Poly(styrene/ α -tertbutoxy- ω -vinylbenzyl-polyglycidol) microspheres for the preparation of novel photonic crystals

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Abstract: We report on the preparation of a novel photonic crystal assembled from poly(styrene/ α -tertbutoxy- ω -vinylbenzyl-polyglycidol) microspheres. The latex particles were fully characterized in terms of size (271 nm, $D_w/D_n=1.004$), electrophoretic mobility ($-3.79 \times 10^{-8} \text{ m}^2 \text{V}^{-1} \text{s}^{-1}$) and surface chemical composition (evidence for a PGL-rich particle surface). They were then assembled into 3D colloidal crystal showing an optical stop band in the visible region. The λ_{max} for the Bragg diffraction peak is 570 nm. As the particles under study are highly hydrophilic and biocompatible, this work opens up new opportunities for the design of photonic crystal-based biosensing devices.

Introduction

Polymer microspheres bearing proteins or other biomolecules find various applications in the bioscience area such as medical diagnostics [1]. Particularly, they prove to be useful for the visualization of biochemical processes (e.g. antigen-antibody, biotin-streptavidin recognition).

For such applications, the microspheres should be monodisperse with, preferably, a hydrophilic surface and should have chemical groups suitable for covalent binding of biomolecules. It was found that hydrophilicity of the surface ensures the resistance of latex particles against the non-specific adsorption of proteins [2], a feature which is especially desired when particles are in direct contact with physiological liquids (human urine or serum). The hydrophilicity of the surface can be usually achieved by direct copolymerization in emulsion of styrene and a water soluble monomer or macromonomer. However, the use of hydrophilic monomers (HEMA, methacrylic acid) has some disadvantages due to a distribution of low molecular weight chains between the polymeric (hydrophobic) and water phase during polymerization process. As a consequence, the composition of the outer layer of particles (surface fraction of hydrophilic polymer chains and their molecular weight) varies from one polymerization to another [3]. In order to control the polymerization process, Dworak and co-workers [4] developed a new type of hydrophilic macromonomers containing both, one side group suitable for copolymerization with a hydrophobic monomer, and a long hydrophilic chain. The macromonomer have also functional groups suitable for the binding of biomolecules. This led to the formation of novel PS latex particles with

a hydrophilic group-rich surface, the size of which could be varied in the range from 200 to 1000 nm [5]. Instead of the commonly used polyethylene glycol, there was interest in using a α -tert-butoxy- ω -vinylbenzyl-polyglycidol. The main chain of polyglycidol has a chemical structure similar to that of poly(ethylene oxide) and in respect to the presence of $-\text{CH}_2\text{OH}$ groups in each monomeric unit, this polymer should be highly hydrophilic. Numerous hydroxyl groups of polyglycidol chains (preferentially oriented to the water medium) allow for an efficient covalent immobilization of biomolecules. The synthetic route for obtaining poly(styrene/ α -tert-butoxy- ω -vinylbenzyl-polyglycidol) (P(S/PGL)) microspheres is presented in Scheme 1.

Scheme 1. Synthetic route for obtaining core/shell P(S/PGL) microspheres.

The presence of polyglycidol chains at the surface of P(S/PGL) microspheres was confirmed by ^{13}C NMR spectra. In the spectra registered for P(S/PGL) microspheres suspended in D_2O , only signals derived from carbon atoms of polyglycidol chains were present. This observation indicated that the polyglycidol chains in the surface layer of P(S/PGL) microspheres were soluble in water [6]. Moreover, the polyglycidol

chains in the surface layer of the microspheres show high mobility [6]. The mobility of the chains is an important factor in protecting the surface from the non-specific adsorption of proteins. It was also found that the longer polyglycidol chains in the particle surface layers were more mobile compared to the shorter ones and provide a better protection against protein adsorption [7].

Furthermore, the hydrophilic polyglycidol segments at the surface of P(S/PGL) particles can be selectively chemically treated in order to covalently bind proteins. The studies revealed that the antigen (*Helicobacter pylori* antigen) covalently immobilized on the surface of (PS/PGL) microspheres was detected by *Helicobacter pylori* antibodies in a broad range of concentrations in the patients sera [8].

Recently, we found in a serendipitous way that drying the P(S/PGL) particles resulted in layers exhibiting a bright iridescence. This opalescence was attributed to the self-assembly of the particles into a crystalline colloidal array. In recent years, the number of publications relevant to photonic crystals has expanded because these fascinating materials have unique optical properties that offer new opportunities in the design and development of optical biosensors [9], magnetophotonic crystals for spin wave propagation [10], and interference pigment particles for paints and displays [11]. Photonic crystals are characterized by a periodic dielectric structure leading to photonic band gaps or stop bands with a depleted photonic density of states [12]. In specific wave length ranges, the propagation of electromagnetic radiation can be suppressed along certain lattice directions. Colloidal photonic crystals are usually self-assembled from monodisperse silica or polystyrene spheres [13], a bottom-up approach which enables low-cost and large-scale fabrication. Nevertheless, even if the design of photonic crystals for applications in the biomedical field is an emerging subject [14], the use of biocompatible particles for such a material has never been reported so far.

The aim of this paper is to explore the propensity of the highly hydrophilic and biocompatible PS/PGL to form tri-dimensional photonic crystals and to characterize their optical properties. The P(S/PGL) particles were prepared by emulsion copolymerization of styrene and α -tert-butoxy- ω -vinylbenzyl-polyglycidol [4], and the 3D colloidal arrays were prepared in a controlled manner by water evaporation at RT. The 3D colloidal arrays were examined by SEM, UV, and standard digital photos taken at different diffraction angles and also in the transmission mode. The physicochemical properties of P(S/PGL) particles were compared to those of commercial negatively charged PS latex particles.

Results and discussion

Physicochemical properties of P(S/PGL) particles

The SEM microphotographs (Figure 1) clearly show that the synthesized PS/PGL microspheres were uniform in shape and monomodal with a number average diameter (D_n) of 271 nm and $D_w/D_n=1.004$. One could note the ordering aspect of the latex particles upon drying: such a self-organization of the particles is an experimental evidence of the presence of repulsive interactions between particles.

The concentration of sulfate groups ($-\text{OSO}_3^-$) derived from the initiator (potassium persulfate), determined by conductometric titration was equal to $6.61 \times 10^{-7} \text{ mol/m}^2$.

The electrophoretic mobility of PS/PGL microspheres suspended in 10^{-3}M NaCl (non buffered solution) was negative and equal to $-3.79 \times 10^{-8} \text{ m}^2 \text{V}^{-1} \text{s}^{-1}$.

Fig. 1. SEM micrographs of P(S/PGL) particles.

The surface chemical composition was assessed by XPS. Figure 2 depicts survey scans and high resolution C1s regions of P(S/PGL) and PS. The main peaks in Figure 2a correspond to C1s (285 eV) and O1s (~ 532 eV). Additionally, there is a minor S2p unresolved doublet centred at ~169 eV which is assigned to the persulfate initiator of the styrene and PGL copolymerization. For PS, the O1s peak due to the sulfate is weak, but for P(S/PGL) it exhibits a much higher relative intensity due to the polyglycidol-rich surface of P(S/PGL). Figure 2b exhibits the high resolution C1s regions from the P(S/PGL) and the reference PS, both exhibiting a main component at 285 eV and a minor one at 291.6 eV assigned to the $\pi \rightarrow \pi^*$ shake-up satellite, a “fingerprint” of the aromatic rings in polystyrene. The main difference between the C1s regions lies in the C-O-C C1s component at 286.7 eV resulting from the polyglycidol chains of P(S/PGL).

Table 1 reports the surface elemental composition of P(S/PGL) and PS, together with the contribution of the C-C/C-H and C-O-C/C-OH carbon types. Using the C/O ratio and the contribution of the C-O-C/C-OH carbon types, it is possible to estimate the surface fraction of PGL chains in P(S/PGL) [5] to ~1.73% PGL. Because there are 35 repeat units of glycidol per PGL, it follows that for 100 styrene repeat units there are about 60 OH groups (1.73 times 35 units). Compared to the initial monomer fractions, the PGL/Styrene molar ratio at the surface is about 4.5 times higher, hence the surface of the particles is PGL-rich.

Tab. 1. Surface elemental composition and contributions to C1s narrow regions for PS and P(S/PGL).

Particles	C	O	S	C-C/C-H ^a	C-O-C/ C-OH
PS	98.3	1.59	0.14	100	-
P(S/PGL)	87.6	12.3	0.1	81.6	18.4

^athe contribution of the $\pi \rightarrow \pi^*$ shake-up satellite is added to that of the main peak component centred at 285 eV.

Fig. 2. Survey scans (a) and high resolution C1s regions (b) taken from P(S/PGL) and PS.

These XPS measurements confirm thus that the PS/PGL microspheres have core-shell morphology, with the surface layer enriched in polyglycidol [5].

Optical properties of P(S/PGL) colloidal arrays

Having established the size, size distribution and surface charges of the particles, we set out to investigate the creation of ordered (crystalline) assemblies. Figure 3 shows a film of the material dried on a hydrophilic glass plate under different illumination conditions. Changing the angle between the light source and the camera allows for the manipulation of the observed color just as changing the sample angle in the spectrophotometer shifts the Bragg peak.

Fig. 3. Photographs of a thin film of colloidal crystal deposited on a hydrophilic glass plate, at various angles between the camera and the film. The variation in color with illumination angle suggests that the color is the result from an ordered material.

SEM images of the surfaces of the colloidal crystals composed of 271 nm P(S/PGL) particles are shown in Figure 4. When viewed at a low magnification (Figure 4a, b), it is seen that the films are composed of many crystalline domains separated by cracks. The domain size can be as large as $20 \times 30 \mu\text{m}$ squared. The formation of the cracks is most likely due to the shrinkage of the self-assembled spherical particles during the drying process. When viewed at a larger magnification (Figure 4c), the spheres appear to be organized into a close-packed face-centered cubic (fcc) structure with the (111) planes parallel to the substrate surface. The crystals are not completely free of point defects. However, these colloidal crystals appear to have a single-crystalline structure.

Fig. 4. SEM micrographs at various magnifications of P(S/PGL) particle assemblies on hydrophilic glass plates.

A representative visible spectrum of this material is shown in Figure 5; it exhibits a "sharp" peak at ~ 570 nm. These optical properties are reminiscent of those observed for colloidal suspensions assembled by charge deshielding of anionic particles.

Fig. 5. Visible spectrum of the photonic crystals of P(S/PGL) particles. The $\lambda_{\max}$ for the Bragg diffraction peak is 570 nm.

In those cases, the observed color was interpreted as diffraction from ordered colloidal arrays that possessed lattice spacing of the order of visible wavelengths, as schematized in Figure 6. The peak at ~ 570 nm represents selective reflection of a narrow band of wavelengths due to Bragg diffraction from the fcc (111) crystal. Because the average refractive index (n_a) of the colloidal crystals should be considered, the basic photonic properties can be quantitatively described by Bragg's law combined with Snell's law:

$$\lambda_{\max} = 1.633(d/m)(n_a^2 - \sin^2\theta)^{1/2} \quad (1)$$

where d is the diameter of the polymer spheres, m is the order of the Bragg reflection, and θ is the angle measured from the normal to the plane of the crystals. The value of n_a is calculated as a weighted sum of the refractive indices of the sphere portion and the gap portion:

$$n_a^2 = \sum n_i^2 \Phi_i \quad (2)$$

where Φ_i is the volume fraction of each i portion. For the closed-packed structure, Φ of the sphere portion is 0.74. At normal incidence, where $\theta = 0$, the spectral position of the peak can be found from

$$\lambda_{\max} = 1.633 (d/m) n_a$$

Judging from the similarity of our results to those reported by others, it is clear that a significant degree of crystalline order must exist for these P(S/PGL) particle assemblies. Crystallinity is also suggested by the decrease in $\lambda_{\max}$ as the angle of the sample with respect to the beam is changed. Such angle dependence brings strong supporting evidence that the color (*i.e.* the diffracted light) is due to crystalline order at optical length scales.

Fig. 6. Schematic illustration of the Bragg diffraction by latex particles assembled in a 3D periodic crystal.

Conclusions

Submicrometer-sized poly(styrene/ α -tert-butoxy- ω -vinylbenzyl-polyglycidol) latex particles, P(S/PGL), were prepared and characterized in terms of surface chemical composition, charge and morphology. These microspheres have a polyglycidol-rich surface and a negatively charged hydrophilic overlayer, which allow them to self-assemble into ordered crystalline arrays. The P(S/PGL) 3D assemblies were found to be organized into face-centered cubic fcc closed-packed structures, with a lattice spacing of the order of visible wavelengths. This structure generates a peak in the visible spectrum, at ~ 570 nm, which represents selective reflection of a narrow band of wavelengths due to Bragg diffraction from the fcc (111). The P(S/PGL) particles assemblies are therefore suitable to be used as photonic crystals and are particularly promising for the design of photonic crystal-based biosensing materials due to the high biocompatibility of polyglycidol.

Experimental part

Preparation of core/shell P(S/PGL) microspheres

The general method of preparation of core/shell P(S/PGL) microspheres has been described in [5]. Briefly, 10 g of styrene (Aldrich) and 1.0 g of α -tert-butoxy- ω -vinylbenzyl-polyglycidol ($M_n=2800$, $M_w/M_n=1.05$), $K_2S_2O_8$ (0.2 g), and H_2O (125 g, 3 times distilled and pH adjusted to 6.8 by the addition of K_2CO_3 and degassed) were put into the reactor. The polymerization was carried out at $65^\circ C$ for 27 h. Then, the microspheres were purified by steam stripping, repeated centrifugation and washing with pure water until the supernatant was free of impurities detected spectroscopically (HP 8452A UV-VIS diode-array spectrometer).

Reference polystyrene microspheres were synthesized and purified in the same way

Determination of sulfate groups at the surface of P(S/PGL) microspheres

The concentration of acidic groups on the surface of PS/PGL microspheres was determined by conductometric titration. Prior to surface charge density

measurements, the sample was flushed through a cation-exchange resin (Dowex 50WX4). Then, the conductometric titrations of the microsphere suspensions (0.10 g of microspheres in 2.0 ml of water) were performed against a solution of potassium hydroxide (with known titer).

Preparation of P(S/PGL) colloidal arrays

The glass plates were rendered hydrophilic following the procedure described by Yan *et al.* [15] The colloidal assemblies were obtained by placing a 40 μ L drop of the colloidal suspension of a given concentration on the hydrophilic glass plates and carefully spreading it to fully cover the substrate surface. The 3D assemblies were left to dry at room temperature for 1 h.

Set up for digital photographs

The P(S/PGL) colloidal arrays were photographed using a digital camera. The set up permitted to catch up the diffracted light from the particles-coated glass plates under various angles.

XPS

Surface chemical compositions were determined by means of X-ray photoelectron spectroscopy (XPS). The dried latex samples were mounted onto a powder holder. XP spectra were recorded using a Thermo VG Scientific ESCALAB 250 system fitted with a monochromatic Al K α X-ray source (1486.6 eV) and a magnetic lens which increases the electron acceptance angle and hence the sensitivity. An X-ray beam of 650 μ m was used at 20 mA and 15 kV. The spectra were acquired in the constant analyzer energy mode with pass energy of 150 and 40 eV for the survey and the narrow regions, respectively. Charge compensation was achieved with an electron flood gun operated in the presence of argon at a partial pressure of 2×10^{-8} mbar. The *Avantage* software, version 2.20 (Thermo Electron), was used for digital acquisition and data processing. Spectral calibration was determined by setting the C1s peak maximum at 285 eV. The O/C atomic ratios were determined by considering the integrated peak areas of C1s and O1s and their respective sensitivity factors corrected for the analyzer transmission.

SEM

Scanning electron micrographs were obtained with a Jeol 35C scanning electron microscope that is completely controlled from a computer workstation. The filament is zirconated tungsten and the accelerating voltage was set at 20 kV. All specimens were coated with gold prior to analysis in order to avoid or limit static charging effects. The diameter of microspheres and polydispersity factor (D_w/D_n) were obtained from the measurement of about 500 microspheres. The electrophoretic mobility of microspheres, in the electric field, suspended in 10^{-3} M NaCl was obtained from measurement performed by photon correlation spectroscopy (Malvern, Zetasizer 3000HS).

UV-visible

The absorption spectra of the photonic crystals were obtained using a Cary 500 spectrophotometer. The incident angles in the transmission spectra detection were 90 $^\circ$.

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