

■ Oxidative mode

- Better reproducibility and robustness
- Superior selectivity
- Larger working range (potential)
- Excellent correlation with metabolic & degradation pathway

■ Reductive mode

- Superior robustness and selectivity
- TiBlue electrodes for highest yield and ease of use
- Works in simple DC mode
- No manual polishing of electrode or cell inlet block necessary

The new μ-PrepCell™ 2.0 (uPC 2.0) is made of a fully inert polymeric inlet block (unlike its predecessor, which was made of titanium). To serve as auxiliary (AUX) electrode, the black polymeric inlet block contains conductive carbon. In addition, the reference electrode (REF) is positioned in a separate chamber and is not in direct contact with the cell volume. The new material of the inlet block and the new position of the REF electrode result in overall improved signal stability and reproducibility. In practice the new uPC 2.0 has distinguished advantages in both oxidative (OX) and reductive (RED) mode.

OX mode:

The new conductive polymeric inlet block results in significant higher selectivity by suppressing unwanted reduction reactions when working under oxidative conditions.

In Figure 1, the pathway of the electrochemical oxidation of amodiaquine (mw 355) is shown. Three major oxidation products also known from literature [1] were generated in high yield with the new uPC 2.0 (blue squares): amodiaquine quinoneimine (mw 325) and amodiaquine quinoneimine aldehyde (mw 296). The known non-specific reduction products, highlighted with red squares, with mw 327, 298 were not formed.

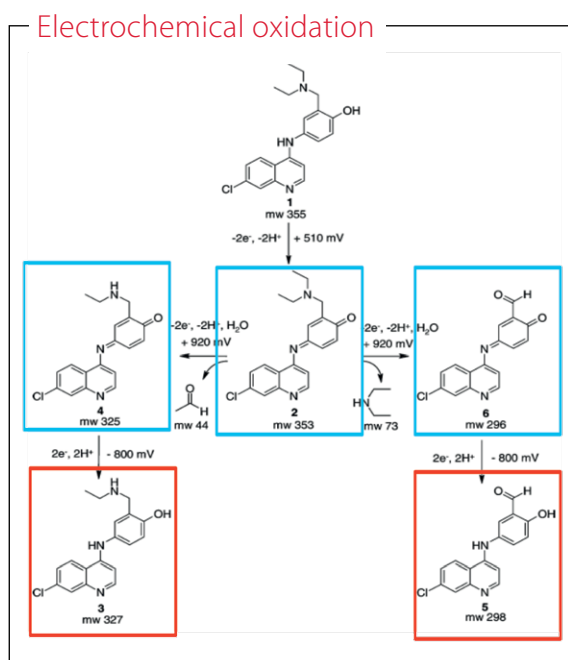


Figure 1: Electrochemical oxidation of Amodiaquine with uPC 2.0 (20 uM Amodiaquine in 20mM Ammonium formate, 1% FA in 50% ACN) and ROXY EC system. Selective generation of oxidation products (blue squares) without generation of non-specific reduction products (red squares). Magic Diamond (BDD), Scan mode from 0 to 2.5V at 20mV/s.

Other advantages of the new cell are the maintenance free inlet block, and the larger potential working range. Cyclic voltammetry was used to illustrate the larger potential range of the new cell. In Figure 2 the scanning voltammogram of dopamine is shown using the new and the former titanium based cell.

With the new cell, a significant larger working range is possible. Under identical conditions, the Ti based cell showed a current overload at around +1.5 V, meanwhile with the new cell no current overflow (air bubble formation) was observed up to +2V. Furthermore, the new cell shows significant better reproducibility.

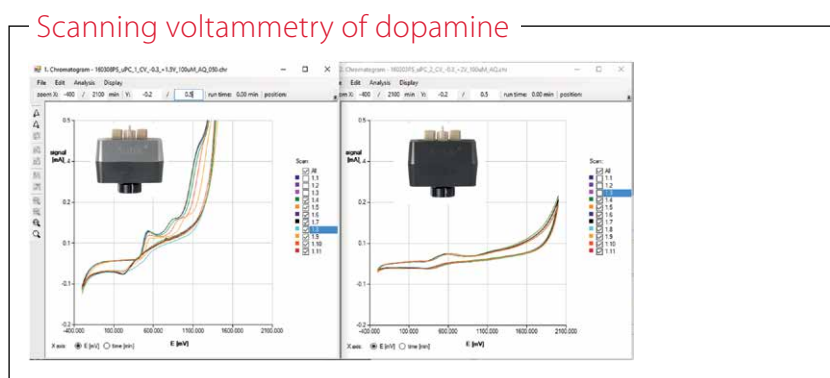


Figure 2: Scanning voltammetry of dopamine with previous (left) and new μ-PrepCell 2.0 (right) using the ROXY potentiostat. Scan mode from -300 mV to +1500 mV for previous cell (left) and -300 mV to +2000 mV for the new μ-PrepCell 2.0 (right); scan rate 20mV/s, Glassy Carbon as WE.

RED mode:

In reductive mode the new μ-PrepCell 2.0 allows to work in simple DC mode for the cleavage of disulfide bonds in proteins/peptides. A potential of ca. -2.0 V is sufficient to reduce selectively the S-S bonds, making the use of square wave pulses obsolete. In combination with the new TiBlue™ [2] electrode a much more stable and reproducible reduction is achieved.



Figure 3: Amino acid structure of insulin with 2 interchain S-S bonds (orange) and 1 intrachain S-S bond (green).

Another advantage of the disposable TiBlue electrode is their maintenance free ease of use (no longer manual polishing). The disposable TiBlue electrode with its active TiO² surface can be used double sides to extend lifetime.

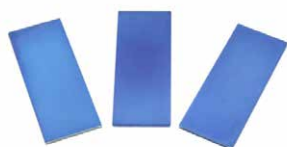


Figure 5: TiBlue electrode for efficient reduction of disulfide bonds in proteins and peptides. The electrodes are made by a proprietary electrochemical surface treatment of a titanium alloy, resulting in a blue-coloured crystalline TiO² layer.

[1] Jurva et al., *Chem. Res. Toxicol.* **2008**, *21*, 928–935
 [2] TiBlue is a registered trademark of Antec, patent pending



Stability and reproducibility

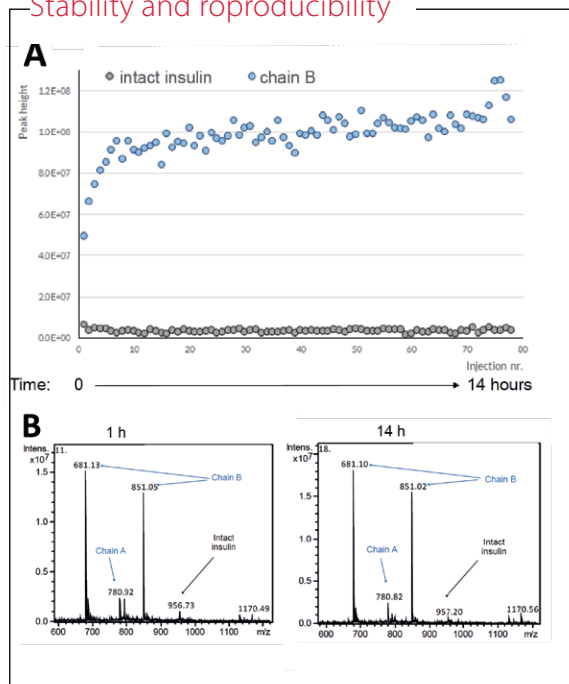


Figure 4: Long term stability and reproducibility. A: Reduction of Insulin over 14 hrs in continuous flow mode using the new uPC 2.0 and TiBlue electrode. Flow rate 20 μL/min, DC mode (E= -2.0V). The sample (5μg/mL insulin in 1% formic acid, 10% acetonitrile, 89% water) was injected every 10 min to check reduction efficiency. The grey dots correspond to peak heights of intact insulin (m/z 956) and blue dots to chain B (m/z 681) obtained after reduction of the two interchain disulphide bonds. B: MS Spectra, representing the 1st and 14th hour of measurements, confirming near complete and stable reduction.

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