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ROXY EC GUIDELINE FOR USE OF S-S REDUCING ELECTRODES

In this document the settings for efficient disulfide bond reduction using µ-PrepCell are described.

Polishing of S-S reducing electrode

The surface of the electrode may be colored (yellowish) in course of experiments. This is normal behavior and when required the electrode may be dry polished on lapping paper (P1000). Rinse the electrode with water and sonicate in 50% isopropanol to remove particulate matter. When the lapping paper is blunt order new one.

The electrode polishing is recommended if performance of the electrode decreased. Lapping paper can be ordered at Antec (P/N 250.1044A Lapping paper SIC1000, 70x70 mm (5 pcs))

Recommended conditions

1. A solution used for reduction of disulfide bonds should contain 1% formic acid in 10 - 50% acetonitrile.
2. Flow rate 50 µL/min
3. Sample: 5µg/mL insulin in solution described in the point 1.

Mobile phase can be optimized if needed. Minimum content of formic acid is 0.5%. Increasing flow rate results in less efficient reduction.

Installing the cell

The installation of µ-PrepCell is described in details in the µ-PrepCell user manual (204.0010).

The µ-PrepCell should be filled according the protocol 204.0016 (µ-PrepCell priming instruction).

An ESI interface of an MS is usually operating at high voltages of typically 3 – 5 kV. In cases where the inlet of the ESI-MS is not grounded, the grounding kit (pn 250.0035) must be used. If not used it may lead to damage of the ROXY potentiostat.

Optimal settings for reduction of disulfide bonds in proteins and peptides

Table 1 and Figure 1 show an optimized square wave pulse settings developed for the efficient reduction of disulfide bonds.

Pulse parameters (E1 and E2) can be adjusted if needed. E1 potential can be optimized in the range of –0.9V to –3V, to reach complete or near to complete reduction of the disulfide bonds. E2 potential must be kept above 0V. t1 and t2 settings can be adjusted, if needed, between 100 and 2000ms. Figure 2 shows mass spectra of Insulin before and after applying the reductive pulse.

Table 1. The settings used for disulfide bonds reduction.

Potential [mV]	Time [ms]
E1= – 1000 E2= + 500 E3= 0	t1= 1500 t2= 500 t3= 0 ts= 40

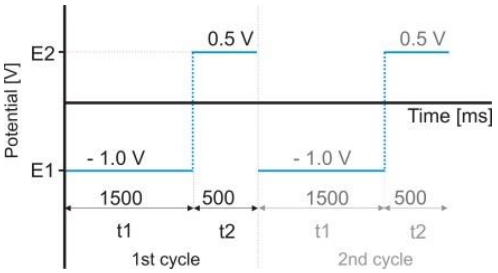

Fig. 1. The square wave pulse settings.

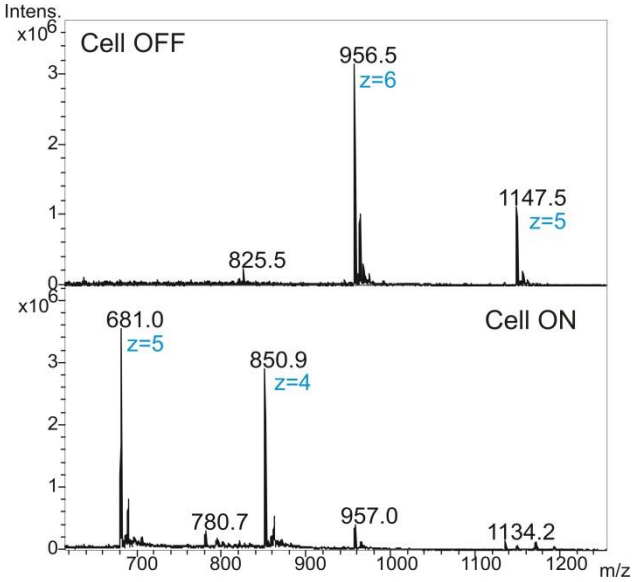

Fig. 2. Electrochemical reduction of disulfide bond in insulin on a specially developed S-S reducing working electrode. (Top) Mass spectrum with Cell OFF. (Bottom) Mass spectrum of Insulin reduced by a square wave pulse with the settings presented in the figure 1. The mass spectrum is an average of 100 scan registered in 50 min of the experiment. The experiment was performed in a direct infusion mode and sample was directly introduced to ESI source via grounded union. 50 µL/min flow rate was used. A HCT plus ion trap MS (Bruker) was used. Insulin was diluted in 1% formic acid in 50% acetonitrile to a final concentration of 5µg/mL.

Troubleshooting

The following actions can be taken at decreased flow cell performance:

1. Switch the cell off. Disconnect outlet tubing and push the sample through the cell by using the syringe. You should observe the gas bubbles leaving the cell. Reconnect tubing and place the syringe in the pump before switching the cell on.
2. Polish the titanium working electrode (See polishing section). Take this step only if the previous was not successful.

APPENDIX

A list of chemicals is shown below as a guideline for the purchase of chemicals at the customer site. The listed brands/purities are not necessarily the best chemicals, but the applications were evaluated/developed at the Antec R&D laboratory using these specific brands/purities. If for any reason alternative chemicals need to be purchased use the following guidelines:

- The chemicals should have at least the same purity or better than the chemicals listed in the table below
- Do not purchase ultra-dry grade or anhydrous chemicals

Table 2. Brands and purities of chemicals used for application development at Antec.

Component	Purity	Brand	Order no:	Mw	kg/L
1. Insulin from bovine pancreas	Meets USP testing specifications	Sigma Aldrich	I8405-100mg		
Formic acid	98% for mass spectroscopy	Fluka	94318-250mL-F	46.03	
Acetonitrile	HPLC grade, 99.9%	Acros	268260025	41.05	D:0.781
Water	TOC <10ppb and deionized, resistivity >18 MOhm-cm (Barnstead Easypure II)				