Supplementary Information

Moving affinity boundary electrophoresis and its selective isolation of histidine in urine

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Supporting Information of Experimental Section

Table S1. The analytical conditions used for the standard amino acid analyses of urine sample.

Time (min)	%B1	%B2	%B3	%B4	%B5	%B6	Pump 1 Flow rate (mL/min)	Column Temp	%R1	%R2	%R3	Pump 2 Flow rate (mL/min)
0.0	100	0	0	0	0	0	0.350	38	50	50	0	0.300
2.0								30				
21.5	100	0	0	0	0	0						
21.6	80	20	0	0	0	0		60				
33.5	70	30	0	0	0	0						
33.6	10	90	0	0	0	0						
36.5								40				
43.5	10	90	0	0	0	0						
43.6	0	100	0	0	0	0						
50.5	0	100	0	0	0	0		70				
50.6	0	0	100	0	0	0						
68.4								45				
69.5	0	0	100	0	0	0						
69.6	60	0	0	40	0	0						
75.0	60	0	0	40	0	0						
75.1	0	0	0	100	0	0						
82.0	0	0	0	100	0	0						
82.1	0	20	0	80	0	0						
92.5								70				

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99.5	0	20	0	80	0	0					
99.6	0	0	0	100	0	0					
112.5	0	0	0	100	0	0					
112.6	0	0	0	0	0	100					
116.0									50	50	0
116.1									0	0	100
121.5	0	0	0	0	0	100					
121.6	100	0	0	0	0	0					
125.0								38			
126.0									0	0	100
126.1									50	50	0
148.0	100	0	0	0	0	0					

Analysis Condition	B1: PF-1	R1: Nin
	B2: PF-2	R2: Nin-Buffer
	B3: PF-3	R3: 5% Ethanol
	B4: PF-4	
	B5: H_2O	
	B6: PF-RG	

Table S2. The composition of buffer solutions used for the standard amino acid analyses of urine sample.

Name	PF-1	PF-2	PF-3	PF-4	PF-RG
Vessel (buffer)	B1	B2	B3	B4	B6
Lithium concentration (N)	0.09	0.255	0.721	1.00	0.20
1. Distilled water (approx.)	700 mL	700 mL	700 mL	700 mL	700 mL
2. Lithium citrate (4 H_2O)	5.73 g	9.80 g	8.79 g	9.80 g	____
3. Lithium chloride	1.24 g	6.36 g	26.62 g	38.15 g	____
4. Citric acid (H_2O)	19.90 g	12.00 g	11.27 g	3.30 g	____
5. Lithium hydroxide	____	____	____	____	____
6. Ethanol	30.0 mL	30.0 mL	100.0 mL	____	30.0 mL
7. Thiodiglycol	5.0 mL	5.0 mL	____	____	____
8. Benzyl alcohol	____	____	3.0 mL	____	____
9. Brig-35*	4.0 mL	4.0 mL	4.0 mL	4.0 mL	4.0 mL
10. pH (nominal)	2.8	3.7	3.6	4.1	____
11. Total (adjust)	1.0 L	1.0 L	1.0 L	1.0 L	1.0 L
12. Caprylic acid	0.1 mL	0.1 mL	0.1 mL	0.1 mL	0.1 mL

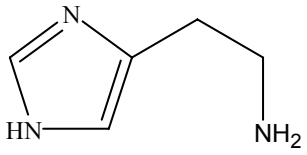
*Dissolve 25 g into 100 mL of distilled water.

Table S3. The composition of Ninhydrin reagents used for the standard amino acid analyses of urine sample.

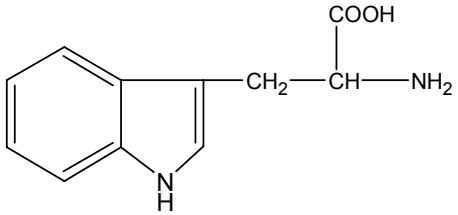
Vessel	Step 1	Reagent	Measurement
R1 (ninhydrin)	1	Propylene glycol monomethyl ether	979 mL
	2	Ninhydrin	39 g
	3	Nitrogen bubbling dissolution	5 min minimum
	4	Sodium borohydride	81 mg
	5	Nitrogen bubbling	30 min minimum
		Density	0.96
R2 for ninhydrin buffer solution	1	Distilled water	336 mL
	2	Lithium acetate dehydrate	204 g
	3	Glacial acetic acid	123 mL
	4	Propylene glycol monomethyl ether	401 mL
	5	Total	1000 mL
	6	Nitrogen bubbling	10 min minimum
		Density	0.96
R3 for 5% of Ethanol	1	Distilled water	900 mL
	2	Ethanol	50 mL
	3	Total	1000 mL
		Density	1.00

Supporting Information of Results and Discussions

Histamine



Trp



Cys

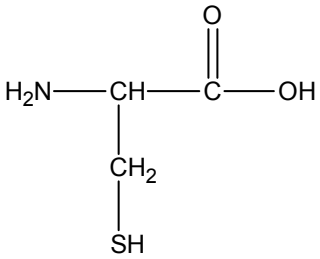
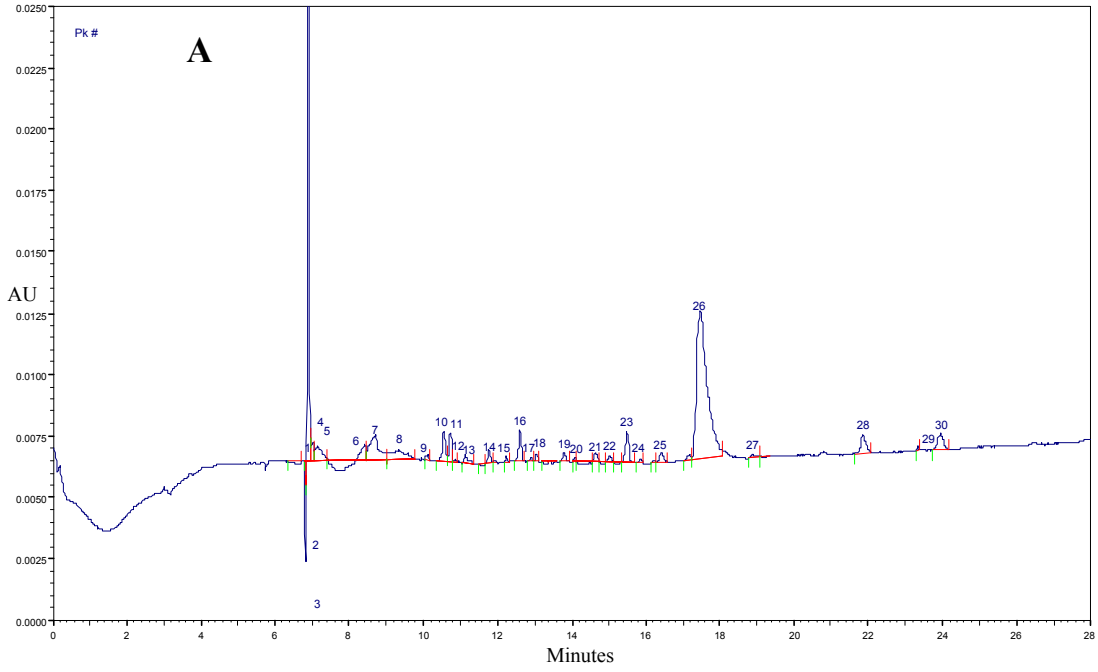
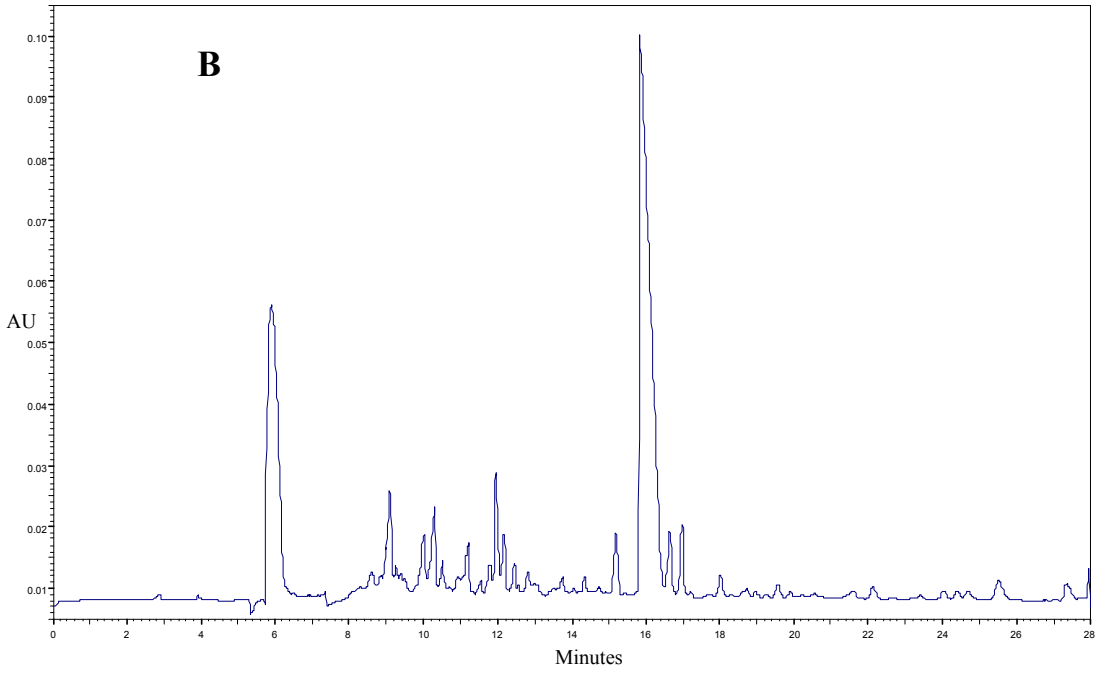


Fig. S1. Chemical structures of Histamine, Trp and Cys that can offer solitary electronic pairs to metal ion of Ni(II).





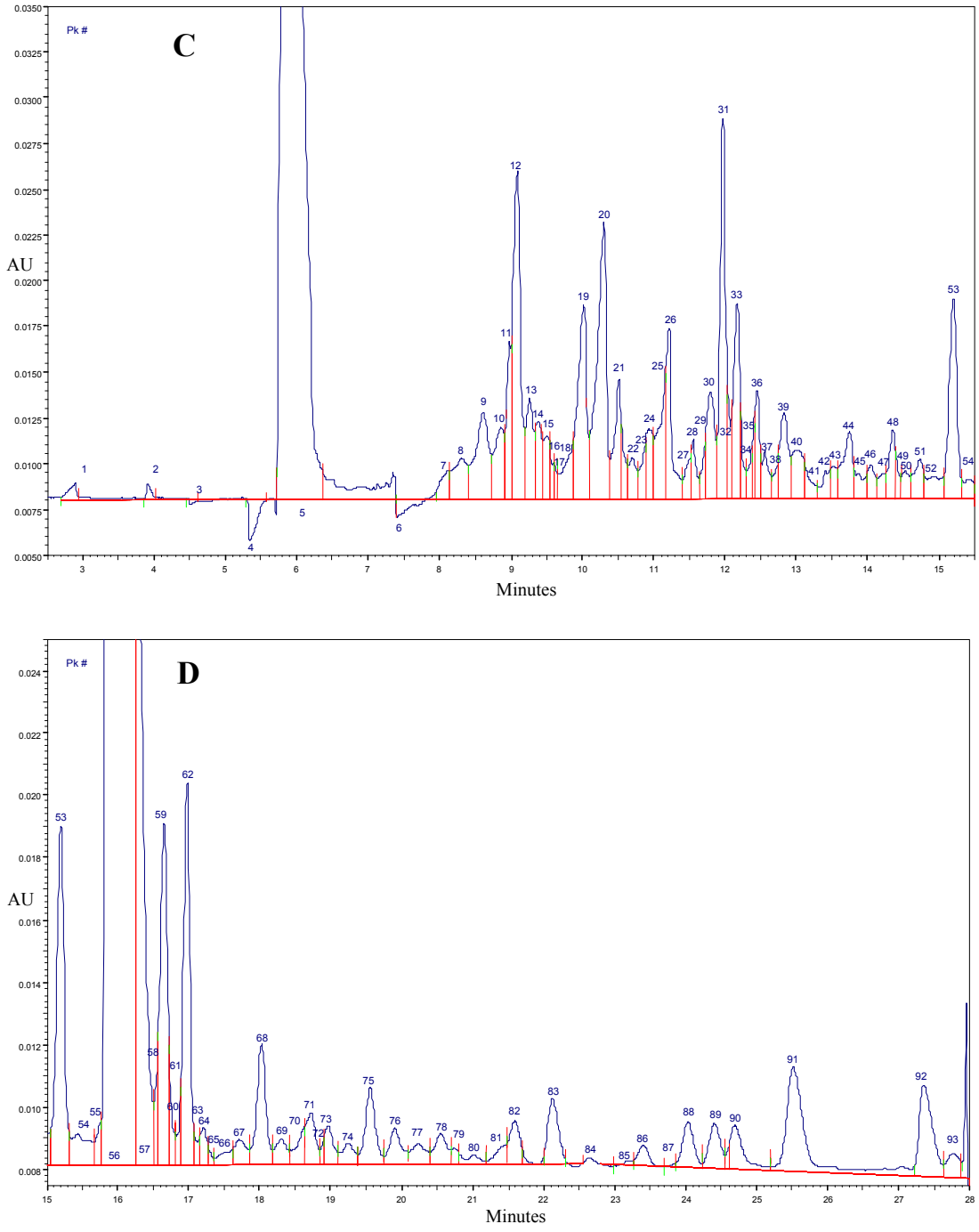


Fig. S2. Comparison of urine profiling achieved with (A) conventional CZE, (B) 0-28 min MRB-induced stacking, (C) 2.5-15.5 min MRB-induced stacking followed by CZE separation, and (D) 15-28 min MRB-induced stacking. In electrophoregram A, the urine sample was prepared in the running buffer and injected for 5 s at 0.2 psi. An insert is included where the minor peaks are enlarged, but it should be noted that the scale of the insert is different from that of electrophoregram A and B. In electrophoregram B and C, the sample was prepared in 25 mM Gly-HCl (pH 2.5) and injected for 10 s at 1.4 psi. An insert is also included to better illustrate the stacking effect. Other conditions are the same in both runs: 50 mM pH 12.3 Gly-NaOH running buffer, 15 kV, capillary 75 μm i.d. × 375 μm o.d. × 60.2 cm length (50 cm to the detector), 214 nm and 24 ℃.

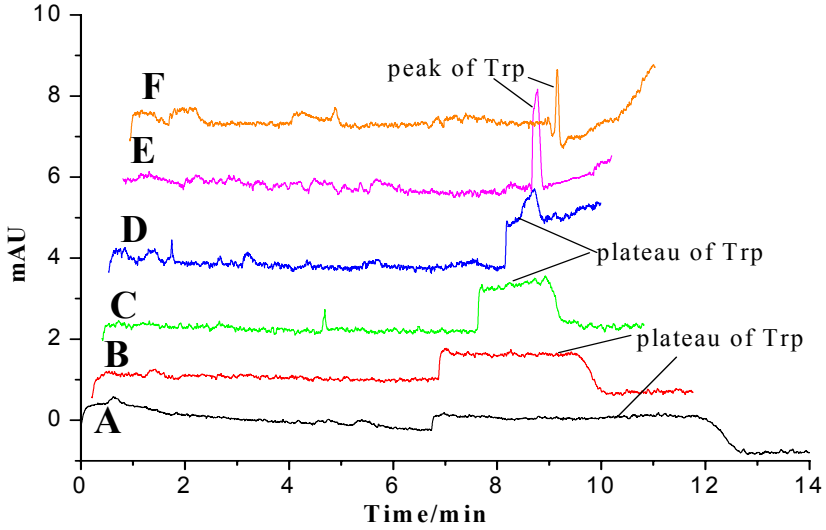


Fig. S3. Capture efficiency of Trp by the MAB-ACE method under different pH value of running buffer with 1.0 mM sodium chloride: (A) pH 5.7, (B) pH 6.0, (C) pH 6.3, (D) pH 6.6, (E) pH 6.9 and (F) pH 7.2. Conditions: 10 μM Trp in 50 mM pH 5.7-7.2 running buffer with 1.0 mM NaCl in whole capillary, 2.0 mM Ni(II) in 50 mM pH 5.7~7.2 running buffer with 1.0 mM NaCl in the anodic vial, 50 mM pH 5.7~7.2 running buffer with 1.0 mM NaCl in the cathodic vial. The other conditions were the same as those in Fig. 3.

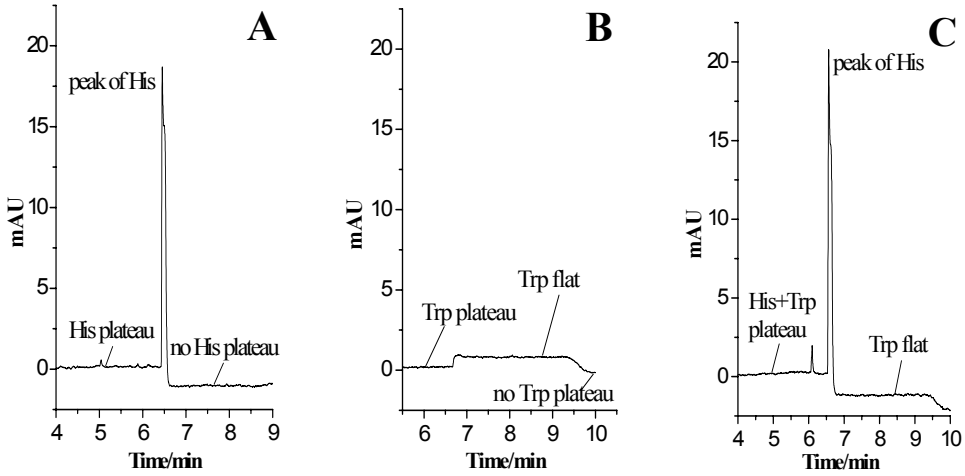


Fig. S4. Selective capture of MAB-ACE to His rather than Trp: (A) electropherogram of 50 μM His; (B) electropherogram of 10 μM Trp; (C) electropherogram of 50 μM His and 10 μM Trp in the whole capillary. Conditions: 2.0 mM Ni(II) in 50 mM pH 6.0 running buffer with 1.0 mM NaCl in the anodic vial. The other conditions are the same as those in Fig. 3.

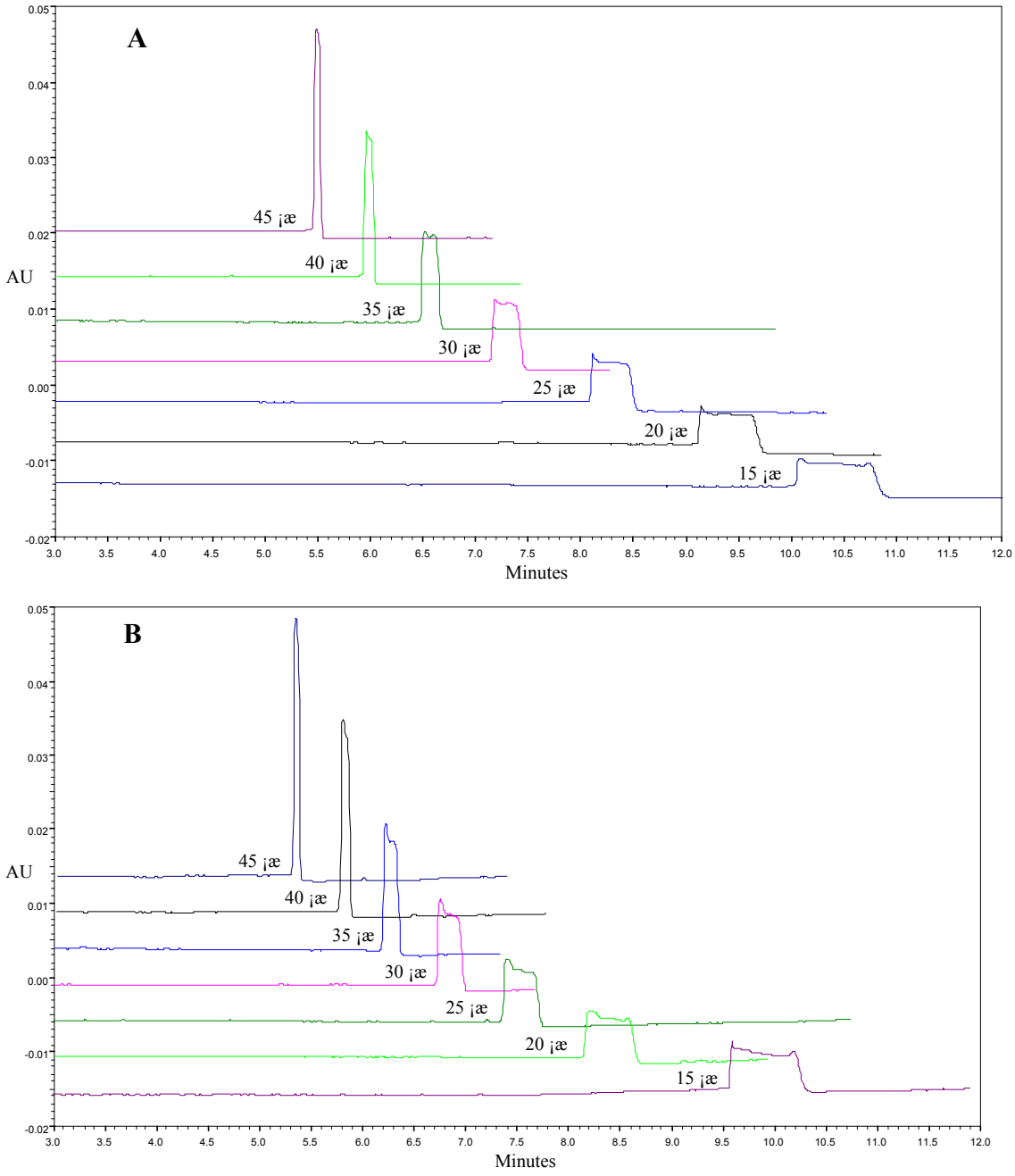


Fig. S5. The affection of temperature on the affinity interaction between His and Ni(II). An apparatus of CE (P/ACE MDQ, Beckman Coulter Co., Fullerton, CA, USA) with temperature control was employed in the experiment, and the wavelength were set at (A) 214 nm, and (B) 200 nm, respectively. Conditions: 50 μM His in 50 mM pH 6.0 running buffer with 1.0 mM NaCl in whole capillary, 2.0 mM Ni(II) in 50 mM pH 6.0 running buffer with 1.0 mM NaCl in the anodic vial. The other conditions are the same as those in Fig. 3.