Journal of Chromatography B, 879 (2011) 1617–1624

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Novel molecularly imprinted polymers based on multi-walled carbon nanotubes with binary functional monomer for the solid-phase extraction of erythromycin from chicken muscle

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ARTICLE INFO

Article history:
Received 23 January 2011
Accepted 25 March 2011
Available online 6 April 2011

Keywords:
Erythromycin
Multi-walled carbon nanotube
High-performance liquid chromatography
β-Cyclodextrin
Surface molecular imprinting

ABSTRACT

A new surface imprinting technique was reported to synthesize multi-walled carbon nanotubes-molecularly imprinted polymers (MWNTs-MIPs) using erythromycin as the template, acryloyl-β-cyclodextrin (acryloyl-β-CD) and methacrylic acid (MAA) as the binary functional monomers. The MWNTs-MIPs were characterized by transmission electron microscopy (TEM), scanning electron micrograph (SEM) and Fourier transform-infrared spectroscopy (FT-IR). Adsorption experiments indicated the MWNTs-MIPs prepared with acryloyl-β-CD and MAA have high selective for erythromycin. The feasibility of the MWNTs-MIPs as solid-phase extraction (SPE) sorbent was evaluated, and the results showed that it can selectively extract erythromycin from chicken muscle samples with the recoveries ranging from 85.3% to 95.8%. The molecularly imprinted solid-phase extraction (MISPE) method could be applied for preconcentration and purification of erythromycin from chicken muscle samples.

1. Introduction

Erythromycin is a macrolide antibiotic and its chemical structure is shown in Fig. 1. Residues of erythromycin in food may lead to allergic reactions in sensitive individuals and spread of antibiotic resistance, posing a potential threat to public health [1]. Up to now, many countries have set maximum residue limits for erythromycin regulation in edible animal tissues [2]. At present, the universal analytical techniques for determination of erythromycin are mainly high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC–MS) [3,4]. However, the sensitivity and reproducibility of these analysis methods are easily affected by the complex nature of the biological matrices. In order to reduce the influence of the matrices and improve the sensitivity of these current methods, novel and rapid pretreatment methods are required for analysis trace-level of erythromycin. Molecular imprinting technology (MIT) could solve this problem.

Recently, MIT has attracted increasing attentions due to its outstanding advantages, involving predetermined recognition ability, mechanical and chemical stability, relative ease and low cost of preparation [5,6]. Owing to these advantages, molecularly imprinted polymers (MIPs) have been applied widely in adsorbents, membranes and sensors [7–9]. Coupling MIT with solid-phase extraction (SPE) is possible to combine the advantages of both molecular recognition and traditional separation method. Thus, molecularly imprinted solid-phase extraction (MISPE) presents higher specificity and selectivity than that of conventional SPE [10]. Although a few studies on the MISPE for erythromycin were reported [11], the MIPs were prepared by bulk polymerization. The main challenge for the traditional imprinted materials is the generated cavities are not at the surface or in the proximity of the materials' surface, and the high resistance to mass transfer will still hinder target species from accessing the deep imprinted cavities, thus reducing the kinetics of binding target analyte. Fortunately, several research groups have made efforts to prepare core–shell structural MIPs, which combine the advantageous properties of both molecular imprinting technology and support material [12,13].

Multi-walled carbon nanotubes (MWNTs) with unique mechanical properties and extremely large surface area can be an excellent candidate as the support material. When the MWNTs were used

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doi:10.1016/j.jchromb.2011.03.054

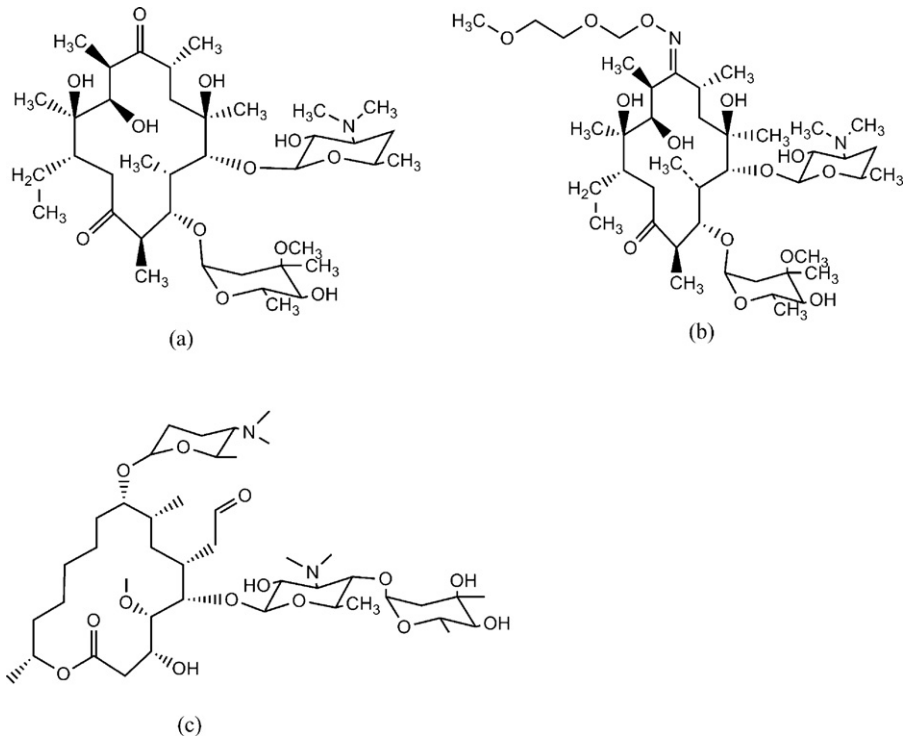

Fig. 1. Chemical structures of erythromycin (a), roxithromycin (b) and spiramycin (c).

as a backbone for the polymerization of MIPs layer, the composite material has mechanical strength and chemical stability. Thus, the binding sites in the outer layer of the composite will improve the accessibility of the template molecule and reduce the binding time. MWNTs as the supported matrix to prepare MWNTs-MIPs have attracted great attentions in recent years. Among many methods for preparation of MWNTs-MIPs, vinyl group functionalized MWNTs selective polymerization of MIPs by covalent bonds on the MWNTs surface reported by Kan et al. [14] using MWNTs as the reinforcement material in the MIPs matrix.

β-Cyclodextrin (β-CD) as a novel functional monomer has been of considerable interest in recent years. Compared with conventional functional monomer, β-CD which is a series of cyclic oligosaccharides with a hydrophilic exterior and hydrophobic cavity, possesses some unique advantages [15]. Due to the rigidity of hydrophobic cavity, β-CD unit can form a complex with the target analyte through various kinds of intermolecular interactions, such as Van der Waals force and hydrophobic interaction, which are helpful to obtain high affinity binding sites [16,17]. Liu's group successfully prepared imprinted polymers with high affinity and selectivity for bilirubin using β-CD as functional monomer [18]. Komiyama's group reported that two kinds of modified β-CD monomers were applied to the imprinting toward amino acid derivatives and oligopeptides, and the MIPs could selectively recognize the template molecules from mixture [19,20]. However, few studies have reported on the preparation of MIPs supported with MWNTs using acryloyl-β-CD and MAA as binary functional monomers.

In this paper, the MWNTs-MIPs were prepared using multi-walled carbon nanotubes as the support matrix, acryloyl-β-cyclodextrin (acryloyl-β-CD) and methacrylic acid (MAA) as binary functional monomers. A series of adsorption studies were conducted to investigate the performance of the MWNTs-MIPs. The results demonstrated that the MWNTs-MIPs can selective recognize erythromycin. Coupling solid-phase extraction (SPE) techniques, the MWNTs-MIPs were used for selective preconcentration of erythromycin from chicken muscle successfully.

2. Experimental

2.1. Materials and reagents

Multi-walled carbon nanotubes (MWNTs, diameters ranging from 20 to 40 nm) were obtained from Shenzhen Bill Corporation. Vinyltriethoxysilane (VTEOS), MAA, β-CD, 3-aminopropyltriethoxysilane (APTES) and ethylene glycol dimethacrylate (EGDMA) were purchased from Sigma (USA). Erythromycin, roxithromycin and spiramycin were obtained from Xi'an Pharmaceutical Factory. Thionylchloride ($SOCl_2$), *N*, *N*′-dimethylformamide (DMF), chloroform ($CHCl_3$), sodium hydroxide (NaOH), hydrochloric acid (HCl), ethanol and methanol were obtained from Changsha Chemical Reagent Company (Hunan, China). Acetonitrile and ammonium acetate were chromatographic grade. The remaining chemical reagents were analytical grade. Ultra pure water used throughout the experiment. The mobile phase used for HPLC experiment was a mixture of ammonium acetate, ultra pure water and acetonitrile (10:65:25, v/v/v), which was filtered through a 0.45 μm polytetrafluoroethylene (PTFE) membrane before use.

2.2. Pretreatment of multi-walled carbon nanotubes

500 mg of MWNTs was dispersed in 50.0 mL of nitric acid solution under sonication for 10 min. Then the mixture was stirred continuously at 80 °C for 24 h. Cooled to room temperature, the mixture was diluted to ten fold with ultra pure water and filtered through a 0.45 μm PTFE membrane. The filtered solid was rinsed with ultra pure water until the pH was neutral. Finally, the filtered solid was dried under vacuum at 80 °C for 24 h to obtain MWNTs-COOH.

2.3. Synthesis of MWNTs ∿∿ CH=CH$_2$

450 mg of MWNTs-COOH suspended in 10.0 mL of $SOCl_2$ was refluxed at 60 °C for 24 h. The solid was washed by anhydrous

Table 1
Components for the preparation of molecularly imprinted polymers.

Polymers	ERY (mmol)	MWNTs-CH=CH_2 (mg)	MAA (mmol)	Acryloyl-β-CD (mmol)	EGDMA (mmol)	AIBN (mg)	ACN (mL)
MWNTs-MIPs	0.2	100	0.8	0.8	4	20	100
MWNTs-MIPs1	0.2	100	0	0.8	4	20	100
MWNTs-MIPs2	0.2	100	0.8	0	4	20	100
MWNTs-NIPs	0	100	0.8	0.8	4	20	100
MWNTs-NIPs1	0	100	0	0.8	4	20	100
MWNTs-NIPs2	0	100	0.8	0	4	20	100

tetrahydrofuran (THF) for several times to remove the excess $SOCl_2$ and dried under vacuum to give MWNTs-COCl. 400 mg of MWNTs-COCl was dispersed in 30 mL of anhydrous THF. Then 1.5 mL of APTES was added into the above solution. After the solution was stirred at 50 °C for 12 h, 1.5 mL of VTEOS was added. Stirred at 50 °C for 12 h, the mixture was collected by centrifugation and washed with anhydrous THF. The solid was dried overnight in a vacuum desiccator to obtain vinyl group functionalized MWNTs (MWNTs ∿∿ CH=CH_2).

2.4. Synthesis of acryloyl-β-cyclodextrins (acryloyl-β-CD)

Acryloyl-β-CD was synthesized reported by Hiroyuki Asanuma et al. [21]. Briefly, β-CD (2.0 g) was dissolved in 400.0 mL of $NaHCO_3$ (1.0 g) aqueous solution. Then 4.0 mL of ACN containing *m*-nitrophenyl acrylate (1.3 g) was added dropwise into the solution at pH 10. After the pH was adjusted to 5 with HCl, the reaction solution was evaporated and acryloyl-β-CD was obtained.

2.5. Preparation of multi-walled carbon nanotubes-molecularly imprinted polymers (MWNTs-MIPs)

The components for preparation of the imprinted polymers are given in Table 1. First, MWNTs ∿∿ CH=CH_2 was incubated with ACN solution in a 250 mL round-bottom flask. Then acryloyl-β-CD, MAA and erythromycin were dissolved in the above solution and the mixture was magnetically stirred at 60 °C for 2 h. Second, EGDMA and AIBN were added and the mixture was purged with nitrogen to remove oxygen for 15 min. The mixture was refluxed under magnetically stirred at 60 °C for 24 h. Finally, erythromycin was removed from the imprinted polymers by washing with a mixture solution of methanol and acetic acid (9:1, v/v) for several times until no erythromycin could be detected by UV–vis (at 210 nm) in the eluent. Multi-walled carbon nanotubes non-imprinted polymers (MWNTs-NIPs) were prepared using the same procedure without addition of the template molecule.

2.6. Adsorption experiment

2.6.1. Adsorption isotherm

A mass of 25.0 mg of the MWNTs-MIPs or the MWNTs-NIPs was suspended in 10.0 mL of ethanol solutions with initial erythromycin concentration ranging from 0.1 to 4.0 mmol/L. The suspensions were sealed and oscillated for 24 h at room temperature. Then the mixture was centrifuged at 10,000 rpm for 2 min. The residual concentration of erythromycin was measured by HPLC. The amount of erythromycin adsorbed by the imprinted polymers was calculated according to the following formula [22]:

$$Q = \frac{(C - C_t)V}{m}$$

where C (mg/mL) and C_t (mg/mL) represent the initial and final erythromycin concentration, respectively. V (mL) is the sample volume and m (g) is the imprinted polymers mass.

2.7. Adsorption kinetic

Adsorption kinetic studies were carried out as follows. 25.0 mg of the MWNTs-MIPs was suspended in 10.0 mL of 2.0 mmol/L erythromycin ethanol solution and the mixture was incubated at room temperature under shaking. Then the imprinted polymers were taken at defined time intervals (20 min), and residual concentration of erythromycin was measured by HPLC.

2.8. Selectivity of MWNTs-MIPs-SPE column

Selectivity of the imprinted sorbent was investigated for erythromycin and the structurally similar compounds such as roxithromycin and spiramycin. The MWNTs-MIPs was packed into column as reported in the studies [23]. Briefly, 100 mg of the MWNTs-MIPs was packed into an empty SPE column. After pretreated with 10.0 mL of methanol and 10.0 mL of pure water in succession, 20.0 mL of 1.0 mmol/L erythromycin, roxithromycin and spiramycin mixture solution was loaded onto the SPE cartridge. Washed with 2.0 mL mixture solution of methanol: water (80:20, v/v), the cartridge was eluted with 2.0 mL of a mixture solution of ethanol:acetic acid (90:10, v/v). The eluent was measured by HPLC.

The recovery of erythromycin is calculated according to the formula:

$$\text{Recovery} \quad \% = \frac{(C - C_t)}{C}$$

where C (mg/mL) and C_t (mg/mL) represent the initial and final erythromycin concentration, respectively.

The specific recognition property of the MWNTs-MIPs is evaluated by imprinting factor (α) which is defined as follows [22]:

$$\alpha = \frac{Q(A)}{Q(B)}$$

where $Q(A)$ and $Q(B)$ are the adsorption capacity of the template and analogue on the MWNTs-MIPs, respectively.

The selectivity factor (β) is defined as follows:

$$\beta = \frac{\alpha_1}{\alpha_2}$$

where α_1 is the imprinting factor toward the template molecule and α_2 is the imprinting factor toward the analogue.

2.9. Real sample determination

Chicken samples used for the preparation of spiked muscle were purchased from local groceries. Skin and bones were removed prior to grinding the muscle. Mince muscle was kept at 4 °C before analysis. Then the tissue was spiked with erythromycin at four levels of 100.0, 200.0, 300.0 and 400.0 μg/kg. The tissue sample was processed as described by Dreassi et al. [24]. Briefly, a total of 20.0 mL of CH_3Cl and 20.0 μL of NaOH (1 mol/L) were mixed with 2.0 g of chicken muscle under vortexing for 5 min. Then the mixture was centrifuged at 10,000 rpm for 20 min and the organic solvent was collected. Afterwards, the extraction was treated again with

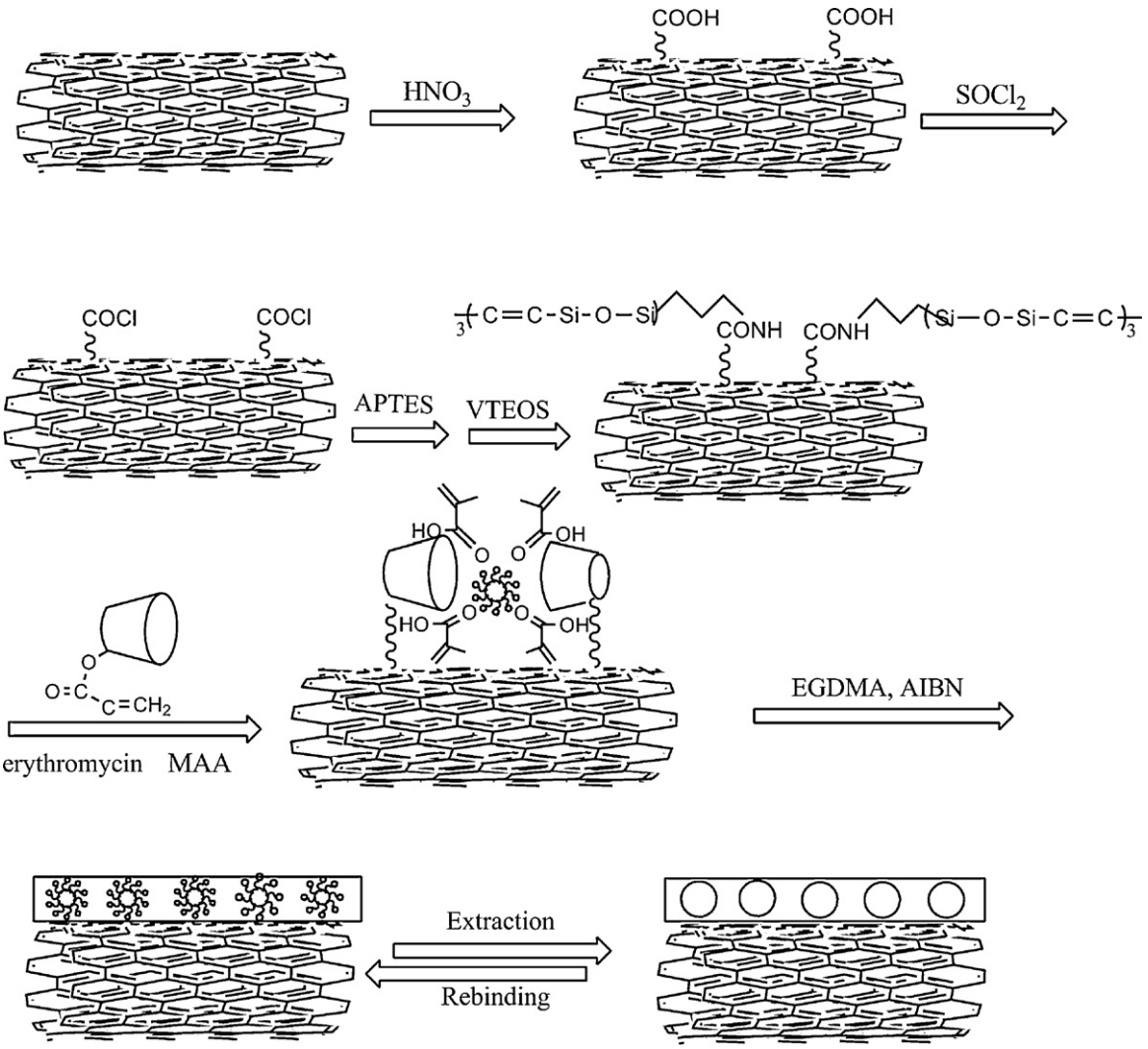

Fig. 2. The protocol for synthesis of MWNTs-MIPs.

another 20.0 mL of CH_3Cl. After the organic solvent was evaporated, the residue was redissolved in 4.0 mL of methanol/water (2:3, v/v) and stored at 4 °C before use. According to MISPE procedure, the extracted chicken muscle samples were passed through the MISPE cartridge. Finally, the columns were eluted with 2.0 mL of methanol and acetic acid (9:1, v/v). A volume of 20.0 μL of sample was analyzed by HPLC.

HPLC measurement was carried out with LC-2010AHT solution system. HPLC conditions are as follows: mobile phase is ammonium acetate: water: acetonitrile (10:65:25, v/v/v, pH 6.8); flow rate is 0.8 mL/min; room temperature; UV detection wavelength is 210 nm.

3. Results and discussion

3.1. Preparation of MWNTs-MIPs

Fig. 2 shows the protocol for synthesis of the MWNTs-MIPs. A novel surface imprinting technique was developed using MWNTs as the support matrix, acryloyl-β-CD and MAA as binary functional monomer to synthesize the MWNTs-MIPs. For comparison, MWNTs-MIPs1 was prepared only using acryloyl-β-CD as functional monomer, and MWNTs-MIPs2 was synthesized only using MAA as functional monomer. The novel monomer acryloyl-β-CD molecule is a torus-shaped cyclic oligosaccharide consisting of 1,4-linked D-glucopyranose units with an internal hydrophobic cavity. This structure enables acryloyl-β-CD to form inclusion compounds with the template through hydrophobic interactions [23]. In the pre-polymerization, each of acryloyl-β-CD molecules binds a portion of the template. Then the positions and mutual conformations of the acryloyl-β-CD molecules were fixed by cross-linkers. Polymerization was carried out in the presence of MAA as an assistant monomer, which could form hydrogen bonding with the template. After removal of the template from the polymers, the formed microcavities by acryloyl-β-CD can selectively bind the template. In the process of recognition, the MWNTs-MIPs, besides the hydrogen bonding recognition sites, the hydrophobic cavities in acryloyl-β-CD polymers formed a specific shape recognition sites for the binding of erythromycin, which were capable of forming inclusion compounds with molecules that fit into their core-shaped hydrophobic cavities [25].

3.2. Characterization of MWNTs-MIPs

3.2.1. Fourier transform-infrared spectroscopy analysis

FT-IR spectroscopy was employed to characterize the crude MWNTs, MWNTs~~CH=CH_2 and MWNTs-MIPs and their spectrograms were shown in Fig. 3. As shown in Fig. 3b, the strong bands around 3600–3000 cm^{-1} resulted from O–H and N–H stretching vibrations. The absorbance at 1620 cm^{-1} was assigned to C=C

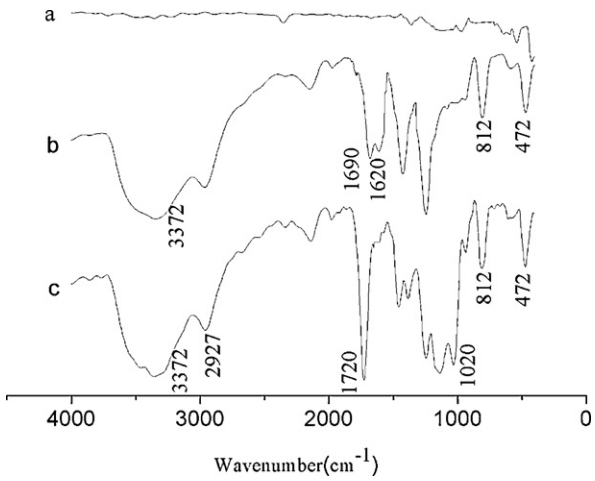

Fig. 3. FT-IR spectra of crude MWNTs (a), MWNTs ∿∿ $CH{=}CH_2$ (b) and MWNTs-MIPs (c).

stretch vibration. The results indicated that VTEOS was grafted on the MWNTs successfully. The presence of the characteristic band at 1690 cm^{-1} of CO–NH indicated that APTES was grafted onto the surface of the MWNTs via the amidation reaction between carbonyl groups of COCl and amino groups of APTES. These bands around 812 cm^{-1} and 472 cm^{-1} resulted from Si–O vibrations. It can be seen from Fig. 3c that the main IR features of acryloyl-β-CD spectrum were the strong peaks at 3370 cm^{-1} and 1020 cm^{-1}, corresponding to O–H and C–O–C absorption bands of glucose units, respectively. The results indicated that acryloyl-β-CD had been grafted on the surface of the MWNTs successfully. The stretching vibration at 1720 cm^{-1} was ascribed to COOH of MAA. The stretching vibration C–O (1720 cm^{-1}) and increase of C–O–C (1020 cm^{-1}) peak intensity revealed the existence of EGDMA [26]. It could be confirmed that the MIPs had been grafted on the surface of MWNTs successfully.

3.2.2. Morphological characterization

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were used to characterize the morphologies of the crude MWNTs and MWNTs-MIPs. As shown in Fig. 4a and b, the crude MWNTs were in the form of small bundles or individual tube. The diameter of the imprinted sorbent increases obviously, which revealed that the MIPs layer was attached on the MWNTs surface successfully. The TEM images revealed that average size of the crude MWNTs was about 30 nm and the length was several micrometers (Fig. 4c). After the imprinted polymerization, the MWNTs were coated drastically with homogeneous imprinted polymers layer (Fig. 4d). The average size increased to 80 nm. Thus it was calculated that the average thickness of the MIPs layer on the surface of MWNTs was about 25 nm.

3.3. Static adsorption

Adsorption capacity is an important factor for the MWNTs-MIPs. The static adsorption experiments for the MWNTs-MIPs and MWNTs-NIPs were carried out in erythromycin solution with the concentration ranging from 0.1 to 4.0 mmol/L. As shown in Fig. 5, at lower concentration of erythromycin, the amount of erythromycin was not enough to saturate the specific binding cavities. However, with erythromycin concentration increased, almost all the specific imprinted sites were occupied by erythromycin and the adsorption capacity of the MWNTs-MIPs reached the highest. The adsorption capacities of the MWNTs-MIPs were better than that of the corresponding MWNTs-NIPs. In addition, the adsorption capacity of MWNTs-MIPs for erythromycin was better than that of MWNTs-MIPs1 and MWNTs-MIPs2. It revealed that the MWNTs-MIPs prepared using acryloyl-β-CD and MAA

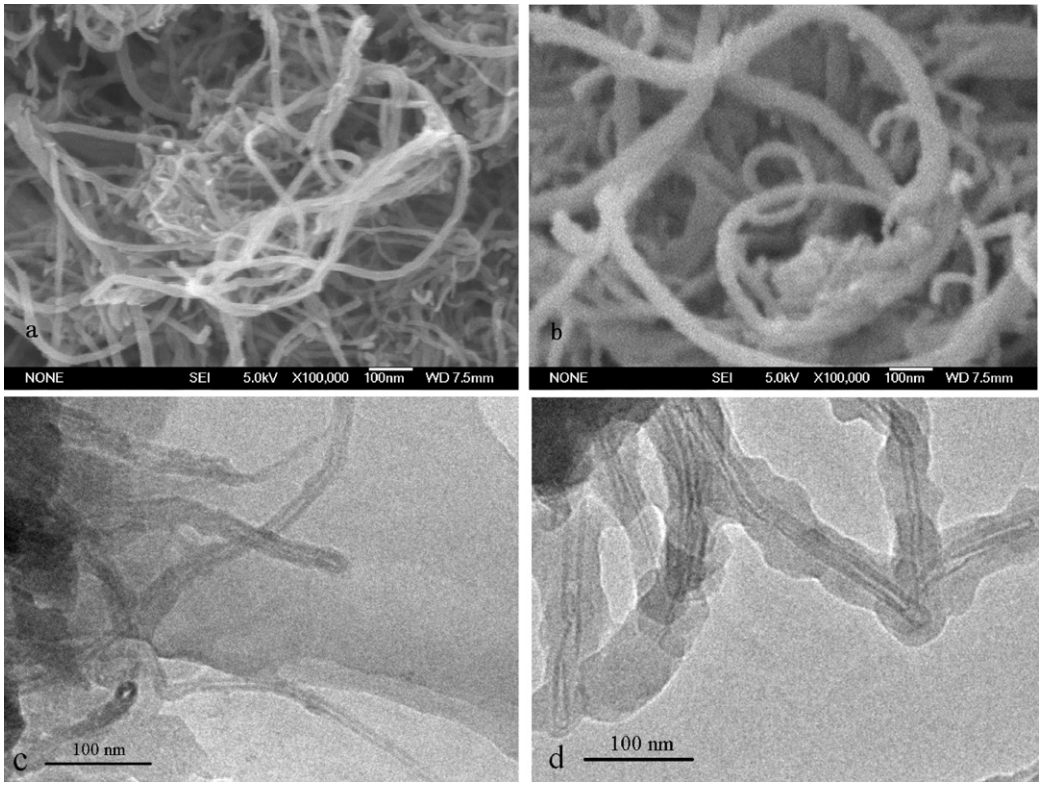

Fig. 4. SEM images of crude MWNTs (a) and MWNTs-MIPs (b); TEM images of crude MWNTs (c) and MWNTs-MIPs (d).

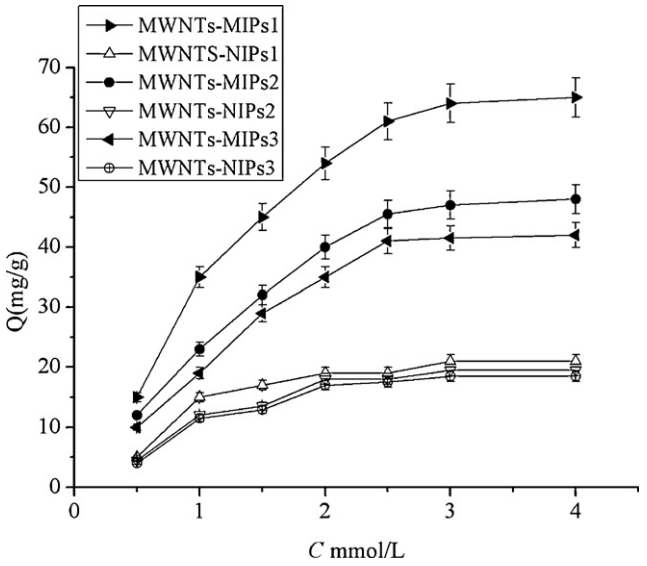

Fig. 5. Adsorption isotherms of erythromycin on the molecularly imprinted polymers and non-molecularly imprinted polymers.

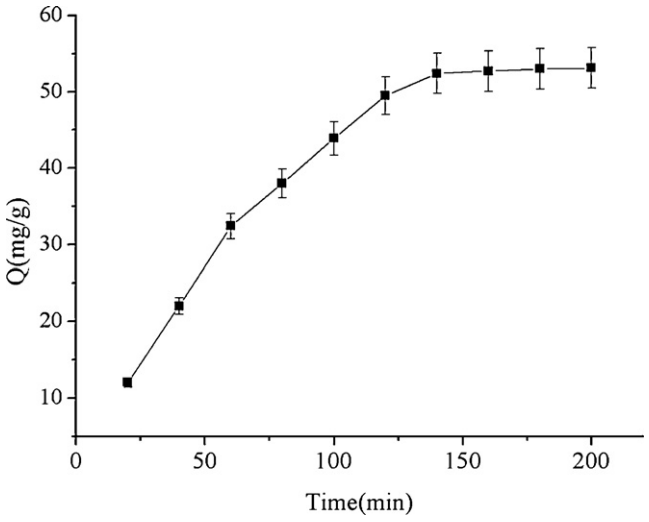

Fig. 6. Adsorption kinetics of MWNTs-MIPs.

had higher affinity than that of other two imprinted polymers prepared using acryloyl-β-CD and MAA, respectively. It owns to the fact that for the MWNTs-MIPs, acryloyl-β-CD and MAA could form a complexes with erythromycin through the hydrophobic and hydrogen bonding interaction simultaneously. MWNTs-MIPs1 recognized the template mainly based on the hydrophobic effect, while MWNTs-MIPs2 recognized the template mainly depending on the hydrogen bonding interaction. Therefore, it was verified that the hydrophobic interaction combined with the hydrogen bonding interaction was stronger than the hydrophobic interaction or the hydrogen bonding interaction [27]. For the MWNTs-MIPs, the templates and acryloyl-β-CD were pre-organized by hydrogen bonding interactions in the pre-polymerization mixture. Then, following the polymerization and subsequent removal of erythromycin, the positions and orientations of the functional monomer were immobilized in the polymers, which were complementary in size and shape to the template [28]. So the formed cavities have high adsorption ability toward erythromycin. However, the MWNTs-NIPs can not form specific recognition sites toward erythromycin in the absence of the template molecule.

3.4. Adsorption kinetics

Adsorption kinetics studies were carried out to investigate the adsorption process. As shown in Fig. 6, the adsorption rate of the MWNTs-MIPs toward erythromycin increased rapidly in the early 60 min, and then the rate of adsorption increased slowly with the time extension. After 140 min, the adsorption process achieved equilibrium. The reason can be explained that a large number of imprinted cavities existed on the MWNTs-MIPs surface, so the template molecules reach easily to the specific binding sites at the early time. When the recognition sites were filled up, the adsorption rate dropped significantly and adsorption process achieved equilibrium. The MWNTs-MIPs took about 140 min to reach adsorption saturation, which is shorter than that of the traditional imprinted polymers (about 3 h to reach adsorption saturation) [18].

3.5. Optimized of SPE

The SPE steps including loading, washing and eluting were optimized to achieve good sensitivity and precision for the extraction and detection of erythromycin.

Sample loading solvent plays an important role in the enrichment of the analyte due to the fact that it determines the microenvironment of the binding reaction and influences the stability of analyte. In order to evaluate the effect of loading solvent in SPE procedure, different loading solvents such as ethanol/H_2O (50%, 70%, 90%, 100%, v/v) were investigated on the MWNTs-MIPs-SPE column. The optimum result was obtained when 100% ethanol was employed, which almost all the loaded analyte was retained by the MWNTs-MIPs-SPE column with recovery being 83.87%. Thus 100% ethanol solution was selected as the sample loading condition in subsequent experiments.

The aim of the washing step is to minimize the interferences for the analysis step. It can activate the binding sites of the MWNTs-MIPs for maximizing their interactions with the target analyte. In this study, six washing solutions with different components were investigated as follow: acetonitrile/water (50:50, v/v), methanol/water (50:50, v/v), acetonitrile/water (40:60, v/v), methanol/water (40:60, v/v), acetonitrile/water (80:20, v/v), and methanol/water (80:20, v/v). As presented in Fig. 7, when the washing solution was a mixture of methanol/water (80:20, v/v),

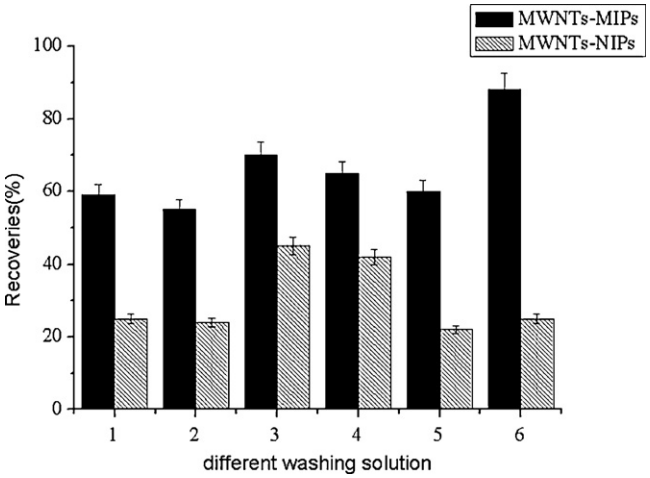

Fig. 7. Recoveries of erythromycin in molecularly imprinted solid-phase extraction column (MISPE) after washing with 2.5 mL of different solvents: 1 acetonitrile/water (50:50, v/v); 2 methanol/water (50:50, v/v); 3 acetonitrile/water (40:60, v/v); 4 methanol/water (40:60, v/v); 5 acetonitrile/water (80:20, v/v); 6 methanol/water (80:20, v/v).

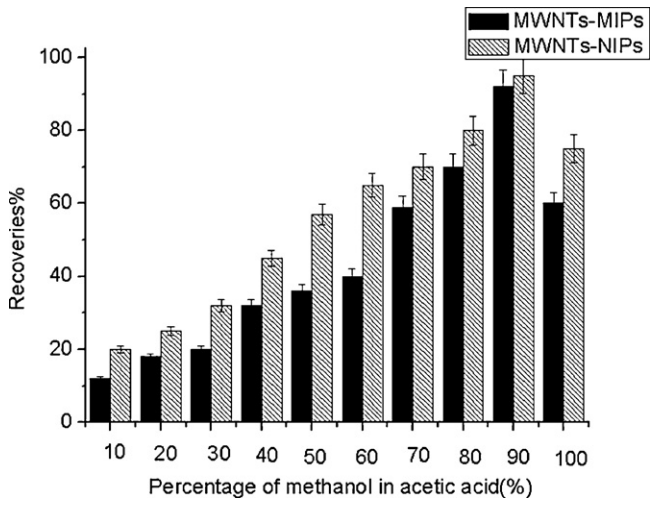

Fig. 8. Recoveries of erythromycin after washed with different percentage of methanol in acetic acid.

more than 88% of the loaded erythromycin was recoveried through the MWNTs-MIPs, while the amount of analyte eluted from the MWNTs-NIPs was less than 25%. Therefore, methanol/water (80:20, v/v) was used as the washing solvent in later experiments.

The effect of different proportions of methanol and acetic acid as the eluting solvent on the recovery of erythromycin was discussed in our study. As shown in Fig. 8, with the increased of methanol in the eluting solution, the recoveries of erythromycin increased steadily, and reached the highest when the amount of methanol was up to 90%. Therefore, methanol/acetic acid (9:1, v/v) was chosen as the eluting solvent in the following study.

3.6. Selectivity

In order to verify the selectivity of the MWNTs-MIPs toward erythromycin, roxithromycin and spiramycin were chosen as competitive molecules because of their similar chemical structures. Chromatograms of erythromycin, roxithromycin and spiramycin mixture solution passed through the MWNTs-MIPs-SPE were displayed in Fig. 9. Fig. 9a shows the chromatogram of directly loading the mixture solution passed through the MWNTs-MIPs-SPE column. It can be seen from Fig. 9a that erythromycin was retained more than roxithromycin and spiramycin. Because of the MWNTs-MIPs-SPE column cannot form specific binding sites for non-template molecule, roxithromycin and spiramycin were washed out from column in washing step. As shown in Fig. 9b, the washing solution contains a large amount of roxithromycin and spiramycin, and the template erythromycin was retained on the MWNTs-MIPs-SPE column. When the MWNTs-MIPs-SPE column was eluted with methanol/acetic acid (9:1, v/v) solution, a large number of erythromycin bound on the MWNTs-MIPs-SPE column was eluted out, thus the chromatogram peak of erythromycin was very high (shown in Fig. 9c). Imprinting factor (α) and selectivity factor (β) of the sorbents were shown in Table 2. The results showed that the MWNTs-MIPs exhibited good selectivity for the template. The adsorption capacity of the MWNTs-MIPs toward erythromycin was much higher than that toward roxithromycin and spiramycin. In the binding process, many specific recognition sites with respect to the template were generated on the MWNTs-MIPs surface, so the template was strongly bound by the imprinted polymers. The results demonstrated that the MWNTs-MIPs-SPE column exhibited specific selectivity for erythromycin in the presence of other structurally related compounds.

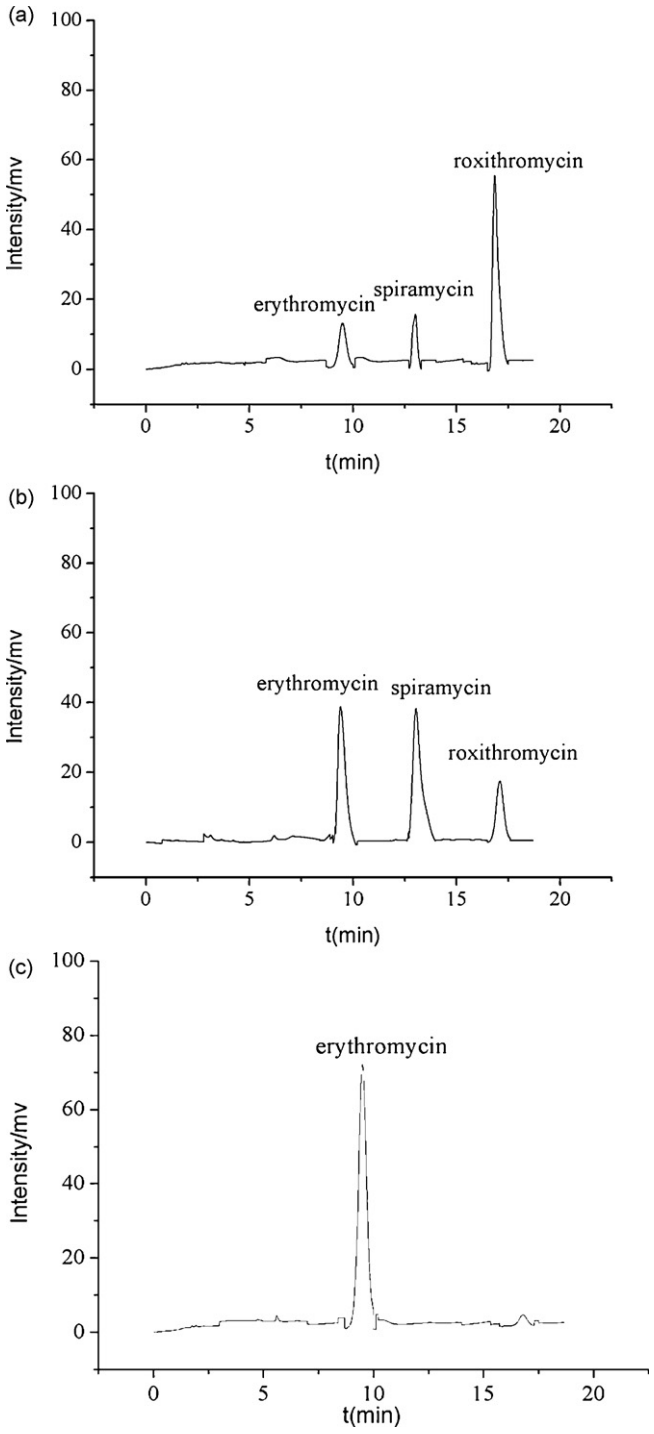

Fig. 9. Chromatograms of MISPE loading (a) washing steps (b) and eluting step (c).

Table 2
Imprinting factor (α) and selectivity factor (β) of the imprinted sorbents.

	Q(MWNTs-MIPs) (mg/g)	Q(MWNTs-NIPs) (mg/g)	α	β
Erythromycin	55.8	18.1	3.08	2.90
Roxithromycin	19.5	18.3	1.06	0.94
Spiramycin	20.1	17.9	1.12	–

Solution concentration: 2.0 mmol/L; $n = 5$.

Table 3
Recoveries at different spiked levels in chicken muscle.

Sample content	Spiked level (μg/kg)	Recoveries (%)	RSD $n = 5$
Chicken muscle	100.0	85.3	3.2
	200.0	92.1	4.0
	300.0	95.8	2.9
	400.0	89.7	4.5
Ethanol	100.0	92.3	2.8
	200.0	94.8	4.1
	300.0	98.1	3.2
	400.0	93.8	2.7

3.7. Application

To demonstrate the potential of the MWNTs-MIPs for the sample clean-up, the MWNTs-MIPs were applied for the purification of spiked erythromycin at the four levels in ethanol and chicken muscle, respectively. In our experiment, spiking was performed by adding a microvolume of solution containing four different concentrations of erythromycin to each portion of the weighed samples. The samples were extracted according to Section 2.7. The recoveries were calculated and summarized in Table 3. As can be seen in Table 3, the recoveries of erythromycin in pure solvent was ranging from 92.3% to 98.1% and the recoveries of erythromycin in chicken muscle samples was ranging from 85.3% to 95.8%, which suggested that the MWNTs-MIP-SPE method could be successfully applied for the purification and enrichment of erythromycin from chicken muscle.

4. Conclusions

A novel MWNTs-MIPs was prepared using multi-walled carbon nanotubes as the support matrix, acryloyl-β-CD and MAA as binary functional monomers. The MWNTs-MIPs were evaluated by transmission electron microscopy, scanning electron microscopy, and Fourier transform infrared spectroscopy. The results suggested that the MIPs were successfully immobilized on the MWNTs surface. Adsorption experiment showed the MWNTs-MIPs exhibited good selectivity toward erythromycin. Employed as a sorbent, the MWNTs-MIPs-SPE can selectively extract erythromycin from chicken muscle. The recoveries ranging from 85.3% to 95.8% indicated that the present imprinting protocol is a promising tool for enrichment and purification of erythromycin.

Acknowledgments

Project supported by the National Natural Science Foundation of China (no. 21005030), the Research Foundation of Education Bureau of Hunan Province, China (no. 10A099) and Hunan Provincial Innovation Foundation for postgraduate (no. CX2010b394) Science and Technology Innovative Research Team in Higher Educational Institutions of Hunan Province.

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