ISSN 0965-545X, Polymer Science, Series A, 2022, Vol. 64, No. 4, pp. 277–289. © Pleiades Publishing, Ltd., 2022.
ISSN 0965-545X, Polymer Science, Series A, 2022. © Pleiades Publishing, Ltd., 2022.
Russian Text © The Author(s), 2022, published in Vysokomolekulyarnye Soedineniya, Seriya A, 2022, Vol. 64, No. 4, pp. 267–279.

MEDICAL POLYMERS

Investigation of the Binding of Lectins with Polymer Glycoconjugates and the Glycoconjugates Containing Silver Nanoparticles by Means of Optical Spectroscopy and Light Scattering

N. A. Samoilova[a],*, M. A. Krayukhina[a], O. V. Vyshivannaya[a], and I. V. Blagodatskikh[a]

[a] *Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, Moscow, 119991 Russia*

**e-mail: samoilova.nadezhda@gmail.com*

Received February 28, 2022; revised March 21, 2022; accepted March 28, 2022

Abstract—The synthesis of glycoconjugates, lectin-specific polymers containing a carbohydrate ligand (spacered residue of N-acetyl-D-glucosamine, β-N-Gly-GlcNAc) has been carried out. Glyconanoparticles (glycol-NPs) containing a label detectable by means of spectrophotometry, silver nanoparticles, have been prepared on the basis of the glycoconjugates. Copolymers of maleic anhydride with ethylene or *N*-vinylpyrrolidone have been used as a carrier to introduce the carbohydrate ligand and a stabilizer of silver nanoparticles. Solutions of the glycoconjugates and the silver glyconanoparticles have been characterized by means of light scattering, UV-visible spectroscopy, and TEM. The interaction of the obtained glycoconjugates and silver glyconanoparticles with N-acetyl-D-glucosamine-specific lectins of *Solanum tuberosum* agglutinin (STA) and wheat germ agglutinin (WGA) has been investigated by means of light scattering and UV-visible spectroscopy. The data obtained via these physical methods using the carbohydrate-containing derivatives labeled with silver nanoparticles have been in agreement. It has been shown that the glycoconjugates and silver glyconanoparticles based on more hydrophilic copolymer of maleic acid with *N*-vinylpyrrolidone are more sensitive than the respective systems based on more hydrophobic copolymer of maleic acid with ethylene. It has been also shown that the considered systems are more sensitive to the STA lectin than to the WGA lectin. The silver glyconanoparticles have allowed more accurate and reliable detection of the lectins by means of light scattering, as compared to the glycopolymer.

DOI: 10.1134/S0965545X22700092

INTRODUCTION

Lectins are proteins not assignable to the class of immune ones and bearing at least one domain for the recognition carbohydrates which specifically and reversibly binds to the carbohydrates of biological membranes of plant, prokaryotic, and eukaryotic cells, including these of higher organisms. Transduction of biological information via the carbohydrate-protein recognition is among the major ones at a cellular level. Lectins are involved in such vital biological processes as cellular adhesion, differentiation and migration of cells, insemination, embryogenesis, protection of an organism from infections, and many others [1, 2]. Lectins are resistant to proteolytic enzymes (including the intestinal ones) and thermally stable, they have been used as biochemical tools in immunology, biotechnology, and pharmaceutical industry [3, 4]. The carbohydrate-lectin interaction has been widely used in biosensors and proteomics. Analytical applications of lectins include histochemistry, cytochemistry, lectin blotting, and microplate analysis. Lectins can be used in the detection of cells and microorganisms, investigation of proteins glycosylation, and identification of glycans as well as characteristic of their structure [5, 6]. The application of lectins in cancer diagnostics and treatment has been recently reported [7]. Moreover, it has been recently demonstrated that plant lectins can be used as promising antiviral biomolecules in relation to the strategies of COVID-19 elimination [8]. It is known that dietary lectins from cereal products do not negatively affect human health. On the contrary, significant decrease in the risk if type II diabetes, cardiovascular diseases, and certain cancer types due to consumption of such products has been marked [9]. Study, isolation, and application of plant lectins have been developed over 130 years [10].

Several highly accurate but quite laborious methods are known for investigation of the lectin-carbohydrate interactions. Such methods include fluorescence-linked immunosorbent assay (FLISA) of lectins

[11], fluorescence spectroscopy [12], piezoelectric probing, electrochemical impedance spectroscopy, surface plasmon resonance (SPR) [13, 14], X-ray structure analysis [15], NMR [16], and other methods.

Polymeric derivatives of carbohydrates (glycoconjugates) have been often used as model compounds for the investigation of the carbohydrate-lectin interactions. The methods of controlled/"living" radical polymerization, click reactions, various types of block copolymerization, and other numerous approaches have been applied for the synthesis of such macromolecular structures [17–24]. Metal-labeled glycoconjugates have been often used in the lectins biosensors, in the investigation of the imitation of the erythrocytes behavior in agglutination, and in other studies. Gold nanoparticles have been mainly used for this purpose, due to their characteristics, especially plasmon resonance. Such glycoconjugates have been usually obtained from the corresponding carbohydrates derivatives (mainly thiolated ones) and the citrate-stabilized gold nanoparticles. The preparation of such labeled glycoconjugate involves several synthetic stages [25–29]. Elaboration of simple methods for the synthesis of model glycoconjugates and the development of sensitive and simple analytical instruments for the identification and detection of lectins have remained topical issues.

Herein we suggested to use the polymeric derivatives of spacered *N*-acetylglucosamine, exhibiting specificity towards a series of plant lectins, as the glycoconjugates. Copolymers of dicarboxylic (maleic) acid were used as the polymer matrix. Such polymeric glycoconjugates, bearing sufficient amount of the carbohydrate units, should provide multivalent binding with lectin. Since the interactions of lectin with carbohydrates are often fairly weak ($K_a = 10^2–10^3$ mol/L) [3], they could thus be significantly enhanced to provide the cluster effect. Most of the soluble lectins exhibit multimer structure with several sites of the carbohydrate binding, which allows the interaction with several carbohydrate ligands and significantly enhances the binding. The main advantages of the maleic acid copolymers in the context of our study is the regularity of the macromolecular chains structure and easy modification (in the form of maleic anhydride copolymers) with carbohydrate derivatives. Such polymers have been successfully used as stabilizers of silver nanoparticles [30, 31]. These nanocomposites can be used in the form of colloidal solutions as well as solid mixtures for prolonged storage. We suggested the use of specific polymeric glycoconjugates based on maleic anhydride copolymers for the investigation of the carbohydrate-lectin binding, either directly or upon the introduction of silver nanoparticles as labels in their structure, thus affording the glyconanoparticles. Silver nanoparticles can be prepared from cheaper and better available precursors in comparison with gold nanoparticles; moreover, they exhibit pronounced plasmon resonance band at 400–420 nm.

In this study, copolymers of maleic acid differing in the hydrophobic-hydrophilic balance were used as polymeric matrices for the preparation of the glycoconjugates and silver glyconanoparticles, whereas the complementary plant lectins: *Solanum tuberosum* agglutinin (STA) and wheat germ agglutinin (WGA) were used as model lectins. Owing to the surface plasmon resonance of the silver nanoparticles label, the carbohydrate-lectin interaction was investigated by means of optical spectroscopy of the lectin complexes with silver glyconanoparticles. The application of static and dynamic light scattering (SLS-DLS) allowed additional investigation of the specific complex formation involving non-labeled glycoconjugates in addition to the silver glyconanoparticles.

EXPERIMENTAL

Materials

The following alternating copolymers of maleic anhydride were used: with ethylene (EM) from Monsanto (USA), ($M_w = 2.5 \times 10^4$) and with *N*-vinylpyrrolidone (VM) obtained as described elsewhere [32] ($M_w = 4.0 \times 10^4$). Hydrolysis of EM and VM in aqueous systems afforded the corresponding copolymers of maleic acid: EMA and VMA. $NaBH_4$, P_2O_5, $AgNO_3$, NaCl, NaOH, $NaNO_3$ (all of the analytical pure grade) from Reakhim were used as received. Milli-Q water was obtained via deionization using a Milli-Q system (Millipore). The lectins: *Solanum tuberosum* agglutinin (STA) and wheat germ agglutinin (WGA) were purchased from Sigma-Aldrich. β-N-Gly-GlcNAc was kindly provided by L.M. Likhosherstov (IOC RAS).

Synthetic Procedures

Synthesis of glycoconjugates of the VMA (glyco-VMA) and EMA (glyco-EMA) copolymers. Activation of the copolymers (conversion into the anhydride form) was performed via vacuum drying over P_2O_5 at 110°C during 3 h.

Thirteen mg (0.047 mmol) of the *N*-acetylglucosamine-containing ligand (β-N-Gly-GlcNAc) was dissolved in 2 mL of H_2O adjusted to pH 9 with 1 M NaOH in H_2O. The activated VM copolymer (62.6 mg, 0.3 mmol) was then added at stirring, and the mixture was stirred during 24 h at 20°C. Completeness of the ligand binding was monitored by means of TLC (Silufol plates, acetone : ethanol : water = 1 : 1 : 5 by volume, development with 1% solution of ninhydrin in acetone), $Rf_{N\text{-}Gly\text{-}GlcNAc} = 0.3$, $Rf_{glyco\text{-}VM} = 0.1$). The obtained solution of glyco-VMA was used without isolation. Glyco-EMA was prepared similarly. The solution of glyco-EMA was freeze-dried for further use as powder.

Synthesis of sols containing silver nanoparticles. Synthesis of silver nanoparticles sols stabilized with VMA (VMA/Ag^0) and EMA (EMA/Ag^0). The VMA/Ag^0 sol was synthesized as described in [30]. To obtain the polymeric silver salt, 1 mL of freshly prepared 0.1 M solution of $AgNO_3$ was added at vigorous stirring to 10 mL of the VMA copolymer solution (0.01 eq/L) in water adjusted to pH 9 with 1 M NaOH (to the copolymer : Ag^+ molar ratio 1 : 1). After 30 min, freshly prepared solution of the reducing agent (0.1 M $NaBH_4$) was added at vigorous stirring in two-fold excess with respect to the silver ions. The reaction mixture was kept during 1 day at room temperature. To perform the elemental analysis, an aliquot of the solution was subject to dialysis and freeze-dried, whereas the sol was used without isolation in further experiments and to synthesize the silver glyconanoparticles. Colloidal solution of the nanosized silver EMA/Ag^0 was obtained similarly.

Synthesis of silver nanoparticles sols stabilized with glyco-VMA (glyco-VMA/Ag^0). A solution of glyco-VMA obtained as described earlier was diluted to concentration of 0.01 eq/L. 1 mL of freshly prepared solution of $AgNO_3$ was added at vigorous stirring to 10 mL of the glyco-VMA solution. After 30 min, freshly prepared solution of the reducing agent (0.1 M $NaBH_4$) was added at vigorous stirring to the solution of the polymeric salt of the glycoconjugate in two-fold excess with respect to the silver ions. The reaction mixture was kept during 1 day at room temperature. The so obtained sol was used without isolation (the sol pH 8.75).

Synthesis of silver nanoparticles sols stabilized with glyco-EMA (glyco-EMA/Ag^0). The glyco-EMA/Ag^0 sol was obtained similarly using a solution of glyco-EMA. Five mg (0.0317 mmol) of the glyco-EMA powder was dissolved in 5 mL of H_2O. Three tens mL of freshly prepared 0.1 M $AgNO_3$ solution was then added at stirring. After 30 min, freshly prepared solution of the reducing agent (0.1 M $NaBH_4$) was added at vigorous stirring to the solution of the polymeric salt of the glycoconjugate in two-fold excess with respect to the silver ions. The reaction mixture was kept during 1 day at room temperature. The so obtained sol was used without isolation (the sol pH 8.84).

Methods for the Specimens Analysis

The solutions pH was determined using a Five FE20 pH-meter (METTLER TOLEDO) equipped with a Micro Pro microelectrode, the measurement accuracy being ±0.02 pH units.

Optical spectra were recorded using a UVIKON-922 (BRD, Germany) or a PE-5400UF (Ekros, Russia) spectrophotometer in 1 cm glass cells (optical path 1 cm; spectral range 190–1000 nm). Optical spectra of the silver glyconanoparticles were recorded at their concentration of 0.05 mg/mL. The spectral changes were monitored upon addition of each portion of a lectin solution (c = 0.5 mg/mL). TEM images of the silver nanoparticles were obtained using a LEO 912 AB transmission electron microscope (OMEGA, Karl Zeiss, Germany) equipped with a magnetic omega spectrometer and an energy filter integrated directly in the optical system of the instrument: accelerating voltage of the electrons E = 100 kV, magnification of 80 to 500000, image resolution 0.2–0.34 nm. For the observation, a droplet of the solution was put on 3 mm formvar-coated copper grid and dried in vacuum. Size distribution of the silver nanoparticles was obtained via analysis of the images (at least 100 particles).

The SLS-DLS experiments were performed using a PhotoCor Complex spectrometer (Russia) equipped with an automated goniometer, a PhotoCor-PC2 pseudo cross-correlation counter, and a PhotoCor-FC single-plate multi-time real-time correlator. A Uniphase1135P He–Ne laser (λ = 633 nm, power 10 mW) was used as the light source. To investigate the particles structure, the scattering intensity and the scattering correlation functions were measured using dilute solutions over the 30°–140° scattering angle range (with 10° step) at 25°C. The titration curves were obtained measuring at the scattering angle 90°. Using the collected data, the plots of the reciprocal relaxation time as function of the wave vector were obtained to determine the diffusion coefficient; the hydrodynamic radius R_h was calculated using the Stokes–Einstein equation. The radius of gyration was determined from the Zimm plot. The specimens were prepared as follows: the VMA and EMA copolymers were dissolved in water (c = 2–3 mg/mL) at stirring during 2 h. The solution pH was adjusted with 0.1 M solution of NaOH. The EMA copolymer concentration equaled c = 2.0 mg/mL. The glyco-VMA solution was prepared via dilution of the reaction mixture to concentration of 1.5 or 3.0 mg/mL. The VMA/Ag^0 and EMA/Ag^0 sols were investigated using a solution in with concentration of c = 0.1 mg/mL adjusted to pH 9 with 0.1 M solution of NaOH. The glyco-VMA/Ag^0 and glyco-EMA/Ag^0 sols were prepared via dilution of the reaction mixture to concentration of 0.1 mg/mL. The prepared solutions were passed through a Durapore membrane filter (nominal pores size 0.22 μm) into an optical cell to remove dust and studied by means of light scattering. The specimens for UV-visible spectroscopy investigation were prepared similarly.

Titration of Glycoconjugates of the Maleic Acid Copolymers and the Silver Sols with Solutions of Lectins

To investigate by means of light scattering, a dust-free (passed through a Durapore membrane with nominal pores size 0.22 μm) sols of glyco-VMA/Ag^0, VMA/Ag^0, or glyco-EMA/Ag^0 with concentration of c = 0.1 mg/mL were put in an optical cell and titrated with a dust-free lectin solution (c = 0.5 mg/mL).

To do so, the titrant was added dropwise at stirring into the optical cell and the intensity of the light scattering at angle of 90° as well as the autocorrelation curve were measured. Using the obtained data, the titration curves were obtained as the relative scattering intensity I/I_0 and hydrodynamic radius as functions of the titrant : analyte volumetric ratio. Similar experiments were performed using glyco-VMA (c = 0.065 mg/mL) and glyco-EMA (c = 0.05 mg/mL).

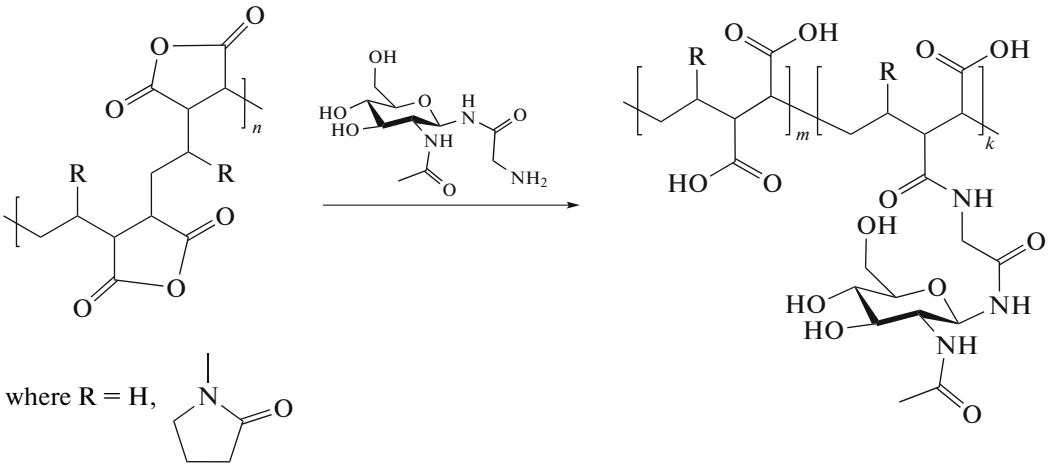
To investigate the silver glyconanoparticles interaction with lectins by means of UV-visible spectroscopy, the glyco-VMA/Ag^0 sol was adjusted to concentration c = 0.05 mg/mL (volume 1 mL, pH 8.87) to ensure the instrument operation in the linear regime and then titrated with a solution of the STA lectin (c = 0.5 mg/mL) at stirring on a magnetic stirrer. Upon addition of each portion, the changes in the absorption spectrum were monitored. The titration of glyco-EMA/Ag^0 with the lectin as well as of the considered glycoconjugates with the WGA lectin was performed similarly.

RESULTS AND DISCUSSION

Synthesis of Glycoconjugates of the Maleic Acid Copolymers and Silver Nanoparticles

The colloidal silver glyconanoparticles were prepared in two stages: synthesis of polymeric glycoconjugates and synthesis of the glycoconjugates containing silver nanoparticles.

Glycoconjugates of the copolymers were prepared from the carbohydrate-containing ligand β-N-Gly-GlcNAc, bearing the *N*-acetylglucosamine residue and a spacer moiety (glycine), and the VMA (or EMA) copolymer. Prior to the conjugation, the copolymers were activated via dehydration (heating in vacuum) for conversion of the maleic acid units formed due to hydrolysis during storage [33] into the active anhydride form. Conjugation of the β-N-Gly-GlcNAc ligand occurred via the following scheme:



The anhydride groups of maleic copolymer readily reacted with the primary amino group of the ligand to form the amide bond, without any additional condensation agents or organic solvents.

The obtained glycoconjugates contained about 10 mol % of the carbohydrate-modified units. It has been earlier stated that higher density of the modified units can reduce the binding ability of the glycoconjugates, i.e., the glycan valency has a threshold limiting the binding with lectin [34].

To obtain the silver glyconanoparticles, sodium borohydride was used as reducing agent in the presence of the glycoconjugate obtained at the previous stage, which served also as stabilizer of the silver nanoparticles. As was revealed by means of transmission electron microscopy, the applied procedure afforded small spherical silver nanoparticles with size of 2–4 nm (Fig. 1), owing to significant difference in the redox potentials of silver and the reducing agent.

Investigation of the Interaction of Silver Glyconanoparticles with Lectins by Means of UV-Visible Spectroscopy

To investigate the interaction between the silver glyconanoparticles with the lectins by means of UV-visible spectroscopy, the glyconanoparticles sol was titrated with the lectin solution in the range of the concentrations and the components ratios ensuring the instrument operation in the linear detection regime. The changes in the absorption spectrum were monitored upon addition of each portion of the titrant. It is known that specific interaction of silver nanoparticles with proteins leads to the decrease in the distance between the metal nanoparticles due to the aggregation induced by the interaction with the biomolecule, which leads to the change in the UV-visible spectrum owing to the interaction of the surface plasmons [35, 36].

Figure 2 displays typical evolution of the spectrum of the starting silver glyconanoparticles sol during its

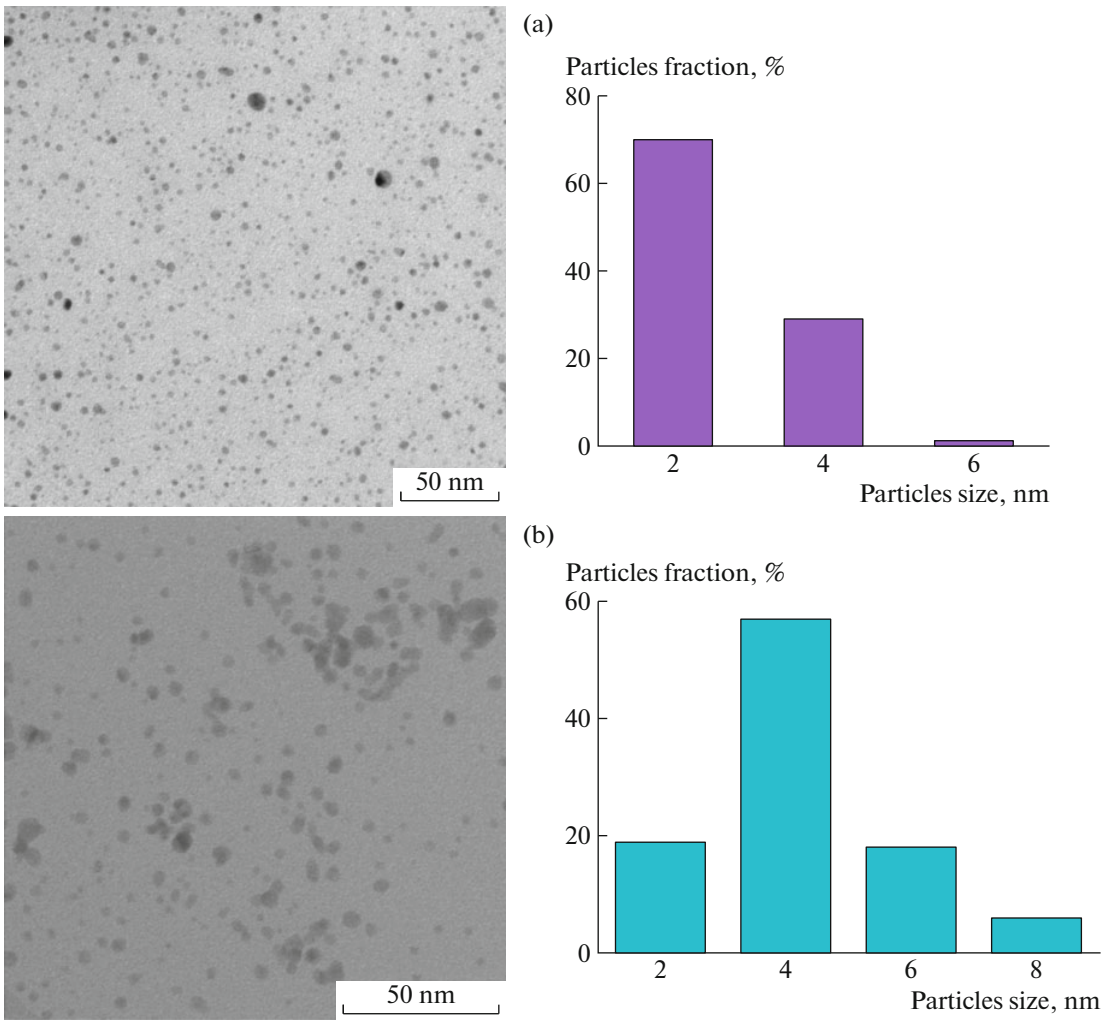


Fig. 1. The results of transmission electron microscopy study of the glyco-VMA/Ag^0 (a) and glyco-EMA/Ag^0 (b) samples. Color figures are available in the online version.

titration with the STA lectin solution, using the glyco-VMA/Ag^0 sample as the example. In that system, the spectrum was changed even at low concentration of the lectin (5–20 μL of STA). The observed shift of the absorption band maximum resulted from the interaction of the surface plasmons during the approach of the nanoparticles due to the carbohydrate-lectin interaction. Significant decrease in the peak height upon keeping the system during 1 h and especially 24 h was due to gradual precipitation of the gel-like sediment of the lectin–specific glycoconjugate complex.

The change in the position of the maximum in the absorption spectra of the glyco-VMA/Ag^0 sol, glyco-EMA/Ag^0 sol, and sol of silver nanoparticles containing no carbohydrate ligand (VMA/Ag^0) as function of the volume of the added STA lectin solution is shown in Fig. 3. The shape of the curves for the silver glyconanoparticles confirmed sufficiently high sensitivity to STA: even at the ratio of 0.2 mg of the protein/mg of silver glyconanoparticles (addition of 20 μL of the protein solution), the shift of the absorption maximum position by 8 nm (glyco-VMA/Ag^0) and 3 nm (glyco-EMA/Ag^0) was observed. For comparison, the test with the non-glycated sol was performed: the UV-visible spectra of the VMA/Ag^0 system were monitored during the titration with the STA solution. The position of the absorption maximum was found unchanged (Fig. 3, curve *3*) over the entire range of the probed protein concentration (up to 1 mg of the protein/mg of VMA/Ag^0). Hence, it was concluded that no specific interaction between the non-glycated silver nanoparticles and the lectin occurred**.** The obtained data on the change in the UV-visible spectra and the light scattering coincided with the earlier described properties of gold nanoparticles [37].

The interaction of the WGA lectin with the silver glyconanoparticles was investigated by means of optical microscopy as well (Fig. 4).

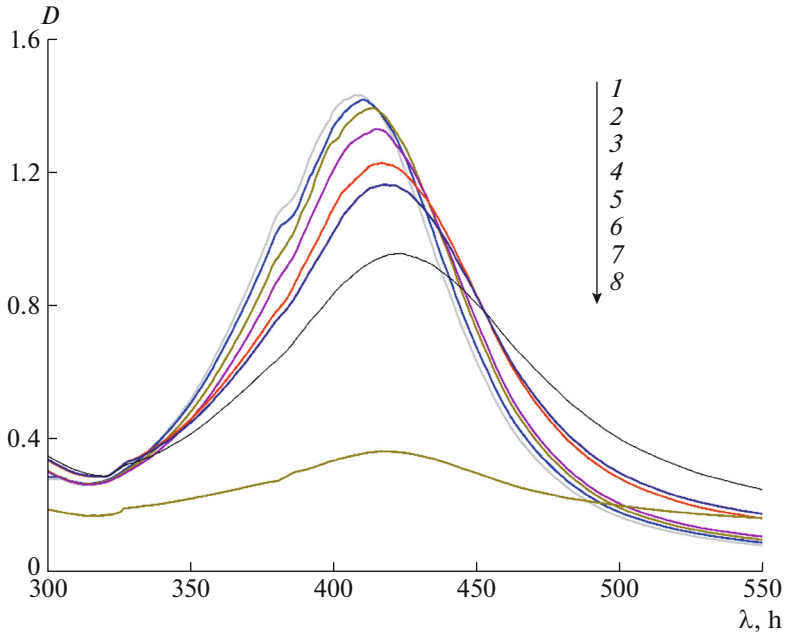


Fig. 2. Evolution of the UV-visible spectrum during the titration of the glyco-VMA/Ag^0 sol with the STA lectin solution. The amount of the added lectin 0 (*1*), 5 (*2*), 20 (*3*), 40 (*4*), 70 (*5*), and 90 μL (*6*); 90 μL upon 1 (*7*) and 24 h (*8*).

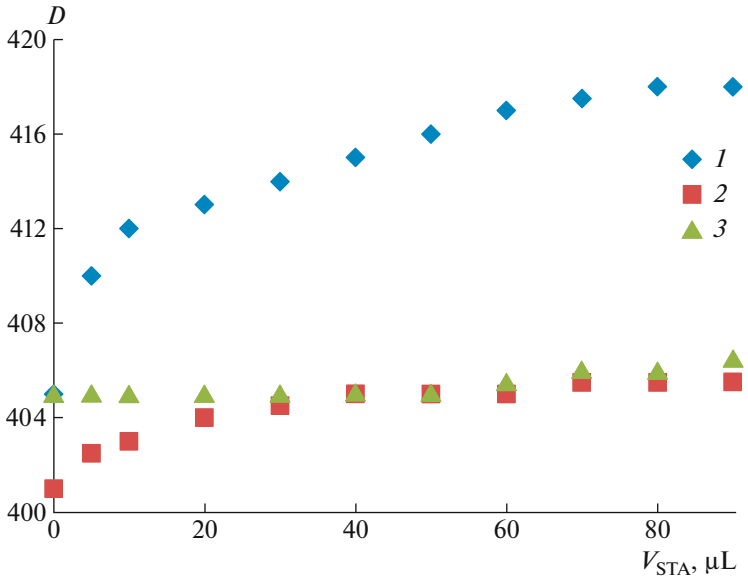


Fig. 3. Change in the absorption band maximum as function of the volume of the STA lectin solution added to glyco-VMA/Ag^0 (*1*), glyco-EMA/Ag^0 (*2*), and VMA/Ag^0 (*3*).

The titration of the glyco-VMA/Ag^0 with the WGA lectin was also accompanied by certain red shift in the absorption maximum (Fig. 4). The magnitude of the change was somewhat smaller in comparison with the interaction with the STA lectin: the addition of 0.2 mg of the protein/mg of silver nanoparticles (20 μL of the lectin solution), the shift of the maximum was as small as 2.5 nm in the case of glyco-VMA/Ag^0 and 1.5 nm in the case of glyco-EMA/Ag^0. The glyconanoparticles based on the more hydrophobic copolymer EMA revealed somewhat weaker change in the absorption spectrum during the interaction with the STA and WGA lectins, in comparison with the VMA-containing glyconanoparticles.

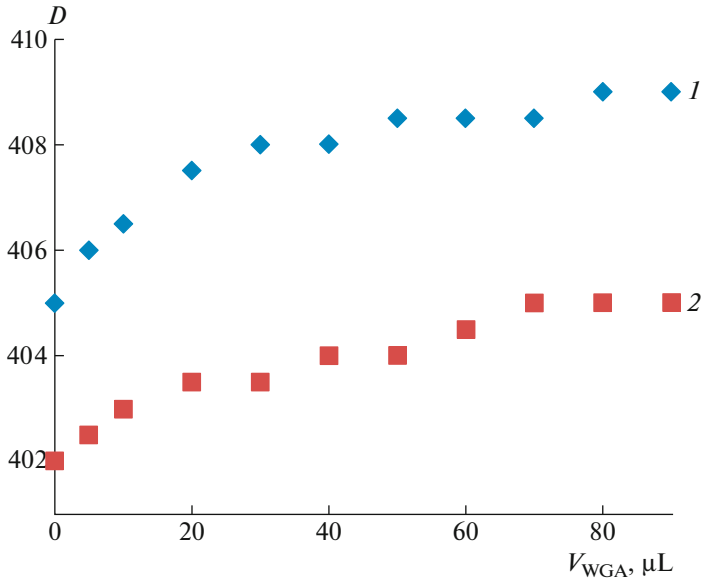


Fig. 4. Change in the absorption band maximum as function of the volume of the WGA lectin solution added to glyco-VMA/Ag^0 (*1*) and glyco-EMA/Ag^0 (*2*).

Light Scattering Method

To investigate the interaction of lectins with polymeric glycoconjugates and silver glyconanoparticles by means of SLS-DLS, we first characterized the solutions of the maleic acid copolymers, their glycoconjugates, the polymer-stabilized silver nanoparticles, and the silver glyconanoparticles via the same method.

Investigation of solutions of the maleic acid copolymers and their glycoconjugates. Investigation of the VMA copolymer and its glycoconjugate performed at different pH values revealed that the starting polymer was practically not aggregated in an alkaline medium (pH 9), whereas the decrease in pH led to the increase in the fraction and the size of the aggregates in the solution (Fig. S1 in the Supplementary Information). At the same time, the glyco-VMA was aggregated in neutral as well as alkaline medium. The size distributions of the VMA and glyco-VMA particles are compared in Fig. 5. The obtained data demonstrated that the introduction of the ligand in the VMA macromolecules enhanced the aggregation, which was likely due to increase in the macromolecule hydrophobicity and the tendency to form hydrogen bonds.

The structure of the glyco-VMA aggregates at two concentrations was assessed by means of SLS-DLS, measuring the scattering intensity and correlation functions at different scattering angles (Fig. S2 in the Supplementary Information). Table 1 lists the apparent (without extrapolation to zero concentration) values of the diffusion coefficient D, hydrodynamic radius R_h of the molecules and the aggregates, and gyration radius R_g and asymmetry factor R_g/R_h of the aggregates for the polymers and their glycoconjugates. The R_g/R_h values of about 2 evidenced the nonspherical shape of the aggregates and could correspond to the ellipsoidal or fractal structures [38]. Mass fraction of the aggregates in the solution could be estimated using the simplified models of Gaussian coil and fractal aggregate. The ratio of the light scattering intensities for those two types of the particles was $I_2/I_1 \approx c_2R_2^2/c_1R_1^2$. Using the respective values of R_h and the experimental $I_2/I_1 \approx 32$ data for the concentration of 1.5 mg/mL, we got $c_2/c_1 \approx 0.05$. Hence, the mass fraction of the aggregates was about 5%.

Investigation of the solutions of more hydrophobic copolymer EMA by means of DLS revealed that it was partially aggregated in the solution in acidic as well as alkaline medium [31]. The structure of the glyco-EMA solutions was similar to that of the starting EMA (Fig. 6), the critical aggregation concentration being below 0.05 mg/mL, in contrast to the glyco-VMA sample, the solutions of which used in the titration with lectins ($c = 0.065$ mol/L) did not reveal the presence of the aggregates.

The ionic strength significantly affected the hydrodynamic radius of the aggregates in the solution for the starting copolymers as well as their glycoconjugates: the addition of low-molecular salt led to the decrease in the observed R_h of the aggregates (Table 1) due to the shielding of the pendant charges on the chain, which is typical of the polymeric particles bearing a polyelectrolyte corona.

Investigation of nanosilver sols stabilized by the maleic acid copolymers and their glycoconjugates. We compared the sols of the polymer-stabilized silver

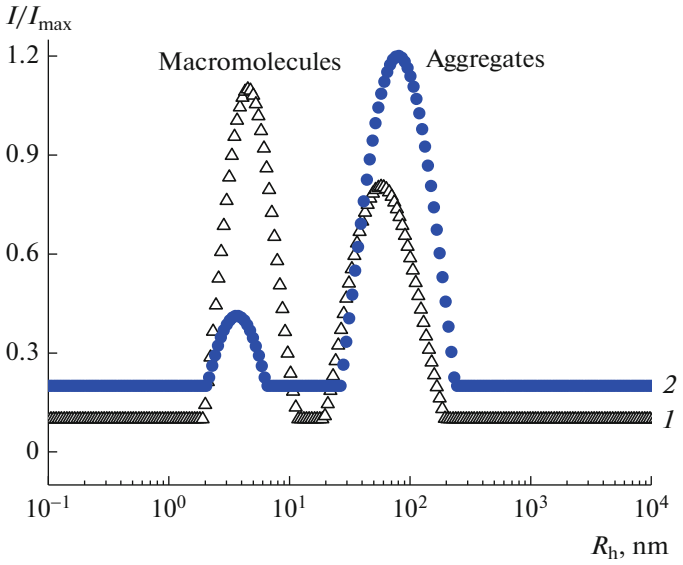


Fig. 5. Distribution of the scattering light intensity over the hydrodynamic radius in dilute solutions of VMA (c = 3 mg/mL, 0.05 M $NaNO_3$) (*1*) and glyco-VMA (c = 3 mg/mL, 0.05 M $NaNO_3$) (*2*).

nanoparticles and the glycosylated derivatives based on them in the cases of the VMA and EMA copolymers differing in the hydrophilicity, using the SLS-DLD data. The particles size distributions are shown in Figs. 7 and 8, respectively.

Earlier study of the silver nanoparticles stabilized by maleic acid copolymers [31] have revealed that the fast mode is assigned to the unimer polymer-stabilized silver nanoparticles and the slow mode corresponds to the clusters of polymer-stabilized silver nanoparticles forming inside the polymer aggregates during the reduction of silver salts. As in the case of the polymer aggregates, the clusters constitute a small fraction of the polymer-stabilized silver nanoparticles, yet giving rise to the prevailing part of the light scattering intensity. As seen from Fig. 7, the sols based on more hydrophobic polymer (EMA) revealed the presence of larger clusters. Stabilization of the metal nanoparticles in unimer micelles and clusters was achieved due to the set of structural-mechanical and electrostatic factors.

Figure 8 displays the hydrodynamic radius distributions of the sols of silver glyconanoparticles. Those sols typically revealed broad multimodal particles size distribution, the strongest maximum corresponding to the clusters with R_h = 45–60 nm. The multimodal nature of the distribution could be ascribed to the presence of the unimer silver nanoparticles and their clusters as well as to the additional manifestation of the inner modes due to internal interference in the clusters [39, 40].

Table 1. Results of the SLS-DLS investigation of the solutions of VMA, EMA, glyco-VMA, and glyco-EMA

Sample	$D_{macromol} \times 10^{-11}$, m^2/s	$D_{aggr} \times 10^{-12}$, m^2/s	$R_{h\ macromol}$, nm	$R_{h\ aggr}$, nm	$R_{g\ aggr}$, nm	R_g/R_h (aggr.)
VMA (pH 6.6 0.05 M $NaNO_3$), 2 mg/mL	5.30*	4.2*	4.6*	58*	–	–
glyco-VMA (0.05 M $NaNO_3$), 3 mg/mL	6.54 ± 0.12	3.29 ± 0.07	3.6 ± 0.2	72 ± 2	159 ± 8	2.21 ± 0.11
glyco-VMA (0.05 M $NaNO_3$), 1.5 mg/mL	5.8 ± 0.2	3.16 ± 0.07	4.0 ± 0.3	75 ± 2	140 ± 7	1.87 ± 0.09
glyco-VMA (H_2O), 3 mg/mL	10.2 ± 0.05	2.84 ± 0.06	2.4 ± 0.3	86 ± 2	164 ± 5	1.9 ± 0.1
glyco-VMA (H_2O), 1.5 mg/mL	9.7 ± 0.7	2.30 ± 0.07	2.4 ± 0.4	102 ± 3	169 ± 8	1.66 ± 0.08
EMA (pH 9), 2 mg/mL	–	–	2.8*	230*	–	–
EMA (pH 9, 0.05 M $NaNO_3$), 2 mg/mL	–	–	4*	80*	–	–
glyco-EMA (pH 9, 0.05 M $NaNO_3$), 0.05 mg/mL	–	–	4.4*	108*	–	–

*Measured at angle of 90°.

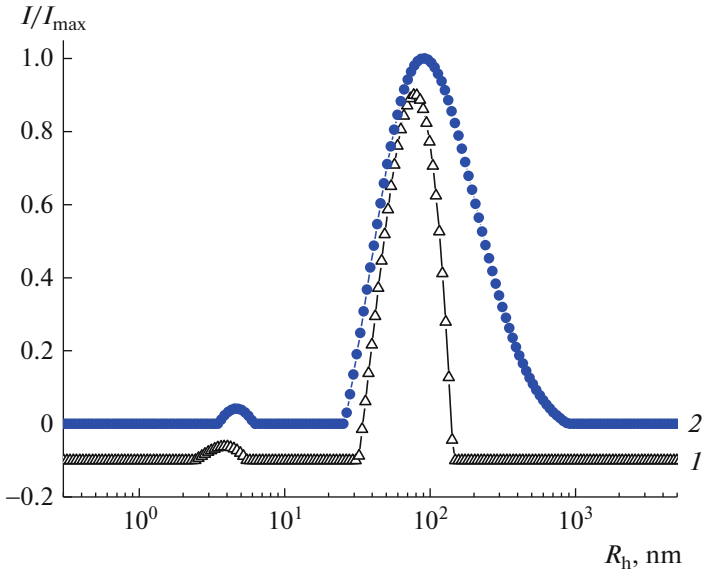


Fig. 6. Distribution of the scattering light intensity over the hydrodynamic radius in dilute solutions of EMA (c = 2 mg/mL, 0.05 M $NaNO_3$) (*1*) and glyco-EMA (c = 0.05 mg/mL, 0.05 M $NaNO_3$) (*2*).

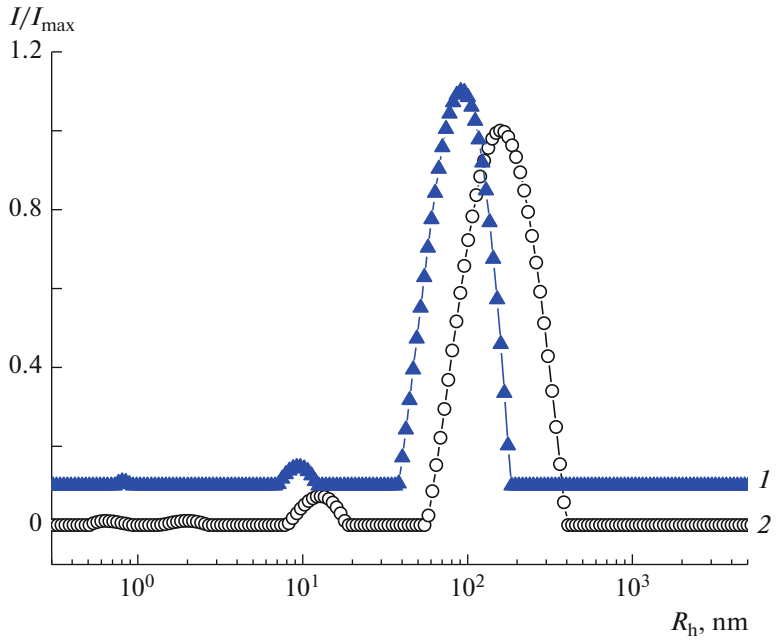


Fig. 7. Distributions of the scattering light intensity over the hydrodynamic radius in the VMA/Ag^0 (*1*) and EMA/Ag^0 (*2*) sols in water with addition of NaOH to pH 9. Scattering angle 90°, c = 0.1 mg/mL.

Investigation of the interactions of the glycoconjugates and silver glyconanoparticles with lectins. To investigate the interaction of the silver glyconanoparticles with the lectins by means of light scattering, we first performed the blank experiment: titration of the non-glycated silver sol VMA/Ag^0 with the STA lectin. The addition of the lectin up to the volumetric ratio 0.10 (the mass fraction 0.5) did not lead to the change in the scattering intensity within the limit of ±5% (Fig. S3 in the Supplementary Information). The same trend was observed regarding the particles size distribution. Hence, it could be concluded that there was no specific interaction between the non-glycated silver nanoparticles and the lectin over the probed concen-

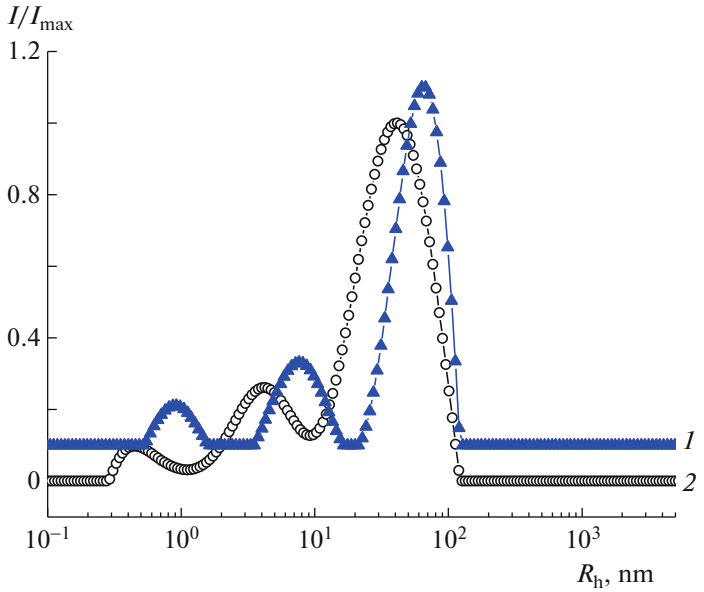


Fig. 8. Distributions of the scattering light intensity over the hydrodynamic radius in the glyco-VMA/Ag^0 (*1*) and glyco-EMA/Ag^0 (*2*) sols. Scattering angle 90°, c = 0.1 mg/mL; freshly prepared sols were diluted without isolation from the reaction mixture.

tration range according to the data of two physical methods: SLS-DLS and UV-visible spectroscopy. Further in this study, we monitored the binding of lectins with the glyconanoparticles at the protein/silver nanoparticles mass fraction not exceeding 0.5, to avoid the nonspecific interactions leading to aggregation. The interaction between the silver glyconanoparticles and the lectins was tracked by the change in the light scattering intensity during the titration with the lectin as well by the change in the size of the aggregates with the strongest contribution into the scattering or mean size of the particles (in the case of non-resolved multimodal distributions).

Figure 9 displays the data in the titration of the glyco-VMA/Ag^0 and glyco-EMA/Ag^0 with the STA lectin. It is to be seen that in the case of the glyco-VMA/Ag^0 sample the introduction of the minimal amount of the protein was accompanied by noticeable increase in the scattering intensity and the shift of the maximum in the particles size distribution. At the V(STA)/V(glyco-VMA/Ag^0) volumetric ratio of 0.02–0.05 (mass ratio 0.10–0.25), the maximum was shifted from 30 to 65–75 nm. The enhancement of the aggregation at that composition range could be attributed to the specific interaction between the silver glyconanoparticles and the complementary protein. Such behavior correlated with the changes in the UV-visible spectra due to the interaction of the surface plasmon during aggregation. Further addition of the protein led to the loss of the colloidal stability of the system, which resulted in the appearance of the micron-sized particles and gradual partial sedimentation, which was also observed by means of the UV-visible spectroscopy. In contrast to the silver glyconanoparticles based on the VMA copolymer, the glyco-EMA/Ag^0 sol revealed weaker reaction on the addition of the STA lectin (Fig. 9a, curve *2*). According to the data from the UV-visible spectroscopy, the response of those silver glyconanoparticles was also weaker than in the case of the glyco-VMA/Ag^0 sample.

To compare the response of different silver glyconanoparticles and the glycoconjugates on the STA and WGA lectins, we performed the corresponding titrations. Table 2 shows the change in the scattering intensity at the lectin : silver glyconanoparticles (or glycopolymer) volumetric ratio equal to 0.02, in comparison with the starting solution of the silver glyconanoparticles or the glycopolymer. Titration of the silver glyconanoparticles sols with the WGA lectin revealed the same trend: the response to the addition of the minimal amount of the lectin (volumetric ratio 0.02–0.05), the increase in the intensity by 1.5 times and the shift of the maximum in the particles size distribution from 65 to 90 nm was observed in the case of the glyco-VMA/Ag^0 (Fig. S6 in the Supplementary Information), whereas almost no changes were observed when using the glyco-EMA/Ag^0 sample (Fig. S5 in the Supplementary Information). Those data also coincided with the UV-visible spectroscopy results.

Titration of the solutions of the polymeric glycoconjugates containing no silver nanoparticles was performed at the polymer concentrations corresponding to its amount in the glyconanoparticles solutions with

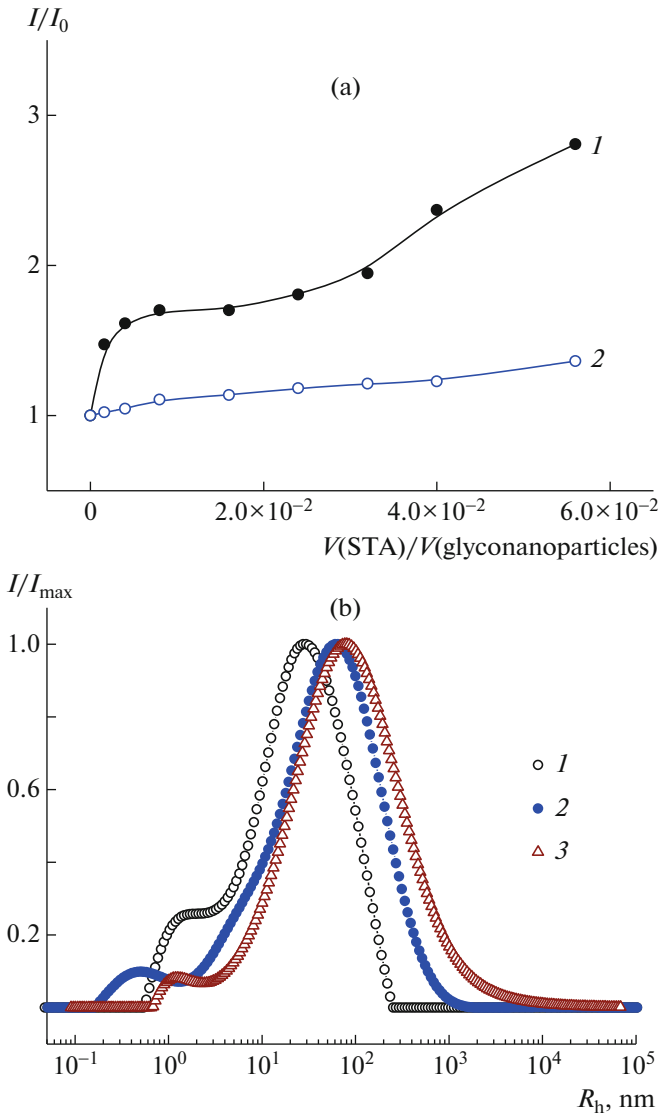


Fig. 9. Change in the scattering light intensity during titration of the glyco-VMA/Ag^0 (*1*) and glyco-EMA/Ag^0 (*2*) sols with the STA lectin solution STA (a) and the change in the particles size distribution at V(STA)/V(glyco-VMA/Ag^0) = 0 (*1*), 0.02 (*2*), and 0.05 (*3*) (b).

concentration of 0.050–0.065 mg/mL. The scattering intensity and the particles size were practically not changed in the course of the titration of the glyco-EMA with the WGA lectin (Fig. S8 in the Supplementary Information), whereas significant increase in the scattering intensity was observed during the titration with the STA lectin, but the change in the size distribution curve was mainly due to the appearance of large micron-sized particles (Fig. S7 in the Supplementary Information). It could be suggested that the interaction of the polymeric glycoconjugates with larger STA macromolecules led to fast loss of the stability of the colloidal particles.

As marked above, the glyco-VMA solutions revealed no aggregation and low scattering intensity, which did not allow analysis of the change in the particles size with the addition of lectin, although certain increase in the scattering intensity was noticed (Fig. S9 in the Supplementary Information).

Hence, the performed study demonstrated that the glyconanoparticles containing silver nanoparticles could be used in the detection of the STA and WGA lectins by means of light scattering with better accuracy and reliability as compared with the corresponding glycopolymer.

CONCLUSION

Glycoconjugates of the maleic acid copolymers with ethylene and *N*-vinylpyrrolidone, specific to certain plant lectins, were prepared. The obtained glyco-

Table 2. Change in the scattering light intensity I/I_0 upon addition of the lectin solution to the silver glyconanoparticles or polymeric glycoconjugates solutions (volumetric ratio 0.02)

Sample	I/I_0 upon addition of lectin	
	STA	WGA
glyco-VMA/Ag^0	1.8	1.5
glyco-EMA/Ag^0	1.2	1.1
glyco-VMA	2.6	2.1
glyco-EMA	2.3	1.1

conjugates were used as stabilizers of the plasmon resonance silver nanoparticles.

Structure of the solutions of the glycoconjugates of the maleic acid copolymers and the glyconanoparticles were investigated by means of light scattering. It was shown that the glycoconjugate solutions revealed stronger tendency to aggregation than the starting polymers. Sols of the silver nanoparticles and the non-modified polymer-stabilized nanoparticles contained the unimer particles and the nanoparticles clusters.

The interaction of the obtained glycoconjugates and the glyconanoparticles with β-N-acetyl-D-glucosamine-specific lectins STA and WGA was investigated by means of light scattering and UV-visible spectroscopy. It was shown that those methods were sensitive to the carbohydrate-lectin interaction and were in line with each other when using the carbohydrate-containing derivatives labeled with the silver nanoparticles.

The glyconanoparticles containing the nanosilver particles allowed the detection of the STA and WGA lectins by means of light scattering with better accuracy and reliability in comparison with the glycopolymer. In contrast to the silver glyconanoparticles, the use of the glycoconjugate without the nanosilver plasmon resonance label did not allow the use of UV-visible spectroscopy as the analytical tool. It was shown that the sensitivity of the glycoconjugates and the silver nanoparticles based on more hydrophilic copolymer of maleic acid with *N*-vinylpyrrolidone was higher in comparison with the corresponding systems based on more hydrophobic copolymer of maleic acid with ethylene. It was also demonstrated that the studied systems revealed higher sensitivity to the STA lectin than to the WGA lectin. The data obtained via two physical methods were in line with each other. The obtained results led to a conclusion that the glyconanoparticles based on the VMA copolymer could serve as the basis for the sensor systems for the STA and WGA lectin exploiting the light scattering and/or UV-visible spectroscopy methods.

ACKNOWLEDGMENTS

The authors are grateful to S.S. Abramchuk for TEM experiments. This study was performed using the equipment of the Center for the Study of Molecules Structure, Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences.

FUNDING

This study was financially supported by the Ministry of Science and Higher Education of the Russian Federation.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at https://doi.org/10.1134/S0965545X22700092.

REFERENCES

1. A. M. Wu, E. Lisowska, M. Duk, and Z. Yang, Glycoconjugate J. **26**, 899 (2008).
2. X. Dan, W. Liu, and T. B. Ng, Med. Res. Rev. **36** (2), 221 (2016).
3. I. E. Liener, N. Sharon, and I. J. Goldstein, *The Lectins: Properties, Functions, and Applications in Biology and Medicine* (Acad. Press, New York, 1986).
4. M. A. Hassan, Int. J. Mol. Sci. **16** (4), 7802 (2015).
5. A. Vojdani, Altern. Ther. Health Med. **21** (1), 46 (2015).
6. O. D. Hendrickson and A. V. Zherdev, Critic. Rev. Anal. Chem. **48** (4), 279 (2018).
7. A. Gupta, Mater. Today: Proc. **31** (2), 651 (2020).
8. Md. N. Ahmed, R. Jahan, V. Nissapatorn, M. Wilairatana, and M. Rahmatullah, Biomed. Pharmacother. **146**, 112507 (2022).
9. V. J. Buul and F. Brouns, J. Cereal Sci. **59** (2), 112 (2014).
10. M. Tsaneva and E. J. M. Van Damme, Glycoconjugate J. **37**, 533 (2020).
11. A. M. Wu and J. H. Liu, Glycoconjugate J. **36**, 175 (2019).
12. K. Kakehi, Y. Oda, and M. Kinoshita, Anal. Biochem. **297** (2), 111 (2001).
13. E. Mahon, Z. Mouline, M. Silion, A. Gilles, M. Pinteala, and M. Barboiu, Chem. Commun. **49**, 3004 (2013).
14. Y. E. Tsvetkov, M. Burg-Roderfeld, G. Loers, A. Arda, E. V. Sukhova, E. A. Khatuntseva, A. A. Grachev, A. O. Chizhov, H.-C. Siebert, M. Schachner, J. Jimenez-Barbero, and N. E. Nifantiev, J. Am. Chem. Soc. **134** (1), 426 (2012).
15. R. A. Palmer and H. Niwa, Biochem. Soc. Trans. **31**, 973 (2003).
16. M. C. Fernández-Alonso, D. Díaz, M. Á. Berbis, F. Marcelo, J. Cañada, and J. Jiménez-Barbero, Curr. Protein Pept. Sci. **13** (8), 816 (2012).

17. K. Godula and C. R. Bertozzi, J. Am. Chem. Soc. **132** (39), 9963 (2010).
18. W. Wang, D. Chance, V. Mossine, and T. Mawhinney, Glycoconjugate J. **31**, 133 (2014).
19. G. Yilmaz and C. R. Becer, Front. Bioeng. Biotechnol. **2**, 39 (2014).
20. D. Pati, A. Y. Shaikh, S. Das, P. K. Nareddy, M. J. Swamy, S. Hotha, and S. S. Gupta, Biomacromolecules **13** (5), 1287 (2012).
21. A. L. Parry, N. A. Clemson, J. Ellis, S. S. R. Bernhard, B. G. Davis, and N. R. Cameron, J. Am. Chem. Soc. **135** (25), 9362 (2013).
22. Q. Zhang, A. Anastasaki, G.-Z. Li, A. J. Haddleton, P. Wilson, and D. M. Haddleton, Polym. Chem. **5**, 3876 (2014).
23. S.-J. Richards, L. Otten, and M. J. Gibson, J. Mater. Chem. B **4** (18), 3046 (2016).
24. P. Sun, ACS Macro Lett. **3** (1), 96 (2013).
25. V. E. Piskarev, V. E. Lutsik-Kordovskii, E. L. Piskareva, and I. A. Yamskov, Appl. Biochem. Microbiol. **39** (5), 512 (2003).
26. M. Toyoshima and Y. Miura, J. Polym. Sci., Part A: Polym. Chem. **47** (5), 1412 (2009).
27. M. Marradi, F. Chiodo, I. Garcia, and S. Penades, Chem. Soc. Rev. **42**, 4728 (2013).
28. A. J. Reynolds, A. H. Haines, and D. A. Russel, Langmuir **22** (3), 1156 (2006).
29. X. Le Gueevel, ACS Appl. Mater. Interfaces **7** (37), 20945 (2015).
30. N. Samoilova, E. Kurskaya, M. Krayukhina, A. Askadsky, and I. Yamskov, J. Phys. Chem. B **113** (11), 3395 (2009).
31. N. Samoilova, I. Blagodatskikh, E. Kurskaya, M. Krayukhina, O. Vyshivannaya, S. Abramchuk, A. Askadskii, and I. Yamskov, Colloid. J. **75** (4), 409 (2013).
32. A. Conix and G. Smets, J. Polym. Sci. **15** (79), 221 (1955).
33. N. Samoilova, M. Krayukhina, A. Naumkin, and I. Yamskov, Monatsh. Chem. **149**, 1179 (2018).
34. K. Godula and C. R. Bertozzi, J. Am. Chem. Soc. **134** (38), 15732 (2012).
35. S. Ashrafpour and T. T. Moghadam, Surf. Interfaces **10**, 216 (2018).
36. S. S. Khan, P. Srivatsan, N. Vaishnavi, A. Mukherjee, and N. Chandrasekaran, J. Hazard. Mater. **192** (1), 299 (2011).
37. M. Toyoshima and Y. Miura, J. Polym. Sci., Part A: Polym. Chem. **47** (5), 1412 (2009).
38. W. Burchard, *Branched Polymers II* (Springer, Berlin; Heidelberg, 1999).
39. P. Stepanek, in *Dynamic Light Scattering, The Method and Some Applications*, Ed. by W. Droun (Clarendron Press, Oxford, 1993).
40. V. A. Bloomfield, Biopolymers **54**, 168 (2000).

Translated by E. Karpushkin