I&EC research
Industrial & Engineering Chemistry Research

RESEARCH NOTE

pubs.acs.org/IECR

Controlled Release of Bis(phosphonate) Pharmaceuticals from Cationic Biodegradable Polymeric Matrices

Konstantinos D. Demadis,* Maria Paspalaki, and Joanna Theodorou

Crystal Engineering, Growth and Design Laboratory, Department of Chemistry, University of Crete, Voutes Campus, Heraklion, Crete, Greece GR-71003

Supporting Information

ABSTRACT: Herein, the controlled release of etidronic acid (hydroxyethylidene-bis(phosphonic) acid), an important drug for osteoporotic conditions, immobilized onto cationic polymeric matrices, such as polyethyleneimine (PEI) or cationic inulin (CATIN) is reported. Several CATIN- and PEI-etidronate composites have been synthesized at various pH regions and characterized. Tablets with starch as the excipient containing the active ingredient (polymer-etidronate composite) were prepared, and the controlled release of etidronate was studied at aqueous solutions of pH 3 (to mimick the pH of the stomach) for 8 h. All studied composites showed a delayed etidronate release in the first 4 h, compared to the "control" (a tablet containing only starch and etidronic acid, without the polymer).

Etidronic acid belongs to the important class of antiresorption agents known as bis(phosphonates) (see Figure 1).[1] Among the important structural features of these molecules, one can single out the position of the two phosphonate moieties that are covalently bonded to the same carbon atom. This geminal position renders the bis(phosphonates) organic analogues of pyrophosphate, and thus, potent chelators of Ca^{2+} ions in solution, or of Ca^{2+} embedded in an inorganic biomaterial, such as bone.[2]

The uses and potential applications of bis(phosphonates) are numerous: they are used in the treatment of osteoporosis,[3] Paget's disease,[4] hypercalcemia,[5] breast cancer,[6] and they are also used in dental materials.[7] The significance of bis(phosphonates) drugs notwithstanding, great interest has been noted in supplying the active ingredient in a controlled fashion. Notably, Xu and co-workers have covalently grafted pamidronate onto polymeric chains based on homo- and copolymers of acrylamide.[8] Other phosphonate incorporation techniques (such as polymerization of suitable phosphonate-containing monomers) have been exploited by other researchers.[9] Results originated from our group have shown that when phosphocitrate (a potent anticalcification inhibitor[10]) is combined with Ca^{2+} an inorganic−organic hybrid material can be prepared which releases the active phosphocitrate in a controlled fashion and results in a 3-fold increase in anticalcification efficiency in Wistar rats[11] and a substantial reduction in osteoarthritic overgrowths in guinnea pigs.[12a] Furthermore, calcium phosphocitrate proved powerful in the inhibition of amorphous calcium phosphate-DNA coprecipitates-induced cell death.[12b]

In this paper, we report the immobilization of etidronic acid (used as a model bis(phosphonate) drug) onto cationic polymers polyethyleneimine (PEI, see Figure 1) or cationic inulin (CATIN, see Figure 1), and the controlled release of the active bis(phosphonate) ingredient at pH ≈ 3 (to mimick the pH conditions of the stomach). PEI has been extensively used in the past as a gene delivery agent.[13] The branched PEI used herein, had a MW 70 kDa and ∼25% primary amines, ∼50% secondary amines, and ∼25% tertiary amines. On the other hand, CATIN is a cationic, inulin-based polysaccharide. Inulin is a polysaccharide composed of β 2,1 linked D-fructofuranose units and end-capped with an α-D-glucopyranose unit. Studies have shown that a carboxylate-modified inulin is nontoxic and environmentally friendly.[14] A family of three CATIN polymers was used as matrices: CATIN-1 (with degree of substitution (DS) = 0.22), CATIN-2 (DS = 0.84), and CATIN-3 (DS = 1.28). DS is defined as the average number of quaternary ammonium moieties per monomeric unit.

The immobilization of etidronic acid can be achieved by ionic cooperative interactions between the cationic polymeric matrix and the deprotonated etidronate. Composite materials (PEI-etidronate or CATIN-etidronate) were prepared in water at appropriate pH regions.[15]

A wide pH range was tested for the syntheses, from 2 to 9, in order to synthesize several composite materials as potential controlled release agents. pH affects the deprotonation of etidronic acid and also the protonation of PEI, but not the CATIN family, as the latter possesses a quaternary ammonium group. Taking into account the pK_a values of branched PEI (4.5 for primary, 6.7 for secondary, and 11.6 for tertiary amine groups[16]) it is obvious that at pH regions <5 PEI will exist in its fully protonated form. On the other hand, the pK_a values of etidronic acid are 1.43, 2.70. 7.02, and 11.20,[17] which means that at pH < 7 etidronic acid will possess a "−2" charge. Its negative charge increases as pH increases. Three PEI-etidronate composites were synthesized (at pH 3, 4, and 5, with corresponding P contents of 5.02%, 4.79%, and 4.59%). We were unable to prepare materials at higher pH regions. This is likely because at

Received: December 21, 2010
Accepted: March 17, 2011
Revised: February 26, 2011
Published: March 22, 2011

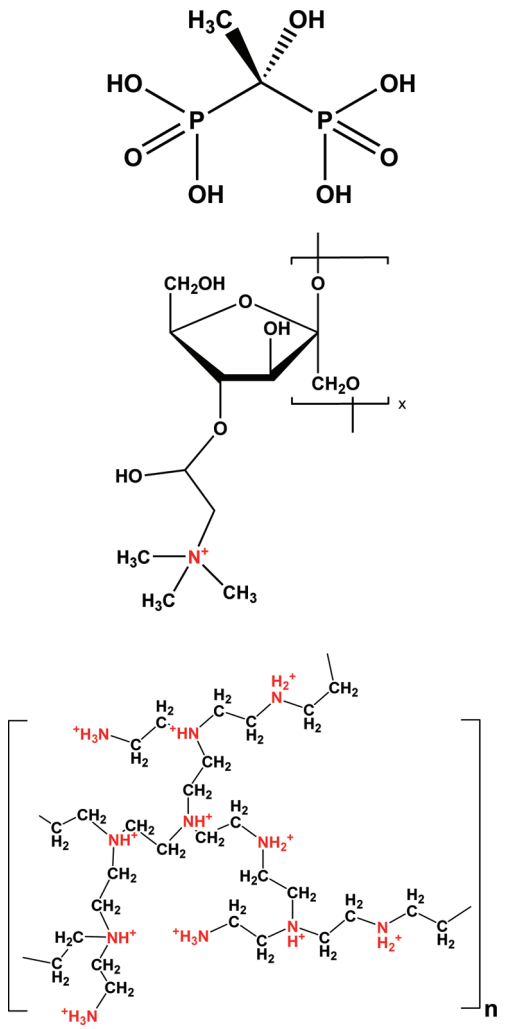

Figure 1. Schematic structures of etidronic acid (hydroxyethylidene-bis(phosphonic) acid, upper), CATIN (middle), and PEI (lower). The positively charged N moieties on CATIN and PEI are highlighted in red.

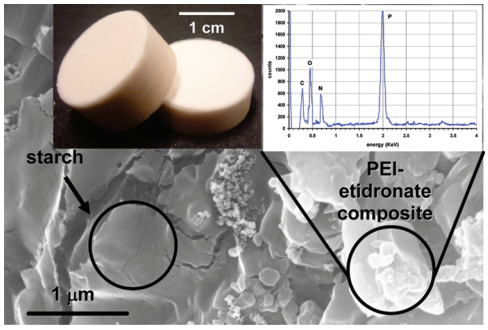

Figure 2. Typical tablet containing polymer−etidronate active ingredient (upper left). SEM image of the tablet surface (lower). Surface particles of the PEI−etidronate composite are highlighted. EDS spectrum of the tablet showing the presence of P (from etidronic acid) and C, O, N from the polymer.

pH > 5 the primary amine groups of PEI are not protonated and cannot interact strongly with etidronate. Apparently, the PEI−etidronate composites that form are too soluble and do not precipitate. We did not pursue such products any further. On the other hand, solid CATIN−etidronate composites were synthesized at pH 7, 8, 9, and 10. A total of 13 composites were prepared (3 PEI- and 10 CATIN-based composites). They were characterized by determining the P content and by FT-IR spectroscopy (see Supporting Information). All CATIN-etidronate composites show bands from the organic polymer chain and those assigned to etidronate in the 900−1200 cm^{-1} region, originating from phosphonate moieties.[18]

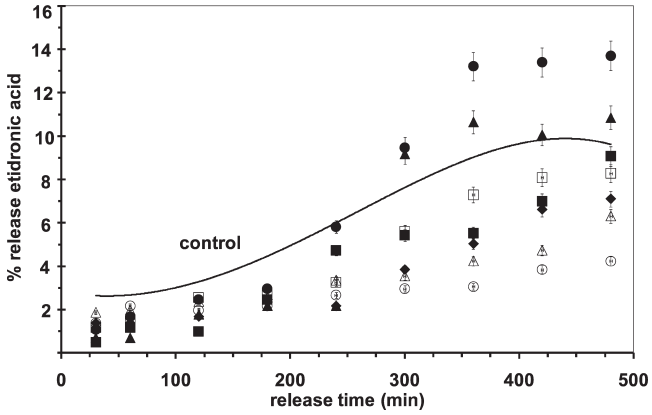

Figure 3. Controlled release curves for the PEI−etidronate and CATIN−etidronate composites. For clarity purposes the average release of free etidronic acid from a starch-only tablet (control) is presented as a continuous line. Filled symbols represent the release of etidronic acid from CATIN matrices and hollow symbols show the release from PEI matrices. Specifically: □, PEI (from pH 3); ○, PEI (from pH 4); △, PEI (from pH 5); ■, CATIN-3 (from pH 9); ♦, CATIN-2 (from pH 7); ●, CATIN-3 (from pH 7); ▲, CATIN-1 (from pH 9).

Controlled release experiments were carried out at pH ≈ 3 and ionic strength 0.1 M (NaCl), in order to mimic the conditions present in the stomach during digestion.[19] Tablets were prepared using starch as the excipient.[20] An image of a typical tablet (typical weight ≈ 1.6 g) is shown in Figure 2. Appropriate amounts of all composites were calculated based on their corresponding P contents, in order for all tablets to contain the same amount of "phosphonate" (see Supporting Information).

The tablets then were used for the controlled release experiments.[21] The released etidronic acid was determined by a well-established spectrophotometric method[22] from aliquots drawn from the experimental solution. Representative controlled release curves for the PEI−etidronate and CATIN composites are shown in Figure 3. A phosphonate release curve for the "control" (starch + etidronic acid) is also included for comparison purposes.

All 13 composite materials exhibited slower phosphonate release than the control, at least in the first 300 min. A number of them produced nearly identical release curves and were not included in Figure 3 (see Supporting Information).

As can be seen, both polymeric cationic matrices enable the controlled release of etidronate. During the first 4 h all tested materials released etidronate at a slower rate than the control. At release times >4 h, the composites CATIN-3-etidronate (from pH 7) and CATIN-1-etidronate (from pH 9) exhibited faster release than the control. All other composites showed a consistently slower release than the control until the last measurement at 8 h. Eventually, these composites release all phosphonate after 2 days. It is noteworthy that the PEI-containing matrices are the most effective, as indicated in Figure 3, showing the slowest release rate.

A few comments regarding the possible underlying mechanism of the demonstrated controlled release of the PEI- and CATIN-etidronate composite materials are in order. Etidronate is immobilized onto the cationic matrices by a combination of electrostatic and hydrogen bonding interactions between the cationic matrix and the anionic bis(phosphonate). In the case of the PEI-based composites etidronate is immobilized mainly by ionic interactions, combined with strong $-P-O^{-}\cdots H-N^{+}-$ hydrogen bonds.[23,24] In the CATIN-based composites the etidronate-matrix ionic interactions have to be weaker, as the cationic charge is "burried" within the quaternary $-N^{+}(CH_3)_3$ group. Hydrogen bonds can also form between the phosphonate groups and the hydroxyl groups in the fructose ring. Another factor that may be important is that the cationic charge density is much higher in PEI than that in CATIN.[25] The cationic groups in the latter are far apart. The more efficient controlled release of etidronate from the PEI-based composites is certainly related to the stability of the composite.

Among the PEI-etidronate composites (prepared at pH 3, 4, and 5), the one synthesized at the lowest pH (3) appears to release etidronic acid faster than the others. This may be because etidronic acid at the synthesis pH 3 may be partially in its monodeprotonated form ("−1" charge), and thus forming weaker electrostatic attractive forces with the PEI matrix. Of the other two PEI−etidronate composites the one prepared at pH 4 appears to release etidronate slower than that prepared at pH 5. This can be explained by re-examining the pK_a values of PEI and etidronic acid. The latter exists in its bis-deprotonated form ("−2" charge) between pH 4−5. However, only at pH 4 all amine groups of PEI are protonated as the pK_a for primary amines is 4.5 (it is 6.7 for secondary, and 11.6 for tertiary amine groups). When synthesis is performed at pH 5, at least some (if not all) primary amine groups are *not* protonated. This certainly creates weaker amine−etidronate interactions, and thus more rapid release.

An interesting theme to discuss is the effect of DS (degree of substitution) on the release rates of the CATIN−etidronate composites. Based in Figure 3, the slower release of etidronic acid is demonstrated for the CATIN-2 (prepared at pH 7) matrix, followed by the CATIN-3 (prepared at pH 9) matrix. CATIN-2 has DS = 0.84 and for CATIN-3 the DS = 1.28. At pH > 7, etidronic acid will have a "−3" charge, whereas the charge of the CATIN polymers will be unaffected by pH. Note that the two composites contain approximately the same loading of etidronic acid.[26] A plausible explanation for the slower release of the CATIN-2-etidronate versus the CATIN-3-etidronate composite is that in CATIN-2 a greater number of hydroxyl groups (from the fructose rings) are available for hydrogen bonding than in CATIN-3. This may induce greater stability of the composite, hence slower release of etidronic acid. The same arguments can be put forth for the CATIN-1-etidronate composite. Lastly, it appears that the overall release rate is determined by a combination of cooperative ionic and hydrogen bonding interactions.

Our current efforts focus on extending this chemistry to other biologically relevant phosphonates of the "−dronate" family, as well as preparing composite materials with other cationic matrices containing branched or linear homo- and copolymers.

■ ASSOCIATED CONTENT

Supporting Information. Controlled release for all composites, FT-IR spectra, P analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: demadis@chemistry.uoc.gr.

■ ACKNOWLEDGMENT

K.D.D. thanks the GSRT and the University of Crete Special Research Account for funding and Dr. Adriana Popa (Institute of Chemistry, Romanian Academy, Timisoara) for technical assistance.

■ REFERENCES

(1) Yarom, N.; Lazarovici, T. S.; Elad, S. Oral Bisphosphonate-Related Osteonecrosis of the Jaw: Incidence, Clinical Features, Prevention, and Treatment Recommendations . *Clin. Rev. Bone Miner. Metab.* **2010**, *8*, 27.

(2) Villa-Bellosta, R.; Sorribas, V. Phosphonoformic Acid Prevents Vascular Smooth Muscle Cell Calcification by Inhibiting Calcium-Phosphate Deposition. *Arterioscler. Thromb. Vasc. Biol.* **2009**, *29*, 761.

(3) Viana, M. P.; Tanck, E.; Beletti, M. E.; da Fontoura Costa, L. Modularity and robustness of bone networks. *Mol. BioSyst.* **2009**, *5*, 255.

(4) Polyzos, S. A.; Anastasilakis, A. D.; Terpos, E. Paget's disease of bone: emphasis on treatment with zoledronic acid. *Expert Rev. Endocrinol. Metab.* **2009**, *4*, 423.

(5) Su, M.; Qiu, Y.; Jia, W. A pilot study of antitumor effect of gallium ethylenediaminetetramethylene phosphonate [Ga(III)-EDTMP] in tumor-bearing rats. *Adv. Therapy* **2005**, *22*, 297.

(6) McCloskey, E.; Paterson, A.; Kanis, J.; Tähtelä, R.; Powles, T. Effect of oral clodronate on bone mass, bone turnover and subsequent metastases in women with primary breast cancer. *Eur. J. Cancer* **2010**, *46*, 558.

(7) Girard, S.; Paqué, F.; Badertscher, M.; Sener, B.; Zehnder, M. Assessment of a gel-type chelating preparation containing 1-hydroxyethylidene-1,1-bisphosphonate. *Int. Endodont. J.* **2005**, *38*, 810.

(8) Wang, L.; Zhang, M.; Yang, Z.; Xu, B. The first pamidronate containing polymer and copolymer. *Chem. Commun.* **2006**, 2795.

(9) Renner, C.; Piehler, J.; Schrader, T. Arginine- and Lysine-Specific Polymers for Protein Recognition and Immobilization. *J. Am. Chem. Soc.* **2006**, *128*, 620.

(10) Sallis, J. D.; Demadis, K. D.; Cheung, H. S. Phosphocitrate, a potential therapeutic agent for crystal deposition diseases. *Curr. Rheum. Rev.* **2006**, *2*, 95.

(11) (a) Demadis, K. D.; Sallis, J. D.; Raptis, R. G.; Baran, P. A Crystallographically Characterized Nine-Coordinate Calcium−Phosphocitrate Complex as Calcification Inhibitor In Vivo. *J. Am. Chem. Soc.* **2001**, *123*, 10129. (b) Demadis, K. D. Structure and In Vivo Anticalcification Properties of a Polymeric Calcium−Sodium−Phosphocitrate Organic−Inorganic Hybrid. *Inorg. Chem. Commun.* **2003**, *6*, 527.

(12) (a) Cheung, H. S.; Sallis, J. D.; Demadis, K. D.; Wierzbicki, A. Phosphocitrate Blocks Calcification−Induced Articular Joint Degeneration in a Guinea Pig Model. *Arthritis Rheum.* **2006**, *54*, 2452. (b) Sun, Y.; Reuben, P.; Wenger, L.; Sallis, J. D.; Demadis, K. D.; Demadis, H. S. Inhibition of Calcium Phosphate−DNA Co−Precipitates Induced Cell Death by Phosphocitrates, Front. *Bioscience* **2005**, *10*, 803.

(13) Vicennati, P.; Giuliano, A.; Ortaggi, G.; Masotti, A. Polyethylenimine In Medicinal Chemistry. *Curr. Med. Chem.* **2008**, *15*, 2826.

(14) Johannsen, F. R. Toxicological profile of carboxymethyl inulin. *Food Chem. Toxicol.* **2003**, *41*, 49.

(15) Synthesis of composite materials. An aqueous solution of etidronic acid (60% w/w from ThermPhos, Belgium), PEI (MW 70 kDa, ∼25% primary amines, ∼50% secondary amines and ∼25% amines, from Polysciences, USA) and CATIN-1, -2, and -3 (MW 2 kD, 3 kD, and 3.5 kDa, respectively, from Cosun Biobased Products, Netherlands), aqueous solutions of NH_4OH and HCl (for pH

adjustments), and deionized water were used for the syntheses. A typical synthesis was as follows for CATIN-1-etidronate composite: In a 20 mL beaker, etidronic acid was added (1.1 mL of the 60% solution, 4.85 mmol) and water was added to a volume of 5 mL. The pH was then adjusted to the desired value (9.0 in this case). In another 20 mL beaker, CATIN-1 was added (2.1 mL of 41.4% w/w solution, 0.5 mmol), water was added to a volume of 5 mL, and then the pH was adjusted to the desired pH (9.0 in this case). The aforementioned solutions are then mixed under rigorous stirring. Within minutes the formation of a white amorphous precipitate is noted. The stirring continues for ∼2 h, and the solid is isolated by filtration, washed with ethanol and acetone, and air-dried. The products were kept in a desiccator to avoid hydration. All products were characterized by P analyses by a published procedure,[26] and FT-IR spectroscopy. All FT-IR spectra are given in Supporting Information. The products show characteristic bands of the phosphonate moieties in the 900−1200 cm^{-1} region, as well as bands due to the organic matrix. The IR spectrum of pure PEI shows a severely broadened band centered around 3500 cm^{-1}, due the N-H stretching modes. This band (albeit less broad) is present in the PEI−etidronate composites. Two other bands (∼1650 and 1450 cm^{-1}, assigned to the C−C and C−N stretches) are also present in the composites. All three CATIN matrices exhibit identical FT-IR spectra. They show a very broad band at ∼3400 cm^{-1} due to the hydroxyl groups and an intense band (among other weaker bands) at ∼1034 cm^{-1}. These bands are also found in the CATIN−etidronate composites. The bands assigned to the phosponate moieties appear shifted compared to those of an authentic sample of etidronic acid, suggesting that etidronic acid is in a deprotonated form in the composites.

(16) (a) Willner, I.; Eichen, Y.; Frank, A. J.; Fox, M. A. Photoinduced electron-transfer processes using organized redox-functionalized bipyridinium-polyethylenimine-titania colloids and particulate assemblies. *J. Phys. Chem.* **1993**, *97*, 7264. (b) Choosakoonkriang, S.; Lobo, B. A.; Koe, G. S.; Koe, J. G.; Middaugh, C. R. Biophysical characterization of PEI/DNA complexes. *J. Pharm. Sci.* **2003**, *92*, 1710–1722.

(17) Popov, K.; Rönkkömäki, H.; Lajunen, L. H. J. Critical evaluation of stability constants of phosphonic acids. *Pure Appl. Chem.* **2001**, *73*, 1641.

(18) Demadis, K. D.; Papadaki, M.; Raptis, R. G.; Zhao, H. Corrugated, Sheet-Like Architectures in Layered Alkaline Earth Metal R, S-Hydroxyphosphonoacetate Frameworks: Applications for Anti-Corrosion Protection of Metal Surfaces. *Chem. Mater.* **2008**, *20*, 4835–4846.

(19) Paoletti, M. G.; Norberto, L.; Damini, R.; Musumeci, S. Human gastric juice contains chitinase that can degrade chitin. *Ann. Nutr. Metab.* **2007**, *51*, 244.

(20) Preparation of polymer-etidronate tablets. The exact amounts of starch and polymer−etidronate composites used to prepare the tablets are given in Table S-2 in the Supporting Information. Here we will provide details for the preparation of CATIN-1-etidronate-containing tablet. An amount of 1.6 g of starch (Aldrich Chemical Co.) was mixed with 0.0167 g of the CATIN-1-etidronate composite. The mixture was ground vigorously in a mortar-and-pestle until it became a free-flowing powder. This powder was then transferred in a stainless steel press, and a force of 10 N was applied. The outcome was a nearly perfect round disk of diameter 1.5 cm and thickness 0.5 cm. This tablet contains 1.03% composite. On the basis of the measured % P of 9.34% (see Table S-1 in SI), the actual amount of etidronic acid in the tablet was found to be 16.7 mg, corresponding to ∼0.15% of the total tablet weight. Tablets prepared in this fashion were used for the controlled release experiments.

(21) Protocol for the controlled release experiments. The controlled release experiments were carried out at pH ≈ 3 and ionic strength 0.1 M (NaCl), in order to mimic the conditions present in the stomach during digestion.[19] A 1 L volume of an aqueous solution containing 0.1 M NaCl at pH ∼3 was placed in a 1 L Erlenmeyer flask. Using long stainless steel tongues, the tablet (prepared as described above) was carefully placed at the bottom of the flask, without any stirring at all. This was $t = 0$ for the release experiment. Fixed aliquots (10 mL) were drawn from the surface of the flask every hour, and they were analyzed by the procedure described in ref 22.

(22) Ormaza-Gonzalez, F. I.; Statham, P. J. A comparison of methods for the determination of dissolved and particulate phosphorus in natural waters. *Water Res.* **1996**, *30*, 2739.

(23) Demadis, K. D.; Barouda, E.; Raptis, R. G.; Zhao, H. Metal Tetraphosphonate "Wires" and Their Corrosion Inhibiting Passive Films. *Inorg. Chem.* **2009**, *48*, 819.

(24) Demadis, K. D.; Barouda, E.; Zhao, H.; Raptis, R. G. Structural Architectures of Charge-Assisted, Hydrogen-Bonded, 2D Layered Amine···Tetraphosphonate and Zinc···Tetraphosphonate Ionic Materials. *Polyhedron* **2009**, *28*, 3361.

(25) Demadis, K. D.; Stathoulopoulou, A. Solubility Enhancement of Amorphous Silica With Polyamine/Polyammonium Cationic Macromolecules: Relevance to Silica Laden Process Waters. *Ind. Eng. Chem. Res.* **2006**, *45*, 4436.

(26) Popa, A.; Parvulescu, V.; Iliescu, S.; Plesu, N.; Ilia, G.; Macarie, L.; Pascariu, A. Synthesis and characterisations of aminophosphonate styrene-divinylbenzene-silica hybrid materials. *Plast. Rubber Compos.* **2008**, *37*, 193.