Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(6):1699-1708

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Highly sensitive detection of morphine based on molecular imprinting polymers using surface plasmon resonance

Hongxia Hao[a*], Hong Zhou[b], Ling Zeng[a], Jianjun Liu[c] and Yao Liu[b]

[a]*Key Laboratory of Evidence Science, China University of Political Science and Law, Ministry of Education, Beijing, China*
[b]*Institute of Forensic Science, Ministry of Public Security, Beijing, China*
[c]*Department of Science, Beijing Institute of Technology ; Beijing, China*

ABSTRACT

A surface Plasmon resonance (SPR) sensor highly sensitive and selective for morphine (MO) was prepared in molecular imprinted technology, in which methacrylic acid (MAA) was used as the functional monomer; Ethyleneglycol dimethacrylate (EGDMA) as the cross-linker; and azobisisobutylnitrile (AIBN) as the initiator. This approach features a rapid response, good selectivity, label-free detection and most notably, this sensor with very low LOQ (0.3ng/ml) can be applied to trace MO determination at ppb order of magnitude. The SPR response is proportional to the concentration of morphine in acetonitrile (R^2=0.9971) in the range of 10^{-9}M to10^{-6}M (0.3–300ng/mL) with LOD (0.001ng/ml). Moreover, the proposed method can discriminate between morphine and very close structural analog codeine.

Keywords: Forensic Science; Surface Plasmon Resonance (SPR); Molecular Imprinted Technology (MIT); Morphine; Sensor; Drug analysis.

INTRODUCTION

Morphine (MO) is well-known not only as a clinical drug for preventing patients from suffering pains, but also as a narcotic when it is used excessive or habitual. Thus, it is important to reliably monitor the concentration of morphine conveniently in the biological samples as forensic evidence. Especially for the requirement of the field-testing, rapid detection of morphine has attracted much attention in recent years. So far as, many efforts have been made for morphine detection using techniques such as HPLC [1], GC-MS [2], capillary electrophoresis [3,4], colorimetric and fluorescent method [5,6], electrochemical analysis [7], immunoassay [8-11]. However, to a certain extent, there exist some limitations related to these methods, the facts that they are relatively time-consuming, require expensive equipment, lack selectivity, the poor stability of biological reagents is also a drawback. Therefore, it is still necessary to find new approaches that could improve the simplicity, selectivity and sensitivity of morphine detection.

Sensory technology based on molecular imprinting polymers (MIP) has attracted considerable interest due to its high specificity for binding analyte through covalent or non-covalent interactions between the functional monomers and the target molecules [12-14]. In the past years, the molecular imprinting method has been applied to the detection of a wide range of analytes including biological molecules, environmental pollutants or explosive compounds [15,16]. Only a few MIP-based sensors for narcotic detection were reported however. Moreover, almost of these sensors relied on an output electrochemical signal and shared a common shortcomings in sensitivity (the limit of detection was restricted to 0.01mM) [17-21]. To improve the sensitivity and selectivity in the detection of MO, we developed a label-free sensing system using a combination of MIP and surface plasmon resonance (SPR), an optical technique with good sensitivity and simplicity for analyte assays [22, 23].

In this work, a molecular imprinting polymer membrane was prepared as a sensing transducer for SPR. It can be applied for on-the-scene determination of MO in the range of 10^{-9}–10^{-6}M. To the best of our knowledge, this is the first example of morphine detection using SPR-MIP-based sensor. This approach features a rapid response, good selectivity, label-free detection and most notably, this sensor has very low limit of qualification (LOQ).

EXPERIMENTAL SECTION

Materials

All chemicals were purchased from Sinopharm Chemical Reagent Corporation (Shanghai, China) and were used as received. Morphine hydrochloride was obtained from Forensic Sciences Institute, Ministry of Public Security (Beijing, China). In order to prepare the morphine free base, 300 mg of morphine hydrochloride were dissolved in 10 mL of water, then 50 mL of concentrated NH_4OH was added and morphine base was precipitated. The solid was filtrated and dried under reduced pressure to give morphine free base.

Sensor membrane and sample preparation

Morphine MIPs were prepared by carefully heating 7.1 mg (0.025mmol) of morphine free base and 8.6 mg (0.1mmol) of methacrylic acid until a homogeneous phase formed. Then, 0.04 g (0.2mmol) of ethylene glycol dimethacrylate, 2mL of acetonitrile, and 3mg of AIBN were successively added and the mixture was purged with nitrogen and polymerized on gold (50nm) SPR chips under nitrogen at 60℃ for 4h. The polymer films were cooled at ambient temperature and the chips were installed in the SPR machine. After that, the films were washed with acetonitrile and 4% acetic acid until a stable baseline was reached. After elution, the morphine solutions (in DMF/acetonitrile 1:99) with different concentration (10^{-9}mol/L, 10^{-8}mol/L, 10^{-7}mol/L, and 10^{-6}mol/L) were injected. In order to test the selectivity of the sensor, a solution of codeine analogue (10^{-4}mol/L, more than 10^5 times to the LOQ of MO) was also injected.

Measurements

The SPR spectrometer used in this study is home-built. From the Linearly polarized He-Ne laser source (DH-HN350P, 632.8nm, DAHENG, CHINA), the light travels through a chopper (M197 AMETAK, USA), polarizer, prism and sample cell, then into the photodiode and Dual Phase DSP Lock-in Amplifier (7265 Signal Recovery, USA). Schematic illustration of the SPR/MIP chip system is showed in Figure1**.** The polymer surface was scanned by Scanning Electron Microscopy (BACPCS4800, Hitachi, Japan) and the polymer thickness was measured using Bruker Profiler Dektak XT. Absorption spectra were collected using a VARIAN CARRY 50 UV-visible spectrometer. ^{1}H NMR spectra were carried out on a JNM-ECA600 spectrometer (JEOL).

RESULTS AND DISCUSSION

Sensing performance study for the MO detection

The MIP film was prepared by polymerizing the functional monomers directly on the gold-coated silylated glass (LaSFN9, Refractive index 1.85002). Surface morphology of the MIP film was observed by scanning electron microscopy, which showed even and closely packing. The polymer thickness was assessed by PROFILOMETER at about 50μm. Figure 2 compares the SPR spectra of the sensor chip in the absence and presence of the MIP film on the gold surface. As shown in Figure 2, the resonance angle without MIP (bare gold) was 25.4^o. As expected, it changed 45.7° after coating of the MIP film. To obtain the cavities of MO, the MIP film was washed with acetonitrile and 4% acetic acid. The SPR spectra before and after washing are shown and compared in Figure 3. Obviously, there is decrease (by 0.8 degree) of the incident angle, which indicated that the recognition sites of MO were regenerated. The MO binding assay of this MIP system was performed by monitoring the SPR resonance angle change of the MIP-coated chip. Upon adding increasing amounts of MO into the injected solution, the angle shift of the SPR response increased gradually, as shown in Figure 4. Notably, there exists an excellent linear relationship between the angle shift ($\Delta\theta$) and the logarithm of the concentration of MO (log [MO]) in the range of 10^{-9} M to 10^{-6} M (Figure 5).

To elucidate the specificity of the MIP film-coated sensor for MO, a structurally related analog codeine, was also tested with the same protocol. It was found from Figure 6 that upon injection of a high concentration of codeine solution, the sensing membrane with MO-cavities detects negligible resonance angle shift, unlike the significant shifts obtained for MO at much lower concentration (10^{-9} M). This result should be attributed to the methyl ether group (-OCH3) substituting the phenol group (-OH) in the 3-position of codeine. Therefore, one can reasonably conclude that hydrogen bonding must play a crucial role in promoting the absorption of MO in our MIP-film. As a result, the MIP film-coated sensor can easily discriminate morphine from codeine at low concentration levels. Further discussion about the sensing mechanism is provided below.

Reproducibility of the MIP-SPR sensor

The remarkable advantage of MIPs is the irrelative stability compared to biological sensor elements. This can be represented by the reproducibility of our measurements upon regeneration of the sensing surface. Figure 7 illustrates the kinetic curves of elution and absorption processes. It was shown that after three repeats under the same conditions, the sensing response for MO exhibited a good reproducibility with a RSD <1%. Further elution and absorption studies of the MO MIPs suggested that this sensor can be regenerated more than ten times with strong response signal.

Sensing mechanism study

To address the mechanism behind the selectivity of the MIP-coated sensor toward MO, the absorption spectra of MO and codeine in the presence of functional monomer MAA in methanol were compared. As shown in Figure 8, the absorption of MO exhibits two major peaks at 211 and 287 nm, similarly to MAA which exhibits an absorption maximum around 212 nm. However, compared to the theoretical overlay spectrum of MO and MAA the absorption maximum of the mixture is red-shifted to 214 nm and is accompanied by an absorbance decreased. Given the fact that hydrogen bonding can lower the energy of π–π* transition in a polar solvent and limit the resonance of the aromatic ring, featuring a absorption red-shift and hypochromicity, one can conclude that a complex between MO and MAA which is driven by hydrogen bond has been formed. To further confirm the importance of the hydrogen bond in the formation of these supramolecular complexes, temperature-dependent absorption spectra of MO and MAA are also collected. It was found from Figure 9 that the magnitudes of the absorption band gradually increased along with temperature conversely to λ_{max} which was blue-shifted. These results can be attributed to the breakage of the hydrogen bond between MO and MAA at higher temperature. Moreover, the differences between the ^{1}H NMR spectra of the carboxyl moiety in MAA and the MO/MAA complex further support this conclusion (Figure 10). In the complex, the protons in the carboxyl moiety are broadened and vanished relative to those for "free" MAA in solution, indicating the significant contribution of hydrogen bond between MAA and MO. It is confirmed that hydrogen bonding plays a crucial role in promoting the formation of MO / MAA complex.

Evaluation of affinity constants

To obtain more insight into the formation mechanism of the MO / MAA complex, the affinity constant between MO and MAA was determined by ratio-dependent absorption spectra (Figure 11). The interaction between MO and MAA can be described by the following equation (1) and (2). Here k is the affinity constant and n is the molar between for MAA and MO. Assuming the initial concentrations of MO and MAA are a_0 and b_0 respectively, equations (4) and (5) can be obtain and the complex concentration [M-M] is given by equation (3) in the case of b_0>>a_0(here n[*M-M*] is ignored and [*MAA*] = b_0).

$$[MO] + n[MAA] = [M-M] \quad (1)$$

$$k = \frac{[M-M]}{[MO][MAA]^n} \quad (2)$$

$$[M-M] = \frac{kb_0^n a_0}{1+kb_0^n} \quad (3)$$

According Beer–Lambert law, the absorbance of the mixture solution is composed of three parts, which is depicted in equation (6) where ε_A, ε_B and ε_C are the molar extinction coefficients for MO, MAA and M-M, respectively.

$$[MO] = a_0 - [M-M] \quad (4)$$

$$[MAA] = b_0 - n[M-M] \quad (5)$$

Considering equations (4) and (5), equation (7) can be got from equation (6). It can be defined that $A_a^0 = \varepsilon_a l a_0$ and $A_b^0 = \varepsilon_b l b_0$, so the absorbance difference (ΔA) should be given by the equation (8).

$$A = \varepsilon_a l[MO] + \varepsilon_b l[MAA] + \varepsilon_c l[M-M] \quad (6)$$

$$A = \varepsilon_a l a_0 + \varepsilon_b l b_0 + (\varepsilon_c - \varepsilon_a - n\varepsilon_b) l[M-M] \quad (7)$$

$$\Delta A = A - A_a^0 - A_b^0 = (\varepsilon_c - \varepsilon_a - n\varepsilon_b) l[M-M] = \Delta\varepsilon l[M-M] \quad (8)$$

Figure1. Schematic illustration of the SPR/MIP chip system

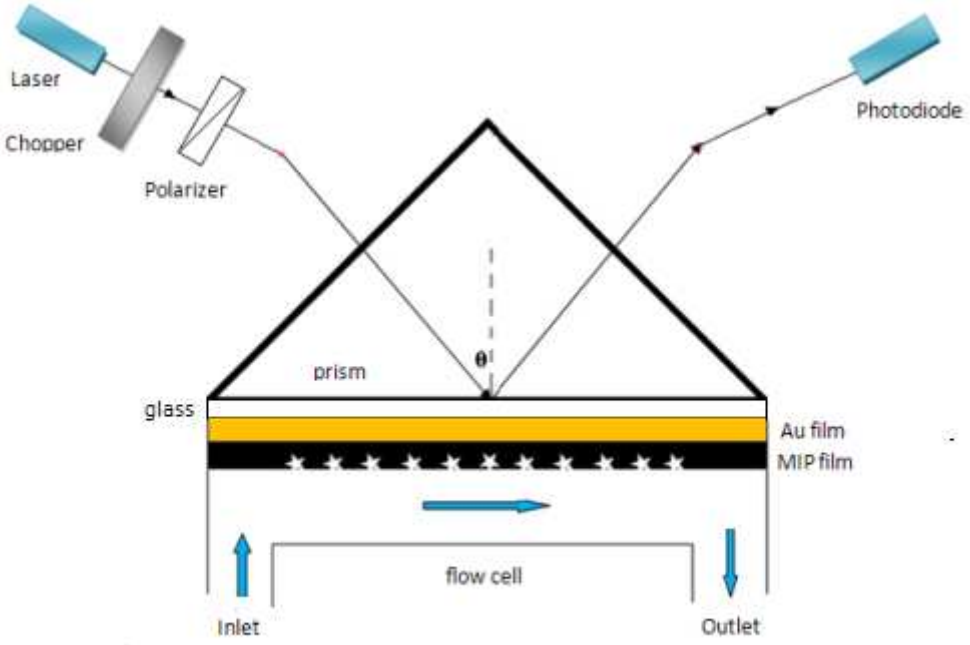
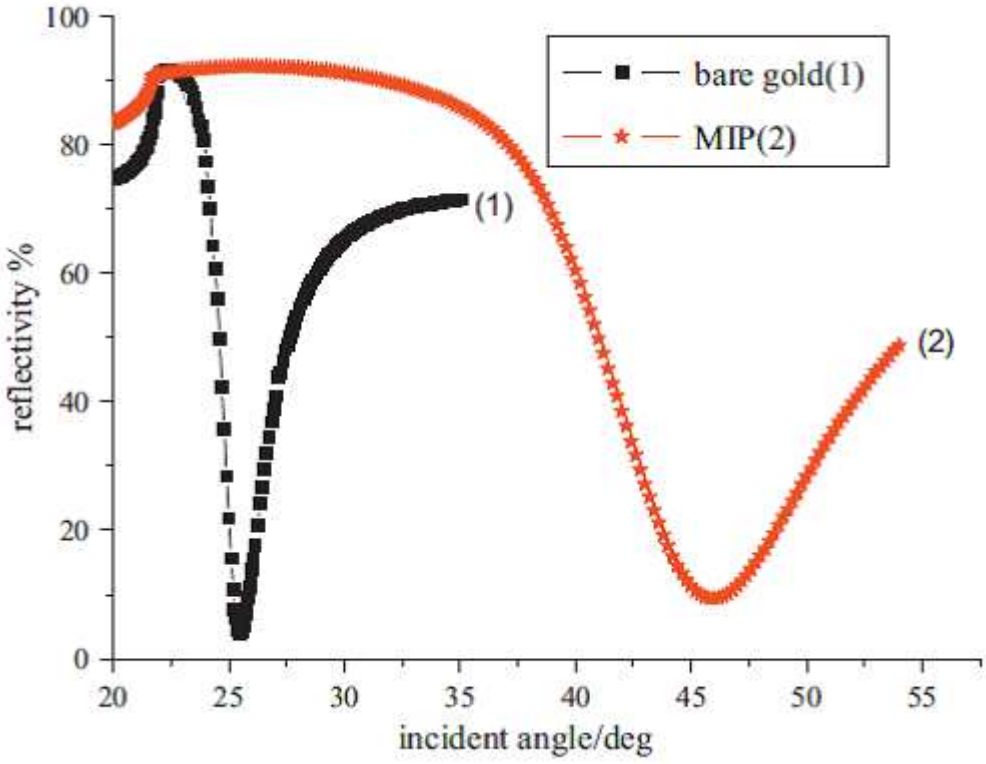


Figure2. SPR spectra of bare gold (1) and MIP (2) in the air



By inserting equation (3) into equation (8) and rearranging, equation (9) is obtained where k, $\Delta\varepsilon$, l and a_0 are constants. From this equation (9), at a reasonable value of n, there will be a good linear relationship between $\frac{\Delta A}{b_0^n}$ and ΔA. Along this approach, several values of n were inserted and certificated.

Figure3. Incident angle of MIPs before and after washing

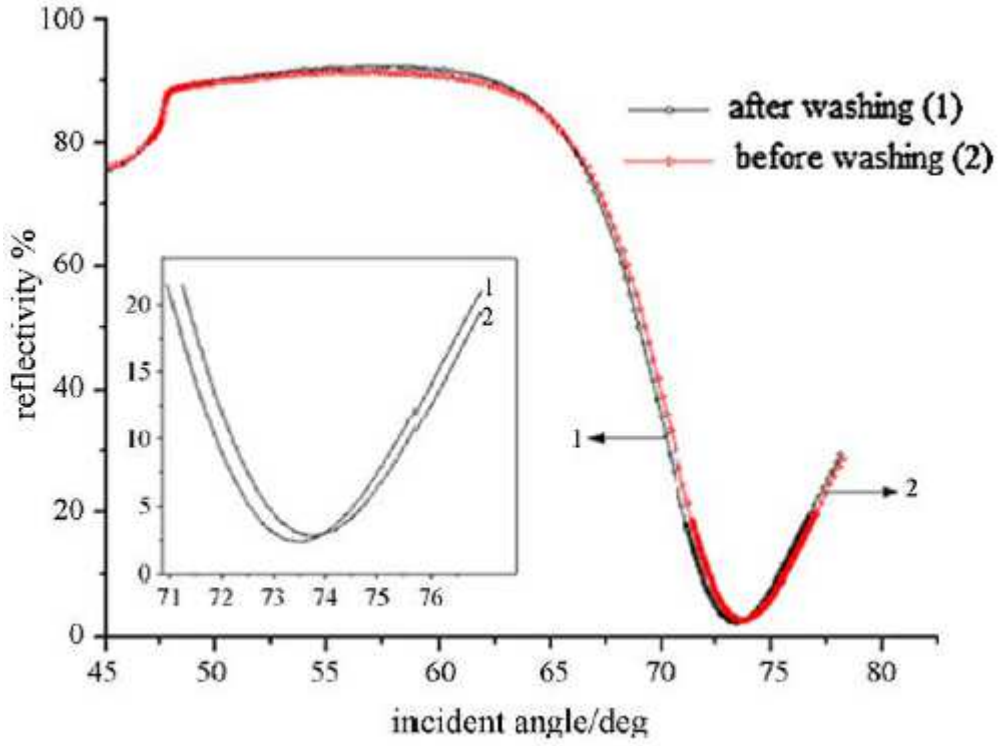
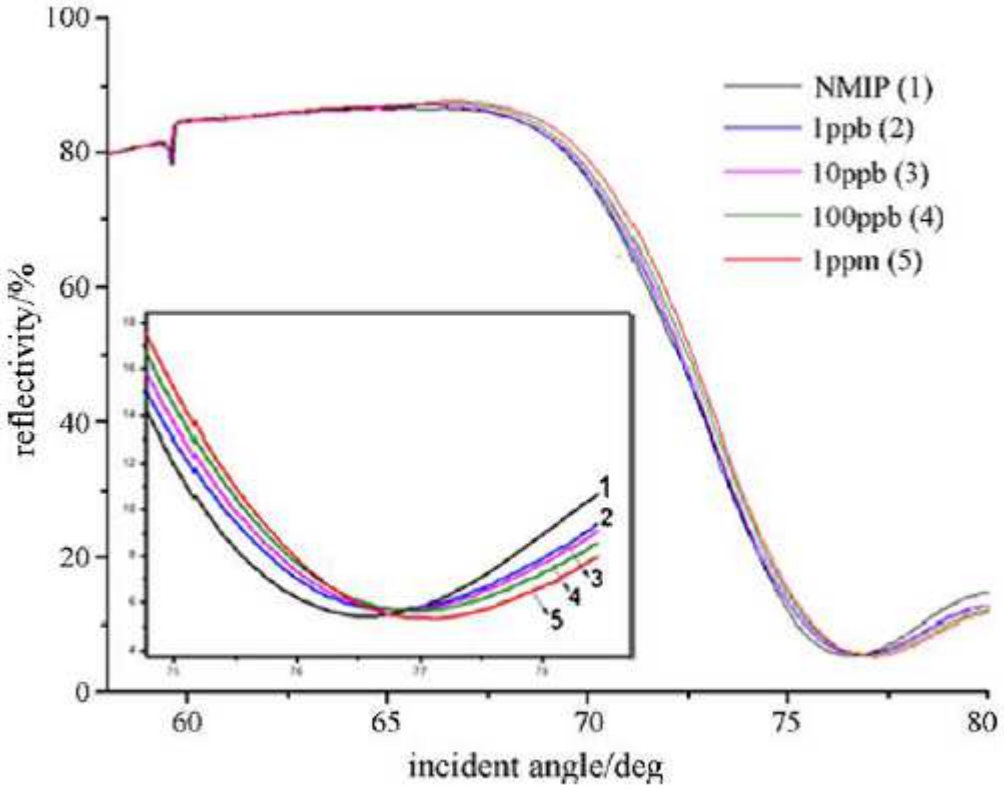


Figure4. SPR spectra of MIPs injecting different concentration of morphine solutions



The data of $\frac{\Delta A}{b_0^n}$ and ΔA in the case of n = 1, 2, and 3 were calculated and repotted in Figure 12. Among them, a linear correlation was obtained in the case of n = 2. Simultaneously, according to the slope and intercept of this fitted lines, the value of k is given(k = 4.17 × 10^6). This result indicated that MO/MAA complex was a formation with the molar ratio of 1/2.

$$\frac{\Delta A}{b_0^n} = -k\Delta A + k\Delta\varepsilon l a_0 \quad (9)$$

Figure5. The liner equation of morphine concentration and changes of resonance angle

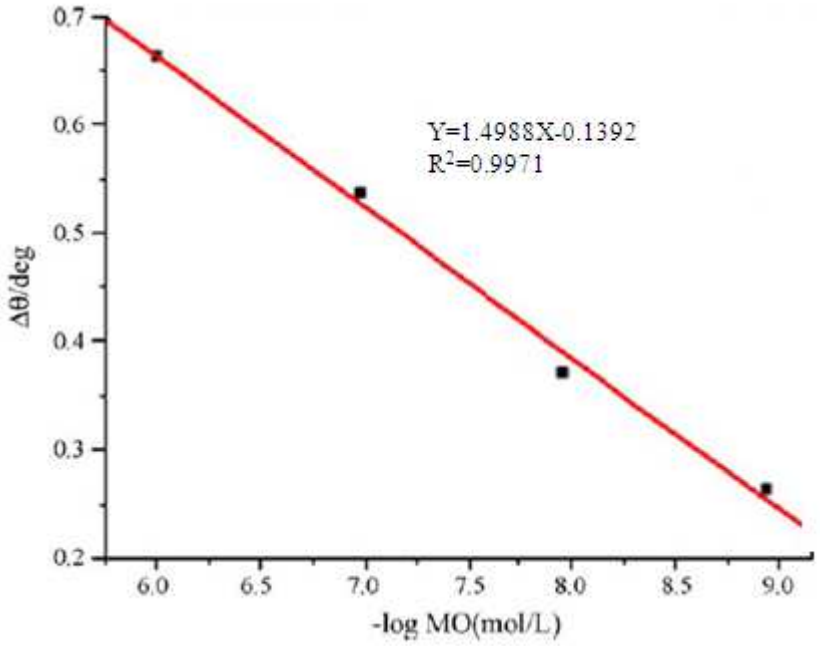


Figure6. SPR spectra of morphine MIP before injecting (1) and after injecting (2) 10^{-4} mol/L codeine solutions

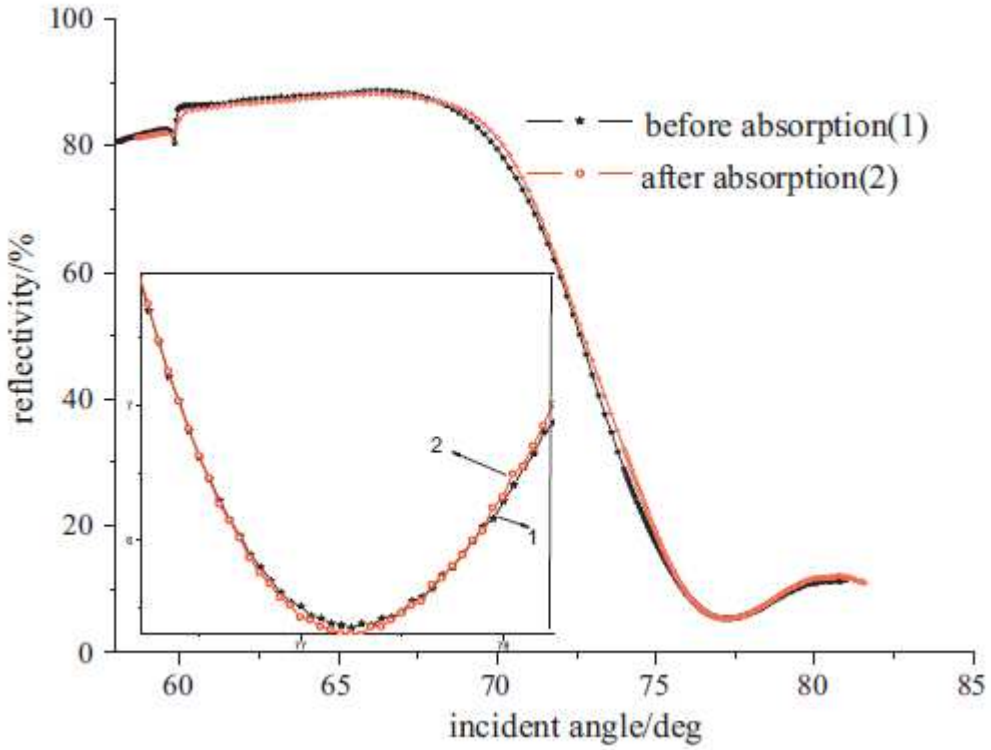


Figure7. The kinetic curves of adsorption and regeneration of MO MIPs

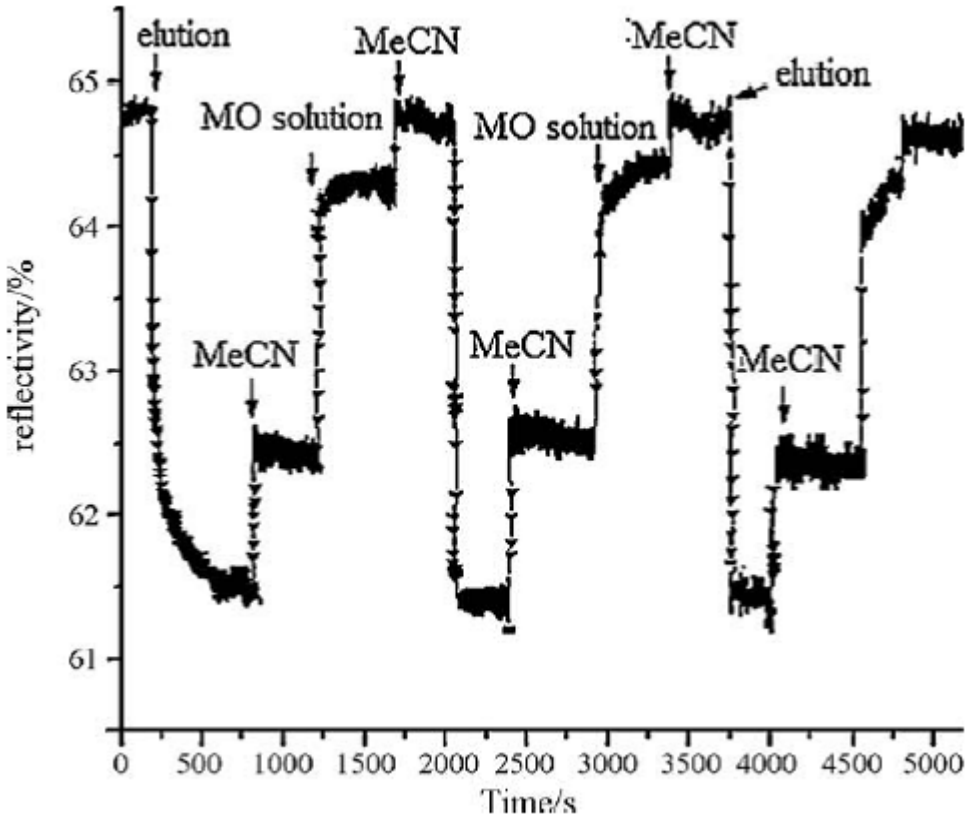


Figure8. Absorption spectra of the MO, MAA and their mixture in methanol as indicated above

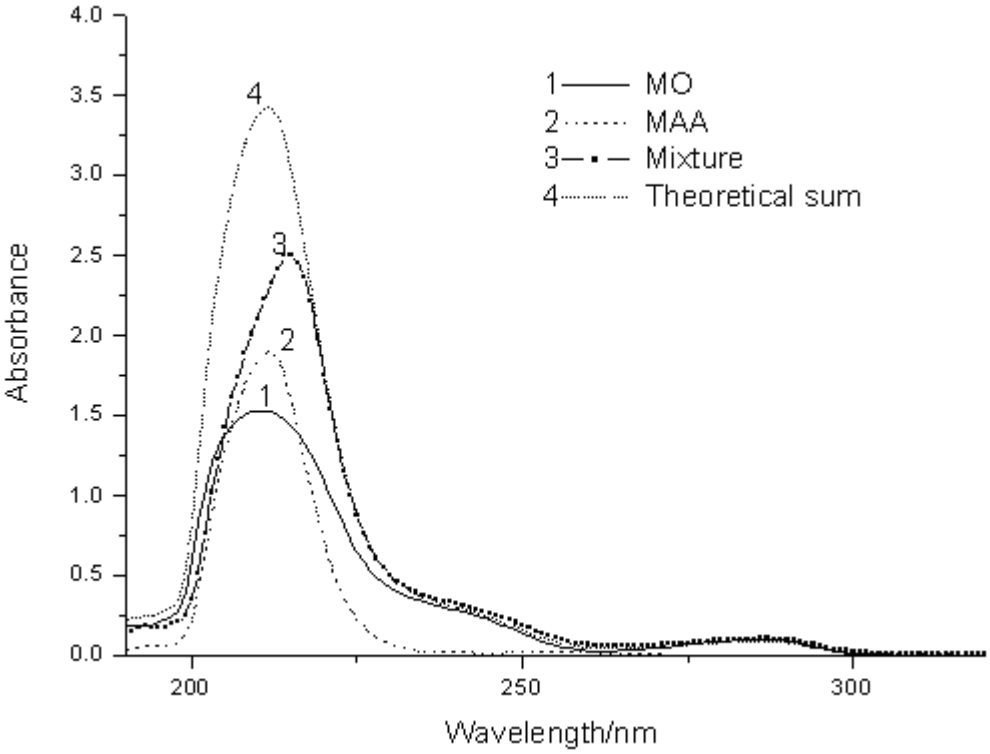


Figure9. Temperature-dependent absorption spectra of MO and MAA (MO: 0.01mmol/L; MAA: 0.04mmol/L) in methanol

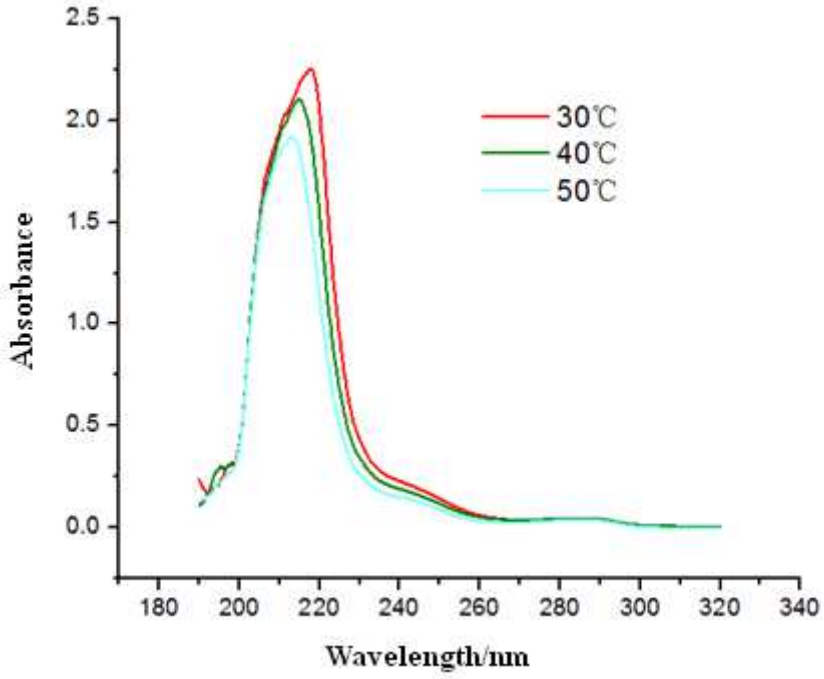


Figure10. ^{1}H-NMR spectra of the MO/MAA complex along with the ^{1}H-NMR spectra of MO and MAA

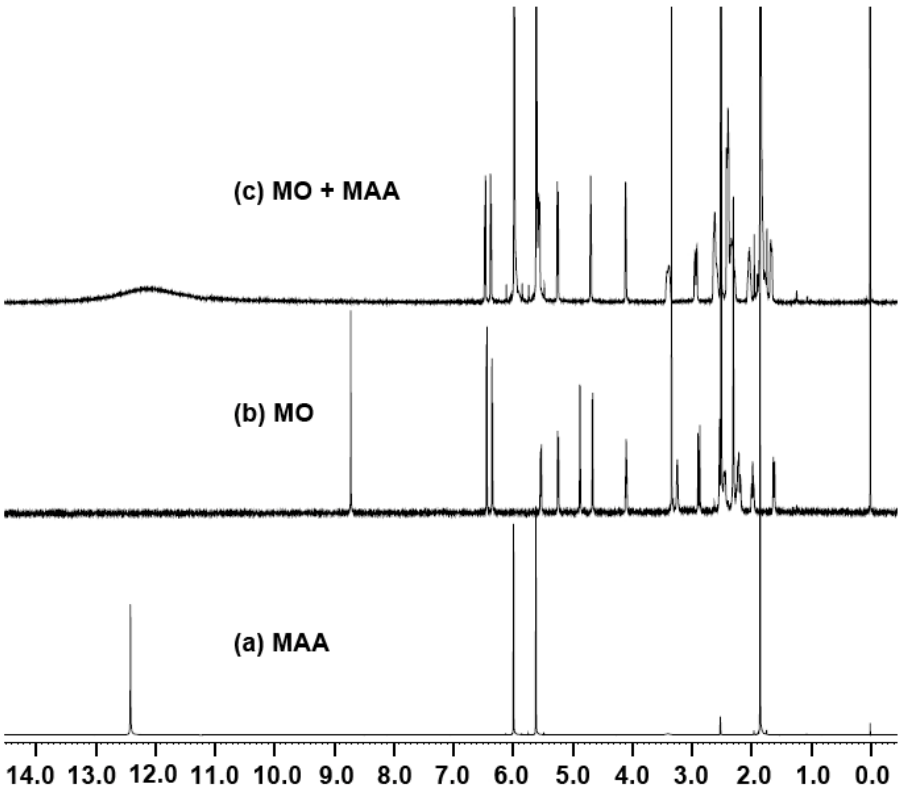


Figure11. Ratio-dependent absorption spectra of MO and MAA in methanol

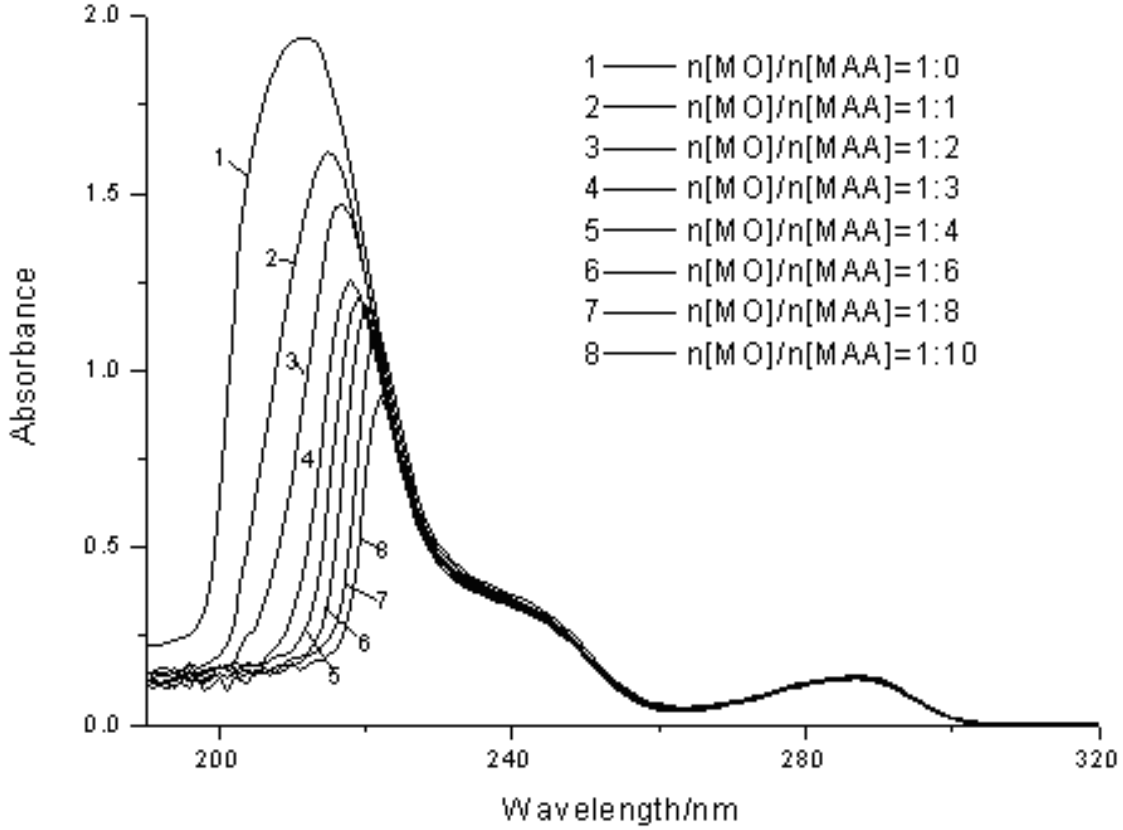
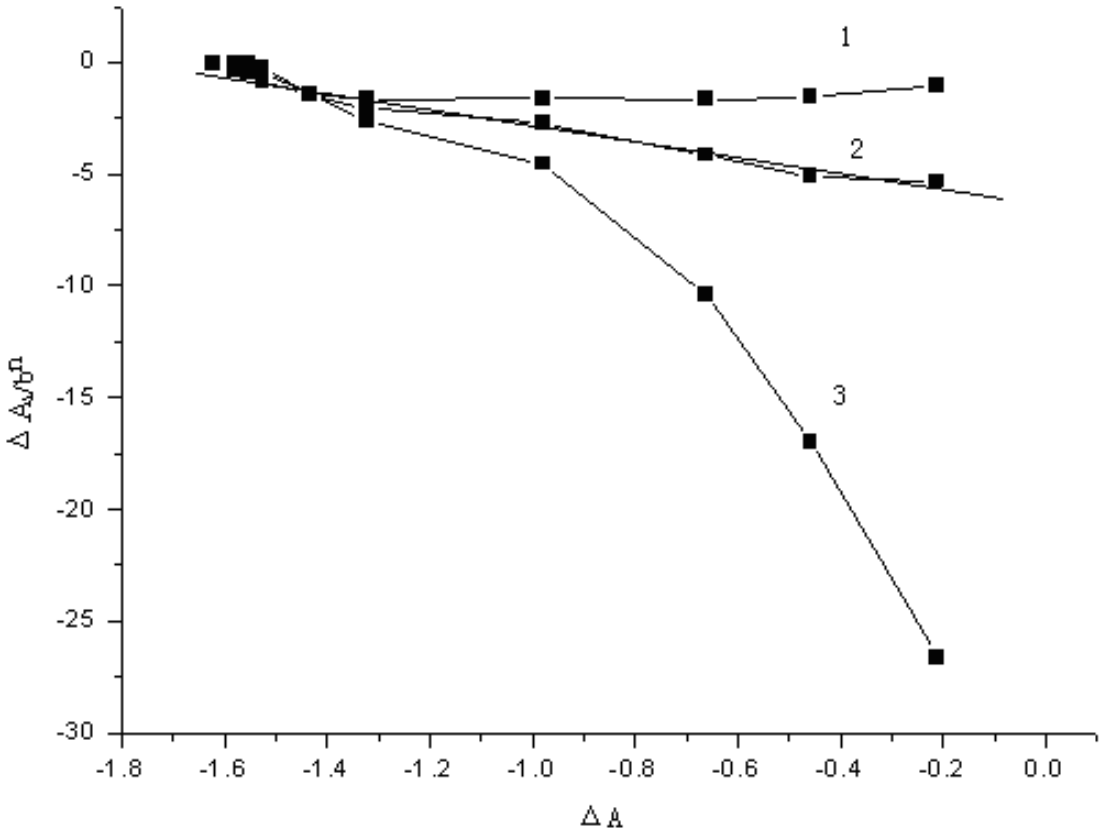


Figure12. The relationship between $\frac{\Delta A}{b_0^n}$ and ΔA



CONCLUSION

In conclusion, we have developed a rapid approach for the detection of morphine based on molecular imprinting polymers using surface plasmon resonance. The sensing membrane was prepared by simply polymerizing MAA and MO directly on gold-coated SPR chips and it was used for the detection of MO after washing by eluents. Upon introduction of MO to the sensing system, MO can be captured specifically by MIP-film unlike its close structure analog codeine. As a result, the resonance angle of the sensor increased dramatically due to the changes of the components of the gold surface. This sensor is label-free, highly sensitive and selective to MO and the driving force for complexation of MAA and MO has been shown to be hydrogen bonding. The MO sensor can measure MO concentrations as low as 1×10^{-11} mol/L and it works well in the range of 1×10^{-9} mol/L to 1×10^{-6} mol/L.

Acknowledgement
This work was supported by the National Natural Science Foundation of China (No. 81001348), the Program for Young Innovative Research Team in China University of Political Science and Law (1000-10814344), Program for Changjiang Scholars and Innovative Research Team in University (IRT0956), and China Scholarship Council Fund.

REFERENCES

[1] Mashayekhi SO, Sattari MG, Hain RDW. *J. Clin. Pharm. Ther*., **2008**. **33**(4)419-427.
[2] Abdi K, Shafiee A, Amini M, Khansari MG, Sabzevari O. *Daru*, **2004**. **12**(2)71-75.
[3] Barnett NW, Hindson BJ, and Lewis SW. *Analyst*, **1999**. **125**(1)91-95.
[4] Alnajjar AO. *Acta Chromatographica*, **2008**. **20**(2)227-238.
[5] Hsu HC, Chen LC, Ho KC. *Anal. Chim. Acta*, **2004**. **504**(1)141-147.
[6] Valimaki HS, Pulli T, Tappura K. *Journal of Fluorescence*, **2010**. **20**(5)1003-1008.
[7] Navaee A, Salimi A, Teymourian H. *Biosens. Bioelectron*., **2012**. **31**(1)205-211.
[8] Yang TB, Yuan YH, Zhong P, Qu LN, Yang B, Li YH, and et al. *Hybridoma and Hybridomics*, **2004**. **23**(1)69-72.
[9] Yuan AS, Rubio FM, Wagner SE. *Clin. Chem*., 19**8**8. **34**(6)1161-1161.
[10] Sakai G, Ogata K, Uda T, Miura N, Yamazoe N. *Sens. and Actuators B (Chemical),***1998**, **49** (1-2)5-12.
[11] Stanley S, Jeganathan A, Wood T, Henry P, Turner S, Woods WE, and et al**. *J. Anal. Toxicol.***, **1991**. **15**(6)305-310.
[12] Steinke J, Sherrington DC, Dunkin IR. *Synthesis and photosynthesis*. **1995**. p. 81-125.
[13] Takeuchi T, Haginaka J. *J. Chromatogr. B,* **1999**. **728**(1)1-20.
[14] Andersson LI. *J. Chromatogr. B*, **2000**. **739**(1)163-173.
[15] Esteban AM. *Fresen. J. Anal. Chem*., **2001**. **370**(7)795-802.
[16] Saloni J, Lipkowski P, Dasary SSR, Anjaneyulu Y, Yu HT, Hill G. *Polymer*, **2011**. **52**(4)1206-1216.
[17] Lee GB, Weng CH, Yeh WM, Ho KC. *Sens. and Actuators B (Chemical),* **2007**. **121**(2).
[18] Kriz D, Mosbach K, *Anal. Chim. Acta,* **1995**. **300**(1-3),71-75.
[19] Ho KC, Yeh WM, Tung TS, Liao JY. *Anal. Chim. Acta,* **2005**. **542**(1),90-96.
[20] Yeh WM, Ho KC. *Anal. Chim. Acta*, **2005**. **542**(1),76-82.
[21] Rana V, Maria VC, Kubic T, Leona M, Lombardi JR. *J Forensic Sci*, **2011**.**56**(1)200-207.
[22] Homola J. *Chem. Rev.*, **2008**. **108**(2),462-493.
[23] Hao HX**,** Zhou H, Chang J**.** *Chin. Chem. Lett.*, **2011**, 22(4),