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Grafting of molecularly imprinted polymers on iniferter-modified carbon nanotube

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ABSTRACT

Molecularly imprinted polymers (MIPs) were grafted on iniferter-modified carbon nanotube (CNT). Tween 20 was first immobilized on CNT by hydrophobic interactions. The hydroxyl-functionalized CNT was modified by silanisation with 3-chloropropyl trimethoxysilane. The iniferter groups were then introduced by reacting the CNT-bound chloropropyl groups with sodium *N*,*N*-diethyldithiocarbamate. UV light-initiated copolymerization of ethylene glycol dimethacrylate (crosslinking agent) and methacrylic acid (functional monomer) resulted in grafting of MIP on CNT for theophylline as a model template. MIPs grafted on CNT were characterized with elemental analysis, scanning electron microscopy, and thermogravimetric analysis. The theophylline-imprinted polymer on CNT showed higher binding capacity for theophylline than non-imprinted polymer on CNT and selectivity for theophylline over caffeine and theobromine (similar structure molecules). The data of theophylline and caffeine binding into the theophylline-imprinted polymer correlated well with the Scatchard plot. These MIPs on CNT can potentially be applied to probe materials in biosensor system based on CNT field effect transistor.

1. Introduction

Molecular imprinting has been established as a new technique which allows the creation of tailor-made binding sites for certain molecules such as chiral molecules (Komiyama et al., 2003; Yan and Ramström, 2005). Molecularly imprinted polymers (MIPs) are usually prepared by polymerizing a mixture of a target molecule (template), functional monomers and an excess of crosslinkers, and then removing the template from the crosslinked polymer network. Due to the several advantages of MIP such as low cost, stability, and easy preparation compared with natural molecular recognition products (e.g. antibody), MIPs have been used in electrochemical sensor system (Haupt and Mosbach, 2000; Blanco-López et al., 2004; Merkoci and Alegret, 2002). The use of MIP for the design of electrochemical sensors based on different signal transduction schemes has been reported. The transduction schemes include capacitive, conductometric, voltametric and potentiometric sensors (Merkoci and Alegret, 2002). However, the coupling of MIP with these transducers is still at an early stage.

Recently, carbon nanotube and semiconductor nanowire field effect transistors were successfully shown to be able to detect proteins, tumor markers, small molecules as well as certain bacteria (So et al., 2005, 2008). Park et al. (2006) fabricated single-walled carbon nanotube biosensors as a tool to detect carcinoembryonic antigens, a tumor-associated marker that increases with the onset of colon cancer. In an effort to develop stable and recyclable biosensor system based on CNT field effect transistor using MIP as a probe material to replace antibody or enzyme, we recently reported the immobilization of MIP on the surface of photoinitiator-treated CNT (Lee et al., 2008). However, some bulk polymerization could not be avoided with this technique. It was suggested that the use of iniferter can overcome the problem of polymerization in solution (Rückert et al., 2002). Iniferter (initiator-transfer agent-terminator) is known to be useful for controlling degree of polymerization by reaction time (Hattori et al., 2004). Iniferters have been successfully immobilized on a cellulosic membrane (Hattori et al., 2004), silica and polystyrene-based supports (Rückert et al., 2002). However, the immobilization of MIP on CNT via iniferter has not been reported.

In this study, we demonstrate that an iniferter-modified CNT can be used to prepare MIP exhibiting molecular recognition properties. After the iniferter was coupled to CNT, MIP was formed on the surface of CNT by UV light-initiated copolymerization of ethylene glycol dimethacrylate (crosslinking agent) and methacrylic acid (functional monomer) using theophylline (template). Theophylline was chosen as a model template because it has been used as a template molecule in many MIP studies and the determination method is well established (Lee et al., 2007; Oh and

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Kim, 2007; Lee et al., 2008). Two similar structure molecules, caffeine and theobromine, were used to compare the binding characteristics of theophylline-imprinted polymers. MIP grafted on CNT was characterized with elemental analysis, scanning electron microscopy, and thermogravimetric analysis. The molecular recognition performance of theophylline-imprinted polymer in comparison with non-imprinted polymer was examined by carrying out batch rebinding tests.

2. Experimental

2.1. Materials

Tween 20, methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), theophylline and caffeine were obtained from Aldrich. 3-Chloropropyltrimethoxysilane was purchased from Fluka Chemie AG (Buchs, Switzerland). Sodium *N,N*-

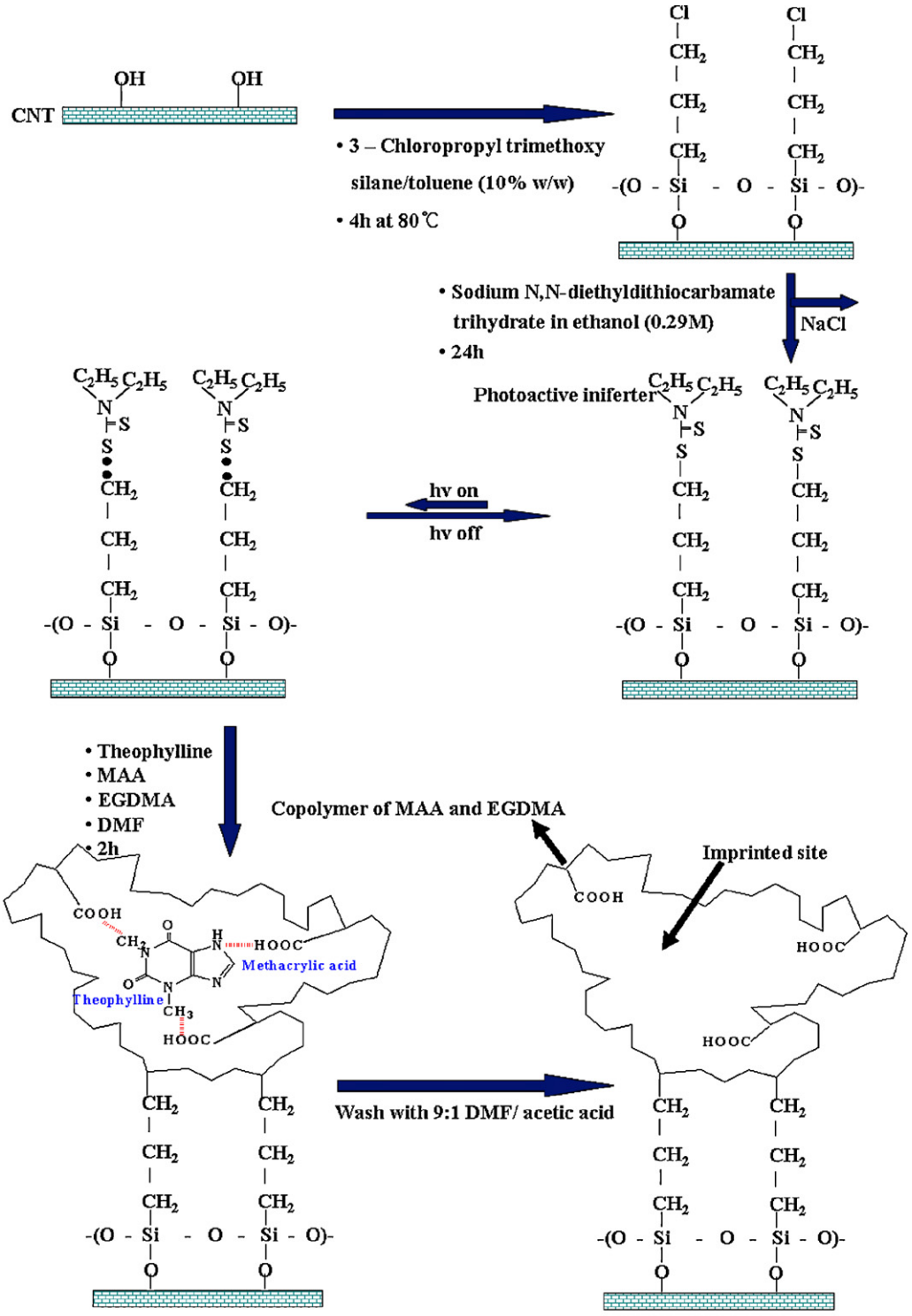

Fig. 1. Procedure for the formation of MIP on CNT.

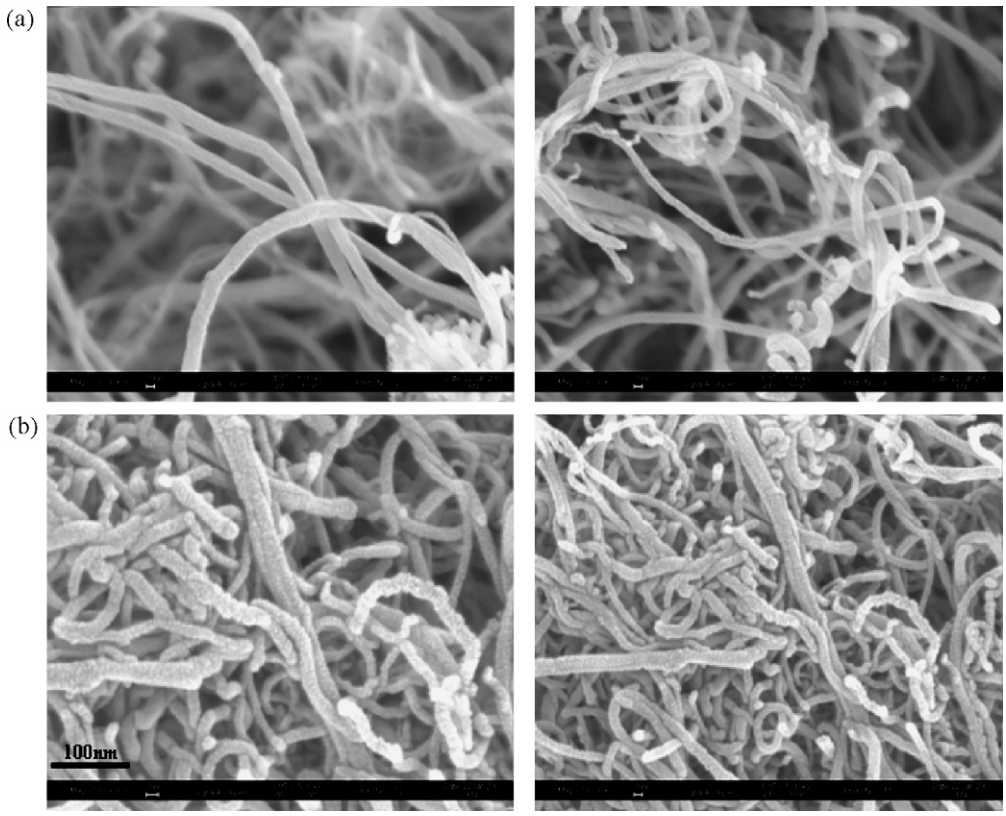

Fig. 2. Scanning electron micrographs of (a) pristine CNT and (b) MIP-grafted CNT.

diethyldithiocarbamate trihydrate was purchased from Wako Pure Chemical Industries (Osaka, Japan). Multi-walled CNT (product No.: CM-95, diameter: 10–15 nm, length: 10–20 μm) was obtained from Hanwha Nanotech Co. (Korea). All other chemicals used were of reagent grade and were used without further purification.

2.2. Grafting of photoactive iniferter on CNT

The method of MIP grafting to the cellulose membrane suggested by Hattori et al. (2004) was modified to immobilize MIP on CNT in this study. First, Tween 20 was immobilized on the sidewalls of multi-walled CNT by hydrophobic interactions between the hydrocarbon chains in Tween 20 and CNT. CNT was mixed with Tween 20 at a ratio of 1:2 (w/w) in distilled water. CNT functionalized with hydroxyl groups was collected by filtration and dried. Then, 3-chloropropyl groups were introduced to the surface of hydroxyl-functionalized CNT using 10% (w/w) solution of 3-chloropropyltrimethoxysilane in toluene treated at 80 °C for 4 h. This activated CNT was soaked in 0.29 M ethanol solution of sodium *N*,*N*-diethyldithiocarbamate trihydrate. The ethanol solution was stirred for 18 h at room temperature, and then the photoactive iniferter was formed on the surface of CNT.

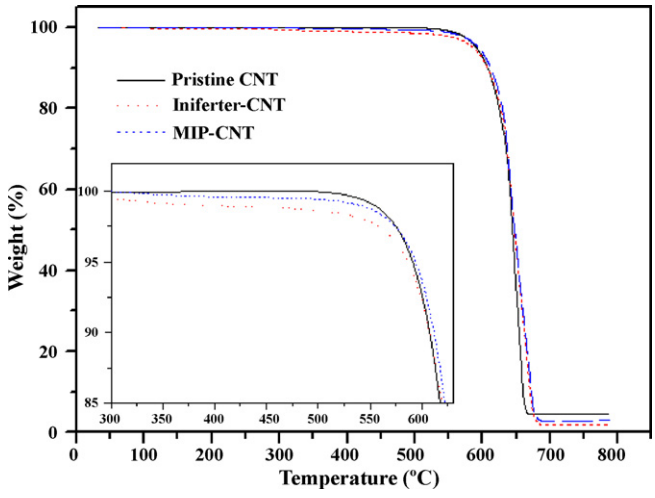

Fig. 3. TGA curves of pristine, iniferter-modified, and MIP-grafted CNT.

2.3. Graft polymerization of the theophylline-imprinted polymer to the surface of CNT

A 1.2 g (13.9 mmol) of MAA, 12.3 g (62.1 mmol) of EGDMA, and 0.63 g (3.5 mmol) of theophylline were dissolved in 33 ml of *N*,*N*-dimethylformamide (DMF). The iniferter-immobilized CNT was soaked in the solution, and then irradiated with a UV lamp GL-20 (Sankyo Denki Co. Ltd., peak intensity at 365 nm) for 2 h to cause radical copolymerization. After photopolymerization, MIP-immobilized CNT was collected by filtration. This was stirred in DMF/acetic acid (9:1, v/v) solution in order to remove weakly adsorbed copolymer and to extract theophylline, and the resultant CNT was dried. Control, non-imprinted polymer (NIP) was prepared without theophylline.

2.4. Characterization

Elemental analyses were performed using elemental analyzer (EA1110, CE Instruments). The polymerized surfaces were examined by scanning electron microscopy (S-2500C, Hitachi). The thermogravimetric analysis (SDT 2960, TA Instruments) was performed under air purging from 30 to 800 °C at heating rate of 10 °C/min.

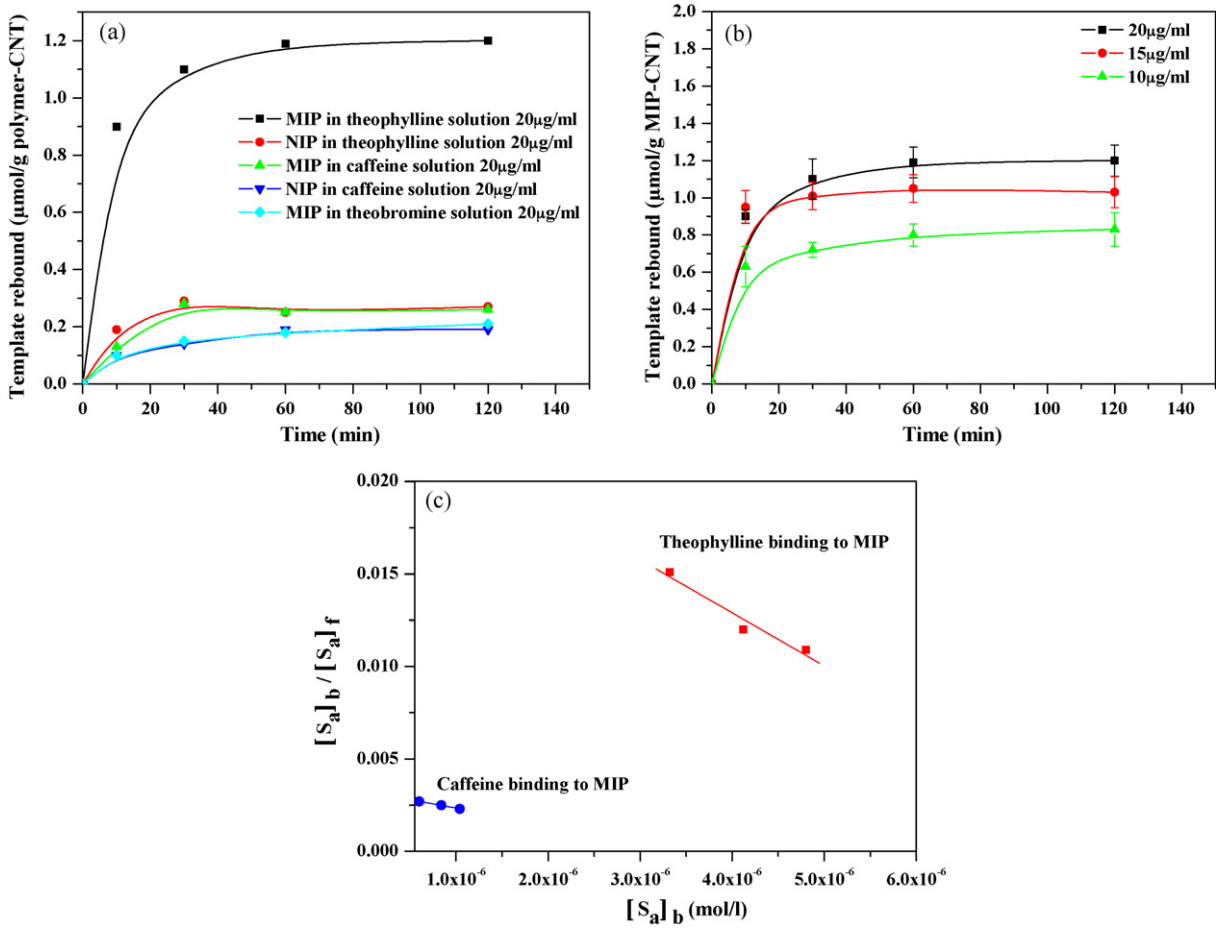

Fig. 4. Binding characteristics of theophylline, caffeine, and theobromine to the theophylline-imprinted polymer (MIP) and non-imprinted polymer (NIP) on CNT. (a) Binding profiles of MIP and NIP with time. (b) Theophylline binding to MIP at various theophylline concentrations ($n = 3$). (c) Scatchard plots of the binding of theophylline and caffeine to MIP.

The rebinding experiment was carried out by incubating MIP and NIP in a 100 ml theophylline, caffeine or theobromine solution (10–20 μg/ml DMF) with shaking at 200 rpm at 25 °C. Theophylline, caffeine and theobromine concentrations were determined by measuring the UV absorbance at 275 nm with spectrophotometer (UV-1650PC, Shimadzu). The binding capacity, defined as μmol template bound per gram polymer-CNT, was calculated by the following equation (Reddy et al., 2002a,b; Lee et al., 2007):

$$[S_a] = \frac{(C_0 - C_t)V}{W}$$

Here $[S_a]$ is binding capacity (μmol/g), C_0 and C_t are the template concentration (μM) measured at initial and after interval time, V and W are the volume of the template solution (100 ml) and the weight of dry polymer-CNT used for the binding experiment (0.4 g), respectively.

3. Results and discussion

3.1. Grafting procedure of MIP on CNT

The whole procedure for the grafting of MIP on CNT is shown in Fig. 1. CNTs were stepwisely functionalized with hydroxyl groups by treating with Tween 20, 3-chloropropyl groups by silane coupling, and then photoactive iniferter by reacting with sodium *N*,*N*-diethyldithiocarbamate. Multi-walled CNT was used because it is easily available and has been successfully employed to immobilize MIP on the surface of photoinitiator-treated CNT in our previous studies (Lee et al., 2008). Tween 20 was chosen as a linking molecule to introduce hydroxyl groups on the surface of CNT due to the following reasons. First, the hydroxyl groups are necessary for introducing 3-chloropropyl groups by silane coupling. Second, it was shown in our previous studies that Tween 20 was successfully immobilized on the sidewalls of CNT by hydrophobic interactions between the hydrocarbon chains in Tween 20 and CNT (Lee et al., 2008). MIPs were formed by photografting polymerization upon UV irradiation in the presence of theophylline with MMA and EGDMA and washing for the removal of theophylline.

The grafting techniques have been suggested to decouple the imprinting step from the generation of a particular morphology, which is important to generate high affinity binding sites while simultaneously controlling the porous properties, morphology or other structural features of the polymers (Rückert et al., 2002). The method of MIP immobilization on the surface of photoinitiator-treated CNT, recently reported by us (Lee et al., 2008), caused some problems because the desired graft polymerization is invariably accompanied by polymerization in solution. Iniferter acts not only as an initiator but also as a retarder, terminator, and transfer agent, inhibiting ordinary bimolecular termination and other transfer reactions (Hattori et al., 2004). Iniferter decomposes to give two different radicals, one able to initiate polymerization and the other being stable but capable of terminating the growing polymer chains by recombination. By immobilizing an iniferter on the CNT surface, we expected that MIP can be formed only on the surface of CNT by the following reason. Polymerization takes place via the active radical attached to the CNT surface, while the

Table 1
Elemental analysis of pristine and iniferter-modified CNT.

	C (%)	H (%)	N (%)	S (%)
Pristine CNT	86	2.4	–	–
Iniferter-CNT	85	1.0	0.3	1.6

non-active radical is present in solution, avoiding polymerization in solution.

3.2. Characterization of MIP grafted on CNT

The iniferter-modified CNT was characterized by elemental analysis, together with pristine CNT (Table 1). While only carbon and hydrogen were found for pristine CNT, nitrogen and sulfur were detected besides carbon and hydrogen for the iniferter-modified CNT (see Fig. 1), indicating that iniferter was grafted onto the surface of CNT. Fig. 2 shows the scanning electron micrographs of pristine and MIP-immobilized CNT. The surface of MIP-immobilized CNT is rougher than that of pristine CNT, suggesting that a thin layer of MIP covers CNT, without changing the morphology of CNT. Fig. 3 shows TGA thermograms of pristine, iniferter-grafted, and MIP-immobilized CNT. The initial decomposition temperatures decreased by grafting of iniferter and MIP, probably due to the thermal decomposition of organic molecules (iniferter and MIP) attached to the surface of CNT.

Fig. 4(a) shows representative binding profiles of theophylline and two similar structure molecules, caffeine and theobromine, to the theophylline-imprinted polymer (MIP) compared with binding to the control, non-imprinted polymer (NIP). Binding amounts of template to the polymer increased with time and became constant. The binding capacities of MIP at 120 min were 1.2 μmol/g polymer-CNT for theophylline, which was 4–6 times higher than that for caffeine (0.26 μmol/g) and that for theobromine (0.21 μmol/g). The binding amounts of theophylline and caffeine to NIP were 0.27 and 0.19 μmol/g polymer-CNT, respectively. The binding experiments to MIP were performed with three theophylline concentrations at 10, 15 and 20 μg/ml. The saturated binding capacity increased as the theophylline concentration was increased as shown in Fig. 4(b). It is known that equilibrium binding constant (K_E) can give a quantitative information about the substrate binding into the polymer (Reddy et al., 2002a,b; Oh and Kim, 2007). Thus, the theophylline and caffeine binding into MIP was evaluated by the following equation of Scatchard relationship.

$$\frac{[S_a]_b}{[S_a]_f} = K_E[PS]_f = K_E\{[PS]_{total} - [PS]_b\} = K_E\{[PS]_{total} - [S_a]_b\}$$

Here $[S_a]_f$ and $[S_a]_b$ are the concentrations of the substrates free and bound to the polymer sites, respectively. $[PS]_f$ is the number of free binding sites of polymer, which equals $[PS]_{total} - [PS]_b$. $[PS]_b$ equals $[S_a]_b$ and $[PS]_{total}$ is the total number of bound sites of polymer. Fig. 4(c) shows Scatchard plot obtained with binding experiments of theophylline and caffeine for the theophylline-imprinted polymer system. Since the plot showed a linear relationship between $[S_a]_b$ versus $[S_a]_b/[S_a]_f$, negative slope and intercept gave each value of K_E and $[PS]_{total}$. The resulted K_E and $[PS]_{total}$ were $2.87 \times 10^3\ M^{-1}$ and 2.12 μmol/g polymer-CNT, respectively, which were higher than those for caffeine binding to MIP ($9.07 \times 10^2\ M^{-1}$ and 0.896 μmol/g polymer-CNT), indicating that the theophylline-imprinted polymer on CNT have a selectivity for theophylline over caffeine.

4. Conclusion

The higher binding capacity of theophylline-imprinted polymer for theophylline than that of non-imprinted polymer and selectivity for theophylline over caffeine, together with elemental analysis, SEM images and TGA thermograms, suggest that MIPs were successfully immobilized on the surface of iniferter-modified CNT. The use of iniferter is very useful for MIP grafting to CNT since MIPs are formed as a thin layer without changing the morphology of CNTs. MIPs as an alternative to protein-based sensing elements can possibly be incorporated into biosensors based on CNT field effect transistor.

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