MASARYKOVA UNIVERZITA

Annex No. 11 to the MU Directive on Habilitation Procedures and Professor Appointment Procedures

Habilitation Thesis Reviewer's Report

Masaryk University	
Faculty	Faculty of Science
Procedure field	Analytical Chemistry
Applicant	RNDr. Jiri Urban, Ph.D.
Applicant's home unit, institution	Masaryk University, Faculty of Science, Department of Chemistry
Habilitation thesis	Polymer-based monolithic stationary phases in the separation of small molecules
Reviewer	Prof. Dr. Michael Lämmerhofer
Reviewer's home unit, institution	Eberhard-Karls-University Tübingen, Institute of Pharmaceutical Sciences, Pharmaceutical (Bio-)Analysis

The habilitation thesis of Dr. Jiri Urban entitled "Polymer-based monolithic stationary phases in the separation of small molecules" reports on the preparation, tailoring of pore size and surface functionality, physical characterization of morphology and pore structure, respectively, and the application of organic polymer monoliths with particular focus on their optimization for small molecule separation by capillary liquid chromatography. It is a cumulative thesis consisting of an introduction part which puts into context the various studies performed on the thesis topic and appended to this thesis as scientific articles in peer-reviewed journals. The work sheds light on various topics of preparation, characterization and application of monolithic capillary columns. In the last 2-3 decades, monoliths have established themselves as one of the main players in the concert of modern chromatographic separation materials besides fully porous particles and superficially porous particles. Thereby, they have shown exceptional chromatographic performances for large bioparticles (viruses, plasmid DNA) and biopolymers (proteins, DNA, oligonucleotides, peptides). Their utility to small organic molecules was limited by some problems in terms of pore structure and morphology. To some extent, this limitation has been partly alleviated by specific optimization of the pore structure (tailoring the pore structure by reaction time to achieve bimodal distribution) and/or hyper-crosslinking approaches. Dr. Urban made significant contributions to this field and to organic polymer monolith technology in general.

In the first part of his work he describes how pore formation occurs in the course of the synthesis of organic polymer monolith structures and how it is possible to tailor their pore structure so that a good chromatographic performance can be achieved for small molecule separations. Crosslinker, functional monomers and porogen compositions all influence the morphology of the resultant monoliths and are parameters that must be fine-tuned to achieve optimal performance. Detailed characterizations of the porous properties of the monoliths using methods such as inverse size exclusion chromatography were carried out. By combining dedicated synthesis with detailed characterization of the hydrodynamic properties of monoliths obtained with altered reaction mixtures allowed to derived empirical models which can be used to produce monoliths with controlled porous structure and thus hydrodynamic properties. This was demonstrated for polymethacrylate monoliths used for size exclusion chromatography. In this part of the work Dr. Urban shows a good understanding of the many factors which have mutual influence on the porous properties of the materials. This work (publications 1 - 4 of the appendix) forms the basis for the many successful monoliths that have been synthesized subsequently.

The second set of articles (publications 6-8) was dealing with adjustment of the monolith surface for various chromatographic modes. Besides already mentioned materials for size exclusion chromatography, monoliths for reversed-phase (RP), hydrophilic interaction chromatography (HILIC) and mixed-modes have been developed. This can be achieved via incorporation of appropriate functional monomers in the polymerization mixture. For instance, RPLC-type monolithic materials have been developed by incorporating butyl, cyclohexyl, ethylhexyl, lauryl and stearyl methacrylate as functional monomer and ethylene dimethacrylate (EDMA) as crosslinker. The resultant monolithic capillary columns have been tested for the separation of small organic test compounds and proteins. Retention increased with chain length of the alkyl substituents especially for proteins. On the other hand there was not such a clear trend in terms of methylene selectivity. In any case the work showed that functional monomers are a proper means to adjust retention and selectivity and that the monolith technology offers flexible tailoring of the surface chemistry and retention properties. In another work, a zwitterionic functional monomer was copolymerized. This monomer imparted completely different retention behavior. Monoliths with this functional monomer exhibited dual mixed-mode RP and HILIC retention behavior for phenolic acids in the same capillary column depending on the organic modifier (acetonitrile) content. This is quite interesting because it increases the flexibility to optimize retentions and selectivities, and presumably offers an expanded application spectrum of such monolithic columns. In yet another work, *N*-isopropylacrylamide was copolymerized. This monomer is known to show thermally responsive retention behavior due to phase transition at a lower critical solution temperature. While such sudden change in the retention behavior was not observed, the new monoliths showed some interesting retention patterns. In all these studies Dr. Urban was able to derive some retention models describing the dependence of retention factors on mobile phase composition, which is quite useful in the course of optimization of the separation and for the understanding of the underlying retention mechanism.

The third part of the thesis (publications 9-13) is dealing with the topic of hyper-crosslinking to adapt the monolith properties for small molecule separations and improve efficiencies as well as decorate surfaces with specific interacting chromatographic ligands. For this purpose, monoliths were prepared from styrene, divinylbenzene, and vinylbenzylchloride. After formation of the monolithic bed the loose vinylbenzylchloride chains on the surface were crosslinked by Friedel-Crafts alkylation. With this additional step, mesopores could be introduced and formed, respectively, on the surface which has led to the significant increase in surface area. The resultant monoliths were better suited for small molecule separations. Experimental variables have been thoroughly investigated and optimized.

Interestingly it could be shown that different monomers could be grafted to the surface in a second step so that a flexible approach of tailoring surface chemistry for distinct chromatographic modes was established. This concept, which was demonstrated for a zwitterionic monomer and HILIC, is of great interest and potentially useful beyond the modes of chromatography shown in the work of Dr. Urban.

In the last part of the thesis (publications 14-17) monolithic capillary columns were utilized for various applications, some of them quite uncommon and innovative. For instance, a monolithic capillary column was hyphenated with an NMR microcoil for detection of the flow profile and for 2D NMR imaging getting distinction of organic molecules in the separation and NMR domain. Interestingly it could be shown that the flow profile in a monolithic bed is less parabolic i.e. more flat than in an open capillary which is susceptible for strong Taylor dispersion. The result is a better efficiency compared to open tubular LC. The work was quite innovative and could be published in Anal. Chem. In another application fast isocratic separation of polymers were developed and this enabled their use as the second dimension in 2D LC. In very recent work Dr. Urban integrates monolithic capillary column separation with sample preparation (i.e. online solid-phase extraction with vinylboronic acid functionalized monolith) for the analysis of neurotransmitters (e.g. dopamine) in urine. Since these analytes are present in very low concentrations, a sensitive electrochemical detection principle was integrated in the monolithic column as well. In a proof of principle application, epinephrine, dopamine, serotonin, homovanillic acid, 5-hydroxyindole-3-acetic acid, 3,4-dihydroxyphenyl acetic acid could be separated and detected in human urine. This work shows that the technology that has been developed over the years is suitable for real life applications. Although this is only the first step in direction of bioanalysis it shows that Dr. Urban is continuously extending the scope of research on monolithic materials.

The monolith technology has matured over the years, yet it is still unclear where there are its major application fields in the market. Dr. Urban provides a short discussion at the end of his thesis in which he argues that new technologies towards monolith preparation are emerging on the horizon such as 3D printing technology which could give the technology a push forward. With great interest we are looking forward to these developments. Dr. Urban also provides a short critical discussion on the performance of monolithic columns in comparison to packed columns with core-shell or sub-2µm particles. It is currently difficult for monolithic columns to compete with those highly efficient columns and it is questionable whether monolith technology can be optimized further to reach such performance. On the other hand, Dr. Urban states that monolith technology is more adaptive to miniaturization. I fully agree, monolithic materials are easier to integrate into microfluidic systems, have many applications beyond classical column chromatography and are expected to find applications in this field.

The thesis of Dr. Urban represents a nice compilation of solid work on a modern topic in separation science. Both miniaturized separation devices and functional materials are of great interest in the analytical community. The work is without flaws and of high technical quality. Without doubt the thesis of Dr. Urban represents a good international standard and the work is of significant interest to the separation science community.

Overall, Dr. Urban submits a thesis with substantial contributions to the field of analytical chemistry, and in particular separation science. He has authored or co-authored already more than 30 publications, some of them received over 100 cites which is exceptional. He has two Analytical Chemistry papers, the top journal in the fields of analytical chemistry, and an *h*-factor of 15 which is formidable considering his age. Most importantly, Dr. Urban is in 9 of the 17 papers, submitted for the cumulative thesis, corresponding author, being responsible for selection of the research topic, the design of the study, controlling the technical quality and quality of the publication. He was also already successful in applications for grants. This documents that he can lead research groups and research projects, respectively. Dr. Urban is also well connected to the international network of separation scientists. He is integrated in the community and well recognized as a leading scientist in monolith technology and monolithic separation materials, respectively. He gained experience by his research stays abroad working together with internationally leading scientists such as Dr. Frantisek Svec or Dr. Peter Schoenmakers. In my opinion he fulfills all requirements to be promoted by acceptance of this thesis.

Reviewer's questions for the habilitation thesis defence (number of questions up to the reviewer)

Please give a short discussion on the advantages, disadvantages of monolithic separation materials (organic polymer monoliths) in comparison to particle packed columns, in particular also focusing on sub-2µm particles and superficially porous particles!

What are the capabilities of monolithic columns in terms of fast chromatography (sub-minute time scale)?

Interestingly, little is reported in the literature on the use of organic polymer monoliths in SFC. Do you see any potential for this combination in the small molecule separation field?

Conclusion

The habilitation thesis entitled "Polymer-based monolithic stationary phases in the separation of small molecules" by Jiri Urban ***fulfills*** the requirements expected of a habilitation thesis in the field of Analytical Chemistry.

In Tübingen on October 26, 2017