

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/282276902>

Polyelectrolyte Microcapsules Dispersed in Silicone Rubber for in Vivo Sampling in Fish Brains

ARTICLE *in* ANALYTICAL CHEMISTRY · SEPTEMBER 2015

Impact Factor: 5.64 · DOI: 10.1021/acs.analchem.5b03036

READS

36

9 AUTHORS, INCLUDING:



Jianqiao Xu

Sun Yat-Sen University

16 PUBLICATIONS 134 CITATIONS

[SEE PROFILE](#)



Yuan Liu

Shanghai Ocean University

30 PUBLICATIONS 240 CITATIONS

[SEE PROFILE](#)



Ruifen Jiang

Sun Yat-Sen University

37 PUBLICATIONS 313 CITATIONS

[SEE PROFILE](#)



Gangfeng Ouyang

Sun Yat-Sen University

109 PUBLICATIONS 2,294 CITATIONS

[SEE PROFILE](#)

Polyelectrolyte Microcapsules Dispersed in Silicone Rubber for in Vivo Sampling in Fish Brains

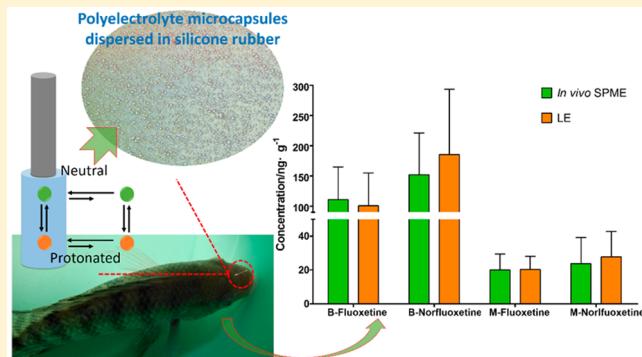
Jianqiao Xu,[†] Rongben Wu,[†] Shuyao Huang,[†] Muzi Yang,[†] Yan Liu,[†] Yuan Liu,[‡] Ruifen Jiang,[†] Fang Zhu,^{*,†} and Gangfeng Ouyang^{*,†}

[†]MOE Key Laboratory of Aquatic Product Safety/KLGHEI of Environment and Energy Chemistry, School of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou, Guangdong 510275, China

[‡]Department of Food Science and Technology, College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China

Supporting Information

ABSTRACT: Direct detection of fluoxetine and its metabolite norfluoxetine in living fish brains was realized for the first time by using a novel solid-phase microextraction fiber, which was prepared by mixing the polyelectrolyte in the oligomer of silicone rubber and followed by in-mold heat-curing. The polyelectrolyte was finally encased in microcapsules dispersed in the cured silicone rubber. The fiber exhibited excellent interfiber reproducibility (5.4–7.1%, $n = 6$), intrafiber reproducibility (3.7–4.6%, $n = 6$), and matrix effect-resistant capacity. Due to the capacity of simultaneously extracting the neutral and the protonated species of the analytes at physiological pH, the fiber exhibited high extraction efficiencies to fluoxetine and norfluoxetine. Besides, the effect of the salinity on the extraction performance and the competitive sorption between the analytes were also evaluated. Based on the small-sized custom-made fiber, the concentrations of fluoxetine and norfluoxetine in the brains of living fish, which were exposed to waterborne fluoxetine at an environmentally relevant concentration, were determined and found 4.4 to 9.2 and 5.0 to 9.2 times those in the dorsal-epaxial muscle. The fiber can be used to detect various protonated bioactive compounds in living animal tissues.



One of the forefronts of the analytical sciences is to minimize and even eliminate the tedious sample preparation procedures for the analysis of complex samples.^{1,2} Solid-phase microextraction (SPME), which substantially simplifies sample preparation by integrating sampling, extraction, and preconcentration into a single step, has been a popular sample pretreatment and preparation method among the scientific community.² Since its conception, the applications of SPME targeting at volatile and semivolatile compounds have been well developed. The methodology of SPME coupled with gas chromatography (GC) has been widely applied in multiple disciplines,^{2,3} even in archeology⁴ and entomology.^{5–7}

The emerging applications of SPME focus on the detection of highly polar exogenous and endogenous bioactive compounds in tissues of living animals, which is promising to facilitate the studies on pharmacokinetics,⁸ metabolomics,⁹ and neurosciences,¹⁰ as well as the studies on the bioaccumulation of pharmaceuticals as emerging pollutants in aquatic biotas.¹¹ However, compared with the studies of detecting volatile and semivolatile analytes with SPME,¹² these studies are still scarce, which is mainly due to the lack of proper fibers for sampling highly polar compounds in living animals.^{8–11,13} Nowadays, the newly explored SPME fiber coatings based on the extractive

materials, such as carbonaceous materials, ionic liquids, and metal–organic frameworks, are seldom used to extract highly polar analytes in living animals.^{12,14–18}

Indeed, the preparation of SPME fibers for sampling highly polar compounds in living animals is quite challenging. First of all, since the highly polar analytes, which contain amino, carboxyl, and/or sulfonic groups, can be ionized at physiological pH, it is difficult to achieve enrichment factors in the fiber coatings for these compounds as high as the nonpolar compounds. In addition, compared with the conventional abiotic and *in vitro* applications of SPME, SPME fibers for *in vivo* sampling should be biocompatible to avoid rejection reactions, matrix effect-resistant to avoid binding of biomacromolecules on the surfaces of the fibers, and small-sized to reduce invasiveness imparted on the living systems.

Polydimethylsiloxane (PDMS) has been applied to sample pharmaceuticals in fish muscle; however, due to the low affinity of PDMS to the analytes, the dimensions of PDMS fibers were quite large (about 640 μm) in order to gain satisfactory

Received: August 7, 2015

Accepted: September 25, 2015

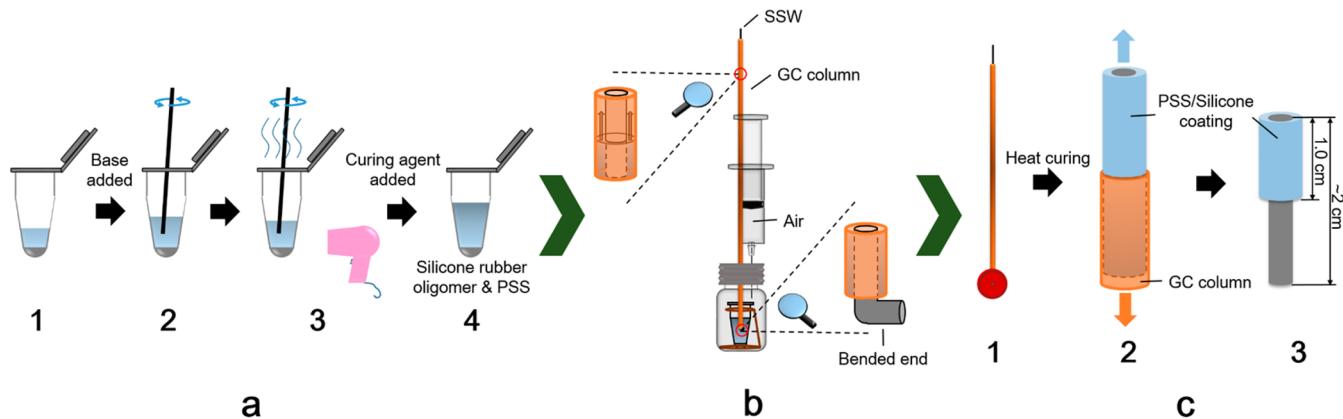


Figure 1. Flow diagram of the preparation procedure of the custom-made fiber. a) Preparation of silicone rubber oligomer/PSS mixture, b) mold filling, and c) in-mold heat-curing and the final product.

sensitivity.¹¹ Recent studies utilized nanofibrous polymers to accelerate the kinetics of sampling pharmaceuticals in living fish and rabbit;^{13,19} however, the fiber dimension was still large,¹³ while the blood needed to be drawn out of the rabbit body.¹⁹ Gluing the commercialized solid phase extraction (SPE) sorbents with biocompatible polymers on metal wires gained a success;^{8–10} however, the sorbent species are quite limited.

In this study, a novel SPME fiber was prepared by dispersing polyelectrolyte containing phenylsulfonate groups in silicone rubber for the extraction of analytes containing amino groups, which are ionized at physiological pH. Different from the conventional fibers which could only extract the neutral species,^{11,13,19} the novel coating could extract both the neutral and the protonated species of the analytes, thus, the sampling efficiencies could be dramatically improved. Owing to the high extraction efficiencies, the fiber dimension was confined to be capable of sampling in the brains of immature fish.

■ EXPERIMENTAL SECTION

Chemicals and Materials. Fluoxetine, norfluoxetine, and the deuterated standards fluoxetine-d5 and norfluoxetine-d5 were all purchased from Toronto Research Chemical Inc. (New York, ON, Canada). HPLC-grade methanol and poly(sodium 4-styrenesulfonate) (PSS, Mw ~ 1,000,000) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Stainless steel wires (SSWs, 127 μm in diameter, medical grade) were purchased from Small Parts Inc. (Miami Lakes, FL, USA). Sylgard 184 silicone rubber (including base and curing agent) was purchased from Dow Corning Co. (Midland, MI, USA). HP-1 gas chromatographic column (I.D. 0.2 mm, film thickness 0.11 μm) was formerly purchased from Agilent Technologies Inc. (CA, USA) and was recycled after being fully used.

Preparation of the SPME Fiber. The novel SPME fiber was prepared as follows: (a) PSS was dissolved in deionized water in a centrifuge tube (PSS: water 1:5 w/w) (Figure 1a-1). After the base of the silicone rubber was thoroughly mixed in the PSS solution by stirring (base: PSS 12:1 w/w) (Figure 1a-2), the water was evaporated by heating the centrifuge tube with a hair drier simultaneously avoiding precipitation of PSS by vigorously stirring (Figure 1a-3). Then the curing agent was added to the centrifuge tube (curing agent: base 2:3 w/w) and mixed under ultrasonic for 5 min (Figure 1a-4). (b) The inner surface of the GC column was washed with acetone and methanol subsequently. Then the column was cut into sections

about 7.0 cm with a GC column cutter and dried at room temperature. SSWs were cut into sections a little longer than the GC columns and also washed with acetone and methanol. SSWs were then soaked in sodium hydroxide solution (2 M) for 2 h to activate the surface and subsequently washed with deionized water and dried under room temperature. The treated SSW was inserted in a washed GC column with both ends of the SSW outside the column, and one end was bent with a pair of tiny tweezers. The centrifuge tube containing PSS/silicone oligomer mixture was set in a 10 mL sample vial, and the GC column with SSW in it was pierced through the septum of the cap with the bent end immersed in the mixture in the centrifuge tube (Figure 1b). The mixture was then pressurized into the GC column by purging air into the vial with a hypodermic syringe (Figure 1b). (c) After the GC column was filled with the mixture, the bent end of the SSW was cut off, and the end was sealed with a GC injector septum (Figure 1c-1). Then, the mixture inside the column was solidified under 100 °C for half an hour and 200 °C for 2 h. After having been cooled down to room temperature, the coated SSW was carefully drawn out from the column (Figure 1c-2). The coated SSW was cut into sections about 2 cm, and the coating was scratched with a knife with 1.0 cm coating remaining at one end (Figure 1c-3). The fiber was cleaned by being soaked in methanol for 15 min prior to use.

Matrix Effect-Resistant Capacity. To validate the matrix effect-resistant capacity of the novel fiber coating, matrix effects were evaluated according to the previous study with a little modification.²⁰ Briefly, the fibers which were exposed to fish muscle or brains for 30 min and the nonexposed fibers were desorbed in the desorption solvent, then standard solutions were added in the desorption solutions to form the matrix-impacted and matrix-free standard solution series (concentrations ranged from 1 to 100 ng·mL⁻¹), and the regression slopes of standard curves derived from the matrix-free and matrix-impacted standard solutions were subsequently compared. In addition, MALDI-TOF MS was used to detect whether there existed biomacromolecules in the matrix-impacted desorption solutions.

Animals and Exposure. Immature tilapias (*Oreochromis mossambicus*, $n = 12$) were purchased from a local fishery and kept in aerated 80 L aquaria containing 40 L of dechlorinated tap water for 2 weeks before experiment. Then, the fish were divided into two groups ($n = 6$ for each) and exposed to spiked tap water for 10 days at two environmentally relevant levels,

respectively. Briefly, 40 L of water was spiked with an aliquot of 25 or 135 μL of a stock solution of fluoxetine ($1000 \mu\text{g}\cdot\text{mL}^{-1}$, in methanol). The nominal water concentrations were 0.63 and $3.38 \mu\text{g}\cdot\text{L}^{-1}$, which were close to the highest concentrations of fluoxetine ($0.54 \mu\text{g}\cdot\text{L}^{-1}$) and the total selective serotonin reuptake inhibitors ($3.2 \mu\text{g}\cdot\text{L}^{-1}$) that were detected in natural waters.²¹ To keep the water concentration steady, the water was changed with fresh water and respiked with the initial amounts every 12 h. The water quality was monitored daily (pH 6.5, dissolved oxygen 6.5 ± 0.2 ppm, and temperature $27.3 \pm 1.3^\circ\text{C}$). Prior to *in vivo* SPME, the lengths and weights of the fish were recorded (length 11.5 to 13.5 cm, median 12.3 cm; weight 29.5 to 45.5 g, median 34.0 g). All of the animal experiments were approved by the Animal Ethical and Welfare Committee of Sun Yat-sen University.

In Vivo SPME. The fish was anaesthetized in dechlorinated municipal water containing 0.1% eugenol until loss of vertical equilibrium. A 26-gauge hypodermic needle was pierced into the fish head along the fish body axis at the middle point of the two eyes to a depth of approximately 0.7 cm to penetrate the skull; then, the needle was removed, and the fiber was inserted into the hole for approximately 1.7 cm to pierce through the whole brain. For comparison, *in vivo* SPME was also conducted in the fish dorsal-epaxial muscle. A 26-gauge hypodermic needle was pierced into the dorsal-epaxial muscle to a depth of approximately 1.4 cm. Subsequently, the needle was removed, and the custom-made SPME fibers were deployed in the punched holes. Two parallel samplings on both sides of the dorsal-epaxial muscle were conducted for mutual reference. The fish was then placed in fresh water to resume vertical balance. At the end of the sampling interval, the fish was reanesthetized, and the fibers were harvested. The total extraction periods were 30 min in both fish muscle and brains. The fibers were then rinsed with deionized water and dried with a Kimwipe tissue. The fibers were desorbed in 80 μL of methanol (containing 0.02% formic acid) for 1 h under a vortex rate of 400 rpm, and 20 μL of deuterated standard solution ($50 \text{ ng}\cdot\text{mL}^{-1}$) was added as internal standards to calibrate the ionization efficiency for LC-MS/MS analysis.

Instrumental Analysis. View the [Supporting Information](#) for details.

Quality Assurance (QA) and Quality Control (QC). View [Table S1](#).

RESULTS AND DISCUSSION

Characterization of the Custom-Made Fiber. The preparation procedure of the novel SPME fiber is described in detail in the [Experimental Section](#) and shown in [Figure 1](#). Polymers with ionizable functional groups are widely used for ion exchange purposes. In this study, PSS was mixed in silicone rubber to simultaneously extract the protonated species in addition to the neutral species of fluoxetine and norfluoxetine, to improve the extraction efficiencies. The fiber coating was prepared by direct heat-curing the mixture of PSS and the base and the curing agent of the Sylgard 184 silicone rubber. Compared with the conventional sol–gel method in which trifluoroacetic acid needs to be used as the catalyst,²² no volatile toxic agent was used in this study. Moreover, the in-mold heat-curing methodology lead to a well-confined fiber dimension.

PSS in solid state was found difficult to be directly mixed into the viscous base of the Sylgard 184 silicone rubber. While since PSS is a surfactant, in this study, PSS was first dissolved in water and then mixed with the base of the silicone rubber

([Figure 1](#)). [Figure S1a](#) shows that before mechanical mixing, the base (the upper layer) and the PSS aqueous solution (the lower layer) were colorless clear liquids. After vigorous stirring, the mixture turned white and cloudy ([Figure S1b, Supporting Information](#)). After the water was thoroughly evaporated (weighting the centrifuge tube until the lost weight was equal to the weight of the previously added water), as well as after the curing agent was added, the mixture remained white and cloudy ([Figure S1c and Figure S1d, Supporting Information](#)). The mixture at all the stages was stable; no separated layers or precipitates were observed even after centrifugation at a rate of 3000 rpm for 10 min.

[Figure 2](#) shows the optical microscope images of the mixture at different stages. After vigorous stirring of the mixture of the

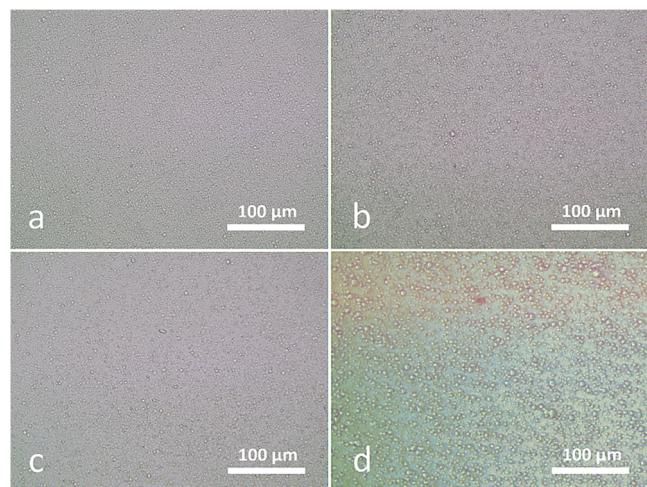


Figure 2. Microscope images of the mixture of PSS and silicone rubber oligomer at different stages. a) After vigorous stirring the mixture of the PSS solution and the base of Sylgard 184 silicone rubber, b) after water being evaporated, c) after the curing agent being added, and d) after being heat cured.

PSS solution and the base, it was observed one phase was dispersed in another phase at micron scales ([Figure 2a](#)). After the water had been thoroughly evaporated, the sizes of the dispersed phase increased ([Figure 2b](#)). Therefore, it might be speculated that the dispersed phase in [Figure 2a](#) was the PSS aqueous solution, and when the water was evaporated, some of the droplets fused ([Figure 2b](#)). The dispersed phase in [Figure 2b](#) might be pure PSS solids at micron scales after the water was thoroughly evaporated. When the curing agent was added, the dispersed phase was diluted, as the number of the particles per unit field of vision decreased apparently ([Figure 2c](#)). While after the mixture being heat-cured on the microscope slide, the sizes of the dispersed phase increased again ([Figure 2d](#)), which might be caused by the heat-induced collision and fusion among the PSS particles.

The scanning electron microscope images of the custom-made fiber are shown in [Figure 3](#). It was observed that the surface of the fiber was quite smooth ([Figure 3a](#)), and the coating was apparently homogeneous that no phase separation or pores were observed ([Figure 3b](#)). These results also demonstrated that the dispersed phase at micron scales observed in [Figure 2d](#) was not pores. From [Figure 3](#), it was also observed that the diameter of the fiber was about 200 μm , and the diameter of the stainless steel wire was about 127 μm .

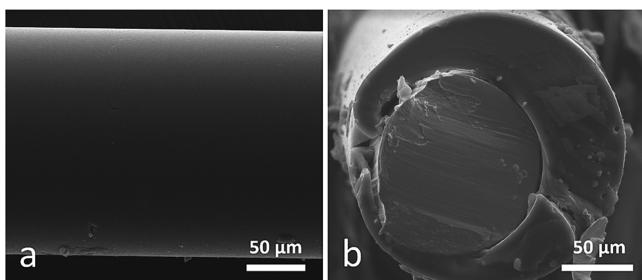


Figure 3. Scanning electron microscope images of a) the surface and b) the cross section of the custom-made fiber.

Figure 4 shows the element mapping results of the surface of the fiber. It was observed that the distribution of S and Na

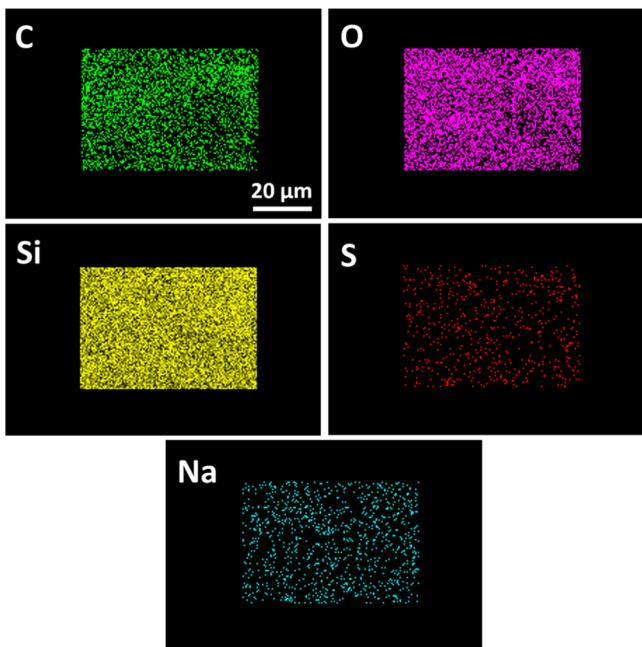


Figure 4. Element mapping results on the surface of the custom-made fiber.

atoms were very similar to each other, which corresponded to the structure of PSS that each monomeric unit contained one S atom and one Na atom. Compared with the dense distribution of C, O, and Si, the distribution of S and Na was separated by some dark spaces, which confirmed that PSS was located in the separated phase in **Figure 2d**.

Extraction Performance of the Custom-Made Fiber. The interfiber reproducibility and intrafiber reproducibility are presented in **Table 1**, which were determined by extraction in the phosphate buffer saline solutions (PBS, 147 mM Na^+). The excellent intrafiber reproducibility demonstrated that the fiber was stable enough for repeated uses. Even though PSS could be easily dissolved in water, the configuration of encasing PSS in

Table 1. Interfiber and Intrafiber Reproducibility (RSD%, $n = 6$) of the Novel Custom-Made Fiber

	interfiber	intrafiber
fluoxetine	5.4	4.6
norfluoxetine	7.1	3.7

the microcapsules in the silicone rubber effectively avoid PSS from being dissolved. Even though as shown in **Figure 3b**, the stainless steel supporting core of the fiber might not be concentric with the coating (the possible locations of the SSWs in the coatings are presented in **Figure S2, Supporting Information**), the interfiber reproducibility was still quite satisfactory. As demonstrated in ref 23, when the surface area and the coating volume are consistent for each fiber (**Figure S2, Supporting Information**), satisfactory interfiber reproducibility could be attained.

Figure 5a shows the comparison of the extraction efficiencies between the coating of the silicone rubber mixed with PSS and the pure silicone rubber coating. The pure silicone rubber coating was also prepared by in-mold heat-curing of the mixture of the base and the curing agent without mixing PSS in it, therein, the ratio of the base to the curing agent was the same as the PSS/silicone coating. Even though PSS was only 5% the weight of the PSS/silicone coating, the extraction efficiencies of the PSS/silicone coating were about two times higher than those of the pure silicone coating at the same dimension. When the PSS/silicone coating was used, the protonated species could be simultaneously extracted through an ion-exchange strategy in addition to the neutral species, as proposed in **Figure 5b**, while only the neutral analytes could be directly extracted when the pure silicone coating was used. As shown in **eqs 1 and 2**, the extracted amount in the pure silicone coating could be expressed as

$$n = C_s K_2 V_f (1 - e^{-(D_n A)/(K_2(1+K_i^*)\delta V_f)t}) \quad (1)$$

while the extracted amount in the PSS/silicone coating at the same dimension could be expressed as

$$n = C_s K_2 (1 + K_i^*) V_f (1 - e^{-(D_n A)/(K_2(1+K_i^*)\delta V_f)t}) \quad (2)$$

where n is the extracted amount of the analyte in the fiber coating, C_s is the concentration of the analyte in the sample matrix, V_f is the volume of the fiber coating, A is the surface area of the fiber, δ is the thickness of the diffusion boundary layer around the fiber which is determined by the fiber dimension and the hydrodynamic conditions around the fiber,²⁴ t is the time duration of extraction, K_2 is the partition coefficient of the analyte between the pure silicone coating and the sample matrix, D_n is the diffusion coefficient of the neutral species in the sample matrix, and K_i and K_i^* are the ratios of the concentration of the protonated species to that of the neutral species at equilibrium in the sample matrix and in the PSS/silicone coating, respectively. The details of the derivation of **eq 1** and **eq 2** are provided in the **Supporting Information**. **Figure 5c** presents the extraction kinetics of the PSS/silicone coating and pure silicone coating with a set of given parameters based on **eq 1** and **eq 2**, where the Y-axis is the ratio of the extracted amount to $C_s K_2 V_f$. Theoretically, no matter if K_i^* were larger or smaller than K_i , the extracted amount in the PSS/silicone coating would always be larger than that in the silicone coating.

In addition, the custom-made fiber also exhibited excellent matrix effect-resistant capacities with no biomacromolecules being detected in the desorption solutions with MALDI-TOF MS, and no significant ionization bias being observed with the liquid chromatography-tandem mass spectrometry analysis (78% to 109% ionization efficiencies of the control, **Table S2, Supporting Information**). Besides, PSS and silicone rubber are both biocompatible materials,^{25,26} which would not lead to rejection reactions in living animals.

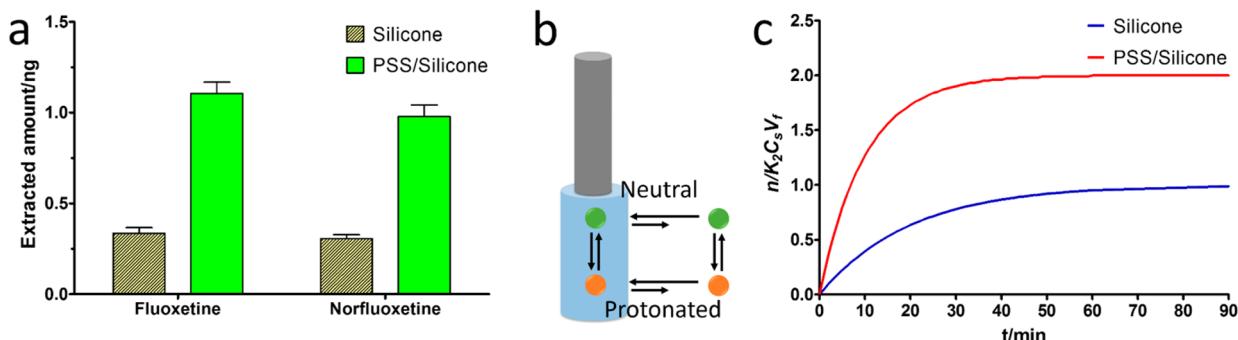


Figure 5. a) Comparison of the extraction efficiencies of pure silicone and PSS/silicone fibers at the same dimension. The concentrations of fluoxetine and norfluoxetine were both $20 \text{ ng}\cdot\text{mL}^{-1}$ (in PBS solutions, 147 mM Na^+), and the extraction durations were 20 min. The error bars represent the standard deviation ($n = 3$). b) Hypothetical extraction mode of the PSS/silicone fiber. Supposing that the neutral and protonated species are in dynamic equilibrium in both the solution and the fiber coating. c) The extraction kinetics of the silicone fiber and the PSS/silicone fiber according to eqs 1 and 2. Where setting $K_2 = 100$, $K_i = 3$, $K_i^* = 1$, $D_n = 3.0 \times 10^{-9} \text{ m}^2\cdot\text{min}^{-1}$, and $\delta = 50 \mu\text{m}$, the ratios of V_f/A of the fibers were calculated to be $2.984 \mu\text{m}$.

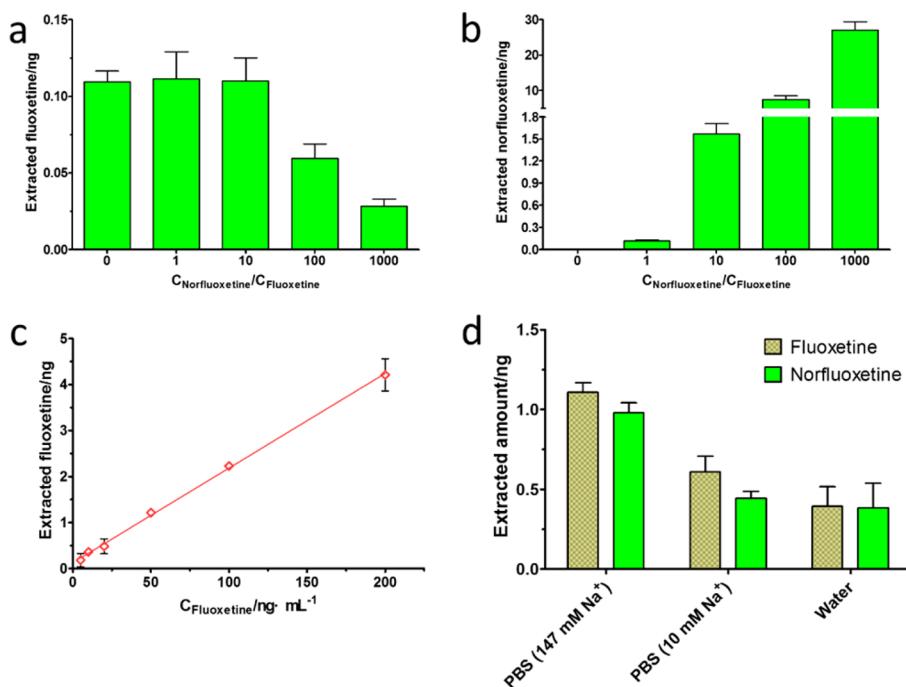


Figure 6. Extracted amounts of a) fluoxetine and b) norfluoxetine in PSS/silicone fibers under different ratios of concentrations (in PBS solutions, 147 mM Na^+). Concentrations of fluoxetine were always $2.0 \text{ ng}\cdot\text{mL}^{-1}$. The error bars represented the standard deviation ($n = 5$). c) Linear range of fluoxetine in PBS solution (147 mM Na^+ , $6 \mu\text{M}$ bovine serum albumin) was from $5 \text{ ng}\cdot\text{mL}^{-1}$ to $200 \text{ ng}\cdot\text{mL}^{-1}$, R^2 was 0.9989. The extraction durations were 20 min. The error bars represent the standard deviation ($n = 3$). d) Effects of salinities on the sorption of fluoxetine and norfluoxetine by PSS/silicone fibers. The concentrations of fluoxetine and norfluoxetine were both $20 \text{ ng}\cdot\text{mL}^{-1}$, and the extraction durations were 20 min. The error bars represent the standard deviation ($n = 6$).

Compared with coatings containing no cation-exchange groups, the competition effects of inorganic and organic cations on the sorption of the target analytes and the competitive sorption between different analytes were likely to occur for the PSS/silicone coating.²⁷ The competitive sorption between fluoxetine and norfluoxetine was evaluated under different concentration ratios (Figures 6a and 6b). There was no decrease in the sorption of fluoxetine until the concentration of norfluoxetine was as high as one hundred times that of fluoxetine in PBS solution (Figure 6a), where the total extracted amount might have already exceeded the loading capacity of the PSS/silicone fiber (no less than 4.2 ng fluoxetine, as shown Figure 6c); in other words, the extracted amounts could no longer increase linearly along with the

concentrations in PBS solutions (Figure 6b). However, the extracted amounts of in vivo sampling in fish tissues (described in the section below, data not shown) were far less than the loading capacity, and the competition between the analytes was ignorable.

Salts in the sample matrix might compete with the ionized analytes for sorption into the coating and exhibit a salting-out effect as well.^{28,29} In this study, it seemed that the salting-out effect was much more significant than the competition effect (Figure 6d).

In Vivo SPME in Fish Brains. In this study, the fish was still alive after deploying the custom-made fiber in its brain (Figure 7a). In vivo SPME in the brains of freely moving mice and rats has been previously reported.^{10,30,31} SPME fibers were

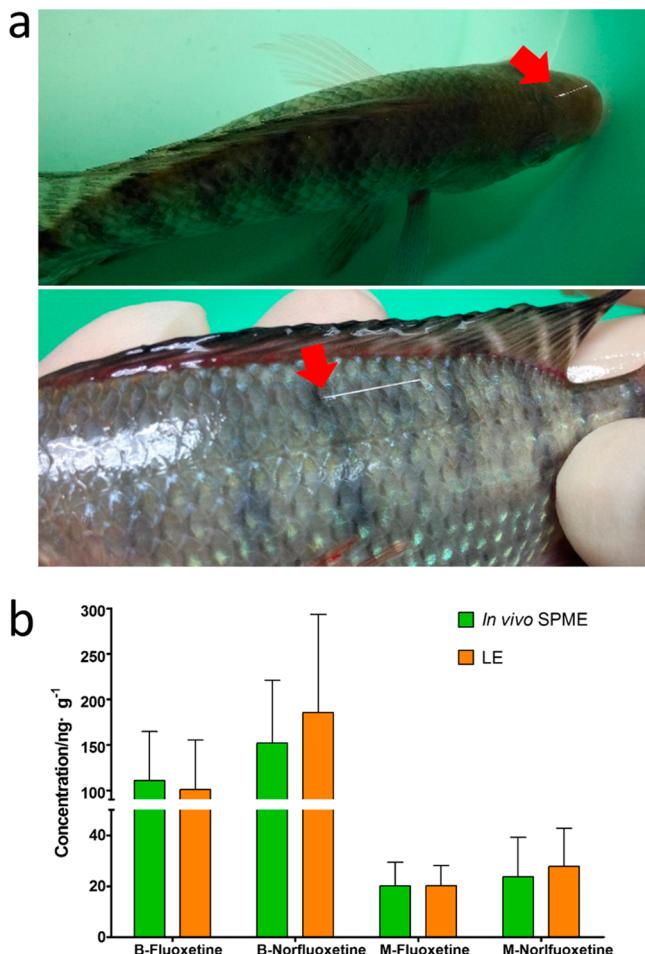


Figure 7. a) In vivo sampling in fish brain and dorsal-epaxial muscle with the custom-made fiber. b) Total concentrations of fluoxetine and norfluoxetine in fish brains and dorsal-epaxial muscle as determined with in vivo SPME and LE. The error bars represent the standard deviation ($n = 6$). “B” and “M” represent the brains and dorsal-epaxial muscle, respectively.

deployed in specific regions of the brains under the guidance of a microdialysis guide cannula, and sophisticated surgery was required.^{10,30,31} Nonetheless, to the authors' knowledge, this was the first time that in vivo SPME in the much smaller immature fish brains was performed (less than 100 mg), and no complicated surgery was needed in the present study (as described in the *Experimental Section*). To confirm that the custom-made fiber was correctly deployed in the fish brain, one fish was sacrificed after the fiber was deployed, and a craniotomy was conducted on its skull. It was found the fiber pierced through the whole brain (Figure S3, *Supporting Information*). Simultaneously, in vivo SPME in the fish dorsal-epaxial muscle was also performed for comparison (Figure 7a). Compared with in vivo SPME in fish brains, in vivo SPME in the fish dorsal-epaxial muscle has been well developed.^{11,13,32,33}

The total concentrations of fluoxetine and norfluoxetine in fish that were exposed to the waterborne fluoxetine at an environmentally relevant concentration (detected to be 0.31 $\mu\text{g}\cdot\text{L}^{-1}$, Table S3, *Supporting Information*) were determined using the sampling rate calibration method.³³ The sampling rates of fluoxetine and norfluoxetine in both fish brains and dorsal-epaxial muscle were determined in another group of fish

($n = 6$) that were exposed at a higher concentration (detected to be 4.18 $\mu\text{g}\cdot\text{L}^{-1}$, Table S3, *Supporting Information*), according to eq 3

$$R_s = \frac{n}{C_s \cdot t} \quad (3)$$

where R_s is the sampling rate; C_s is the concentration in the fish brain or dorsal-epaxial muscle determined with liquid extraction after in vivo sampling (Figure S4 and Table S3, *Supporting Information*); and t was the in vivo sampling duration. The relative standard deviations (RSDs) of the sampling rates as determined in fish brains and dorsal-epaxial muscle ranged from 13.8% to 43.0% ($n = 6$, Figure S5, *Supporting Information*). Based on the sampling rates, the total concentrations that were determined with in vivo SPME were close to those that were determined with liquid extraction (LE) (Figure 7b), demonstrating that the sampling rate calibrated in vivo SPME was accurate. The total concentrations of fluoxetine and norfluoxetine as determined with in vivo SPME in fish brains were 4.4 to 9.2 and 5.0 to 9.2 times those in the dorsal-epaxial muscle, respectively, which were similar to the results of the previous in vitro study.³⁴

CONCLUSIONS

The present study reported the first application of in vivo SPME in the sampling of dominantly ionized analytes (the pK_a values of fluoxetine and norfluoxetine are 8.7 and 9.1, respectively³⁵) in immature fish brains. This is contemporarily the smallest organ that has ever been sampled by direct deploying SPME fiber in it. To achieve this goal, a novel SPME fiber was prepared with a facile methodology that effectively dispersed polyelectrolyte at micron scales in the silicone rubber and well confined the fiber dimension. This fiber is also the smallest fiber that has ever been used for sampling the highly polar analytes in living animals. In addition, the high affinities of the custom-made fiber to partially protonated analytes was theoretically explained.

Since most artificially synthesized pharmaceuticals and many endogenous bioactive compounds in animals contain amino groups, the custom-made fiber might be very useful in various studies, such as the studies on pharmacokinetics, the detection of the neurotransmitters, and the studies on the bioaccumulation of waterborne pharmaceuticals in aquatic animals. Moreover, the methodology proposed in the present study might be used to prepare fibers containing anion-exchange polyelectrolytes, which has the potential to efficiently sample deprotonated analytes in living animals.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.analchem.5b03036](https://doi.org/10.1021/acs.analchem.5b03036).

Instrumental analysis conditions, derivation of eq 1 and eq 2, pictures of the preparation procedure of the silicone rubber oligomer/PSS mixture, possible locations of SSWs in the coatings, location of the fiber in the fish brain, sampling rates, total concentrations that were determined with LE, and QA/QC data (PDF)

AUTHOR INFORMATION

Corresponding Authors

*Phone/Fax: 86-20-84110845. E-mail: cesoygf@mail.sysu.edu.cn (G.O.).
*Phone/Fax: 86-20-84110954. E-mail: ceszhuf@mail.sysu.edu.cn (F.Z.).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We acknowledge financial support from the projects of NNSFC (21225731, 21377172, 21477166) and the NSF of Guangdong Province (S2013030013474).

REFERENCES

- (1) Cooks, R. G.; Ouyang, Z.; Takats, Z.; Wiseman, J. M. *Science* **2006**, *311*, 1566–1570.
- (2) Ouyang, G.; Vuckovic, D.; Pawliszyn, J. *Chem. Rev.* **2011**, *111*, 2784–2814.
- (3) Pawliszyn, J. *Handbook of Solid Phase Microextraction*; Chemical Industry Press: Beijing, 2009.
- (4) McGovern, P. E.; Mirzoian, A.; Hall, G. R. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 7361–7366.
- (5) Syed, Z.; Leal, W. S. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 18803–18808.
- (6) Syed, Z.; Leal, W. S. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 13598–13603.
- (7) Liebig, J.; Peeters, C.; Oldham, N. J.; Markstädter, C.; Hölldobler, B. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 4124–4131.
- (8) Musteata, F. M.; Musteata, M. L.; Pawliszyn, J. *Clin. Chem.* **2006**, *52*, 708–715.
- (9) Vuckovic, D.; de Lannoy, I.; Gien, B.; Shirey, R. E.; Sidisky, L. M.; Dutta, S.; Pawliszyn, J. *Angew. Chem., Int. Ed.* **2011**, *50*, 5344–5348.
- (10) Cudjoe, E.; Bojko, B.; de Lannoy, I.; Saldivia, V.; Pawliszyn, J. *Angew. Chem., Int. Ed.* **2013**, *52*, 12124–12126.
- (11) Zhang, X.; Oakes, K. D.; Wang, S.; Cui, S.; Pawliszyn, J.; Metcalfe, C. D.; Servos, M. R. *TrAC, Trends Anal. Chem.* **2012**, *32*, 31–39.
- (12) Xu, J.; Zheng, J.; Tian, J.; Zhu, F.; Zeng, F.; Su, C.; Ouyang, G. *TrAC, Trends Anal. Chem.* **2013**, *47*, 68–83.
- (13) Xu, J.; Huang, S.; Wu, R.; Jiang, R.; Zhu, F.; Wang, J.; Ouyang, G. *Anal. Chem.* **2015**, *87*, 3453–3459.
- (14) Zhang, S.; Du, Z.; Li, G. *Anal. Chem.* **2011**, *83*, 7531–7541.
- (15) Zewe, J. W.; Steach, J. K.; Olesik, S. V. *Anal. Chem.* **2010**, *82*, 5341–5348.
- (16) Pena-Pereira, F.; Marcinkowski, Ł.; Kłoskowski, A.; Namieśnik, J. *Anal. Chem.* **2014**, *86*, 11640–11648.
- (17) He, C.; Tian, J.; Liu, S.; Ouyang, G.; Zhang, J.; Chen, X. *Chem. Sci.* **2013**, *4*, 351–356.
- (18) Li, Y.; Yang, F.; Liu, Z.; Liu, Q.; Dong, Y. *J. Mater. Chem. A* **2014**, *2*, 13868–13872.
- (19) Wu, Q.; Wu, D.; Guan, Y. *Anal. Chem.* **2013**, *85*, 11524–11531.
- (20) Zhang, X.; Oakes, K. D.; Luong, D.; Metcalfe, C. D.; Servos, M. R. *Anal. Chem.* **2011**, *83*, 6532–6538.
- (21) Mennigen, J. A.; Sassine, J.; Trudeau, V. L.; Moon, T. W. *Aquat. Toxicol.* **2010**, *100*, 128–137.
- (22) Ke, Y.; Zhu, F.; Zeng, F.; Luan, T.; Su, C.; Ouyang, G. *J. Chromatogr. A* **2013**, *1300*, 187–192.
- (23) Ouyang, G.; Cui, S.; Qin, Z.; Pawliszyn, J. *Anal. Chem.* **2009**, *81*, 5629–5636.
- (24) ter Laak, T. L.; van Eijkeren, J. C. H.; Busser, F. J. M.; van Leeuwen, H. P.; Hermens, J. L. M. *Environ. Sci. Technol.* **2009**, *43*, 1379–1385.
- (25) Shao, M.; Bai, H.; Gou, H.; Xu, J.; Chen, H. *Langmuir* **2009**, *25*, 3089–3095.
- (26) Ye, S.; Marston, G.; McLaughlan, J. R.; Sigle, D. O.; Ingram, N.; Freear, S.; Baumberg, J. J.; Bushby, R. J.; Markham, A. F.; Critchley, K.; Coletta, P. L.; Evans, S. D. *Adv. Funct. Mater.* **2015**, *25*, 2117–2127.
- (27) Peltenburg, H.; Groothuis, F. A.; Droke, S. T. J.; Bosman, I. J.; Hermens, J. L. P. *Anal. Chim. Acta* **2013**, *782*, 21–27.
- (28) Chen, C.; Liang, X.; Wang, J.; Zou, Y.; Hu, H.; Cai, Q.; Yao, S. *J. Chromatogr. A* **2014**, *1348*, 80–86.
- (29) Jeon, J.; Kannan, K.; Lim, H. K.; Moon, H. B.; Ra, J. S.; Kim, S. *D. Environ. Sci. Technol.* **2010**, *44*, 2695–2701.
- (30) Nakajima, D.; Win-Shwe, T.-T.; Kakeyama, M.; Fujimaki, H.; Goto, S. *NeuroToxicology* **2006**, *27*, 615–618.
- (31) Win-Shwe, T.-T.; Mitsushima, D.; Nakajima, D.; Ahmed, S.; Yamamoto, S.; Tsukahara, S.; Kakeyama, M.; Goto, S.; Fujimaki, H. *Toxicol. Lett.* **2007**, *168*, 75–82.
- (32) Xu, J.; Luo, J.; Ruan, J.; Zhu, F.; Luan, T.; Liu, H.; Jiang, R.; Ouyang, G. *Environ. Sci. Technol.* **2014**, *48*, 8012–8020.
- (33) Ouyang, G.; Oakes, K. D.; Bragg, L.; Wang, S.; Liu, H.; Cui, S.; Servos, M. R.; Doxin, D. G.; Pawliszyn, J. *Environ. Sci. Technol.* **2011**, *45*, 7792–7798.
- (34) Brooks, B. W.; Chambliss, C. K.; Stanley, J. K.; Ramirez, A.; Banks, K. E.; Johnson, R. D.; Lewis, R. J. *Environ. Toxicol. Chem.* **2005**, *24*, 464–469.
- (35) Togunde, O. P.; Oakes, K. D.; Servos, M. R.; Pawliszyn, J. *Environ. Sci. Technol.* **2012**, *46*, 5302–5309.