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Physico-chemical properties of physiologically active polysaccharides from wheat tissue culture

Abstract: Polysaccharides (PS) from wheat cell culture were isolated by liquid-liquid extraction. The molecular mass distribution was determined by gel-permeation chromatography (GPC) using dual detectors for the simultaneous detection. It was supposed that PS sample from wheat cell culture has molecular weight of 1632 Da. The physic-chemical properties of PS such as solubility in different solvents, surface activity, ξ-potential, the pH value, polydispersity (PDI) were determined. The PS sample was soluble in water and insoluble in ethanol, acetone and chloroform. ξ-potential of PS was evaluated in order to determine its charge at different pH value from 3 to 9. As a result, the ξ values for the PS solution were negative throughout the pH range studied, varying from -2.85 mV (pH 3.0) to -21.1 (pH 9). Using tensiometry method, surface tension of the PS at the liquid/air interface was investigated. At 0.05% concentration interfacial tension decreases slowly and reaches an equilibrium value after ~ 8-8.5 hours. The pH was equal to 5.6±0.05. For a PS solution of 0.001% at pH 5.5 PDI was equal to 0.595.
Key words: polysaccharide, surface activity, average molecular weight

Introduction

All cell wall polysaccharides except cellulose are water-soluble compounds; their anchorage in the cell wall exists by use of different types of bonds [1]. The main monosaccharides that are part of the cell wall are: glucose, galactose, mannose, rhamnose and fucose, which contain six carbon atoms, as well as arabinose and xylose containing five carbon atoms. Common component of plant cell wall polysaccharides are uronic acids – modified sugars that have not closed in the ring – CH_2OH group is replaced by a carboxyl group – COOH. Most frequent uronic acids are presented by galacturonic acid, which is a derivative of galactose [2].

One of the main physic-chemical parameters characterizing a macromolecule – whether it is naturally occurring or synthetically produced – is its «molecular weight» [3].

Determination of the molecular weight of water-soluble polysaccharides is usually carried out by gel-permeation chromatography (GPC), which effectively allocates the molecules based on their hydrodynamic volume. GPC is used in carbohydrate research to determine molecular weight distributions of polysaccharides. The columns are calibrated using commercially available standards which are commonly dextrans or pullulans [5].

GPC of polysaccharides is often based on size exclusion mechanism: physical exclusion of molecules that are unable to penetrate the pore structure of the resin. Sample molecules with a size greater than the pore diameter of the support matrix cannot enter the pores. They are excluded and eluted rapidly from the column in the void volume. Molecules with a size smaller than the pore diameter enter the pores and elute differentially in volumes that are in size between the void volume and the void volume plus pore volume [6].

Alternative approaches to determine the molecular weight is the analysis of light scattering and viscometer [7].

Polysaccharides consisting of one type of sugar unit uniformly linked in linear chains are usually water insoluble even when the molecules have a low molecular weight with degrees of polymerization (DP) 20-30. Insolubility results from the fit of molecules and their preference for partial crystallization. An exception to the rule is in (1→6)-linked homoglycans, which because of the extra degrees of freedom provided by the rotation about the C-5 to C-6 bonds gives higher solution entropy values. Homoglycans

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with two types of sugar linkages or heteroglycans composed of two types of sugars are more soluble than purely homogeneous polymers. Ionized linear homoglycans are soluble but like all soluble linear polymers easily form gels because of segmental association which sometimes may be in a double helix formation. As these junction zones develop a stronger tertiary structure, gel hardness increases [8].

Zeta Potential analysis is a technique for determining the surface charge of particles in solution (colloids) [9]. ξ-potential has values that typically range from +100 mV to -100 mV. The magnitude of the zeta potential is predictive of the colloidal stability. Particles with zeta potential values greater than +25 mV or less than -25 mV typically have high degrees of stability. Dispersions with a low zeta potential value will eventually aggregate due to Van Der Waal inter-particle attractions [10].

Substances that capable to decrease the surface tension of the system (dy/dc< 0) are referred to as surface active substances (SAS), or surfactants [11]. It follows from the Gibbs equation that the adsorption of such compounds is positive, i.e. their concentration within the surface layer is higher than that in the bulk. For example, at air-water and water-hydrocarbon interfaces the surface active compounds are the ones containing hydrocarbon (non-polar) chain and a polar group (-OH, -COOH, -NH_2 etc.) in their structure. Such an asymmetric (diphilic) structure of surfactant molecules accounts for their similarity to the nature of both contacting phases: a well-hydrated polar group has the strong affinity towards the aqueous phase, while the hydrocarbon chain has the affinity towards the non-polar phase [12].

The aim of present work was to reveal physicchemical properties of extracellular polysaccharides (PS) isolated from wheat cell suspension culture, including determination of molecular weight, surface activity and zeta potential of total PS fraction.

Materials and methods

The molecular mass distribution was determined using size exclusion system at the Max Planck Institute of Colloids and Interfaces (Potsdam, Germany). The chromatograph was equipped with a reflective index (RI) detector (GE, Sweden) operating at 265 nm and two column in series, packed with Suprema 30 and Suprema 3000 (both from GE, Sweden. The range for Suprema 30 column is from 100-30000 Da, whereas Suprema 3000 column is suitable for range from 1000-3 000 000 Da. The analysis of molecular mass was carried out by this method [13]. In order to determine the molecular weight the polysaccharides samples were analyzed by gel-permeation chromatography (GPC) using dual detectors for the simultaneous detection. The system was calibrated with pullulan standards. A mixture of standard pullulans with different molecular weights (342, 1460, 5600-710 kDa) were dissolved in 0.1 N NaNO3 and applied on the same size exclusion Suprema 30 and Suprema 3000 columns and the same chromatography condition for the samples. After that, 7 mg of polysaccharide fraction was dissolved in 35 ml 0.1 N NaNO3 solution at a flow rate of 1.000 ml/min and then filtrated through 0.22 μ

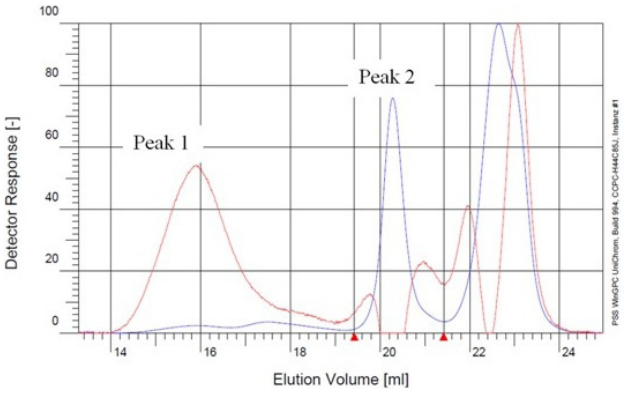

Figure 1 – The dependence of detector response on the elution volume

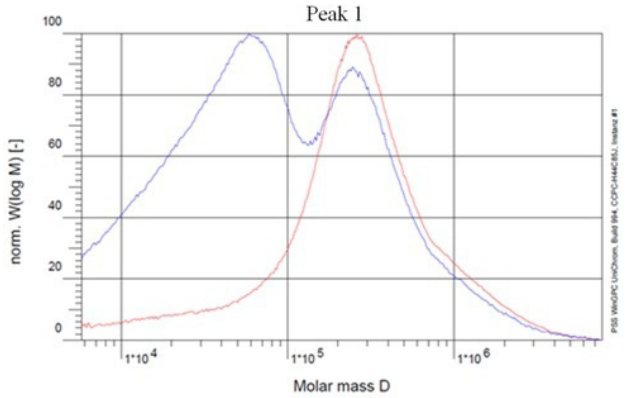

Figure 2 – The chromatogram of UV signal of PS sample

According to the literature review, proteoglycans consist of a core protein with one or more covalently attached glycosaminoglycan (GAG) chains. So, UV signal corresponding to the high molecular weight (430400 Da) can be explained by the structure of proteoglycan. If we reckoned in that polysaccharides can be detected only by RI detector

we can suppose that our PS sample has molecular weight of 1632 Da.

Determination of PS solubility and pH of PS solutions. The polysaccharide was soluble in water and insoluble in ethanol, acetone and chloroform. The PS sample was dissolved in distilled water in concentration of 1% (w/v) and measurements of pH value were conducted in three replications. As a result, it was found that pH of PS solutions was equal to 5.6±0.05.

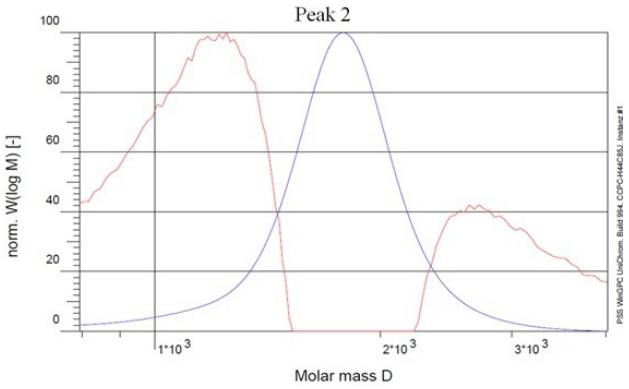

Figure 3 – The chromatogram of RI signal of PS sample

Determination of surface activity. The ability of the molecules of surfactants adsorb at interfaces is well known due to their amphiphilic structure. As a result of this process, the adsorption layers are formed, which in the equilibrium condition can be characterized by surface tension isotherms.

To clarify the characteristics of the formation of interfacial adsorption layers of PS solutions kinetic dependencies of the interfacial tension decrease at the time have been studied (Figure 4, 5). Figure 4 shows that the interfacial tension of the PS at 0.05% concentration decreases slowly and reaches an equilibrium value after ~ 8-8.5 hours. Duration of the interfacial tension decrease was due to the slow diffusion of the macromolecular coil to the surface, as well as conformational rearrangement of statistical balls` macromolecules on the surface of the active segment, which is reflected in the values of the relaxation time.

For the 1% solution the surface tension should be lower than for 0.05%. We have the opposite, which can be explained only by a certain multi-component composition of the substance, and consequently a completely different composition of the interfacial layer (Figure 5).

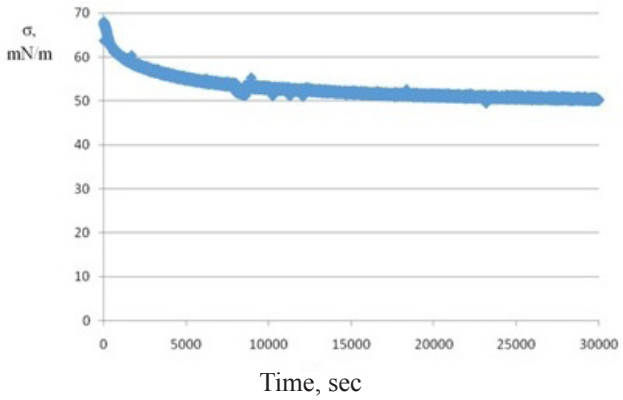

Figure 4 – Surface tension (σ, mN/m) as a function of time (s) for 0.05% PS solution as measured by dynamic drop volume method.

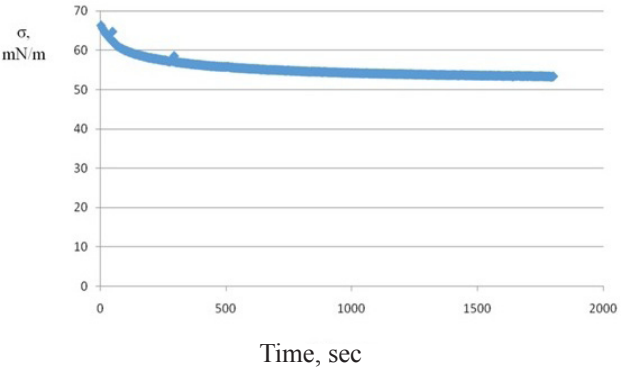

Figure 5 – Surface tension (σ, mN/m) as a function of time (s) for 1% PS solution as measured by dynamic drop volume method

According to the results obtained by tensiometry method, it was established that investigated cell culture polysaccharides have the features of surface-active substances (SAS), which characterized by capability to decrease the surface tension of solutions.

ξ-potential and dynamic light scattering. Electrostatic forces are usually the major driving force for the interaction of charged biopolymers in aqueous solutions, and so it was important to determine the electrical characteristics of the biopolymers used in this work. ξ-potential of PS was evaluated in order to determine the charge of their molecules at different pH value – from 3 to 9. The electronegativity of the solution increased with increasing pH from – 2, 85 mV (pH 3.0) to -21.1 (pH 9), respectively. At pH 3 ξ-potential value of PS was equal to -2.85±0.27

mV (Figure 6), thus showing that polysaccharide isolated from wheat is a practically neutral polysaccharide. The polysaccharides may be constituted either by polycations or by polyanions, depending on their functional group, and may also be neutral, which is the case of different types of polysaccharides with a higher content of mannose and galactose units. In neutral medium at pH 5.5 it was established that ξ-potential has higher negative value of -18.8±2.05 (Figure 7). At pH 9 the value of ξ-potential corresponded to the -21.7±0.55 (Figure 8).

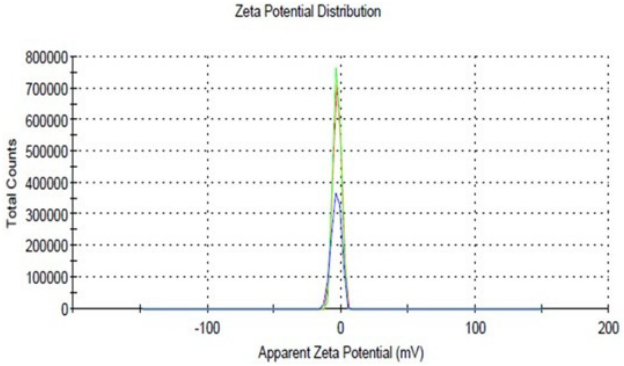

Figure 6 – Determination of ξ-potential at pH 3 value

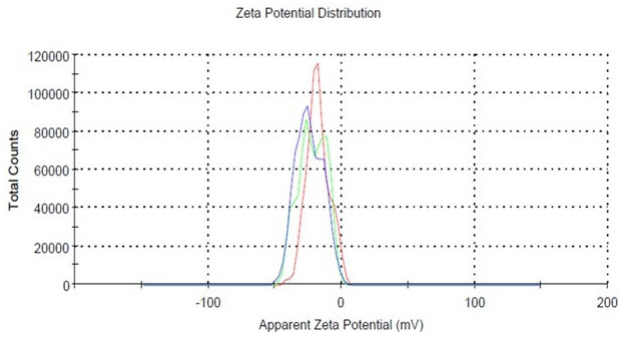

Figure 7 – Determination of ξ-potential at pH 5.5 value

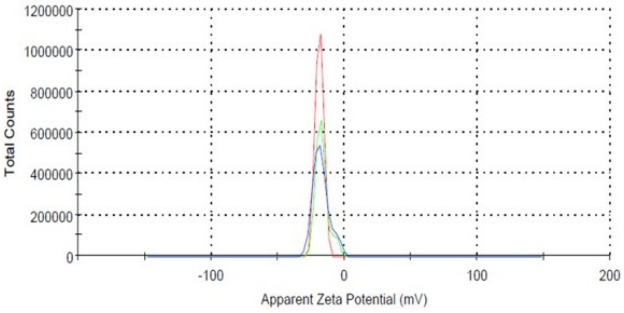

Figure 8 – Determination of ξ-potential at pH 9 value

The pH dependence of the zeta potential (ξ) for the PS solution is shown in Figure 9. The ξ values for the PS solution were negative throughout the pH range studied, varying from – 2.85 mV (pH 3.0) to -21,1 (pH 9). The increase of negative ξ values that occurred by the increase of pH values can be attributed to the ionization of the carboxylic moieties (–COOH) giving rise to carboxylate groups ($–COO^-$), while the decrease of negative ξ values at pH=3 value are due to the protonation of the amino moieties ($–NH_2$) giving rise to ammonium groups ($–NH_3^+$).

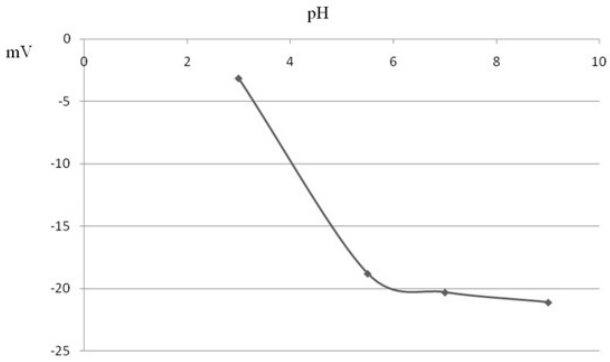

Figure 9 – Dependence of zeta potential (mV) of the PS solution with concentration of 0,001% on pH values ranging from 3 to 9

ξ-potential values are also related to the stability of solutions. As a general rule, absolute values of ξ-potential above 60mV indicate an excellent stability, from 60 to 30 mV are physically stable, from 30 mV to 5 mV are at the limit of stability and below 5 mV are not stable and there are aggregates can be formed. According to this general rule, the results obtained with at different pH show that PS solutions are stable.

The information obtained by ξ-potential and Dynamic Light Scattering (DLS) are crucial to indicate the occurrence of stable functional nanostructures. Polydispersion index (PDI), obtained by DLS is a measure of the size distribution width (Figure 10)

When polydispersity equals zero, the sample is monodisperse. Values of PDI close to or above 0.5 represent heterogeneous solutions in relation to the particle size and are characteristic of samples outside the standards. The term «particle» represents the molecule of polysaccharide, which stay disperses into diluted solution. For a PS solution of 0.001% at pH 5.5, Z-average and PDI were 926.8 and 0.595, respectively. It was established that PS sample is polydisperse.

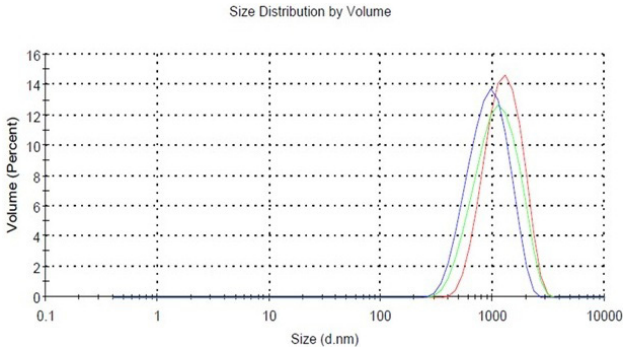

Figure 10 – The size distribution by volume

In conclusion, physic-chemical properties of extracellular polysaccharides from wheat cell culture have been determined for the first time: molecular weight, solubility in different solvents, the pH value, surface activity, ξ-potential, polydispersity (PDI).

It was revealed that total fraction of PS consists of high molecular weight proteoglycan (430400 Da) and low molecular weight glycan (1632 Da).

Investigated PS are soluble in water, insoluble in ethanol, acetone and chloroform; pH of 1% solutions is equal to 5.6±0.05.

Parameters of PS surface tension as well as dependency from concentrations of PS solution have been determined. Obtained results suggest that PS are high-molecular non-ionogenic compounds. It was shown that investigated PS have the characteristics of surface active substances.

Established dependence of ξ- potential from pH allow to assume the neutral nature of investigated polymers. Obtained results from dynamic light scattering (DLS) show the polydispersity of investigated PS.

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