Summary - *In vitro* optimization of microdialysis for pharmacokinetic applications

Quantitative microdialysis of lipophilic compounds has always been problematic in the past. Due to adherence to the membrane and tubing used, observed recoveries are often low, and the time needed to reach steady state variably long. This thesis aims to develop an *in vitro* set up to test the suitability of these materials for pharmacokinetic applications, and to suggest numerical parameters to describe the observed response to concentration changes. A range of tubing materials and microdialysis probes are tested with a model lipophilic compound (ZK 975, $LogP_{OW} = 4$) and a model hydrophilic compound (ZK 894, $LogP_{OW} = 1.5$).

Five tubing materials were tested and compared: fluorinated ethylene propylene (FEP), FEP/Teflon, polyethyleneethyleneketone (PEEK), fused silica and silicone. Each material was first 'primed' by flushing with a 5-10 µM test solution for 1 hour, then rinsed by flushing with Ringer's solution for 1 hour, both at 2 µL/min. During the rinsing phase, samples were collected every 3 minutes, and the results presented graphically. From the data, the 'amount eluted' (Ae), or washed out, from the tubing material was evaluated and compared.

For the lipophilic compound ZK 975, only fused silica showed no elution and therefore no binding. For ZK 894, FEP, FEP/Teflon and fused silica showed no elution, with PEEK and silicone only eluting minimal amounts.

Eleven microdialysis probes for intracranial use were tested and compared. The probes differed in their membrane material (polycarbonate (PC), polyethylenesulfone (PES), polyacrylonitrile (PAN), cuprophane (Cu) or cellulosic (Cell)), membrane pore size (6, 15, 35 or 100 kDa), membrane surface area (3 or 7.5 mm^2), membrane thickness (5, 20 or 30 µm) and outlet material (stainless steel or PEEK). Fused silica was used as outlet tubing. Each probe was first immersed in a 1 µM test solution for 1 hour (Phase A), then in Ringer's solution for 1 hour (Rinse B). The same was repeated with a 10 M test solution (Phase B) and a 1 hour rinsing phase (Rinse B). All probes were perfused with Ringer's solution at 2 µL/min, and all experiments were carried out in a water bath set at 37°C, with the medium stirred at maximum setting (1500 rpm). Samples were collected every 6 minutes throughout this 4-hour test. The results were presented graphically, and the recovery and mass transfer coefficient K were calculated. Also, a hypothetical ideal area under the data (iAUD) was calculated for both concentration phases, for a probe that reached steady-state immediately, and had the same mass transfer coefficient K. The actual AUD of the probe tested was related to this iAUD, to obtain the percentage of the iAUD achieved (%iAUD). For the rinsing phases A and B, the amount eluted from the exposed membrane surface was calculated, and related to the total membrane volume.

For ZK 975, only probes with cellulosic or cuprophane membranes displayed a %iAUD of > 95% and an Ae of < 5 pmol/mm^3. All other materials showed clear delays in response to increasing concentrations (Phase A and B) as reflected by a low %iAUD, and to decreasing concentrations (Rinse A and B) as reflected by a high Ae. For ZK 894, most materials had a %iAUD of > 95% and an Ae of < 5 pmol/mm^3. Only the PC-membrane showed considerable bleeding during the rinsing phases, and the PAN-membrane behaved differently at the two concentrations tested.

It is concluded that the presented method and parameters are useful in testing and describing the suitability of tubing and microdialysis probe materials for pharmacokinetic applications. Materials are identified that allow quantitative microdialysis of the lipophilic compound (fused silica tubing and cellulosic or cuprophane membranes). It is also shown that even for a hydrophilic compound, not all materials lend themselves for microdialysis, confirming the importance of always testing microdialysis materials for quantitative studies *in vitro*, in an experimental setting representative of the pharmacokinetic study to be carried out *in vivo*.