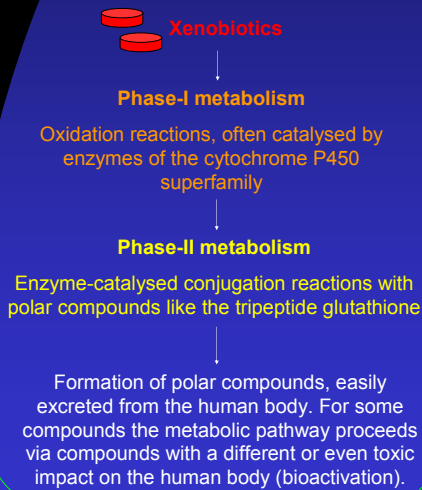




# Implementation of Wall-Jet Cells for the Simulation of Oxidative Phase-I Metabolism in Online EC/HPLC/MS

## Degradation pathway of xenobiotics



## Conventional methods for metabolic studies

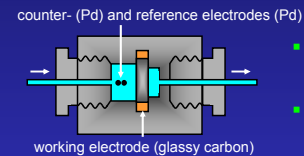
- In vivo and in vitro methods, using isolated liver cells and microbodies
- ⇒ Complex and time consuming
- ⇒ Reactive metabolites may form adducts with the cell matrix, hence isolation and identification of metabolites is hampered

## Instrumental approach for metabolic studies

- Coulometric **flow-through cells** for the simulation of the oxidative phase-I metabolism
- ⇒ Fast method for direct identification of reactive metabolites
- ⇒ High potential for high-throughput-screening of metabolites in drug development

**New approach:** Implementation of **wall-jet cells** with variable electrode material

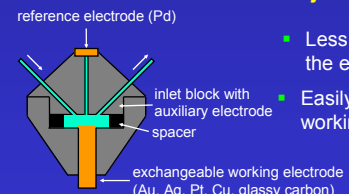
## Simulation in coulometric flow-through cells



- Complete conversion even at high flow rates
- Little maintenance required

Fig. 2: Coulometric flow-through cell (ESA Biosciences Inc., Chelmsford, MA, U.S.A.)

## Simulation in wall-jet cells



- Less adsorption on the electrode surface
- Easily exchangeable working electrode

Fig. 3: Electrochemical wall-jet cell (Antec Leyden, Zoeterwoude, The Netherlands)

## Instrumental set-up for the simulation of the oxidative phase-I metabolism

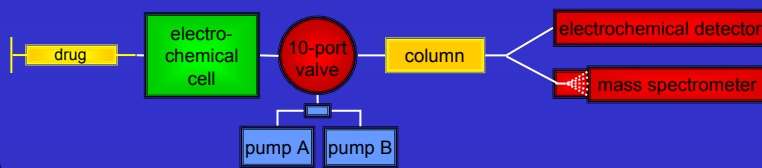


Fig. 4: Instrumental set-up for the electrochemical simulation of the oxidative phase-I metabolism. A 10-port switching valve allows variations in the mobile phase or the flow rate between the electrochemical cell and the column.

## Electrochemical oxidation of the model-system paracetamol

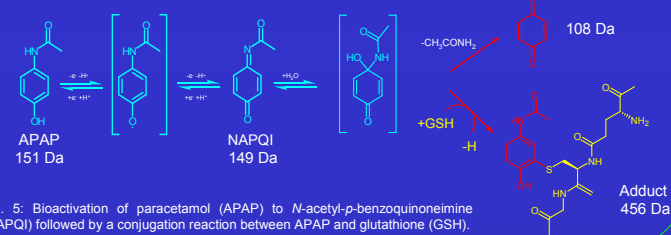


Fig. 5: Bioactivation of paracetamol (APAP) to *N*-acetyl-*p*-benzoquinoneimine (NAPQI) followed by a conjugation reaction between APAP and glutathione (GSH).

## Results

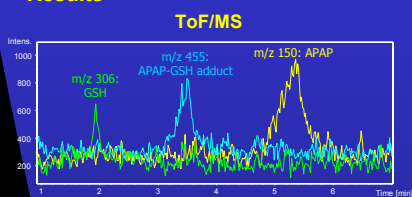


Fig. 6: Identification of the electrochemically generated metabolites of APAP and GSH by ToF/MS.

## Electrochemical detection

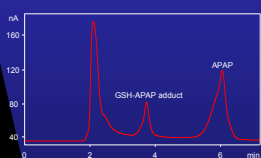


Fig. 7: APAP as well as the APAP-GSH adduct are electrochemically active and show well separated peaks. Therefore these peaks are used for the comparison between the electrochemical cells.

## Wall-jet cell

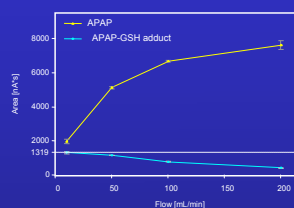


Fig. 8: Peak area of APAP and APAP-GSH adduct at increasing flow rates in the wall-jet cell.

- Maximum adduct formation at a flow rate of 10 μL/min
- Higher flow rates: short dwell time in the cell and less adduct formation

## Coulometric flow-through cell

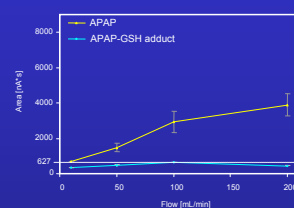


Fig. 9: Peak area of APAP and APAP-GSH adduct at increasing flow rates in the coulometric flow-through cell.

- Maximum adduct formation at a flow rate of 100 μL/min
- Low flow rates: decreasing amount of adduct and high standard deviation, indicating adsorption effects in the cell

## Electrode material

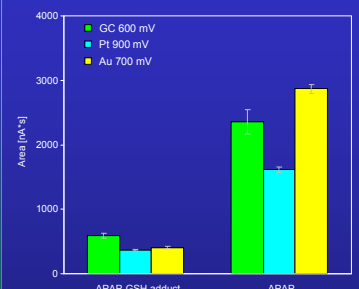


Fig. 10: Peak area of APAP and APAP-GSH adduct at different electrode material. Glassy carbon (GC), Au and Pt was implemented in the wall-jet cell and the optimum potential was determined.

- Maximum adduct formation at glassy carbon, but glassy carbon also shows the highest standard deviation

## Conclusion

- Oxidative phase-I metabolism for paracetamol was successfully simulated in a coulometric flow-through cell as well as in a wall-jet cell
- The wall-jet cell shows stronger adduct formation and less standard deviation than the flow-through cell
- In comparison to the electrode materials Au and Pt, glassy carbon shows the strongest adduct formation, but also the highest standard deviation

## Outlook

- Wall-jet cell and different electrode materials will be tested for a larger number of model systems in order to examine the influence of the cell geometry and the material on different metabolic reaction types
- Implementation of immobilised enzymes in the instrumental set-up, for a complete simulation of the phase-I and phase-II metabolism