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In Vivo Fast Equilibrium Microextraction by Stable and Biocompatible Nanofiber Membrane Sandwiched in Microfluidic Device

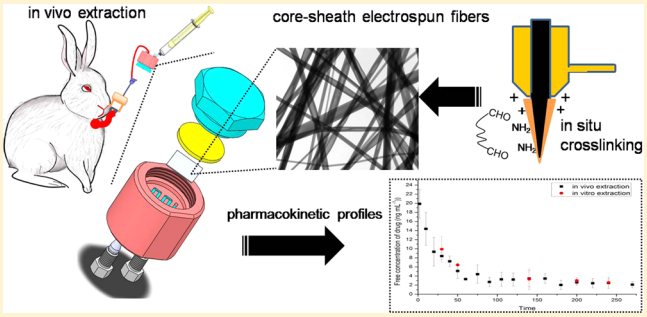
Qian Wu,[†,‡] Dapeng Wu,*[,†] and Yafeng Guan*[,†]

[†]Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457 Zhongshan Road, Dalian 116023, P. R. China

[‡]Dalian Institute of Chemical Physics, Graduate School of the Chinese Academy of Sciences, Beijing 100039, P. R. China

Supporting Information

ABSTRACT: In vivo analysis poses higher requirements about the biocompatibility, selectivity and speed of analytical method. In this study, an in vivo fast equilibrium microextraction method was developed with a biocompatible core−sheath electrospun nanofiber membrane sandwiched within a microfluidic unit. The polystyrene/collagen core−sheath nanofiber membrane was coaxially electrospun and strengthened with in situ glutaraldehyde cross-linking. This membrane not only kept high mass transfer rate, large extraction capacity and biomatrix resistance as our previously proposed membrane (Anal. Chem. 2013, 85 (12), 5924−5932), but also got much better mechanical strength and stability in water. The microfluidic device was designed to sandwich the membrane, and the blood in vivo can be introduced into it and get contact with the membrane repetitively. With this membrane and device, a 2-min equilibrium in vivo extraction method was established, validated in a simulated blood circulation system, and was used to monitor the pharmacokinetic profiles of desipramine in rabbits. The free and total concentration of desipramine in vivo was monitored with 10-min interval almost without rabbit blood consumed. The results met well with those of in vitro extraction, and a correlation factor of 0.99 was obtained.



Most in vitro analysis methods may not be able to accurately indicate or predict the real processes occurring in a complex living system because sample transport and storage and subsequent in vitro sample preparation could significantly change the real composition of the measured analytes, and the blood or tissue sampling could largely deplete or damage the living system and make temporal and spatial studies extremely difficult (such as rapid pharmacokinetics study and drug distribution mapping in tissue).[1] To address these problems, a lot of in vivo analysis or sampling methods were developed, such as microdialysis (MD) sampling,[2] implantable sensors,[3] in vivo microfluidic chip,[4] and in vivo solid-phase microextraction (SPME).[1] Among them, in vivo sampling methods such as MD sampling and in vivo SPME coupled with chromatography analysis was more suitable for the complex biological system.

In vivo SPME was developed in recent years by Janusz Pawliszyn and co-workers.[1a] Series of biocompatible SPME coatings had been developed and exposed directly in the living system such as blood circulation system, animal tissues, and plant tissues by the needlelike device.[1a,5] This method was demonstrated to have minimum destruction for the living system and to have wider adaptation to hydrophobic analytes than MD.[6] Unfortunately, the extraction equilibrium time of most biocompatible SPME coatings with satisfactory extraction capacity was not short enough (>15 min) for high temporal resolution analysis, so pre-equilibrium extraction (PE-extraction) instead of equilibrium extraction (E-extraction) was commonly used, and kinetic calibration methods were needed to compensate the kinetic variation of extraction caused by the biomatrix. However, PE extraction usually results in low sensitivity and requires accurate timing.[1a,7] Kinetic calibration usually needs deuterated standards preloaded on the solid phase[8] and distribution coefficients of analytes between phases,[8,9] which would complicate the whole calibration process. Moreover, free concentration of analytes in biofluids, such as blood, could not be measured directly by using kinetic calibration without the binding constant of the drugs to the blood matrix.[9b,10] Thus, a biocompatible extraction material with a faster mass transfer rate and larger extraction capacity to ensure fast E-extraction and higher sensitivity is still pursued actively.

Recently, our group proposed a biocompatible extractive material of polystyrene (PS)/poly(*N*-isopropylacrylamide) (PNIPAAm) core−sheath nanofiber, which could fulfill in

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vitro 2 min equilibrium extraction with wide linear ranges.[11] Thus, it had the potential to be a better alternative for in vivo extraction with high temporal resolution than SPME coatings. However, the in vivo application was prevented by its limited stability in water,[11] resulting in dissolution of sheath polymer into the in vivo system and disturbing the living system. Thus, a nanofiber with more stable sheath was needed, but improving stability of hydrophilic polymer electrospun fibers without damaging their nanostructure was always a challenge for the electrospinning technique.[12] Moreover, for in vivo extraction, a new form of interface is urgently needed for this new material to be introduced into the living system.

In this study, we developed a much more stable biocompatible core−sheath nanofiber membrane as extractive material and successfully utilized it for fast equilibrium in vivo extraction with a typical microfluidic device. Collagen as a native extracellular matrix component of tissues with good biocompatibility, nonimmunogenicity, and biodegradability[13] was used as the sheath and polystyrene as the core. Coaxial electrospinning[14] with in situ cross-linking was used for the first time to prepare the core−sheath nanofibers, and glutaraldehyde (GTA)[12c] was used as the cross-linking reagent. In situ cross-linking was demonstrated to be able to stabilize the collagen sheath without destroying the nanostructure of fibers. Experimental results showed that the membrane had high stability (weight loss of as-spun fiber in water <10%, no weight loss in methanol and reused 20 times in plasma), rapid mass transfer rate for three drugs being tested (equilibrium time ∼2 min), antifouling ability to biomatrix, and good repeatability. To introduce it into the in vivo system, a flow-through microfluidic device was designed to sandwich the membrane and to connect it to the in vivo blood circulation. Finally, the in vivo extraction method was validated by the simulated blood circulation system and then was successfully used to study the pharmacokinetics of rabbits. The in vivo studied results were compared with the conventional in vitro analysis results, and a correlation coefficient was around 0.99.

EXPERIMENTAL SECTION

Chemicals and Materials. Amitripyline (95% pure) was purchased from Sigma−Aldrich (St. Louis, MO). Propranolol (95% pure) was purchased from Acros Organics (Morris Plains, NJ). Desipramine (95% pure) and berberine chloride (95% pure) were purchased from J&K Scientific Ltd. (Beijing, China). Aripiprazole (90% pure) were provided by Jiangsu Vcare Pharmatech. Company (Nanjing, China). Their structures and physical chemical properties are summarized in Figure S1 and Table S1 of the Supporting Information. NaH_2PO_4 and NaOH (analytical grade) used to prepare phosphate buffer saline (PBS, pH = 7.4) were bought from Shenyang Chemical Reagent Company (Shenyang, China). PS (M_w = 250000−270000 Da, general type I) was purchased from Aladdin Industrial Company (Shanghai, China), and collagen (M_w = 80000−100000 Da) was purchased from Sichuan Mingrang Bio-Tech Company Ltd. 1,1,1,3,3,3-Hexafluoroisopropanol (HFIP) and aqueous GTA solution (50%) were purchased from Aladdin Reagent Company (Shanghai, China), and chloroform were obtained from Kermel Chemical Reagent Company (Tianjing, China). The materials were used without any purification. Acetonitrile and methanol of HPLC grade were purchased from Merck Company (Darmstadt, Germany). Human plasma of healthy adults was obtained from the Second Affiliated Hospital of Dalian Medical University (Dalian, China).

Preparation of Electrospun Fibers. 10% (w/v) PS solution in HFIP/chloroform (2:5, v/v) (A solution) and 5% (w/v) collagen solution in HFIP (B solution) were prepared at room temperature and stirring for 5 h, respectively. The schematic illustration of coaxial electrospinning device is shown in Figure S2 of the Supporting Information. The spinneret was composed of two coaxial capillaries and are connected to the electrical potential (20 kV) applied by a high voltage power supply (ADW300-0.5, Dongwen high voltage power supply Inc., Tianjing, China). The tip-to-collector distance was set to 15 cm, and a grounded aluminum foil (5 × 4 cm) was used for the fiber collection. B and A solutions were introduced from the outer and inner capillary with flow rate at 0.2 mL/h and 0.4 mL/h, respectively. The spinneret and collector were housed in a transparent polymethyl methacrylate room. 25% GTA solution was put closely to the collector for at least 1 h before electrospinning.

The detailed characterization processes of the nanofibers are described in method S1 of the Supporting Information.

Microfluidic Device for In Vivo Extraction. The components of the microfluidic module are shown in Figure 1. The substrate block was made of polytetrafluoroethylene (PTFE) with micromachined serpentine microchannels of 1 mm width and 0.5 mm depth. The cover block was made of nylon. During device assembly, one piece of electrospun nanofiber membrane, a PTFE/silicone septum, and the cover were placed on the substrate, respectively. The two blocks were then screwed together, and the nanofiber membrane was sandwiched tightly due to the compression between the PTFE/silicone septum and the PTFE substrate. For liquid connection, two holes on the substrate block were drilled with inner screw threads down to the microchannel end. The inner screw threads were used to connect two PTFE tubes to the outside by combining the standard bite type fittings. One tube was connected with a syringe, and the other tube was connected with a needle.

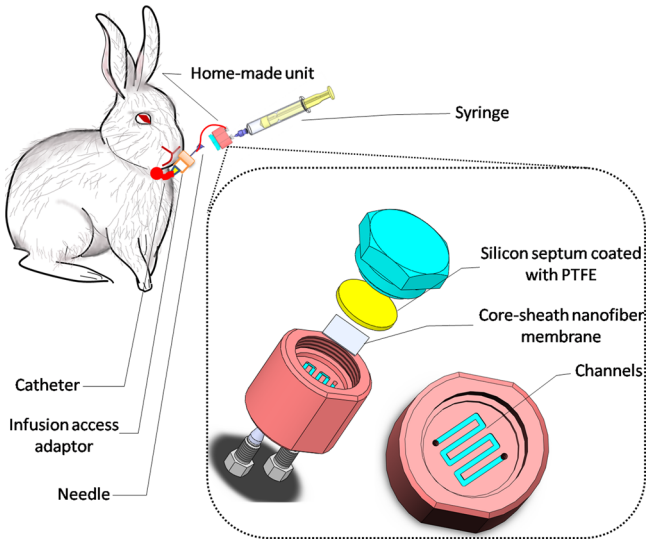

Figure 1. Schematic illustration of in vivo microextraction with a biocompatible nanofiber membrane sandwiched in a microfluidic device.

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In Vitro Extraction Experiments. Stock solutions of 1 mg mL^{-1} investigated drugs were prepared in methanol and kept at −20 °C until analysis. Rabbit whole blood was maintained at 4 °C within 1 week and plasma was kept frozen at −20 °C until use. For analysis, plasma was thawed at room temperature and transferred into clean vials. Appropriate amounts of drug standards were added in plasma samples, whole blood samples, and PBS (12 mmol L^{-1}, pH 7.4) to final concentrations, followed by vortex mixing for 30 min. The fiber membranes were first sterilized by immersing them in 70% ethanol for 18 h and then dried in vacuum.

For in vitro static extraction of drugs with the membrane directly, 2 mg extracting phase of electrospun fibers was put into the samples (1 mL) placed on a temperature-controlled shaking bed (SHZ-82A, Weier experimental equipment Ltd., Suzhou, China) for a precise period of time.

For in vitro extraction with the microfluidic unit, an artificial circulatory system (Figure S3 and method S2 of the Supporting Information) similar as that in other references[9b,15] was used to simulate the blood circulation, and the extraction procedure was the same as the in vivo extraction.

In Vivo Extraction Experiments. All animal experiments were carried out in the College of Medical Laboratory of Dalian Medical University (DMU, Dalian, China) with the assistance of the animal experiment expert. The rabbits were housed in the animal facility and maintained on a 12 h light−dark cycle. Rabbits had access to water and to Lab Diet. Surgical placement of catheters was performed under ethyl caronate anesthesia. Briefly, an infusion access adaptor (Jierui Medical Products Company Ltd., Weihai, China) was collected to a catheter implanted in the common carotid artery of rabbits. Animals were allowed to recover and acclimatize individually to their cage for at least one day prior to dosing and in vivo sampling experiments. Before dosing, 500 μL of blank blood was withdrawn for in vitro blood analysis, and 2 min in vivo extraction was conducted as a blank extraction. Then, desipramine was dosed at 0.5 mg/kg into the rabbit ear vein using desipramine dosing solution prepared in physiological saline. At each time point, in vivo E-extraction was conducted for 2 min, and immediately after in vivo extraction, 500 μL of blood was withdrawn for in vitro blood analysis. For in vivo extraction, the fiber membranes were first sterilized as the above-mentioned. Then, the microfluidic device was used to clamp the sterilized fibers mat (0.8 mg) and was further connected to the infusion access adaptor by piercing the septum of the adaptor with the needle on the device. The blood was introduced into the channel of the unit and contacted with the membrane by the push/pull action of the syringe. Usually, 0.5 mL of blood was withdrawn at a flow rate of 1 mL/min and then pushed back at the same flow rate. This flow rate was well below the normal flow rate in the rabbit carotid artery and produced minimal disturbance.

Desorption Procedure. After extraction, the fiber was then briefly rinsed with water, and then centrifuged at 4000 rpm for 1 min to remove excess water in the fibers. Dried fibers were desorbed in 50 μL of methanol for 30 min without stirring (desorption solution and time was chosen according to Figure S4 of the Supporting Information). Two microliters of desorption solution was introduced to LC−MS/MS for analysis.

For repeat use, the fibers should be washed in methanol for three times (one time for 10 min) to keep the residues content all below 10%.

LC-MS/MS System. The details for the LC−MS/MS system are described in method S3 of the Supporting Information, and the parameters of MS/MS detection are summarized in Table S2 of the Supporting Information.

RESULTS AND DISCUSSION

Preparation and Characterization of Core−Sheath Electrospun Fibers. *Preparation and Morphology of Core−Sheath Electrospun Fibers.* Collagen is a well-known biocompatible and hydrophilic polymer and easy to be cross-linked by dialdehyde compounds to get more stable and coriaceous in water.[13a−c] This sheath could improve the long time stability and mechanical strength of the biocompatible sheath, which was an unsettled problem of our previous study.[11]

Conventionally the cross-linking process was carried out after electrospinning by GTA vapor or solution exposing,[12c,16] and it would make the nanofibers thicker and melt into each other, resulting in a decrease of porosity and surface area (as seen in Figure 2B). In our study, the coaxial electrospinning with an in situ cross-linking process was carried out. As seen in Figure S2 of the Supporting Information, the coaxial electrospinning with collagen solution as sheath feed and PS solution as the core feed was carried out in GTA vapor (saturated vapor pressure of 25% GTA−water), so the collagen could be cross-linked during elongation and whipping of a single fiber in the air. This cross-linking before the fibers collected on the receiving plate could stabilize the single fiber and avoid melting among fibers. Fibers just collected on the receiving plate were still undergoing the cross-linking reaction with GTA vapor to further stabilize the sheath layer. With this method, nanofibers without melting with each other were obtained (Figure 2A) and showed higher special surface area, higher hydrophilicity (Figure S5A of the Supporting Information), and thus higher extraction efficiency (Figure S5B of the Supporting Information).

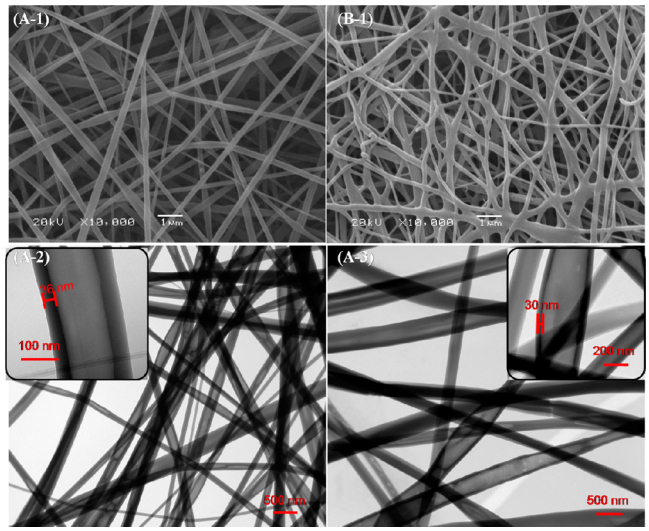

Figure 2. SEM images of PS/collagen core−sheath nanofibers with in situ cross-linking (A-1) and conventional cross-linking (B-1), TEM images of PS/collagen core−sheath nanofibers with in situ cross-linking before (A-2) and after (A-3) 1 day of water washing.

The core−sheath structure was demonstrated by the TEM images in Figure 2A-2. It can be seen that in almost every fiber there was dark region outside of the light region. It was known

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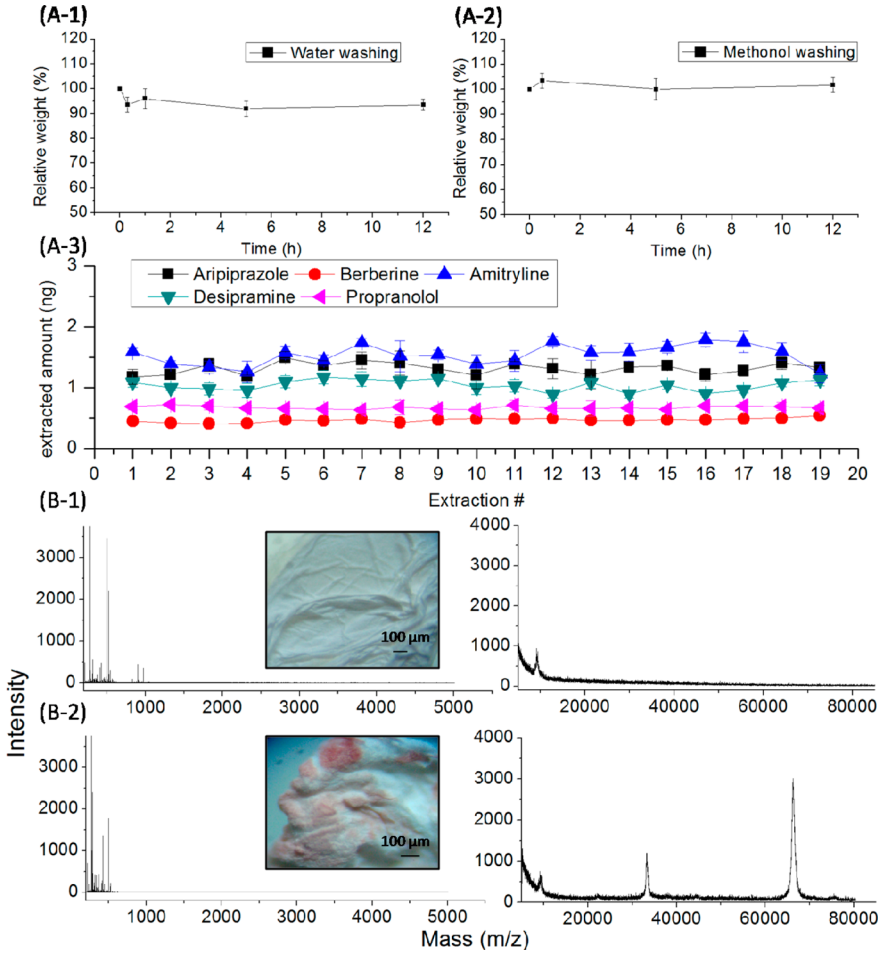

Figure 3. Weight loss of PS/collagen core–sheath nanofibers in water (A-1) and methanol (A-2) and its reusability for extraction of drugs from plasma (A-3) (n = 3). MALDI-TOF MS analysis of eluate from plasma (B) with (1) PS/collagen core–sheath nanofibers and (2) PS nanofibers treatment. (Insets are microscopic pictures of two nanofibers after exposure to whole blood.)

that the nitrogen element in collagen could enhance the TEM contrast because of its higher conductivity,[17] which confirmed that the dark region as the sheath of the fiber is rich in collagen.

Stability and Reusability of Core–Sheath Electrospun Fibers. The stability of the fibers in water is the most important property for in vivo application. The weight loss of the as-spun fibers in water and methanol was investigated as shown in Figure 3A. It can be seen that the weight loss was <10% in water and no weight loss in methanol after 12 h washing, so the dissolution of cross-linked collagen was negligible. The TEM of fibers before and after 1 day of water washing was shown in Figure 2A-3. It can be seen that the core–sheath structure was well-maintained after 1 day of water washing except for the slight increase of fiber diameter. In our following experiments, the fibers with 30 min water washing were used for extraction.

The stability of the fibers in real samples can be proven by the reusability of the fibers for extraction of five drugs from plasma, as shown in Figure 3A-3. It can be seen that the relative standard deviations (RSDs) were less than 16% for the 20 extraction–desorption cycles from pure plasma.

Biomatrix Resistance of the Core–Sheath Electrospun Fibers. Protein resistant ability of the material was investigated by MALDI-TOF analysis of the desorption solution of the core–sheath fibers packed solid phase extraction (SPE) column (100 mg fibers/3 mL column) after loaded with whole blood (method S4 of the Supporting Information and Figure 3B). The blood cell-repelling property was investigated by optical microscopic pictures of fibers after exposure to whole blood for 3 h (insets of Figure 3B). Though the collagen sheath can ensure the biocompatibility of the core–sheath fibers,[13a,15] the antifouling ability for proteins and blood cells of collagen were not reported previously. Actually, in some references it even had been pointed out that the modification of collagen on the biomaterial surface is proper to promote its affinity to cells, such as smooth muscle cells and neural stem cells.[18] However, in our study, no molecules larger than 1000 was observed under MALDI-TOF analysis for collagen/PS fibers (Figure 3B-1), while large amount of HSA (M_w = 66000) were observed for pure PS fibers (Figure 3B-2). Moreover, it can be seen that the blood cell attachment of the composite fibers was negligible, while that of PS fibers was obvious. This antifouling ability of both proteins and cells may be caused by the hydrophilicity of the fiber sheath and the microscale roughness of the fiber mat surface, which could form a strong hydration layer to resist the proteins adsorption and cell adhesion.[19]

Besides the large amount of proteins and cells in blood, other coextracted molecules from blood could also significantly bias free-concentration measurements through affecting the MS response by matrix-induced ionization suppression/enhancement.[20] Here it was evaluated by the relative response (RR) of matrix-matched standards to matrix-free standards after extraction (method S5[20] as shown in Figure S6 of the

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Supporting Information). RRs of all drugs were among 90%–120% for core–sheath fibers but varied from 50% to 130% for PS fibers.[11] Thus, the coextracted matrix with core–sheath fibers was negligible to affect the LC–MS/MS responses of target drugs. It may be attributed to the antifouling of the fiber sheath discussed above and the selectivity of the fiber to small polar drugs due to the hydrophilicity enhancement of the fiber by the collagen sheath.

Reproducibility of the Core–Sheath Electrospun Fibers. To investigate the reproducibility of fibers, fiber mats (2 mg) from one batch of electrospinning and among different batches of electrospinning were used for 2 min extraction of five commercial drugs from pure plasma. From Table 1, the RSDs of extraction results with fibers from one batch were from 1%–15%, and RSDs of average results from different batches were all below 13%. It means there was no significant difference between fibers from one batch and different batches.

Table 1. Relative Standard Deviations (RSDs) of Extraction Results with Membrane Inner Fiber Mat and Inter Fiber Mats

RSD (%)	Mat 1	Mat 2	Mat 3	intermats
aripiprazole	7	7	13	5
berberine	4	15	10	12
amitriptyline	7	3	2	4
desipramine	6	1	2	5
propranolol	6	2	5	13

Extraction Device and Strategy for In Vivo Analysis. *Microfluidic Device.* To introduce this core–sheath membrane into the in vivo analysis, the microfluidic device (Figure 1) was designed. Within this device, the extraction area (the membrane area exposed to the blood flow) was controlled precisely by the microchannel size. The flow rate of blood within the channel could be tuned precisely via the syringe. Thus, the repeatability of the in vivo extraction was dependent on the uniformity of the nanofiber membrane. In the pre-experiments, it was found that the membrane uniformity increased with increasing the tip-to-collector distance of electrospinning, but the Taylor cone was not stable if the distance was too large. The optimization result was 15 cm for a 5 × 4 cm alumina receive plate to form a membrane with uniform thickness. The uniformity was demonstrated by cutting the membrane into a 1 × 1 cm piece and then weighing them one-by-one (controlled in the range of 0.7–0.8 mg). The uniform 0.8 mg core–sheath fiber mats were used in all the following experiments.

Fast Extraction Using the Microfluidic Device. To simulate the in vivo blood circulation, an artificial circulatory system was established (Figure S3 of the Supporting Information), and three drugs (aripiprazole, desipramine, and propranolol) were used as the target analytes.

First, the time profiles of extraction using the microfluidic device with a push/pull flow rate of 1 mL/min were obtained (Figure 4A). It can be seen that the equilibration time (expressed as the time required to extract at least 95% of the maximum amount) for most of the drugs was around 2 min, and the time constant (a) of three drugs in this extraction system was summarized in Table 2. On the contrary, the equilibration time for pure PS fibers was all longer than 10 min (Figure S7 of the Supporting Information). The higher extraction rate of PS/collagen core–sheath fibers had been

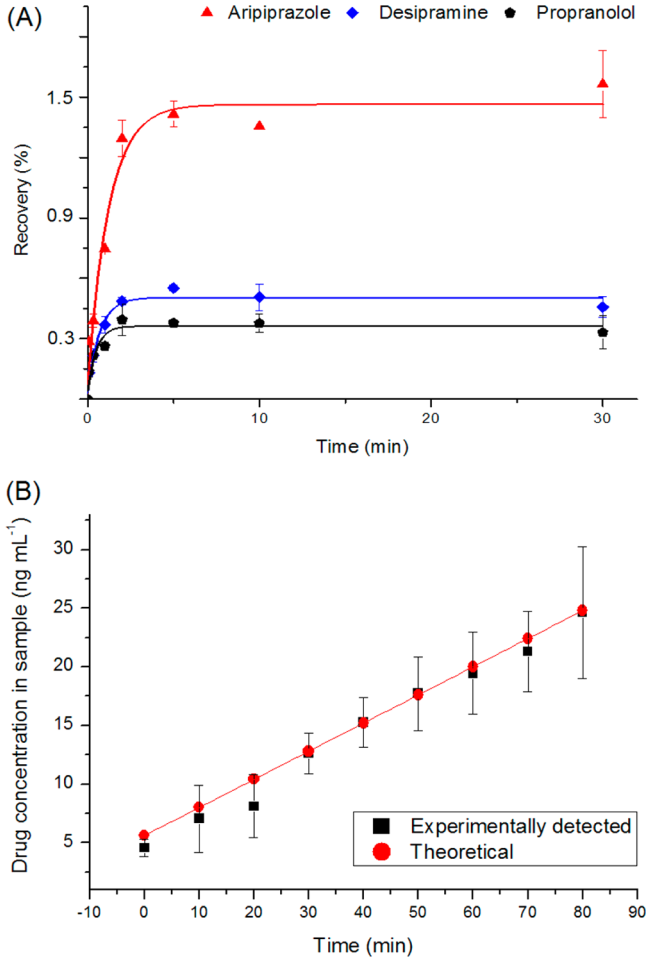

Figure 4. The time profiles of microextraction recovery with (A) PS/collagen core–sheath nanofibers and comparison of the detected and theoretical drug amount (aripiprazole) in (B) the linear dynamic sample system (n = 3).

demonstrated before[11] that nano hydrophilic sheath layer of electrospun fibers can greatly enhance the mass transfer rate of analytes between the bulk sample and solid extraction phase. We chose 2 min as the extraction time in the following study owing to the advantages of E-extraction.[11] Push/pull flow rate also affected the mass transfer kinetic and equilibrium time of extraction, and 1 mL/min of flow rate was chosen because it was well below the normal flow rate in the rabbit carotid artery and produced minimal disturbance.

With this extraction method, the calibration curves of three drugs in PBS and rabbit whole blood in the artificial circulatory system were obtained, respectively. The detection and quantification limit of method (LOD and LOQ), slope, linearity coefficient, RSD, and linear range of the calibration curves and distribution coefficients of free drugs between the fibers surface and the sample matrix (K_{fs}) are summarized in Table 2. The data showed that a very good linear relationship (r^2 > 0.99) was obtained for a seven- or eight-point calibration (n = 3), and the linear range covered 2 orders of magnitude for all the drugs both in the plasma and in the PBS buffer. Moreover, the LOD/LOQs (0.01–0.18 ng/mL) are low enough for free drug detection in conventional pharmacokinetic study. K_{fs} determined the extraction ability of fibers to analytes. From Table 2, the extraction mechanism could be demonstrated to be majorly the hydrophobic interaction of the

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Table 2. Analytical Performance of Extraction Method with Microfluidic Device in Artificial Circulatory System for Three Drugs

drugs		aripiprazole	desipramine	propranolol
standard sample	LOD (ng mL^{-1})[a]	0.047	0.013	0.053
	LOQ (ng mL^{-1})[a]	0.15	0.042	0.18
	linear range (ng mL^{-1})	0.5−50	0.5−50	0.5−50
	slope[b]	3.29×10^2	3.18×10^3	2.95×10^2
	R^2	0.9989	0.9992	0.9952
	RSD[c]	9%	11%	2%
	a (min^{-1})	0.81	1.43	2.00
	K_{fs}	493	239	127
whole blood	linear range[b] (ng mL^{-1})	2−200	2−200	2−200
	slope[b]	1.99×10^1	1.55×10^1	1.35
	R^2	0.9992	0.9933	0.9933
	RSD[c]	20%	7%	14%
B%[d]	whole blood	93.9%	99.4%	99.4%
	plasma[e]	76%	81%	87%
	literature value[f]		82%	63−74.9%

[a]LOD and LOQ are calculated as S/N = 3 and 10 at a concentration of 0.5 ng/mL. [b]The fitting expression is $y = ax + b$. X is the concentration of analytes in sample (ng mL^{-1}), and y is the peak area of analytes in sample after extraction. [c]Repeated three times. [d]%PPB is the drug plasma protein binding value. [e]These results were calculated by the ratio of calibration curve slopes in 1 mL of standard and plasma with static equilibrium extraction. [f]Ref 22.

PS core because K_{fs} increases with the increase of logP/D of analytes (Table S1 of the Supporting Information).

Quantitative Calibration Method for In Vivo Analysis. E-extraction could avoid the detection deflection from the variation of mass transfer kinetics between different sample matrixes. For in vivo extraction, if K_{fs} was obtained and the sample volume was large enough to neglect the sample depletion during extraction, the free concentration of drugs could be calculated by eq 1,[9b]

$$C_s = \frac{n_e}{K_{fs}V_f} \quad (1)$$

where C_s is the concentration of the analyte in the sample matrix, V_f is the volume of extractive material, and n_e is the amount of analyte in the extraction phase at equilibrium.

Equation 1 shows that the extraction results at equilibrium are independent of the volume or the flow condition of the sample and could be determined just with the K_{fs} and n_e values. In addition, the antifouling ability of material ensured that its extraction behavior was unaffected by the different biomatrix, so K_{fs} of free drugs in PBS could be directly used as that in the sample matrix for calculation of free concentration of drugs in whole blood sample by eq 1, which largely facilitated the calibration of in vivo extraction. Moreover, with the determination of free concentrations and the protein binding percent of drugs in whole blood (% B), the total concentration of drugs could also be calculated directly.

From the slope of the calibration curves in the PBS samples in Table 2, the K_{fs} value of the three drugs could be obtained. From a comparison of the slopes of calibration curves in the PBS sample and in the whole blood sample, %B could be calculated (Table 2). The data in Table 2 showed that the %B was higher than the plasma protein binding percent reported.[11] It may be caused by the additional absorption of the drugs by blood cells which was also reported in other literature.[9b]

Validation of the Analytical Method in Dynamic System. The extraction and calibration method discussed above was first validated in a simulated dynamic sample system, which was established based on the artificial circulatory system. From the previous studies,[6,11] the mass-uptake model of equilibrium microextraction in a dynamic system can be deduced as eq 2.

$$n = C_s V_f K_{fs} + (bt - b/a)V_f K_{fs} \quad (2)$$

where b is the concentration change rate in the sample.

To determine the real-time concentration, the second term in eq 2 should be insignificant (less than 10% of the first term). From the (a) equilibration time (2 min) and time constants in our system summarized in Table 2, the temporal resolution of this extraction method was only suitable for the dynamic system with b smaller than $0.065C_s$ (min^{-1}). Thus, the simulated dynamic system was set at a 5.6 ng/mL initial concentration of three drugs, C_0, with a positive concentration change rate of 0.24 ng mL^{-1}/min. Drug concentration in the dynamic system could be calculated by eq 1 and the K_{fs} value as mentioned above. The monitored concentration in the dynamic system versus time is shown in Figure 4B and Figure S8 of the Supporting Information and was compared with the theoretical concentration. It can be seen that the experimental results had a good agreement with the theoretical data, and a correlation coefficient of 0.97−0.99 was obtained.

Application to In Vivo Pharmacokinetic Study of Rabbit. For in vivo microextraction experiments, desipramine pharmacokinetics in rabbits was studied in vivo to evaluate the performance of our method. Desipramine is frequently used in animal studies of antidepressant action and is useful therapeutically.[21] With the 2 min E-extraction method in vivo, the pharmacokinetic profiles of desipramine were monitored over 6 h. The results were calculated by eq 1 and the parameters summarized in Table 2. Because the fibers were able to resist protein and protein-bound drugs, only the free drugs could be extracted.[11] Thus, the amount of analyte extracted is inherently related to the free concentration, and measurements of the free concentration were reliable. The values for the in vivo total concentration were calculated if assuming that the blood in the simulated system used for calibration has the same binding properties as the in vivo blood, which is usually the case for healthy individuals. For comparison, the results of in vitro blood analysis for five time points (30, 50, 140, 200, 240 min) are presented in Figure 5. All of the in vivo results were

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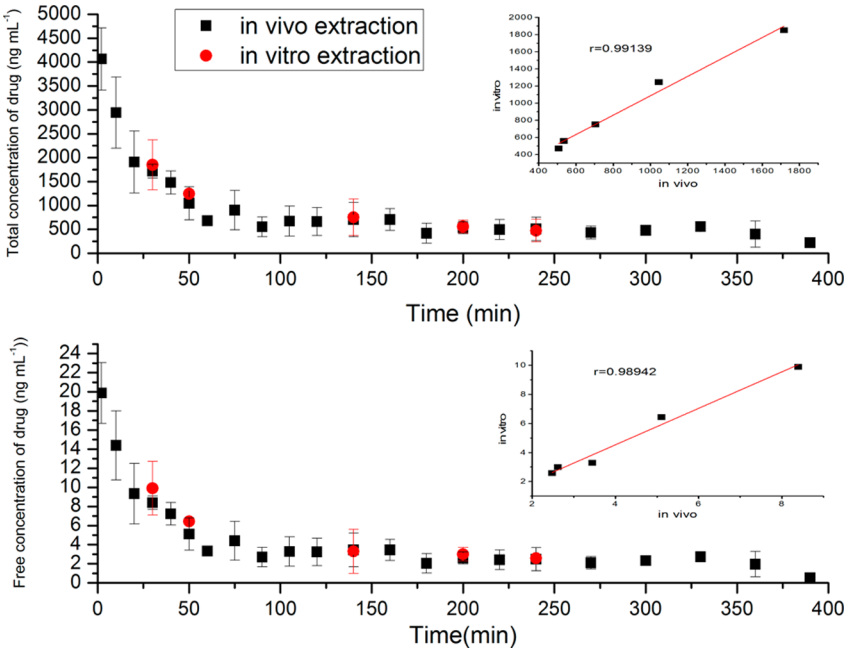

Figure 5. Averaged pharmacokinetic profiles of desipramine monitored by in vivo extraction over 6 h on three rabbits (n = 3). The insets show the correlation of analysis results with in vivo extraction and in vitro extraction.

consistent with the results from the in vitro assay, and a correlation coefficient of around 0.99 was obtained. However, the in vivo method obtained more advantages, such as short sampling interval, almost no consuming of the rabbit blood, and no need for cold preservation of blood samples for metabolism quenching. From the pharmacokinetic profiles it can be seen that the metabolism rate in rabbits was rapid, and it only took around 1 h to metabolize 90% of the drugs being tested.

CONCLUSION

An in vivo extraction method with a PS/collagen core−sheath nanofiber membrane and microfluidic extraction device was developed. The nanofibers with collagen sheath have all the advantages of previously proposed core−sheath material, including biocompatibility, good matrix (proteins and cells) resistance, and high hydrophilicity of surface which resulted in a high mass transfer flux of analytes (2 min equilibrium time). Moreover, with in situ cross-linking during the electrospinning process, the stability and mechanical strength of the sheath were largely enhanced without sacrifice of its nanostructure and surface area of the core−sheath membrane, which then made the in vivo extraction realizable. With the microfluidic device sandwiching the core−sheath membrane and connecting it to the living system, the in vivo E-extraction method was used to measure the pharmacokinetic profiles of desipramine in rabbits, and the free and total concentration could be simultaneously monitored with a short interval and almost without the rabbit blood consumed.

In addition, compared with other biocompatible extractive material, this biocompatible electrospun nanofiber membrane could offer both high temporal resolution of in vivo E-extraction and large extraction capacity. These merits will be of great benefit for in vivo application to the future discovery of more trace and variant metabolites.

ASSOCIATED CONTENT

Supporting Information

Methods S1−S5, Figures S1−S8, Tables S1 and S2). This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: guan_yafeng@yahoo.com.cn
*E-mail: dpwu@dicp.ac.cn

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) (a) Ouyang, G.; Vuckovic, D.; Pawliszyn, J. *Chem. Rev.* **2011**, *111* (4), 2784−2814. (b) Vuckovic, D.; de Lannoy, I.; Gien, B.; Shirey, R. E.; Sidisky, L. M.; Dutta, S.; Pawliszyn, J. *Angew. Chem., Int. Ed.* **2011**, *50* (23), 5344−5348.

(2) (a) Shou, M.; Ferrario, C. R.; Schultz, K. N.; Robinson, T. E.; Kennedy, R. T. *Anal. Chem.* **2006**, *78* (19), 6717−6725. (b) Watson, C. J.; Venton, B. J.; Kennedy, R. T. *Anal. Chem.* **2006**, *78* (5), 1391−1399.

(3) (a) Hu, Y.; Mitchell, K. M.; Albahadily, F. N.; Michaelis, E. K.; Wilson, G. S. *Brain Res.* **1994**, *659* (1−2), 117−125. (b) Cui, J.; Kulagina, N. V.; Michael, A. C. *J. Neurosci. Methods* **2001**, *104* (2), 183−189. (c) Brown, F. O.; Lowry, J. P. *Analyst* **2003**, *128* (6), 700.

(4) (a) Cellar, N. A.; Burns, S. T.; Meiners, J. C.; Chen, H.; Kennedy, R. T. *Anal. Chem.* **2005**, *77* (21), 7067−7073. (b) Li, T.; Barnett, A.; Xiao, D. Q.; Zhong, M.; Gianchandani, Y. B. *An in Vivo Blood Microsampling Device for Pharmacokinetic Applications*; IEEE: New York, 2007; p U116−U117. (c) Wu, H. M.; Sui, G.; Lee, C. C.; Prins, M. L.; Ladno, W.; Lin, H. D.; Yu, A. S.; Phelps, M. E.; Huang, S. C. *J. Nucl. Med.* **2007**, *48* (5), 837−845.

(5) Vuckovic, D.; Zhang, X.; Cudjoe, E.; Pawliszyn, J. *J. Chromatogr., A* **2010**, *1217* (25), 4041−4060.
(6) Zhang, X.; Oakes, K. D.; Luong, D.; Wen, J. Z.; Metcalfe, C. D.; Pawliszyn, J.; Servos, M. R. *Anal. Chem.* **2010**, *82* (22), 9492−9499.
(7) (a) Ai, J. *Anal. Chem.* **1997**, *69* (6), 1230−1236. (b) Zhang, X.; Oakes, K. D.; Luong, D.; Metcalfe, C. D.; Pawliszyn, J.; Servos, M. R. *Anal. Chem.* **2011**, *83* (6), 2371−2377.
(8) Zhou, S. N.; Zhao, W.; Pawliszyn, J. *Anal. Chem.* **2008**, *80* (2), 481−490.
(9) (a) Zhang, X.; Oakes, K. D.; Hoque, M. E.; Luong, D.; Metcalfe, C. D.; Pawliszyn, J.; Servos, M. R. *Anal. Chem.* **2011**, *83* (9), 3365−3370. (b) Zhang, X.; Es-haghi, A.; Musteata, F. M.; Ouyang, G.; Pawliszyn, J. *Anal. Chem.* **2007**, *79* (12), 4507−4513.
(10) Musteata, M. L.; Musteata, F. M.; Pawliszyn, J. *Anal. Chem.* **2007**, *79* (18), 6903−6911.
(11) Wu, Q.; Wu, D.; Guan, Y. *Anal. Chem.* **2013**, *85* (12), 5924−5932.
(12) (a) Muthiah, P.; Hoppe, S. M.; Boyle, T. J.; Sigmund, W. *Macromol. Rapid Commun.* **2011**, *32* (21), 1716−1721. (b) Meng, L.; Arnoult, O.; Smith, M.; Wnek, G. E. *J. Mater. Chem.* **2012**, *22* (37), 19412. (c) Chen, Z. G.; Wang, P. W.; Wei, B.; Mo, X. M.; Cui, F. Z. *Acta Biomater.* **2010**, *6* (2), 372−382.
(13) (a) Chen, D. W.; Hsu, Y. H.; Liao, J. Y.; Liu, S. J.; Chen, J. K.; Ueng, S. W. N. *Int. J. Pharm.* **2012**, *430* (1−2), 335−341. (b) Prabu, P.; Dharmaraj, N.; Aryal, S.; Lee, B. M.; Ramesh, V.; Kim, H. Y. *J. Biomed. Mater. Res., Part A* **2006**, *79A* (1), 153−158. (c) Gomathi, K.; Gopinath, D.; Ahmed, M. R.; Jayakumar, R. *Biomaterials* **2003**, *24* (16), 2767−2772. (d) Matthews, J. A.; Wnek, G. E.; Simpson, D. G.; Bowlin, G. L. *Biomacromolecules* **2002**, *3* (2), 232−238.
(14) (a) Li, D.; Xia, Y. N. *Adv. Mater.* **2004**, *16* (14), 1151−1170. (b) McCann, J. T.; Li, D.; Xia, Y. *J. Mater. Chem.* **2005**, *15* (7), 735.
(15) Yeung, J. C. Y.; Vuckovic, D.; Pawliszyn, J. *Anal. Chim. Acta* **2010**, *665* (2), 160−166.
(16) Chen, R.; Huang, C.; Ke, Q.; He, C.; Wang, H.; Mo, X. *Colloids Surf., B* **2010**, *79* (2), 315−325.
(17) Zhang, Y.; Huang, Z. M.; Xu, X.; Lim, C. T.; Ramakrishna, S. *Chem. Mater.* **2004**, *16* (18), 3406−3409.
(18) (a) Fujie, T.; Furutate, S.; Niwa, D.; Takeoka, S. *Soft Matter* **2010**, *6* (19), 4672. (b) Kuo, Y. C.; Yeh, C. F. *Colloids Surf., B* **2011**, *82* (2), 624−631.
(19) (a) Svetushkina, E.; Puretskiy, N.; Ionov, L.; Stamm, M.; Synytska, A. *Soft Matter* **2011**, *7* (12), 5691. (b) Yu, Q.; Li, X.; Zhang, Y.; Yuan, L.; Zhao, T.; Chen, H. *RSC Adv.* **2011**, *1* (2), 262.
(20) Zhang, X.; Oakes, K. D.; Luong, D.; Metcalfe, C. D.; Servos, M. R. *Anal. Chem.* **2011**, *83* (17), 6532−6538.
(21) Deupree, J. D.; Montgomery, M. D.; Bylund, D. B. *Eur. J. Pharmacol.* **2007**, *576* (1−3), 55−60.
(22) (a) Benet, L. Z.; Oie, S.; Schwartz, J. B., *Pharmacological Basis of Therapeutics*; McGraw Hill: New York, 1996; (b) Musteata, F. M.; Pawliszyn, J.; Qian, M. G.; Wu, J. T.; Miwa, G. T. *J. Pharm. Sci.* **2006**, *95* (8), 1712−1722.