LED-induced In-column Molecularly Imprinting for Solid Phase Extraction/Capillary Electrophoresis

Xinfeng Zhang[a, b], Shuxia Xu[a, b], Yong-Ill Lee[b,c *], Steven A. Soper[c]

[a] *College of material and chemistry & chemical engineering*

Chengdu University of Technology, Chengdu 610059, China;

[b]*Anastro Laboratory, Department of Chemistry, Changwon National University,*

Changwon 641-773, The Republic of Korea

[c]*Department of Biomedical Engineering, School of Medicine, University of North*

Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

Content

* Corresponding author: Department of Chemistry, Changwon National University, Changwon 641-773, The Republic of Korea. Tel.: +82 55 213 3436; fax: +82 55 213 3439. E-mail: yilee@changwon.ac.kr (Y.-I. Lee).

1. Experimental details

1.1 Chemicals. All chemical reagents were of analytical grade. Epitestosterone, methyltestosterone, testosterone acetate, bisphenol A (BPA) and Phe-Phe peptide standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). The stock solutions (1 mg mL^{-1}) of analytes, epitestosterone, methyltestosterone, testosterone acetate, BPA, and Phe-Phe peptide, were prepared in methanol and kept at 4 ^{o}C. A mixture of 10 mM borate and 50 mM cholate solution at pH 9.20 was used as a running buffer. NaOH and HCl solutions (both 1 M) were utilized to adjust pH to the desired value. Ethylene dimethacrylate (EDMA), methacrylic acid (MAA), and azo(bis)-isobutyronitrile (AIBN) were also obtained from Sigma-Aldrich. Acetonitrile (ACN) and methanol were purchased from Daejung Chemical & Metals Co. (Gyeonggi-do, South Korea). Purified water prepared by a Milli-Q water purification system (Daihan Labtech Co., Gyeonggi-do, South Korea) was used for all experiments.

1.2 Instrumentation. All of the experiments were conducted using a self-assembled CE instrument, which consisted of a high power supply system (CZEPN10, High voltage Electronic Co., USA), an UV detector (Sappire 800 CE, Ecom Ltd., USA), and a Labview data collection system (Labview 7.1, National instruments, USA). An uncoated 100 μm silica capillary (Hebei Reafine Chromatography LTD., Hebei, China) with a total length of 56 cm and an effective length of 51 cm was used for the separation of the analytes. The detection wavelength was set at 254 nm (for methyltestosterone and BPA) or 210 nm (for Phe-Phe).

1.3 Procedure of On-line Preparation of MISPE Concentrator. A 3 mm irradiation window was made by burning the inlet end of a capillary as illustrated in Figure 1. The inner wall of the capillary (i.d. 100 μm, Hebei Reafine Chromatography LTD., Hebei, China) was silylated to enable covalent attachment of the polymer by the following procedure: (1) The capillary was washed with 0.1 M NaOH and HCl in sequence, and then flushed with water and then acetone for 5 min; (2) The capillary was dried with nitrogen gas; (3) The capillary was filled with about a 1.5 cm length of 30% of 3-(trimethoxysilyl)propyl methacrylate in acetone and then sealed with two pieces of rubber followed by reaction at room temperature for 1 h; and (4) The silylated capillary was then washed with acetone and dried using a stream of nitrogen. It should be noted that the silylation length of the capillary should be as short as possible to minimize the obstruction of electroosmotic flow.

After completing the silylation process, the capillary was filled with the polymerization solution for an approximate 5 cm length from the inlet. The polymerization solution contained the templating molecule (Methyltestosterone/bisphenol/Phe-Phe, 0.05 mmol), the monomer (MAA, 0.2 mmol), and cross-linker (EDMA, 1 mmol) in 3 mL ACN (methanol for Phe-Phe), which was degassed with a gentle stream of nitrogen for 5 min prior to capillary filling. Both capillary ends were closed with two small pieces of rubber. Then, a 4.2 mW UV LED (360-370 nm, T5F, Seoul Optodevice, Korea) was placed in front of the window with the capillary irradiated for 20 min. Subsequently, the unpolymerized solution was flushed out

with nitrogen gas, and the capillary was closed again with two small pieces of rubber for further polymerization under UV LED irradiation for 1 h. After the reaction, the capillary was carefully rinsed with methanol for 10 min to remove the template.

1.4 SPE and CE Procedure. Before SPE, the capillary was preconditioned with 0.1 M NaOH for 1 min and then with deionized water for 1 min. The sample solution was flowed through the MIP concentrator for 15 min at a flow rate of 1.02 μL min^{-1} to extract the target analytes. Then, the running buffer was used for washing the MIP concentrator for 30 s. Subsequently, a plug of eluent was injected by raising the inlet end of the capillary 7 cm higher than the outlet end for 10 s (about 20 nL). After injection, the CE system was waited for 20 s to desorpt the analytes. Then, A 12 kv (18 kv for Phe-Phe) voltage was applied for CE separation. Prior to the next run, the capillary was washed and reconditioned with 0.1 M NaOH, water, methanol, and water for 30 s in sequence.

1.5 Sample Preparation. A human urine sample was donated by a drug-free and healthy volunteer, and stored at 4 ^{o}C in a refrigerator. A 2-mL volume of spiked human urine sample was adjusted to neutral pH by adding 200 μL of 300 mM pH 7.0 phosphate buffer. The sample solution was centrifuged at 6000 rpm for 10 min to remove any particulates. The supernatant was filtered through a 0.45 μm membrane filer and used for analysis.

2. Effects of parameters on in-column MISPE

2.1 Effect of porogen volume and prepolymerziation time

Both porogen volume and preplolymerization time are important parameters that affect the thickness of the MIP layer. Using a 20 min preplolymerization time, the capillary was found to be blocked and yielded high backpressure when 0.75 mL or 1.5 mL of ACN was used as the porogen reagent. Using 3 mL of porogen, the unpolymerized solution could easily be flushed out with N_2 gas, resulting in a thin layer of coated MIP. The thin layer of MIP led to a low back pressure during CE process. However, increasing the porogen volume led to a sparse MIP network and also less active sites. The prepolymerization time was investigated from 0 to 40 min. The longer the prepolymerization time, the thicker MIP layer became (data not shown), which can help to improve the MISPE capacity. However, the thicker MIP layer made the analyte difficult to diffuse from the MIP to the CE running solution and therefore larger band broadening, which was noted by the electropherograms of methyltestosterone in Figure S3. A 20 min prepolymerization time was considered to be an optimum because of little sacrifice in separation efficiency and low backpressure problem, but still with high extraction efficiency.

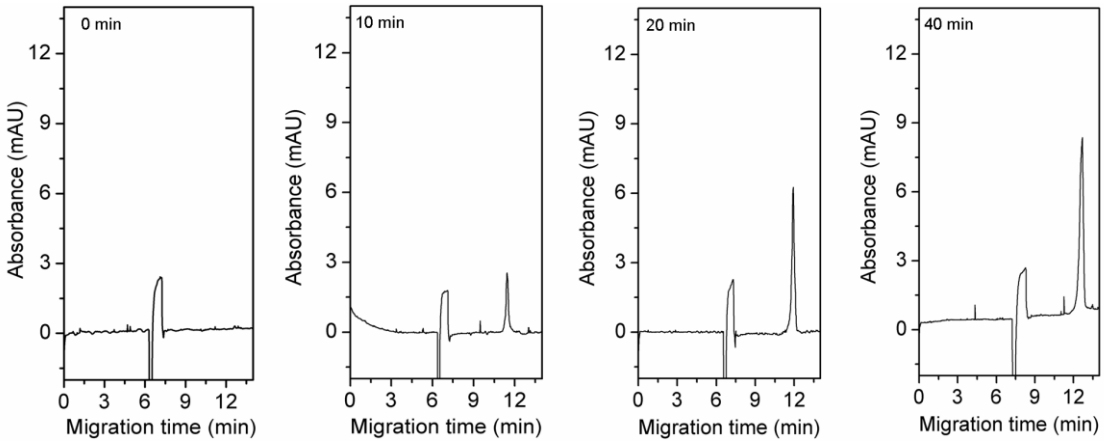

Figure S2. Effect of prepolymerziation time on extraction efficiency and CE separation. Other extraction and CE condition was the same as Figure S1

2.2 Effect of Desorption solvent

The desorption solvent can affect not only the elution efficiency, but also the peak shape during the electrophoresis phase of the assay. Initially, we used methanol or ACN as the desorption solvent. However, distorted peak shapes were found for both solvents, but higher CE signal was obtained with ACN. The addition of a running buffer to ACN was helpful for improving the peak shape, which revealed that the distorted peak shapes were due to the different electrophoretic mobilities in the desorption solvent zone and running buffer zone. Figure S4 showed that the peak shape becomes sharpened as increasing the percentage of running buffer in desorption solvent, the low percentage of ACN in the solvent can also decrease the desorption efficiency of analytes. A lower and tailing peak was found when using 1:3 ratio of ACN and running buffer. Thus, the mixture of ACN and running buffer (1:2) was chosen for efficient separation and desorption of analyte, methyltestosterone.

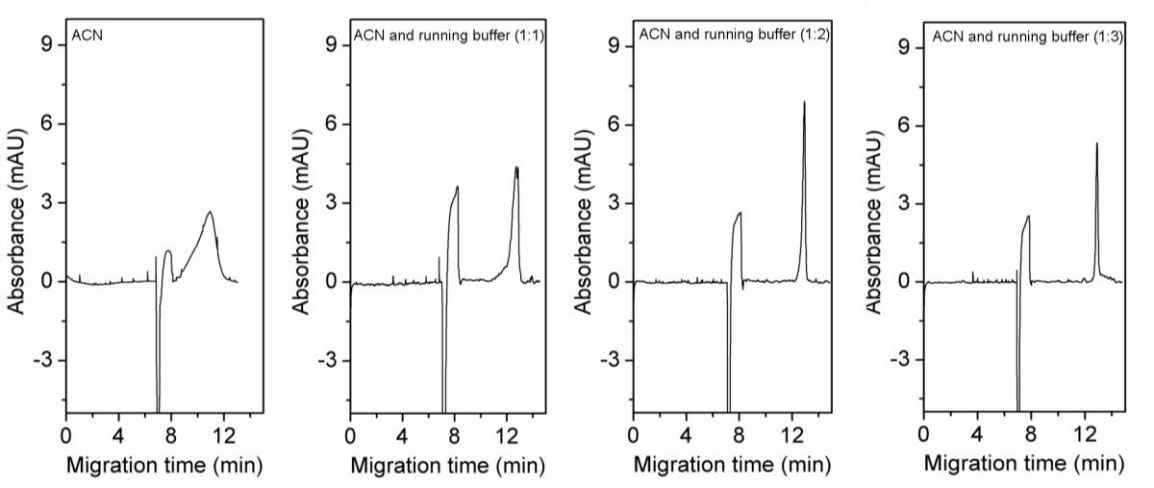

Figure S3. Effect of eluents on desorption efficiency and CE separation. Other extraction and CE condition was the same as Figure S1.

2.3 Effect of elution plug length

The length of elution plug is also important factor affecting desorption. The electropherograms were evaluated by increasing the injection time of eluent from 5s to 20s as shown in Figure S5. It was revealed that an unsatisfactory peak with a tailing and low intensity was observed when a short eluent plug of 5 s injection was applied. The longer injection time, the broader peak we obtained. The optimum time for eluent injection was found to be 10s, and the injected plug of eluent about 3 mm long (20 nL).

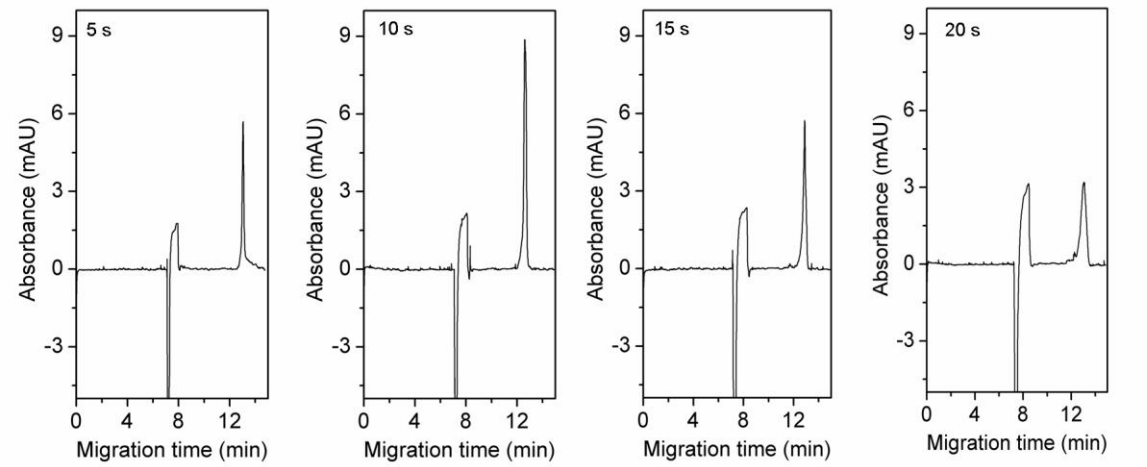

Figure S4. Effect of injection time of eluent on desorption efficiency and CE separation. Other extraction and CE condition was the same as Figure S1

Desorption efficiency

To estimate desorption efficiency, the desorption process and CE running was repeated continuously until no CE signal was found, which indicate the total desorption of analytes. After extraction of 100 ng mL^{-1} of methyltosterence, we found the total desorption .of analtes can be achieved with three times repeated desorption process. Based on the following equation, the desorption efficiency (E_d) can be calculated to be 78. 3%.

$$E_d = \frac{A_1}{A_1 + A_2 + A_3} \times 100\%$$

Where A_1, A_2 and A_3 stands for the CE peak areas of the 1st, 2nd and 3rd desorped analytes, respectively.

3. Stability of in-column MISPE concentrator

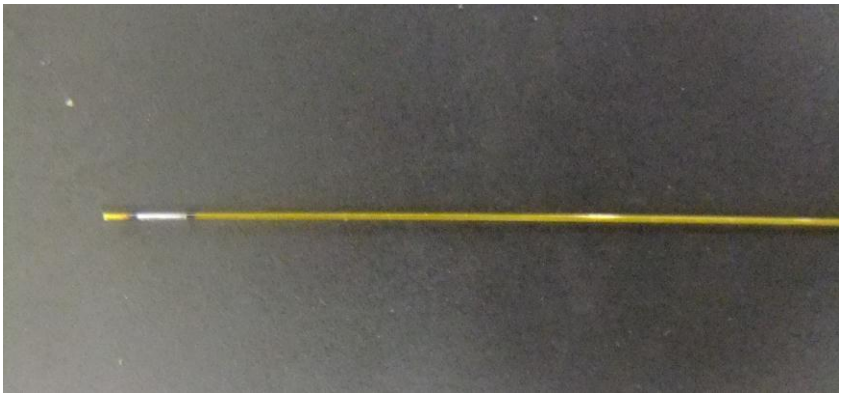

Figure S4. The picture of in-column MISPE concentrator after 100 times use

4. Extraction of BPA and Phe-Phe using in-column MISPE

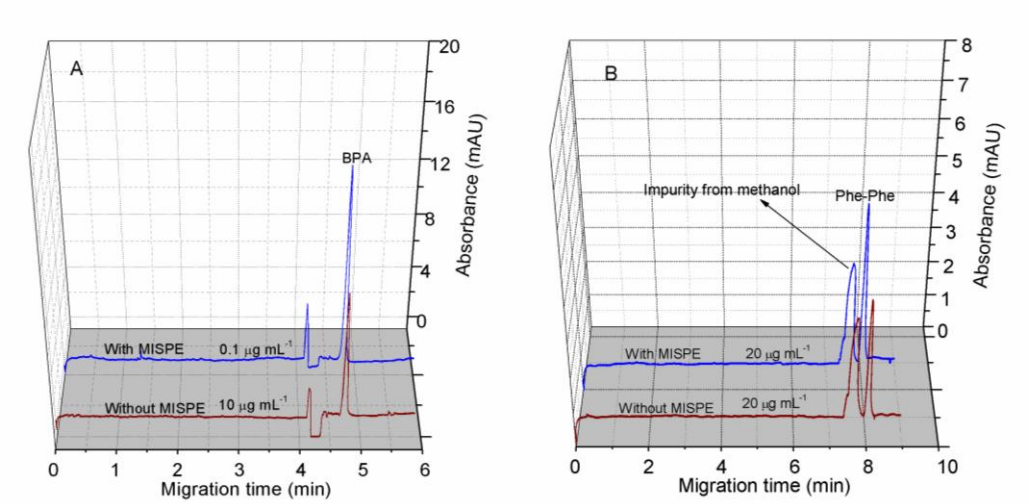

Figure S5. Electropherograms of BPA (A) and Phe-Phe (B) with MISPE or without MISPE. Conditions for determination of BPA: running buffer, 25 mM pH 9.20 borate buffer; separation voltage, 12 kV; eluent, ACN; Conditions for determination of Phe-Phe: running buffer, 20 mM pH 4.30 phosphate buffer; separation voltage, 15 kV; eluent, methanol.