A MEMBRANE MICROCONTACTOR AS A TOOL FOR SAMPLE PRE-TREATMENT OF PHARMACEUTICAL COMPOUNDS

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ABSTRACT

A membrane microcontactor is presented that allows sample pre-treatment by means of extraction and operates in parallel flow mode, hence avoiding an emulsification and compulsory phase separation step. The extraction kinetic behaviour of the device was first evaluated by extraction of a drug candidate, proving equilibrium is reached within 10 min. Next the device was evaluated under more challenging working conditions, a homogenized mouse kidney sample containing the drug candidate was cleaned and characterized by HPLC. The system has the potential to be operated in a continuous fashion making it appealing to be implemented in screening or high throughput applications.

KEYWORDS: micro-membrane liquid-liquid extraction, membrane contactor, sample pre-treatment

INTRODUCTION

An important part of drug development consists of quantifying drug uptake in test subjects. Despite the availability of advanced analytical instrumentation, manual sample pre-treatment is usually necessary to extract, isolate and concentrate the analytes, as most instruments cannot handle complex matrices directly. [1] Procedures for organic non-volatile analytes generally involve a solvent extraction step during sample preparation. Classical macroscopic liquid-liquid extraction (LLE) has been traditionally the first choice of preparative technique and is still widely used in many available (regulatory) protocols, but has lost importance over the last years due to increasing sensitivity for certain aspects of the technique. The large consumption of pure solvents, the need for relatively large sample volumes and the difficulty to automate the technique constitute the major drawbacks. [2] This has lead to gradually replacement of the conventional extraction methods by solid phase extraction (SPE), which is currently the most applied extraction technique. In SPE, analytes (generally in an aqueous sample) are adsorbed to a solid sorbent and subsequently eluted with an organic solvent. SPE sorbents are available in (expensive) disposable cartridges and in columns. Disadvantages of SPE is the limited selectivity, low retention for highly polar components, dependence on a commercial supplier of SPE columns and the occurrence of sample crossover and contamination. [3] Considerable time is typically spent to LLE and SPE, making the sample preparation often the bottleneck of an entire analytical method. It is estimated that sampling and preparation steps constitute over 80 % of the total analysis time. [1] Some of the claimed advantages of SPE over LLE can be regarded critically, as laborious operations like conditioning, washing and solvent evaporation are often required as well. Moreover, evaporation of the solvents typically involved in LLE occurs faster than the protic solvents typically used in SPE, that have a higher vapour pressure.

In the present study, sample pre-treatment is performed using a membrane equipped microcontactor operated in parallel flow mode, hence avoiding an emulsification and compulsory phase separation step. [4] The sample pre-treatment of homogenized mice kidney samples in which MPB (4-(2,5-dimethyl-pyrrol-1-yl)-2-hydroxybenzoic acid) was administered in life condition is used as a case study. MPB is a drug candidate for the treatment of amyotrophic lateral sclerosis (ALS). An important aspect of the effectiveness of a drug is the ability to transport it to the zone where it has to exert its function. [5, 6] By intraperitoneal injection in mice and evaluating the in vivo distribution, it can be assessed if the drug is transported towards the zone of interest. To study the kinetics of the microcontactor, MPB was extracted out of matrix free samples as well as out of homogenized mice kidney samples in which MPB was administered in life condition. To increase the concentration after the extraction step, the sample was concentrated by evaporating the solvent using a second microcontactor, where nitrogen was flowing at the other side of the membrane to remove the evaporating solvent in continuous flow mode.

EXPERIMENTAL

The microcontactor consists of two milled aluminium (AA2024) plates that are clamped together and sandwich a PTFE-membrane (0.1 µm pore size, 68% porosity, 70 µm thickness, Frisenette Aps, Denmark) (Fig 1). In order to validate the microcontactor operating in co-flow with a flow rate ratio of 1:1, the extraction kinetics of pure MPB solution were measured. For this purpose, 100 mg/L MPB was dissolved in 15/85 m% methanol/water (pH = 5,2) and extracted with ethyl acetate at different residence times.

The samples were analysed by High Performance Liquid Chromatography (HPLC). This was performed isocratically using a Thermo Hypersil ODS-column (250 x 4.6 mm i.d., d_p 3 µm), a mobile phase consisting of methanol and 20 mM KH_2PO_4-K_2HPO_4 pH 5.5 (52:48 v/v%) at a flow rate of 1.0 ml/min, $V_{injection}$ 10 µl, $\lambda_{UV\text{-}detection}$ 210 nm.

Tissue samples (kidney) were collected from mice 1 hour after treatment with 1 mg MPB (dissolved in DMSO, with 200 µl extra added physiological water). Before collection the mice were perfused by phosphate buffered saline and all blood was removed from the organs. In order to operate above the lower detection limit of the HPLC the acceptor phase was enriched after extraction by using a second microcontactor in which ethylacetate is evaporated at following operating conditions: 150 µl/min, pressure N_2 0.3bar, room temperature.

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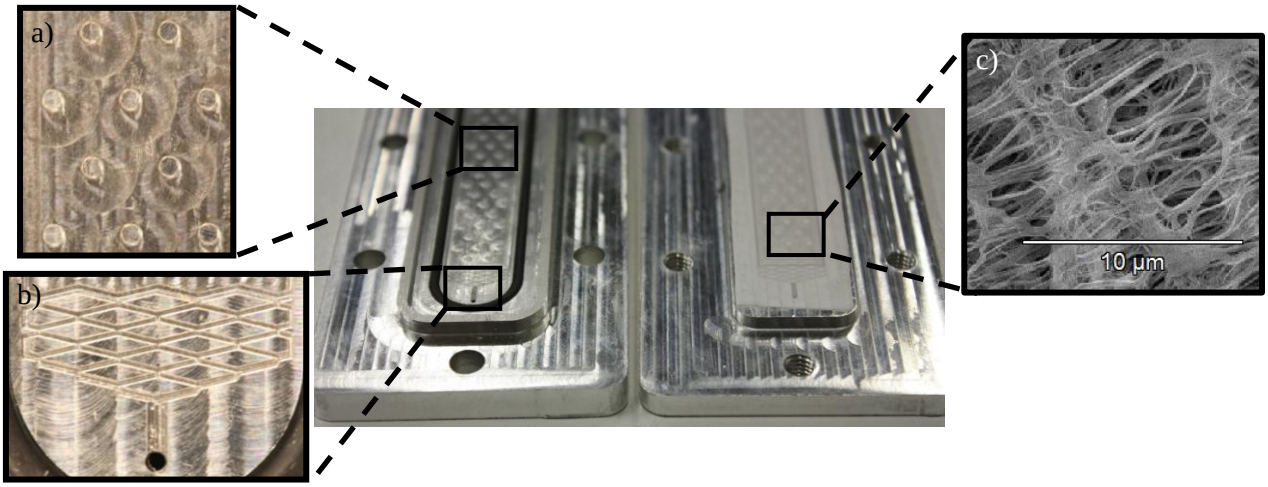

Figure 1: Schematic representation of the membrane micro-extractor (width 13 mm, length 11 cm). a) membrane support pillars to ensure equally spacing (d_{pillar} 1mm) b) fluid distributor to guarantee a uniform velocity profile c) PTFE membrane.

RESULTS AND DISCUSSION

The extraction kinetics of pure MPB were first measured in a microcontactor with a channel depth of 200 µm ($V_{microcontactor}$ 237.4 µl) (Fig 2). After a residence time of 30 min, the equilibrium was still not reached. In order to reach the equilibrium faster and lower the sample consumption the channel depth was lowered to 100 µm ($V_{microcontactor}$ 118.7 µl). Equilibrium was reached in 10 min within 10% deviation with a partition coefficient of 4.9 (Fig 2), in agreement with a shaking test.

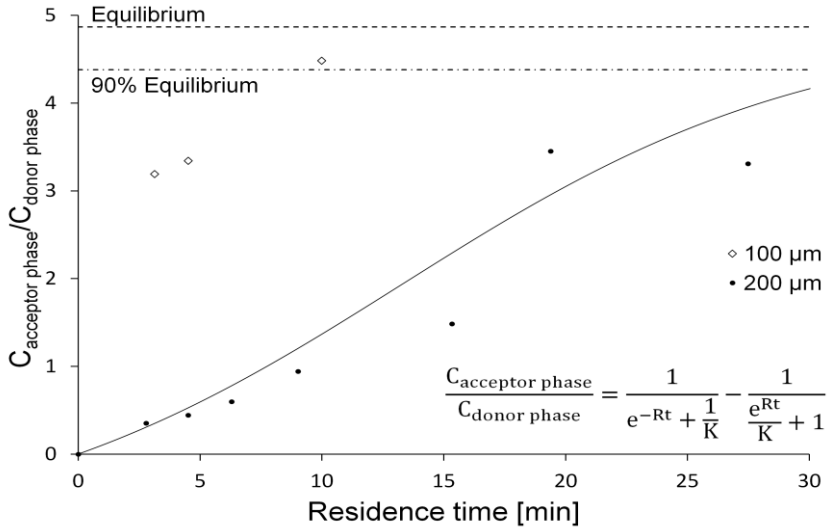

Figure 2: Representation of the extraction kinetics by the ratio $C_{Rafinate}/C_{Extract}$ in function of the residence time in the micro-extractor with a flow rate ratio of 1:1 (co-flow) and with a channel depth of 200 µm and 100 µm were breakthrough is not observed. Equilibrium is then always reached in the micro-extractor with a channel depth of 100 µm. The solid line is a theoretically predicted exponential curve, characterized by an overall mass transfer coefficient R and K the partition coefficient.

After the extraction kinetics were determined the microcontactor was exposed to more challenging samples, namely homogenized mice kidney samples containing solid entities. Despite the presence of these solid particles clogging did not occur.

To work in the detection range of the HPLC first a blank mice kidney sample is spiked with 100 mg/L MPB and passed through the microcontactor with a residence time of 10 min. The chromatogram of the acceptor phase is depicted in figure 3a. Second a homogenized kidney sample from MPB administered mice were passed through the microcontactor under the same conditions, no peak is visible (Fig 3b). Subsequently this acceptor phase is enriched by evaporating ethyl acetate in a second microcontactor. With a flow rate of 150µl/min for the acceptor phase and a N_2 pressure of 0.3bar the acceptor phase is enriched with a factor of 4.4. A peak for MPB is now visible in the chromatogram (Fig 3c) and corresponds to a concentration of 0.68 mg/L.

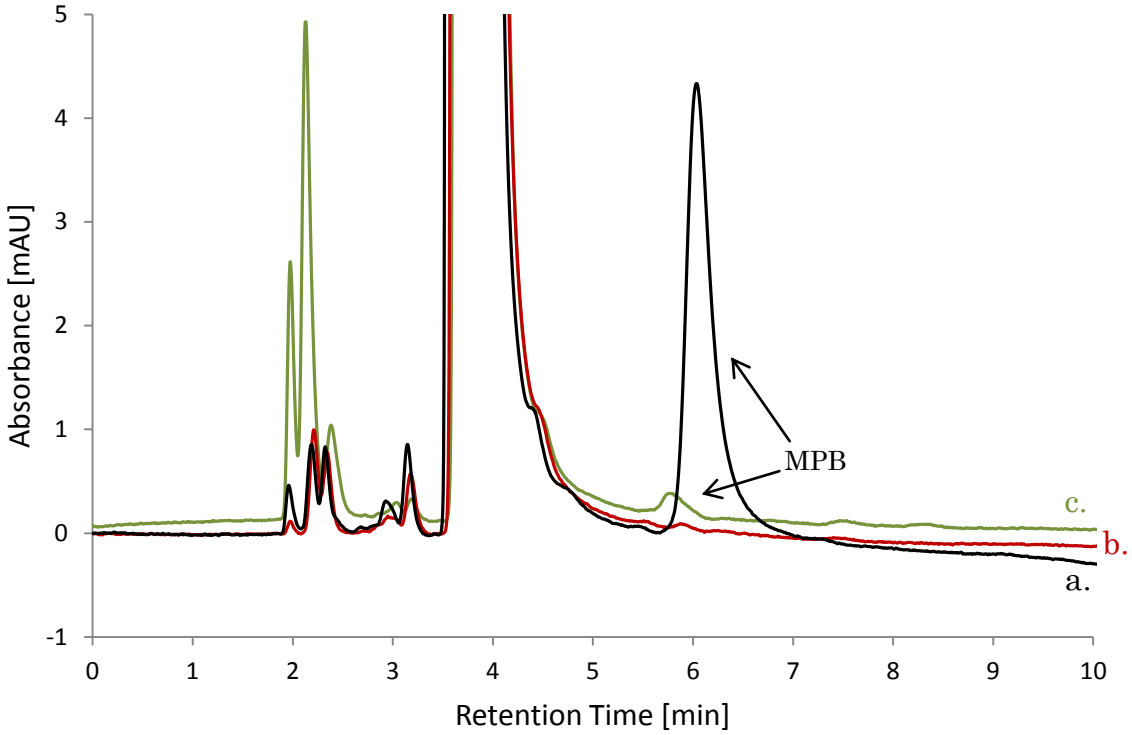

Figure 3: a) Chromatogram of acceptor phase of blank kidney sample spiked with 100 mg/L MPB b) Chromatogram of acceptor phase of the MPB administered kidney sample c) Chromatogram of evaporated acceptor phase of the MPB administered mice kidney sample.

CONCLUSION

The microcontactor was demonstrated to be a well suited tool for sample pre-treatment of tissue samples. A purification protocol could be developed, with the perspective to further automate the analysis set-up. The low sample amount needed for sample pre-treatment with a microcontactor, presents a valuable asset for drug analysis in early stage drug development, where potential drug candidates often have to be studied in small sample volumes. By investigating the extraction kinetics, a working range was defined where on the one hand equilibrium is reached and where on the other hand a compulsory phase separation step is avoided. By evaporating the acceptor phase an enrichment of a factor 4.4 could be achieved. The microcontactor has also proven capable to handle challenging solid samples.

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