



Original article

Rapid and sensitive analysis of trace β -blockers by magnetic solid-phase extraction coupled with Fourier transform ion cyclotron resonance mass spectrometry



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ABSTRACT

A rapid and sensitive method for analyzing trace β -blockers in complex biological samples, which involved magnetic solid-phase extraction (MSPE) coupled with Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS), was developed. Novel nanosilver-functionalized magnetic nanoparticles with an interlayer of poly(3,4-dihydroxyphenylalanine) (polyDOPA@Ag-MNPs) were synthesized and used as MSPE adsorbents to extract trace β -blockers from biological samples. After extraction, the analytes loaded on the polyDOPA@Ag-MNPs were desorbed using an organic solvent and analyzed by FTICR-MS. The method was rapid and sensitive, with a total detection procedure of less than 10 min as well as limits of detection and quantification in the ranges of 3.5–6.8 pg/mL and 11.7–22.8 pg/mL, respectively. The accuracy of the method was also desirable, with recoveries ranging from 80.9% to 91.0% following the detection of analytes in human blood samples. All the experimental results demonstrated that the developed MSPE-FTICR-MS method was suitable for the rapid and sensitive analysis of trace β -blockers in complex biological samples.

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1. Introduction

β -blockers are widely used to treat various cardiovascular disorders such as hypertension, heart arrhythmias, and ischemic heart disease [1]. Apart from their clinical uses and therapeutic values, β -blockers are known to improve heart function by relaxing heart muscles and reducing heart rate. However, they are often misused by athletes in professional competing sports and are thus forbidden by the Medical Commission of the International Olympic Committee [1]. In addition, fatal adverse drug events following the use of β -blockers have been observed in hospitalized patients in internal medicine [2]. Furthermore, a series of deaths suspected to be strongly associated with the use of metoprolol [3,4] and propranolol [5,6] have been reported. The inherent relevance of β -blockers in doping, forensic science, and toxicological contexts, as well as the need for optimum dosage adjustment in clinical settings, has led

researchers to develop analytical methods that can be used to accurately monitor these drugs.

To date, analytical methods including high-performance liquid chromatography (HPLC) [7,8], two-dimensional HPLC [9], and HPLC coupled with mass spectrometry (HPLC-MS) [10–17] have been developed for use in the analysis of β -blockers. However, following administration, β -blockers are present at very low concentrations in organisms. In addition, the matrices of biological samples are very complex [7–17], making it cumbersome and time-consuming to analyze these trace-molecules in biological samples. To overcome these limitations, various sample pretreatment methods such as protein precipitation (PP) [18], matrix solid-phase dispersion [19], liquid-liquid extraction (LLE) [20], solid-phase microextraction (SPE) [21,22], and magnetic solid-phase extraction (MSPE) [23,24] have been applied. Among these sample preparation methods, an obvious matrix background is still observed in the sample extracts following the use of PP, and a large volume of organic solvents is used in LLE and SPE. However, these drawbacks are avoided with MSPE, in which analytes are isolated from the sample matrix by a magnetic field, thus simplifying the sample pretreatment process [25,26].

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Magnetic nanoparticles (MNPs) are a series of easily synthesized MSPE materials with large surface-to-volume ratios. However, bare MNPs have limited interaction with organic analytes [23], and chemical modifications of the particles are usually required to enhance their extraction capacity. 3,4-dihydroxyphenylalanine (DOPA) is easily self-polymerized in weakly alkaline aqueous media, forming a polyDOPA coating [27]. PolyDOPA shows excellent adhesive ability on various material surfaces through metal bidentate coordination and hydrogen bonding [28], and polyDOPA-coated MNPs (polyDOPA-MNPs) have been widely applied as extraction adsorbents [29]. Importantly, polyDOPA-modified surfaces provide abundant active sites to immobilize molecules and grow shells through the Michael addition/Schiff base reactions [30], coordination [31], and redox [32] owing to the presence of many functional groups such as catechol, amine, carboxyl, and quinone moieties on the surface. In addition, the catechol group of DOPA can be used as a reducing agent to anchor the surface of metal nanoparticles [33]. Wen et al. [34] prepared Au@polyDOPA@Ag nanoparticles to form a surface-enhanced Raman spectroscopy tag for imaging in the human lung adenocarcinoma cell line A549. A one-step in situ reduction process has been reported for the preparation of Ag@DOPA@Hg nanostructures to detect Hg²⁺ using a colorimetric method [35]. Au@DOPA nanoparticles have also been synthesized for catalytic and environmental applications [36].

Based on these reports, we developed novel nanosilver-functionalized MNPs with an interlayer of polyDOPA (polyDOPA@Ag-MNPs) and used them as MSPE adsorbents for the analysis of trace β -blockers (i.e., acebutolol, metoprolol, and propranolol) in complex biological samples. The adsorption performance of polyDOPA (polyDOPA@Ag-MNPs) towards β -blockers was investigated, and a method involving MSPE coupled with Fourier transform ion cyclotron resonance mass spectrometry

(FTICR-MS) for the analysis of the investigated β -blockers was developed.

2. Experimental

2.1. Materials and reagents

Ferric chloride hexahydrate (FeCl₃·6H₂O), sodium acetate (NaAc), diethylene glycol (DEG), ethylene glycol (EG), and acetic acid (HAc) were obtained from Tianjin Damao Chemical Reagent Factory (Tianjin, China). Sodium acrylate (Na acrylate) was purchased from Beijing Universal Century Technology Company (Beijing, China). Sodium dihydrogen phosphate dihydrate (NaH₂PO₄·2H₂O) and disodium hydrogen phosphate dodecahydrate (Na₂HPO₄·12H₂O) were purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China). DOPA was purchased from Alfa Aesar Chemical Company (Shanghai, China). Bupivacaine hydrochloride, metoprolol tartrate salt, acebutolol hydrochloride, and propranolol hydrochloride were supplied by Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). HPLC-grade acetonitrile and methanol were supplied by Burdick & Jackson (Muskegon, MI, USA).

2.2. Preparation of polyDOPA@Ag-MNPs

The procedure for the preparation of polyDOPA@Ag-MNPs is illustrated in Fig. 1. First, carboxyl-modified MNPs (COOH-MNPs) were synthesized using a solvothermal method according to a previous study [37]. Briefly, 1.08 g of FeCl₃·6H₂O was dissolved in a mixture of 30 mL of DEG and 10 mL of EG. Next, 3.0 g of NaAc and 3.0 g of Na acrylate were added, and the mixture was vigorously stirred to form a homogeneous dark yellow solution. Afterwards, the mixture was transferred to a 50 mL Teflon-lined stainless-steel

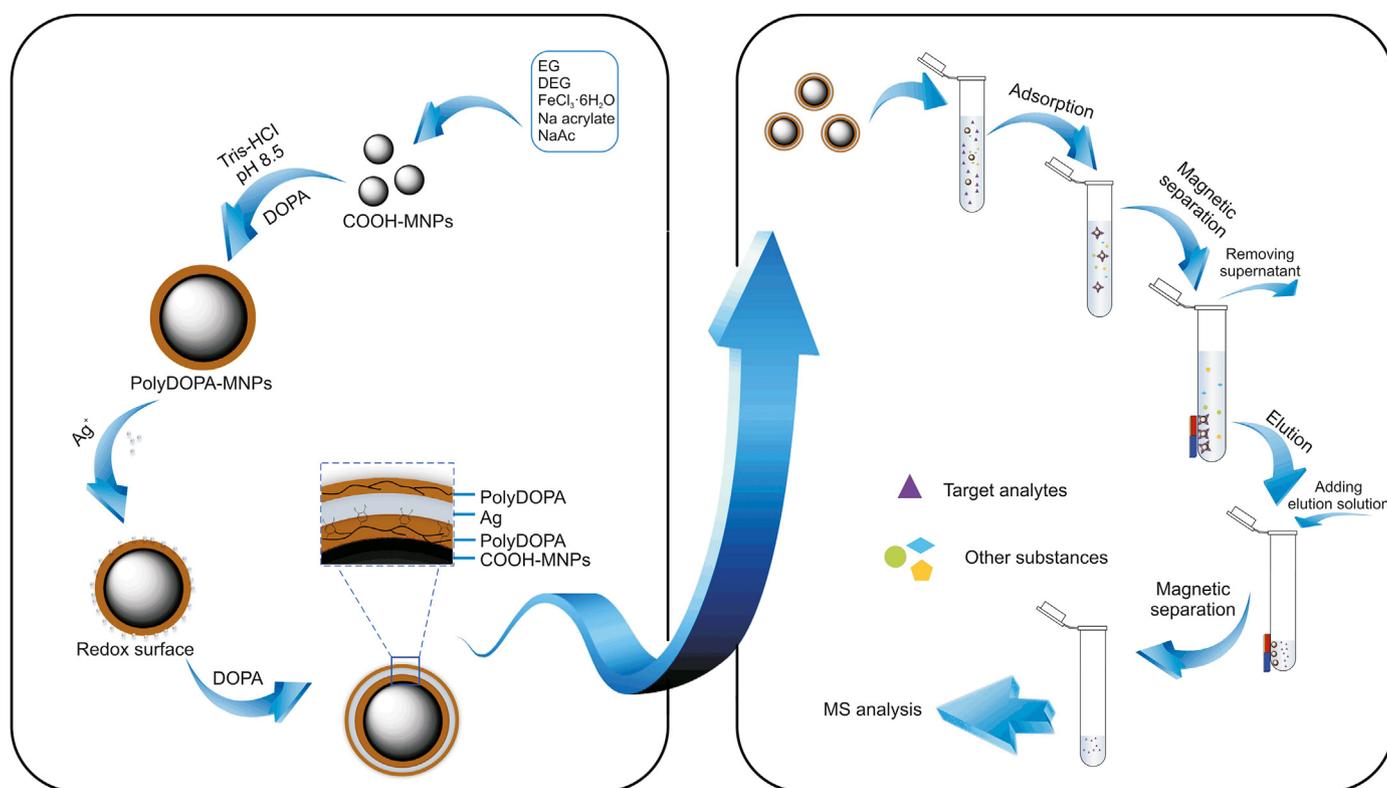


Fig. 1. Schematic illustration of the synthesis of polyDOPA@Ag-MNPs and their application as MSPE adsorbents for the analysis of trace β -blockers in biological samples using FTICR-MS. DOPA: 3,4-dihydroxyphenylalanine; MNPs: magnetic nanoparticles; MSPE: magnetic solid-phase extraction; FTICR: Fourier transform ion cyclotron resonance; MS: mass spectrometry; EG: ethylene glycol; DEG: diethylene glycol.

autoclave and heated at 200 °C for 10 h. The obtained products were washed three times with ethanol and deionized water, and dried at 60 °C under a nitrogen stream.

Following this, the obtained COOH-MNPs were then functionalized with polyDOPA [29]. Briefly, 160 mg of DOPA was dissolved in 160 mL of an aqueous solution of Tris-HCl (10 mM, pH 8.5) to prepare the DOPA solution, and 40 mg of COOH-MNPs were dispersed in the above mentioned DOPA solution and stirred for 10 h at 25 °C. The obtained polyDOPA-MNPs were rinsed with water for further use.

Finally, the synthesized polyDOPA-MNPs were dispersed in 3 mL of Tris-HCl aqueous solution (pH 8.5), and 200 μ L of AgNO₃ (40 mM) was slowly added dropwise under sonication at 25 °C, followed by the addition of 40 μ L DOPA (40 mM). The mixture was sonicated for another 15 min, and the final product, polyDOPA@Ag-MNPs, was obtained after washing with water three times and stored in 3 mL of H₂O at 4 °C.

2.3. MSPE procedure

The MSPE procedure for the extraction of β -blockers using polyDOPA@Ag-MNPs is depicted in Fig. 1. First, 1 mL of the liquid

sample was diluted with 10-fold volumes of phosphate solution (10 mM, pH 7.0). To analyze whole blood samples, 1 mL of blood was deproteinized by adding the same volume of acetonitrile, and the supernatant was collected via centrifugation at 5,000 r/min for 2 min, followed by a 10-fold dilution with phosphate solution (10 mM, pH 7.0).

PolyDOPA@Ag-MNPs (4 mg) were dispersed in the above sample solution and then mixed by sonication for 2 min. Next, polyDOPA@Ag-MNPs were separated from the solution using a magnet and washed three times with water. Subsequently, 1 mL of MeOH containing 1% (V/V) HAC was utilized to desorb β -blockers from the MNPs by sonicating the mixture for 2 min, and then the eluates were filtered through a 0.22 μ m filter for subsequent MS analysis.

2.4. MS detection

All MS analyses were performed using a Sarix X 7T FTICR mass spectrometer (Bruker Daltonics, Bremen, Germany) in positive ion detection mode. Accurate mass measurement was acquired with a 4 M recording mode, and mass resolutions of more than 100,000 for target analytes were obtained.

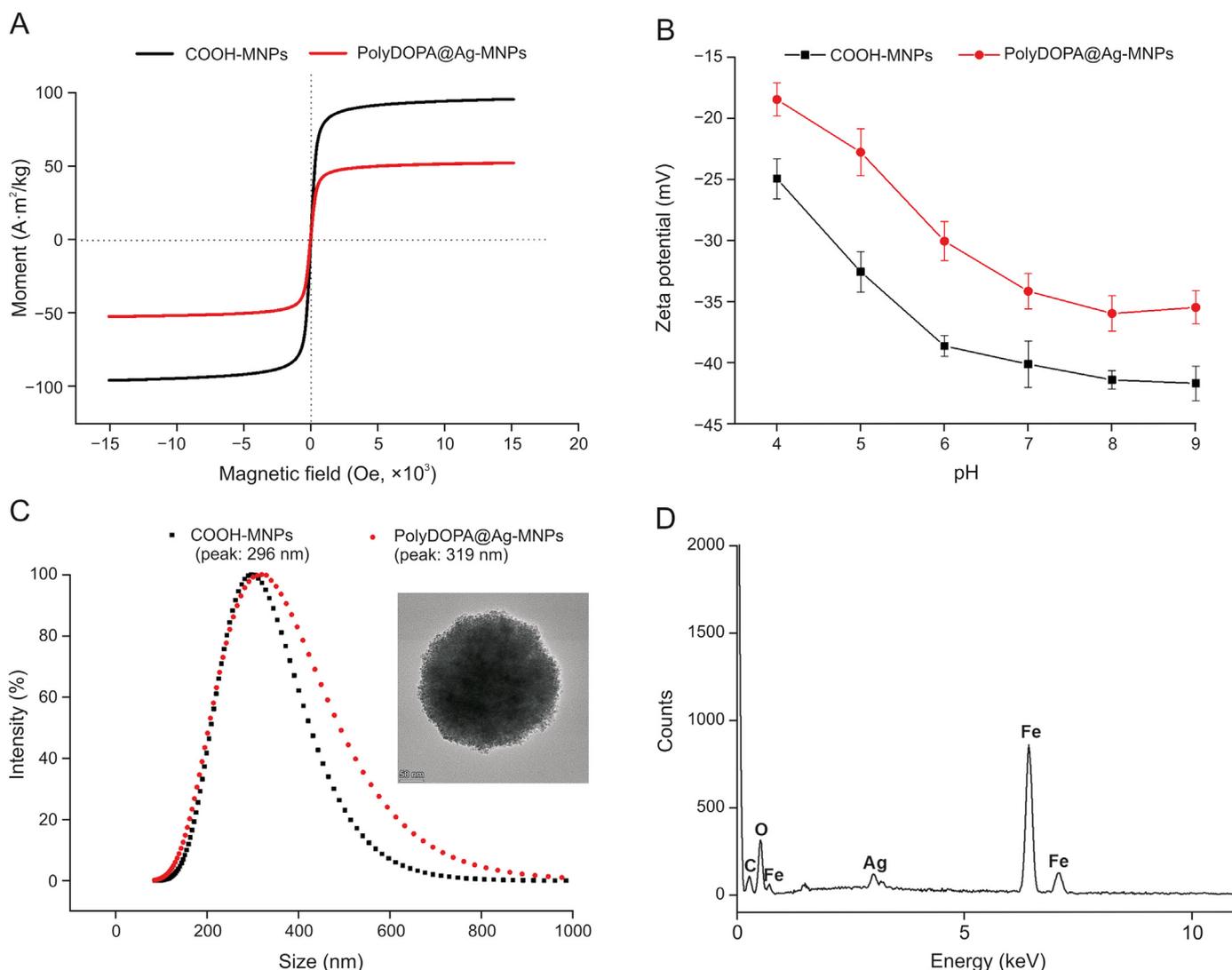


Fig. 2. (A) Vibrating sample magnetometer curves of COOH-MNPs and polyDOPA@Ag-MNPs; (B) zeta potential of COOH-MNPs and polyDOPA@Ag-MNPs at different pH values ($n=3$); (C) size distribution of COOH-MNPs and polyDOPA@Ag-MNPs (inset: particle size of polyDOPA@Ag-MNPs); and (D) energy dispersive X-ray spectrum of polyDOPA@Ag-MNPs.

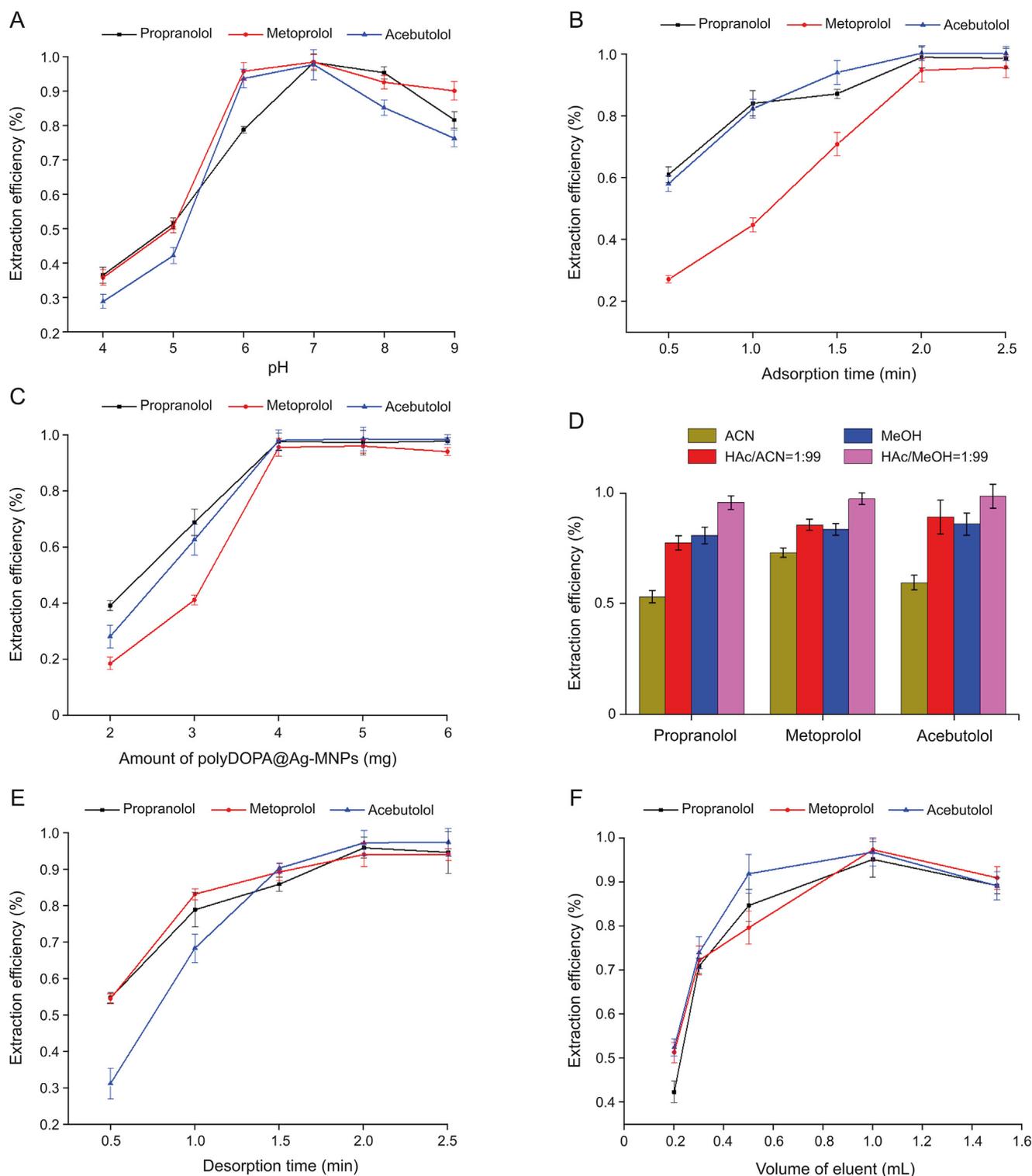


Fig. 3. Effect of (A) pH, (B) adsorption time, (C) amount of polyDOPA@Ag-MNPs, (D) type of eluent, (E) desorption time, and (F) volume of eluent on the extraction efficiencies of polyDOPA@Ag-MNPs for β -blockers.

3. Results and discussion

3.1. Preparation and characterization of polyDOPA@Ag-MNPs

The synthesis of polyDOPA@Ag-MNPs involved the reduction of silver cations into neutral silver atoms using polyDOPA, and the

subsequent production of reactive quinones for the deposition of nanocomposites to immobilize molecules and grow shells. First, polyDOPA-MNPs were prepared according to a previous study [29]. The catechol in polyDOPA films is easily oxidized to quinone, inducing a localized reduction of metal ions [35,38]. Simultaneously, a polyDOPA film is spontaneously deposited outside the

structure, acting as a protective layer to prevent the oxidation of the generated Ag [34]. Therefore, DOPA was the reductant and stabilizer for the formation of AgNPs during the reaction process, and polyDOPA@Ag-MNPs were synthesized in situ.

The magnetic characterization of COOH-MNPs and polyDOPA@Ag-MNPs was performed using a vibrating sample magnetometer with a LakeShore7404 instrument (Lake Shore Cryotronics, Inc., San Diego, CA, USA) at an applied field of 15 kOe and a temperature of 25 °C. As shown in Fig. 2A, the saturation magnetization values of COOH-MNPs and polyDOPA@Ag-MNPs were 95.5 A·m²/kg and 52.2 A·m²/kg, respectively. These results indicated that the particles exhibited an excellent magnetic response. Compared with COOH-MNPs, the decrease in the saturation magnetization value of polyDOPA@Ag-MNPs demonstrated that polyDOPA@Ag had been successfully modified on the surface of COOH-MNPs.

Next, the zeta potential and size distribution characteristics were determined using a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK), and the zeta potentials of COOH-MNPs and polyDOPA@Ag-MNPs at pH values from 4 to 9 were compared (Fig. 2B). With increasing pH, the zeta potential of COOH-MNPs and polyDOPA@Ag-MNPs both decreased gradually and finally plateaued out. The zeta potential magnitude of polyDOPA@Ag-MNPs was lower than that of COOH-MNPs at each pH, indicating the successful immobilization of polyDOPA@Ag on COOH-MNPs.

The particle size and surface morphology of the COOH-MNPs and polyDOPA@Ag-MNPs were determined by transmission electron microscopy (TEM; Talos™ F200x; ThermoFisher Scientific, Waltham, MA, USA). Both particles were spherical, with the diameter of approximately 300 nm. There seemed to be no significant difference between COOH-MNPs and polyDOPA@Ag-MNPs following visualization of their TEM images. However, the size distribution of the particles was different (Fig. 2C).

To further prove the successful preparation of polyDOPA@Ag-MNPs, the composite of the particles was verified by energy dispersive X-ray (EDX) spectroscopy (JEM-2100 HR; JEOL, Tokyo, Japan). The results obtained are shown in Fig. 2D. The EDX graph of the

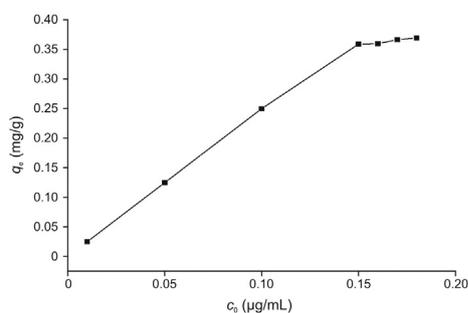


Fig. 4. Correlation of initial propranolol concentration and amount of propranolol adsorbed by polyDOPA@Ag-MNPs.

Table 1
Reproducibility, linearity and sensitivity for β -blockers by MSPE-FTICR-MS analysis.

Analyte	Linear range (ng/mL)	Regression equation	r^2	LOD ^a (pg/mL)	LOQ ^a (pg/mL)	Repeatability (RSD, %, n=6) ^b	Reproducibility (RSD, %, n=6) ^b
Acebutolol	0.02–20	$y = 1.511x + 0.2327$	0.9928	3.5	11.7	4.1	10.3
Metoprolol	0.05–20	$y = 0.7002x + 0.1033$	0.9901	6.8	22.8	5.6	9.8
Propranolol	0.02–20	$y = 0.8936x + 0.1299$	0.9924	5.9	19.6	4.2	10.6

^a LOD and LOQ were determined as the concentrations producing signal-to-noise ratios of 3 and 10, respectively.

^b Analysis of samples spiked with 1.0 ng/mL. MSPE: magnetic solid-phase extraction; FTICR: Fourier transform ion cyclotron resonance; MS: mass spectrometry; LOD: limit of detection; LOQ: limit of quantification; RSD: relative standard deviation.

polyDOPA@Ag-MNPs revealed that Ag was present in the particles. The weight percentage of Ag in the polyDOPA@Ag-MNPs was 4.25%.

3.2. Optimization of MSPE conditions

To achieve a good yield of β -blockers during the extraction process, factors affecting MSPE, including sample pH, adsorption time, amount of polyDOPA@Ag-MNPs, type of eluent, desorption time, and volume of eluent, were optimized using acebutolol, metoprolol, and propranolol as target analytes for examination.

The sample pH, which determines the charge of the analyte and the adsorbent, was investigated first. As shown in Fig. 3A, the highest extraction efficiency was achieved when the pH of the sample solution was 7. The dissociation constant (pK_a) values of acebutolol, metoprolol, and propranolol are 9.20, 9.68, and 9.45, respectively [17]. Therefore, these β -blockers are primarily cationic below their pK_a values. Consequently, the electrostatic attraction between the analytes and the adsorbents should play a role in the adsorption process. If the electrostatic interaction plays a key role in the adsorption, theoretically, the extraction efficiencies should be higher at a pH lower than 7.0. However, in the pH range of 4.0–6.0, the extraction efficiencies were lower than those at pH 7.0, suggesting that non-specific interactions between the analytes and the adsorbents, such as π - π stacking and hydrogen bonding forces, were also at play in the adsorption process. Therefore, a sample pH of 7.0 was used in this study.

Next, the adsorption time was investigated, and 2 min was found to be the optimal adsorption time (Fig. 3B). Following this, the effect of the amount of polyDOPA@Ag-MNPs on the extraction efficiency of β -blockers was investigated. As shown in Fig. 3C, the extraction efficiency of polyDOPA@Ag-MNPs for each β -blocker increased with increasing amounts of the adsorbent, with a plateau attained at 4 mg. Therefore, 4 mg polyDOPA@Ag-MNPs was used for further experiments.

The conditions of desorption, including the type of eluent, desorption time, and volume of eluent, were investigated. As depicted in Fig. 3D, MeOH containing 1% HAc exhibited the best extraction performance. The extraction efficiencies increased with increasing eluent time until a plateau of 2.0 min (Fig. 3E). Subsequently, the eluent volume associated with the optimal extraction efficiency was recorded. The extraction efficiency reached a maximum with an eluent volume of 1 mL, and further increase in the eluent volume was unfavorable (Fig. 3F).

3.3. Adsorption capacity of polyDOPA@Ag-MNPs

Propranolol was selected as the target analyte to evaluate the adsorption capacity of polyDOPA@Ag-MNPs, and the adsorbed amount of the β -blocker was calculated according to Eq. (1).

$$q_e = \frac{(c_0 - c_e)V}{m} \quad (1)$$

Where q_e (mg/g) is the amount of propranolol adsorbed at equilibrium, c_0 and c_e are the initial and equilibrium concentrations of

Table 2
Recovery and RSD values in analysis of the spiked blood samples at two concentration levels by MSPE-FTICR-MS ($n=3$).

Analyte	Spiked β -blockers concentration					
	1 ng/mL			10 ng/mL		
	Measured value (ng/mL)	Recovery (%)	RSD (%)	Measured value (ng/mL)	Recovery (%)	RSD (%)
Acebutolol	0.90	90.3	13.4	9.10	91.0	12.9
Metoprolol	0.81	80.9	14.9	8.59	85.9	14.3
Propranolol	0.88	87.8	14.2	8.99	89.9	11.8

propranolol ($\mu\text{g/mL}$), respectively, V (mL) is the volume of solution, and m (g) is the mass of polyDOPA@Ag-MNPs used.

The Langmuir model was used to analyze the adsorption process of propranolol on polyDOPA@Ag-MNPs, and the model is expressed using Eq. (2).

$$\frac{c_e}{q_e} = \frac{c_e}{q_m} + \frac{1}{K_d \times q_m} \quad (2)$$

where K_d refers to the energy of adsorption and q_m (mg/g) is the maximum adsorption amount of propranolol. The experiment was performed under the optimized conditions, and the obtained results are shown in Fig. 4. The relationship between c_e/q_e and c_e was expressed as an equation, $c_e/q_e = 2.57c_e + 0.0043$, with a determination coefficient (R^2) of 0.9973. The maximum adsorption capacity of polyDOPA@Ag-MNPs for propranolol was calculated as 0.39 mg/g after applying the Langmuir model.

3.4. Method validation

Acebutolol, metoprolol, and propranolol were added into phosphate solution (10 mM, pH 7.0) to form a series of matrix-spiked samples (with concentrations of 0.02, 0.05, 0.1, 0.5, 1, 5, 10, and 20 ng/mL) for construction of calibration curves. Quantitative calculations were performed using the internal standard (IS)

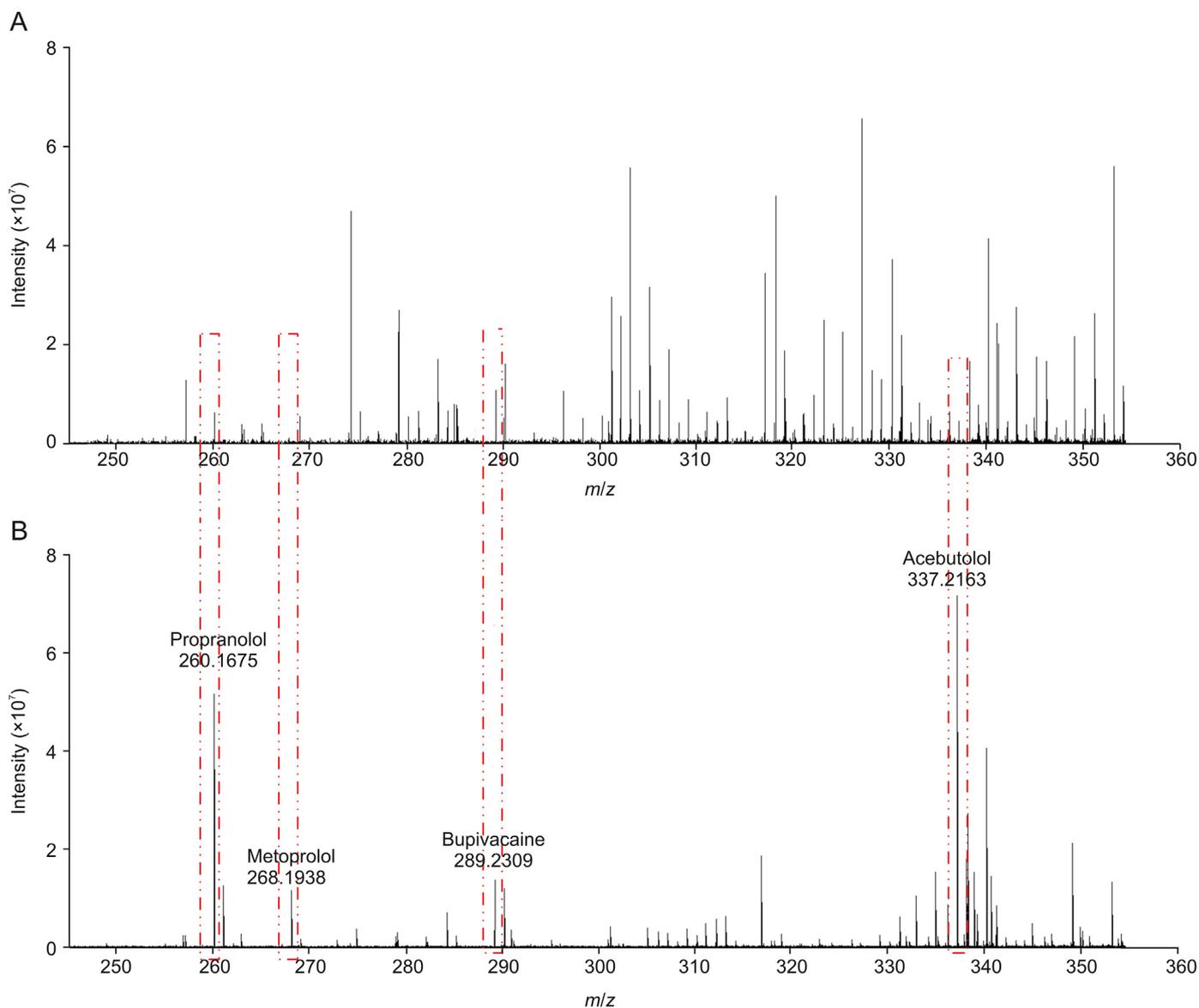


Fig. 5. Mass spectra of (A) spiked human blood samples (10 ng/mL of each β -blocker) without extraction and (B) spiked human blood samples after extraction with polyDOP@Ag-MNPs.

calibration curve method. Bupivacaine was used as the IS and was added into the eluent with a concentration of 10 ng/mL.

Good linearities were obtained in the concentration range of 0.02–20 ng/mL, with correlation coefficient values (r^2) >0.9901 for analyses of the three target β -blockers (Table 1). The limits of detection (LOD) and quantification (LOQ) were from 3.5 to 6.8 pg/mL and 11.7–22.8 pg/mL, respectively, indicating desirable sensitivity of the developed MSPE-FTICR-MS method.

The repeatability experiment was performed by conducting six consecutive analyses of phosphate sample solutions (10 mM, pH 7.0) spiked with 1.0 ng/mL of each β -blocker in a single day. For reproducibility, the experiment was performed by analyzing six phosphate solution (10 mM, pH 7.0)-spiked samples (1.0 ng/mL of each β -blocker) on six different days. The obtained relative standard deviation (RSD) values of the ion intensity ratio of analyte to IS ($I_{\text{analyte}}/I_{\text{IS}}$) for each β -blocker are summarized in Table 1. The results indicated that our developed MSPE-FTICR-MS method had good repeatability, with RSD values ranging from 4.1% to 5.6% for six replicate analyses. In addition, our method showed desirable reproducibility, with RSD values ranging from 9.8% to 10.6% for six parallel experiments conducted on different days.

3.5. Real sample analysis

The feasibility of applying our MSPE/FTICR-MS method in the analysis of real biological samples was verified by analyzing the β -blockers in spiked blood specimens from healthy human volunteers. The recovery (%) of the analyte was calculated from the measured versus added amounts according to Eq. (3).

$$\text{Recovery} = \frac{C_{\text{found}} \times V_1}{C_{\text{spiked}} \times V_0} \times 100\% \quad (3)$$

In Eq. (3), C_{found} and C_{spiked} are the concentrations of analyte in the eluate and spiked standard solution, respectively, and V_1 and V_0 are the volumes of the eluate and spiked standard solution, respectively. To determine the recoveries of the three investigated β -blockers (i.e., acebutolol, metoprolol, and propranolol) in the blood, three blank human plasma samples were spiked with 1 and 10 ng/mL of the mixed standard. Using the above equation, the recoveries were calculated to be in the range of 80.9%–91.0% (Table 2).

The analytical performances of the spiked blood samples (10 ng/mL) with and without undergoing extraction using polyDOPA@Ag-MNPs were compared, and the results are shown in Fig. 5. When the spiked blood sample was directly detected by the ESI-FTICR-MS method, the signals of the three investigated β -blockers were very weak (Fig. 5A). However, the signals of the investigated β -blockers in the blood sample were clearly observed using the MSPE-FTICR-MS method (Fig. 5B). As is known to all, the composition of deproteinized blood is very complex and contains components such as carbohydrates, lipids, amino acids, and inorganic salts. Matrix components in biological samples often affect the sample preparation step and the ionization efficiency of MS [39]. Overall, our results indicate that this method provides a novel technique for the sensitive detection of trace β -blockers in human blood samples.

4. Conclusions

In conclusion, a polyDOPA@Ag-MNP-based MSPE coupled with FTICR-MS method was developed for the rapid analysis of trace β -blockers in biological samples. The redox-active polyDOPA facilitated in situ formation of polyDOPA@Ag-MNPs, which not only effectively removed matrix interferences from biological samples,

but also enhanced detection sensitivity through the enrichment of targets. By using our developed method, rapid and sensitive analysis of trace β -blockers in blood samples was successfully achieved, with an overall detection process of less than 10 min as well as LOD and LOQ in the ranges of 3.5–6.8 pg/mL and 11.7–22.8 pg/mL, respectively. Considering the excellent properties and broad application of the synthesized nanocomposites, this strategy provides a novel method for use in pharmacokinetic and pharmacodynamic studies on β -blockers.

CRedit author statement

Xue Xiao: Investigation, Writing - Original draft preparation, Methodology, Formal analysis; **Kaili He:** Formal analysis; **Ya-Jun Hou:** Validation; **Zhangmin Xiang:** Project administration; **Yunyun Yang:** Writing - Reviewing and Editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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References

- [1] M. Caban, P. Stepnowski, M. Kwiatkowski, et al., Determination of β -blockers and β -agonists using gas chromatography and gas chromatography-mass spectrometry – a comparative study of the derivatization step, *J. Chromatogr. A* 1218 (2011) 8110–8122.
- [2] O. Lamy, A. Jenzer-Closuit, P. Burckhardt, Hypercalcaemia of malignancy: an undiagnosed and undertreated disease, *J. Intern. Med.* 250 (2001) 73–79.
- [3] A. Mozayani, P. Singer, G. Jones, Distribution of metoprolol enantiomers in a fatal overdose, *J. Anal. Toxicol.* 19 (1995) 519–521.
- [4] T.P. Rohrig, D.A. Rundle, W.N. Leifer, Fatality resulting from metoprolol overdose, *J. Anal. Toxicol.* 11 (1987) 231–232.
- [5] N. Fucci, C. Offidani, An unusual death by propranolol ingestion, *Am. J. Forensic Med. Pathol.* 21 (2000) 56–58.
- [6] R. Gault, J.R. Monforte, S. Khasnabis, A death involving propranolol (Inderal), *Clin. Toxicol.* 11 (1977) 295–299.
- [7] S. Asadi, H. Tabani, S. Nojavan, Application of polyacrylamide gel as a new membrane in electromembrane extraction for the quantification of basic drugs in breast milk and wastewater samples, *J. Pharmaceut. Biomed. Anal.* 151 (2018) 178–185.
- [8] A. Mahmoudi, M. Rajabi, Selective determination of some beta-blockers in urine and plasma samples using continuous flow membrane microextraction coupled with high performance liquid chromatography, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 1128 (2019), 121768.
- [9] V.M.F. Gonçalves, P. Rodrigues, C. Ribeiro, et al., Quantification of alprenolol and propranolol in human plasma using a two-dimensional liquid chromatography (2D-LC), *J. Pharm. Biomed. Anal.* 141 (2017) 1–8.
- [10] J.V. Shah, D.P. Patel, P.A. Shah, et al., Simultaneous quantification of atenolol and chlorthalidone in human plasma by ultra-performance liquid chromatography-tandem mass spectrometry, *Biomed. Chromatogr.* 30 (2016) 208–216.
- [11] J.Q. Cheng, T. Liu, X.M. Nie, et al., Analysis of 27 β -blockers and metabolites in milk powder by high performance liquid chromatography coupled to quadrupole orbitrap high-resolution mass spectrometry, *Molecules* 24 (2019), 820.
- [12] R. AminiTabrizi, D. Hassan, R. Davis, et al., Analysis of beta-blocker bio-concentration in brown planaria (*Girardia dorotocephala*) and its effects on regeneration, *Environ. Sci. Pollut. Res. Int.* 26 (2019) 27435–27443.
- [13] M.A. Rosa, H.D. De Faria, D.T. Carvalho, et al., Biological sample preparation by using restricted-access nanoparticles prepared from bovine serum albumin: application to liquid chromatographic determination of β -blockers, *Mikrochim. Acta* 186 (2019), 647.
- [14] V. Kertesz, T.M. Weiskittel, M. Vavrek, et al., Extraction efficiency and implications for absolute quantitation of propranolol in mouse brain, liver and kidney

- tissue sections using droplet-based liquid microjunction surface sampling high-performance liquid chromatography/electrospray ionization tandem mass spectrometry, *Rapid Commun. Mass Spectrom.* 30 (2016) 1705–1712.
- [15] H. He, L. Li, L. Zhao, et al., An improved HPLC-MS/MS method for simultaneous quantification of propranolol and its two phase I metabolites in plasma of infants with hemangioma and its application to a comparative study of plasma concentrations, *RSC Adv.* 8 (2018) 37286–37294.
- [16] X. Xiao, M.-M. Zhang, Z.-Q. Wang, Determination of β -blockers in bovine and porcine tissues and bovine milk by high-performance liquid chromatography-tandem mass spectrometry for the analysis of β -blockers: experimental and theoretical studies, *Microchem. J.* 146 (2019) 258–269.
- [17] H.A. Al Shaker, N.A. Qinna, H. Al Hroub, et al., RP-HPLC-UV method for the quantification of propranolol in rat's serum and Krebs buffer using one-step protein precipitation, *Acta Chromatogr.* 30 (2018) 147–152.
- [18] G. Castro, I. Carpinteiro, I. Rodriguez, et al., Determination of cardiovascular drugs in sewage sludge by matrix solid-phase dispersion and ultra-performance liquid chromatography tandem mass spectrometry, *Anal. Bioanal. Chem.* 410 (2018) 6807–6817.
- [19] J.M. Peña-Herrera, N. Montemurro, D. Barceló, et al., Development and validation of an analytical method for determination of pharmaceuticals in fish muscle based on QuEChERS extraction and SWATH acquisition using LC-QTOF-MS/MS system, *Talanta* 199 (2019) 370–379.
- [20] S. Fan, J.H. Zou, H. Miao, et al., Simultaneous and confirmative detection of multi-residues of β_2 -agonists and beta-blockers in urine using LC-MS/MS/MS coupled with β -receptor molecular imprinted polymer SPE clean-up, *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.* 30 (2013) 2093–2101.
- [21] X. Hu, J. Pan, Y. Hu, et al., Preparation and evaluation of propranolol molecularly imprinted solid-phase microextraction fiber for trace analysis of beta-blockers in urine and plasma samples, *J. Chromatogr. A* 1216 (2009) 190–197.
- [22] T. Ballesteros-Esteban, E.M. Reyes-Gallardo, R. Lucena, et al., Determination of propranolol and carvedilol in urine samples using a magnetic polyamide composite and LC-MS/MS, *Bioanalysis* 8 (2016) 2115–2123.
- [23] S. Jamshidi, M.K. Rofouei, G. Thorsen, Using magnetic core-shell nanoparticles coated with an ionic liquid dispersion assisted by effervescence powder for the micro-solid-phase extraction of four beta blockers from human plasma by ultra high performance liquid chromatography with mass spectrometry detection, *J. Sep. Sci.* 42 (2019) 698–705.
- [24] A.A. Asgharinezhad, H. Ebrahimzadeh, F. Mirbabaei, et al., Dispersive micro-solid-phase extraction of benzodiazepines from biological fluids based on polyaniline/magnetic nanoparticles composite, *Anal. Chim. Acta* 844 (2014) 80–89.
- [25] H. Yan, M. Gao, C. Yang, et al., Ionic liquid-modified magnetic polymeric microspheres as dispersive solid phase extraction adsorbent: a separation strategy applied to the screening of sulfamonomethoxine and sulfachloropyrazine from urine, *Anal. Bioanal. Chem.* 406 (2014) 2669–2677.
- [26] B.P. Lee, P.B. Messersmith, J.N. Israelachvili, et al., Mussel-Inspired adhesives and coatings, *Annu. Rev. Mater. Res.* 41 (2011) 99–132.
- [27] Y. Ai, J. Nie, G. Wu, et al., The DOPA-functionalized bioadhesive with properties of photocrosslinked and thermoresponsive, *J. Appl. Polym. Sci.* 131 (2014), 41102.
- [28] X. Xiao, J. Wu, Z. Li, et al., Enantioseparation and sensitive analysis of ofloxacin by poly(3,4-dihydroxyphenylalanine) functionalized magnetic nanoparticles-based solid phase extraction in combination with on-line concentration capillary electrophoresis, *J. Chromatogr. A* 1587 (2019) 14–23.
- [29] H. Lee, J. Rho, P.B. Messersmith, Facile conjugation of biomolecules onto surfaces via mussel adhesive protein inspired coatings, *Adv. Mater.* 21 (2009) 431–434.
- [30] B. Yang, C. Lim, D.S. Hwang, et al., Switch of surface adhesion to cohesion by dopa- Fe^{3+} complexation, in response to microenvironment at the mussel plaque/substrate interface, *Chem. Mater.* 28 (2016) 7982–7989.
- [31] J. Zhou, P. Wang, C. Wang, et al., Versatile core-shell nanoparticle@metal-organic framework nanohybrids: exploiting mussel-inspired polydopamine for tailored structural integration, *ACS Nano* 9 (2015) 6951–6960.
- [32] X. Zhong, K. Yang, Z. Dong, et al., Polydopamine as a biocompatible multifunctional nanocarrier for combined radioisotope therapy and chemotherapy of cancer, *Adv. Funct. Mater.* 25 (2015) 7327–7336.
- [33] H. Wen, P. Jiang, Y. Hu, et al., Synthesis of Au@Ag core-shell nanostructures with a poly(3,4-dihydroxy-L-phenylalanine) interlayer for surface-enhanced Raman scattering imaging of epithelial cells, *Mikrochim. Acta* 185 (2018), 353.
- [34] Y. Hu, D. Wang, G. Li, Mussel inspired redox surface for one step visual and colorimetric detection of Hg^{2+} during the formation of Ag@DOPA@Hg nanoparticles, *Anal. Methods* 7 (2015) 6103–6108.
- [35] R. Subair, B.P. Tripathi, P. Formanek, et al., Polydopamine modified membranes with in situ synthesized gold nanoparticles for catalytic and environmental applications, *Chem. Eng. J.* 295 (2016) 358–369.
- [36] S. Xuan, Y.X. Wang, J.C. Yu, et al., Tuning the grain size and particle size of superparamagnetic Fe_3O_4 Microparticles, *Chem. Mater.* 21 (2009) 5079–5087.
- [37] Y. Liu, K. Ai, L. Lu, Polydopamine and its derivative materials: synthesis and promising applications in energy, environmental, and biomedical fields, *Chem. Rev.* 114 (2014) 5057–5115.
- [38] M. Caban, N. Migowska, P. Stepnowski, et al., Matrix effects and recovery calculations in analyses of pharmaceuticals based on the determination of β -blockers and β -agonists in environmental samples, *J. Chromatogr. A* 1258 (2012) 117–127.