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Imprinted Microspheres Doped with Carbon Nanotubes as Novel Electroresponsive Drug-Delivery Systems

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ABSTRACT: Novel molecularly imprinted polymers (MIPs) suitable for the electroresponsive release of diclofenac were synthesized by precipitation polymerization in the presence of carbon nanotubes (CNTs). Both conventional and electroresponsive imprinted polymers were synthesized with methacrylic acid as the functional monomer and ethylene glycol dimethacrylate as the crosslinker. Preliminary experiments were performed to fully characterize the conventional MIPs and composite materials in terms of their morphological properties, recognition behavior, and electric resistivity. *In vitro* release experiments were performed in aqueous media to elucidate the ability of the MIPs and spherical imprinted polymers doped with CNTs to release the loaded template in a sustained manner over time in comparison to the that of the corresponding nonimprinted materials. Furthermore, a 20-V direct-current voltage was applied through the releasing media to evaluate how the electric field influenced the drug release to demonstrate the suitability of the proposed macromolecular system as an electroresponsive drug-delivery device. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 130: 829–834, 2013

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INTRODUCTION

The development of new polymeric biomaterials for medical and pharmaceutical purposes is of great interest to lifecare science and engineering.[1,2] In this regard, over the past few decades, several different drug-delivery systems have been proposed, developed, and studied in great depth to improve the curative effect of drugs.[3,4] Among them, environmentally responsive materials have been investigated widely to release drugs into their target organs at desired times.[5,6] These materials have earned a reputation as intelligent materials because of their ability to alter their structures and physical properties in response to external stimuli, such as electric fields, pH, and temperature.[7–9]

Molecularly imprinted polymers (MIPs), stable polymers with molecular recognition abilities due to the presence of specific recognition sites for a desired target molecule (the template), are highly promising materials for application in the drug-delivery field.[10–13] In several studies, MIPs have indeed been successfully applied as base excipients for the fabrication of innovative drug-delivery devices for the sustained/controlled release of selected drugs in response to environmental stimuli.[14–16]

A key stimulus to be used for the modulation of drug release is the electrical field, and several different strategies have been proposed to enhance the electroresponsive behavior of selected materials. Among them, carbon nanotubes (CNTs) have recently attracted much attention as conducting materials that are suitable for use in biomaterial fabrication. In particular, multiwallled carbon nanotubes (MWCNTs) have been used in the preparation of polymer/MWCNT composites with peculiar mechanical and electrical properties.[17–19] As an emergent class, with these materials, researchers seek to creatively combine the inherent properties of constituent materials to give rise to technologically relevant properties for devices and systems.[20]

On the basis of these considerations, in this study, spherical imprinted polymers doped with carbon nanotubes (CNT_MIPs) that were suitable to release a selected model drug [diclofenac sodium salt (DS)] in response to an electric field were synthesized by the noncovalent imprinting approach with methacrylic acid (MAA) as the functional monomer, ethylene glycol dimethacrylate (EDGMA) as the crosslinking agent, and MWNTs as the electroresponsive element. Specifically, imprinted spherical

polymers were prepared by precipitation polymerization and were characterized in terms of their electric properties, recognition ability, and drug-delivery profile. The results show the increased electroresponsivity in the hybrid CNT_MIPs and their ability to control the delivery of diclofenac in response to the application of an external electric field.

EXPERIMENTAL

Chemicals

EGDMA, MAA, 2,2′-azoisobutyronitrile, DS, phenylacetic acid (Pha) ferrocene, 2-aminophenylacetic acid, tetrahydrofuran, acetonitrile, methanol, and cyclohexane were obtained from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO). All of the solvents were reagent grade or high-pressure liquid chromatography (HPLC) grade. The monomers were purified before use by distillation under reduced pressure.

Instruments

The HPLC analyses were carried out with a Jasco PU-2080 liquid chromatograph (Tokyo, Japan) equipped with a Rheodyne 7725i injector (fitted with a 20-μL loop), a Jasco UV-2075 HPLC detector, and a Jasco–Borwin integrator (Massachusetts, USA). A reversed-phase C18 column (μBondapak, 10 μm of 150 $\times$ 4.6 mm^2 *i.d.*, Waters) was used. The HPLC conditions were a mixture of an aqueous solution of ammonium acetate, methanol, and acetonitrile (40 : 30:30 v/v). The pH of the aqueous mobile phase portion of the ammonium acetate buffer (pH 7.0, 10^{-3} *M*) was adjusted with glacial acetic acid. The mobile phase was filtered, degassed, and pumped isocratically at a flow rate of 0.6 mL/min; UV detection was done at 284 nm.[21] The morphology of the samples was analyzed with a scanning electron microscope (NOVA MicroSEM 200 [0–30 kV], FEI Co., Hillsboro, OR) and a transmission electron microscope (HRTEM/Tecnai F30 [300 kV] FEI Co.). The samples were deposited onto self-adhesive, conducting carbon tape (Plano GmbH, Wetzlar, Germany) for scanning electron microscopy (SEM) analysis. Transmission electron microscopy (TEM) samples were prepared by insertion of the powdery CNT composite between two small slides of aluminum foil on a Cu TEM grid (200 mesh, Plano GmbH). The approximate range of particle size was determined by the measurement of 300 particles for each sample with the use of an image processing and analysis system (Leica DMRB equipped with a Leica Wild 3D stereomicroscope, Wetzlar, Germany). The Raman experiments were performed in a Raman Fourier Transform Spectrometer IFS 100 (Bruker Massachusetts, USA) with a wavelength of 633 nm and a laser power of 8 mW. The resolution of the spectrometer was 2 cm^{-1}. The samples were carefully prepared on aluminum foil. The electrical measurements of the powder samples were investigated by four-point methods under pressure at room temperature. A suitable amount of the powder sample was pressed in insulting ceramic under constant pressure of 100 MPa. It is important to note that this pressure value was not sufficient to cause any deformation in the sample structure. The electrical measurements were done in the presence of argon gas.

Synthesis of the CNTs

The synthesis of the MWCNTs was performed by the application of the so-called aerosol-assisted chemical vapor deposition method as described previously with ferrocene as the metal organic catalyst precursor (ferrocene) and cyclohexane as the carbon source. An excitation frequency of 850 kHz and a carrier gas flow consisting of 100 sccm Ar were used. The as-grown material was subsequently purified to eliminate the amorphous carbon and catalyst particles with a two-step method, including a thermal treatment at 450°C in air for 1 h and an acid treatment with hydrochloric acid.[22]

Preparation of MIPs

The CNT_MIPs were prepared by precipitation polymerization with DS as the template, MAA as the functional monomer, and EGDMA as the crosslinking agent.[23] The general synthetic procedure was as follows. MWNT (20 mg), the template (1 mmol), and MAA (8 mmol) were dissolved by sonication in a mixture of acetonitrile (20 mL) and methanol (20 mL) in a 100-mL, round-bottomed flask, and then EGDMA (10 mmol) and 2,2′-azoisobutyronitrile (50 mg) were added. The polymerization mixture was degassed in a sonicating water bath and purged with nitrogen for 10 min while cooling in an ice bath. The flask was then gently agitated (40 rpm) in an oil bath. The temperature was increased from room temperature to 60°C over 2 h and then kept at 60°C for 24 h. At the end of the reaction, the particles were filtered and washed with 100 mL of ethanol, 100 mL of acetone, and then 100 mL of diethyl ether. The template was extracted by a Soxhlet apparatus with a methanol–acetic acid mixture (1 : 1 v/v, 100 mL) for 48 h and then by methanol for another 48 h. We monitored the drug concentration in the extraction solvent by HPLC. Particles were successively dried *in vacuo* overnight at 40°C.

Conventional spherical MIPs were synthesized with the same synthetic procedure in the absence of CNTs.

Conventional blank polymers [nonimprinted polymers (NIPs)] and composite materials (CNT_NIPs), which acted as controls, were also prepared with the corresponding polymerization process carried out in the absence of the template.

Binding Experiments

The binding efficiency of polymeric matrices toward template and analogue molecules was evaluated by specific rebinding experiments in water media (PBS 10-3 mol/L, pH 7.4). The experiments were performed as follows. An amount of 30 mg of polymer particles was mixed with 1 mL of a DS solution with a concentration of 1.0 mol/L in a 1-mL Eppendorf. The Eppendorf tubes was oscillated by a wrist action shaker (Burrell Scientific, Pennsylvania, USA) in a water bath at 37 $\pm$ 0.5°C for 24 h. Then, the samples were centrifuged for 10 min (10,000 rpm) in an ALC microcentrifugette 4214 (ALC, Milano, Italia), and the template concentration in the supernatant was measured by HPLC analyses. The amount (percentage) of DS adsorbed by the polymer was obtained by the comparison of the template concentration in the CNT_MIPs and MIP samples to that in the CNT_NIP and NIP samples. The same rebinding experiments were performed with solutions of Pha (analogue) with the aim of verifying the selectivity (ε) of the process.

Water Contents (WRs) of the Spherical Polymers

Aliquots (40–50 mg) of the microspheres dried to a constant weight were placed in a tared, 5-mL sintered glass filter (Ø10

mm; porosity, G3), weighed, and left to swell by the immersion of the filter plus a support in a beaker containing the phosphate buffer swelling media (pH 7.4, 0.01 mol/L, simulated biological fluids). After 2 h, the excess water was removed by percolation at atmospheric pressure. Then, we placed the filter in a properly sized centrifuge test tube by fixing it with the help of a bored silicone stopper; then, the sample was centrifuged at 3500 rpm for 15 min and weighed. The filter tare was determined after centrifugation with water only. The weights recorded at the different times were averaged and used to give the WR percentage by the following equation:

$$\mathrm{WR}(\%) = \frac{W_s - W_d}{W_d} \times 100 \quad (1)$$

where W_s and W_d are weights of the swollen and dried spherical microparticles, respectively.

The same experimental protocol was applied when a 20-V direct-current (dc) voltage was applied through the swelling media by means of platinum electrodes (distance = 1.0 cm).

In Vitro Release Studies

The polymeric matrix (2.0 g) was immersed in a DS solution in a 1 : 1 v/v acetonitrile/methanol mixture (20 mL, 5.5*mM*) and soaked for 3 days at room temperature. During this time, the mixture was continuously stirred, and then the solvent was removed *in vacuo*. Finally, the powder was dried *in vacuo* overnight at 40°C. The same experiments were performed with Pha solution.

Release studies were carried out with the dissolution method described in USP XXIV (apparatus one-basket stirring element). Two separate experiments were performed. First, the release profile was recorded in the absence of an electric field, and subsequently, a 20-V dc voltage was applied through the releasing media by means of platinum electrodes (distance = 1.0 cm).

In each experiment, the samples (10 mg) were dispersed in flasks containing a 10*mM* phosphate buffer solution (pH 7.4, simulated biological fluids, 10 mL). Thus, the samples were drawn from the dissolution medium at appropriate time intervals to determine the amounts of drug released by HPLC. The amount of DS released from the five samples of each formulation was used to characterize drug release. The same experiments were performed with particles loaded with the Pha molecule.

Statistical Analyses

All of the experiments were done in triplicate, and the data are expressed as means plus or minus the standard deviation. A one-way analysis of variance was performed to assess the significance of the differences among the data of the rebinding experiments. A Tukey–Kramer posttest was used to compare the means of different treatment data. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Rationale of the Synthesis of the Imprinted Microspheres

For the synthesis of the spherical imprinted microparticles, the aim of this research work, an extensive analysis of the literature data was performed to determine the most adequate synthetic strategy and the optimal molecular composition.

First, with the aim of obtaining microparticles to be applied as drug-delivery devices, we selected the noncovalent imprinting approach because of its fast kinetics of binding, absence of toxic reaction products, and compatibility with a wide range of functional monomers.[24] To prevent the swelling anisotropic behavior of the microparticles, a spherical shape is advisable, and the precipitation polymerization method was selected for this purpose because of the possibility of obtaining spherical monodisperse microparticles and preserving the integrity and stability of the recognition sites. Other synthetic strategies (e.g., aqueous two-step swelling polymerization, suspension polymerization in water) require water or highly polar organic solvents and result in decreased specific interactions between the functional groups and template molecule and a subsequent reduction of the imprinting efficiency both in the prepolymerization and recognition steps. Furthermore, by this procedure, imprinted microspheres with a hydrogel-like behavior can be prepared, and this is of dramatic importance in an effective DDS. We found a suitable compromise between the rigidity and flexibility of the polymers by carrying out imprinted cavities with stability adequate to retain the steric conformation but, at the same time, flexible enough to facilitate the attainment of a fast equilibrium between the release and reuptake of the template.[25]

Subsequently, a core point was the choice of functional monomer and crosslinking agent. As functional monomer, MAA was selected by virtue of its great ability to form hydrogen-driven interactions with the template molecule and EGDMA as the crosslinking agent to stabilize the spatial geometry of the binding sites.[24]

Finally, as previously reported, CNTs were selected as tailored materials to increase the electroresponsivity of the imprinted polymers. In this regard, several different strategies have been proposed for the fabrication of composites based on polymeric and CNT materials.[26,27] Most of these approaches involve a multistep procedure including the preliminary chemical modification of the CNT surface by means of oxidation processes and/or cycloaddition reactions.[28,29] A simpler protocol involving a single-step, free-radical polymerization of monomers around CNTs was recently developed and was selected for the preparation of the CNT_MIPs in this work. This strategy involves the radical coupling of the growing polymer chain onto the π-based surface of the dispersed CNTs.[22]

Characterization of the Imprinted Microspheres

Conventional imprinted polymers were characterized by means of morphological, dimensional analyses and the determination of the imprinting efficiency.

SEM pictures [Figure 1(a)] show the spherical shape and the monodisperse behavior of the microparticles in the dimensional ranges of 0.92 ± 1.2 and 0.97 ± 0.9 nm for the MIPs and NIPs, respectively. The water affinity of the materials was tested in water media at pH 7.4 (0.01 mol/L PBS), and the results show a high hydrophilicity with values around 380% (Table I).

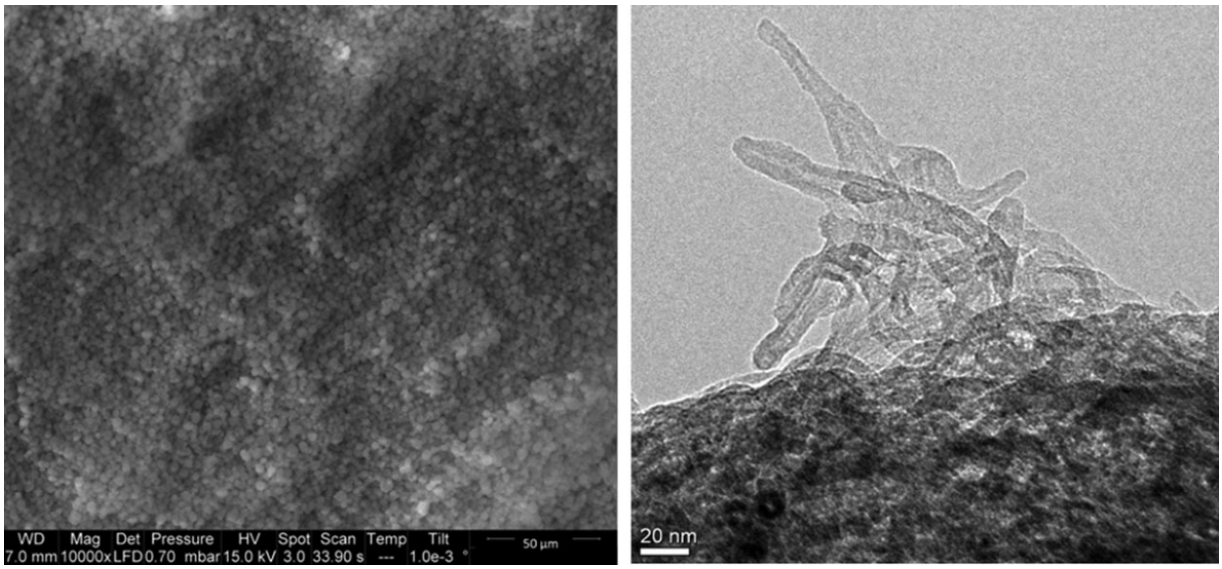

Figure 1. Representative (a) SEM images of the MIPs and (b) high-resolution TEM images of CNT_MIPs surface.

With regard to the imprinting efficiency, the high affinity and ε of the polymeric microparticles toward the template DS were proven by the results of specific binding experiments, in which the MIP particles were found to retain 70% of the template, whereas only 53% of DS interacted with the NIP particles (Table II). This effect was highlighted by the introduction of two specific parameters, α and ε, which are reported in Table II. The α value is a quantitative determination of the recognition properties because it is calculated as the ratio between the amount (percentage) of analyte (template or analogue) bound by the MIPs and NIPs, whereas the ε value is a quantitative measurement of the imparted ε within the imprinted cavities because it is the ratio between the amount (percentage) of the template and the Pha molecule bound by the MIPs.[20] As reported in Table II, the recorded α value was 1.29 for the template and 0.98 for Pha; this confirmed the higher affinity of the MIPs for the template. The ε value was 1.34; this means that the polymeric sample was 1.34 times more selective for DS than for Pha.

The second step of the research was the preparation of the composite microspheres. The shape of the microspheres was confirmed by SEM, and no significant differences were recorded by the comparison of the samples with MIPs (CNT_MIPs) and without MIPs (CNTs). The dimensional analyses of the sample proved diameters of 1.12 ± 1.0 and 1.09 ± 1.1 μm for the CNT_MIPs and CNT_NIPs; this confirmed that neither the shape nor the dimension of the microparticles were affected by the presence of CNTs. Furthermore, the presence of CNTs was highlighted by TEM analyses [Figure 1(b)].

The strong interconnection between the CNTs and polymeric particles was proven by Raman analyses, which proved the modification of the intensity of the graphitic and disorder bands, which moved from pristine to functionalized CNTs (Figure 2). Specifically, an increase in the relative intensity of the disorder band was observed, whereas the graphitic band was somewhat suppressed. As a result, the intensity of D-Raman peak and G-Raman peak (ID/IG) ratio was modified from 1.2 in the pristine CNTs to 3.1 in the CNT_MIP sample.[30]

As reported for the MIPs, the water affinity of the CNT_MIPs was tested in phosphate buffer solution, and the data proved a swelling degree around 320% (Table I). The reduction of the water affinity from the MIPs to the CNT_MIPs was due to the presence of CNTs conferring hydrophobic behavior to the whole system.

After this preliminary physicochemical characterization, the imprinting efficiency was determined (Table I) by means of binding experiments and the determination of α and ε values. From the observation of the binding percentages, it was clear that the presence of CNTs conferred more hydrophobicity to the samples and resulted in increased nonspecific hydrophobically driven interactions. Nevertheless, this effect did not negatively interfere with either ε or the recognition properties of the

Table I. Polymer Characterization: Mean Particle Sizes (d_n's) and WR (%) Values for the Synthesized Polymers

Matrix	d_n (nm)	WR at 0 V (%)	WR at 20 V (%)
MIP	0.92 ± 1.2	383 ± 1.4	387 ± 1.3
NIPs	0.97 ± 0.9	388 ± 1.6	392 ± 1.7
CNT_MIPs	1.12 ± 1.0	321 ± 1.2	542 ± 1.1
CNT_NIPs	1.09 ± 1.1	326 ± 1.1	545 ± 1.4

Table II. Imprinting Efficiency: Binding Percentages and α and ε Values

Matrix	Bound DS (%)	Bound Pha (%)	α DS	α Pha	ε
MIP	71 ± 2.1	53 ± 1.7	1.23	0.98	1.34
NIPs	55 ± 1.8	54 ± 1.6			
CNT_MIPs	80 ± 1.9	62 ± 2.0	1.25	0.95	1.29
CNT_NIPs	64 ± 2.2	65 ± 2.1			

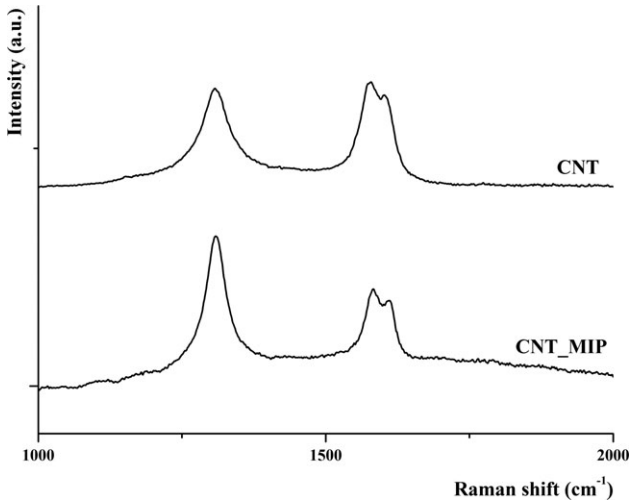

Figure 2. Raman spectra of the CNT-MIPs and CNTs.

imprinted materials, as highlighted by the small variations in the α (1.25) and ε (1.29) values.

In Vitro Release Studies

To test the ability of the polymeric sample to release DS in response to an electrical stimulus, two separate experiments were performed with the MIPs and CNT_MIPs loaded with the same amount of DS.

In the first series of experiments, polymeric samples were immersed in a buffered solution simulating biological fluids, and the release amount of DS was recorded over time [Figure 3(a)]. The analysis of the release profile proved that the MIPs and CNT_MIPs were more effective in controlling the template release compared to the respective NIPs (NIPs and CNT_NIPs) because of the presence of specific functional groups in the imprinted cavities, which strongly interacted with DS. The release from the NIPs and CNT_NIPs, indeed, was faster than that observed from the MIPs and CNT_MIPs. The differences in the release profile form the MIPs and CNT_MIPs were due to the higher hydrophobicity of the composite microspheres as a consequence of the presence of CNTs; this resulted in a greater hydrophobic interaction with the template and, thus, a slower release.

A second kind of experiment was performed when a 20-V dc voltage was applied through the releasing media. Under these conditions, the release profile from the MIPs was not significantly affected by the electrical field, whereas a significant increase in the release (in terms of kinetics and percentage) was recorded for the CNT_MIPs [Figure 3(b)]. This was due to the specific release mechanism observed in the imprinted polymers. The release for the MIPs, indeed, was due to the progressive ionization of the carboxylic groups into the imprinted cavities, which reduced their ability to interact with the template.[16] As a result, the affinity between the polymers and DS decreased, and the drug was released. This phenomenon was faster in the NIPs than in the MIP sample because, in the nonimprinted samples, the interaction between DS and COOH was mainly on the particle surface, whereas in the MIPs, the presence of specific cavities in which the template was located increased the ionization time and thus reduced the release. When a dc voltage was applied, the ionization was promoted by the electrical field, the whole process required a shorter time, and an increase in the DS release was observed. In Figure 4, the comparison between the release behavior of the MIPs and MIP_CNTs with and without the application of an electrical field is shown. In the conventional MIPs [Figure 4(a)], no significant changes were recorded in the release profile, the increase (in percentage) at 24 h, indeed, was below 10%, whereas in the CNT_MIPs, much more DS was released when a dc voltage was applied, and the increase (in percentage) at 24 h was around 40%.

The presence of CNT drove the different behaviors of the MIPs and CNT_MIPs, as confirmed by the specific resistivity measurements. Resistivity values of 48.8×10^7 and 15.8×10^7 ohm/cm were recorded for the MIPs and CNT_MIPs, respectively; this proved that the presence of CNTs greatly enhanced the electroresponsivity of the polymeric microparticles. A further confirmation of the increased ionization of the CNT_MIPs compared to the MIPs was obtained by swelling measurements performed in the presence of the 20-V dc voltage. As a result, an increase in WR from 321 to 542% was recorded as a consequence of the formation of carboxylate anions in the CNT_MIPs.

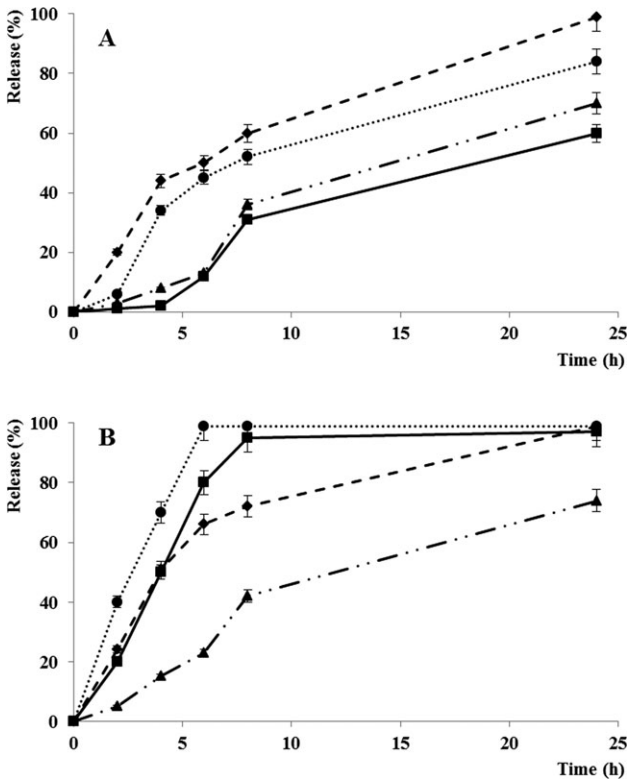

Figure 3. DS release profile of the (—••▲—••) MIPs, (——◆——) NIPs, (——■——) CNT_MIPs, and (••●••) CNT_NIPs in (a) simulated biological fluids (PBS, pH 7.4) and (b) simulated biological fluids with a 20-V dc voltage (PBS, pH 7.4).

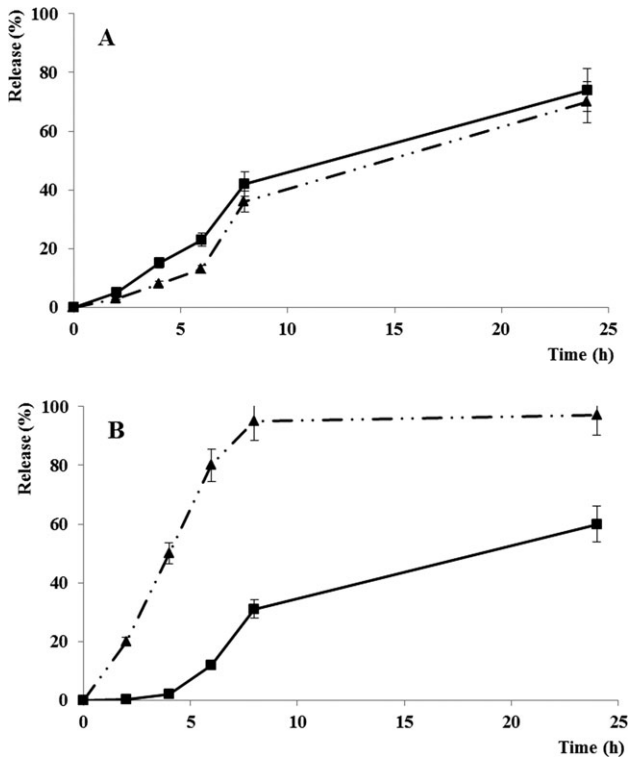

Figure 4. (a) DS release profile of the MIPs in (— ••▲— ••) simulated biological fluids and (——■——) in simulated biological fluids with a 20-V dc voltage. (b) DS release profile of the CNT_MIPs in (——■——) simulated biological fluids and (— ••▲— ••) simulated biological fluids with a 20-V dc voltage.

CONCLUSIONS

The ability of CNTs to impart electroresponsivity to an MIP drug-delivery system was evaluated. MIP microspheres were prepared by means of polymerization precipitation in the presence of DS as the template, whereas a free-radical grafting reaction technique was used to introduce CNTs into the molecular composition of CNT_MIPs. The presence of CNTs in the composite materials was evaluated by Raman spectroscopy, morphological analyses, and electroresistivity measurements, whereas the recognition properties were evaluated by binding experiments. Both the MIPs and CNT_MIPs were found to be highly selective toward the template ($\alpha = 1.23$ and 1.25 and $\varepsilon = 1.34$ and 1.29, respectively). Release experiments in simulated biological fluids indicated that the proposed imprinted polymeric devices were able to release DS in a sustained manner over time compared their nonimprinted counterpart. Finally, the application of an external electrical field was found to greatly enhance the release kinetics in the CNT_MIPs sample.

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