Nanostructured gold: applications in the study of drug metabolism
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2017

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Chapter 7

Summary and Perspectives

The theme of this thesis is to develop new methods for the highly selective synthesis of drug metabolites with high yield. Although most of the drug metabolites generated by cytochrome P450 can be synthesized electrochemically, in most cases, a mixture of different drug metabolites is obtained in an electrochemical batch cell, and the yield is usually quite low. In this thesis, we developed different methods for circumventing this problem. First, a hemin modified electrode was prepared and characterized in an in situ system in chapter 3, nanoporous gold (NPG) was developed as N-dealkylation catalyst in chapter 5, and a continuous-flow reactor was developed in chapter 6.

In chapter 2, we reviewed different approaches that have been taken to mimic oxidative drug metabolism as executed by members of the cytochrome P450 (CYP450) family of enzymes in humans. A complete array of drug metabolites can be produced on a modified electrode surface with enzymes from the CYP450 family, immobilized through a self-assembled monolayer. On the electrode, the use of NADPH and CYP reductase can be obviated. However, immobilization of active CYP450s is not straightforward and keeping the enzyme active is even more challenging. Strategies for the preparation of different CYP450-modified electrodes are discussed, such as gold electrodes, carbon-based electrodes and indium-tin oxide electrodes. In addition, synthetic metalloporphyrins are discussed for the preparation of modified electrodes.

In chapter 3, a hemin modified electrode was prepared, and was characterized by an integrated surface enhanced Raman scattering spectroelectrochemical (SERS SEC) analysis system, which allows for the simultaneous SERS and electrochemical investigation of any modified electrode surface with very low laser power. A nanostructured gold working electrode was used, which enables highly sensitive in situ SERS spectroscopy through large SERS enhancements, eliminating the need for resonant wavelength matching of the laser excitation source with the electronic absorption of the target molecule. In situ SEC measurements showed shifts of the iron oxidation marker band v4 on the nanostructured Au working electrode under
precise potential control. This result is a strong indication that the hemin layer is indeed electrochemically active on the gold surface, and that the absence of product formation may be due to the low surface coverage.

In chapter 4, different methods for the preparation of nanoporous gold (NPG), such as electrodeposition and chemical dealloying, were discussed. Special attention was paid to the catalytic properties of nanoporous gold, such as the catalytic oxidation of CO and ethanol and electrochemically-assisted catalysis. The mechanism of NPG catalysis was also discussed in this chapter. The fact that the size of the nanopores varies while changing the amount of Ag by etching complicates mechanistic studies. While most applications use dealloyed NPG, electrodeposition methods hold considerable promise for mechanistic studies, because the nanoporous structure can be prepared without Ag, or with strictly controlled amounts of Ag or other metals on the surface.

In chapter 5, we report a new catalytic reaction of NPG, the N-dealkylation of drug molecules. A nanoporous gold surface with a nanopore size of about 38 nm was prepared by chemical etching, with a roughness factor of 174. High temperature (60 °C) and air purging were found to be the conditions leading to the highest N-dealkylation yield. Lidocaine and metoprolol were used for the catalysis study on NPG, both producing only the N-dealkylated product at high yield and selectivity. The catalytic mechanism probably depends on the unique nanoporous structure, especially the surface defect regions. However, we cannot eliminate the effect of the presence of residual Ag.

In chapter 6, following the in-batch NPG catalysis work, a continuous-flow reactor was designed for the catalytic N-dealkylation of lidocaine, metoprolol and atropine on NPG by flow chemistry. The results showed the highly selective conversion of lidocaine and metoprolol. Of note, for atropine, the selectivity of the N-dealkylation reaction was improved from 25% in a batch system to 88% in the continuous-flow reactor.

There is no doubt that modified electrodes will not replace existing in vivo and in vitro methods, since modified electrodes cannot mimic the complexity of all CYP450-mediated reactions taking place in vivo. However, with the modified electrodes as electron supplier, the need for NADPH and CYP reductase in complex biological matrices is obviated. It is noteworthy that that all studies to date were performed at the laboratory scale, thus the amount of metabolites is quite limited. In
the future, more effort should be put into the design of robust modified electrodes, which can be reused for extended periods of time, and thereby produce the desired drug metabolites selectively and in high amounts.

In the case of the in situ SERS SEC work, future work should focus on the preparation of different modified electrodes, for example, electrodes modified with CYP450s, for in situ SEC and drug metabolite studies. On the other hand, it is clear that the low surface area of a gold electrode in a regular electrochemical cell is not sufficient for synthesis of mg amounts of drug metabolites by direct electrochemistry and even less on a hemin-modified surface. High-surface area gold, such as gold nanoparticles or nanoporous gold provide options to achieve better yields.

Regarding NPG, adding small amounts of Pt or metal oxides (TiO$_2$, Al$_2$O$_3$) was reported to result in improved catalytic activity in terms of conversion yield and/or selectivity. This provides new ideas to prepare efficient NPG catalysts. Clarifying the catalytic mechanism at the atomic level is of vital importance for the design of highly active and sustainable NPG catalysts. High resolution in situ morphology measurements have provided new insights, notably that catalytic activity is located at surface defect regions and that other elements like Ag play an important role. Secondly, different nanoporous gold surfaces can be prepared from different alloys containing different ratios of Au/Ag, or from Au/Cu, or Au/Al alloys, for the systematic study of how residual elements influence NPG catalysis. NPG has the potential to help the chemical industry meet the need for sustainable and energy-efficient production processes, a challenge for the decades to come.

The continuous flow system is particularly suitable for screening reaction conditions prior to scaling up owing to the merits of fast response and ease of operation. For the upscaling of drug metabolites, a more efficient design of the flow reactor should be developed, for example, by tuning dimensions, improving the heating system and adding a high pressure system.