 40-90 kDa) at the expense of a decrease in high molecular fraction 3 (range 240-550 kDa, Table 5). It was concluded that specimens of"Chitopharm" M and especially S are gradually enriched in polymer chains of lower molecular weight as compared with Chitofarm L specimen due to a more profound cleavage of chitin chains during deacetylation process by treatment with a strong alkali.

Solutions of high molecular chitosans at concentrations 2-3% w/v are highly viscose, which is inconvenient in their application, especially in biomedicine. This drawback can be eliminated by mild acid hydrolysis, which markedly reduces the viscosity of obtained derivatives. We investigated changes in molecular weight profile of chitosan after mild acid hydrolysis. It was revealed, that treatment of chitosan by 0.5 M HCl in 0.5 M acetic acid at 80°C during 1 h led to a highly significant increase in content of very low molecular weight fraction 20-

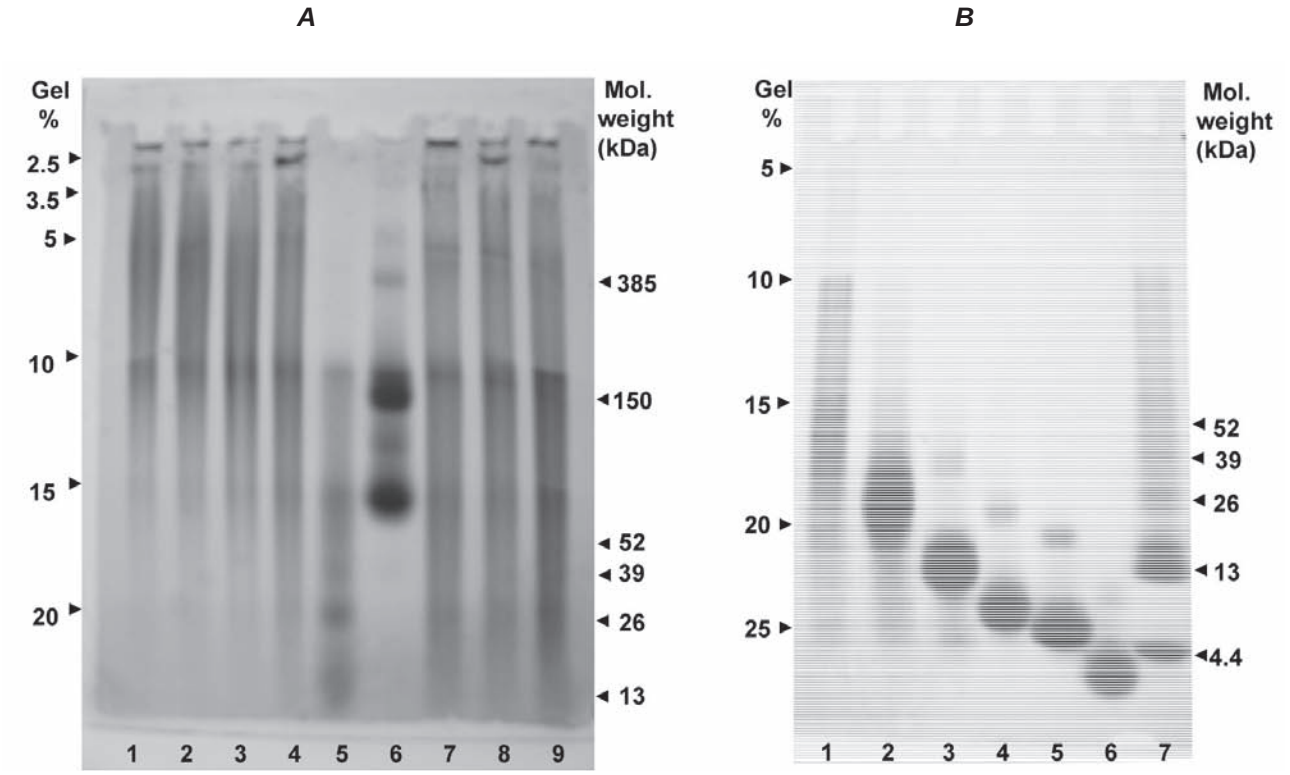

*Fig. 2. Electrophoretic pattern of selected native chitosan specimens in gel type 1 (**A**) and of low molecular fragments of honeybee chitosan in gel type 2 (**B**). Molecular weight scale is shown on the right margin of images, concentration of acrylamide in %w/v in gel layers is indicated on the left margin at the level of borders between layers. In **A** samples are enumerated as follows: "Chitopharm" L (1), "Chitopharm" M (2), "Chitopharm" S (3), chitosan "Aldrich" practical grade (4), cytochrome c oligomers (5), human immunoglobulins (6), chitosan "Organica" (7), chitosan "BioProgress 300", native (8) and after treatment with 0.5 M HCl in 0.5 M acetic acid 1 hr at 80°C (9). **B**. Electrophoresis of low molecular fragments of honeybee chitosan after its hydrolysis and fractionation by gel filtration on "Acrylex" P-30 column. Samples numeration. 1. Initial sample of honeybee chitosan, 2-6 – fractions of hydrolysate eluting consecutively from the column "Acrylex" P-30; 7 – markers of molecular weight: clupein (4.4 kDa), cytochrome c (13 kDa) and its oligomers.*

40 kDa at the expense of high molecular fractions, mainly of fraction 3, decrease (Table 5). Besides, there was noticed an important decrease in very high molecular fractions which are retarded in stacking gel (Fig 2, lane 9). It was suggested, that just these minor fractions make a significant contribution to the high viscosity of chitosan solutions and a decrease in their quantity after mild acid hydrolysis leads to the considerable reduction of viscosity.

An example of the application of type 2 gradient porosity gel for monitoring the purification of honeybee chitosan fragments after more profound hydrolysis with HCl and subsequent fractionation by SEC on column of "Acrylex" P-30 is shown in Fig. 2, *B*. The introduction of gel layer with 25% w/v acryl amide concentration and increased cross-linking was necessary for the achievement of clear resolution of low molecular chitosan fragments in the range of 3-40 kDa.

An important feature of the proposed method of chitosan electrophoresis is application of stepwise gradient porosity of polyacrylamide gel, which permits to separate a complex mixture of chitosan chains into several distinct fractions with definite limits of molecular weight. This can not be achieved by the use of uniform polyacrylamide gel or gels with a continuous gradient of porosity as in these conditions chitosan migrates as evenly extended strip without visible peaks due to very high heterogeneity in DP of polysaccharide chains.

Concerning the application of a modern method of capillary electrophoresis (CE) in the investigations on chitosan, we met several publications on determination of chitosan in different cosmetic and

Table 5. The percentage ratio of electrophoretic fractions in selected chitosans

Digit of electrophoretic fraction	Limits of molecular weight areas	Specimens of chitosan				
		Chitopharm L	Chitopharm M	Chitopharm S	Aldrich (pract. grade)	Bio-Progress 300	Bio-Progress hydrolyzed
1	Over 1.000 kDa	3.9 ± 0.1	5.0 ± 0.2	3.2 ± 0.1	2.8 ± 1.1	6.9 ± 1.4	6.4 ± 0.8
2	1-0.6 MDa	8.4 ± 2.2	8.7 ± 1.9	12.2 ± 2.0	9.4 ± 1.2	8.4 ± 2.8	7.9 ± 0.7
3	550-240 kDa	61.7 ± 2.3	58.2 ± 1.7	45.8 ± 2.1**↓	34.7 ± 2.2	29.7 ± 3.8	18.9 ± 1.3**↓
4	240-90 kDa	22.1 ± 0.4	25.0 ± 1.0*↑	32.8 ± 1.2**↑	38.1 ± 2.1	33.4 ± 1.4	34.0 ±1.5
5	90-40 kDa	4.0 ± 0.1	4.6 ± 0.1*↑	6.2 ± 0.3**↑	13.0 ± 1.5	16.7 ± 1.9	19.7 ±1.8
6	40-20 kDa	0	0	0	2.0 ± 0.4	5.6 ± 1.4	13.3±1.2**↑

Notes. Chitopharm M and Chitopharm S were compared with Chitopharm L, BioProgress hydrolyzed with BioProgress 300 native. Significance of differences: *$P < 0.05$, **$P < 0.01$, $n = 4$. Arrows indicate the direction of changes

*Fig. 3. Electrophoretic plots of three types of "Chitopharm" chitosan samples. **A** – "Chitopharm"L (lane 1 in Fig. 2, A); **B** – "Chitopharm"M (lane 2 in Fig. 2, A); **C** – "Chitopharm"S (lane 3 in Fig. 2, A). Dashed lines mark limits of peaks in quantitative analysis*

pharmaceutical preparations [18-20]. The chitosan as a polycation with a high positive charge exhibits the highest electrophoretic mobility in free aqueous solutions and migrates in the leading peak. As for the efficiency of CE for determination of heterogeneity in DP in the whole population of chitosan chains in the specimen, we found no reports. On the other side, it was communicated the usefulness of CE for determination of the DDA of chitosan chains due to minute differences in charge in special conditions [4, 5].

The resolution of chitosan into fractions according to differences in DP permits to determine an important characteristic of sample heterogeneity defined as polydispersity index (PDI). At present, it is determined by HP-SEC, for which a high expensive equipment and special requirements for the analyte are expected [10-12]. The proposed method of electrophoresis can be considered as alternative to the HP-SEC method. The comparison of PDI values obtained by electrophoresis and the referred data obtained with HP-SEC is under investigation.

Conclusion. The method of electrophoresis of chitosan in the step-gradient porosity of polyacrylamide gel was developed. This method permits to characterize the molecular weight profile of polymer chains in chitosan specimens. The method is also effective in monitoring the isolation of chitosan fragments by SEC in a range of molecular weights from 3 to 400 kDa.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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АНАЛІЗ ПРОФІЛЮ МОЛЕКУЛЯРНОЇ МАСИ ХІТОЗАНУ МЕТОДОМ ЕЛЕКТРОФОРЕЗУ В СТУПІНЧАСТОМУ ГРАДІЄНТІ ПОРИСТОСТІ ПОЛІАКРИЛАМІДНОГО ГЕЛЮ

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Хітозан є біосумісним і здатним до біодеградації природним біополімером, який широко застосовується у різних галузях біології, медицини і фармації, проте його біологічна дія суттєво залежить від ступеня полімеризації (DP) та ступеня деацетилювання (DDA) полімерних ланцюгів. Оцінка ланцюгів хітозану за показником DP потребує застосування вартісного методу високоефективної ексклюзійної хроматографії (HP-SEC). Метою нашого дослідження було визначення профілю молекулярної маси зразків хітозану шляхом електрофорезу у ступінчастому градієнті пористості поліакриламідного гелю, а також оцінка ефективності цього методу для моніторингу очищення фрагментів хітозану і його похідних. Застосували 2 типи гелів із ступінчастим градієнтом пористості: 1) гель із шарами акриламіду концентрації 2,5; 3,5; 5,0; 10,0; 15,0; 20,0% w/v для нативного хітозану або його високомолекулярних фрагментів; 2) гель із шарами акриламіду концентрації 2,5; 5,0; 10,0; 15,0; 20,0; 25,0% w/v для низькомолекулярних фрагментів хітозану. Переважна кількість молекул із пулу хітозану локалізувалась у гелі типу 1 у області 550-40 кДа та розподілялась між трьома бендами, які у різних зразках значно відрізнялись за відсотковим співвідношенням. Електрофорез фрагментів хітозану, фракціонованих із допомогою гель-проникної хроматографії, забезпечував чітке розділення фрагментів середньої молекулярної маси (50-400 кДа) у гелі типу 1 та низькомолекулярних фрагментів (3-40 кДа) у гелі типу 2. Отже, розроблено метод електрофорезу хітозану у ступінчастому градієнті пористості поліакриламідного гелю, який дозволяє охарактеризувати молекулярну масу полімерних ланцюгів у зразках хітозану, а також є ефективним у моніторингу очищення фрагментів хітозану з молекулярною масою в діапазоні від 3 до 400 кДа, отриманих методом гель-проникної хроматографії.

Ключові слова: хітозан, визначення молекулярної маси, електрофорез, поліакриламідний гель, ступеневий градієнт пористості.

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