Research Article

Multifunctional Silica Nanoparticles Modified via Silylated-Decaborate Precursors

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A new class of multifunctional silica nanoparticles carrying boron clusters (10-vertex closo-decaborate) and incorporating luminescent centers (fluorescein) has been developed as potential probes/carriers for potential application in boron neutron capture therapy (BNCT). These silica nanoparticles were charged \textit{in situ} with silylated-fluorescein fluorophores via the Stöber method and their surface was further functionalized with decaborate-triethoxysilane precursors. The resulting decaborate dye-doped silica nanoparticles were characterized by TEM, solid state NMR, DLS, nitrogen sorption, elemental analysis, and fluorescence spectroscopy.

1. Introduction

Boron neutron capture therapy (BNCT) has been, for many decades, advocated as an innovative form of radiotherapy for diffuse tumors [1–4]. In BNCT, a nuclear reaction occurs when a low energy thermal neutron is absorbed by a nonradioactive boron-10 atom (20% of natural elemental boron). This reaction yields an unstable intermediate, \(^{11}\text{B}\), which immediately undergoes fission \((\sim 10^{-12} \text{ sec})\) generating high linear energy transfer particles \((7\text{Li}^{3+} \text{ and } 4\text{He}^{2+})\) and low energy gamma \((\gamma)\) rays [5, 6]. The advantage of this protocol over other therapeutic procedures lies in the small distance \((\sim 9-10 \mu m)\) traveled by the particles to dissipate their energy, which, thus, confines the damage to the tumor cells. Successful BNCT protocols highly depend on the sufficient and selective boron delivery to the targeted cells. Therefore, a preferential accumulation of boron-10 atoms in the desired location with effective concentrations \((10–35 \mu g \text{ of boron atoms per g of tumor mass})\) is required [6]. Various boron carrier agents have been developed to this end including liposomes [7, 8], dendrimers [9], carbon nanotubes (CNTs) [10], boron nanotubes (BNTs) [11, 12], and magnetic nanoparticles [13, 14] but none have been fully integrated so far.

Although BNCT has demonstrated substantial results in several preclinical studies, this technique has not yet been fully accepted in the armory of tools for tumor treatment. Such delay could be attributed to the differences in the uptake and distribution of \(^{10}\text{B}\) among patients and to the uncertain determination of tumor-to-blood \(^{10}\text{B}\) concentration ratio. Consequently, the real time localization of the BNCT agents and estimation of the boron content inside tumors are required [1]. As such, entities that both contain as many boron atoms as possible (which could be triggered by neutron irradiation and are optically, magnetically, or radioactively active so as to indicate its exact location) and are able to obtain information in view of accumulated dose
are highly desirable. Among the compounds developed for BNCT, momentous attention has been devoted to carboranes. Carboranes are icosahedral cages including carbon, boron, and hydrogen atoms [15–17]. The importance of these cages in BNCT is accentuated by their resistance to biodegradation and facile functionalization with lipophilic moieties in order to permit their specific and efficient delivery. Recently, several approaches have been developed for the synthesis of dual MRI (Magnetic Resonance Imaging)/BNCT agents containing carborane derivatives which allow their localization and the determination of $^{10}$B concentration ratio [18–21]. For example, Goswami et al. have recently described the synthesis of a polyfunctional MRI contrast agent based on single icosahedral-closo-dodecaborate cluster $[\text{B}_{12}(\text{OH})_{12}]$ carrying eleven Gd$^{3+}$-DOTA chelates arms (DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) [22]. A similar strategy has been also developed by Akimov et al. describing the coordination of gadolinium (III) salts by closo-decaborate-based polydentate ligands [23]. Unfortunately, such compounds encounter several drawbacks such as their presumed dissociation upon introduction into in vivo or in vitro systems and their limitations to MRI. To overcome such drawbacks, we propose to establish a covalent link between the decaborate cluster and the luminescent silica carrier agent through the grafting of silylated-decaborate derivatives on the surface of luminescent silica nanoparticles.

Silica-based nanoparticles (SNPs) are considered as viable candidates in theranostics applications (diagnostic and therapy protocols gathered into a single entity) [24–27]. Due to their morphological and structural versatility, their ease of synthesis, and their biocompatibility, these multifunctional systems have an escalating and synergistic alliance with the medicinal and pharmacological fields [28]. For instance, SNPs loaded with fluorophores are considered to be powerful tools for imaging [29] whereas mesoporous silica nanoparticles (MSNs) have dominated the field of drug carriers since their introduction [28]. Moreover, even though their possible toxicity (i.e., liberation of reactive oxygen species (ROS)) and biodegradability remain an issue of debate [30], these nanoparticles have shown numerous advantages over conventional therapy [31]. To date, more than two dozen multifunctional nanoparticle-based agents have been approved for clinical trials [32–34].

We have recently reported the synthesis of the first two members of the decaborate-silane precursor family by exploiting the amino $[\text{B}_{10}\text{H}_{8}\text{N}_{2}]^-$ and carboxyl 2-$\text{B}_{10}\text{H}_{6}\text{CO}^-$ anion derivatives. These silylated precursors were then immobilized on mesoporous SBA-15 silica in significant percentages [35]. Based on these results, we report herein the immobilization of decaborate-silane precursors through covalent grafting to silica (SiO$_2$) probe/carrier agent charged with silylated-FITC (fluorescein isothiocyanate) luminescent fluorophore. The implantation of a luminescent tracer in these decaborate-silica-based nanoparticles will allow obtaining crucial information on the pathway and the localization of these potential boron vehicles in future BNCT applications.

2. Experimental Section

2.1. Materials. All synthetic reactions were performed under an argon atmosphere using vacuum line and Schlenk techniques. All solvents were dried and distilled unless stated otherwise. (NH$_4$)$_2$B$_{10}$H$_{10}$ was purchased from Katchem Ltd., Prague, and was dried under vacuum for 24 hours at 80°C prior to use. 3-Aminopropyltriethoxysilane (98%), N,N-diisopropylthylamine (99%), fluorescein isothiocyanate (98%), 3-isocyanoatopropyltriethoxysilane (95%), oxalyl chloride (2.0 M in CH$_2$Cl$_2$), and tetraethyl orthosilicate (98%) were purchased from Sigma-Aldrich and used as received [36]. The silylated closo-decaborate clusters [PPh$_4$]$_{12}$[B$_{12}$H$_8$NH$_2$CH$_2$CH$_2$NHCONH(CH$_2$)$_3$Si(OCH$_2$H$_2$)$_3$] (PI) and [PPh$_4$][((Pr)$_2$(Et)NH$_2$)][B$_{10}$H$_8$CONH(CH$_2$)$_3$-Si(OCH$_2$H$_2$)$_3$] (P2) were synthesized as previously reported [30].

2.2. Characterization Methods. Elemental analyses were performed on a FLASH EA 1112 CHNS analyzer. Mass spectrometry was carried out on Synapt G2-S (Waters) equipped with an ESI source. The mass spectrum was recorded in the positive mode, between 100 and 1500 Da. The capillary voltage was 1000 V and the cone voltage was 30 V. The temperature sources and dissolution are, respectively, 120°C and 250°C. $^{11}$B, $^{31}$P, and $^{29}$Si NMR spectra in solution were recorded using an AMX 400 Bruker spectrometer operating, respectively, at 128 MHz, 162 MHz, and 80 MHz. Chemical shifts were externally calibrated to TMS in $^{29}$Si, $^3$H$_2$PO$_4$ for $^{31}$P, and EtO$_2$BF$_3$ for $^{11}$B nucleus. Deuterated chloroform or acetonitrile was used as solvent. Transmission Electron Microscopy (TEM) observations were carried out at 100 kV on a JEOL 1200 EXII microscope. Solid state NMR spectra for $^{29}$Si, $^{11}$B, and $^{29}$P were recorded on a Varian VNMRS 300 solid spectrometer with a magnetic field strength of 7.05 T equipped with 75 mm MAS probe at 5 kHz as a spinning rate. Dynamic light scattering analysis was performed using a Courodon Technologies DL 135 particle size analyzer instrument. Cumulant analysis of the correlation function was used to determine the mean hydrodynamic diameter (Z-average) and the polydispersity of each sample. UV-Visible spectra were recorded on a Jasco V-650 spectrophotometer in quartz cells. Fluorescence spectra were recorded at room temperature on an Edinburgh FS920 spectrophotometer. Emission and excitation spectra were corrected for the wavelength response of the system using correction factors supplied by the manufacturer.

2.3. Synthesis of N-(3-(Triethoxysilyl)propyl)-N-fluorescein-thioruea (Si-FITC). Si-FITC was obtained according to a slightly modified literature procedure [37]. To a solution of fluorescein isothiocyanate (FITC) (150 mg, 0.385 mmol) in dry DMF (10 mL), 3-aminopropyltriethoxysilane (85 mg, 0.400 mmol) was added. The mixture was stirred for 4 h at room temperature under argon. The reaction progress was monitored by LCMS and $^{29}$Si NMR. When the reaction was considered to be complete, the solvent was evaporated and the excess of 3-aminopropyltriethoxysilane was removed.
by washing with pentane. After drying under vacuum for 6 hours, 216 mg (92%) of Si-FITC was obtained for a bright yellow to orange solid. $^{29}$Si$^4$H (δ ppm, 79.5 MHz, DMSO-d6): −45 (s). $^1$H NMR (δ ppm, 400 MHz, DMSO-d6): 0.51 (2H, t), 1.21 (9H, t), 1.56 (2H, m), 3.60 (2H, t), 3.91 (6H, q), 6.49–7.60 (9H, m), 10.01 (1H, s), 12.38 (1H, s). LCMS m/z calcd for $\text{C}_{30}\text{H}_{24}\text{N}_2\text{O}_7\text{Si}$ [M+H]$^+$: 611.19, found: 611.19. Anal. Calcd for $\text{C}_{30}\text{H}_{24}\text{N}_2\text{O}_7\text{Si}$: C, 59.00; H, 5.61; N, 4.59. Found: C 58.74; H 5.46, S 4.48.

2.3.1. Synthesis of Fluorescein-Doped Silica Nanoparticles (F$_x$@SiO$_2$Nps) ($x =$ Mole Percentage of Si-FITC). Silica nanoparticles were prepared according to the Stöber method [38]. To a water/absolute ethanol mixture (150 mL: 15 mL), ammonium hydroxide solution (25%) (3 mL) was added. When the stirred solution was stabilized at 30°C, TEOS (9 mL) and, simultaneously, varying amounts of the silylated FITC (Si-FITC) (0.05, 0.10, and 0.15% mole percent, resp.) in DMF (1 mL) were added. The mixture was vigorously stirred for 6 hours at room temperature. Then, the particle dispersion was centrifuged at 15°C (20000 r.p.m.). The resulting yellow solids were washed repeatedly by ethanol (10 mL), sonicated, and centrifuged again. Finally, the products were dried under vacuum at 60°C.

$^{29}$Si CP MAS-NMR: −94 ppm (Q$^2$), −104 ppm (Q$^3$), and −110 ppm (Q$^4$).

Nanoparticles are as follows:

- F$_{0.05}$@SiO$_2$Nps - $S_{\text{BET}}$ (m$^2$/g): 56; $V_p$ (cm$^3$/g): 0.10.
- F$_{0.10}$@SiO$_2$Nps - $S_{\text{BET}}$ (m$^2$/g): 62; $V_p$ (cm$^3$/g): 0.12.
- F$_{0.15}$@SiO$_2$Nps - $S_{\text{BET}}$ (m$^2$/g): 64; $V_p$ (cm$^3$/g): 0.12.
- F$_{0.20}$@SiO$_2$Nps - $S_{\text{BET}}$ (m$^2$/g): 68; $V_p$ (cm$^3$/g): 0.12.

Elemental analyses are as follows:

- F$_{0.05}$@SiO$_2$Nps - Calcd (found): Si 37.91 (37.30), B 3.07 (3.10), C 11.48 (11.81), N 1.32 (1.29).
- F$_{0.10}$@SiO$_2$Nps - Calcd (found): Si 31.64 (31.83), B 5.24 (5.21), C 19.70 (19.57), N 2.25 (2.34).
- F$_{0.15}$@SiO$_2$Nps - Calcd (found): Si 27.03 (27.51), B 6.79 (6.48), C 25.70 (25.90), N 2.93 (2.91).
- F$_{0.20}$@SiO$_2$Nps - Calcd (found): Si 24.44 (24.80), B 3.98 (4.00), C 16.63 (16.90), N 1.14 (1.18).
- F$_{0.25}$@SiO$_2$Nps - Calcd (found): Si 25.90 (25.41), B 6.79 (7.01), C 28.99 (28.49), N 1.93 (1.89).
- F$_{0.30}$@SiO$_2$Nps - Calcd (found): Si 21.46 (22.10), B 8.19 (8.34), C 35.20 (35.97), N 2.36 (2.71).

3. Results and Discussion

Prior to the preparation of luminescent silica nanoparticles (F$_x$@SiO$_2$Nps), the silylated fluorophore (Si-FITC) was synthesized by the reaction of fluorescein isothiocyanate (FITC) with 3-aminopropyltrimethoxysilane (APTES) in dry DMF at room temperature (Scheme 1). The conversion of FITC into the corresponding silylated compound was followed by NMR spectroscopy (ESI, see Figure S1 in Supplementary Material available online at http://dx.doi.org/10.1155/2015/608432) and LCMS. A complete conversion of FITC was achieved in 4 h.

The synthesis of the dye-doped silica particles was performed using a modified Stöber method (Sol-gel process) where tetraethoxysilane (TEOS) and Si-FITC (0.05, 0.10, and 0.15% per mole) were hydrolyzed in the presence of ethanol/water/ammonium hydroxide mixture and maintained for six hours at room temperature under stirring (Scheme 2).

The resulting mixtures were centrifuged and washed repeatedly by ethanol and acetonitrile to yield quantitatively yellow dye-doped silica nanoparticles. The repetitive washing cycles allow the removal of low weight oligomers, adsorbed and unreacted species. The concentration of TEOS to ethanol and water was kept moderate, thus permitting the aggregation of primary particles into a single uniform size with sufficient surface area (or diameter) adequate for post-synthesis surface modification (grafting).

The resulting dye-doped SNPs (F$_x$@SiO$_2$Nps) were analyzed by nitrogen sorption (ESI, Figure S2). A relatively small surface area ($S_{\text{BET}}$ = 56–61 m$^2$/g) and an insignificant pore volume ($V_p$ = 0.09–0.11 cm$^3$/g) were obtained indicating...
Scheme 1: Synthesis of Si-FITC.

Scheme 2: Postsynthesis modification of FITC-charged SNPs with silylated-decaborate precursors P1 and P2.

Table 1: Size of the SiO₂ NPs estimated by TEM and DLS analysis.

<table>
<thead>
<tr>
<th>x</th>
<th>TEM (nm)</th>
<th>DLS (nm)</th>
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<tbody>
<tr>
<td>0.05</td>
<td>225</td>
<td>257</td>
</tr>
<tr>
<td>0.10</td>
<td>250</td>
<td>269</td>
</tr>
<tr>
<td>0.15</td>
<td>258</td>
<td>372</td>
</tr>
<tr>
<td>0.05</td>
<td>230</td>
<td>372</td>
</tr>
<tr>
<td>0.10</td>
<td>270</td>
<td>407</td>
</tr>
<tr>
<td>0.15</td>
<td>275</td>
<td>418</td>
</tr>
<tr>
<td>0.05</td>
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<td>390</td>
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<tr>
<td>0.10</td>
<td>277</td>
<td>412</td>
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<tr>
<td>0.15</td>
<td>285</td>
<td>427</td>
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</table>

The hydrodynamic radius of the decaborate-surface-modified silica nanoparticles was measured by DLS analysis in EtOH and was found to be in the range 350–450 nm with a polydispersity index (PI) between 0.05 and 0.15 (ESI, Figure S4). This underlines the uniform distribution of the particle diameter as observed by TEM (Table 1). The increase in the hydrodynamic diameter after grafting precursors
Figure 1: TEM images of F_{0.10}@SiO\textsubscript{2} Nps.

Figure 2: TEM images of F_{x}P1@SiO\textsubscript{2} Nps (x = 0.05 (a), 0.10 (b)) and F_{x}P2@SiO\textsubscript{2} Nps (x = 0.05 (c), 0.10 (d)).

P1 and P2 indicates covalent coupling of the triethoxysilylated precursors P1 and P2 to the hydroxyl group present on the SiO\textsubscript{2}Nps surface.

\textsuperscript{29}Si, \textsuperscript{31}P, and \textsuperscript{11}B solid state CP-MAS NMR spectra were recorded for all surface-modified nanoparticles. Solid state \textsuperscript{11}B CP-MAS NMR spectra of the decaborate-functionalized luminescent silica nanoparticles F_{x}P1@SiO\textsubscript{2}Nps and F_{x}P2@SiO\textsubscript{2}Nps are quite consistent with the \textsuperscript{11}B NMR spectra of P1 and P2 in solution (Figure 3). These results further confirm the presence of the silylated-decaborate clusters on the surface of the nanoparticles. For instance, \textsuperscript{11}B CP-MAS NMR spectrum of F_{0.15}P1@SiO\textsubscript{2}Nps reveals the presence of three distinct peaks: a peak at \(\sim -8\) ppm (versus 7.5 ppm in liquid NMR) corresponding to the substituted B\textsubscript{1} atom at an apical position, another peak at \(\sim -1.8\) ppm (versus \(-1.2\) ppm in liquid NMR) for B\textsubscript{10} atom position, and a broad peak between \(-10\) and \(-37\) ppm for the remaining eight equatorial B atoms (versus \(-27\) to \(-32\) ppm in liquid NMR). In contrast, two peaks at \(\sim -0.5\) ppm (corresponding to B\textsubscript{1,10}) and between \(-18\) and \(-35\) ppm (B\textsubscript{2} is overlapped with the remaining B\textsubscript{3-9}) are observed for F_{0.15}P2@SiO\textsubscript{2}Nps.

The \textsuperscript{29}Si CP-MAS solid state NMR spectra of F_{0.15}P1@SiO\textsubscript{2}Nps and F_{0.15}P2@SiO\textsubscript{2}Nps give information on the different silicon environment present, where siloxane
peaks $Q^2$, $Q^3$, and $Q^4$ are observed at $-94$, $-105$, and $-111$ ppm, respectively. However, rather small but distinguished peaks corresponding to $T^2$ and $T^3$ environment can be observed at $-56$ and $-69$ ppm (ESI, Figure S5). This is generally attributed to the low percentage of grafted precursors on the surface of the silica nanoparticles. The $^{31}$P CP-MAS NMR spectra of $F_{0.15}P1@SiO_2$ Nps and $F_{0.15}P2@SiO_2$ Nps exhibit a singlet at $-23$ ppm relevant to the presence of the $PPh_4^+$ counterion.

The percentage of incorporated boron atoms was determined from Elemental Analyses (Table 2). The data disclosed a moderate anchoring of the precursors where a maximum value of 1.0% (corresponding to 10% of boron atoms) was attained. It is interesting to note that the anchoring rate for $P2$ exceeds that of $P1$ due to the steric hindrance and longer chain for $P1$.

The optical properties of the Si-FITC-doped particles ($F_x@SiO_2$ Nps) were studied before and after surface modification with decaborate. FITC was chosen since it is a suitable and well-known fluorescent probe for biological studies [39–41]. Indeed, FITC absorbs and emits the visible region of the spectrum, respectively, at 490 nm and 520 nm. The optical properties of fluorescein are inherent to the presence of the xanthene and benzoic acid moieties, which can exist in multiple ionization states [42]. Despite its widespread use in bioassays, this organic dye has well-known deficiencies, such as low resistance to photodegradation, decreased fluorescence intensity upon conjugation with biomolecules, and pH dependent fluorescence properties. The incorporation of Si-FITC into silica matrices has been known to effectively circumvent these issues by improving its photostability and preventing the decrease in fluorescence intensity.

The absorption and emission spectra of the prepared Si-FITC-doped silica nanoparticles are shown in Figure 4. An absorption band at $\lambda = 490$ nm is noticed in the absorption spectra of $F_x@SiO_2$ Nps which corresponds to the fluorescein absorption. The emission spectrum of the $F_x@SiO_2$ Nps exhibits an emission band centered at 520 nm where intensity increases with the Si-FITC content in the silica matrix. It is interesting to note that the modification of the SNPs with closo-decaborate does not significantly modify the absorption and emission spectra, thus maintaining the inherent luminescent properties of the $F_x@SiO_2$ Nps (ESI, Figure S6).

### 4. Conclusion

A new class of decaborate-surface-modified luminescent SNPs has been developed for potential applications in boron neutron capture therapy. To this aim, different percentages (2–10% of boron atoms) of silylated-decaborate clusters ($P1$ and $P2$) were grafted at the surface of SNPs incorporating a fluorescein-based fluorophore in 0.05, 0.10, and 0.15% per mole, respectively. The covalent grafting of the closo-decaborate cluster onto the dye-doped SNPs does not alter the photoluminescence properties of the prepared nanoparticles. These decaborate-modified/luminescent silica nanoparticles...
could be promising prototypes for future BNCT applications allowing the precise localization of these potential boron vehicles. However, improvements have to be considered since only a low content of P1 and P2 was grafted. To increase the content of boron in silica nanoparticles, we propose the cocondensation of TEOS and decaborate-triethoxysilane to produce boron-enriched SiO$_2$ core in addition to surface-modification. Such a modification requires the design and synthesis of bisilylated-decaborate silane precursors which are currently under investigation. Additional research will also be carried out to further increase the solubility of the decaborate-silica nanoparticles in biological media by PEG functionalization for future testing in BNCT.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References


