Polymer Nano- and Microparticle Based Systems for Medical Diagnostics

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Summary: Synthesis, properties and medical diagnostic applications of hydrophilic nano- and microspheres with carboxyl, aldehyde and hydroxyl groups on their surface are described. The particles were obtained by emulsion copolymerization of styrene, acrolein, methyl methacrylate, methacrylic acid, and 2-hydroxyethyl methacrylate carried on in water media and initiated with potassium persulfate. Stabilization of particles' suspensions was provided by addition of sodium dodecyl sulfate to polymerizing mixture or by formation of surfactants *in situ* in copolymerization involving acrolein or α-*tert*-butoxy-ω-vinylbenzyl-polyglycidol macromonomer (PGL). Relations between interfacial properties of these particles and their ability for covalent immobilization of proteins, with eliminated or at least reduced nonspecific adsorption of these species were investigated. The particles with covalently attached proteins (antigens or antibodies) were used for preparation of diagnostic tests based on visual or turbidimetric observation of particles' aggregation or by monitoring changes in their electrophoretic mobility accompanying specific antigen (or antibody) binding. The later test was directed toward determination of antibodies against Helicobacter pylori. Principle of a new type of diagnostic test based on photonic crystals of microspheres are described.

Keywords: diagnostic test; functional microspheres; hydrophilic microspheres; immobilized proteins

Introduction

Majority of diagnostic tests is based on specific interactions between biomolecules, most often antibodies and antigens or between complementary strands of nucleic acids. However, direct observation of these interactions is difficult due to small dimensions of biomolecules. There was developed a variety of indirect methods suitable for detection of antigen-antibody interactions. However, properties of nano- and microspheres** make these particles especially valuable as candidates for development of diagnostic tests.

Diameters of nano- and microspheres are from about 50 nm to a few micrometers, this means they are noticeably (from ca 10 to 500 times) larger than biomacromolecules (e.g. proteins and oligonucleotides) to be detected. Thus, interactions involving proteins attached to microspheres may be seen as interactions involving particles, the much larger objects which could be easier monitored.

Nano- and microparticles and their aggregates could be observed using a variety of well known methods: microscopic,[1] turbidimetric/nephelometric or other methods based on light scattering,[2–4] fluorescence,[5–8] surface plasmon resonance,[9] and piezoelectric balances techniques.[10] Thus, nano- and microparticles with immobilized biomolecules, which are able for binding specifically selected molecules, can be used as convenient tools for detection of

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**The therm ''microspheres'' refers to polymeric spherical particles with diameters in the range 0.1–100 μm. Particles with diameters below 0.1 μm are called nanospheres.

the latter.[11–14] The most often used are diagnostic tests based on optical detection.[15,16] In last years significant attention was concentrated on so-called ''dry tests'' developed using microspheres.[17]

In this paper we summarize briefly results of our studies on synthesis, properties and selected applications of three kinds of functional, hydrophilic nano- and microspheres. Our attention was concentrated on relations between adsorption, covalent immobilization of proteins and chemical composition of the interfacial layer of microspheres. Applicability of synthesized microspheres for standard tests and for the new class of tests based on electrophoretic mobility of particles and on using colloidal crystals made from microspheres will be discussed. Following monomers were used for synthesis of functional hydrophilic segments of nano- and microspheres:

Particles for medical applications are often synthesized from monomer mixtures containing acrolein and methacrylic acid. Synthesis of polyglycidol containing microspheres was elaborated recently in our laboratory.[18] The main chain of polyglycidol is similar to that of poly(ethylene oxide), presence of which in the interfacial layer reduces protein adsorption. However, there is a significant difference between these polymers. Whereas poly(ethylene oxide) chains tethered to the surface contain only one reactive group (end-group) the polyglycidol chains contain hydroxyl groups in all monomeric units. This opens new opportunities for design of tests based on microspheres suitable for controlled covalent protein immobilization.[19,20]

Materials and Methods

Syntheses of Nano- and Microspheres

Poly(methyl methacrylate/methacrylic acid/2-hydroxyethyl methacrylate)/ethylene glycol dimethacrylate) (denoted as P(ACRYL) nanospheres, poly(styrene/acrolein) (P(S/A)) and poly(styrene/α-*tert*-butoxy-ω-vinylbenzyl-polyglycidol) (P(S/PGL) microspheres were prepared by emulsion polymerization of the corresponding monomers initiated with potassium persulfate ($K_2S_2O_8$) and carried on in water. Detailed descriptions of these syntheses were given in our earlier papers, here are only presented briefly.[18,21,22]

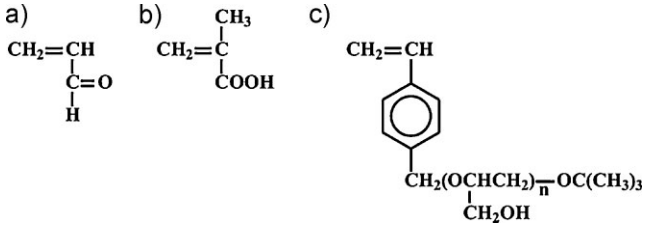
Synthesis of P(S/A) Microspheres

Ingredients: styrene (10 ml, purified by distillation under reduced pressure), acrolein (in various syntheses from 0.3 to 1.5 ml), $K_2S_2O_8$ (0.045 g, initiator), and water (100 ml, distilled three times, pH adjusted to 6.7 with K_2CO_3).

Polymerizations were carried on at 65 °C for 27 h with stirring (600 rpm) under argon. Unreacted monomers were removed by steam stripping. Microspheres were purified by repeated centrifugation, isolation and resuspension in new portions of water.

Synthesis of P(ACRYL) Nanospheres

Ingredients: methyl methacrylate (4.6 g), methacrylic acid (0.8 g), 2-hydroxyethyl



Scheme 1.
Monomers used for synthesis of microspheres with hydrophilic interfaces containing functional groups used for covalent immobilization of proteins: a – acrolein, b – methacrylic acid, c – α-*tert*-butoxy-ω-vinylbenzyl-polyglycidol macromonomer.

methacrylate (2.4 g), ethylene glycol dimethacrylate (0.24 g), sodium dodecyl sulfate (0.03 g), $K_2S_2O_8$ (6×10^{-3} g, initiator), and water (42 ml, distilled three times).

For the first 15 minutes the polymerization was carried on at 70 °C. Then, during 1 h the temperature was raised to 96 °C and kept at this level for the next two hours. Obtained nanoparticles were purified by repeated centrifugation and exchange of supernatant with fresh portions of three times distilled water.

Synthesis of P(S/PGL) Microspheres

Ingredients: styrene (10 g), α-*tert*-butoxy-ω-vinylbenzyl-polyglycidol (PGL2800) (0.1 g, $M_n = 2800$, $M_w/M_n = 1.05$), water (125 ml, distilled three times), and $K_2S_2O_8$ (0.2 g).

Macromonomer was prepared as it was described earlier.[23] Synthesis was carried on at 70 °C for 27 hours (600 rpm) under argon. The microspheres were purified by repeated centrifugation and replacement of supernatant with fresh portions of water.

Determination of Diameters of Nano- and Microspheres

Diameters of microspheres were determined from SEM microphotographs registered using a JEOL 5500LV apparatus. Number average diameters of microspheres (D_n) and diameter dispersity parameter (D_w/D_n) were calculated sampling at least 600 microspheres.

Determination of Surface Concentration of Acidic Groups

Concentration of $-OSO_3^-$ anions (end-groups in polymer chains formed during initiation with $K_2S_2O_8$) in interfacial layer of microspheres was determined by conductometric titration with KOH.[18,21]

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

Chemical composition of interfacial layer of the P(S/PGL) microspheres was based on analysis of X-ray photoelectron spectra registered for dry particles (XPS method). Spectra were registered using a Thermo VG Scientific ESCALAB 250 system equipped with a monochromatic Al Kα X-ray source (1486.6 eV) and magnetic lens. Atomic ratio of carbon and oxygen atoms was calculated from the ratio of the intensity of corresponding XPS signals (for carbon atoms in a range from 285 to 291.6 eV, including a shake-up signal due to the polystyrene aromatic ring, for oxygen atoms at 532 eV) corrected for the relevant sensitivity factors. The details of calculations of fractions of hydrophilic components were described elsewhere.[18,21]

Binding of Proteins onto the Surface of P(S/A), P(ACRYL), P(S/PGL) Microspheres

Immobilization of HSA onto P(S/A) microspheres

Human serum albumin was immobilized covalently onto the surface of P(S/A) microspheres by simple incubation of microsphere suspension with protein solution. The reaction of amino groups of proteins with aldehyde groups of microspheres formed Schiff-base linkages. For example, incubation of P(S/A) microspheres and of HSA (7 mg/ml and 8.75 mg/ml, respectively) was carried on for 24 hours at room temperature. Particles with attached protein purified by centrifugation, separation of supernatant, an exchange for fresh PBS (phosphate buffered saline, pH = 7.4, I = 0.2 M) were stored at 4 °C.

Immobilization of Antibodies Against Plasminogen (anti-Plg) on the Surface of P(ACRYL) Nanoparticles

Rabbit antibodies against plasminogen (anti-Plg) were immobilized on the surface of P(ACRYL) particles in a process involving reaction of carboxyl groups of poly(acrylic acid) units, ε-aminocaproic acid (EACA) linker (carboxyl groups activated with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC)) and amino groups of protein. Briefly, a mixture containing protein, suspension of particles purified by dialysis (0.23 g), EDC (15 mg), and EACA (6 mg) was shaken for 2 h at 4 °C. The unbound EACA was separated

by dialysis. Then, anti-Plg was added. The particles with immobilized anti-Plg were purified by dialysis. The remaining carboxyl groups activated with EDC were blocked by incubation of P(ACRYL) nanoparticles containing immobilized anti-Plg with bovine serum albumin (BSA). Finally, the nanoparticles were isolated by centrifugation, resuspended in fresh portion of three times distilled water and stored at 4 °C.

Immobilization of Proteins onto P(S/PGL) Microspheres

Description of procedures used for binding proteins onto the surface of particles containing polyglycidol were published elsewhere.[24] Briefly, the water suspension containing P(S/PGL) particles (0.4 g) was incubated with 1,3,5-trichlorotriazine (TCT, 0.18 g) at room temperature for 2 hours. The, unbound TCT was removed by repeated centrifugation and resuspension. Finally, protein was covalently immobilized on the surface of TCT activated particles. After 12 h of incubation, the microspheres were isolated by repeated centrifugation and resuspension in PBS ($I = 2 \times 10^{-3}$ M).

Detected Proteins

The goat anti-HSA (Sigma) was dissolved in 2 ml PBS and from this stock solution several dilutions were prepared. Plasminogen was isolated from the human plasma by the affinity chromatography on the Lys-Sepharose 4B (Sigma) and purity was checked by the polyacrylamide gel electrophoresis.[22] Sera containing Helicobacter pylori antibodies were obtained from children patients and the procedure for titer determination (using indirect ELISA) was described in details in our earlier work.[24]

Determination of Concentration of Proteins Attached (Adsorbed or Covalently Immobilized) onto P(S/A), P(ACRYL), P(S/PGL) Microspheres

Concentrations of proteins in solutions were determined spectrophotometrically or by using the standard Lowry method.[25] The amounts of proteins immobilized on the surface of particles were obtained by measuring the difference of protein concentrations before and after immobilization or directly on particles by using the modified Lowry method.[26]

Measurements of Electrophoretic Mobility of P(S/PGL) Microspheres

Electrophoretic mobility of P(S/PGL) microspheres without and with immobilized 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).

Detection and determination of antibodies against Helicobacter pylori on blood sera using P(S/PGL) microspheres with immobilized Helicobacter pylori antigens

Suspension of microspheres (2.9 ml) with immobilized Helicobacter pylori antigens (concentration 640 μg/ml) was incubated for 30 minutes with 100 μl of diluted serum. Thereafter, the particles were separated by centrifugation and resuspended in a fresh portion of PBS ($pH = 7.4$, $I = 2 \times 10^{-3}$ M). Titer of the serum before dilution was estimated by ELISA test. Depending on the patient the titer was in the range from 1:500 to 1:32000.

The concentration of antibodies against Helicobacter pylori in the 100-fold diluted serum with a titer 1:1000 was chosen as a unit concentration (1 U). Thus, the concentration could be calculated according to formula (1):

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

[anti-H. pylori] denotes concentration of antibodies against Helicobacter pylori, T and α denote the titer of serum estimated by ELISA and the degree of dilution, respectively.

Results and Discussion

Basic Characteristics of Nano- and Microspheres

X-Ray photoelectron spectroscopy (XPS) allows studies of the composition of particles' interfacial layer about 5 nm thick.

Data in Table 1 indicate that fraction of hydrophilic polyacrolein or polyglycidol segments in the interfacial layer (from XPS measurements) are significantly higher than content of the corresponding comonomers in the polymerizing mixture. Moreover, the higher fractions of hydrophilic comonomer in the polymerization mixture lead to microspheres with lower diameters. This allows for thermodynamically most favorable maximal contact between the hydrophilic component of microspheres and water. The particles have core-shell morphology with shells enriched in hydrophilic compound (polyacrolein or polyglycidol) and cores enriched in polystyrene. Described polymerizations yielded particles with a very narrow dispersity (D_w/D_n was close to 1).

For P(S/A) particles the surface fraction of polyacrolein was controlled in the whole range 0-100% (100% for polyacrolein particles). We found that regardless how high was polyglycidol macromonomer content in the polymerizing mixture the fraction of polyglycidol units in the interfacial layer of microspheres did not exceed 45%. Apparently, copolymer chains with polyglycidol content higher than 45% are water soluble and thus not incorporated into surface layer of particles.

All synthesized microspheres were negatively charged. In the case of P(ACRYL) particles the negative charge is due not only to the sulfate anion end groups but also to the carboxyl groups in the poly(methacrylic acid) units.

Immobilization of Proteins onto P(S/A), P(ACRYL), P(S/PGL) Microspheres

Most suitable for diagnostic applications are particles with covalently immobilized proteins, and simultaneously, with eliminated undesirable and not controlled proteins adsorption. However, it is well known that very often the covalent immobilization is accompanied with protein adsorption. The adventitious adsorption from the analyzed liquid may significantly perturb determination of the compound to be detected.

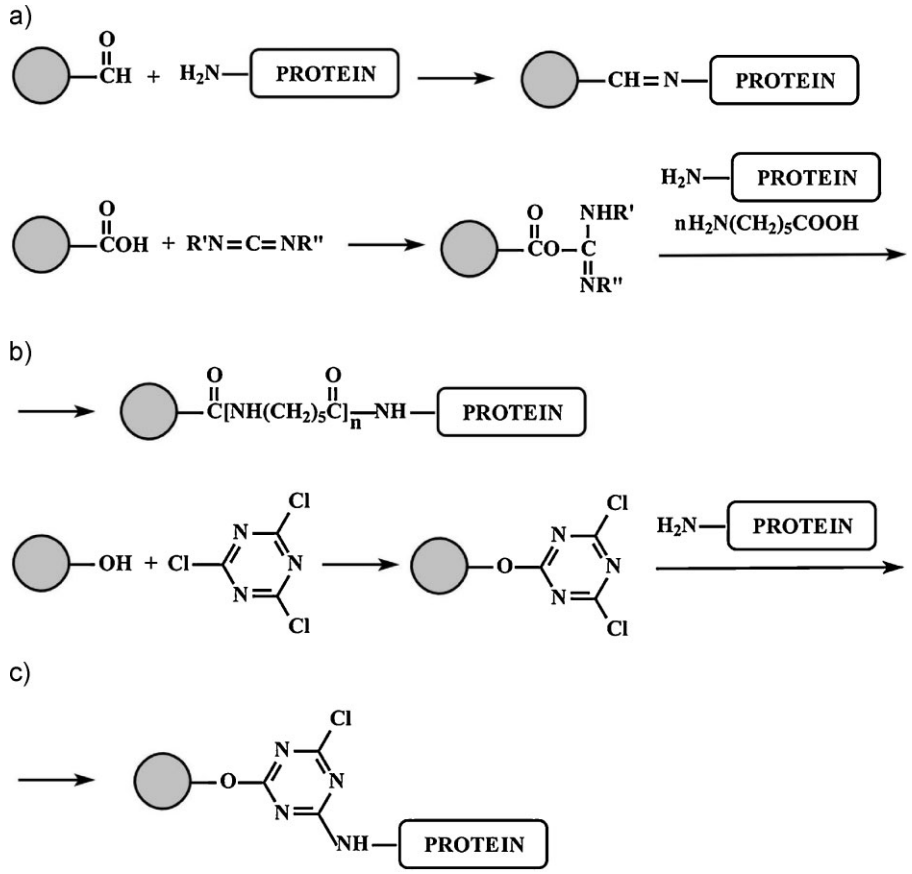
Reactions involved in covalent binding of proteins onto P(S/A), P(ACRYL) and P(S/PGL) microspheres are shown in Scheme 2. From the three classes of investigated particles only the P(S/A) microspheres with aldehyde groups did not require any activation before protein immobilization.

Plot of surface concentration of immobilized protein as a function of protein concentration in the mixture during protein immobilization is usually characterized by the initial rapid increase which at higher protein concentrations becomes slower and eventually the plot approaches plateau.[13,27]

Table 1.
Parameters characterizing P(S/A), P(ACRYL) and P(S/PGL) microspheres.

Symbol of particles	$f_{styrene}$:$f_{acrolein}$ or $f_{styrene}$:$f_{PGLmacromonomer}$ in monomer feed,[c] mol:mol	D_n, μm	D_w/D_n	[$-OSO_3^-$], mol/m^2	f_{PA} or f_{PGL} determined by XPS,[d] mol%
P(S/A)	1:0.052	0.51	1.010	2.52×10^{-6}	50
	1:0.100	0.49	1.002	1.38×10^{-6}	63
	1:0.260	0.38	1.007	2.14×10^{-6}	84
	0:1	0.30	1.003	3.24×10^{-6}	100
P(ACRYL)	–	0.14	1.2	8.41×10^{-6}	–
P(S/PGL)2700[a]	$1:2.89 \times 10^{-5}$	0.65	1.008	1.65×10^{-6}	21.6
P(S/PGL)2700[a]	$1:5.78 \times 10^{-4}$	0.35	1.007	8.77×10^{-7}	25.5
P(S/PGL)2800[b]	$1:2.88 \times 10^{-4}$	0.27	1.010	4.13×10^{-7}	34.0

[a]P(S/PGL) microspheres polymerized from α-*tert*-butoxy-ω-vinylbenzyl-polyglycidol macromonomer with $M_n = 2700$ ($M_w/M_n = 1.03$).
[b]P(S/PGL) microspheres polymerized from α-*tert*-butoxy-ω-vinylbenzyl-polyglycidol macromonomer with $M_n = 2800$ ($M_w/M_n = 1.05$).
[c]$f_{styrene}$:$f_{acrolein}$ and $f_{styrene}$:$f_{PGLmacromonomer}$ denote ratios of styrene and acrolein and styrene and α-*tert*-butoxy-ω-vinylbenzyl-polyglycidol macromonomer mol fractions in the monomer feed.
[d]f_{PA} and f_{PGL} denote molar fraction of polyacrolein and polyglycidol units, respectively.



Scheme 2.
Reactions involved in covalent immobilization of proteins onto: a – P(S/A), b – P(ACRYL) and c – P(S/PGL) microspheres.

This feature of protein (HSA and γ-globulins) attachment was observed for P(S/A) and P(S/PGL).[13,27] In each case there were determined fractions of the covalently immobilized and of the adsorbed protein. The determination was possible by ''washing out'' the loosely bound – adsorbed protein – with surfactant solution (3% of sodium dodecyl sulfate in PBS buffer). Plot of maximal surface concentration (at saturation) of covalently immobilized protein as a function of fraction of polyacrolein or polyglycidol monomeric units in the particles' interfacial layer is shown in Figure 1.

From plots in Figure 1 it is clear that for P(S/A) microspheres the fraction of covalently immobilized proteins (HSA and γ-globulins) gradually increased with the

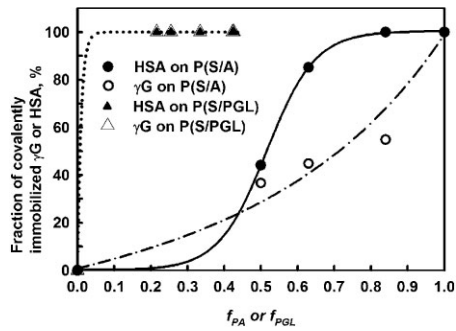


Figure 1.
Fraction of covalently immobilized γ-globulins and/or HSA on P(S/A) and P(S/PGL) microspheres (at saturation) as a function of the fraction of polyacrolein or polyglycidol monomeric units in particles' interfacial layer.

increasing fraction of polyacrolein in particles' interfacial layer up to 100% for the pure polyacrolein microspheres. However, for P(S/PGL) microspheres with polyglycidol fraction exceeding 0.2 the whole amount of attached protein was covalently immobilized. This observation indicates, that even in the case of particles with the small content of polyglycidol units in the interfacial layer properties of particles' surface do not allow for physical protein adsorption.

Selected Applications of P(S/A), P(ACRYL), P(S/PGL) in Medical Diagnostics

The simplest diagnostic tests are based on monitoring with a naked eye aggregation of particles with attached antibodies (or antigens) in the presence of specific antigens. Although many years passed from elaboration of the first diagnostic test involving particles with adsorbed proteins the new and better tests based on various applications of microspheres with covalently immobilized proteins are needed and still developed.

An example of a model aggregation test for detection of goat antibodies against human serum albumin is described below. The tests were based on polystyrene microspheres with physically adsorbed human serum albumin (P(S)-HSA) and on P(S/A) microspheres with covalently immobilized HSA (P(S/A)-HSA). Typically, 20 μl of a stable suspension of particles with attached human serum albumin ($c = 1.5\,mg/m^2$) was mixed with 20 μl of anti-HSA diluted serum on a glass plate. Aggregation was monitored with a naked eye. Results of the above described investigations are given in Table 2.

Data in Table 2 show that the sensitivity of the test in which the particles with covalently bound protein were used is higher (detection limit is close to the 4000-fold dilution) than for the particles with adsorbed protein (detection limit 2000-fold).

In many instances very important is not only detection of biomolecules but also determination of their concentration. For this purpose tests based on measurements of various parameters characterizing microspheres and microsphere aggregates (for example, size of particles and particle aggregates, particle charge, light scattering by particle random aggregates and crystal-like assemblies) are developed.

The simplest quantitative test is based on using UV-VIS spectroscopy for monitoring aggregation of microspheres. As an example there is described here the test developed in our laboratory for detection of plasminogen. The P(ACRYL) particles bearing antibodies against plasminogen (anti-plasminogen), immobilized covalently via the ε-aminocaproic acid linkers (EACA), were used as the main reagent. The particles are denoted shortly as P(ACRYL)-EACA-anti-Plg. Concentration of plasminogen in solutions used for preparation of the calibration curve was in

Table 2.
Aggregation of P(S)-HSA and P(S/A)-HSA microspheres mixed with anti-HSA solution (20 μl of particle suspension and 20 μl of anti-HSA solution).

Dilutions of goat anti-HSA serum	P(S)-HSA (concentration 12.8 mg/ml)	P(S/A)-HSA (concentration 8.9 mg/ml)
1:2	++	+
1:4	++	++
From 1:8 to 1:256	+++	+++
1:512	+++	++
1:1024	+++	++
1:2048	+	++
1:4096	−	+
1:8192	−	−

Legend: +++ denotes very strong aggregation, large aggregates are formed, ++ denotes well developed aggregation clearly visible with naked eye, + denotes weak aggregation (visible with magnifying glass), - absence of aggregation, readout 4 minutes after mixing particle suspension and analyte.

the range from 14 ng/ml to 2 μg/ml. Measurements were performed in the following way. The suspension of P(ACRYL)-EACA-anti-Plg particles (80 μl, particles' concentration 1.92 mg/ml) and solution of plasminogen (20 μl of solution with known plasminogen concentration) were added to 1 ml of PBS and optical density of this mixture was monitored at $\lambda = 600$ nm (OD(600)). Initially OD(600) rapidly increased and eventually after a few minutes became steady. The finally established value was denoted as $OD_f(600)$. The plot of the concentration of plasminogen as a function of measured $OD_f(600)$ is shown in Figure 2.

Plot in Figure 2 shows that determination of the concentration of plasminogen in the range from 0.75 to 74 μg/ml is possible on the basis of the $OD_f(600)$ measurements. For concentrations of plasminogen lower than 0.75 μg/ml values of $OD_f(600)$ were too low for sufficiently precise measurements, whereas for the concentrations higher than 75 μg/ml $OD_f(600)$ was practically independent of the concentration of plasminogen.

Progress in synthesis of particles with surfaces allowing for covalent immobilization of antibodies (antigens) and for specific binding to them complementary proteins, without particle aggregation did open possibilities for design of new tests based on properties of single particles. Some of these tests are characterized by very high sensitivity. Others allow for detection of various compounds during one analytical procedure. Most often such multi-potent tests are based on special fluorescent particles.[28] Fluorescent particles used in the abovementioned tests are labeled with at least two different dyes or quantum dots in such a way each type of particles is marked in an unique manner resulting in emission spectrum which can be used as a 'fingerprint''. Different types of such particles are used for binding antibodies against various compounds to be detected. Tests consist in combining mixtures of fluorescent microspheres with analyzed liquid and in subsequent additions of fluorescently labeled antibodies against analyzed compounds. In this way the specifically labeled particles with bound searched compound and specific fluorescent antibodies are formed. Flow of such particles one-by-one during capillary illuminated by lasers with wavelengths suitable for excitation of fluorescent tags specific for particles and for antibodies and measurements of emission of light at corresponding wavelengths allows for unambiguous detection of various compounds in the mixture. For each selected compound the unique type of construct combining particularly labeled fluorescent particle and specific particularly labeled antibodies is formed.

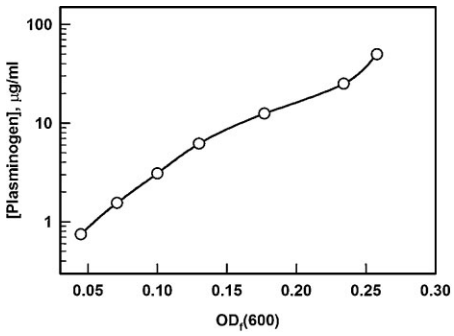


Figure 2.
Calibration curve (concentration of plasminogen as a function of $OD_f(600)$) for determination of plasminogen. Based on data from ref.[22].

Very high sensitivity was noticed also in the tests based on measurements of electrophoretic mobility of microspheres.[24] These assays are based on the following observations: a) proteins are electrically charged in solutions (except at isoelectric point at which the protein molecule as a whole is electrically neutral), b) there are several types of particles which are electrically charged too (for example P(S/PGL) particles obtained in polymerization initiated with $K_2S_2O_8$ leading to polymers with anionic sulfate end-groups), c) immobilization of proteins on the surface of charged particles results in a change of the overall charge of these particles and finally, d) after specific binding of antibodies the charge should be changed again.

On the basis of observations presented above there was developed a test for determination of antibodies against Helicobacter pylori in blood serum. Helicobacter pylori causes stomach and duodenum ulcers and stomach cancer. Increased level of antibodies against Helicobacter pylori in patient blood serum is an indicator of an infection.

The test was elaborated using P(S/PGL) particles with fraction of polyglycidol in particles' interfacial layer equal 34 mol%.[24] Helicobacter pylori antigens were covalently immobilized on microspheres activated with 1,3,5-trichlorotriazine (TCT; see Scheme 2). Surface concentration of antigens was equal $1.63 \pm 0.3\,mg/m^2$. Titer of Helicobacter pylori antibodies in patients sera used for development of the test was determined by ELISA test.

Electrophoretic mobility of P(S/PGL) particles with immobilized Helicobacter pylori antigens measured in the presence of sera containing antibodies against Helicobacter pylori was strongly dependent on the latter. It is worth to note that an increase of anti-Helicobacter pylori from 0 to 10 U was accompanied with the change of electrophoretic mobility equal $0.31\,m^2 \times 10^{-8}$/Vs whereas for higher concentrations of antibodies the 24 times larger increase of anti-Helicobacter pylori (from 10 to 250 U) led only to the two times larger change in electrophoretic mobility (equal $0.6\,m^2 \times 10^{-8}$/Vs). Thus, in contrary to the commonly used ELISA test the test based on measurements of electrophoretic mobility is most sensitive at low concentrations of detected antibodies.

There was developed also a very convenient diagnostic test based on changes of optical properties of colloidal crystals.[29] The device designed for determination of glucose was made from gel with embedded colloidal crystals of polystyrene microspheres ($D_n = 140\,nm$). Macromolecules in the gel were cross linked, which resulted in hydrogen bonds involving hydroxyl groups and boronic acid moieties linked to polymer chains. In the presence of glucose the competition between hydroxyl groups of polymer chains and glucose for binding of boronic acid groups results in less efficient cross linking allowing for more efficient swelling of the gel and subsequently for an increase of distances between microspheres in colloidal crystals. In effect light scattering from colloidal crystalline elements is changed what is manifested by change of their color. The described above material was used for fabrication of the contact lenses like devices with embedded colloidal crystal spots. Applied onto eye balls these devices allow monitoring of glucose in the tears, the quantity related to concentration of glucose in the blood.

Recently we found that the P(S/PGL) microspheres ($D_n = 270\,nm$, $D_w/D_n = 1.004$) with polyglycidol rich interfacial layer ($f_{PGL} = 34$ mol%) easily self-assembly into colloidal crystals during drying of particles' suspension.[19]

Distances between particles in particle arrays determine the intensity of light with a given wavelength which is scattered at a given angle – Bragg diffraction peak. For polymer nano- and microspheres the Bragg peak is noticed for light in the visible range. In the case of the described P(S/PGL) microspheres the Bragg peak was registered at 570 nm. Incorporation of any compound (also biomolecules) into the interfacial layer of microspheres changes distance between particles in colloidal crystal and consequently shifts position of the Bragg peak. Indeed, the covalent immobilization of ovalbumin on the surface of P(S/PGL) microspheres (surface concentration of ovalbumin was $2 \times 10^{-4}\,g/m^2$) induced hypsochromic shift of the Bragg peak for 10 nm. The diffraction spectra registered for photonic crystals patterns prepared from bare P(S/PGL) and P(S/PGL) with attached ovalbumin are shown in Figure 3.

Results of described above investigations indicate suitability of P(S/PGL) microspheres for development of novel biosensing devices based on the photonic crystal assemblies.

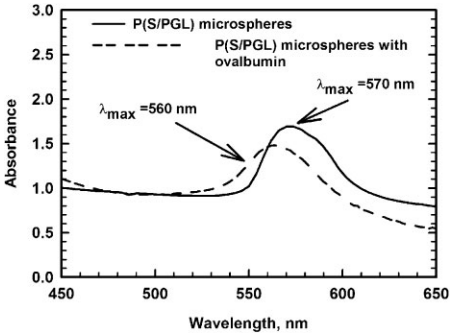


Figure 3.
UV spectrum of scattered light from colloidal crystal from bare P(S/PGL) particles and P(S/PGL) particles with bound ovalbumin.

Conclusion

Our studies revealed that all three kinds of investigated microspheres (P(S/A), P(ACRYL) and P(S/PGL)) can be effectively used for development of diagnostic tests. However, the highest reduction of the adventitious protein adsorption was noticed for P(S/PGL) microspheres which not only were hydrophilic but did contain mobile polyglycidol chains in the interfacial layer. Combination of the properties of P(S/PGL) microspheres (high uniformity of particle diameters, their electric charge, elimination of protein adsorption and possibility of effective covalent adsorption of these biomolecules) makes them an excellent candidate for design of various types of diagnostic tests.

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