

**O‘ZBEKISTON RESPUBLIKASI
OLIV TA‘LIM, FAN VA INNOVATSIYALAR VAZIRLIGI
NAMANGAN MUXANDISLIK TEXNOLOGIYA INSTITUTI**



**«TA‘LIM, FAN VA ISHLAB CHIQRISHNING DOLZARB
MUAMMOLARI»
MAVZUSIDAGI RESPUBLIKA ILMIY – AMALIY
KONFERENSIYA**

MATERIALLARI TO‘PLAMI



Namangan 2023 yil 7-8 noyabr

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«Ta'lim, fan va ishlab chiqarishning dolzarb muammolari» mavzusidagi respublika ilmiy-amaliy konferensiya materiallari

«Ta'lim, fan va ishlab chiqarishning dolzarb muammolari» mavzusidagi respublika ilmiy-amaliy anjuman materiallar to'plami (2023 yil 7-8 noyabr).

Anjuman materiallari to'plami professor-o'qituvchilar, katta ilmiy xodimlanuvchilar, mustaqil tadqiqotchilar, magistrantlar hamda talabalar uchun mo'ljallangan.

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SIZE-EXCLUSION CHROMATOGRAPHY OF PULLULANS AND ARABINOGALACTANS

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Abstract. *The validity of the Universal calibration concept in aqueous Size-exclusion chromatography for arabinogalactan and pullulans is presented. The electrostatic effects of these polysaccharides during separation process were eliminated by utilizing of a water-salt eluent as a mobile phase, and the size-exclusion separation mechanism of this method was realized.*

Annotatsiya. *Arabinogalaktan va pullulanlar uchun suvli eksklyuziv xromatografiyada universal kalibrlash kontseptiyasining bajarilishi isbot etilgan. Ajratish jarayonida bu polisaxaridlarning elektrostatik ta'siri suv-tuzli eritmani suyuq faza sifatida ishlatish yo'li bilan bartaraf etildi va bu usulning o'lcham bo'yicha ajratish mexanizmi amalga oshirildi.*

Аннотация. *Представлена обоснованность концепции универсальной калибровки в водной эксклюзионной хроматографии для арабиногалактана и пуллуланов. Электростатические эффекты этих полисахаридов в процессе разделения были устранены за счет использования водно-солевого элюента в качестве подвижной фазы и реализован механизм разделения по размеру.*

Size-exclusion chromatography (SEC) or Gel permeation chromatography (GPC) is one of the powerful and fast methods for the determination and characterization of the average molar mass of polymers [1]. The chromatographic behavior of macromolecules separated by SEC can be described by the general chromatographic equation: $K_d = (V_R - V_0) / (V_t - V_0)$, where V_R is the measured peak elution volume, V_t the total column volume, and V_0 the exclusion (or void) volume. The concept of ГТШМУКЫФД СФДШИКФЕШЦТ (UC) in GPC was introduced by Benoit in 1967 [2]. Instead of plotting the log molecular weight of a series of narrow polymer standards vs. retention volume, the logarithm of the product of the intrinsic viscosity $[\eta]$ and molecular weight M is plotted vs. retention volume. The product $(M[\eta])$ has dimension unit dl/mole and can characterize the hydrodynamic volume (V_h) of macromolecules. The GPC plot between $\log (M[\eta])$ and V_R , represents a universal calibration of Benoit and retention volume V_R can be determined from the following equation: $V_R = A - B \lg (M[\eta])$, where A and B - constants. Using UC

constructed for polymers having different conformation in solution (random coil, rodlike, branched, star, copolymers, etc) and their average molar masses can be determined. This study aims to analyze the applicability of the UC to pullulans, arabinogalact GPC measurements were performed on the liquid chromatograph Agilent 1260 Infinity, consisting of a syringe pump, differential refractive index (RI) detector, and degasser of the eluent. For aqueous GPC the chromatographic column PL Aquagel-OH Mixed (300x8 mm, Polymer laboratories, UK) thermostated at 250C was used. GPC analysis was performed using NaNO_3 in the water with a concentration of 0.1 mol/l as eluent. Pullulan standards were purchased from Showa Denko, Japan. Larch AG was provided by Megazyme Int, Ireland. The injection volume of the sample and the flow rate of eluent was equal to 20 microliters and 0.8 ml/min. correspondingly. The injected sample concentration of polymers was always equal to 3 g/l. Elution volumes were determined at the peak maximum of GPC curves, since all chromatograms obtained in aqueous salt solutions gave quite sharp, symmetric curves of Gaussian type. Molar mass and polydispersity were determined using UC, and chromatograms were evaluated according to the size-exclusion separation mechanism of GPC. According to UC two polymers with the same hydrodynamic volume ($V_h \propto [\eta]M$) will be separated with the same elution time V_t . The use of $(M[\eta])$ as a universal parameter representing the hydrodynamic volume of polymer chains in good solvents was proposed by various authors [3,4]. Using $V_{h1}=V_{h2}$, $V_{h1} = [\eta]_1 M_1$ (for pullulans), $V_{h2} = [\eta]_2 M_2$ (for AG), and Mark-Kuhn-Houwink-Sakurada equation ($[\eta]=KM_a$) for both polymers we can determine molar mass for AG at fixed elution time that point lies on UC curve (figure 1).

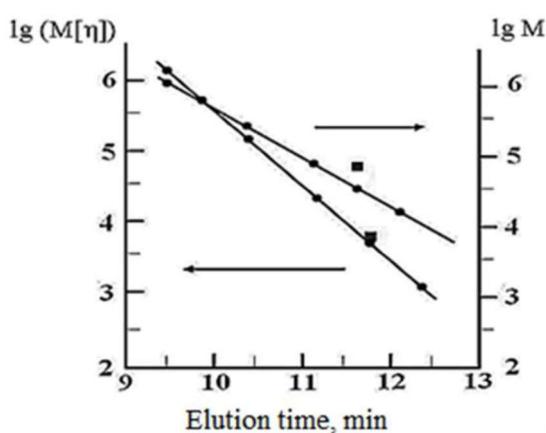


Figure 1. Combined dependences $\lg (M[\eta])$ and $\lg M$ from elution time (universal and primary calibration correspondingly) for Pullulan standards (●) and larch arabinogalactan (Megazyme, Ireland) (■). Column: PL Aquagel OH Mixed. Mobile phase: water

+ 0.1MNaNO₃.

From equality of hydrodynamic volumes for both polymers $K_1M_1(1+a_1) = K_2M_2(1+a_2)$ molar mass of AG can be determined: $\lg M_2 = \lg (K_1/K_2) / (1+a_2) + \lg M_1 (1+a_1) / (1+a_2)$. Aqueous GPC measurements indicated that pullulans (standards) and larch AG in water-salt solution (0.1M NaNO₃) as eluent demonstrated the validity of UC for both polymers. It should be noted that AG has a small number of galacturonic acids in its chain dissociating to anionic groups in pure water. Before construction UC for AG and pullulans polyelectrolyte expansion effect of AG was suppressed by using the aqueous salt solution as eluent. Figure 1 represents overlapped universal and primary calibrations for pullulan standards and larch AG after eliminating electrostatic effects. As shown in figure 1 point for AG lies on the curve $\lg (M[\eta])$ from retention time (left curve). For the construction of UC, we used the following constants of Mark-Kuhn-Houwink's equation for pullulans: $K_1 = 1.91 \cdot 10^{-4}$ dl/g and $a_1 = 0.67$; for AG: $M_2 = 42$ kDa [5] and $[\eta]_2 = 0.1$ dl/g (data from Megazyme, Ireland). For the determination of the molar mass of CMCh, the following constants of the Mark-Kuhn-Houwink equation were used: $K = 7.92 \cdot 10^{-5}$ dl/g and $a = 1$ [6].

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