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Research Article

pH Responsive Self-Assembly of Cucurbit[7]urils and Polystyrene-Block-Polyvinylpyridine Micelles for Hydrophobic Drug Delivery

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Polystyrene-block-polyvinylpyridine (PS-b-P4VP) polypseudorotaxanes with cucurbit[7]urils (CB[7]) were prepared from water soluble PS-b-P4VPH$^+$ polymer and CB[7] in aqueous solution at room temperature. At acidic and neutral pH, the pyridinium block of PS-b-P4VP is protonated (PS-b-P4VPH$^+$) pushing CB[7] to preferably host the P4VP block. At basic pH (pH 8), P4VP is not charged and thus is not able to strongly complex CB[7]. This phenomenon was verified further by monitoring the release of pyrene, a hydrophobic cargo model, from a PS-b-P4VPH$^+$/CB[7] micellar membrane. Release study of UV active pyrene from the membrane at different pH values revealed that the system is only operational under basic conditions and that the host-guest interaction of CB[7] with P4VPH$^+$ significantly slows down cargo release.

1. Introduction

Self-assembly of polystyrene-block-poly(4-vinylpyridine) PS-b-P4VP amphiphilic block copolymer has been studied extensively with different metals (because a variety of metal salts can be selectively coordinated to the PVP block) and at different temperatures and pH values [1–8]. PS-b-P4VP is a well-known block copolymer that is commercially available and can be easily synthesized by sequential anionic polymerization of styrene followed by 4-vinylpyridine (4VP) in tetrahydrofuran (THF) at −78°C under nitrogen [9]. Multiple changes in aggregate morphology (spheres, rods, lamellae, and mixture of aggregates) of this block copolymer have been described as a function of pH [7]. The reason for this behavior or morphological complexity can be ascribed to the amphiprotic nature of P4VP where the addition of acid or base introduces ionic groups into the corona chains. Polymer micellar morphologies are useful in the field of controlled drug release, and so we reported the morphological changes and Dox release from PS-b-P4VP micellar membrane at different pH values [8].

Cucurbit[n]urils (CB[n], n = 5–8 of glycoluril units) are a class of barrel-shaped macrocyclic hosts with symmetric carbonyl-lined portals [10–16]. They are capable of forming inclusion complexes with appropriately sized guest compounds in water with high affinity ($K_a > 10^5\,M^{-1}$) [17]. Cucurbit[7]uril (CB[7]) has been shown to form a variety of strong, stable complexes with pyridinium cation type compounds. Polymers with such aromatic moieties promote the creation of a wide range of macromolecular architectures in water, which are held together by host-guest interaction with CB[7]. Pseudorotaxanes, a class of supramolecular species in which a molecular thread is encircled by a molecular bead, have been studied extensively [18–21]. Many reports on CB[7] have been published, including the recognition of CB[7] towards the aliphatic amines ions, pyridylmethyl-ended alkylammonium ions, and other guest molecules. The formation of pseudorotaxanes, rotaxanes, pseudopolyrotaxanes, polyrotaxanes, molecular machines, molecular necklaces, and so forth from CB[7] has also been reported [18–20, 22–24]. Inclusion complexes with CB[7] can induce self-assembly of block copolymer complexes

and result in the formation of systems of pseudorotaxane with different morphologies and physical properties [25]. By complexing stimuli-responsive polymers with CB[7], a wide variety of polymer/CB[7] complexes with amphiphilic properties may be produced. We recently reported the pH sensitive behavior of PS-b-P4VP micelles; however, the host-guest interaction and self-assembly of this block copolymer with macrocycles such as CB[7] have not been reported before [8]. Herein, self-assembly of PS-b-P4VPH$^+$ with CB[7] to afford a controlled release system for hydrophobic cargo is reported. It is a supramolecular approach using CB[7] to slow cargo release, which can be very crucial for drug delivery systems. PS-b-P4VPH$^+$ was used in this study because its self-assembly and morphology at different pH values are extensively studied in the literature. Pyrene was chosen as the cargo as it is a neutral, UV active, and hydrophobic molecule (most drugs are hydrophobic) that is too big to be enclosed by CB[7]. Moreover, CB[7] does not absorb at 245 nm, so it will not affect UV measurements [26]. PS-b-P4VP/CB[7] micellar solution was casted into a membrane and tested under different pH values (Figure 1).

2. Experimental Section

2.1. Materials. Polystyrene-b-poly(4-vinylpyridine) block copolymer (PS-b-P4VP, 81 000-b-57 000 g/mol) was purchased from Polymer Source, Inc. Dimethylformamide (DMF), CB[7], pyrene, and other buffer solutions were used as purchased from Sigma-Aldrich, USA.

2.2. PS-b-P4VPH$^+$·CB[7] Polypseudorotaxanes Complex for NMR. To a stirred solution of 5 mg CB[7] in 1 mL, D_2O/DCl (pD 3) was added a solution of 10 mg of PS-b-P4VP in 0.5 mL d_7-DMF and stirred for 10 hours at room temperature. The solution was filtered and the filtrate was characterized by ^{1}H-NMR, SEM, and TEM.

2.3. pH Responsive Micellar Membrane Preparation. A micellar solution containing 20 wt% PS-b-P4VP block copolymer was loaded with pyrene (0.005 μg per 2 μg polymer) in DMF. Water (0.5 mL) was then added dropwise to the DMF solution and stirred overnight. The mixture was then transferred into a dialysis bag where it was dialyzed in water (pH 3) and CB[7] (5 mg) aqueous solution at room temperature (20°C) overnight. The dialysis bag was then removed and washed with water three times before the contents were lyophilized for 48 hrs. The mixture was then redissolved in a minimum amount of DMF and casted on a glass plate using casting blades with 200 μm gate height. The room temperature was 20°C with 60% relative humidity. The glass plate was then immersed in water to peel the membrane off. The membrane was finally air dried overnight before UV testing. The control solution was prepared using the same method but without incorporating CB[7]. The release test was carried out using 2 mg of the casted membrane and using UV spectrometer Cary 5000, at 245 nm at a series of pH values (pH 4.0 to 8.0) two times on duplicate.

2.4. Instruments. Pyrene release from micellar membrane was measured using UV-Vis spectrophotometer Cary 5000, at 245 nm. Scanning electron microscopy (SEM) images were obtained using a Quanta 600 FEG scanning electron microscope (SEM) from FEI company, USA. The ^{1}H NMR spectrum was recorded on Bruker Avance III (400 MHz) spectrometer, using DMF-d_7 as the solvent. Dynamic light scattering (DLS) measurements were done using the Zetasizer Nano from Malvern Instruments. Transmission electron microscope (TEM) images were obtained using Titan 3 80–300 electron microscope (TEM) from FEI company, USA.

3. Results and Discussion

The complexation of CB[7] with PS-b-P4VPH$^+$ was first verified by NMR. Figure 2 revealed (i) disappearance of styrene peaks, (ii) shifting of pyridine peaks in acidic media, and (ii) broadening of all peaks. This suggests that the motion of both blocks in an aqueous solution is highly restricted and the absence of the styrene peaks (Figure 2(b)) proves the formation of what is called a "frozen micelle" [22, 23], as the NMR cannot detect the hydrophobic core of the particles. When CB[7] was added (Figure 2(c)), a clear shift of the pyridinium block was observed which suggests a complexation between the protonated pyridine and CB[7].

PS-b-P4VPH$^+$ and PS-b-P4VPH$^+$/CB[7] micelles were then characterized using SEM and TEM. More aggregates were observed using TEM in PS-b-P4VPH$^+$/CB[7] sample (Figure 3(b)) versus PS-b-P4VPH$^+$ (Figure 3(a)). A slight difference in terms of morphology between the nanoparticles with and without CB[7] can be seen using SEM. The surface of the polymeric micelles was smooth (Figure 4(a)), while that of the complex was coarse (Figure 4(b)).

DLS measurements showed a distribution of the hydrodynamic diameter with mean values of 76 and 66 nm for PS-P4VPH$^+$ nanoparticles and PS-P4VPH$^+$/CB[7] nanoparticles, respectively (Figure 4). The difference in size of nanoparticles in the dried and solvated phase is consistent with that of a swollen composite polymeric system in aqueous media. The diameter distribution observed by DLS can be explained by interparticle interactions or the statistical distribution of the number of macromolecules within individual particles [27].

3.1. Payload Release Investigation. To investigate the host-guest effect of CB[7] complexation on hydrophobic cargo release, pyrene was loaded into PS-P4VPH$^+$ micelles as described in the experimental section. UV spectrometer was employed to measure the amount of pyrene released into the aqueous solution at a series of pH values (pH 4.0 to 8.0) at room temperature (Figure 5).

Two types of pyrene-loaded membranes were prepared, one with (pH 4′–8′) and the other without CB[7] (pH 4–8) (control experiment). PS-b-P4VP micellar membrane (2 mg) was placed in the bottom of a UV spectrometer cell without stirring. At pH 4-5, negligible amount of pyrene was released from both samples, where at pH 6-7, a very small amount of pyrene <5% was released during a long period of time (Figure 5), which suggests that (i) the encapsulation efficiency

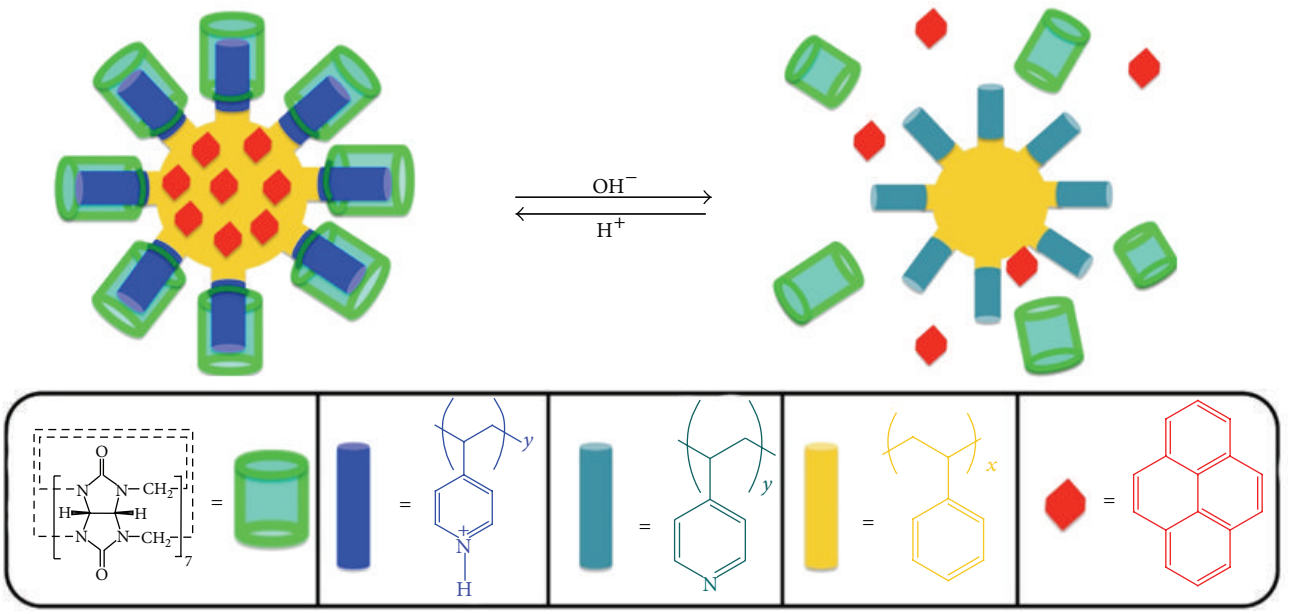

Figure 1: Schematic representation of pyrene-loaded micelles and their release mechanism.

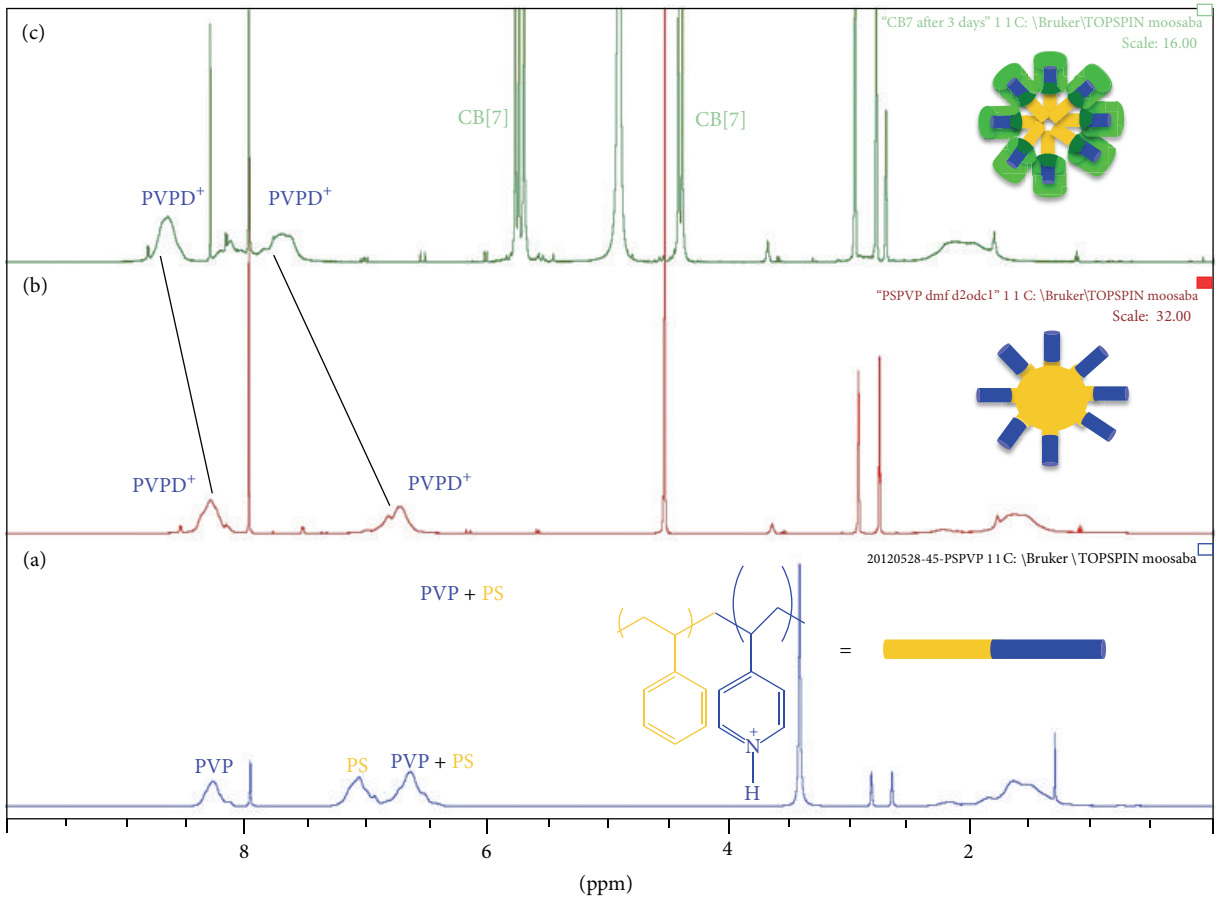

Figure 2: ^{1}H-NMR spectra (500 MHz) of (a) PS-P4VPH (d_7_DMF), (b) PS-P4VPH$^+$ (1 mM in D_2O), and (c) PS-P4VPH$^+$ (1 mM in D_2O) + 0.4 equiv CB[7].

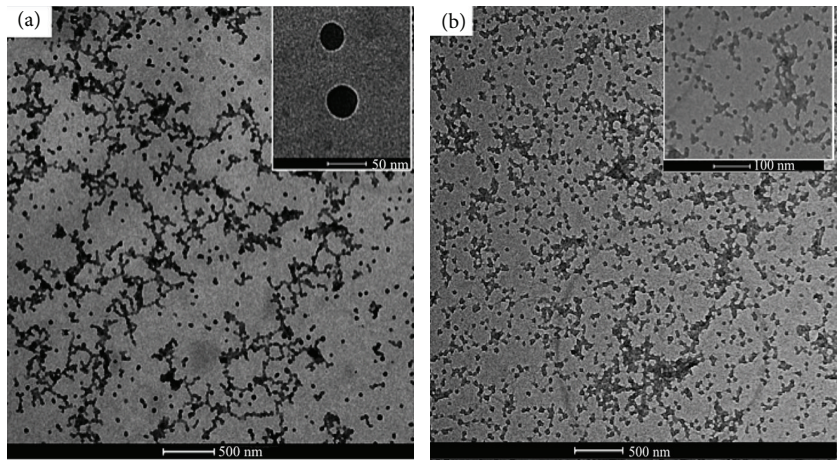

Figure 3: TEM images of (a) PS-P4VPH$^+$ and (b) PS-P4VPH$^+$CB[7] complex where more aggregates can be observed.

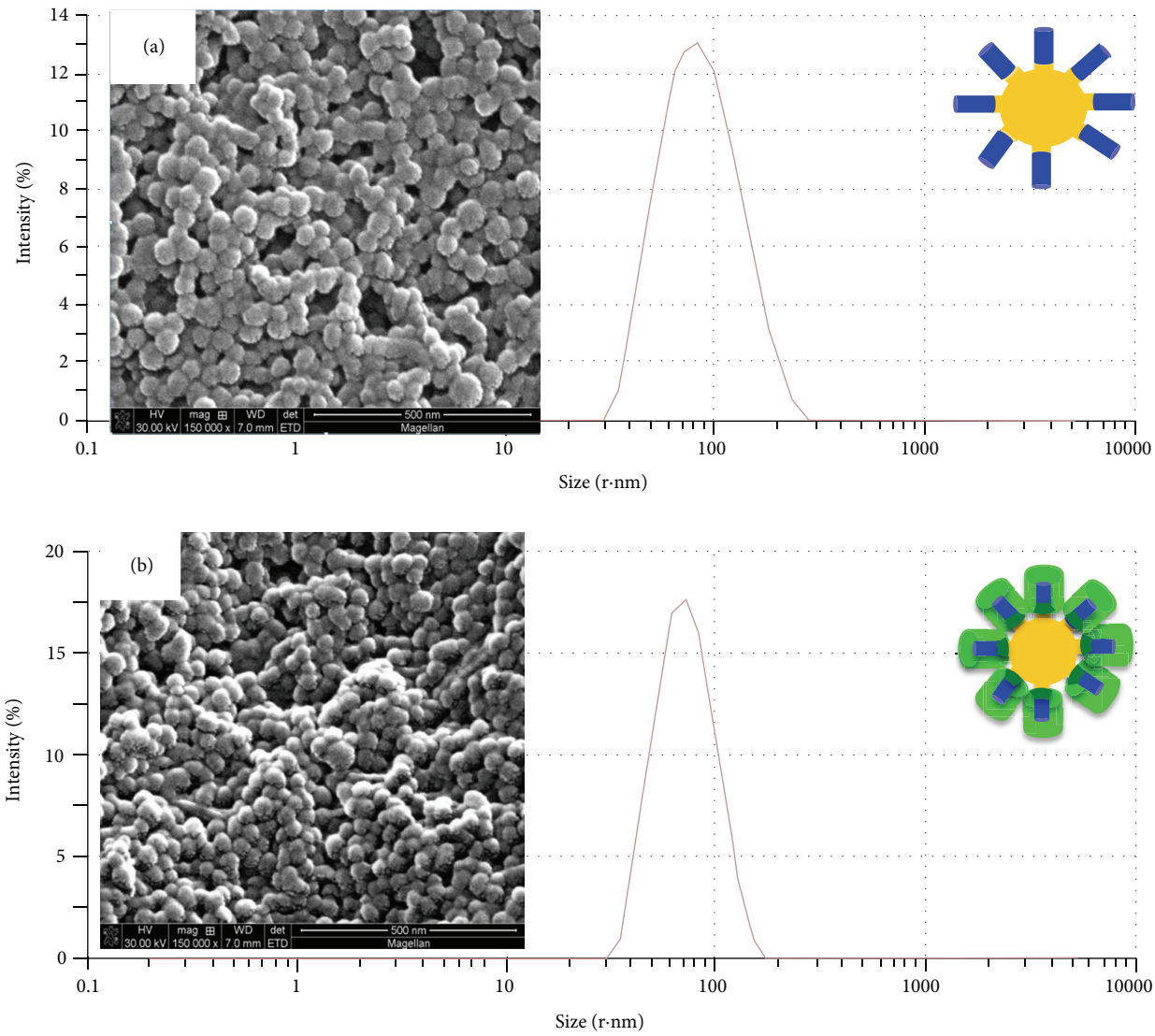

Figure 4: SEM images of (a) PS-P4VPH$^+$ and (b) PS-P4VPH$^+$CB[7] complex.

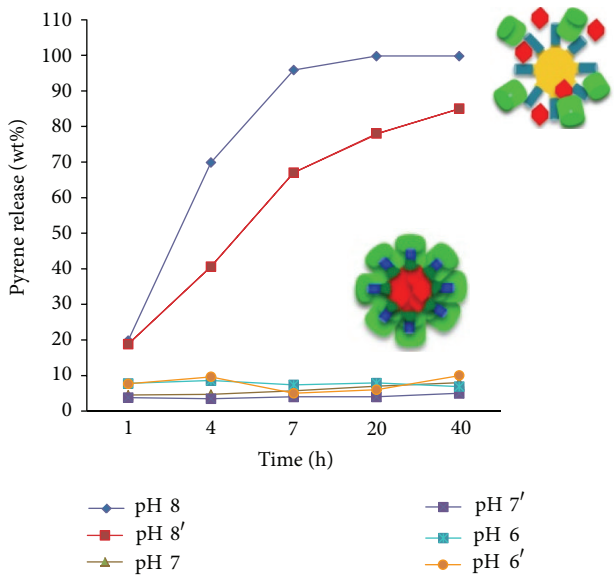

FIGURE 5: Release profile of pyrene at different pH with CB[7] pH(4′–8′) and without CB[7] pH(4–8).

of this membrane is ~95% (no major leakage) and (ii) it is very stable at physiological pH 7.4. At pH 8, a clear release of pyrene was observed which is due to the deprotonation of the pyridinium ion block, which forms the corona shell of the micelle at high pH as we previously reported [8]. The pyridinium molecules shrink and expose the pyrene-loaded PS core to the buffer solution, resulting in a substantial pyrene release (Figure 5). The major difference between the two profiles pH 8 and pH 8′ is that the pyrene release was slower in the presence of CB[7]. This is due to CB[7] threading the pyridinium block which will decrease the deprotonation rate and thus hinders the release of pyrene. To calculate the loading efficiency, the membrane was digested at pH 1 and the amount of released pyrene was measured by UV. Pyrene was loaded into the micellar membrane with a loading efficiency of about 0.02 wt%, which means that every 2 mg of the micellar membrane contains 0.4 μg of pyrene.

4. Conclusion

A supramolecular approach for building a potential controlled drug delivery system is both simple and efficient. CB[7] strongly complexes P4VPH$^+$, making up the corona shell of the micelle, at acidic and neutral pH which affords a closed delivery system with minimal leakage. Under basic conditions, the micelles will shrink and cargo release will be initiated. The presence of CB[7] slows down the release rate and thus provides more control over this system. Designing drug delivery systems that offer prolonged drug release and are only operational under basic pH, such that the pancreatic cancer duct can afford both controlled and targeted release to diseased tissues [28, 29]. Further studies are now underway to incorporate this concept into biocompatible block copolymers for *in vivo* applications.

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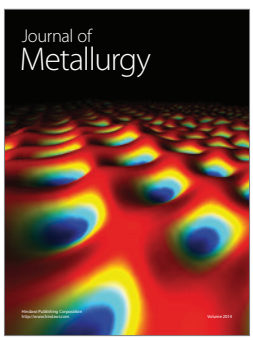

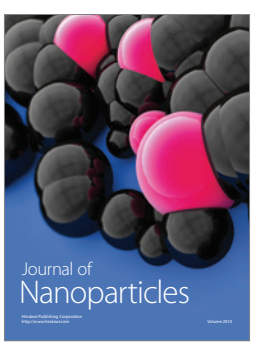

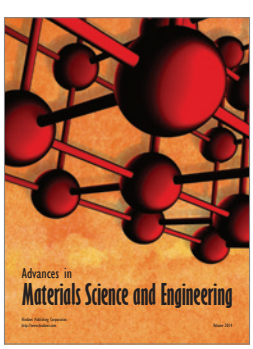

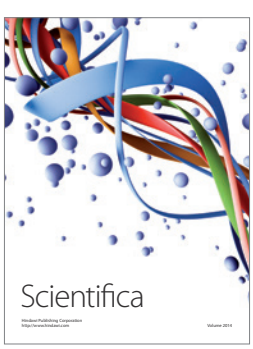

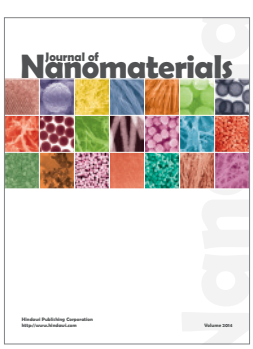

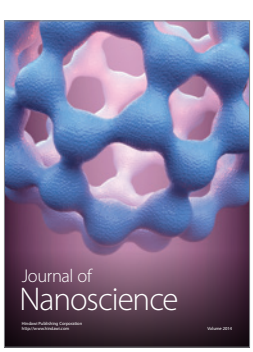

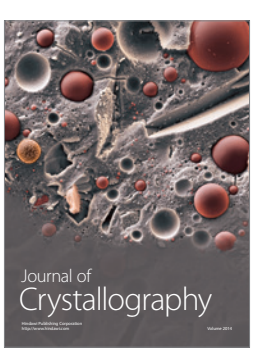

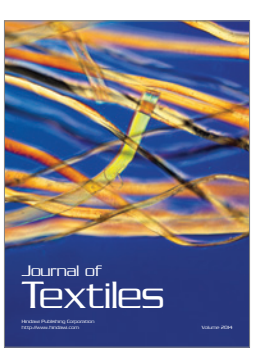

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