



OLIGOPEPTIDE BRUSHES GENERATED ONTO SILICA MICROPARTICLES

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A novel route to synthesize oligopeptide chains (oligo(S-benzyl-L-cysteine)) attached onto organic/inorganic hybrid material was developed. Poly[*N*-(β -aminoethylene)acrylamide] (PAEA) adsorbed onto silica particles surface was cross-linked by the reaction between the accessible primary amino groups of the PAEA and 3,3',4,4'-benzophenone tetracarboxylic dianhydride (BTCDA). After the adsorption of PAEA onto microporous silica particles and stabilization by cross-linking, fifteen repeated coupling reactions of boc-S-benzyl-L-cysteine were performed. X-ray photoelectron spectroscopy (XPS), elemental analysis, and thermogravimetric analysis were employed to obtain information about the amount of the amino acid S-benzyl-L-cysteine that was covalently bound to the hybrid particle surface.

INTRODUCTION

Chemical modification of solid surfaces (metal oxides, zeolites, magnetic nanoparticles, colloidal carbon, etc.) by creation of new functional groups produces materials with new properties, which can be utilized in nanofabrication, catalysis, nanolithography, control drug delivery, chromatography, etc. A simple method for controlling and anticipation of desired properties is the adsorption of polyelectrolytes, in monolayer or multilayer, onto various kinds of solid materials.¹⁻¹¹ The presence of reactive groups such as primary amino and carboxylic groups onto solid surface is a prerequisite for further functionalization reactions. Spange and coworkers¹²⁻¹⁷ deposited the poly(vinylformamide-*co*-vinylamine) [P(VFA-*co*-VAm)] onto silica, TiO₂, colloidal carbon, silicon wafers. The authors used fullerene, (4,4'-diisocyanate)diphenyl methane, and other bifunctional cross-linkers to irreversibly fix the polyelectrolyte layer onto the inorganic particle surfaces.¹² It was shown that P(VFA-*co*-VAm) is a highly interesting polyelectrolyte for surface functionalization of inorganic particles because, after coating the inorganic surface with the

polymer, a large number of reactive primary amino groups remain available for subsequent functionalization reactions.^{4,6}

The aim of this work was to investigate the adsorption of poly[*N*-(β -aminoethylene)acrylamide] (PAEA) onto silica particles and step-by-step coupling reactions of a protected amino acid, boc-S-benzyl-L-cysteine, on the silica/PAEA hybrid until an oligopeptide with 15 amino acids in the backbone was formed.

RESULTS AND DISCUSSION

1. Characterization of silica and PAEA

Silica, SiO₂, is a giant atomic structure in which each silicon atom is bonded to four oxygen atoms, each silicon atom being situated in the center of a regular tetrahedron. In the presence of water, the silanol groups (Si-OH) ionize, producing mobile protons that associate-dissociate with the surface. In a wide range of pH, the two species, Si-O⁻ and Si-OH₂⁺, may coexist on the silica surface because the acidity of surface silanol groups can be quite different. The primary amino groups of PAEA can

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be protonated by hydronium ions resulting $-\text{NH}_3^+$ groups. Hence, the adsorption could be driven by a balance of forces, electrostatics ($-\text{NH}_3^+$ and Si-O^-) and hydrogen bondings ($-\text{NH}_2$ and Si-OH).

Potentiometric titrations in water showed that the point of zero charge (pzc) (pH where the potential is zero) was reached at $\text{pH} = 2.5$ for bare silica, and at $\text{pH} = 9.2$ for PAEA (Figure 1).

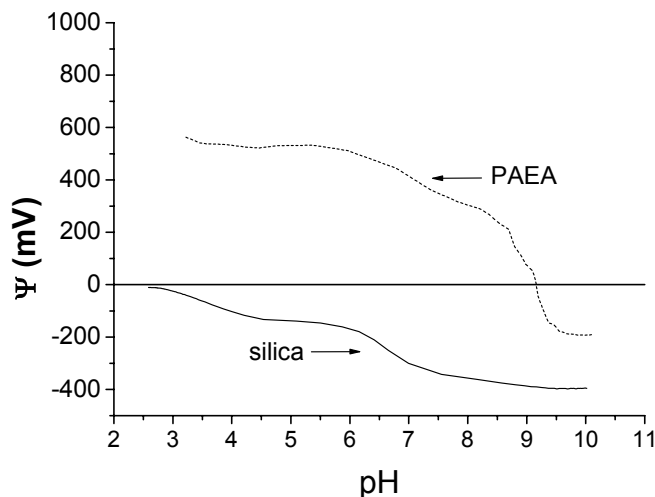


Fig. 1 – Potentiometric titration of bare silica (—) and PAEA (····).

Therefore, at $\text{pH} 7-8$ the silica is negatively charged and PAEA, positively charged, thus the adsorption is driven by electrostatic interactions.

2. Adsorption and cross-linking of PAEA onto silica

The adsorption of PAEA from salt-free aqueous solution increased with PAEA concentration, the plateau region being situated at $\sim 8 \text{ mg/g silica}$.⁵

After 24 hours of PAEA adsorption, the adsorbed amount was nearly the same like after 2 hours (Figure 2), that means the majority of pores ($\sim 5 \text{ nm}$) are inaccessible to the polycation molecules.

The three main thermodynamic parameters include enthalpy of adsorption (ΔH), free energy change (ΔG) due to the transfer of one mole of solute from solution to the solid-liquid interface, and entropy (ΔS) of adsorption. The thermodynamic parameters of the PAEA adsorption from salt-free aqueous solutions onto silica particles were determined using the total organic carbon (TOC) analysis. The standard free energy change ΔG^0 ($\text{kJ}\cdot\text{mol}^{-1}$) was calculated by equation (1):

$$\Delta G^0 = -RT \ln K \quad (1)$$

where: K is the equilibrium constant of the adsorption process, calculated with equation (2):

$$K = (C_i - C_e)/C_e \quad (2)$$

where: C_i and C_e are the initial and equilibrium concentration of the polycation in solution.

The values of ΔG^0 were calculated for $C_{\text{PAEA}} = 197 \text{ mg}\cdot\text{L}^{-1}$ at 24 and 40 °C (Table 1).

The standard enthalpy change (ΔH^0) was calculated using equation (3):

$$\ln \frac{K_2}{K_1} = \frac{\Delta H^0}{R} \left[\frac{1}{T_1} - \frac{1}{T_2} \right] \quad (3)$$

where: K_1 and K_2 are the equilibrium constants of the adsorption process at two different temperatures (T_1 and T_2). The value of ΔH^0 was calculated to be $-63.3 \text{ kJ}\cdot\text{mol}^{-1}$. The negative value of ΔH^0 indicates that the process is exothermic and the sorption behavior is rather physical in nature. The standard entropy change (ΔS^0) was calculated by equation (4):

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad (4)$$

The average value of ΔS^0 was $-222.9 \text{ J}\cdot\text{mol}^{-1}\cdot\text{grad}^{-1}$. It is evident that the enthalpic factor favors the adsorption process ($\Delta H^0 < 0$), but the entropic factor did not ($\Delta S^0 < 0$).

The instability of PAEA adsorbed onto silica is a disadvantage which was eliminated by inter- or intramolecular cross-linking. As we have already shown in a previous article, the silica/PAEA hybrid particles cross-linked with 0.02% BTCDA

appeared to be stable and possessed a sufficient number of free amino groups on the surface.⁵ BTCDA has two anhydride groups that can link

two amino groups belonging to two polymer chains, the idealized structure being shown in Figure 3.

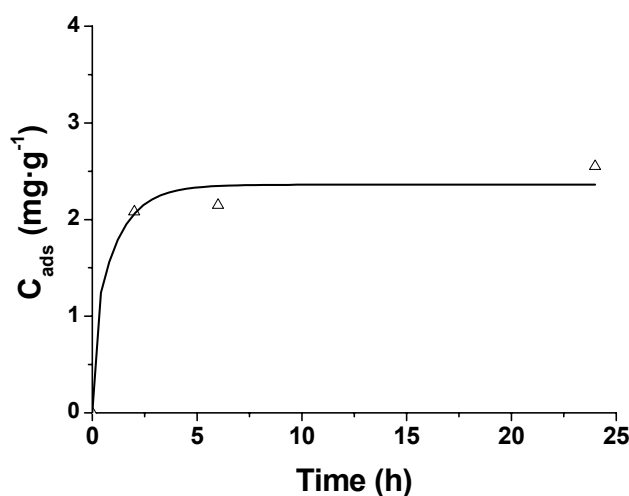


Fig. 2 – Influence of adsorption time on the PAEA adsorbed amount onto silica ($C_{\text{PAEA}} = 2.3 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$). Adsorption was performed from salt-free aqueous solution at $T = 23 \text{ }^{\circ}\text{C}$.

Table 1

ΔG^0 values for PAEA adsorption at two temperatures

$C_i \text{ (mg}\cdot\text{L}^{-1}\text{)}$	$C_e \text{ (mg}\cdot\text{L}^{-1}\text{)}$		K		$\Delta G^0 \text{ (kJ}\cdot\text{mol}^{-1}\text{)}$	
	24 °C	40 °C	24 °C	40 °C	24 °C	40 °C
197	151	182	0.304	0.082	2.940	6.508

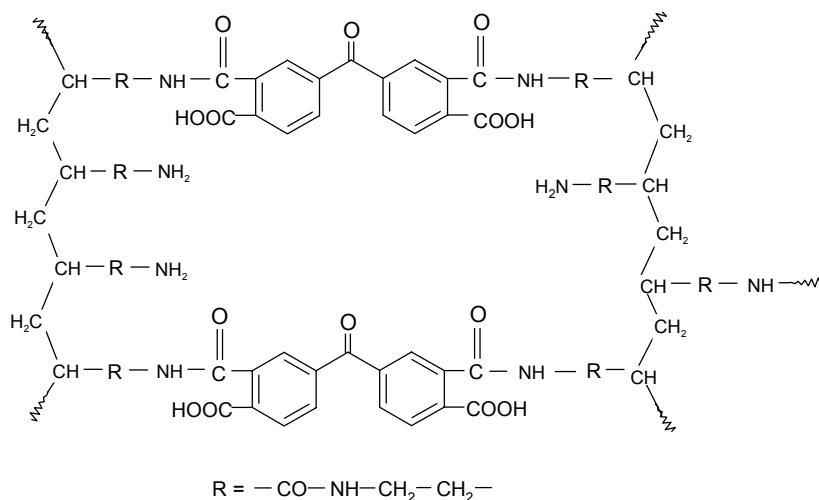


Fig. 3 – The idealized structure of the PAEA-BTCDA network.

3. Step-by-step reaction of coupling of boc-S-benzyl-L-cysteine with the free amino groups on the surface of silica/PAEA hybrid surface

After cross-linking of silica/PAEA hybrid microparticles, the functionalization with S-

benzyl-L-cysteine was carried out according to Figure 4, where DCC was used as the dehydrating agent. XPS, elemental analysis and thermogravimetry were employed to obtain information about the amount of amino acid S-benzyl-L-cysteine which reacts with the free amino

groups on the silica hybrid surface. In the XPS spectra the main important peak is the sulfur 2s, which indicates qualitatively and quantitatively the

presence of the amino acid on the surface of hybrid material.

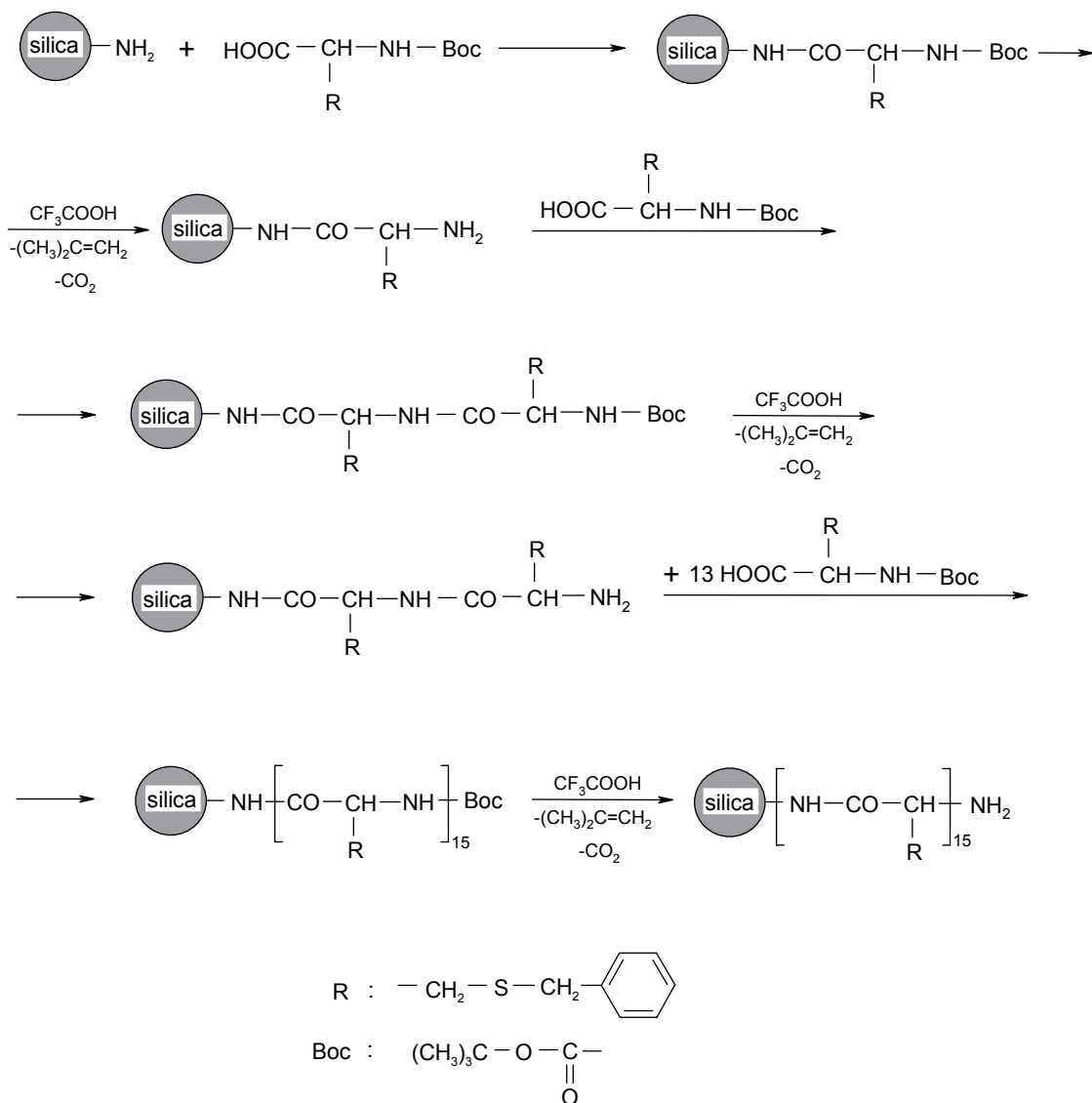


Fig. 4 – The oligopeptide formation onto silica/PAEA surface by step-by-step coupling of boc-S-benzyl-L-cysteine.

After 5-th and 15-th coupling reaction, the hybrid microparticles were characterized using an Phi 5000 VersaProbe photoelectron spectrometer (Figure 5).

The sulfur peaks (S 2s) were quantified to demonstrate the formation of oligo(*S*-benzyl-L-cysteine) in a step-by-step reaction between amino acid *S*-benzyl-L-cysteine and the free amino groups from the surface of silica particles. Each coupling reaction was followed by a deprotection reaction in trifluoroacetic acid (TFA) to form free new amino groups onto the hybrid surface. The oligopeptide formation was supported by the increase of the intensity of the XPS S 2s peak. The

ratio between S 2s peak after the fifteenth and fifth coupling was 3:1, the same like the ratio between number of couplings (15:5 = 3:1), showing a constant and almost linear increase of oligopeptide on the solid surface.

To underline this, the silica/PAEA hybrid microparticles were characterized by elemental analysis. Considering that the amino acid *S*-benzyl-L-cysteine contains 13.44% sulfur, the content of amino acid in each sample (samples obtained after 5-th, 10-th and 15-th couplings of amino acid) was calculated, as it is shown in Figure 6.

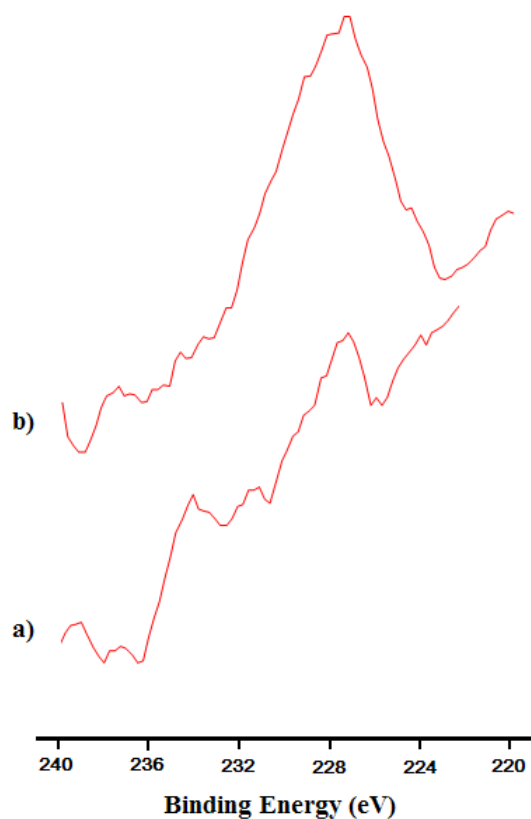


Fig. 5 – The S 2s XPS spectra of the PAEA/silica particles after the fifth (a) and fifteenth (b) coupling of boc-S-benzyl-L-cysteine.

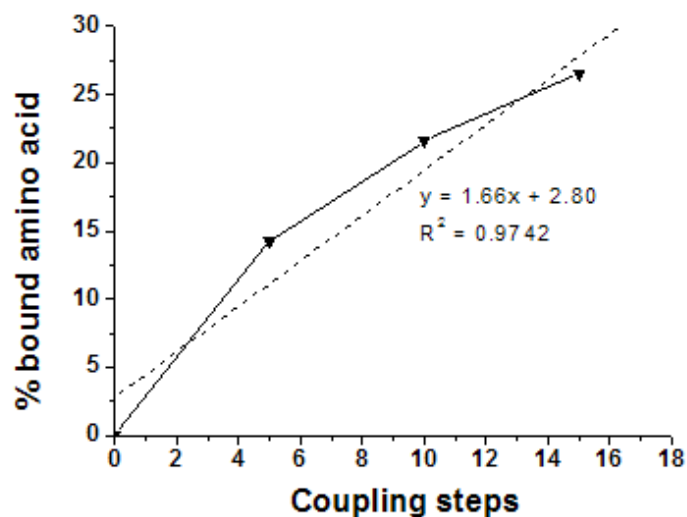


Fig. 6 – The amount of S-benzyl-L-cysteine bound to the silica/PAEA surface.

From the linear fit of the curve represented in Figure 6, a linear regression of 0.9742 was obtained. From this value we can conclude that the synthesis of oligopeptide oligo(S-benzyl-L-cysteine) with 15 amino acids in the backbone depends almost linear on the number of amino acid coupling steps, each amino acid reacting with previously bound amino acid.

Thermal degradation behavior of silica/PAEA before coupling was compared with the silica/PAEA after the 5-th and 10-th coupling of the amino acid S-benzyl-L-cysteine. The TG curves, recorded in inert atmosphere, are presented in Figure 7.

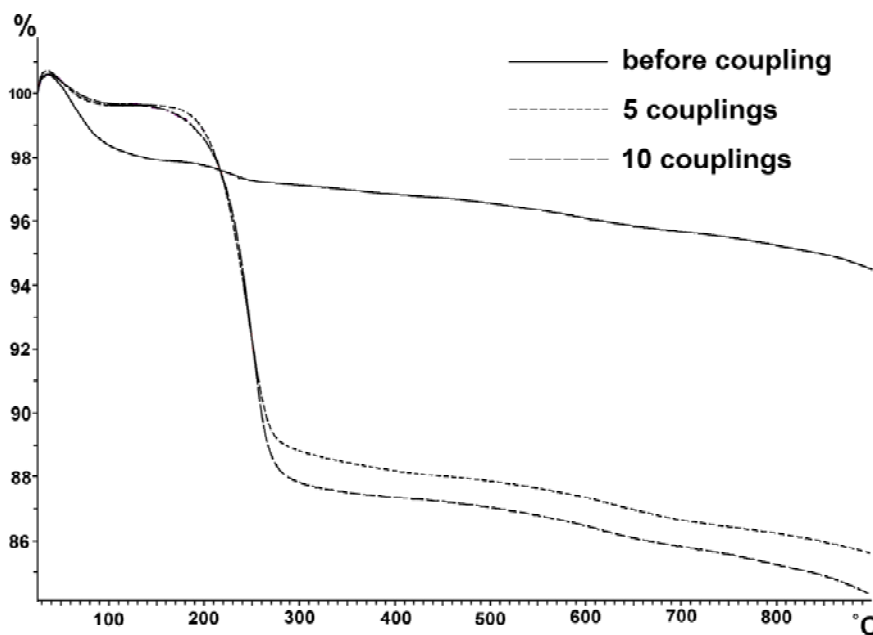


Fig. 7 – TG curves of the silica/PAEA hybrid microparticles before coupling, after the 5th and 10th coupling of the amino acid *S*-benzyl-L-cysteine onto solid surface.

The initial water loss stage below 100 °C has been ignored from discussion. The silica/PAEA with five amino acids on the surface had a mass loss of 11.76% in stage II of degradation (169–267 °C), lower than the sample with 10 amino acids, where the mass loss in stage II was 13.85%. The hybrid microparticles before coupling had a mass loss of 3.28% in stage II (206–900 °C). Thus, the step-by-step coupling reactions of the amino acid *S*-benzyl-L-cysteine on silica surface, forming the oligopeptide, was confirmed.

EXPERIMENTAL

Materials. Silica particles from Merck, Germany, was used as substrate material. The main diameter of particles ranged between 15 and 40 μm. The PAEA was synthesized according to a previous paper.¹⁸ BTCDA, *N,N'*-dicyclohexyl carbodiimide (DCC) from Merck, Germany, and *boc-S*-benzyl-L-cysteine, trifluoroacetic acid and triethyl amine from Fluka, Germany, were used as received.

Adsorption and cross-linking of PAEA onto silica microparticles.

Samples of 3 g of silica were suspended in 150 mL PAEA solution ($5 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$). During the adsorption process over 3 h, the suspension was gently shaken at room temperature. The hybrid materials were carefully washed with distilled water and dried in vacuum at 40 °C for 24 h. The silica/PAEA hybrid particles cross-linked with 0.02% w/w BTCDA (weight of BTCDA per gram PAEA/silica $\times 100\%$) were used in the synthesis of a oligopeptide (oligo-*S*-benzyl-L-cysteine) with 15 amino acids.

Synthesis of oligo(*S*-benzyl-L-cysteine). The formation of a small brush consisting of a short peptide, oligo(*S*-benzyl-L-

cysteine), on the surface of PAEA/silica hybrid particles was carried out according to the Merrifield procedure.¹⁹ For the step-by-step coupling reaction of the protected amino acid *boc-S*-benzyl-L-cysteine, 3 g of the PAEA/silica hybrid particles were suspended into 20 mL solvent mixture dichloromethane (DCM): dimethylformamide (DMF) (1:1). After 10 min, a solution containing 0.64 mmol of *boc-S*-benzyl-L-cysteine was added, followed by the addition of 0.64 mmol DCC. The suspension was shaken for 3 h. After the coupling reaction, the hybrid particles were washed three times with 10 mL of DMF and three times with 10 mL of DCM to remove the excess of reagents and byproducts. To remove the protected group of the amino acid bound to the PAEA/silica hybrid particles, the reaction with a solution of trifluoroacetic acid (TFA) ($c = 25\%$) was carried out. The deprotection reaction was followed by three times washing with triethylamine ($c = 10\%$).

Methods. Total organic carbon (TOC) analysis is a method which allows to indirectly measure the adsorbed polymer amount by determining the amount of carbon in the supernatant solution. The TOC analysis was performed using the TOC 5000-A (Shimadzu of Kyoto, Japan), oxidizing the carbon to CO₂ and measuring the gas concentration by its infrared absorption. TOC analysis is effective to measure carbon concentrations of over 15 ppm (equivalent to about 0.005% of polymer per sample). X-ray photoelectron spectroscopy (XPS) measurements were performed using a Phi 5000 VersaProbe. This system consists of a monochromatic focused Al K α X-ray (1486.7 eV) source and a hemispherical analyzer. The X-ray beam was incident normal to the sample, and the emitted photoelectrons were collected at an emission angle of 45° relative to the sample normal. High-resolution scans were obtained using pass energy of 11.75 eV. Quantitative elemental compositions were determined from peak areas using experimentally determined sensitivity factors and the spectrometer transmission function. The elemental analysis were performed with a PerkinElmer 2400 Series II CHNS/O Elemental Analyzer which determine

the sulfur content in the hybrid silica microparticles. Thermogravimetric analysis (TGA) was performed under nitrogen flow ($20 \text{ cm}^3 \cdot \text{min}^{-1}$) at heating rate $10 \text{ }^\circ\text{C}/\text{min}$ from 25 to $900 \text{ }^\circ\text{C}$ with a Mettler Toledo model TGA/SDTA 851. The initial mass of the samples was 3–5 mg. Operating parameters were kept constant for all the samples in order to obtain comparable data.

CONCLUSION

By the synthesis of an oligopeptide, oligo(S-benzyl-L-cysteine), with 15 amino acids in backbone, on the surface of silica/PAEA hybrid microparticles, we showed the potential application of the new substrate in obtaining peptide brushes.

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