TOPICAL REVIEW

Engineering of poly(ethylene glycol) chain-tethered surfaces to obtain high-performance bionanoparticles

Yukio Nagasaki\textsuperscript{1,2,3,4}

1 Graduate School of Pure and Applied Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan
2 Research Center for Interdisciplinary Materials Science (TIMS), University of Tsukuba, Tsukuba, Ibaraki, Japan
3 Center for Tsukuba Advanced Research Alliance (TARA), University of Tsukuba, Tsukuba, Ibaraki, Japan
4 Master’s School of Medical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Satellite Laboratory, International Center for Materials Nanoarchitectonics (MANA), National Institute of Materials Science (NIMS), Tennoudai 1-1-1, Tsukuba, Ibaraki 305-8573, Japan
E-mail: yukio@nagalabo.jp

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Abstract

A poly(ethylene glycol)-b-poly[2-(N,N-dimethylamino)ethyl methacrylate] block copolymer possessing a reactive acetal group at the end of the poly(ethylene glycol) (PEG) chain, that is, acetal-PEG-b-PAMA, was synthesized by a proprietary polymerization technique. Gold nanoparticles (GNPs) were prepared using the thus-synthesized acetal-PEG-b-PAMA block copolymer. The PEG-b-PAMA not only acted as a reducing agent of aurate ions but also attached to the nanoparticle surface. The GNPs obtained had controlled sizes and narrow size distributions. They also showed high dispersion stability owing to the presence of PEG tethering chains on the surface. The same strategy should also be applicable to the fabrication of semiconductor quantum dots and inorganic porous nanoparticles. The preparation of nanoparticles \textit{in situ}, i.e. in the presence of acetal-PEG-b-PAMA, gave the most densely packed polymer layer on the nanoparticle surface; this was not observed when coating preformed nanoparticles. PEG/polyamine block copolymer was more functional on the metal surface than PEG/polyamine graft copolymer, as confirmed by angle-dependent x-ray photoelectron spectroscopy. We successfully solubilized the C\textsubscript{60} fullerene into aqueous media using acetal-PEG-b-PAMA. A C\textsubscript{60}/acetal-PEG-b-PAMA complex with a size below 5 nm was obtained by dialysis. The preparation and characterization of these materials are described in this review.

Keywords: nanoparticle, nanodiagnistics, poly(ethylene glycol) tethered chain, gold, quantum dot, porous clay, fullerene

1. Introduction

Recently, nanoparticles have attracted considerable attention as a key material in nanomaterial science and technology [1–6]. Their application is particularly important in biological fields such as bioseparation [7, 8], diagnostics [9, 10], bioimaging [11, 12] and therapy [13–15]. Gold nanoparticles (GNPs) with a size ranging from several
to hundreds of nanometers show a bright pinkish color due to plasmon resonance; they have been used in stained glasses for several hundred years. A simple means of anchoring different ligand molecules onto particle surfaces allows their utilization as colloidal sensors [16–18]. In particular, color changes induced by the aggregation of nanometer-sized gold particles provide a basis for a simple yet highly selective method for detecting specific biological events between anchored ligand molecules and receptor molecules in the milieu. Mirkin and co-workers have shown that colloidal gold particles modified with oligonucleotides form large assemblies through hybridization with complementary oligonucleotide strands, providing a new method for the colorimetric detection of targeted DNA sequences [19, 20].

The growing interest in colloidal GNPs in the bioanalytical field has stimulated the development of their novel preparation methods [21–24]. Most of these methods are based on the reduction of tetrachloroauric acid (HAuCl₄) in an aqueous medium. The thus-prepared GNPs are dispersed in solution via the ion repulsion of ions, such as AuCl₄⁻, adsorbed on their surface [25, 26]. Under physiological salt concentration, however, the ionically stabilized GNPs tend to aggregate because of charge shielding. To improve their dispersibility in solutions with appreciably high ionic strength, various types of low-molecular-weight stabilizers, as well as water-soluble polymers such as starches, are often added to the solution [27]. An alternative way to stabilize such a dispersion is to form a brush layer of hydrophilic polymer strands on the surface of the GNPs. Indeed, a brush layer of poly(ethylene glycol) (PEG) was successfully prepared on GNPs using end-thiolated PEG [28, 29]. Functionalization of the α-end of ω-thiolated PEG was further accomplished so that specific ligands could be added for molecular sensing [30, 31]. α-Lactosyl-ω-PEG-anchored GNPs easily aggregate in response to a lactose-specific lectin, RCA120, and this aggregation can be calibrated for quantitative measurements [29]. Recently, a grafting method that significantly increases the tethered chain density on the surface and paves the way for a new surface modification technology has emerged for the construction of tethered polymer chains on a nanoparticle surface [30, 31].

Sulfanyl (thiol) chemistry has been widely used for the stable modification of gold surfaces. However, the functionality of sulfanyl(thiolate)-modified GNPs in vivo is limited to only a few days because of the limited oxidative stability of sulfanyl species [32–34] and in vivo exchange reactions with sulfanyl compounds such as glutathione. Sulfanyl-modified surfaces are also damaged by exposure to light, high temperatures and oxygen [35]. Thus, the in vivo stability of PEGylated GNPs at the sulfanyl-gold bond is poor.

An alternative method was proposed using water-soluble polymers possessing coordination ability with metal surfaces, such as poly(2-vinylpyridine) and poly(ethyleneimine), as stabilizers for metal colloids [27, 36]. To improve the long-term stability of PEGylated GNPs under physiological conditions, we have used PEG-b-polyamine block copolymers. The polyvalent coordination of amino groups in the side chain of the block copolymer plays an important role in the stabilization of GNPs. This review describes the synthesis of PEG/polyamine block copolymers via our original polymerization methodology and their application to GNP preparation. Other nanoparticles, such as semiconductor quantum dots (QDs), upconversion nanophosphors and inorganic porous clay nanoparticles, can also be prepared using actal-PEG-b-poly[2-(N,N-dimethylamino)ethyl methacrylate] (actal-PEG-b-PAMA) block copolymers. The unique solubilization of fullerenes using actal-PEG-b-PAMA is described.

2. Synthesis of acetal-PEG-b-PAMA via anionic polymerization technique

It is well known that alkali metal alcoholates cannot initiate the anionic polymerization of methacrylate monomers owing to the low nucleophilicity of the alkoxide anion [37]. The alkoxide anion, having a relatively high nucleophilicity, however, initiates the polymerization of methyl methacrylate (MMA). For example, Tomoi et al reported that lithium 2-methoxy ethanolate initiates the anionic polymerization of MMA because the 2-methoxy group in the initiator increases the nucleophilicity of the alkoxide anion due to the intramolecular chelation of the lithium cation [38]. Therefore, a slight change in the initiation reactivity markedly alters the polymerizability of methacrylate monomers during the anionic polymerization.

We have revealed that methacrylate monomers possessing an electron-donating atom at the β-position of the ester group, such as 2-(trialkylsiloxy)ethyl methacrylate (SEMA) [39] and 2-(N,N-diethylamino)ethyl methacrylate (AMA) [40], improve the reactivity of the alcoholate initiator, thereby increasing its polymerization ability (scheme 1). For example, SEMA and AMA are polymerized by potassium ethoxide as an initiator with a controllable molecular weight and its distribution. Thus, several types of methacrylate

![Scheme 1. Schematic diagrams of some of the nanoparticles described in the text. (One of the pictures was reproduced from [45].)](image-url)
polymerization initiated with alcoholates can be achieved by improving the nucleophilelicity of the initiator.

This reaction was extended to the synthesis of PEG-polyamine block copolymers. We have previously demonstrated several types of heterotelechelic PEG synthesis. For example, acetal-PEG-OK was easily prepared via the anionic polymerization of ethylene oxide initiated with potassium 3,3-diethoxypropanolate [41]. Because the end group of the product is potassium alcoholate, it is possible to prepare acetal-PEG-b-PAMA by simply adding AMA monomer to the reaction mixture after the complete consumption of ethylene oxide monomer [42]. The molecular weight, its distribution and composition were confirmed by size exclusion chromatography and nuclear magnetic resonance. Because an acetal group can be easily converted into an aldehyde group through treatment with an acid, the acetal group at the end of the PEG segment in acetal-PEG-b-PAMA can be utilized as a ligand-installation site via a reductive amination reaction. The obtained acetal-PEG-b-PAMA could be applied to a polyeon complex micelle with plasmid DNA to realize targetable gene delivery systems [43, 44].

3. Preparation of bioenvironmentally applicable gold nanoparticles by self-reduction with acetal-PEG-b-PAMA

Wuelfing et al [28] and our group [45] have reported the preparation of highly dispersion-stable GNPs by PEGylation with thiold-ended PEG. Although the S–Au linkage is sufficiently strong (196 kJ mol\(^{-1}\)) [46] for the construction of a dense PEG brush on the colloid surface, it can be easily cleaved through the oxidation of the thiold group [32–34]. This strategy is not suitable when PEGylated GNPs are used under physiological conditions, because biofluid contains numerous oxidation species such as active oxygen groups [35]. Our idea was to explore alternative methods of synthesizing PEGylated GNPs, which can eventually be utilized \textit{in vivo}. A lone pair of an amino group can be coordinated on a gold surface and thus can be used instead of the Au–S linkage. Because the N–Au bond is weaker (25 kJ mol\(^{-1}\)) [46] than the S–Au bond, we employed PEG-polyamine block copolymers, anticipating the polyvalent interaction of amino groups in the polyamine segment of the block copolymer. Our new block copolymer, acetal-PEG-b-PAMA, was applied for the surface modification of GNPs. An unprecedented finding is that the block copolymer facilitated the self-reduction of the auric cations to obtain GNPs without using any additional reducing reagent [47]. The tetrachlorauric acid solution changed from colorless to bright red owing to the formation of colloidal GNPs when an appropriate amount of acetal-PEG-b-PAMA was added to the solution. The tertiary amino groups in the PAMA segment play a crucial role in the reduction of auric cations and anchoring PEG on the surface of the GNPs.

The right insert of figure 1 shows a typical absorption spectrum of a GNP surface plasmon. The GNPs were prepared using acetal-PEG-b-PAMA block copolymers and a molar concentration ratio of the amino group in block copolymer to the aurate cation of [N]/[Au\(^{3+}\)] = 8. The left insert of figure 1 is a transmission electron microscopy (TEM) image of the obtained GNPs. From the TEM image, the average size and the distribution index (D\(_w\)/D\(_n\)) of the obtained GNPs were deduced to be 11.8 nm and 1.01, respectively. Figure 1 is a plot of the GNP size and its distribution index as a function of the [N]/[Au\(^{3+}\)] molar ratio. When this ratio is increased from 4 to 16, the average particle size decreases from 16 to 6 nm. Note that these acetal-PEG-b-PAMA-stabilized GNPs have a substantially narrower size distribution than those prepared by conventional methods, including that using the PEI/PEG graft copolymer. This narrow distribution is an obvious advantage of using block copolymers as stabilizers because they can form a multimolecular micelle structure with a definite association number and a well-defined core-shell architecture, namely, an aurate-complex core covered by a hydrophilic shell. We also found that irradiation with low-frequency atmospheric-pressure plasma of a solution containing tetrachloroauration cations and PEG-b-polyamine block copolymer accelerates the reduction of auric cations to form PEGylated GNPs [48]. The obtained GNPs were purified by centrifugation at 45 000 g for 30 min at 20 °C. The precipitated particles were resuspended in water or in various buffer solutions with a wide range of pH. The centrifugation was repeated several times to remove free block copolymers. No color change was observed, and the transparency of the solution was retained. This behavior clearly indicates the high dispersion stability of acetal-PEG-b-PAMA-anchored GNPs.

Commercially available GNPs prepared using citrates were dispersed in an aqueous solution through the ionic repulsion of surface-adsorbed ions. The citrate-reduced GNPs have an appreciably negative \(\zeta\)-potential (below \(-20\) mV) at neutral pH, yet they undergo aggregation in the acidic region (\(\text{pH} < 5\)) owing to the neutralization of the negative surface charge. On the other hand, the \(\zeta\)-potential of GNPs prepared with acetal-PEG-b-PAMA was close to zero regardless of the pH value. This result indicates that the PAMA segment in the block copolymer coordinates with the gold surface, allowing
the PEG segment to be tethered from the surface into the aqueous exterior to shield the surface charge. The clear phase separation of the PEG layer and the anchored PAMA segment should be an important factor in shielding the cationic charge of the PAMA segment.

To further characterize the state of the polymer on the GNP surface, commercial GNPs were modified using PEG-b-PAMA prepared under various PEGylation conditions [49]. In alkaline media (pH > 10) and with a [N]/[GNP] ratio above 3300, the dispersions of PEGylated GNPs were highly stable against coagulation. The amino groups of the PAMA segment of the block copolymers were completely deprotonated at pH > 10. This means that the PEG-b-PAMA interacted with the GNP surface via multipoint coordination of the tertiary amino groups of PAMA, and not electrostatically. The effect of the number of amino groups in the PAMA segment on GNP surface modification was investigated by ζ-potential and dynamic light-scattering measurements. When the PEGylated GNPs were prepared in an excess polymer solution, almost the same diameter was observed regardless of the PAMA chain length. After the PEG-GNPs were purified by centrifugation, the ζ-potential of all PEG-GNPs was reduced by shielding to almost 0 mV, indicating the effective modification of the GNP surface with PEG-b-PAMA regardless of the chain length. However, the particle size and its distribution for the purified PEG-GNPs were strongly affected by the PAMA chain length. PEG-GNPs with longer PAMA segments underwent coagulation after purification, while PEG-GNPs with shorter PAMA segments had increased dispersion stability. Thermogravimetry measurements confirmed that the PEG density on the GNP surface increased as the number of AMA units decreased to 3. Thus, the dispersion stability depended significantly on the PEG density on the GNP surface. GNPs modified with PEG-b-PAMA having a few AMA units had excellent dispersion stability even in bovine serum albumin (BSA) solution or 95% human serum.

The conformation of PEG-b-PAMA block copolymer on a gold surface was analyzed by angle-resolved x-ray photoelectron spectroscopy (ARXPS) [50]. Conventional PAMA-g-PEG was employed as a control. Figures 2(a) and 2(b) show the ARXPS data for gold surfaces modified with PAMA-g-PEG and PEG-b-PAMA, respectively. The y-axis is the atomic ratio of nitrogen to carbon (N/C) and the x-axis represents the take-off angle of photoemission. As shown in figure 2(a), in the case of the PAMA-g-PEG-modified gold surface, the N/C value was almost the same for take-off angles between 0° and 90°, indicating that the PAMA and PEGA segments homogeneously migrated to all regions of the constructed copolymer layer, as illustrated in the inset of figure 2(a). Different behavior is observed for the PEG-b-PAMA-modified gold surface. The N/C ratio is 0.95 for take-off angles between 45° and 90°, which is almost the same as that for the PAMA-g-PEG-modified gold surface; however, the N/C value decreases for smaller angles. These results suggest a decrease in the number of nitrogen atoms at the top polymer layer, indicating the formation of a phase-separated polymer. In this polymer, the polyamine segments are concentrated at the interface between the PEG layer and the gold surface as depicted in the inset of figure 2(b).

4. Preparation of CdS quantum dots by co-precipitation in the presence of PEG-b-PAMA

The optical properties of quantum dots (QDs) have been intensively studied for the past several decades. Since the use of ligand-conjugated QDs as fluorescent biolabeling reagents was reported in 1998 by the groups of Alivisatos [51] and Nie [52], many approaches to QD applications have been realized in the bioanalytical field such as DNA sequencing, tissue immune-diagnostics and single molecular imaging [53–66]. The advantages of QDs as biolabeling agents are (i) tunability of the emission wavelength by...
changing the particle size, (ii) a sharp and symmetrical emission peak, (iii) the high intensity and long lifetime of the fluorescence and (iv) a wide range of excitation wavelengths. Although the QDs are expected to be important nanomaterials in the bioanalytical field, their dispersion stability in liquids significantly decreases with their decreasing size, particularly in the nanometer range owing to their increased surface area. Another problem is the deterioration of the fluorescence characteristics of QDs in aqueous media, which may be due to surface erosion.

We recently found that acetal-PEG-b-PAMA can be utilized for both the preparation and stabilization of CdS QDs [57]. Figure 3 shows the results of the co-precipitation of CdCl_2 with Na_2S with and without various water-soluble polymers. When CdCl_2 and Na_2S were mixed in water, CdS crystals were formed. However, their size was too large for them to be dispersed in aqueous solutions without any additive. The same result was obtained in the presence of PEG. In the presence of the PAMA homopolymer with a molecular weight of 5000, no precipitation was observed at low salt concentrations. The solution was transparent with a pale yellow color. However, CdS particles precipitated when the salt concentration was increased, indicating the low dispersion stability of the QDs for the PAMA homopolymer. In addition, almost no fluorescence could be detected under 400 nm excitation. In contrast, strong 540 nm emission was observed under the same excitation when CdS QDs were prepared in the presence of the acetal-PEG-b-PAMA block copolymer. The prepared CdS QDs were reasonably stable, and no precipitation was observed in 0.3 M NaCl solution for at least several days.

It is known that the emission from CdS QDs is affected by the surface charge state [58]. Several approaches to fluorescence activation based on the confinement of charge carriers within the QD cores have been reported [59–62]. For example, the coordination of electron-donating compounds such as amines and phosphine oxide significantly increases the fluorescence intensity [63, 64]. In the case of QD stabilization by the PAMA homopolymer, the amino groups in the side chain of PAMA were used not only for coordination on the CdS surface but also for solubilization in the aqueous phase. Thus, PAMA homopolymer is weakly bound to the surface of CdS QDs. For acetal-PEG-b-PAMA block copolymer stabilization, in contrast, the PAMA segments are strongly coordinated to the surface of CdS QDs. This is probably due to the immiscibility of the block copolymer segments with each other, viz., PAMA segments are segregated from PEG segments, settling on the QD surface to minimize interfacial free energy, as in the case of the acetal-PEG-b-PAMA-immobilized GNP described above. Biotin-installed PEGylated CdS QDs also exhibited specific biorecognition, which was monitored via the transfer of fluorescence energy [57].

The preparation of PEGylated nanoparticles using acetal-PEG-b-PAMA can be applied to other materials such as inorganic porous nanoparticles [65], upconversion nanophosphors [66], fullerenes and so forth.

5. Porous clay as a drug delivery

Clay materials have a large surface area, a nanoporous structure and the ability to incorporate various substances in the nanopores to form organoclay composites. These porous clay materials interact with guest compounds mostly via electrostatic forces [67] and hydrogen bonding [68]. The formation of inclusion complexes by these interactions has been widely investigated for various types of guest compounds containing polar functional groups, particularly organic cations.

In pharmaceutical fields, the use of nanoporous clay minerals as a drug delivery system has received considerable attention in view of their structural suitability, inclusion
capacity and spontaneous dispersion in aqueous solutions. However, colloidal dispersions of clay particles tend to flocculate and precipitate in ion-containing solutions. Under physiological conditions, clay dispersions are unstable owing to the high salt concentration and the presence of polyelectrolytes such as proteins. Dispersion stability is one of the important factors for drug carriers because it plays a key role in absorption and bioavailability. Thus, clays do not yet have practical pharmaceutical uses although some of their properties are clearly beneficial. Acetal-PEG-b-PAMA can help solve the above problem, as the coordination of the PEMA segment of the block copolymer on the surface improves the dispersion stability of clay particles.

The dispersion stability of the obtained hybrid was estimated from the optical transmittance, the surface charge determined via ζ-potential measurements and the particle size distributions obtained from dynamic light scattering. The turbidity of the clay/acetal-PEG-b-PAMA solution is dependent on the polymer content, and the solution is transparent when it contains a sufficient amount of acetal-PEG-b-PAMA. Under the same conditions, the size of the complex was almost the same as that of the initial clay particles (about 30 nm). These findings indicate that a suitable amount of the block copolymer disperses clay in aqueous media without any flocculation. The surface charge was completely shielded when the acetal-PEG-b-PAMA/clay ratio reached 2.5, and exhibited a constant value with increasing polymer content. This result is in good agreement with the dispersion stability measured via optical transmittance. In other words, shielding of the surface charge by the PEG segment plays a key role in the dispersion stability in an aqueous solution under the condition of physiological ionic strength. The effect of salt concentration on the dispersion stability was estimated via the transmittance at 500 nm (figure 4). Colloidal particles flocculate and precipitate at salt concentrations greater than 0.1 M in the absence of the polymer, whereas the PEG-treated clay particles are stable at the physiological salt concentration, and the dispersion is completely transparent and independent of the salt concentration.

To evaluate the drug loading capacity, the incorporation of pyrene—a model compound for a hydrophobic drug—in clay was characterized via fluorescence spectra. The fluorescence intensity at 373 nm increased with increasing pyrene concentration up to 5 wt%. Above 5 wt%, the intensity at 373 nm was almost constant, but the intensity at 475 nm gradually increased, which is ascribed to pyrene excimer formation. In the absence of clay, no 373 nm peak was detected because of the low solubility of pyrene in water. Figure 5 shows the release kinetics of pyrene from the acetal-PEG-b-PAMA/clay complex. A sustained release profile was obtained owing to a relatively strong interaction between the pyrene and clay nanocrystals. The release of pyrene from the clay hybrid was slow until day 3 and then became almost linear with time. The pyrene molecules released during the initial stage (until day 20) were in the excimer state. A decline in the release rate was observed after that owing to the consumption of the excimer-forming species.

Its high dispersion stability, even at high salt concentrations, and slow and sustainable drug release kinetics make the acetal-PEG-b-PAMA/clay complex a promising drug carrier.

6. Fullerene-based nanomedicine

The C60 fullerene has attracted much attention owing to its unique cage structure and chemical and physical properties. In addition, fullerenes and their derivatives exhibit high biological activity in vitro. However, their poor solubility in aqueous media hinders their biological application. The modification of fullerenes is one way to improve their solubility. For example, several tens of hydroxyl groups were added to a fullerene molecule to increase its solubility in aqueous media. The functionalization of the C60 fullerene, however,
impairs its properties. We have improved the solubility of fullerene compounds using acetal-PEG-b-PAMA. Since polyanime interacts with fullerenes both hydrophobically and electrostatically, acetal-PEG-b-PAMA effectively solubilizes fullerenes. Figure 6 shows the solubilization characteristics of C_{60} by acetal-PEG-b-PAMA. When C_{60} in water was stirred in the presence of acetal-PEG-b-PAMA, the solution gradually acquired color. With increasing concentration of acetal-PEG-b-PAMA, the size of the complex decreased as demonstrated in figure 6(b). When 1 mg of polymer was added to a 1 mg ml^{-1} solution of C_{60}, the size of the complex was about 150 nm. Almost no light scattering was observed upon the addition of more than 4 mg of polymer to 1 mg ml^{-1} C_{60} solution, implying that the agglomerate size was smaller than the light wavelength. TEM revealed the size of the particles to be below 5 nm under these conditions, as shown in figure 6(a). The solubility of C_{60} in acetal-PEG-b-PAMA aqueous solution reached 214 mg l^{-1} as evaluated by UV spectra, which was much higher than previous reported solubilities, such as 3 mg l^{-1} in lecithin, 58 mg l^{-1} in cyclodextrin and 100 mg l^{-1} in fluoroalkyl end-capped water-soluble oligomers [76].

The bioactivity of C_{60} has been reported. One of its most promising features is its scavenging of reactive oxygen species (ROS) [77–79]. The ROS-scavenging activity of C_{60}/acetal-PEG-b-PAMA was evaluated via a hypoxanthine (HPX)/xanthine oxidase (XOD) reaction using 5-dimethyl-1-pyrroline N-oxide (DMPO) as a spin trapping agent [80–82] (see figure 7). With increasing concentration of the complex, the concentration of super oxide decreased. The half maximal inhibitory concentration (IC_{50}) of the complex was about 360 mg ml^{-1}. Although this was not as high as that of superoxide dismutase (SOD, about 0.27 mg ml^{-1}), it is still promising because the complex was prepared by simply mixing in aqueous media without any chemical functionalization.

7. Conclusions

We have developed the synthesis of a PEG-b-polyamine block copolymer possessing a reactive group at the PEG chain end; the synthesis uses an improved oxyanion as the initiating species. The obtained acetal-PEG-b-PAMA acts as both a reducing and stabilizing agent when mixed with AuCl_{3} in aqueous media. Gold nanoparticles immobilized with PEG-b-polyamine form stable dispersions under physiological conditions. The bonding of nitrogen to the gold surface plays a crucial role in the stable immobilization, which does not occur via electrostatic interaction. Three nitrogen atoms at the end of the PEG chain are sufficient for polyvalent coordination on the gold nanoparticles. The same strategy can be employed for the preparation of PEGylated nanoparticles such as quantum dots, porous clay and nanoparticle materials for light upconversion. Acetal-PEG-b-PAMA also solubilizes the C_{60} fullerene in aqueous media. The size of the C_{60}/acetal-PEG-b-PAMA complex was less than 5 nm under the suitable preparation conditions. The obtained complex showed high dispersion stability and the scavenging of reactive oxygen species. PEG-b-polyamine-immobilized nanoparticles can be utilized
in various applications such as calorimetric bioanalysis, bioimaging, as siRNA carriers and for the stabilization of enzymes.

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