Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Investigation of metformin hydrochloride–bovine serum albumin interaction by narrow-bore capillary zone electrophoresis

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Experimental Section

Reagents and materials: Tris(hydroxymethyl)aminomethane (Tris) was purchased from Sangon Biotech (Shanghai, China). Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from Beijing Chemical Works (Beijing, China). MET and BSA were purchased from Solarbio (Beijing, China) and Sigma-Aldrich (Shanghai, China), respectively. Dimethylsulfoxide (DMSO) was obtained from Aladdin (Shanghai, China). Fused silica capillaries with outer and inner diameters of 360 and 2 μm, respectively, were specially produced by Polymicro Technologies (USA). Fused silica capillaries with outer and inner diameters of 360 and 50 μm, respectively, were produced by Yongnian Ruifeng Chromatographic Devices (Hebei, China). The pH meter was purchased from Sartorius (model PB-10, Germany), whereas the water-circulation multifunction vacuum pump was obtained from Beijing Shentai Weiye Instrument and Equipment Co., Ltd. (Beijing, China). Zeta potential analyzer and the high-voltage DC power supply and capillary UV detector were purchased from NanoBrook (USA), Tianjin Dongwen High Voltage Power Supply Co. (DW-P303-1ACF0, Tianjin, China)， and Ecom Co. (Sapphire 800CE, Czech Republic), respectively. The ultrapure water used in the experiment was provided by a GenPure UV/UF system (USA). All solutions used in the experiments were filtered through a 0.22 μm filter membrane (Derian Instrument, Shanghai, China).

Instrumentation: The narrow-bore capillary zone electrophoresis/UV detector device mainly consisted of the drive, sample, separation, and detection modules. The drive module used a high-voltage DC power supply to move the sample forward. The sample module also used a high-voltage DC power supply to control the injection; different injection volumes could be achieved by changing the voltage value and injection time. The separation module was a bare capillary column with a length of 30 cm, an outer diameter of 360 μm, and an inner diameter of 2 μm. The detection window was set 10 cm away from the end of the capillary and prepared by scraping the polyimide coating on the capillary wall with a blade, generally ~ 2 mm. A commercial capillary UV detector was used as a detection module. The detector receives the UV spectrum generated by the sample and stores it on a computer in real-time through a data acquisition card.

Preparation of running buffer and samples: Tris-HCl (100 mmol L^{-1}, pH = 7.4) was used as the running buffer. The buffer solution was sterilized at high temperature and stored in a refrigerator at −4 °C for later use. MET solutions of different concentrations (2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 mmol L^{-1}) were prepared and dissolved in the running buffer. MET solutions with concentration gradients of 2, 4, 6, 8, and 10 mmol

L^{-1} were used to draw the MET standard curve. BSA solutions with mass concentrations of 100, 8, and 4 mg mL^{-1} were prepared and dissolved in the running buffer. Nine MET solutions with different concentrations (20, 18, 16, 14, 12, 10, 8, 6, 4 mmol L^{-1}) and BSA (100 mg mL^{-1}) were incubated in a 1:1 volume ratio at room temperature for 30 min. All solutions were degassed by the water-circulation multifunction vacuum pump.

Capillary zone electrophoresis

Capillary activation: one end of the capillary was inserted into a pressure chamber containing 20 µL 1 mol L^{-1} NaOH. The other end of the capillary was inserted into a 200 µL centrifuge tube filled with ultrapure water. NaOH was injected into the capillary at 6894.76 kPa by a gas pressure-driven device (N_2 was used in the experiment) for 2 h. After that, NaOH was replaced with 20 µL ultrapure water and washed for 1 h under the same pressure. To further rinse the residual NaOH on the tube wall, 20 µL of 1 mol L^{-1} HCl was injected into the capillary under the same pressure for 30 min. Then, HCl was rinsed with 20 µL ultrapure water under the same pressure for 1 h. The ultrapure water was replaced with 20 µL acetonitrile, and the capillary was flushed under the same pressure for 30 min. Finally, the capillary was dried overnight with nitrogen. All operations were performed at room temperature.

Monitoring of EOF migration direction: Dimethylsulfoxide (DMSO) was used as a neutral marker for EOF monitoring. The injection voltage and time of DMSO were 8 kV and 10 s, respectively. The running buffer and voltage were 100 mmol L^{-1} Tris-HCl (pH =7.4) and 12 kV, respectively. And the detection wavelength was 200 nm.

Detection of different concentrations of MET: a capillary with a detection window was placed in the narrow-bore capillary electrophoresis/UV detector device. The capillary was filled with running buffer by a gas pressure-driven device. The two ends of the capillary and the anode and cathode of the high-voltage DC power supply were inserted into a vial containing 20 µL of running buffer. The height of the capillary inserted into the running buffer should be the same on both ends. The baseline was stabilized at 12 kV for 20 min. After the signal became stable, MET solutions of different concentrations (2, 4, 6, 8, and 10 mmol L^{-1}) were used as samples, and a small-plug sample was injected into the capillary. The injection voltage and time were 8 kV and 10 s, respectively. Three parallel experiments were performed for each concentration of MET. After the sampling was completed, the capillary was replaced with the running buffer, which was injected at 12 kV.

Analysis of MET–BSA interaction: MET (4 mmol L^{-1}), BSA (4 mg mL^{-1}), and a mixed MET (8 mmol L^{-1})-BSA (8 mg mL^{-1}) solution incubated for 30 min in a volume ratio of 1:1 were separately injected as samples. The voltage and time used in the sampling procedure were 8 kV and 10 s, respectively. The total length of the separation column was 30 cm (20 cm effective) and the ID was 2 μm and 50 μm. The separation conditions were 100 mmol L^{-1} Tris-HCl (pH =7.4) at 12 kV. The detection wavelength was 200 nm.

Zone electrophoresis was used to separate MET, BSA, and MET-BSA. Under the same sampling procedure and separation conditions, the mixed samples with different concentrations of MET and 100 mg mL^{-1} BSA were separated. The MET–BSA binding parameters were obtained by investigating the binding of different concentrations of MET and BSA.

Supplementary figures

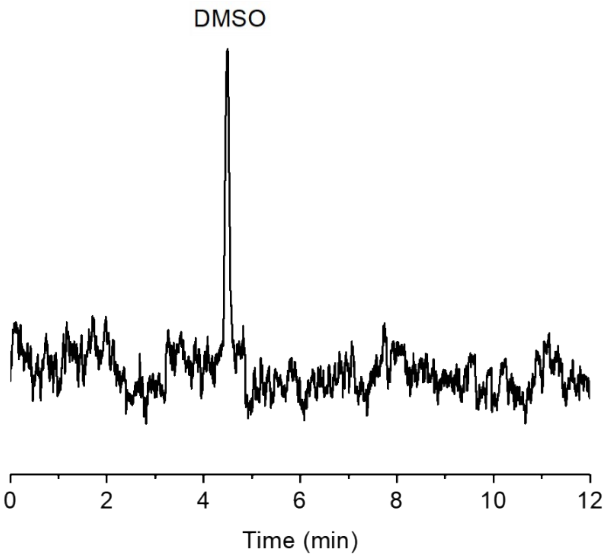


Figure S1 Monitoring of EOF migration direction. Sampling conditions: DMSO for 10 s at 8 kV; separation column: total length = 30 cm (20 cm effective) and ID= 2 μm; separation conditions: 100 mmol L-1 Tris-HCl (pH = 7.4) injected at 12 kV; detection wavelength: 208 nm.

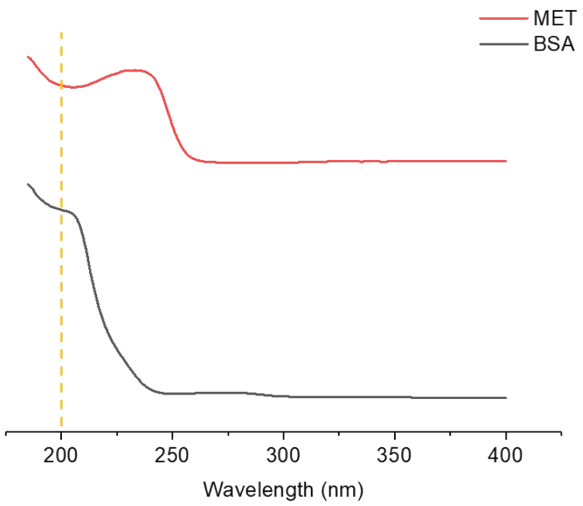


Figure S2 The UV absorption spectra of MET and BSA

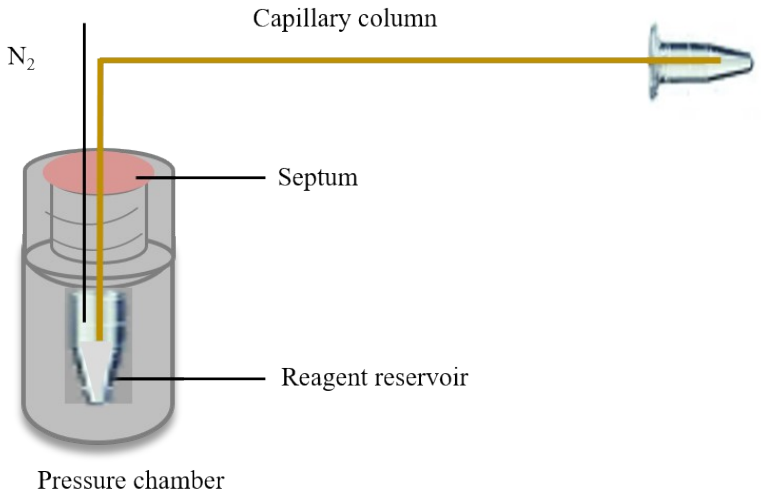


Figure S3 Schematic illustration of pressure-driving device.

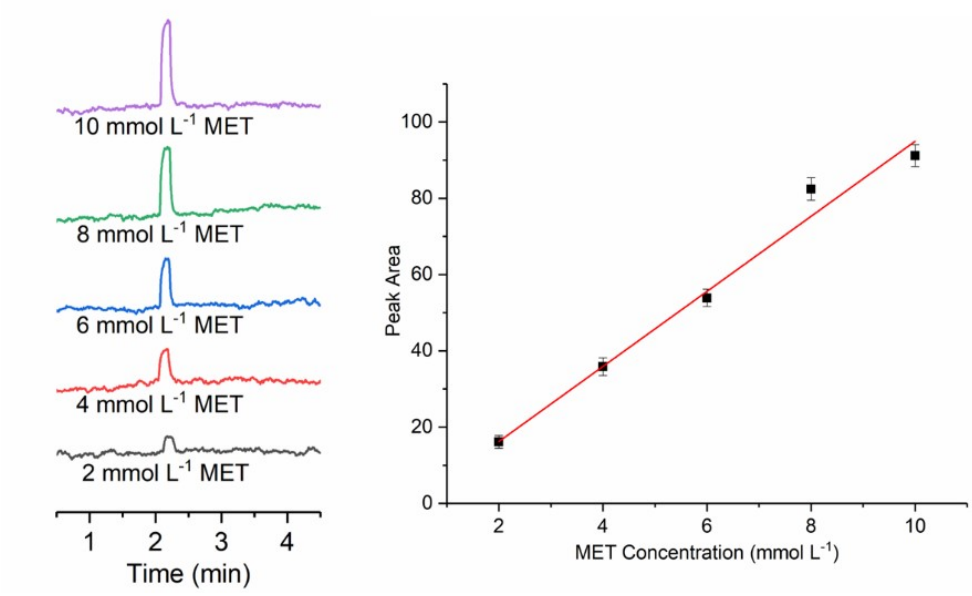


Figure S4 Electrophoresis diagram of MET solutions of different concentrations (A) and standard curve of MET solution (B). Sampling conditions: 2, 4, 6, 8, and 10 mmol L^{-1} MET solution were injected for 10 s at 8 kV; separation column: total length = 30 cm (20 cm effective) and ID= 2 μm; separation conditions: 100 mmol L^{-1} Tris-HCl (pH = 7.4) injected at 12 kV; detection wavelength: 200 nm.

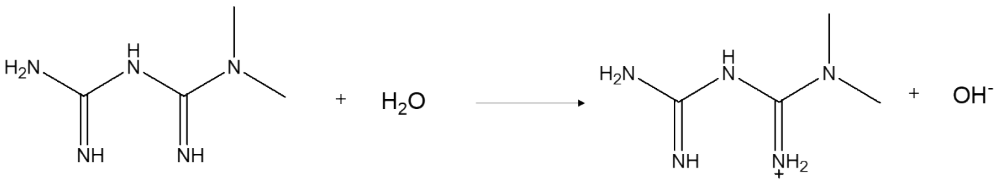


Figure S5 Hydrolysis equation of MET

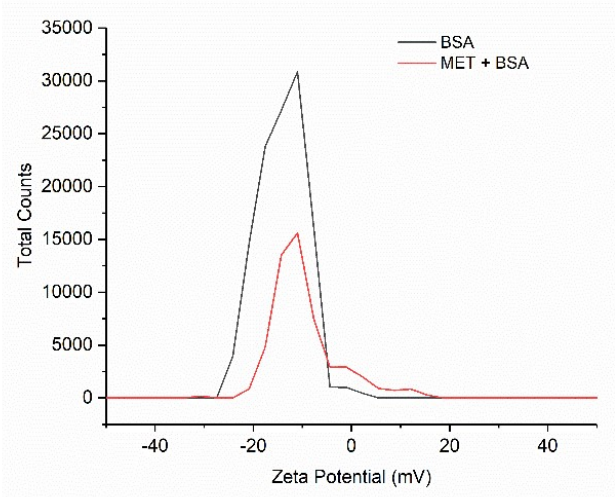


Figure S6 Zeta potential distribution of 4 mg mL^{-1} BSA and mixed MET (8 mmol L^{-1}) + BSA (8 mg mL^{-1}) solution incubated for 30 min at a volume ratio of 1:1.

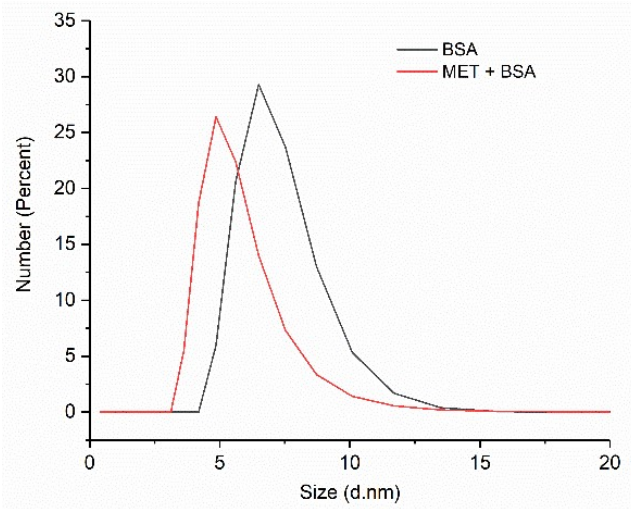
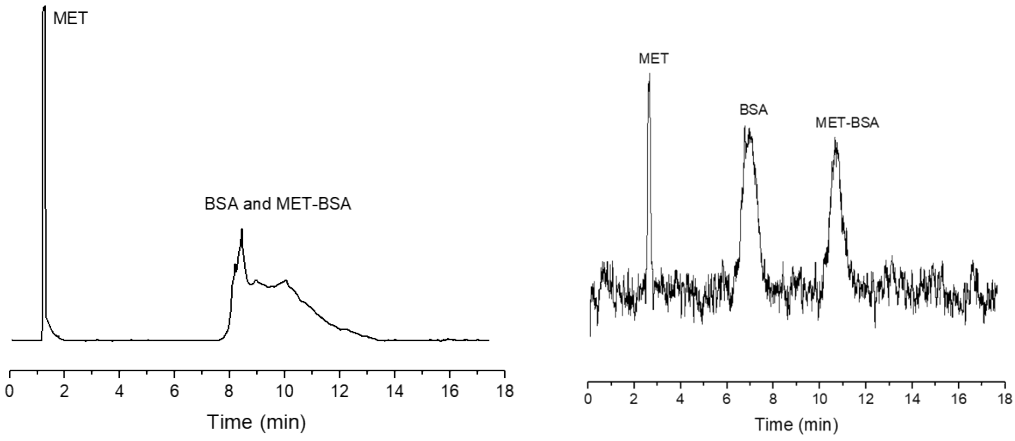


Figure S7 Particle size distribution of 4 mmol L^{-1} BSA and mixed MET (8 mmol L^{-1}) + BSA (8 mg mL^{-1}) solution incubated for 30 min at a volume ratio of 1:1.



(A) (B)

Figure S8 Electrophoresis diagram of MET-BSA interaction in ID of 50 μm (A) and 2 μm (B). Sampling conditions: the mixed solution of 8 mmol L^{-1} MET and 8 mg mL^{-1} BSA was incubated for 30 min at a volume ratio of 1:1 and injected for 10 s at 8 kV; separation column: total length = 30 cm (20 cm effective); separation conditions: 100 mmol L^{-1} Tris-HCl (pH = 7.4) injected at 12 kV; detection wavelength: 200 nm.

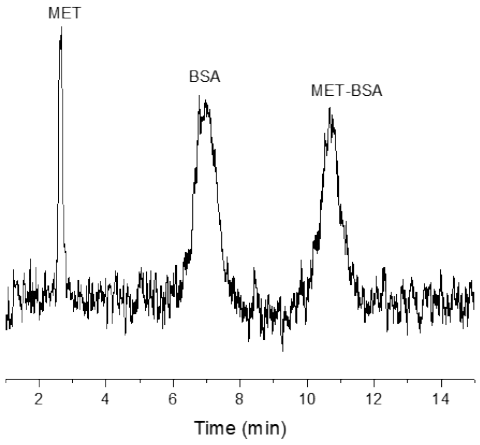


Figure S9 Limit of detection electropherogram for simultaneous detection of free MET, free BSA, and MET-BSA complex in the MET-BSA interaction. Sampling conditions: the mixed solution of 6 mmol L^{-1} MET and 6 mg mL^{-1} BSA was incubated for 30 min at a volume ratio of 1:1 and injected for 10 s at 8 kV; separation column: total length = 30 cm (20 cm effective) and ID= 2 μm; separation conditions: 100 mmol L^{-1} Tris-HCl (pH = 7.4) injected at 12 kV; detection wavelength: 200 nm.

Table S1 Peak areas of MET, BSA, and MET–BSA following the interaction of different concentrations of MET with 100 mg mL^{-1} BSA.

	4 mmol L^{-1} MET + BSA	8 mmol L^{-1} MET + BSA	12 mmol L^{-1} MET + BSA	16 mmol L^{-1} MET + BSA	20 mmol L^{-1} MET + BSA
Free MET	10.88	27.96	40.90	65.11	82.09
Free BSA	1105.61	698.25	329.07	312.07	301.12
MET-BSA	0	90.55	112.34	127.27	145.01

Table S2 Comparison of advantages and limitations of drug-protein interaction methods

	Advantages	Limitations	References
Spectroscopy	binding parameters obtained more comprehensive	high sample consumption and high sample purity	[1-2]
Isothermal titration calorimetry	direct access to thermodynamic parameters	high sample consumption and high sample purity	[1-2]
Surface plasmon resonance	binding kinetic information obtained	higher sample consumption, high sample purity and complex immobilization step	[3-4]
Narrow-bore CZE	low sample consumption and low sample purity	slow-dissociating system required	In our work

1. S. Yasmeen, Riyazuddeen, S. Khatun and F. Abul Qais, *Journal of Biomolecular Structure and Dynamics*, 2020, **38**, 722-732.
2. A. Shamsi, M. Al Shahwan, S. Ahamad, M. I. Hassan, F. Ahmad and A. Islam, *Journal of Biomolecular Structure and Dynamics*, 2020, **38**, 1094-1102.
3. F. Fathi, H. Mohammadzadeh-Aghdash, Y. Sohrabi, P. Dehghan and J. Ezzati Nazhad Dolatabadi, *Food Chemistry*, 2018, **246**, 228-232.
4. D. Sun, Y. Wu, S.-J. Chang, C.-J. Chen and J.-T. Liu, *Talanta*, 2021, **222**, 121466.