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Hydrophilic Microspheres Containing α-*tert* Butoxy-ω-vinylbenzyl-polyglycidol for Immunodiagnostics: Synthesis, Properties and Biomedical Applications

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Summary: Synthesis, characteristics and medical diagnostic application of core-shell poly(styrene/α-*tert*-butoxy-ω-(vinylbenzyl)polyglycidol) (P(S/PGL)) microspheres are described. The particles were prepared by soap-free emulsion polymerization of styrene and α-*tert*-butoxy-ω-(vinylbenzyl)polyglycidol macromonomer (PGL) initiated with potassium persulfate. The polymerization in water yielded microspheres with diameters in the range from 220 to 650 nm, depending on concentration of the macromonomer. Polydispersity was usually below 1.06. The fraction of PGL in interfacial layer of microspheres ranged from 0 to 42 mol.% (estimated by XPS). Microspheres suspended in NaCl solutions manifested fully reversible swelling-deswelling properties of their interfacial layer with characteristic transition temperature (T_t) slightly dependent on NaCl concentration. The adsorption of human serum albumin on the surface of P(S/PGL) microspheres was highly reduced when the PGL surface fraction exceeded 40 mol.%. P(S/PGL) microspheres with immobilized antigens *Helicobacter pylori* were used for detection of antibodies against. The test was based on monitoring differences in electrophoretic mobility of P(S/PGL) microspheres covalently bound *H. pylori* antigens and antibodies against *H. pylori* in blood serum.

Keywords: aggregation test; hydrophilic microspheres; immobilized proteins

Introduction

Water is the main component of biosystems; thus, all kinds of interactions (ligand-receptor, antigen-antibody, enzyme-substrate, etc.) which occur in living organisms take place in water environment. Therefore, the nano- and microspheres designed for detection of bioreagents (ligands, antigens, antibodies) should be hydrophilic and should mimic living cells with respect to the presence of specific receptors of biomolecules. The particles designed for medical diagnostics should be also monodisperse.

A few years ago, we elaborated a synthesis of microspheres with hydrophilic interfacial layer rich in α-*tert*-butoxy-ω-(vinylbenzyl)polyglycidol (PGL). The main chains of PGL and poly(ethylene oxide) are similar, but PGL contains additionally hydroxymethyl groups in each repeating unit. The hydroxy groups at the surface of microspheres allow for efficient binding of biomolecules.

Surface properties of polymer particles designed for biomedical applications are very important. It is known that their surface should be highly hydrophilic and the molecules in hydrophilic layer should be mobile. These properties stimulate efficient elimination of adventitious protein adsorption. It is known that hydrophilic surfaces containing poly(ethylene oxide) are protein repellents mostly for entropic reasons.[1]

Diagnostic tests are based on monitoring effects of specific binding of such bioreagent pairs such as antigen/antibody, ligand/receptor, DNA – complementary DNA

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strand, which occurs in an aqueous medium. Typically, one component is covalently bound to polymersupport (e.g. nano- or microspheres). Usually, the above interactions between biomacromolecules (known as agglutination) result in particles aggregation. The aggregation can be observed even by naked eye or determined quantitatively by techniques like turbidimetry or nephelometry,[2] Coulter counter[3] and flow cytometry.[4,5] The degree of aggregation is quantified by determination of size distribution of particles and their aggregates.[6]

Here, we briefly present a new method of detection of antibodies against *Helicobacter pylori*. The method is based on changes in hydrodynamic diameter and electrophoretic mobility of microspheres resulting from interactions of *H. pylori* antigens immobilized on microspheres with antibodies against *H. pylori* in blood sera.

Materials and Methods

Synthesis of α-*tert*-Butoxy-ω-(vinylbenzyl)polyglycidol (PGL) Macromonomer

The procedure for synthesis of macromonomer is described elsewhere.[7] Briefly, PGL was obtained by anionic polymerization of 2-ethoxyethyl glycidyl ether, initiated with potassium *tert*-butoxide and terminated with 4-(chloromethyl)styrene. The hydrolysis of ethylethoxy blocking groups in the presence of oxalic acid and subsequent neutralization with $Ca(OH)_2$ resulted in water-soluble PGL macromonomer.[8] The macromonomer was characterized by ^{1}H NMR, ^{13}C NMR and GPC. Molecular weight of PGL used in this work was $M_n = 2700$ ($M_w/M_n = 1.07$) and $M_n = 2800$ ($M_w/M_n = 1.05$).

Synthesis of Poly[styrene/α-*tert*-butoxy-ω-(vinylbenzyl)polyglycidol] Microspheres P(S/PGL)

Components: styrene (10 g), α-*tert*-butoxy-ω-(vinylbenzyl)polyglycidol macromonomer ($M_n = 2700$, $M_w/M_n = 1.07$ or $M_n = 2800$, $M_w/M_n = 1.05$) (PGL) (0-1.0 g), $K_2S_2O_8$ (0.22 g), triple distilled water (120 ml).

Procedure: The comprehensive description of synthesis of P(S/PGL) particles was described in our earlier paper.[9] The reactor was charged with triple distilled water, styrene and macromonomer. The mixture was purged with argon for 1 h. Polymerization was carried out for 28 h at 65 °C with stirring (600 rpm). A suspension of synthesized microspheres was purified by five times repeating the sequence of steps comprising centrifugation, decantation and resuspension in distilled water.

Diameters of microspheres were determined by analysis of scanning electron microscopy (SEM) microphotographs recorded using a JEOL 5500LV apparatus. The number-average diameter of microspheres (D_n) and dispersity parameter (D_w/D_n) were calculated by sampling randomly at least 700 microspheres.

Determination of Surface Concentration of Acid Groups

The concentration of -OSO_3^- anions in the interfacial layer of microspheres (anions formed as end-groups in initiation with K_2SO_8) were determined by conductometric titration with KOH.[9]

Determination of Hydrodynamic Diameter of P(S/PGL) Microspheres (D_h)

Hydrodynamic diameter of P(S/PGL) microspheres was determined by photon correlation spectroscopy measurements (PCS) using a Zetasizer 3000 HSa apparatus (Malvern Instruments) equipped with a He-Ne laser emitting light at 632.8 nm and detector recording the intensity of light scattered at 90°. The hydrodynamic diameters of P(S/PGL) microspheres were measured at different temperatures, and at different concentrations of NaCl. Samples of P(S/PGL) microspheres were suspended in water or water containing NaCl. The suspensions of microspheres were equilibrated for 3 hours prior to measurements. The hydrodynamic diameter (D_h) of particles was evaluated from the determination of diffusion coefficient of spherical particles

measured by PCS and calculated according to the Einstein-Stokes equation (1):

$$D = kT/3\pi\eta D_h \quad (1)$$

where D denotes diffusion coefficient, D_h – hydrodynamic diameter of microspheres, k – Boltzmann constant, T- temperature and η - viscosity of the medium. The data were analyzed using a cumulant method on the basis of 60 measurements for each sample at a given temperature and concentration of NaCl. According to ISO13321, the cumulant method is recommended for calculation of hydrodynamic diameters of spherical particles with low polydispersity.

Determination of Electrophoretic Mobilities (μ) of P(S/PGL) Microspheres

Electrophoretic mobilities (μ) of P(S/PGL) microspheres with attached *Helicobacter pylori* antigens were measured in a cell equipped with electrodes to which a controlled potential (up to 400 V) was applied. Measurements were carried on using a Zetasizer 3000 HSa (Malvern Instruments). The electrophoretic mobility measurements were performed at room temperature with 6-ml samples of suspensions of microspheres with bound protein. The average of the results of 10 measurements recorded at a required number of counts (usually cca 1500 kCps) was used for analysis.

Determination of Chemical Composition of Interfacial Layer of P(S/PGL) Microspheres

Chemical composition of interfacial layer of P(S/PGL) microspheres was based on analysis of X-ray photoelectron spectra recorded for dry particles (XPS method). The spectra were recorded using a Thermo VG Scientific Escalab 250 system equipped with a monochromatic Al-Kα X-ray source (1486.6 eV) and a magnetic lens which increases the sensitivity. The atomic ratio of carbon and oxygen atoms was calculated from the ratio of the intensities of corresponding XPS signals (for carbon atoms in the range from 285 to 291.6 eV, including a shake-up signal due to the polystyrene aromatic rings, for oxygen atoms at 532 eV) corrected for the relevant sensitivity factors. Taking into account that each styrene monomeric unit contains 8 carbon atoms and there are three carbon and two oxygen atoms the glycidol unit, the mole fraction of polyglycidol and polystyrene could be found from the relative carbon and oxygen atom contents.[9]

Determination of Surface Concentration of Proteins Immobilized on P(S/PGL) Microspheres

The procedures used for studies of protein immobilization (adsorption on the surface of P(S/PGL) microspheres and covalent immobilization after modification of hydroxy groups of PGL) were described in our earlier papers.[10,11] Generally, the experiments consisted of incubation of a protein solution (human serum albumin or *Helicobacter pylori* antigen) and suspension of microspheres in a buffered medium (phosphate buffered saline PBS, with pH 7.4, $I = 0.2$ M). Incubation was carried out at room temperature for 24 h. The amount of attached protein was determined as the difference of the initial and final (after incubation of protein) concentration in solution. Covalent immobilization of protein onto P(S/PGL) microspheres required activation of hydroxy groups. The detailed recipe was described earlier.[12] Briefly, 1,3,5–trichlorotriazine (TCT, 0.18 g) was added to 8 ml of aqueous suspension containing 0.4 g of P(S/PGL) particles. After 12-h incubation at room temperature, microspheres were isolated by centrifugation and resuspended in triple distilled water. Centrifugation and resuspension were repeated 4 times in order to remove unbound TCT. Then, the protein (human serum albumin, *H. pylori* antigen, etc.) was covalently immobilized onto activated microspheres. Usually, to suspension of microspheres (3×10^{-3} g/ml), a protein solution (in phosphate buffered saline (PBS, pH 7.4, $I = 0.2$ M) was added. The samples were incubated overnight at room temperature and then, the unbound protein was removed by centrifugation. The surface concentrations of attached proteins

were determined according to the procedures established earlier.[10,11] The amounts of proteins attached onto microspheres were determined either by measuring the protein contents in solution before and after incubation or directly on microspheres by the modified Lowry method.[13,14] A diode array Hewlett-Packard 8452A spectrometer was used for the measurements.

Preparation of P(S/PGL)-*H. pylori* Complexes for Detection of *H. pylori* Antibodies in Human Sera and Determination of Content Rating of *H. pylori* Antibodies in Human Sera

Usually, the suspension of microspheres (2.9 ml) with bound *H. pylori* antigens (640 μg/ml) was mixed with 100 μl of diluted serum, incubated for 30 min at room temperature which was followed by separation of particles by centrifugation and resuspension in a fresh portion of PBS buffer (pH 7.4, $I = 2 \times 10^{-3}$ M). The titer of the serum before dilution estimated by the indirect ELISA depended on the patient and was in the range from 1:500 to 1:32000. As a unit concentration of *H. pylori* antibodies (1 U) in a serum, the amount of *anti-H. pylori* in 100-fold diluted serum with a titer 1:1000 was chosen, according to equation (2):

$$[anti - H.pylori] = 1/10T\alpha \quad (2)$$

where T and α denote titer of serum estimated by ELISA and degree of dilution, correspondingly.

Results and Discussion

Basic Properties of P(S/PGL) Microspheres

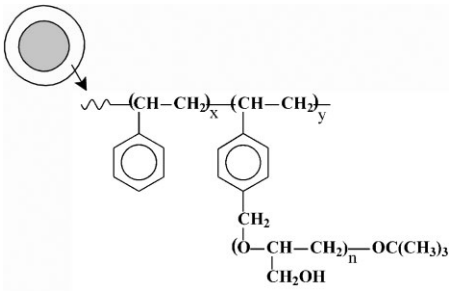
The data characterizing P(S/PGL) microspheres obtained using various fractions of PGL in the polymerizing mixture are collected in Table 1. Polymerization yields spherical particles with a low diameter dispersity. The diameter of microspheres decreased with increasing concentration of PGL in polymerizing mixture. It is worth noting that the fraction of PGL macromonomer in interfacial layer of particles, estimated by XPS analysis is ca seven times higher than the average fraction of PGL in the bulk of particles (obtained from elemental analysis). This means that the particles have core-shell morphology. Their shells are enriched in polyglycidol and cores in polystyrene. Structure of P(S/PGL) microspheres is shown in Scheme 1.

The average content of PGL in ca 5 nm thick interfacial layer of particles (measured by XPS) equals 42 mol.%. The assuming that the external part of the 5-nm layer is covered with pure PGL one could estimate the thickness of the PGL layer to be only ca 2 nm thick. Thus, it is reasonable to expect that PGL penetrates in the interfacial layer much deeper than 2 nm; however, the concentration of chains diminishes along the radius.

It is worth noting that above a certain fraction (5.78×10^{-4}) of PGL macromonomer in the polymerizing mixture, the interfacial fraction of PGL does not exceed 42%. Apparently, polystyrene-*graft*-polyglycidol copolymers with higher PGL contents are

Table 1.
Parameters characterizing P(S/PGL) microspheres.

PGL in monomer feed, mol.%	D_n, nm (SEM)	D_w/D_n	D_h (PCS)	PGL in the particle bulk, mol.%	PGL in interfacial layer, mol.%	$[-OSO_3^-]$, mol/m²
PGL with $M_n = 2700$ ($M_w/M_n = 1.03$)						
2.89×10^{-5}	650	1.008	765	0.0229	0.216	1.65×10^{-6}
5.78×10^{-4}	350	1.007	410	0.0280	0.255	8.77×10^{-7}
3.85×10^{-4}	260	1.007	294	0.0532	0.423	4.85×10^{-7}
2.88×10^{-4}	220	1.022	259	0.0705	0.426	4.13×10^{-7}
PGL with $M_n = 2800$ ($M_w/M_n = 1.05$)						
2.88×10^{-4}	270	1.010	282	0.048	0.340	6.61×10^{-7}



Scheme 1.
Core-shell structure of P(S/PGL) microspheres.

water-soluble. The P(S/PGL) microspheres are negatively charged due to the presence of persulfate end-groups introduced in initiation with $K_2S_2O_8$. However, one has to remember that swelling of hydrophilic interfacial PGL layer penetrated with potassium counterions would result in the negative charge screening.

Swelling-Deswelling Behavior of Surface Layer of P(S/PGL) Microspheres

The hydrophilic, PGL rich interfacial layer may swell in water media. Therefore, effective diameters of particles in suspension should be larger than in the dry state. Hydrodynamic diameters of P(S/PGL) microspheres with various fractions of PGL in interfacial layer, measured by photon correlation spectroscopy (PCS), are given in Table 1.

One has to take into account, that PCS provides information on diffusion coefficient of the observed objects. Calculations of diameters are usually based on the assumption that particles are spherical, rigid and not permeable. Thus, in the case of microspheres with swollen interfacial layer, PCS provides only ''equivalent'' hydrodynamic diameters (i.e. diameters of rigid particles with the same diffusion coefficient as that of the investigated particles). Values of diameters of P(S/PGL) microspheres are shown in Table 1. The degree of swelling of the interfacial layer in particles may depend on temperature therefore the dependence of microsphere diameters on temperature was investigated. The particles ($D_n = 270$ nm) with interfacial fraction of PGL $f_{PGL} = 0.34$ were chosen for investigations.

According to Figure 1, diameters of particles do not change for temperatures increasing up to ca 45 °C. Then, in a narrow temperature range, the diameters decrease slightly but significantly and again do not change with a further increase in temperature. This observation conforms to the assumption that below 45 °C the interfacial

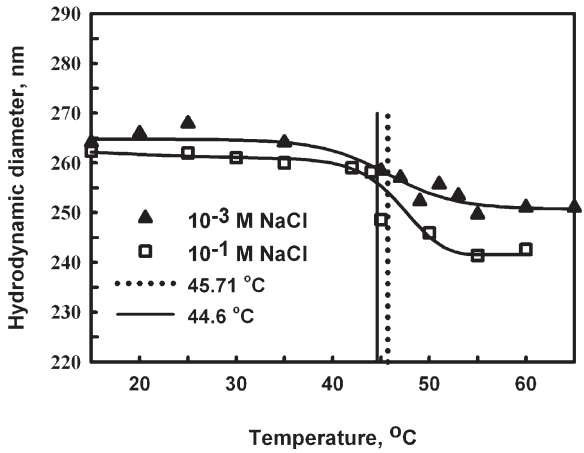


Figure 1.
Hydrodynamic diameter of P(S/PGL) microspheres ($D_n = 270$ nm, SEM) suspended in water containing [NaCl] = 0.1 and 0.001 M versus temperature. Vertical lines indicate transition temperatures.

layer is swollen with water and above this temperature desolvation of polyglycidol-rich polystyrene-*graft*-polyglycidol macromolecules leads to the chain-globule transition resulting in collapse of interfacial layer. Moreover, plots in Figure 1 suggest that, the decrease in particle diameters is higher for higher salt concentration. This is presumably due to an increase in osmotic pressure as a result of addition of salt. The differences between hydrodynamic diameters of particles at 25 °C and at 55 °C, suspended in 0.001 M and 0.1 M of NaCl, were small and equal 7.9 nm and 8.2 nm, respectively. Similarly, the transition temperature (T_t), corresponding to the inflexion point measured at half-height of the sigmoidal curve, was only slightly shifted with changing salt concentration and the hundred-fold increase in NaCl concetration resulted in ΔT_t about 1 °C.

It is worth mentioning that swelling and deswelling of the interfacial layer are typical of core-shell particles with shells composed of polymers with LCST (lower critical solubilization temperature) for example, for particles with shells composed of poly(*N*-isopropylacrylamide) (PNIPAM), poly(2-hydroxyethyl methacrylate), and copolymers of NIPAM with acrylic acid[15] and poly(vinylimidazol).[16]

Attachment of Proteins to P(S/PGL) Microsphere Surface

The natural environment of proteins and other biomolecules is water. In aqueous medium proteins are synthesized and function. The three-dimensional structure of proteins, hydrophilic and hydrophobic domains and charge distribution allow for the optimum fitting of these biomolecules to their environment. Therefore, the design of protein-resistant, antifouling surfaces is still a big challenge. The general rules for synthesis of microspheres with protein repellent surfaces, are well known; however, we are still far from the needed reliability and accuracy of structure-properties predictions. Studies of this subject are still required. Also antifouling surfaces are needed which, after appropriate activation, are suitable for covalent and thus irreversible protein immobilization.

It is worth reminding that surface of microspheres for medical tests should be protected against spontaneous and unwanted non-specific adsorption of proteins from the investigated biological material. It is known that this requirement is fulfilled in the case of particles containing interfacial layers with high density of hydrophilic, flexible chains with sufficient length. For example, comprehensive studies revealed that surfaces with grafted PEO chains containing 129 monomer units ($M_n = 5680$) are highly resistant to fibrinogen adsorption.[17]

It has been found that relaxation times (T_1) of carbon atoms of polyglycidol in interfacial layer of particles and those of polyglycidol dissolved in D_2O, used as a measure of chain mobility, are very close. This suggests that the mobility of polyglycidol chains in the interfacial layers of particles is almost the same as the chain mobility in solution.[8]

Studies of adsorption of human serum albumin (data presented) and of other proteins (γG and fibrinogen) on P(S/PGL) microspheres revealed a substantial decrease in the amount of the adsorbed protein with increasing fraction of polyglycidol in the interfacial layer.[11] A plot of maximum surface concentration (Γ_{max}) of adsorbed human serum albumin (HSA) versus the fraction of polyglycidol in particles surface layer is shown in Figure 2 (Γ_{max} was determined by extrapolation of the dependence of the surface concentration of adsorbed protein on reciprocal of the protein concentration in solution extrapolated to 0). It is worth noting that Γ_{max} of HSA adsorbed on polystyrene surface equal to 1.24 mg/m^2 is almost an order of magnitude higher than for particles with $f_{PGL} = 0.42$ which is 0.181 mg/m^2.[11]

A similar tendency was found for HSA adsorbed on polystyrene particles before and after grafting of poly[*N*-(2-methoxyethyl) acrylamide] PMEA (M_n of PMEA = 3300). The surface concentration of adsorbed protein was 4 mg/m^2 and 1.4 mg/m^2, respectively.

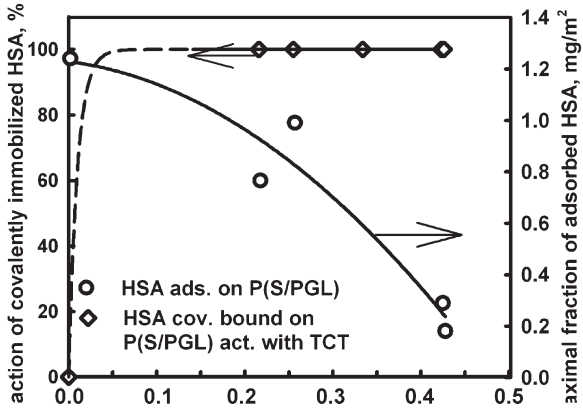
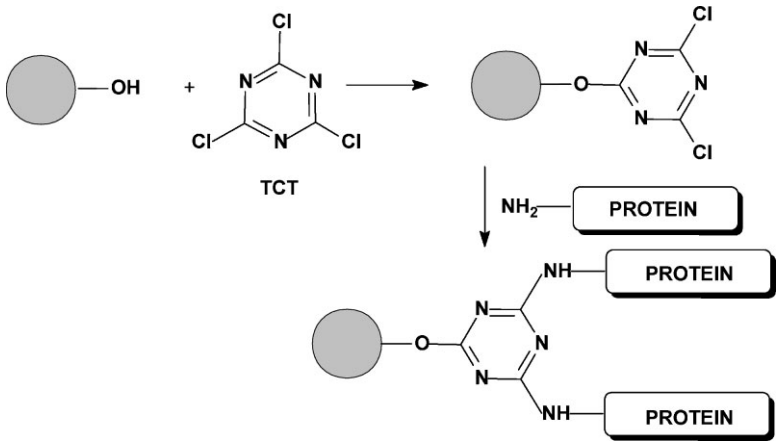


Figure 2.
Fraction of HSA covalently immobilized on P(S/PGL) microspheres activated with TCT (left) and maximum fraction of adsorbed HSA on non-activated particles (right) as a function of the fraction of polyglycidol (f_{PGL}) in interfacial layer.

The subsequent modification of particles with polyNIPAM reduced the surface concentration of adsorbed protein to 0.8 mg/m^2.[18]

Particles needed for diagnostic applications do not adsorb proteins but can be used for their controlled covalent immobilization. In the case of P(S/PGL) particles proteins could be covalently immobilized by activation of polyglycidol hydroxy groups with 2,4,6-trichloro-1,3,5-triazine (TCT). Subsequent reactions involving protein amino groups lead to their covalent immobilization (see Scheme 2).[19]

It is worth noting that for particles with surfaces containing hydrophobic domains and hydrophilic patches with active groups there is a possibility of covalent immobilization accompanied by some protein adsorption. Thus, for each type of particles it is desirable to determine not only the total amount of the attached protein but also its covalently immobilized fraction.[19] The fraction of protein loosely bound (adsorbed) to the surface is usually determined by washing out with solutions of surfactants (e.g. sodium dodecyl sulfate).[10] It is also worth noting that the immobilization



Scheme 2.
Covalent binding of proteins onto P(S/PGL) microspheres.

of the protein onto surface of poly(styrene/acrolein) particles for any fraction of polyacrolein, covalent attachment of protein was always accompanied by a fraction of adsorbed protein.[19,20]

The dependence of the HSA fraction covalently immobilized on P(S/PGL) on interfacial fraction of polyglycidol is shown in Figure 2. The plot indicates 100% covalent immobilization of HSA attached to particles activated with TCT. This means that the proteins can be bound to the surface enriched in polyglycidol chains, just ''on request'', after activation of hydroxy groups with TCT, without nonspecific adsorption. This feature is especially valuable for particles for medical diagnostic tests in which any nonspecific and uncontrolled attachment of antibody (or antigen) decreases the accuracy of analysis.

Application of P(S/PGL) Microspheres with Bound Antigens Against Helicobacter Pylori for Determination of its Antibodies in Blood Serum

Even though various diagnostic tests based on microspheres as analytical reagents are present on the market (usually aggregation tests), new microspheres enabling better sensitivity and accuracy of analysis are still needed. Synthesis of core-shell particles that are protein repellent but allow controlled covalent protein immobilization after activation opens such possibility.

The particles synthesized with potassium persulfate as an initiator are electrically charged (containing $-OSO_3^-$ groups, from the initiator). Any attachment of proteins (polyelectrolytes that are charged, except at isoelectric point) change their charge. Thus, binding of specific antibodies by antigens immobilized on the particle surface should also result in a change of particle charge. This process can be monitored by measuring of the electrophoretic mobility particles.[21] Therefore, mixing of a suspension of particles bearing covalently bound antigens with a biological liquid containing specific antibodies should affect not only aggregation of particles but also a change in electrophoretic mobility of nonaggregated particles. The principle presented above was used as a basis for elaboration of a new selective and sensitive test in which P(S/PGL) microspheres were used as a key reagent.

A test was designed for determination of *Helicobacter pylori* antibodies in blood serum.[21] *Helicobacter pylori* ranks among the bacteria that cause various diseases of the digestive tract, in particular stomach ulcers and cancers. Therefore, an increased level of antibodies against *Helicobacter pylori* in blood serum indicates an infection and potential high risk of serious diseases.

A test was developed using P(S/PGL) microspheres with molar fraction of polyglycidol in interfacial layer $f_{PGL} = 0.34$. Particles were activated with TCT and in the second step *H. pylori* antigens were covalently bound to these microspheres (surface concentration of *H. pylori* antigens $\Gamma_{H.\ pylori}$ $1.63 \pm 0.3\,mg/m^2$). Suspensions of microspheres with *H. pylori* antigens were incubated with dilute sera containing *H. pylori* antibodies. It has been found that after incubation the nanoparticles were slightly swollen (by ca 80 nm) but the aggregation of particles was absent. This finding proved that in this case, the test based only on the determination of particle size would fail. However, it was observed that the electrophoretic mobility of the particles with immobilized antigen incubated with solutions containing *H. pylori* antibodies changed significantly. It is worth to know, that the electrophoretic mobility of particles with immobilized *H. pylori* antigens incubated with sera free from anti-*H. pylori* (diluted in the same way as sera with *H. pylori* antibodies) did not change. This finding indicated that the change of electrophoretic mobility of particles is induced by biospecific interaction between immobilized *H. pylori* antigens and antibodies.

The dependence of electrophoretic mobility of poly(styrene/α-*tert*-butoxy-ω-(vinylbenzyl)polyglycidol) microspheres with covalently bound *H. pylori* antigens on the concentration of *H. pylori* antibodies in solution is presented in Figure 3. The plot

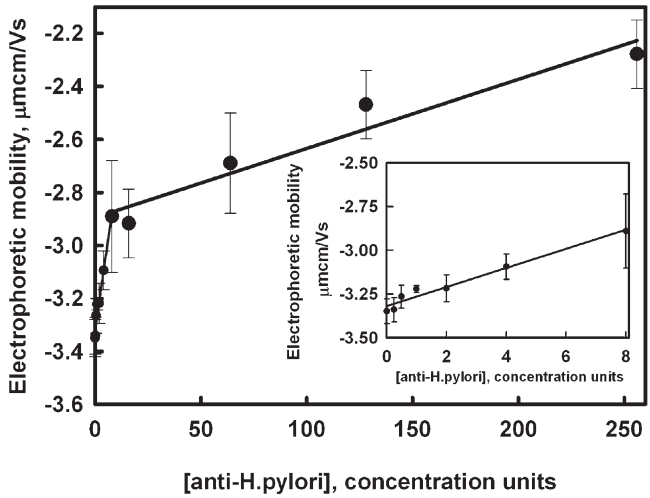


Figure 3.
Dependence of *electrophoretic* mobility of P(S/PGL) microspheres with immobilized *H. pylori* antigens on the concentration of *H. pylori* antibodies in blood sera (reproduced with permission from ref.[21])

clearly indicates that at low (below 10 U) and high (10-250 U) concentrations of antibodies, slopes of the plots differ significantly. This means that the test based on monitoring changes in electrophoretic mobility of microspheres is especially sensitive at low concentrations of antibodies and superior to the commonly used ELISA tests.[21]

Conclusion

The particles synthesized by radical emulsion copolymerization of styrene and α-*tert*-butoxy-ω-(vinylbenzyl)polyglycidol macromonomer show a core-shell morphology with shells enriched in polyglycidol units. The syntheses yielded microspheres with a narrow diameter dispersity ($D_w/D_n \leq 1.06$) and with number-average diameters in the range 220–650 nm. Measurements of hydrodynamic diameters of microspheres at various temperatures and NaCl concentrations revealed the swelling-deswelling behavior of the particle shell with a slight shift of transition temperature depending on the salt concentration. Increasing NaCl concentration resulted in a decrease in transition temperature T_t (salting out of polymer chains in particle shells).

Particles with the highest fraction of hydrophilic polyglycidol in the interfacial layer ($f_{PGL} = 0.3$) were resistant to protein adsorption. However, activation of hydroxy groups of polyglycidol component on particle surface with TCT allowed an efficient covalent binding of proteins (non-specific binding did not occur, 100% of the protein was covalently immobilized). The particles with covalently bound antigen from *Helicobacter pylori* were suitable for development of a new type of a diagnostic test, based on electrophoretic mobility measurements, for detection of specific antibodies in blood sera of patients infected with *H. pylori*. The test is especially sensitive at low concentrations of *H. pylori* antibodies.

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