Development and validation of an imprinted polymer based DGT for monitoring β-blocker drugs in wastewater surveillance

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Abstract

Wastewater surveillance is an effective and objective approach to monitor contaminant releases and drug usage in the catchment, the estimation requires accurate measurement. In this study, a novel diffusive gradients in thin-film (DGT) technique based on molecularly imprinted polymers (MIPs) for selective measurement of a class of widely prescribed cardiovascular drugs (β-blockers) in wastewater was developed. The synthesized MIPs showed strong affinity and selectivity for the target compounds. The MIP-DGT had large effective capacities, its performance was independent of a wide range of environmental conditions, including pH (4.58 – 8.89), ionic strength (0.01 – 0.5 M) and dissolved organic matter (< 20 mg L^{-1}). Biofouling had little effect on the uptake of target compounds within 7 days. MIP-DGT devices were applied in a Chinese urban WWTP alongside an auto-sampler. Metoprolol concentrations detected were one-fold higher than other β-blockers. Concentrations obtained using MIP-DGT were comparable to the 24 h composite samples using an autosampler. The estimated daily consumption calculated based on the data obtained with MIP-DGT implied that metoprolol and propranolol were the most popular β-blockers in the studied area. Overall, the results in this study demonstrate that the MIP-DGT is a cost-effective, reliable and efficient tool for *in situ* wastewater monitoring.

Key words:

Diffusive gradients in thin-films (DGT); β-blocker drugs; Molecularly imprinted polymer (MIP); Wastewater monitoring

1. Introduction

Wastewater-based epidemiology (WBE) is an objective approach used to monitor

environmental and public health impacts within a population. It is helpful for medical research to investigate drug usage and back-estimate regional consumption [1]. The sampling approach has great influence on the accuracy of wastewater surveillance. Conventionally, wastewater samples from the influent of wastewater treatment plants (WWTPs) are collected using active sampling techniques by auto-samplers for 24 h [2]. In some circumstances, grab sampling of snapshot water samples is also employed [3]. During the storage and transport of large volumes of water, some of the target chemicals may be lost by degradation and/or sorption on suspended particles. Moreover, the samples captured by these approaches only provide information for the time of sample collection; episodic contaminant events may be missed, which may lead to inaccurate estimation in WBE [4]. The auto-sampler instruments are expensive and sometimes are not easy to access. This restricts the number of locations and frequency of sampling. Therefore, the development of a cost-effective sampling approach that provides more representative (time-integrated) information is required.

Compared to the aforementioned sampling approaches, passive sampling has a large number of advantages. The *in situ* sampling does not affect the environment, the loss of target chemicals is limited during transport. Retained chemicals in the samplers are preconcentrated, so that no extra pre-treatment procedure is required and the detection limit decreases. Moreover, time-weighted average (TWA) concentrations of chemicals obtained are more representative [5]. Passive sampling techniques, such as POCIS (polar organic chemical integrative sampler), have been applied to WBE in recent years [6-8]. However, the measurements by POCIS are highly depended on hydrodynamic conditions in the field deployments. They need to be calibrated and/or corrected for the effects of flow through laboratory uptake experiments, or kinetic models, or through the loss of performance reference compounds (PRCs) added to the sampler. In contrast

to POCIS, the diffusive gradients in thin-films (DGT) sampler can be used for field measurements without calibration prior to deployment. The thick diffusive gel layer which controls the uptake of chemicals minimizes the effect of the diffusive boundary layer (DBL), such that sampling is not affected by changes in water flow rates (dynamic conditions) [9]. The measurements using DGT were reported to have higher accuracy and precision compared to POCIS. The concentrations of 130 pharmaceuticals measured by both DGT and POCIS samplers in a river showed narrower range of variation for DGT results than POCIS results [10]. Several studies reported under-estimations of target compound concentrations in water monitoring using POCIS, due to using laboratory-derived uptake rates (R_s) obtained from the literature, to the field conditions [11, 12], which has been further proven by studies that using PRCs for correction [13].

Typical DGT devices are comprised of three layers (from back to front) [14]: a binding resin-impregnated hydrogel layer, a diffusion hydrogel layer, and a protective filter membrane. These three layers are sandwiched between a plastic base and piston. The chemicals diffuse through the top two layers and are accumulated in the binding layer. The DGT measured concentration of the analytes can be calculated based on Fick's first law of diffusion and expressed as Eq.1:

$$C_{DGT} = \frac{M \Delta g}{D_e A t} \quad (1)$$

Where M stands for the mass of target compound accumulated on the binding gel (ng), Δg is the thickness of diffusion layer (diffusive gel plus filter membrane, cm), D_e refers to the diffusion coefficient of the analyte in the diffusion layer (cm^2 s^{-1}), A is the exposure window area of DGT devices (cm^2), and t is the deployment time (s).

Since 2012, the application of DGT has been extended from trace metals and nutrients to organic contaminants. It has been employed in wastewater monitoring for antibiotics

[15], PFAS [16], PPCPs [17], EDCs [18], synthetic musks [19], etc. Its application for WBE has just started [20]. A binding layer with large capacity for target compounds is vital for accurate measurement. Several binding resins have been employed for the detection of organic compounds, such as HLB, XAD-18, XDA-1, activated charcoal and PEP-2 [21-25]. Binding gels based on these non-selective resins can adsorb multiple compounds simultaneously, but the effective capacity may be reduced by the co-adsorption of non-target substances. Moreover, these co-existing substances may bring interference to the analysis. Hence, a new DGT device with high selectivity could be advantageous for accurate measurement of specific organic compounds, particularly in wastewater sampling because of the huge range of co-existing substances present.

Molecularly imprinted polymers (MIPs) are a class of synthetic material used to selectively remove specific target molecules with pre-designed imprinted cavities [26, 27]. Several types of MIPs have been used as DGT binding materials for specific organic compounds, such as fluoroquinolones antibiotics [28], ciprofloxacin [29], tetrabromobisphenol [30] chlorophenols [31], 4-chlorophenol [32] and PAHs [33]. The selectivity, precision and robustness of MIP-based DGT have been demonstrated, which laid the foundation of this study.

β-blocker drugs are among the most prescribed pharmaceuticals globally with significant annual consumption worldwide due to their crucial role in treating cardiovascular diseases such as angina, arrhythmias, hypertension, and myocardial infarction [34]. In addition, they may also be abused by athletes to reduce the cardiac rhythm and farmers to prevent anxiety in animals [35]. They are not fully absorbed by the body following ingestion, with a large fraction of the drugs being excreted faeces. Due to their growing consumption and incomplete removal by WWTP [36], β-blockers have been widely detected in the environment, with concentrations ranging from ng L^{-}

^{1} to μg L^{-1} in waters and from 10s to 10000s of ng kg^{-1} in soils and sediments [37, 38]. Harmful effects from β-blockers have been reported on fish, algae, and invertebrates. The drugs could be accumulated in fish and reduce their egg release [39]. Cellular damage was observed in tissues of clams and oysters after being exposed to metoprolol and propranolol [40]. After accumulating in food chains, they can be ingested by humans. Hence, it is important to monitor the usage of β-blockers and evaluate their consumption. As described above and shown in Table S1, the monitoring methods for β-blockers have mainly been through grab sampling followed by SPE treatment. Most studies have used adsorption resins, such as HLB for preconcentration [41], although some studies have used synthesized MIP materials and employed them for preconcentration [42]. Apart from grab sampling, passive sampling approaches such as POCIS [43] have been adopted for measuring β-blockers in rivers and WWTP . To overcome the *ex situ* sampling problems of active sampling and measurement error induced by R_s values of POCIS, the DGT technique has also been developed for a large number of pharmaceuticals, including 3 β-blockers (ATL, MTL, PPL) using HLB as the binding material [44]. However, the analytical interferences in the complex matrices sampled in WWTPs may be a problem in wastewater surveillance. Moreover, so far there has been no systematic study or selective measurement specific for the β-blocker group using DGT.

The aims of this study were therefore to synthesize cost-effective MIP material and develop a selective MIP-DGT sampler for measuring β-blocker drugs in wastewaters. The binding properties of the MIP materials and the effective capacity of MIP-DGT devices were evaluated. The performances of MIP-DGT were tested under different environmental conditions including pH, ionic strength (IS), dissolved organic matter (DOM) and biofouling in the laboratory. The dependence of diffusive gel thickness and

deployment time were also assessed. After systematic assessment, the novel DGT devices were deployed in an urban WWTP alongside active sampling, to evaluate its reliability and robustness for measuring β-blocker drugs in wastewater, so as to more efficiently and accurately support WBE studies.

2. Materials and methods

2.1 Chemicals and regents

Standards (> 98%) of atenolol (ATL), acebutolol (ABL), bisoprolol (BSL), betaxolol (BTL), metoprolol (MTL), nadolol (NDL), propranolol (PPL), sotalol (STL), carvedilol (CVL), sulfamethazine (SMZ), ractopamine (RTP) and cotinine (COT) were all purchased from Sigma-Aldrich. ATL-d7 was used as internal standard (IS). Details of regents and materials are given in the Supporting Information (SI), physicochemical properties of all compounds are presented in SI Table S2.

2.2 Synthesis and characterization of molecular imprinted polymers (MIP)

The MIP material was synthesized by a bulk polymerization method. CVL was selected as the template, due to its similar molecular structure to target compounds, low environmental detection rate [37] and low cost (~$13/g, HPLC). A schematic diagram of the synthesis of MIP materials is presented in Fig. 1. In summary, 406 mg CVL (1 mmol) and 0.34 mL (4 mmol) methacrylic acid (MAA) were added into a 50 mL glass tube containing 5.6 mL ACN, sonicated for 5 min, then the solution was stored in 4 °C for 4 h. Then 3.8 mL (20 mmol) ethylene glycol dimethacrylate (EGDMA) and 40 mg 2,2'-azobisisobutyronitrile (AIBN) were dissolved in the solution. The mixture was sonicated and then deoxidized with high purity N_2 for 10 min. The tube was heated at 60 °C in a water bath for 24 h under stirring. The resulting materials were collected, then crushed and sieved to 35 - 60 μm. The template molecules were removed by Soxhlet extraction with MeOH: HAc = 9: 1 (v:v) until no templates were detected. The

wet materials were washed with pure MeOH to remove residual HAc and dried under vacuum at 60 °C to obtain dry MIP particles. Meanwhile, the non-imprinted polymer (NIP) materials were prepared by the same procedures described above in the absence of the templates.

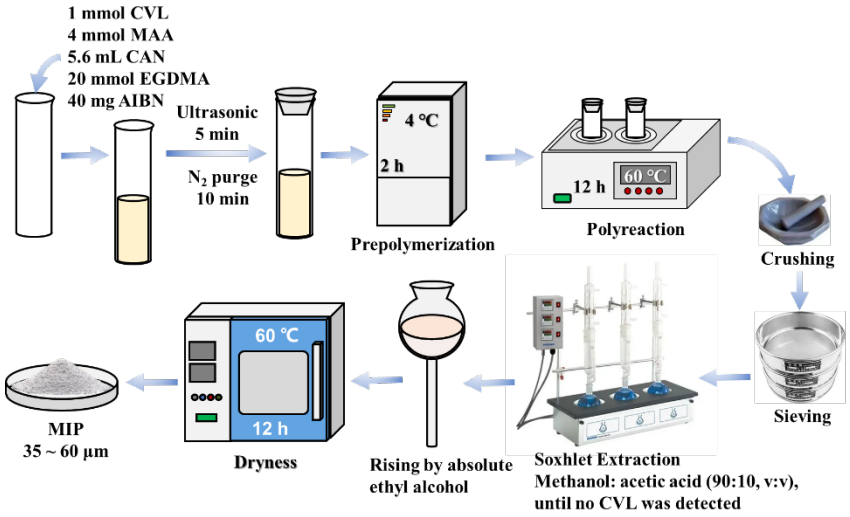


Fig. 1. The schematic diagram of the synthesis of MIP materials

The size and surface morphologies of the MIP and NIP particles were examined by scanning electron microscopy (SEM, JEOL LSM-7800F, USA). The surface functional groups of MIP and NIP were recorded with a Fourier transform infrared spectrometer (FTIR, Nicolet iS5, USA) in a spectral range of 4000 – 400 cm^{-1}. Specific surface areas were evaluated using the Brunauer-Emmett-Teller (BET) method.

2.3 Adsorption experiments

A series of batch adsorption experiments were carried out to evaluate the binding properties of the MIP, including the adsorption capacity and binding selectivity. 10 mg MIP or NIP particles were soaked in a series of 5 mL CVL solutions with concentrations ranging from 5 - 150 mg L^{-1} in 20 mL amber tubes, the tubes were shaken at 150 rpm in a water bath for 24 h (25 °C). After centrifugation, particles were removed and the amount adsorbed onto MIP and NIP was determined. The equilibrium adsorption capacity (Q_e) was calculated using the following equation:

$$Q_e = \frac{(C_o - C_e) \times V}{m} \quad \text{(Eq. 2)}$$

Where C_o and C_e are the initial and equilibrium concentrations (mg L^{-1}) of CVL respectively. V (in L) is the solution volume, and m (in g) is the mass of MIP or NIP.

To ensure the selectivity of MIP, competitive adsorption experiments were conducted by adding 10 mg MIP and NIP particles into 5 mL solutions containing mixed target β-blockers, together with two competitor compounds (SMZ, highly detectable for co-existing with β-blockers and has been used as a competitor for testing the selectivity of MIP material for ATL [45]; RTP, structural analogue) at 3 mg L^{-1}. Tubes with solutions were shaken for 24 h. The distribution coefficient K_d (mL g^{-1}), selectivity coefficient (α) and relative imprinting coefficient (IF) were determined from the following equations:

$$K_d = \frac{Q_e}{C_e} \quad \text{(Eq. 3)}$$

$$\alpha = \frac{K_d \text{ (target)}}{K_d \text{ (competitor)}} \quad \text{(Eq. 4)}$$

$$IF = \frac{\alpha_{MIP}}{\alpha_{NIP}} \quad \text{(Eq. 5)}$$

where Q_e is the amount of the compound adsorbed onto the MIP/ NIP (mg g^{-1}) at equilibrium, C_e is the concentration of the target compound needed to reach adsorption equilibrium (mg L^{-1}), K_d (target) and K_d (competitor) are the distribution coefficients of the targets and the interfering compounds, α_{MIP} and α_{NIP} are selectivity coefficients of MIP and NIP, respectively.

2.3 Gel Preparation and DGT Assemblies

To avoid the possible degradation of agarose gels, which were often used in DGT devices for organic chemicals, polyacrylamide (PA) gels were used for this work. PA binding gels were prepared by mixing 1 g MIP material, 10 mL gel solution (provided by DGT Research Ltd., UK), 60 μL of freshly prepared ammonium persulfate, and

initiated by 15 μL of TEMED (N,N,N′,N′-tetramethylethylenediamine). The gel solution was pipetted into two glass plates with 0.25 mm spacer in between and gel was set under 42 - 45 °C for 40 min. PA diffusive gels were prepared using a 0.5 mm spacer in the same way as for the binding gels in the absence of the MIP materials. All gels were hydrated in MQ water for at least 24 h, the thicknesses of the binding gels expanded to 0.4 mm and diffusive gels to 0.78 mm.

Possible adsorption to diffusive gel, filter membrane and DGT moulding has been assessed in our previous work (data not shown), so the DGT device was assembled with a 0.4 mm thick MIP binding gel, a 0.78 mm thick PA diffusive gel and a PTFE filter membrane (0.065 mm) sandwiched between the standard plastic moulding (a base and a cap).

2.4 Effective adsorption, uptake kinetics and elution efficiencies of DGT

The effective capacities of DGT were measured by deploying DGT devices in 0.01 M NaCl solutions with concentrations of mixed compounds ranging from 100 to 1000 μg L^{-1}. Each DGT device was immersed in a 50 mL solution (triplicate) and was shaken for 24 h. The amount of target compounds taken up by DGT devices were obtained by measuring the difference between concentration before and after shaking.

To investigate uptake kinetics of the target compounds, each DGT device with binding gel in the front was immersed in 50 mL solution containing 20 μg L^{-1} of mixed compounds and 0.01 M NaCl. All solutions were shaken for 24 h, 0.2 μL subsamples of solution were collected from 5 min to 24 h.

The elution efficiencies were evaluated by immersing MIP binding gels separately in 10 mL solutions containing 5 (for laboratory samples) and 0.5 (for field samples) μg L^{-1} mixed-compounds and shaken for 24 h. Three different elution solutions were tested: i) 10% acetic acid (HAc) in methanol (MeOH); ii) acetonitrile (ACN) in MeOH and iii)

5% NH_3 in MeOH. Binding gels were eluted with 5 mL of elution solution for 30 min sonication. For 0.5 μg L^{-1} target compounds, the elution were only carried out using 10% acetic acid (HAc) in MeOH. The elution efficiencies were calculated using the ratio of masses of target compounds in the eluent to their masses adsorbed on the binding gels.

2.5 Time and diffusive layer thickness dependence

To test if the performance of DGT follows the DGT principle, DGT devices were deployed in a well-stirred solution (pH = 6.5 ± 0.2, 0.01M NaCl, T = 26 ± 0.5 °C) containing mixed compounds at 10 μg L^{-1} for different time periods up to 168 h. The masses accumulated on the binding gels for different time periods were determined. DGT devices with various thicknesses of diffusive gels (0.5 to 2.0 mm) were deployed in a well-stirred 10 μg L^{-1} mixed target β-blocker solution for 24 h (pH = 6.4 ± 0.3, 0.01M NaCl, T = 24.1 ± 0.6 °C).

2.6 DGT performance tests under different conditions

To investigate if the performances of DGT devices were affected under different environmental conditions, DGT devices were deployed in 5 μg L^{-1} mixed-compound solutions for 24 h with (a) various pH (ranging from 4 – 9), 0.01 M NaCl, no DOM addition, T = 26.3 ± 0.3 °C; (b) different ionic strength (IS) (from 0.01 to 0.5 M), pH = 6.4 ± 0.3, no DOM addition, T = 25.2 ± 0.5 °C; (c) DOM (humic acid) ranging from 0 to 20 mg L^{-1}, pH = 6.5 ± 0.2, IS = 0.01 M, T = 23.4 ± 0.6 °C.

As previously reported, biofilm would form and grow on the surface of the filter membrane when DGT devices were deployed for a long time. Biofouling could potentially influence the DGT measurement by interacting with target compounds, impeding the diffusion or increasing the thickness of the diffusion layer. To investigate the influence of biofilm on the performance of DGT, fouled filter membranes were collected from WWTP deployment and reassembled with clean diffusive gels and

binding gels. Reassembled DGT devices with clean filters, fouled filters from 7-day and 14-day deployment were deployed in 5 μg L^{-1} mixed-compound solutions (pH = 6.8 ± 0.2, IS = 0.01 M, no DOM addition, T = 25.8 ± 0.4 °C) for 24 h.

2.9 Application of DGT *in situ* in WWTPs

To test whether MIP-DGT can be used for wastewater surveillance and can be applied for WBE, a 7-day sampling campaign was conducted in the influent of a WWTP in Dalian, China in December 2023. An auto sampler (pumping 50 mL every half hour) was employed together with the deployment of MIP-DGTs (shown in Fig. S4 (a) and (b)). The 24h composite water samples were collected into glass bottles in duplicate daily. MIP-DGTs were fixed in a protective plastic cage, then deployed 30 cm under the water surface in triplicate for 1, 3, 5 and 7days. After retrieval, DGT devices were rinsed thoroughly with MQ water and placed in a clean plastic bag before transportation to the laboratory. Temperature and pH in the influent were recorded every day. The devices were treated as mentioned above. Details of the extraction procedures and sample preparations are described in the SI.

2.10 Estimated drug consumption based on wastewater-based epidemiology (WBE)

Nicotine is the main addictive substance of tobacco and can be a biomarker in WBE estimation. Cotinine (COT), a metabolite of nicotine, has a longer half-life than nicotine and is not affected by dietary factors. It has been reported to be more stable in wastewater than other human biomarkers [46]. It has been proposed to reflect the number of inhabitants (inh) served by the WWTP [47]. As MIP-DGT can only measure β -blockers, DGT with HLB binding gels were used to provide TWA COT concentrations in WWTP. The population served by the WWTP was calculated using Eq. 5:

$$P_{(\mathrm{COT})} = \frac{C_{\mathrm{COT}} \times F}{E} \qquad \text{(Eq. 5)}$$

where $P_{(COT)}$ is the population estimated according to COT consumption (1000 inh), C_{COT} (ng L^{-1}) represents the TWA concentration of COT measured by HLB-DGT in a 5-day deployment, F (m^3 d^{-1}) is the average daily flow of the WWTP during 5-day deployment, E (mg d^{-1} inh^{-1}) is the COT discharge coefficient.

The consumption of target β-blocker per capita can be estimated using Eq. 6:

$$m_i = \frac{C_i \times F \times f_i}{P_{(COT)}} \quad \text{Eq. 6}$$

where m_i stands for daily consumption of target β-blocker i (mg (1000 inh)$^{-1}$ d^{-1}), C_i is the concentration of compound i in WWTP measured by MIP-DGT (ng L^{-1}), f_i represents the correction factor for compound i (shown in Table S8).

2.11 Quality assurance /quality control (QA/QC)

All DGT deployments in the laboratory and the WWTP were conducted in triplicate, results are expressed as the average ± standard deviation (SD). Active samples were collected in duplicate from WWTP and pre-treated with SPE cartridges. Parallel blank and control samples were performed with laboratory experiments. Field blank DGTs were used with field applications.

The instrumental detection limits (IDLs) for UPLC-MS/MS, method detection limits (MDLs), diffusion coefficients of target β-blockers, recoveries for DGT and water samples are given in Table S4.

3. Results and Discussions

3.1 Characterization of MIP

The surface morphology of MIP and NIP particles were characterized using scanning electron microscopy (SEM). As shown in Fig. 2a and c, the particles were irregularly shaped with a particle size of 35 – 60 μm. Micropores in the material skeleton increased the specific surface area. High specific surface area of 325.6 m^2 g^{-1} (MIP) and 342.8 m^2 g^{-1} (NIP) were obtained through nitrogen adsorption data (shown in Fig. 2e). Fig. 2f

shows the results of Fourier transform infrared (FTIR) analysis of the MIPs and NIPs. The peaks at 3560 and 1730 cm^{-1} were attributed to stretching vibration of -OH and C=O from MAA. The evidence for successful attachment of MAA and EGDMA appeared at 2990 cm^{-1} for stretching vibrations of C–H, at 1160 and 1260 cm^{-1} for C-O. These characterization results indicated that the MIP and NIP materials were synthesized as proposed.

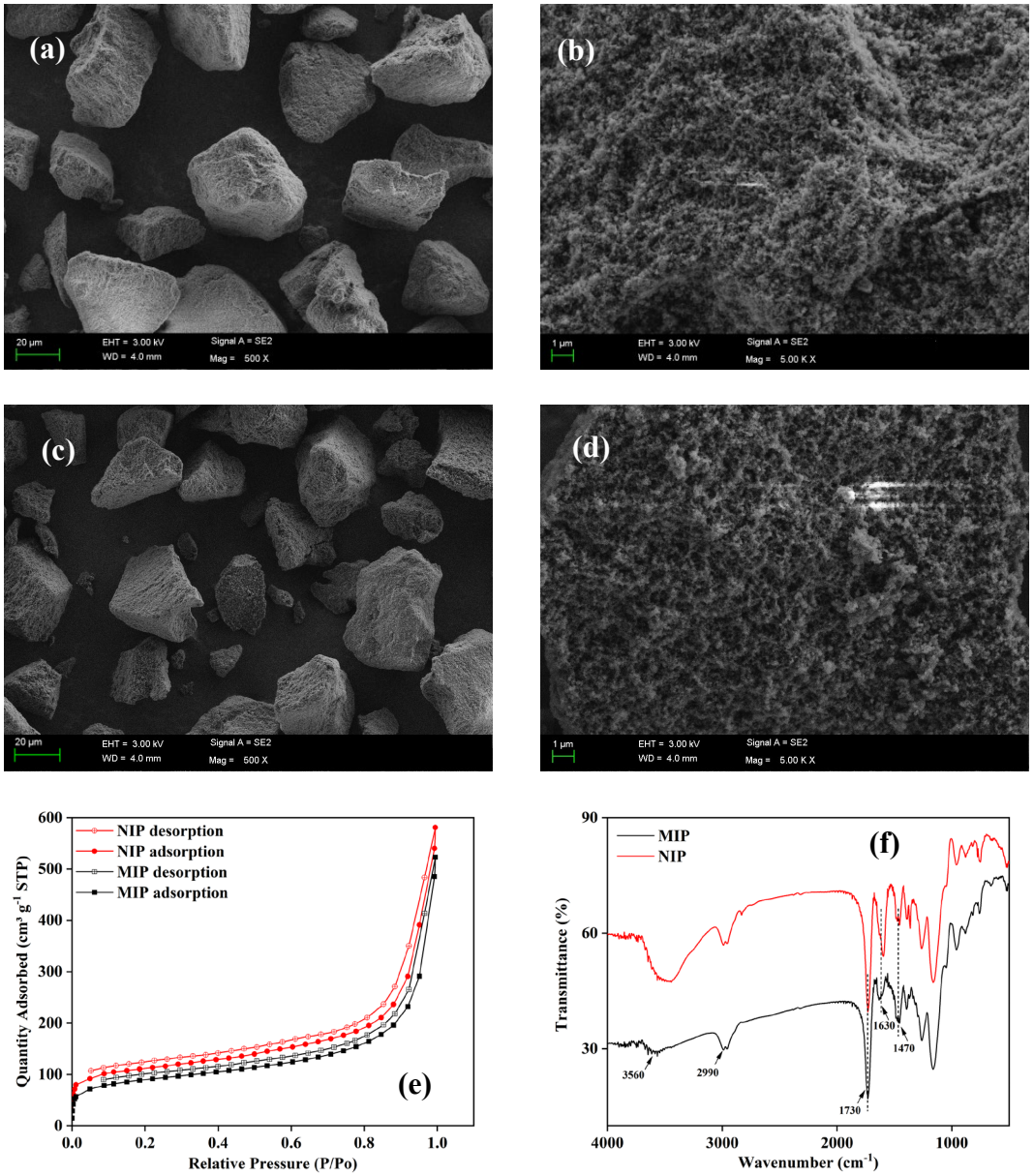


Fig. 2. SEM images of MIP at magnification of (a) × 500, (b) × 5000; SEM images of NIP at magnification of (c) × 500, (d) × 5000; (e) Nitrogen adsorption-desorption isotherms of MIPs and NIPs; (f) FTIR spectroscopy of MIPs and NIPs.

3.2 Adsorption performance of MIPs

The MIP material was immersed in a series of CVL solutions with concentrations ranging from 5 - 150 mg L^{-1}. As presented in Fig. 3(a), the adsorbed masses of CVL increased rapidly with increasing of test concentration and slowed down when the test concentration was over 100 mg L^{-1}. Langmuir and Freundlich isothermal models were used to evaluate the maximum capacity of MIP. The results show that the adsorption process was better fitted with the Langmuir model, indicating a monolayer adsorption in the material. The adsorption capacity of MIP for CVL was around 29 mg g^{-1}. Comparing to the adsorption capacities (ranging from 0.1 – 31 mg g^{-1}) of previously developed MIP materials using the bulk polymerization method for β-blockers (atenolol, carvedilol, pindolol, sotalol etc.) [48], the MIPs synthesized in this study have a high capacity.

A highly selective binding material can ensure the effective uptake of target compounds in complex environments. A competitive adsorption experiment was conducted; distribution coefficients (K_d), selective factor (α) and relative imprinting coefficient (IF) were used to evaluate the selectivity of MIPs. As shown in Fig. 3(b) and Table S5, the uptake of target compounds onto the MIP material were much more than that onto the NIP material, with IF values ranging from 7.2 – 15.6, which were relatively high values compared to reports on MIPs for ATL [49, 50]. Meanwhile, the adsorption of interfering compounds (SMZ and RTP) was much less than the target compounds, indicating that the MIP material had strong affinity and selectivity to the target β-blockers.

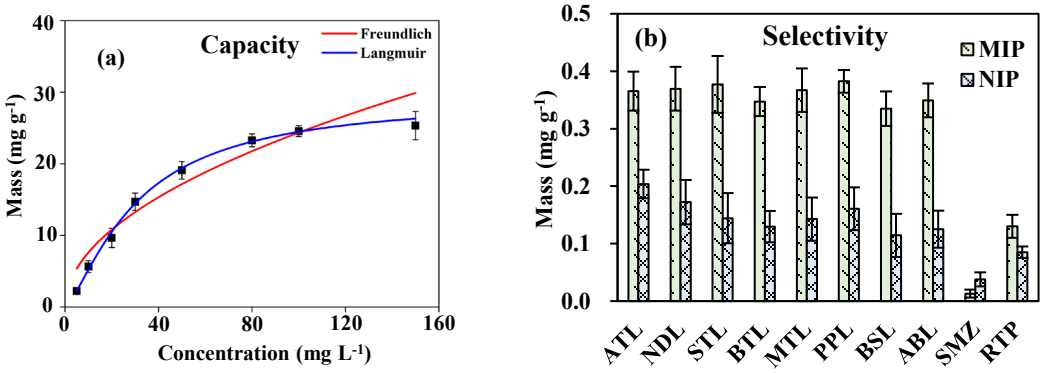
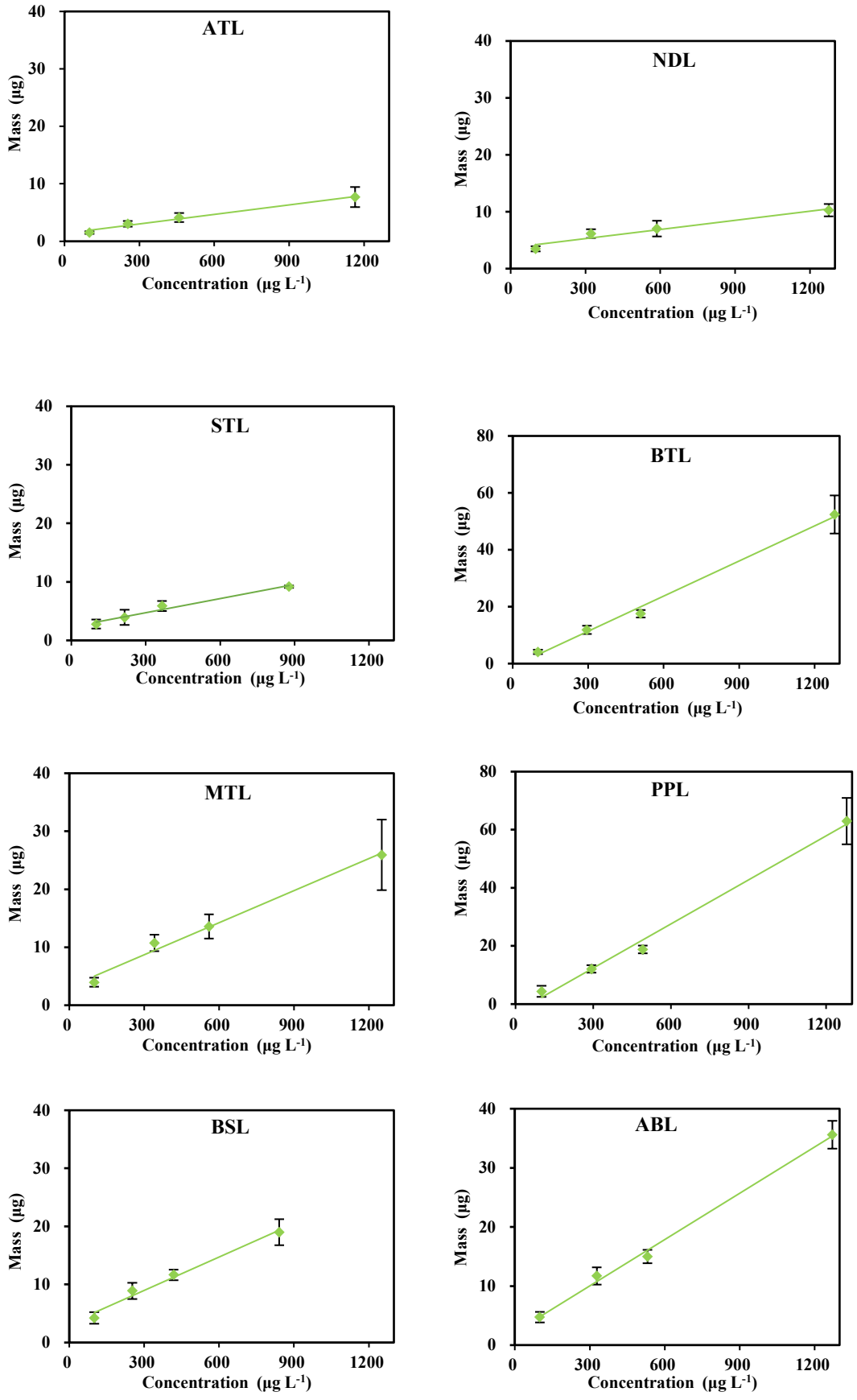


Fig. 3. (a): Adsorption isotherms of CVL on MIPs and (b): Selectivity of target β-blockers on MIP and NIP under competitive conditions.

3.3 Effective capacity, uptake kinetics and elution efficiency of DGT devices

Although the binding material is highly selective, a large capacity for target compounds on the binding gel still needed to be evaluated for the measurement in heavily polluted environments. The effective capacities of DGT were determined with binding gels in the front of the devices with one-side exposed directly to the bulk solution. According to Fig. 4, the accumulated masses of all compounds were linearly correlated with the increasing solution concentrations. The slopes were steep for 5 target compounds and smaller for ATL, NDL and STL, demonstrating a slower uptake of these 3 compounds. Within the test concentrations, the effective capacity of all target compounds was at least in the range of 8 μg (ATL) - 63 μg (PPL) per device. If the deployment time was 1 week, as is often the case in WWTP studies, the maximum concentration of β-blockers that could be measured ranges from 67 to 530 μg L^{-1}. These were much higher than the normal concentrations in WWTP (e.g. ATL: 0.0056 – 11.2 μg L^{-1}, MTL: 0.012 – 8.3 μg L^{-1}, PPL: 0.005 – 0.59 μg L^{-1} etc.) [37], and the maximum capacities were not reached in this study, confirming that the effective binding capacities of MIP-DGTs were large enough for monitoring β-blockers in wastewater.



359 **Fig. 4.** Effective capacities of 8 target β-blockers at different concentrations.

As shown in Fig S1, the uptake of target compounds by MIP-DGT increased sharply and linearly within 60 min, then the uptake rate slowed down slightly. After 24 h of adsorption, 5 target compounds were taken up by > 90% of the total amount added. The uptake amount of NDL, STL and ATL after 24 h were 80%, 77% and 42% respectively, demonstrating there was competition between target compounds.

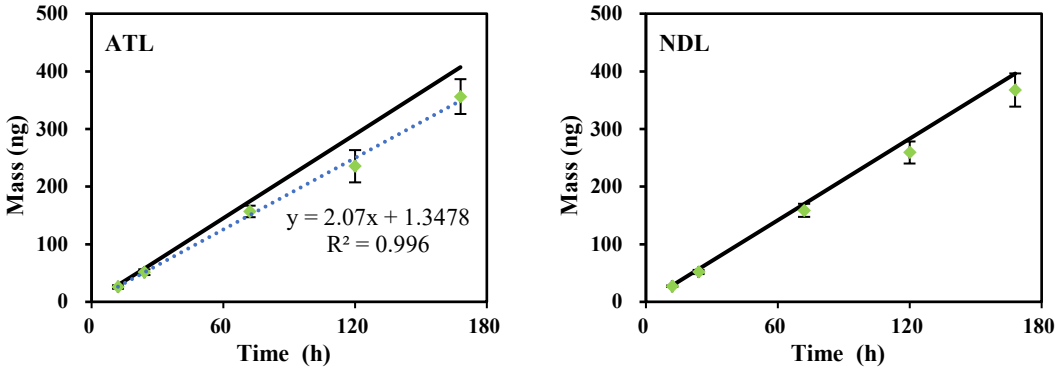
According to Fick's first law, the minimum amount of target analytes diffused through the diffusion layer in the first 5 min was about 1 ng. The actual amounts of target compounds taken up by MIP binding gel were all > 70 ng (see Figure XX in SI). The results indicated that the target β-blockers bound onto MIP binding gels sufficiently fast, which should enable good performance of DGT.

Several trials were carried out to obtain high and stable elution efficiencies for target compounds. For MIP binding gels immersed in solutions with 5 μg L^{-1} mixed compounds, when ultrasonic elution was conducted once with 5 mL 5%NH_3 in MeOH, most of the β-blockers were eluted over 90% from binding gels, much higher than using ACN or 10%HAc in MeOH, except for BTL and PPL with only ~65% (shown in Table S6). To improve the elution efficiencies for all target compounds, elution processes were carried out twice sequentially with 3 mL eluant each time. The recoveries of all target compounds were over 85% using 10% HAc in MeOH. Therefore, this double elution procedure was adopted for the following experiments. The elution efficiencies were further tested for lower concentration of target compounds in solutions of 0.5 μg L^{-1}. The results were similar to those obtained for higher concentrations when using 10%HAc in MeOH (Table S6).

The detection limits of MIP-DGT for target β-blockers were within the range from 0.5 – 1.6 ng L^{-1} in a 7-day deployment (shown in Table S4), which can meet the requirement for detecting low concentrations of target β-blockers in WWTPs.

3.5 Time and diffusive layer thickness dependence

Two laboratory experiments were carried out to validate the principle of DGT for measuring β-blocker drugs. Multiple DGT devices were deployed in mixed-compound solution at 5 μg L^{-1} to test the relationship between uptake mass and deployment time. As shown in Fig. 5, the accumulation of all target compounds increased linearly with the deployment time up to 168 h. The uptake of 7 compounds agreed well with theoretical predictions, except for ATL, which displayed deviations from the theoretical line. It was reported that the linear uptake of ATL went on for only 4 days in a 5 μg L^{-1} solution with 30 mixed compounds using DGT with HLB resin [51]. The poor uptake of ATL could be caused by the competitive adsorption between target compounds. In our study, although the binding material was selective, ATL still competed with the other β-blockers. This phenomenon could also be reflected from the slow uptake rate of ATL compared to other target β-blockers in the effective capacity experiment. However, after 7 days, 86% of the predicted mass of ATL was detected, which was still acceptable.



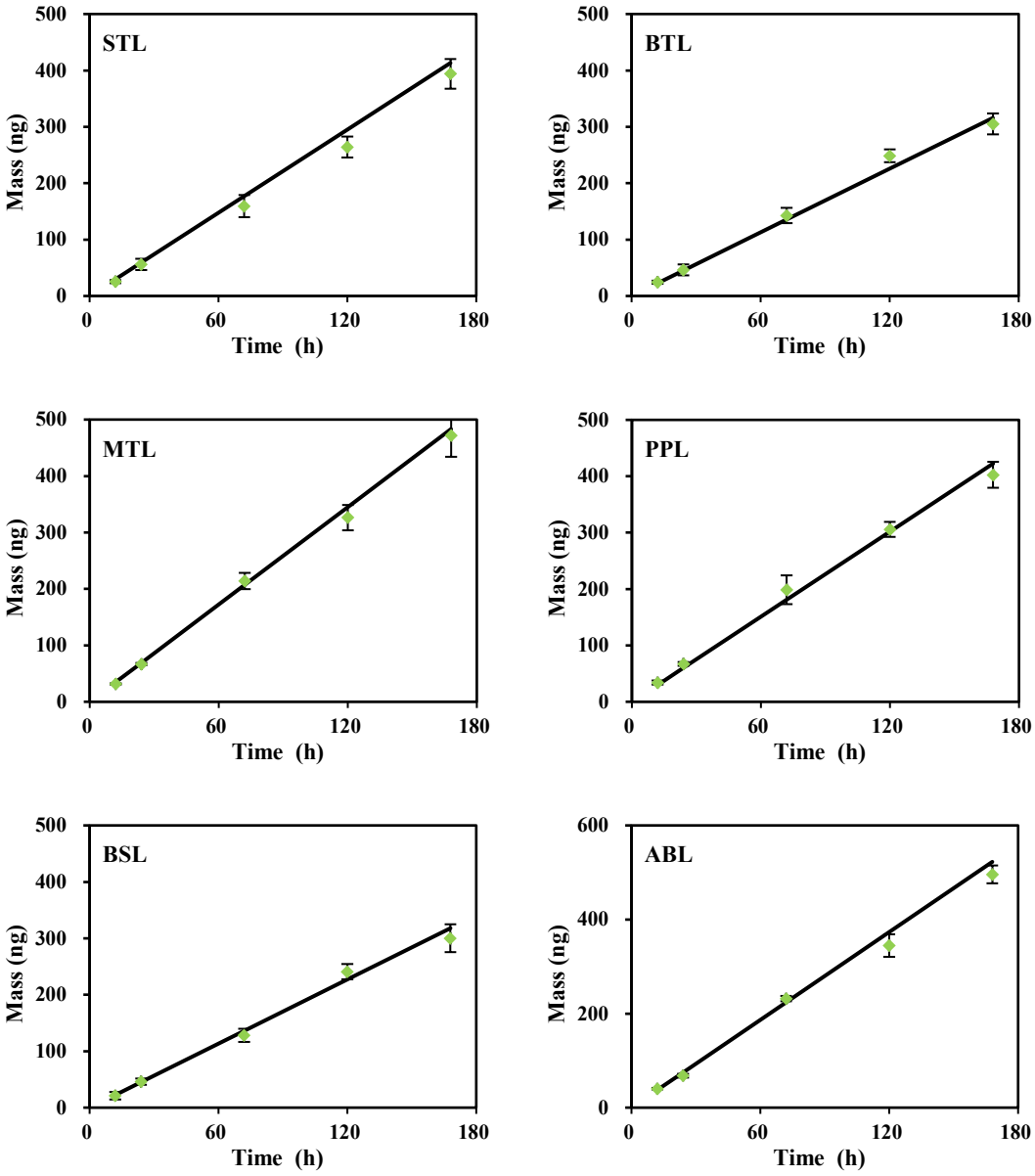


Fig. 5. Measured masses of 8 target β-blockers in the MIP binding layer of DGT devices for different times. The lines are theoretical lines obtained using Eq. 1.

The masses of target compounds that diffuse through the diffusion layer (including diffusive gel and filter membrane) should be inversely proportional to the diffusion layer thickness as shown in Eq. 1 (diffusion coefficients were measured in our previous study which is under review and shown in Table S4). Almost all the test data agreed well with theoretical lines calculated from the test solution concentration (see Fig. S2), showing that concentration of target compounds could be accurately measured and the diffusion boundary layer (DBL) was insignificant under fast stirred conditions. The

409 only exception was ATL diffusing through 0.5 mm diffusive gel, for which the
410 accumulated masses on the binding gel was ~88% of the predicted value. This may
411 again be attributed to the lower uptake efficiency of ATL.

412 **3.6 DGT performance tests under different conditions**

413 In the complex wastewater environment, physicochemical properties of target
414 compounds may change with the environment conditions and affect DGT measurement.
415 Biofilm grown on the surface of the filter membrane sometimes affects the uptake of
416 compounds in natural waters and it may be more serious in wastewaters. The effects of
417 pH, IS DOM and biofouling on the DGT measurement were evaluated using C_{DGT}/C_{soln}
418 ratios.

419 Most C_{DGT}/C_{soln} values were within the acceptable range (0.9 - 1.1) when pH increased
420 from 4.58 to 8.89 (shown in Fig. 6(a)), indicating that pH had no significant effect on
421 the measurement using DGT, satisfying most cases in wastewaters. However, when pH
422 decreased to 3.75, the ratio of ATL and NDL dropped a little to 0.83 and 0.87. This may
423 due to the slightly less efficient and less effective uptake of these two compounds to
424 MIP binding gel at more acidic pH range. Similar decreasing trends were observed for
425 ATL detection using HLB-DGT. Adsorption significantly declined from pH 8.5 to pH
426 5 [51]. Several studies found negative effects of high pH on the performance of DGT
427 measuring EDCs using SXLA resin gels [18], pesticides by HLB resin gels [52], and
428 tetrabromobisphenol A through MIP binding gels [30]. Comparing to these widely used
429 resins, performance of MIP-DGT was more resistant to extreme pH environmental
430 conditions.

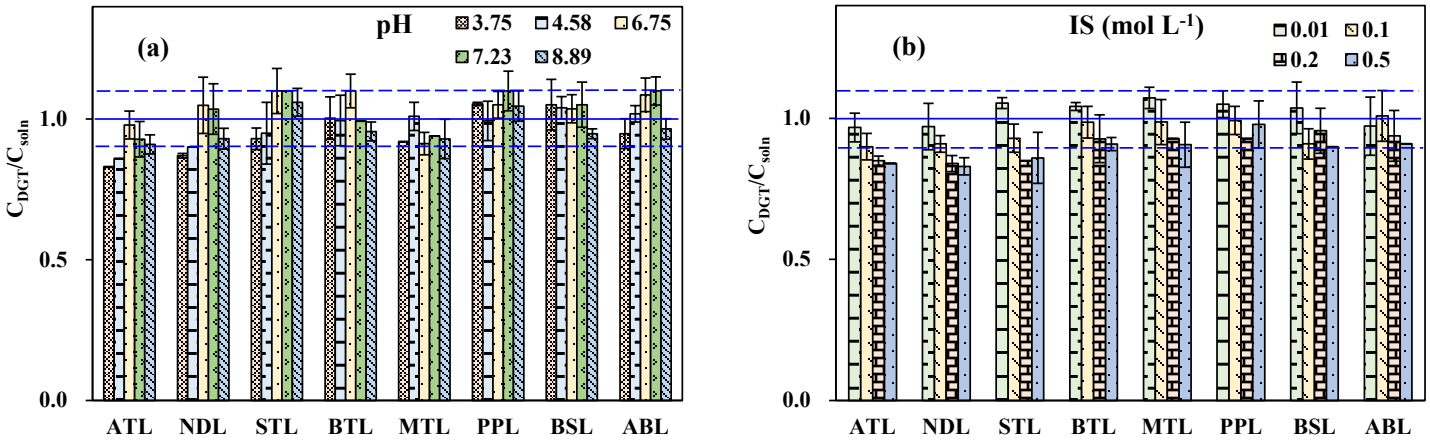
431 As presented in Fig. 6(b), a slight reduction of C_{DGT}/C_{soln} values was observed for most
432 target compounds when the IS increased from 0.01 to 0.5 M, but most of them still fell
433 into the range 0.9 – 1.1. For ATL, NDL and STL, the ratio values dropped to ~0.85

when the IS was 0.2 M. The effect of IS on the uptake of target compounds varied due to different mechanisms. In contrast to our study, some studies reported increasing adsorption of target compounds to the MIP adsorbent, due to the salting-out effect [53]. In our study, this inhibition is compound dependent and may due to interference of mass transfer caused by the increasing salinity [54] and the competition between ionized forms of compounds [55], since these three compounds had slow uptake rates to the MIPs.

DOM can reduce uptake of compounds by DGT, since it may bind with target compounds, and/or compete with target compounds for adsorption sites [56]. It was reported that the adsorption of fluoroquinolone antibiotics onto MIP binding gels was greatly suppressed, for example [28]. In our study, DOM did not have obvious impacts on uptake to the DGT samplers in the range of 0 – 20 mg L^{-1}, except for ATL, which was slightly affected by high DOM content with a C_{DGT}/C_{soln} ratio of 0.85 (shown in Fig. 6(c)). More hydrophobic compounds are more likely to adsorb to DOM. The logK_{ow} values of target β-blockers were all < 4, which could be defined as 'hydrophilic'. Thus, the sampling of target β-blockers by MIP-DGT was independent of DOM.

In general, the performances of DGT devices were independent of various environmental conditions. Although the uptake of ATL was slightly inhibited (< 15%). The growth of biofilms on the surface of the filter membrane when DGT was deployed in WWTP is a consequence of exposure. The influent of WWTP is considered as a nutrient- and microorganism-rich sampling environment [57], so attention should be paid to the biofouling effect. DGT devices with PTFE filter membranes (PALL, thickness: 0.065 mm, pore size: 0.22 μm) were deployed in the influent of a WWTP in Dalian, China for 7 and 14 days to let biofilm grow on the surface of the filter membrane. After retrieval and jet washing with MQ, new DGT devices were assembled with fouled

filter membranes for further experiments. As seen in Fig. S3, unlike some previous studies where the surface of the filter membrane was covered with yellow or brown biofilm after 10 days [58], the colour of biofilm in this study was light brown, implying that biofilm had been built up, but any change in thickness of the filters was not observed. As seen in Fig. 6(d), C_{DGT}/C_{soln} ratio values were still within the range of 0.9 – 1.1 with 7-day fouled filter membrane. Other studies also observed no effect of biofouling on the DGT measurement of tetracyclines by biofilm cultivated in wastewater < 5 days [59] and PFAS for 21 days [60]. As no systematic decrease was observed, the change of the membrane thickness could be ignored. However, the uptake of 3 target compounds using DGT with 14-day fouled filter membrane was suppressed, especially for PPL, where the C_{DGT}/C_{soln} ratio dropped to 0.6. That may due to the relatively high log*K*ow value (3.48) of PPL, which is consistent with the detection of some HPCPs (BPA) and EDCs (ethylparaben and propylparaben). Although considered as hydrophilic, some of the compounds ($\log K_{ow}$ > 2.5) could still be affected by biofouling while the uptake was independent of DOM [17, 18, 61]. The results therefore demonstrated that a 7-day (or less) deployment time for measuring β-blockers in the WWTP is recommended.



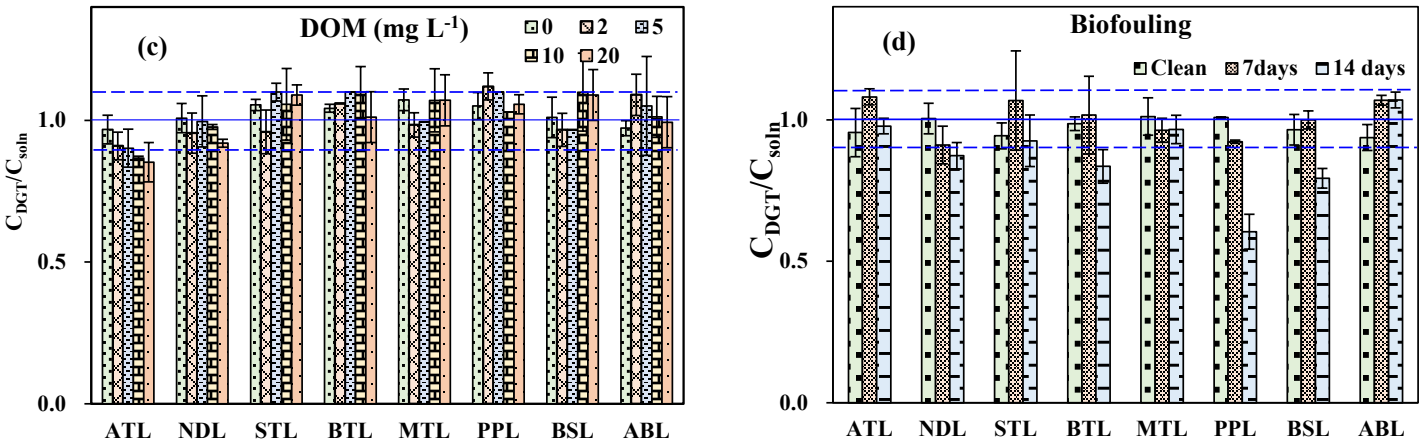


Fig. 6. Effect of (a): pH; (b): ionic strength (IS); (c): dissolved organic matter (DOM); (d): biofouling on the performance of MIP-DGTs.

3.7 Application in WWTP and estimated drug consumption through WBE

3.7.1 Application of MIP-DGT in the WWTP

MIP-DGT devices were deployed at the influent of a WWTP in Dalian, China. A 24 h daily composite water sample was collected with an autosampler alongside with DGT, as a commonly used sampling approach for WBE (shown in Fig. S4(a-c)). The pH and temperatures measured are listed in Table S7. All target β-blockers were detected using both approaches (see Fig. 7). Concentration of MTL was an order of magnitude higher than the other β-blockers in the influent, with a concentration ranging from 130 – 440 ng L^{-1}. Usually ATL, MTL and PPL are three important β-blockers receiving most attention in wastewater monitoring studies [62]. ATL and MTL together account for more than 80% of total β-blocker consumption in Europe [63]. However, in our wastewater sampling campaign, the concentration of ATL was < 20 ng L^{-1}; PPL was also relatively low (< 10 ng L^{-1}), which may due to the prescription differences.

Concentrations of BSL were the second highest, ranging from 19 – 64 ng L^{-1}. This was similar as the situation in some WWTPs in Serbia, where the concentrations of MTL detected was even over 700 μg L^{-1} [64].

In the MIP-DGT deployment, the uptake masses of most target compounds increased gradually with the increasing deployment time in the first 5 days (shown in Fig. S5), after which a plateau or decline occurred. A similar phenomenon was observed in measuring EDCs using HLB-DGT after 18 days [18] and fluoroquinolone antibiotics by MIP-DGT after a week [28]. In our study, the rate of increase slowed after 5 days, which is mainly attributed to the sludge attached to the surface of the filter membrane as seen in Fig. S4(d). It is believed that the sludge and sand covered part of the filter membranes, impeding further uptake of target compounds. As shown earlier, the selective adsorption capacities for target compounds were much greater than the accumulated masses in this campaign, so this phenomenon is not thought to be caused by saturated adsorption of the MIP binding gels.

Given these observations, to avoid the underestimation of the measured concentration of target compounds, we suggest that deployment for 5 days or less is a suitable pre-cautionary approach. As Fig. 7 shows, comparable concentrations were obtained by 5-day DGT deployment to 5-day average concentration measured using the auto-sampler. The day-on-day variations in concentrations measured with the autosampler give a larger variation (standard deviations) than those measured by DGT. Thus, DGT can be used as an effective and efficient (time integrative) sampling tool for WBE.

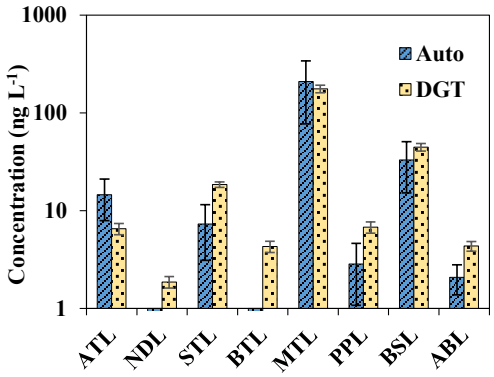


Fig. 7. 5-day TWA concentration measured by MIP-DGT and average concentration of 24 h composite samples (taken each day for 5 days) for target β-blockers in the influent of a WWTP.

3.7.2 Estimated daily drug consumption through WBE

Several laboratory tests have been conducted to validate the measurement of COT using HLB-DGT. The elution efficiency of COT using 5 mL 5% NH_3 in MeOH was 98.6 ± 4.5 %. 3 HLB-DGT devices with PA diffusive gels and PTFE filter membranes were immersed in a 2 L solution for 24 h to measure the diffusion coefficient of COT. Through calculation using Eq. 1, it was 3.62 × 10^{-6} cm^2 s^{-1} at 25 °C. The detected concentration of COT during the 5-day deployment was 1690 ± 132 ng L^{-1}, the discharge coefficient (*E*) of COT in Dalian was estimated as 0.7 mg d^{-1} inh^{-1} [65]. The mean COT equivalent population (P_{COT}) was calculated as 157 ± 12 (× 10^3) in the WWTP. According to Eq. 6, the estimated daily consumptions of different target β-blockers varied greatly as shown in Fig, 8, ranging from 0.9 (NDL) to 1463 (MTL) mg (1000 inh)$^{-1}$ d^{-1}, indicating that β-blocker drugs such as MTL and PPL (140 mg (1000 inh)$^{-1}$ d^{-1}) are still the most prescribed cardiovascular drugs, accounting for > 95% of the total consumption. The extremely high consumption of MTL was consistent with the situation in most parts of China [65], as it had a high utilization rate for hypertension patients.

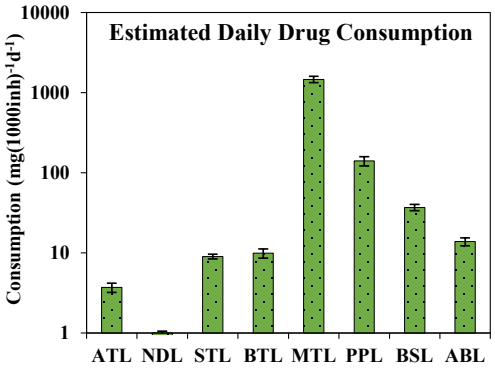


Fig. 8. Estimated daily consumption of target β-blockers per 1000 people

4. Conclusion

A new DGT device based on MIP with high selectivity for β-blocker drugs was developed for accurate measurement of those compounds in wastewater. The MIP binding material synthesized by polymerization had strong affinity and a large effective capacity to target compounds. The performances of MIP-DGT for most target compounds were independent of pH (in the range 4.58 – 8.89), ionic strength (0.01 – 0.5 M), DOM (< 20mg L^{-1}), with only ATL as an exception, but the accuracies were still acceptable (< 15% errors). The uptake of all target compounds was not affected by biofilm grown for 7 days, which ensured the reliable sampling and measurement of β-blockers in WWTP. During the sampling campaign in the influent of a WWTP, all target compounds were detectable. The calculation based on MIP-DGT data showed that MTL and PPL were the two most popular cardiovascular drugs in the studied area.

Comparing to HLB resin and commercial MIP material (~$270/g) which is no longer in the market, the MIP material synthesized in this study has economic benefits with the cost < $20/g. The detection limit of DGT devices in field deployment can reach 0.5 – 1.6 ng L^{-1} for target β-blocker drugs, which could meet the demand for wastewater monitoring. It preconcentrates target compounds *in situ*, reduces the treatment procedures in the laboratory, saves labour and time. Therefore, compared to

conventional auto-sampling, DGT has advantages in cost, time integration, efficiency and detection limit, making it an ideal tool for wastewater surveillance.

Notes

The authors declare no conflict of interest.

CRediT authorship contribution statement

Yanying Li: Investigation, Project administration, Writing – original draft. Mingzhe Wu: Investigation, Methodology. Xinyu Yin: Methodology, Validation. Yansong Wang: Methodology. Dongqin Tan: Methodology, Funding acquisition. Peng Zhang: Data curation, Funding acquisition. Zhimin Zhou: Funding acquisition, Validation. Degao Wang: Conceptualization, Supervision. Kevin C. Jones: Supervision, Writing – review & editing. Hao Zhang: Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgement

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Supporting Information

Supplementary data associated with this article can be found in the online version at

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