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Preparation and Evaluation of Florfenicol Imprinted Polymers by Using Nano-Titanium Dioxide

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Surface florfenicol-imprinting technique combined with a sacrificial-support process was established. Taking nano-titanium dioxide as sacrificial carrier and using acrylamide as the functional monomer, the hollow molecularly imprinted polymers (H-MIPs) with specific performances of recognition and adsorption towards to florfenicol were prepared. The microstructures of the molecularly imprinted polymers were characterized by the scanning electron microscopy and energy dispersive X-ray. A series of performance measurement including adsorption kinetics, adsorption isotherm and adsorption selectivity have been studied. In comparison with the polymers synthesized with traditional polymerization method, the results showed that the molecularly imprinted polymers obtained in this study had relatively homogenous structure with numerous mesopores. The results showed that the adsorption approached the equilibrium in 210 min. The research on binding performances of hollow molecularly imprinted polymers demonstrates better specific adsorption ability and selective adsorption to florfenicol. The k' of the hollow molecularly imprinted polymers was nearly 3.95-4.15 times greater than that of the non-imprinted polymer (H-NIPs).

Key Words: Molecularly imprinted polymers, Florfenicol, Nano-titanium dioxide, Sacrificial carrier.

INTRODUCTION

Florfenicol is a broad-spectrum antibiotic currently used in salmon aquaculture and proposed for use in channel catfish. As the injectable formulation, florfenicol has been approved for use in cattle (US and European Union) and swine (European Union only) for treatment of respiratory disease and/or foot rot[1]. Because of the lack of scientific management, the problem we face now is that animal drug residues had effected serious on human health. Florfenicol residues may include the unaltered parent compound and its metabolites and/or conjugates and may have direct toxic effects on consumers. The complexity of food matrices and the presence of many potential interferences, require specific and selective methods of analysis[2].

There is a constant requirement for accurate, simpler, faster and improved analytical methods. Various analytical methods have been reported for the determination of florfenicol in animal tissues, such as capillary electrophoresis[3], liquid chromatography (LC)[4], GC-mass spectrometry (MS)[5] and LC-MS[6]. But these approaches usually need to isolate and concentrate the target analytes from sample matrices, the methods require a large amount of organic solvents and many steps, so the pretreatment of the sample is not easy. Molecularly imprinted polymers is one of the most powerful tools for preparing materials that can bind analytes reversibly and selectively in the presence of their interferents[7]. Molecularly imprinted polymers (MIPs) are extensively cross-linked polymers containing specific recognition sites with a predetermined selectivity for analytes. It has been developed as separation media and affinity supports for the recognition of target molecules, such as drugs[8] and proteins[9].

According to various application requirements, many types of inorganic materials like SiO_2, TiO_2 and silica gel, *etc*. It can be used as support matrixes for surface imprinting technique[10-12]. In this work, we prepared florfenicol-functionalized molecularly imprinted polymers *via* a surface molecularly imprinting technique using TiO_2 as the support material. Compared with SiO_2 and silica gel, there is a growing consensus that TiO_2 possesses a high potential for the environmental applications due to its physical and chemical stability, lower cost, nontoxicity and the resistance to corrosion[13]. Strong oxidizing power of the holes, redox selectivity, high photostability, easy preparation and high affnity make TiO_2 a good candidate for environmental remediation[14]. Thus, in this study we are presenting a facile method for the synthesis of molecularly imprinted polymer *via* surface imprinting and a sacrificial support matrix molecular imprinting techniques (Fig. 1). The

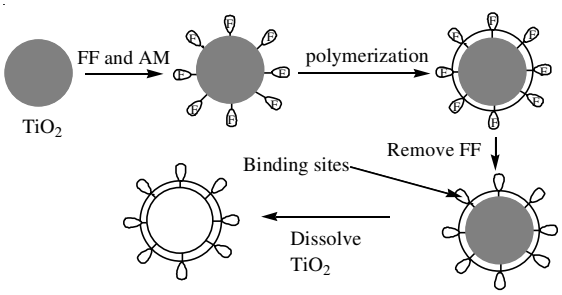


Fig. 1. Schematic illustration of the preparation of H-MIPs

design and synthesis of hollow functional materials with surface-imprinting technique and sacrificial-support concept had attracted intense interest because of their novel applications in advanced separation technologies[15].

EXPERIMENTAL

Nano-titanium dioxide was purchased from Jiangsu Taibai Group Co., Ltd. (Zhenjiang, China). Florfenicol was purchased from Jiangsu Provincial Institute for Veterinary Drug and Food Quality Control (Nanjing, China). Ethylene glycol dimethacrylate (EGDMA) were purchased from Sigma chemical company (USA). Azodiisobutyronitrile (AIBN), acrylamide, methanol, acetone, hydrofluoric acid (40 %) and methanol-acetic acid were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). These reagents were analytical grade unless specially stated. Distilled water was used throughout this work.The concentrations of florfenicol after the sorption were recorded by a UV-9600 spectrophotometer (Beijing, China).The morphology of the materials was obtained by scanning electron microscopy (SEM) energy dispersive X-ray (EDX) (S-4800).

Preparation of florfenicol-imprinted polymers (H-MIPs): A solution of 0.5 mmol florfenicol in a glass tube of 10 mL acetone was added to 4.0 mmol acrylamide. The monomer and template degassed and fully dispersed ultrasonically. After 0.5 h, TiO_2 (500.0 mg) was added to the contents and the latter maintained for 3 h at room temperature with continuous stirring at 300 rpm. The cross-linker monomer (EGDMA, 15.0 mmol) and the initiator (AIBN, 0.2 mmol)) were then added. First the mixture was sonicated for 10 min under a N_2 atmosphere and placed in a water bath at 50 ºC for 3 h, then the mixture was placed in a water bath at 60 ºC for 24 h. The polymer generated was separated and washed three times with ethanol to remove all impurities. The obtained polymers (FF-TiO_2-MIPs) were dried in vacuum for 12 h at 60 ºC.

The polymers was transferred to the PTFE beaker, then adding the appropriate amount of 40 % aqueous HF so that TiO_2 could be completely dissolved.This suspension was cooled in a water-ice bath and stirred at room temperature for 12 h. The suspension was diluted with deionized H_2O and washed extensively until neutrality. Then the obtained polymers were dried in vacuum for 12 h at 60 ºC. The resultant polymer was washed with methanol-acetic acid (9:1, v/v 200 mL) repeatedly until florfenicol cannot be detected in the filtrate. The polymer complex (H-MIPs) was dried in vacuum at 60 ºC overnight. As a reference, non-imprinted polymer (H-NIPs) was also prepared with absence of the template in parallel with the MIPs by using the same synthetic protocol.

Preparation of traditional florfenicol-molecularly imprinted polymers (FF-MIPs), the difference is that the polymerization system did not add the TiO_2 and without the HF treatment.

Adsorption experiments

Measurement of adsorption isotherm: To examine the adsorption isotherm of the imprinted (H-MIPs), 10 mg polymer was equilibrated with varied initial concentrations (20-26 μg/mL) of adsorbate in each conical flask. The conical flasks were placed in a shaker at a presettled temperature and shaken.The adsorption time was maintained for 6 h. After the adsorption reached equilibrium, the concentration of florfenicol in mixed solution was determined using ultraviolet visible absorption spectra at a wavelength of 223 nm. The equilibrium adsorption capacity (Q_e, mg/g) was calculated as:

$$Q_e = \frac{(C_0 - C_e)V}{m} \quad (1)$$

where C_0 (μg/mL) and C_e (μg/mL) are the initial and equilibrium concentration of florfenicol, respectively. V (mL) and m (mg) are the solution volume and the weight of adsorbent H-MIPs, respectively.

Adsorption kinetics: 10 mg polymer was equilibrated with varied initial concentrations (26 μg/mL) of adsorbate in each conical flask, the conical flasks were placed in a shaker at a presettled temperature and shaken at different times, the florfenicol solution was determined. The adsorption capacity (Q) was calculated as:

$$Q = \frac{(C_0 - C_t)V}{m} \quad (2)$$

where Q (mg/g) is the adsorption capacity in different time; C_t (μg/mL) is the equilibrium concentration of florfenicol, V (mL) is the volume of the florfenicol solution; m(mg) is the weight of the absorbent H-MIPs.

Study of selectivity: In order to estimate the selectivity of synthesized polymer for florfenicol, several drugs such as: thiamphenicol and chloramphenicol were chosen due to their molecular structures being similar to florfenicol to some extent. In each test, the initial concentration of each solution was 10 μg/mL. After adsorption equilibrium was reached, the concentrations of florfenicol,thiamphenicol and chloramphenicol in the solutions were determined, respectively. Distribution coefficient (K_d, mL/mg) was defined as:

$$K_d = \frac{Q_e}{C_s} \quad (3)$$

where K_d (mL/mg) represents the distribution coefficient; Q_e (mg/g) is the equilibrium adsorption capacity; C_s (μg/mL) is the equilibrium concentration.

$$k = \frac{K_{d(FF)}}{K_{d(B)}} \quad (4)$$

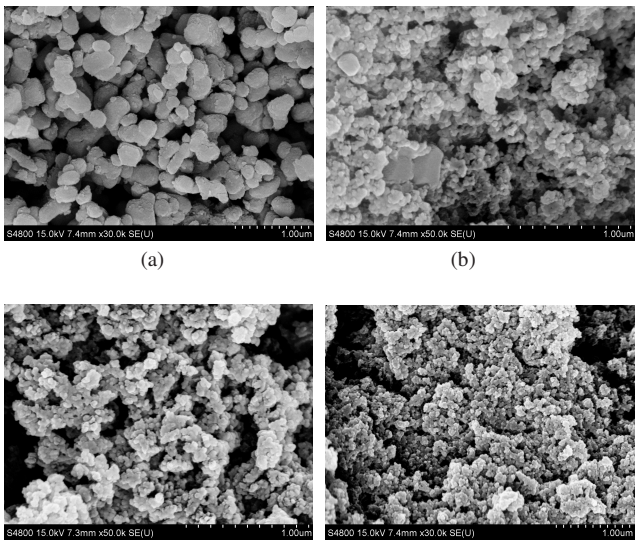
where k is the selectivity coefficient and B represents the thiamphenicol and chloramphenicol. The value of k allows an estimation of selectivity of H-MIPs for florfenicol. A relative selectivity was defined as

$$k' = \frac{k_{H\text{-}MIPs}}{k_{H\text{-}NIPs}} \quad (5)$$

The value of k' can indicate the enhanced extent of adsorption affinity and selectivity of H-MIPs for the template with respect to H-NIPs.

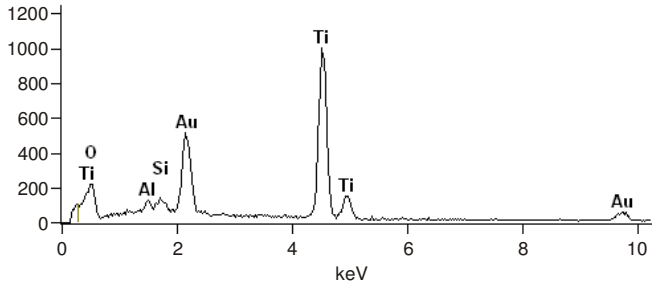
RESULTS AND DISCUSSION

SEM analysis: The SEM images of nano-titanium dioxide and the molecular imprinted polymers are shown in Fig. 2(a) was TiO_2, (b) and (c) show images of the polymers before sacrificial carrier (FF-TiO_2-MIPs)and after sacrificial carrier. To the certain extent, the process of sacrificial carrier had effected the surface and shape of the polymers, but the polymer particles were still homogeneous. The holes on the surface of polymers increase the surface area, polymers with more specific affinity sites for a target molecule. There are some disadvantages about FF-MIPs(d) where materials must be sieved prior to use, leading to the partial destruction of the recognition sites. The energy dispersive X-ray (EDX) are shown in Fig. 3(a) was nano-titanium dioxide, (b) and (c) was the polymers before sacrificial carrier and after sacrificial carrier. Through the comparison of (b) and (c), titanium atoms are still residues, but were significantly reduced with the process of sacrificial carrier. The results of EDX measurements evidenced that the process of sacrificial carrier was successful.

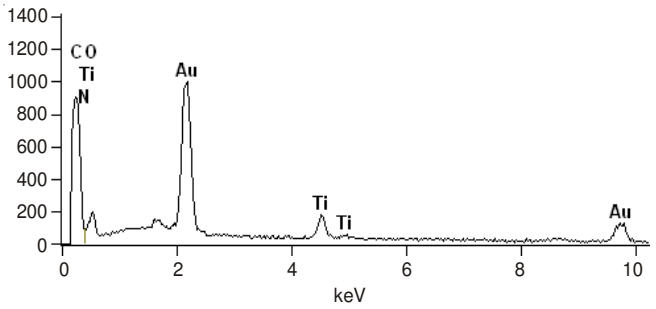


(a) (b) (c) (d)

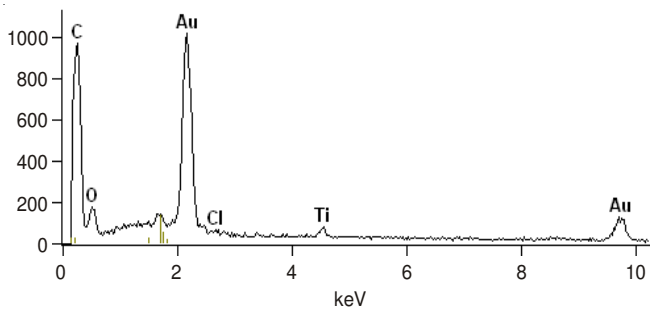
Fig. 2. SEM images of TiO_2 (a), FF-TiO_2-MIPs (b), H-MIPs (c) and FF-MIPs (d)



(a)



(b)



(c)

Fig. 3. EDX of TiO_2 (a), FF-TiO_2-MIPs (b) and H-MIPs (c)

Adsorption kinetics: The kinetic adsorption curve is shown in Fig. 4. The adsorption capacity for florfenicol on H-MIPs increases sharply within the first 150 min. At the beginning, as H-MIPs possesses large quantities of unoccupied imprinted rebinding sites, florfenicol molecules are adsorbed preferentially to react with the rebinding sites present on the polymers, displaying rapid adsorption. Then the adsorption approached equilibrium gradually in the last 90 min.

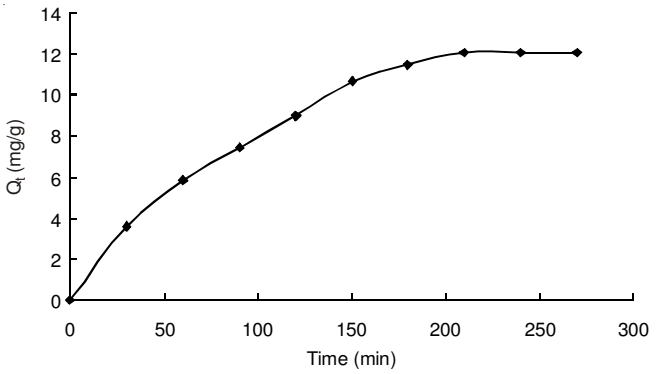


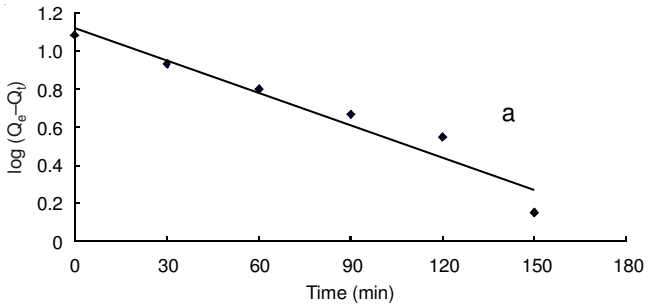
Fig. 4. Kinetic adsorption curve of H-MIPs for FF (C_0: 26 µg/mL; m = 10 mg; V = 10 mL; temperature: 318 K)

To study the adsorption kinetics for florfenicol over MIPs, the pseudo-first-order[16] (eqn. 6) and pseudo-second-order[17] (eqn. 7) models were selected to fit the experimental data to clarify the adsorption mechanism for florfenicol.

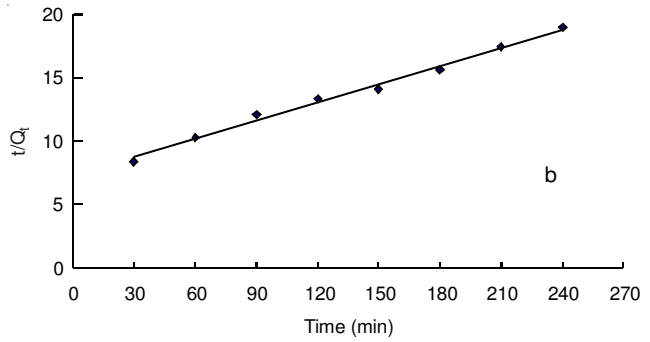
$$\log(Q_e - Q_t) = \log Q_e - \frac{k_1(t)}{2.303} \quad (6)$$

$$\frac{t}{Q_t} = \frac{1}{k_2 Q_e^2} + \frac{t}{Q_e} \quad (7)$$

where Q_e and Q_t (mg/g) represent florfenicol adsorption capacities for MIPs at equilibrium and at time t, respectively; k_1 is rate constant, the slope of the linear plotting of log (Q_e-Q_t) in respect of t, k_2 is rate constant,the slope of the linear plotting of t/Q_t in respect of t. A linear equation of florfenicol adsorption over MIPs were obtained in Fig. 5(a-b) (where MIPs equilibrium adsorption capacity is 12.1 mg/g. Table-1 shows the rate constant $k_1 = 0.0129$ min^{-1}, with a correlation coefficient $R^2 = 0.9428$; the rate constant $k_2 = 0.00032$ min^{-1}, with a correlation coefficient $R^2 = 0.9906$). These suggest that MIPs adsorption obeys the pseudo-second-order reaction kinetic.



(a)



(b)

Fig. 5. Linear plot of log (Q_e-Q_t) *versus* t(a) and t/Q_t *versus* t(b) temperature: 318 K; initial concentration: 26 µg/mL

TABLE-1
COEFFICIENTS OF THE PSEUDO-FIRST-ORDER AND PSEUDO-SECOND-ORDER KINETIC MODELS AS APPLIED TO THE ADSORPTION OF FF BY H-MIPS (318 K)

T (K)	Pseudo-first-order kinetics		Pseudo-second-order kinetics	
	k_1 (min^{-1})	R^2	k_2 (g mg^{-1} min^{-1})	R^2
318	0.01290	0.9428	0.0003173	0.9906

Adsorption isotherms: Fig. 6 shows the florfenicol adsorption isotherms for the H-MIPs, with the increase of florfenicol concentration, the adsorption capacity increases significantly. The adsorption capacity increased with increasing temperature, but did not increase significantly. To further assess the success of molecular imprinting, Table-2 summarizes all the fitting parameters (Q_m, K_L, K_F, n and R^2)[18,19], all of these were calculated from eqs.

$$\frac{C_e}{Q_e} = \frac{C_e}{Q_m} + \frac{1}{(Q_m K_L)} \quad (8)$$

$$\ln Q_e = \left(\frac{1}{n}\right) \ln C_e + \ln K_F \quad (9)$$

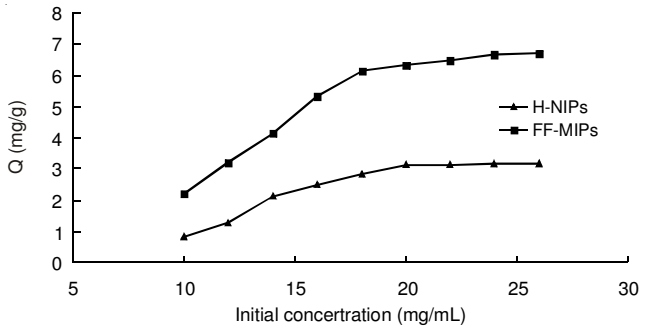


Fig. 6. Adsorption isotherms of florfenicol onto H-MIPs (298-318 K), FF-MIPs (318 K) and H-NIPs (318 K) (C_0: 10-26 µg/mL; m = 10 mg; V = 10 mL)

TABLE-2
PARAMETERS OF THE LANGMUIR ADSORPTION EQUATION AND THE FREUNDLICH ISOTHERMAL EQUATION AS APPLIED TO THE EXPERIMENTAL ADSORPTION DATA

T (K)	Langmuir parameters			Freundlich parameters		
	Q_m (mg/g)	K_L (L/mg)	R^2	K_F	1/n	R^2
298	35.71	0.02313	0.5121	1.019	0.8132	0.9648
308	43.86	0.02035	0.5999	1.046	0.8481	0.9716
318	65.36	0.01426	0.7257	1.058	0.8849	0.9931

where Q_m (mg/g) is the theoretical maximum adsorption capacity by monolayer adsorption, C_e (µg/mL) is the equilibrium concentration of florfenicol. K_L (mL/µg) and K_F, respectively are Langmuir and Freundlich constants, n is Freundlich constants related to temperature.

The Freundlich adsorption equation gives a better fit to the equilibrium adsorption data. It is more suitable in Freundlich adsorption isotherm than in Langmuir isotherm. So the adsorption of florfenicol onto H-MIPs occurs *via* a multiple layer process. In addition, adsorption capacity of H-MIPs increased with the increase of temperature.Adsorption capacity of H-MIPs towards florfenicol was higher than that of FF-MIPs and H-NIPs.

Adsorption selectivity: As shown in Fig. 7, the adsorption capacity of H-MIPs for florfenicol was about 2 times than FF-MIPs,because H-MIPs was synthesized by the surface molecular imprinting technique,with a sacrificial matrix process,it has more specific cavities.

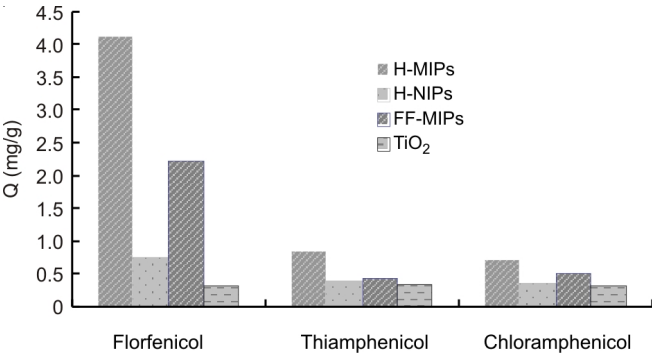


Fig. 7. Adsorption selectivity of H-MIPs

In Table-3, H-MIPs also exhibited a certain binding affinity to thiamphenicol and chloramphenicol, because of its similar size, shape and action sites to the imprinted cavities, but the selectivity of synthesized polymer dependence to the

TABLE-3
SELECTIVE RECOGNITION OF THE TiO_2, FF-MIPS,H-NIPS AND H-MIPS FOR FLORFENICOL, THIAMPHENICOL AND CHLORAMPHENICOL

	TiO_2		FF-MIPs		H-NIPs		H-MIPs		k'
	K	k	K	k	K	k	K	k	
Florfenicol	0.034	–	0.285	–	0.081	–	0.72	–	–
Thiamphenicol	0.035	0.97	0.045	6.33	0.041	1.98	0.092	7.83	3.95
Chloramphenicol	0.033	1.03	0.053	5.38	0.036	2.25	0.077	9.35	4.15

presence of specific cavities designed for florfenicol, the K of the H-MIPs to florfenicol was about 7.83 and 9.35 times that of thiamphenicol and chloramphenicol, respectively. TiO_2 and H-NIPs exhibits relatively lower adsorption capacity; it should be attributed to the absence of suitable recognition sites and imprinted cavities to the target molecules.The results showed that the k' of the H-MIPs was nearly 3.95-4.15 times greater than that of the H-NIPs.

Conclusion

Molecularly imprinted polymers (MIPs) using florfenicol as the template, acrylamide (AM) as the monomer and ethylene glycol dimethacrylate (EGDMA) as the crosslinker were prepared by polymerization. Preparation and adsorption behaviour of florfenicol imprinted polymers by using nano-titanium dioxide as sacrificed support material. The results obtained using scanning electron microscopy indicated that with a sacrificial matrix process, H-MIPs have more larger specific surface area and pore features. An appropriate increase in temperature enhanced the adsorption process. The adsorption process followed pseudo-second-order model by kinetics analysis and Freundlich equation by isotherm analysis. The H-MIPs showed higher uptaking capability to florfenicol, in aqueous solution, in comparison to NIPs. The selectivity of H-MIPs for florfenicol was noticeably more than that for other similar compound like thiamphenicol and chloramphenicol. The results showed that the k' of the H-MIPs was nearly 3.95-4.15 times greater than that of the H-NIPs.

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REFERENCES

1. C. Wrzesinski, L. Crouch, P. Gaunt, D. Holifield, N. Bertrand and R. Endris, *Aquaculture*, **253**, 309 (2006).
2. B. Boyd, H. Björk, J. Billing, O. Shimelis, S. Axelsson, M. Leonora and E. Yilmaz, *J. Chromatogr. A*, **1174**, 63 (2007).
3. P. Kowalski, L. Konieczna, A. Chmielewska, I. Oledzka, A. Plenis, M. Bieniecki and H. Lamparczyk, *Pharm. Biomed. Anal.*, **39**, 983 (2005).
4. A. Posyniak, J. Zmudzki and J. Niedzielska, *Anal. Chim. Acta*, **483**, 307 (2003).
5. P. Li, Y. Qiu, H. Cai, Y. Kong, Y. Tang, D. Wang and M. Xie, *Chin. J. Chromatogr.*, **24**, 14 (2006).
6. S. Zhang, Z. Liu, X. Guo, L. Cheng, Z. Wang and J. Shen, *J. Chromatogr. B*, **875**, 399 (2008).
7. P.S. Sharma, F. D'Souza and W. Kutner, *TrAC Trends Anal. Chem.*, **34**, 59 (2012).
8. U. Athikomrattanakul, M. Katterle, N. Gajovic-Eichelmann and F.W. Scheller, *Biosens. Bioelectron.*, **25**, 82 (2009).
9. J. Gao, H. Tian, Y. Wang, Q. Yang, D. Liu and H. Mi, *J. Biomater.*, **33**, 3344 (2012).
10. W. Zhao, N. Sheng, R. Zhu, F. Wei, Z. Cai, M. Zhai, S. Du and Q. Hu, *Hazard. Mater.*, **179**, 223 (2010).
11. D.H. Yang, N. Takahara, S.W. Lee and T. Kunitake, *Sens. Actuators B*, **130**, 379 (2008).
12. T.P. Hu, Y.M. Zhang, L.H. Zheng and G.Z. Fan, *J. Fuel Chem. Technol.*, **38**, 722 (2010).
13. W.C. Hung, S.H. Fu and J.J. Tseng, *Chemosphere*, **66**, 2142 (2007).
14. T. Balaji and H. Matsunaga, *Anal. Sci.*, **18**, 1345 (2002).
15. E. Yilmaz, O. Ramstrom, P. Moller, D. Sanchez and K.J. Mosbach, *Mater. Chem.*, **12**, 1577 (2002).
16. E. Tütem, R. Apak and Ç.F. Ünal, *Water Res.*, **32**, 2315 (1998).
17. O. Aksakal and H.J. Ucun, *Hazard. Mater.*, **181**, 666 (2010).
18. M.J. Mazzotti, *J. Chromatogr. A*, **1126**, 311 (2006).
19. S.J. Allen, G. McKay and J.F. Porter, *J. Colloid Interf. Sci.*, **280**, 322 (2004).