PREFACE

Welcome to the 18th International Symposium on Flow- and Field Based Separations (FFF2016). Established in 1989 by the inventor of the field flow fractionation technique, John Calvin Giddings, the FFF symposium have become the leading international platform in the field.
In the last twenty years FFF has increasingly been used in branches such as materials research, pharmacy, environmental research, and toxicology. The diversity of applications requires and inspires exchange on methodology, recent results and challenges for scientists using FFF in research or industrial applications and engineers working on further development of the equipment.
We hope that FFF2016 will provide a pleasant and stimulating forum of interdisciplinary exchange and give new impetus to the FFF community!

Topics (related to FFF)

- Nanomaterials & Related Technology
- Synthetic & Natural Macromolecules
- Cells, Viruses & Bioparticles
- Proteins & Drug Delivery
- Environment & Toxicology
- Food & Agriculture
- Theory & Instrumentation Development
- Industrial Application of FFF

ORGANIZATION

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Organizing Institute
Leibniz-Institut für Polymerforschung Dresden e. V.

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The organizers gratefully acknowledge the generous financial support by

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GENERAL INFORMATION

Venue and registration/conference office
Hotel Elbflorenz
Rosentraße 36, 01067 Dresden
Phone: +49 351 8640-500 www.hotel-elbflorenz.de

The conference office is located in the first floor.
Phone: 0160/97216924

Opening hours:

Sun, May 22, 2016:	from 17:00 to 20:00
Mon, May 23	from 7:40 throughout all sessions
Tue, May 24 to Thu, May 26, 2016:	from 8:00 throughout all sessions

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Coffee and lunch breaks
Drinks, coffee breaks and a lunch buffet are included and will be served in the lobbies adjacent to the lecture hall and the poster display and exhibition areas.

Internet
WLAN/WiFi access is possible for participants during the meeting and is free of charge.
Participants may get individual access data at the hotel reception upon presentation of their identity card or passport.
Participants accommodated in hotel Elbflorenz may login with the room number and the reservation number.
In addition, you may use an internet terminal at the conference office.

Book of abstracts
The book of abstract (hard copy) includes the short abstracts of all papers and posters and will be published with the ISBN number 978-3-9816007-3-5. A pdf file of all presentations will be made available to the participants

EXHIBITION AND PRODUCT SEMINARS

Relevant instruments are presented by Postnova and Wyatt
in an exhibition throughout the symposium

and in product seminars:	Postnova	Tuesday, May 24, 11:20
	Wyatt	Wednesday, May 25, 11:30

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PRESENTATIONS

Oral presentations
To upload your oral presentation, please contact one of our student helpers in the session hall. You should do that in advance, at the latest in the break before your presentation.

Posters
Posters will be up for the whole duration of the symposium.
Please fix your poster as early as possible after your check-in and remove it by 14:00 on Thursday.

Each poster author is requested to introduce his/her poster in a short oral presentation of 2 to 3 minutes (max. 2 to 3 charts). For precise presentation times please cf. assignment of poster numbers.

AWARDS

Awards will be handed over for the best oral presentations by students and for the best posters. The awards are sponsored by Springer Analytical & Bioanalytical Chemistry and Wiley VCH Macromolecular Journals.

SOCIAL EVENTS

- **Welcome Mixer**
  Sunday, May 22, 2016, 18:00 to 20:00
  Hotel Elbflorenz (symposium venue)
  You may enjoy some snacks and drinks while having the first talks to your colleagues.

- **Sightseeing and Conference Dinner**
  Tuesday, May 24, 2016, 18:00
  Tour of the Transparent Factory/Gläserne Manufaktur of VW and subsequent dinner (19:30) in the Restaurant Lesage located in the complex

No special dressing code. Registration is required (no extra charge for active participants and officially registered accompanying persons) – make sure you have the voucher in your conference bag and bring them along.

From the symposium venue you reach the dinner location in a 35 minutes' walk
or
by tram line 10 from stop Hp Freiberger Straße (in front of Hotel Elbflorenz, direction to Striesen) to stop Straßburger Platz (6 stops, 10 minutes, trams leave in 10 minutes' intervals: ...:10; ...:20; ..:30...)

Monday, May 23		
07:40	Registration	
08:30	Opening	
	Chair: W. Kok	
08:50	PL1 - Francoise Winnik	Characterization of responsive polymersomes and nanogels by asymmetrical flow field-flow fractionation
09:30	K1 - Lars Nilsson	Asymmetric flow field-flow fractionation: A powerful tool in food technology
10:00	O1 - Ryan R. Manning	Use of AF4 in peptide formulation and stabilization
10:20	O2 - Claudia Zielke	Separation and analysis of polysaccharide mixtures utilizing asymmetric flow field-flow fractionation coupled to multiangle light scattering (MALS)
10:40	*Break and Poster Discussion*	
	Chair: M. H. Moon	
11:10	K2 - Kim Williams	Thermal diffusion, soret coefficients, and thermal FFF of polymers and nanoparticles
11:40	O3 - Guilaume Greyling	Multidetector thermal field-flow fractionation as a unique tool for the characterisation of block copolymer micelles
12:00	O4 - James D. Oliver	FFF of branched polymers: Pushing FFF further with a second dimension of capillary electrophoresis
12:20	O5 - Stepan Podzimek	Characterization of the molecular and chemical structure of acrylic emulsion polymers by asymmetric flow field-flow fractionation coupled with a multi-angle light scattering detector
12:40	Poster Presentation nr. 1 - 10	
13:10	*Lunch and Poster Discussion*	
	Chair: H. Pasch	
14:10	K3 - Michael Maskos	Characterization of nanoparticles: Contributions of different field-flow fractionation methods
14:40	O6 - Manuel Correia	Simultaneous on-line detection of SiO_2, TiO_2 and Al_2O_3 particles in toothpaste by asymmetric flow field-flow fractionation hyphenated to triple-quadrupole inductively coupled plasma mass spectrometry
15:00	O7 - David Müller	Inverse supercritical fluid extraction as efficient tool for FFF sample pretreatment and its application to the analysis of sunscreens
15:20	O8 - Aljosha-Rakim Jochem	Ligand dependent particle losses of gold nanoparticles during AF4: Electrostatic versus polymeric stabilization
15:40	O9 - William C. Smith	Composition and size-based separation of metal hybrid nanoparticles by thermal field-flow fractionation
16:00	*Break and Poster Discussion*	
	Chair: M. Maskos	
16:30	O11 - Jone Omar	Characterisation of polydisperse TiO_2: A step towards the implementation of the EC definition on nanomaterial
16:50	O12 - Catia Contado	Does a centrifugal field-flow fractionation system equipped with an UV-Vis absorption detector allow characterizing silver nanoparticle suspensions for sizes and concentration?
17:10	O13 - Carina A. Sötebier	Characterization of silver nanoparticles: Limitation and advantages of field-flow fractionation
17:30 - 17:50	O14 - Haruhisa Kato	Characterization of size and size distribution of nanomaterials: A comparison of scanning electron microscopy, dynamic light scattering, and flow field-flow fractionation methods

Tuesday, May 24		
08:00	Registration	
	Chair: K. Williams	
08:30	PL2 - Ulrich Schubert	The need of novel characterization methods in the advanced materials science
09:10	K4 - Martin Brandl	Flow field-flow fractionation: A tool for liposome size-analysis and drug-release/-transfer studies
09:40	O15 - Johannes Fingernagel	Biohybrid structures of proteins and dendritic glycopolymers characterized by AF4
10:00	O16 - Sandrine Huclier-Markai	Characterization of gadolinium nanohydrogels for MRI by field-flow fractionation techniques
10:20	Poster Presentation nr. 11 - 20	
10:50	*Break and Poster Discussion*	
	Chair: M. Baalousha	
11:20	Product Seminar Postnova	
11:50	O17 - Florian Meier	Poly(lactic-co-glycolic acid) nanoparticles in cell medium used as bio-compatible substrates in pharmaceutical applications: Comprehensive characterization with centrifugal field-flow fractionation coupled with online dynamic light scattering
12:10	O18 - Ugo Till	Poly-ion complexes analysis with Frit Inlet flow field-flow fractionation systems
12:30	O19 - Simona Sitar	Size characterization and quantification of exosomes by AF4
12:50	Poster Presentation nr. 21 - 30	
13:20	*Lunch and Poster Discussion*	
	Chair: F. Winnik	
14:30	K5 - Myeong Hee Moon	Flow field-flow fractionation with mass spectrometry for subcellular proteins and metalloproteins
15:00	O20 - Lee Moore	Continuous magnetic depletion of red blood cells from whole blood by magnetic SPLITT
15:20	O21 - Carmen Bria	Impact of asymmetrical flow field-flow fractionation on protein aggregate stability
15:40	O22 - Bruce Gale	Separation of exosomes with electrical field-flow fractionation
16:00 - 16:30	K6 - Serge Battu	Field-flow fractionation for stem cells sorting: From normal, embryonic and cancer stem cells to a new development in the field of human induced pluripotent stem cells

18:00 - 00:00	Tour of the Transparent Factory/Gläserne Manufaktur of VW and subsequent dinner in the Restaurant Lesage

Wednesday, May 25		
08:00	Registration	
	Chair: M. Martin	
08:30	Tribute to Francesco Dondi	
08:40	PL3 - Helmut Coelfen	Analytical ultracentrifugation: Multiwavelength UV-Vis analysis in high resolution analysis of functional nanoparticles and polymers - possibilities and limits and a comparison to Fl-FFF
09:20	K7 - Oleg Iliev	Numerical simulation as a powerful tool to understand and improve flow-FFF separation
09:50	O23 - Petr S. Fedotov	Sedimentation field-flow fractionation of nano- and microparticles in rotating coiled columns: Theory and applications
10:10	O24 - Maria Marioli	Continuous AF4 for the fractionation and purification of biomolecules and nanoparticles with the use of microstructured membranes
10:30	Poster Presentation nr. 31 - 40	
11:00	*Break and Poster Discussion*	
	Chair: V. Hackley	
11:30	Product Seminar Wyatt	
12:00	O25 - Norbert Löwa	A novel magnetic FFF detector for the quantification and characterization of magnetic nanoparticles
12:20	O26 - Timo F. Beskers	Online coupling of FFF and FTIR spectroscopy
12:40	O27 - Christoph Johann	Combination of electrical and flow field-flow fractionation to measure electrophoretic mobility of nanoparticles and proteins
13:00	Poster Presentation nr. 41 - 50	
13:30	*Lunch and Poster Discussion*	
	Chair: S. Boye	
14:40	K8 - Julien Gigault	Asymmetric-flow field-flow fractionation: A powerful technique for characterizing nanomaterials (with many cautions)
15:10	O28 - Sachin Vilas Nehete	Uranium speciation in soft water using asymmetrical flow field-flow fractionation coupled with UV and inductively coupled plasma mass spectrometry (AsFlFFF-UV-ICP-MS)
15:30	O29 - Florian Dutschke	Development of an analytical approach using centrifugal field-flow-fractionation hyphenated to ICP-MS/MS for the detection and
15:50	O30 - Björn Meermann	Tracing and quantification of isotopically modified iron nanoparticles in a sediment slurry matrix via AF4/ICP-SFMS
16:10	O31 - Vaughn Mangal	Utilizing asymmetrical flow field-flow fractionation and high resolution mass spectrometry to assess the role of dissolved organic matter size and composition on mercury bioavailability
16:30	*Break and Poster Discussion*	
	Chair: L. Nilsson	
17:00	K9 - Antje Potthast	AsFlFFF for characterisation of polymers from renewable resources - Challenges and (some) solutions
17:30	O32 - Tomasz Kowalkowski	Application of self-adjustable split-flow lateral-transport thin channel for separation of environmental microparticles
17:50	O33 - Zhiqiang Tan	Study on environmental effects of nanomaterials based on hollow fiber flow field-flow fractionation
18:10 - 18:30	O34 - Zhiyuan Gao	Molecular weight distribution of marine dissolved organic matter in highly stratified Arctic Ocean

Thursday, May 26		
08:00	Registration	
	Chair: E. Mes	
08:30	K10 - Wei Gao	Characterization of colloidal particles in water using asymmetrical flow field-flow fractionation with advanced detection: Challenges and progresses
09:00	O35 Mikhail S. Ermolin	Preparative separation of particulate functional materials using field-flow fractionation in rotating coiled columns
09:20	O36 - Irina Sulaeva	Applicability of AsFlFFF for industrial lignosulfonate analysis
09:40	*Break*	
	Chair: B. Gale	
10:10	K11 - Vincent A. Hackley	Electrospray-differential mobility analysis: An introductory overview and applications in nanomedicine
10:40	O37 - Xiaotong Fu	Microfluidic free flow electrophoresis using tunable conductive PDMS polymer membranes
11:00	O38 - Robert Stange	Biomagnetic separation with variable electromagnetic induced force fields
11:20	O39 - Torsten Kreer	Translocation of macromolecules through polymer-brush covered microchannels
	Chair: A. Lederer	
11:40	PL4 - Stefan Diez	Separation and detection of analytes by biomolecular transport systems
12:20	Discussion session and presentation of the best posters	
12:50	Closing session	
13:20 - 14:20	Lunch and Farewell	

List of posters

#	Title	Authors
1	Characterization of stimuli responsive microgels with flow field-flow fractionation	P. Kodlekere, **Wenjing Xu**, A. Pich
2	Nanogels based on poly(methacrylic acid)/ poly-acrylamide interpenetrating polymer networks as drug delivery system for verapamil hydrochloride	M. Simeonov, **Susanne Boye**, A. Lederer, E. Vassileva
3	Generic sample preparation procedure for isolating inorganic engineered nanoparticles from complex matrixes	**Milica Velimirovic**, St. Wagner, F. Abdolahpur Monikh, F. von der Kammer, T. Hofmann
4	Asymmetric flow field-flow fractionation for characterization of highly concentrated conjugates of gold nanoparticles and antibodies	**Irina V. Safenkova**, E. S. Slutskaya, V. G. Panferov, A. V. Zherdev, B. B. Dzantiev
5	Determination of mass and density of nanomaterials using centrifugal field-flow fractionation, single particle ICP-MS and transmission electron microscopy	**Soheyl Tadjiki**, F. Meier, T. Pfaffe, E. Moldenhauer, Th. Klein
6	Large-scale synthesis and size characterization of silica nanoparticles using ssymmetrical flow field-flow fractionation (AF4)	**Sujeong Han**, J. Choi, Y. Yoo, W.-j. Kim, E. Chang Jung, S. Lee
7	El-FFF separation of nanoparticle mixtures	**Farhad Shiri**, K. E. Petersen, B. K. Gale
8	Hyphenation of field-flow fractionation and single particle ICP-MS for the assessment of number-based particle size distributions at ultratrace levels	**Soheyl Tadjiki**, T. Pfaffe, E. Moldenhauer, F. Meier, Th. Klein
9	AF-4 to characterize sulfated GAG building blocks for cell instructive biohybrid hydrogels	**Susanne Boye**, P. Atallah, U. Freudenberg, C. Werner
10	Analysis of thermoreversibly crosslinking polymer networks by temperature dependent size exclusion chromatography	**Johannes Lenz**, J. Brandt, K. Pahnke, Ch. Barner-Kowollik, F. Georg Schmidt, A. Lederer
11	Temperature dependent size exclusion chromatography for the in situ investigation of dynamic bonding/debonding reactions	**Josef Brandt**, N. K. Guimard, K. Pahnke, K. K. Öhlenschläger, Ch. Barner-Kowollik, F. Georg Schmidt, A. Lederer
12	A biohybrid topological diversity investigated by asymmetrical flow field-flow fractionation	**Susanne Boye**, F. Ennen, D. Appelhans, A. Lederer
13	Hydrodynamic characterization of functional poly(ethylene glycol)s by means of analytical ultracentrifugation and viscosimetry	**Ivo Nischang**, I. Perevyazko, U. S. Schubert
14	Synthesis and characterisation of a polyothio-phene-oligodeoxynucleotide block copolymer for the site-specific attachment to DNA origami	**Johanna Zessin**, S. Boye, F. Fischer, A. Heerwig, A. Kiriy, M. Mertig
15	Polysaccharide characterization by HF5 with on-line multi-angle static light scattering and differential refractometry	**Leena Pitkänen**, A. M. Striegel
16	Characterization of natural rubber samples via thermal field-flow-fractionation	G. Heinzmann, F. Meier, **Evelin Moldenhauer**, Th. Klein, S. Tadjiki
17	Multi-detector thermal field-flow fractionation of elastomers: Relating elastomer properties to the thermal diffusion coefficient	**James D. Oliver**, K. P. Bierbaum, S. K. R. Williams
18	Simultaneous in-situ monitoring of polymerization reactions via AF4 or SEC coupled with multidetectors	**Chiu-Hun Su**, C. T. Wang, C. H. Chen, J. Y. Hwang, L. D. Tsai

19	Multidetector thermal field-flow fractionation as an innovative tool for microstructure separation of synthetic polymers	**Guilaume Greyling**, H. Pasch
20	Study on microgel-containing butadiene rubbers (BR) using thermal field-flow fractionation coupled with multi-angle light scattering (ThFFF-MALS)	**Jaeyeong Choi**, A-J. Kim, S. H. Lee, S. Lee
21	Optimisation and characterisation of the synthesis of mono- methoxy poly (ethylene glycol)-block-poly(4-vinyl pyridine) (PEG-b-P4VP) by asymmetric flow field-flow fractionation (AF4) and multiangle light scattering (MALS)	**Catalina Fuentes**, J. Castillo, L. Nilsson
22	Characterization of variously branched dendritic polyethylene by SEC-LS and AF4-LS	**Laura Schlechte**, S. Boye, R. Mundil, J. Merna, A. Juriju, J.-U. Sommer, A. Lederer
23	Straightforward analysis of PEG-peptide conjugates	**Marcus Binner**, M. Tsurkan, C. Werner
24	Characterization of polymeric vesicles by AF4	**Susanne Boye**, D. Appelhans, A. Lederer
25	Characterization of plasma proteins and lipo-proteins using microchannel asymmetrical flow FFF	**Soheyl Tadjiki**, R. Reed, R. Welz, T. Pfaffe, F. Meier, R. Drexel, Th. Klein
26	Use of AF4 to study the conformation and stability of interferon-tau	**Ryan R. Manning**, R. E. Holcomb, R. W. Payne, B. M. Murphy, A. Tellechea, R. J. Krammes, N. S. Krammes, G. A. Wilson, M. C. Manning
27	Analysis of urinary exosomes by asymmetrical flow field-flow fractionation	**Joon Seon Yang**, M. H. Moon
28	Asymmetric flow field flow fractionation methods for virus purification	**Katri Eskelin**, M. Lampi, D. H. Bamford, H. M. Oksanen
29	Profiling of metalloproteins from plasma using miniaturized AF4 coupled with ICP-MS	**Jin Yong Kim**, H. B. Lim, M. H. Moon
30	Programming considerations in modified full feed depletion magnetic SPLITT device	**Benno Kraft**, P. Joshi, E. Boschke, J. J. Chalmers, M. Zborowski, L. R. Moore
31	Tunable polymersomes by pH-triggered encapsulation of rhodamine	**Johanna Kerber**, S. Boye, A. Lederer, H. Gumz, D. Appelhans
32	Analyzing enzyme encapsulation in smart polymersome nanoreactors	**Hannes Gumz**, V. Krönert, S. Boye, B. Voit, D. Appelhans
33	Bile salt micelles and phospholipid vesicles present in artificial and aspirated human intestinal fluids: A flow field-flow fractionation/ multi-angle laser light scattering-study	**Philipp A. Elvang**, A. H. Hinna, J. Brouwers, B. Hens, P. Augustijns, M. Brandl
34	AF4 characterization of nanoemulsions of lipid droplets covered by a monolayer of sphingomyelin and cholesterol	**Valerija Vezočnik**, S. Sitar, K. Kogej, M. Tušek Žnidarič, K. Sepčič, D. Pahovnik, P. Maček, E. Žagar
35	Analysis of the protein corona using asymmetrical flow field-flow fractionation	**Claudia Weber**, C. Rosenauer, K. Mohr, K. Landfester, S. Winzen
36	Combining AF4-ICP-MS and SP-ICP-MS with XAS techniques for the characterization of soil colloids involved in the mobilization of arsenic	**Miguel Angel Gomez-Gonzalez**, E. Bolea, J. García-Guinea, F. Garrido, F. Laborda
37	Towards trace level analysis of silver nanoparticles in environmental samples using asymmetrical flow field-flow fractionation coupled with UV, DLS and ICP-MS	**Florian Meier**, A.-L. Grün, Ch. Emmerling, A. Antipov, S. Straskraba, M. Schmidt, C. Büchel, P. Diehl, Y. Kohl

38	Analysis of low and intermediate sized beta-glucan from barley products and their relation to proteins and the consumers' health	**Claudia Zielke**, C. Teixeira, M. Nyman, L. Nilsson
39	Study of aptamer - Tetracycline complex by asymmetric flow field-flow fractionation	**Irina V. Safenkova**, E. S. Slutskaya, A. V. Samokhvalov, A. V. Zherdev, B. B. Dzantiev
40	Charaterization of macromolecules in beer using asymmetrical flow field-flow fractionation (AF4) coupled with multi-angle light scattering (MALS)	**Jaeyeong Choi**, H. Dou, C. Zielke, L. Nilsson, S. Lee
41	Compositional effects on the association of casein	**Norbert Raak**, S. Boye, A. Lederer, H. Rohm, D. Jaros
42	Structural and conformational analysis of β-glucan from oat and barley using asymmetric flow field-flow fraction (AF4)	**Claudia Zielke**, M.-L. Ainalem, A. Stradner, L. Nilsson
43	Recent advances in El-SPLITT: A flow addition with porous electrode	K. E. Petersen, B. King, T. White, F. Shiri, J. L. Hood, S. A. Wickline, **Bruce K. Gale**
44	Development of continuous two-dimensional asymmetrical flow field-flow fractionation for particles: Principle, instrument development and applications	M. Jussila, K. Moslova, **Pertti Vastamäki**, M.-L. Riekkola
45	Semi-preparative asymmetrical flow field-flow fractionation for nanoparticle characterization	**Carmen R. M. Bria**, P. W. Skelly, A. A. Ashames, S. K. R. Williams
46	Ionic strength effect on retention behavior in sedimentation field-flow fractionation (SdFFF)	**Sujeong Han**, J. Choi, K. Rah, S. Lee
47	What are the assumptions behind the basic retention equation in FFF ?	**Michel Martin**
48	Covalent modification of ultrafiltration membranes for flow field-flow fractionation	**Carmen R. M. Bria**, F. C. Prehn, C. Martin, A. Sledgianowski, St. G. Boyes, S. K. R. Williams
49	Length selection and replication in a thermal flow chamber	**Lorenz Keil**, S. Lanzmich, M. Kreysing, M. Hartmann, D. Braun
50	Characterization of synthetic polymers in organic media using asymmetrical flow field-flow fractionation: Development of industrial applications	**Mubasher A. Bashir**, W. Gao, E. Mes

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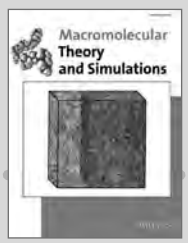

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Abstracts of the plenary lectures

PL1

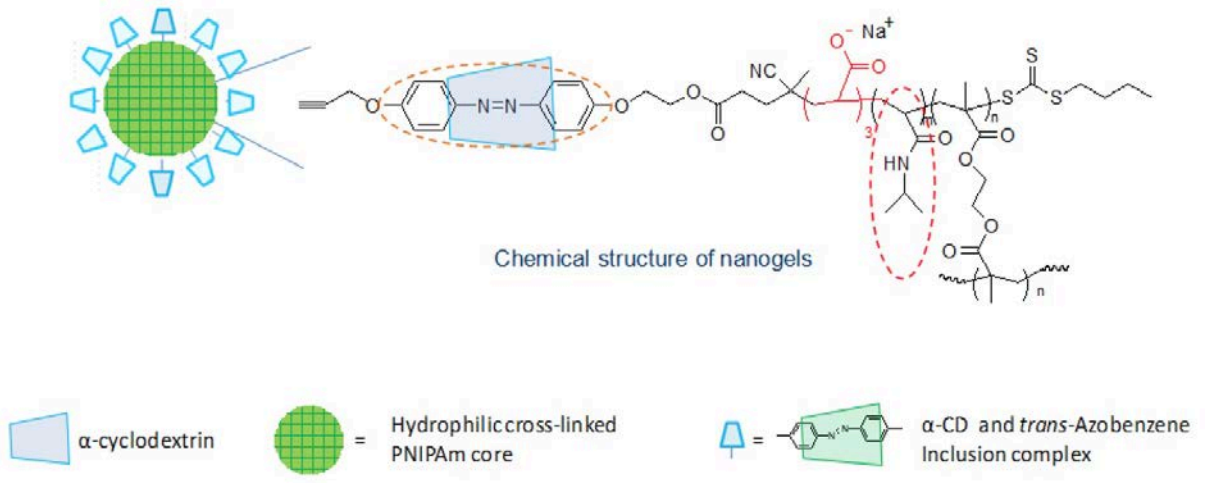
CHARACTERIZATION OF RESPONSIVE POLYMERSOMES AND NANOGELS BY ASYMMETRICAL FLOW-FIELD FLOW FRACTIONATION

D. Ma, Francoise M. Winnik
University of Montreal, Department of Chemistry, Canada and NIMS, WPI International Center for Materials Nanoarchitectonics, Tsukuba Japan

francoise.winnik@umontreal.ca

We have made extensive use of asymmetrical flow-field flow fractionation (AF4) coupled online to UV-Visible absorption, fluorescence, multiangle light scattering (MALS) and dynamic light-scattering (DLS) detectors to characterize macromolecular nanoparticles consisting of biomacromolecules and synthetic polymers.

To illustrate the advantages of AF4 over other characterization methods, we will present its use as part of a study of the interactions between polymersomes of poly(isopropyloxazoline)-b-poly(dimethylsiloxane)-b-poly(isopropyloxazoline) with di-myristoylphosphatidylcholine liposomes. The encapsulation and release of dyes and proteins entrapped in polymersomes and in hybrid copolymer/phospholipid vesicles were moniotored by AF4. In a second study, AF4 was used to characterize multiresponsive nanogels formed upon of complexation of α-cyclodextrin and azobenzene groups linked to poly(N-isopropylacrylamide). Perspectives on the use of AF4 to study waterborne polymeric dispersions will be discussed with emphasis on materials of use in the food and drug formulations.



Structure of the nanogels

PL2

THE NEED OF NOVEL CHARACTERIZATION METHODS IN THE ADVANCED MATERIALS SCIENCE

Ulrich S. Schubert
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The area of advanced materials has diversified rapidly with the inception of a variety of synthetic methods to create soft matter components for life science and energy application platforms. The structure and chemical identity of such components is crucial for the resultant implementation. In the case of macromolecular and multi-functional constructs, this requires exact knowledge and detailed reconciliation of the absolute molar mass, functionality, and conformation in solution. Methods to determine molar masses based on most commonly pursued approaches such as size exclusion chromatography (SEC) fall short in their suitability for more complex macromolecular systems. Notwithstanding, together with mass spectrometry (MS) and the diversity of spectroscopic techniques, these are standard to almost any laboratory. The inherent roadblock for a fundamental characterization of advanced material precursors urgently needs to be overcome.

Several sophisticated options of choice for characterization of macromolecules are found in the overarching family of hydrodynamic principles. These methods find full emphasis in techniques such as analytical ultracentrifugation (AUC) and the family of flow field fractionation implementations, e.g. asymmetric flow field-flow fractionation (AF4). AF4 as well relies on first principles and its coupling to multi-angle laser light scattering (MALLS) is increasingly recognized as a valuable tool for the determination of absolute molar masses and dispersities.

This contribution aims at showing the diversity of such and related solution characterization approaches for reconciliation of the structure of colloidal matter including large macromolecules, macromolecular assemblies, e.g. in the form of micelles, as well as more complex constructs such as nanoparticles. The diversity is enriched by contemporary and new examples for which common characterization approaches such as SEC and MS hit fundamental roadblocks.

PL3

ANALYTICAL ULTRACENTRIFUGATION: MULTIWAVE-LENGTH UV-VIS ANALYSIS IN HIGH RESOLUTION ANALYSIS OF FUNCTIONAL NANOPARTICLES AND POLYMERS - POSSIBILITIES AND LIMITS AND A COMPARISON TO FL-FFF

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Analytical Ultracentrifugation (AUC) is a classical method of colloid and polymer analysis, which nowadays is almost exclusively applied to the solution of biophysical problems although its resolution of nanoparticles is superior and separations with Angström resolved particle size distributions have meanwhile even reached the realm of technical routine. The recent development of a CCD array based UV-Vis multiwavelength detector (MWL) for AUC now enables the detection of entire UV/Vis spectra instead of single wavelengths and allows for the detection of a three dimensional data space directly showing spectral changes with the sample size or composition. This is first demonstrated for the case of a protein-DNA mixture as common case in biophysical characterizations. However, the baseline resolution can be extremely high in the range of a few Angströms as will be demonstrated for CdTe semiconductor nanoparticles. Here, a mixture of more than 20 species can be hydrodynamically resolved and spectra of the individual compounds are also available revealing the size dependence of semiconductor optical properties in a single experiment. Experimental raw data from this detector can even yield significant information about the sample without any evaluation as will be demonstrated for different industrial β-carotene samples. Such high-resolution particle size analysis calls for the detection of early nucleation species. Application of MWL-AUC to nucleation of nanoparticles will be demonstrated. Also, the possibilities of MWL-AUC will be compared with the possibilities of AF4 equipped with a MWL detector.

PL4

SEPARATION AND DETECTION OF ANALYTES BY BIO-MOLECULAR TRANSPORT SYSTEMS

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We are interested in the application biomolecular motor systems in a synthetic, engineered environment for the generation and manipulation of nanostructures as well as novel approaches in molecular detection. Thereby, our main emphasis is on the development of methods to control the nano-transport systems by external signals in a spatio-temporal manner. Towards this end we investigate novel bio-template-based nano-structuring techniques and fabricate smart composite surfaces, where motor proteins are embedded in stimuli-responsive polymer layers. Current applications of our biomolecular transport systems include approaches towards nanoscopic surface imaging and parallel biocomputation as well as the separation and detection of analytes for future molecular-diagnostics devices.

Abstracts of the keynote lectures

K1

ASYMMETRIC FLOW FIELD-FLOW FRACTIONATION: A POWERFUL TOOL IN FOOD TECHNOLOGY

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Most formulated foods contain macromolecules and/or colloidal particles and these play an important role in influencing the properties of foods. The properties include technological and nutritional as well as consumer perceived properties. Characterization of such macromolecules and colloidal particles can prove to be a substantial challenge due their often large molar mass and size as well as high dispersity. Food macromolecules are often present in highly aggregated structures which further complicate the analysis. Asymmetric flow field-flow fractionation (AF4) is a powerful separation technique with many applications in food technology. By using AF4 in conjunction with multiangle light scattering (MALS) (and other detectors) molar mass and size can be obtained over a large size distribution but also different conformational properties can be analyzed. AF4 is also valuable as a preparative tool for further off-line analyses. Results and challenges from several food related systems are discussed in this talk. These include characterization of ultra-high molar mass polysaccharides, proteins and lipid vesicles.

K2

THERMAL DIFFUSION, SORET COEFFICIENTS, AND THERMAL FFF OF POLYMERS AND NANOPARTICLES

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Thermal field-flow fractionation (ThFFF) has the distinction of being both the oldest and the least well understood of the FFF family of techniques. The latter makes ThFFF a very interesting technique with capabilities that are remarkably different from those of other FFF techniques. The externally applied field is a temperature gradient that evokes the thermal diffusion of analytes and solvent molecules. Differences in the ratio of the thermal diffusion coefficient to the diffusion coefficient (DT/D, also known as the Soret coefficient ST) can lead to polymer separations by molecular weight, composition, and tacticity [1-3].

We have taken a combined theory and experimental approach to understanding thermal diffusion and to developing new capabilities for complex polymers and nanoparticles analyses. The role of solvents has been examined and a universal calibration approach has been established for determining composition distributions of copolymers. A route towards obtaining the number of polymer chain ends has also been devised and applied to different polymer architectures such as stars and bottlebrushes. Very importantly, these ThFFF chain end determinations are accomplished without the use of linear polymer analogues and represent a significant breakthrough in the analysis of non-linear polymer architectures. Furthermore, the Soret coefficient which is readily calculated from retention time can provide insights into the type of polymer architecture. This presentation addresses recent developments in ThFFF pertaining to polymer analyses and touches on our current work with nanoparticles.

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K3

CHARACTERIZATION OF NANOPARTICLES: CONTRIBUTIONS OF DIFFERENT FIELD-FLOW FRACTIONATION METHODS

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The succesful characterization of dispersed nanoparticles is still a challenging task. Typically, a multitude of different analytical methods and tools are needed to obtain a somewhat clear picture of the nanoparticle properties. Due to the fact that dispersed nanoparticles (comparable to colloids) most of the time are sensitive to changes of their environment, characterization methods that need additives are increasingly challenging. For example, surfactants are typically and frequently employed in Asymmetrical Flow Field-Flow Fractionation (AF-FFF), having the potential to alter the properties of an analysed sample. This contribution will discuss some examples of different nanoparticles and their characterization with different types of FFF techniques, namely AF-FFF and thermal FFF (Th-FFF) and what kind of information these methods can successfully deliver.

K4

FLOW FIELD FLOW-FRACTIONATION: A TOOL FOR LIPOSOME SIZE-ANALYSIS AND DRUG-RELEASE/-TRANSFER STUDIES

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AF4-MALLS-derived size distributions of filter-extruded vesicles of egg phosphatidylcholine (evtl with cholesterol) are set in relation to extrusion parameters (pore-size, number of filter passages, and flow-rate) and compared to data of dynamic light scattering, cryo-transmission electron microscopy and 31P-NMR-analysis of lamellarity. Liposome sizes and decrease with sequential extrusion through smaller pore size filters, starting at a size range of 70 – 415 nm upon repeated extrusion through 400 nm pore-filters, eventually ending with a size range of 30 – 85 nm upon extrusion through 30 nm pore filters. While for small pores (50 nm), increased flow rates resulted in smaller vesicles, no significant influence of flow rate on mean vesicle size was seen with larger pores. Cholesterol at increasing mol-fractions up to 0.45 yielded bigger vesicles (at identical process conditions). For a cholesterol mol-fraction of 0.5 in combination with small filter pore size, a bimodal size distribution was seen indicating precipitation of cholesterol micro-crystallites.

The 2nd aim of the presentation is to introduce AF4 as a tool for in vitro drug-release / drug-transfer studies. The porphyrin derivative p-THPP (5,10,15,20-Tetrakis(4-hydroxyphenyl)21H,23H-porphine; as hydrophobic model drug. Small, p-THPP-containing liposomes (diameter ≈70 nm) were prepared and their drug-retention established. To this end, they were blended with large acceptor (diameter ≈ 400 nm) liposomes, the latter serving as a model for lipophilic sink compartments. p-THPP- liposomes could be easily separated from the larger acceptor liposomes and the p-THPP content was determined in both fractions by on-line UV/VIS-detection under careful evaluation of and compensation for light scattering/turbidity. By fractionating donor- and acceptor-vesicles after distinct periods of incubation, the extent of drug-release (to the aqueous phase) and kinetics of drug-transfer (to the model lipid sink) were established.

K5

FLOW FIELD-FLOW FRACTIONATION WITH MASS SPECTROMETRY FOR SUBCELLULAR PROTEINS AND METALLOPROTEINS

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Analysis of subcellular proteins is of interests in functional proteomics but requires an accurate and fast separation/isolation of subcellular organelles such as nucleus, lysosomes, mitochondrion, peroxisomes, Golgi apparatus, etc which are essential components in cells due to their own specific roles. Organelle separation has widely been conducted by centrifuge based methods, fluorescence-activated organelle sorting, and affinity purification methods. This presentation will show recent studies for the subcellular protein characterization and for the metalloprotein analysis with off-line or on-line hyphenation of asymmetrical flow FFF (AF4) with mass spectrometry (MS). Separation of subcellular organelles from human embryonic kidney 293 T (HEK293T) cell line was accomplished with AF4 without any pre-processing steps. It was conducted in steric/hyperlayer mode first and then few incompletely resolved fractions were re-injected to AF4 for improved separation in normal mode. Confirmation of organelles were made with SEM, Western blot, and proteomic analysis using LC-MS/MS. A new approach to analyze metals in metalloproteins will be introduced by directly hyphenating a miniaturized AF4 system with inductively coupled plasma mass spectrometry (mAF4-ICP-MS). Selective analysis of metals in biological systems is an emerging research field and is important to understand the biological processes such as signal transduction and metabolic pathways. Though metalloproteins are reported to be potential biomarkers of diseases, critical roles of these metal ions remain unknown. mAF4-ICP-MS was demonstrated not only to separate metalloproteins in blood plasma directly, but to quantitatively analyze metals and few non-metal elements simultaneously. Optimizations in the analysis of blood plasma will be discussed together with applications to lung cancer plasma samples.

K6

FIELD FLOW-FRACTIONATION FOR STEM CELLS SORTING - FROM NORMAL, EMBRYONIC AND CANCER STEM CELLS, TO A NEW DEVELOPMENT IN THE FIELD OF HUMAN INDUCED PLURIPOTENT STEM CELLS.

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FFF (Field Flow-Fractionation) applications in stem cell research started in the middle 90's [1]. Such important studies have been done with GrFFF (Gravitational FFF) or DEP-FFF (dielectrophoretic-FFF) [2-3], we widely used SdFFF (Sedimentation FFF) to sort various types of normal: embryonic neural stem cells [4]; genetically modified mouse embryonic stem cells (ES cells) to more efficiently obtain transgenic mice [5]; and cancer stem cells [6]. After an overview of FFF implication in stem cells sorting, we further go in a new and important application concerning human induced Pluripotent Stem Cells (hiPSC). hiPSC are a highly interesting solution to create diseases models. Once obtained, hiPSC could give all cellular types after several weeks of a costly differentiation process. However, the main problem is to get as much as possible an enriched specific cell population, in order to better understand the pathophysiology of a disease and provide a good model for further investigation and drug screening. In order to rapidly and efficiently sort hiPSC without disturbing their potentialities, SdFFF appeared as a good solution. Compared to canonical way (30 days), SdFFF elution of hiPSC could be performed only after 10 days culture in a basic medium without growth factors. On the basis of proteomic analysis, SdFFF was able to prepare two distinct progenitor populations, one of endothelial progenitors, the second of neural progenitors. The isolation of progenitors involved in various lineages is a major interest for the rapid and efficient preparation of complex models for development of new therapeutic tools.

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K7

NUMERICAL SIMULATION AS A POWERFUL TOOL TO UNDERSTAND AND IMPROVE FLOW-FFF SEPARATION

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The amazing progress in the computer hardware together with the new achievements in applied mathematics, physics and chemistry, allowed the computational experiments to expand from areas as aerospace and automotive engineering and to take new territories in process engineering, nano science, analytical chemistry, etc. The concept of performing computational experiments based on solving for 3D flow and nano particles transport has been adapted to the specifics of the fractionation processes, and computational experiments have been performed toward better understanding the flow-FFF processes, optimizing the shape of the channel and optimizing the flow control. Commercial software have been used for the regimes were it provided reliable results, while own software was used for the more sensitive computations. The setting of the computational experiment allows to observe the details of the flow and the pressure in the channel, including, e.g., areas with very slow flow, boundary layers, etc. Furthermore, particle tracking at all stages of injection, focusing and elution is possible. Proper visualization tools allow getting visual impression about the flow and particle transport. The quantitative result in the form of a fractogram is calculated as well and allows evaluating the impact of conditions of the experiment on the quality of the separation. The model has been validated with standard channel geometries and standard samples. It is shown how the model can be used to optimize the geometry and flow programming of a separation. The method can be adapted to visualize transport and separation of macromolecules in a flow stream and might find applications in other areas outside FFF.

K8

ASYMMETRIC-FLOW FIELD-FLOW FRACTIONATION: A POWERFUL TECHNIQUE FOR CHARACTERIZING NANOMATERIALS (WITH MANY CAUTIONS)

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Asymmetric flow field flow fractionation has been increasingly utilized in recent years to fractionate and characterize nanoscale particles, including metals such as gold and silver, carbon nanotubes, and polymers and proteins. A4F has demonstrated very high efficiency to fractionate and characterize particulate analyte according to physical parameters such as size and shape, and coupled with various on-line detectors, A4F can provide researchers with a powerful analytical tool with respect to on-line high-resolution multidimensional characterization and quantitative analysis. Nevertheless, in A4F, a considerable complication exists in regard to the correct determination of the analyte diffusion coefficient, the principal determinant of retention time. There are obvious benefits to the use of on-line size measurements (e.g., using dynamic light scattering, multi-angle light scattering, mass spectrometry); however, despite this coupling opportunity, the accuracy and representativeness of the physical parameters determined for the nanoscale materials can still be discussed. While some techniques, such as batch-Dynamic Light Scattering and other Electronic Microscopy, rapidly became standard methods and reference techniques for characterizing nanomaterials, A4F still appears as a complex technique that required never-ending-optimization procedures and excessive control experiments. Based on our personal results and on the literature concerning the nanoparticle characterization by A4F, the aim of my presentation is to critically discuss the real potentiality of A4F with its advantages and limitations and the gap that needs to be filled in order to make this technique one of the inescapable measurement methods for nanoscale materials.

K9

ASFLFFF FOR CHARACTERISATION OF POLYMERS FROM RENEWABLE RESOURCES – CHALLENGES AND (SOME) SOLUTIONS

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In most instances, biorefinery products are often complex mixtures of biomass-derived biopolymers and their degradation products, their ratios, purity and modifications depending on origin, treatment, and purification level. Classical separation approaches and chromatographic techniques are often not fit-for-purpose in the special cases of biorefinery product streams. The paper will discuss the specific needs of biopolymer analysis in such scenarios and will highlight approaches to solve some of these problems by AsFlFFF methods designed to cope with the peculiarities of lignocelluloses.

Molar mass analysis of polymers derived from renewable resources is challenged by their structural complexity. The traditional molar mass analysis approach - size exclusion chromatography (SEC) - is often hard to implement for lignocellulosics due to the necessity of additional sample purification or insolubility problems. Asymmetric flow field-flow fractionation (AsFlFFF) appears to be a suitable means to tackle some of the problems.

We have investigated the potential of AsFlFFF for the analysis of various renewable materials, such as lignosulfonates, alginates, water-soluble cellulose and polysaccharide derivatives and cellulosic nanoparticles of different shapes.

Interference arising from the membrane-sample interactions was controlled by tuning the mobile phase. For lignosulfonate analysis, the interference of fluorescence or absorption effects with MALLS detection is still of great concern for most of the industrial samples. Hence, calibration of the chromatographic system with polystyrene sulfonate standards was evaluated. The positive sides as well as possible gaffes of a conventional calibration are critically compared. In the analysis of differently shaped cellulose nanoparticles, AsFlFFF was able to demonstrate its superiority by combining analysis of soluble and insoluble parts with the ability to retrieve information on particle geometries.

K10

CHARACTERIZATION OF COLLOIDAL PARTICLES IN WATER USING ASYMMETRICAL FLOW FIELD-FLOW FRACTIONATION WITH ADVANCED DETECTION: CHALLENGES AND PROGRESSES

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Colloidal refers to a state of subdivision, implying that the molecules or polymolecular particles dispersed in a medium have at least in one direction a dimension roughly between 1 nm and 1 µm, or that in a system discontinuities are found at distances of that order[1]. The colloids systems of solid particles dispersed in liquids are called sol, and water is the most common liquid dispersion medium in both natural and man-made products. Colloids play a very significant role in nature and in our daily life. The applications of colloids are almost unlimited.

The Dow Chemical Company produces millions of pounds of colloid-based products and materials that are processed by customers into complex formulated dispersions. The analytical capability for colloidal particle characterization is essential to product development, quality control, intellectual property protection, and fundamental research. Many analytical techniques have been used for colloidal particle characterization; however, there are still many challenges to obtain necessary information with high precision and/or accuracy in a reasonable time frame. Asymmetrical flow field-flow fractionation (AF4) with on-line advanced detectors such as multi-angle light scattering (MALS), dynamic light scattering (DLS) and differential refractive index (dRI) have proven their strength in colloid particle characterization. In this presentation, we will use real industrially relevant examples to demonstrate what AF4-MALS-DLS-RI can offer in terms of particle size distribution, particle quantification, particle mass, particle morphology information, and probing the interaction and stability of the particles. Meanwhile, challenges in characterizing these colloidal systems will also be discussed to stimulate future developments in this arena.

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K11

ELECTROSPRAY-DIFFERENTIAL MOBILITY ANALYSIS: AN INTRODUCTORY OVERVIEW AND APPLICATIONS IN NANOMEDICINE

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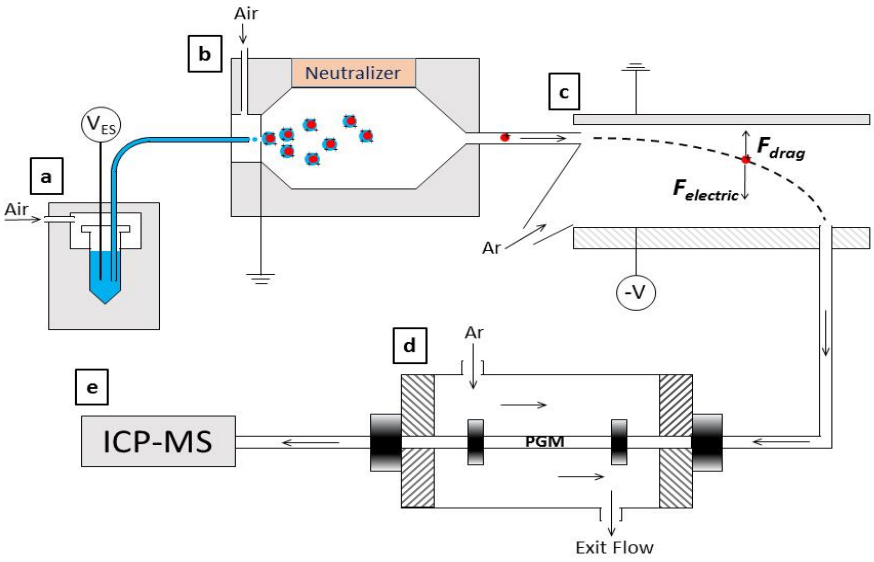
Electrospray-differential mobility analysis (ES-DMA) is a field-driven separation and discrete sizing technique that yields aerosolized particles that are classified based on their electrical mobility in an applied DC field. The ES aerosol generator converts a colloidal suspension into small liquid droplets. The droplets, which nominally contain a single particle or suspending medium, are evaporated as they mix with a stream of air. Nonvolatile salts within the droplets condense to form small salt particles, while colloid containing droplets yield single colloids with a coronal layer of nonvolatile salts. The aerosol particles pass thru a static eliminator, which converts most particles to a charge of +1, 0 or -1. The aerosolized particles then enter the DMA, a cylindrical column consisting of a flow channel between two electrodes. An applied voltage is scanned in the DMA column, driving the positively charged particles across the channel. A small port at the end of the column allows particles of a specific electrical mobility to pass through. The electrical mobility is determined by the balance of the electrical and drag forces on the particles. For particles carrying a charge of +1, and knowing the applied voltage, gas properties and assuming or knowing the particle shape, the mobility diameter can be obtained. For particles small compared to the mean free path of the gas, a slip correction is applied; otherwise the measurement is based on first principles and requires no calibration. A size resolution of about 3.5% can be achieved, and size can be selected with sub-nm precision. The selected stream of monodisperse particles can be transmitted to a particle counter to generate particle number concentration. Recent advances in coupling of DMA with ICP-MS (see figure) have enabled quantitative size-differentiated analysis of anti-tumor drug loading on gold nanoparticle conjugates. The utilization of ES-DMA for nanomedicine applications will be highlighted.



Schematic illustration of coupled ES-DMA-ICP-MS system: (a) ES, (b) neutralizer chamber, (c) DMA column, (d) gas exchange device to replace air with argon for ICP compatibility, and (e) ICP-MS.

Abstracts of the oral presentations

O1

USE OF AF4 IN PEPTIDE FORMULATION AND STABILIZATION

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There are relatively few AF4 studies on therapeutic peptides, which typically have molecular weights of 2 to 5 kD. Using a membrane with a 1 kD cut-off, the separation of peptides using AF4 is illustrated. In some cases, oligomers and higher molecular weight aggregates are clearly seen. The appearance of these species was found to correlate to the appearance of subvisible particles in some cases. These studies demonstrate that AF4 can be employed to study the solution behavior of polypeptides with molecular weights of < 5 kD. The use of AF4 in peptide formulation development will be discussed.

O2

SEPARATION AND ANALYSIS OF POLYSACCHARIDE MIXTURES UTILIZING ASYMMETRIC FLOW FIELD-FLOW FRACTIONATION (AF4) COUPLED TO MULTIANGLE LIGHT SCATTERING (MALS)

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Polysaccharides are biopolymers which often have complex characteristics regarding structure, size, molar mass, properties in aqueous solution etc. Many of the characteristics influence the functionality of these substances and, thus, understanding of them is essential. Separation and analysis of polysaccharides can be very challenging and few analytical techniques are suitable for the task. The challenges are typically related to the broad distributions in terms of size and molar mass but often also to the tendency of the substances to form supra-molecular aggregates. AF4-MALS-dRI is a highly suited method but molar mass and size determination can still be challenging when, for instance, analyzing complex samples and heterogeneous mixtures of polysaccharides. In order to obtain high quality data, careful and systematic method development is a requirement.

In this work we separate and analyze common polysaccharides utilizing AF4-MALS-dRI. One often observed phenomenon in AF4 is a downturn in the molar mass vs. elution time at, for instance, high retention. This result is often thought of as an artifact attributed to various errors in data obtained from detectors and the subsequent data processing. However, the results in this work illustrate that this phenomenon may be a correct measurement and that it can also be caused by poorly chosen separation conditions in the AF4. Furthermore, we illustrate and discuss disturbances such as shifts in retention time and peak distortion which can arise in the separation of mixtures of chemically heterogeneous polysaccharides in relation to the separation of the individual substances.

O3

MULTIDETECTOR THERMAL FIELD-FLOW FRACTIONATION AS A UNIQUE TOOL FOR THE CHARACTERISATION OF BLOCK COPOLYMER MICELLES

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Micelles are appealing for applications in fields such as colloid stabilisation, drug delivery and microreactor applications due to their stability and versatility and although micelles are traditionally characterized by various techniques such as electron microscopy (SEM and TEM), NMR, fluorescence spectroscopy and dynamic light scattering, these self-assemblies still lack suitable analytical techniques to provide comprehensive information on corona composition as well as on size and shape distributions.

To address this problem various FFF techniques such as asymmetric flow field-flow fractionation and flow field-flow fractionation were successfully applied to characterise self-assemblies in terms of shape, size and molecular weight distributions. However, unlike other FFF techniques, thermal field-flow fractionation (ThFFF) is uniquely suited to fractionate analytes according to composition as well as size.

It is shown for the first time that a multidetector ThFFF setup can fractionate micelles according to corona composition while providing comprehensive information on important micelle characteristics as a function of temperature. Moreover, it is shown that ThFFF serves a unique characterisation platform to monitor the formation of mixed micelles in terms of size, molecular weight and composition distributions.

O4

FFF OF BRANCHED POLYMERS: PUSHING FFF FURTHER WITH A SECOND DIMENSION OF CAPILLARY ELECTROPHORESIS

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The necessity to accurately analyse branched polymers has increased over recent years. Natural branched polymers such as starch and glycogen play an important role in our diets as well as the future of bioenergy. Synthetic branched polymers such as poly(acrylic acid) have aided in the development of a number of new materials such as superabsorbents and paints. Asymmetrical field-flow fractionation (AF4) has been shown to separate these polymers by size [1]. However, in the case of branched polymers, co-elution occurs with polymers that have a distribution of molar mass in addition to a distribution of branching. The utilization of a complementary separation technique may further characterize the branching distribution of such polymers; however, they are currently limited to chromatography based techniques which limits the size range. Capillary electrophoresis in the critical conditions (CE-CC) has recently been shown to separate branched polymers independent of size and is not restricted by a stationary phase [2]. The aim of this work is to couple AF4 to CE-CC for the analysis of branched polymers.

This talk will focus on the combined capabilities of AF4 and CE-CC to characterize two complex polymers: cyanobacterial glycogen and PAA. Glycogen from genetically modified cyanobacteria was analysed by both AF4 and CE-CC to determine the effect that genetic manipulation had on glycogen size and branching. PAA is a branched smart polymer and a 2D separation was used to characterize PAA by size and branching. One of the significant challenges in coupling AF4 to CE-CC is the dilution of polymers after AF4 and the small injection volumes used in CE. This talk will show strategies to overcome this issue with CE focusing techniques. The talk will then explore the potential future of other FFF x CE-CC separations.

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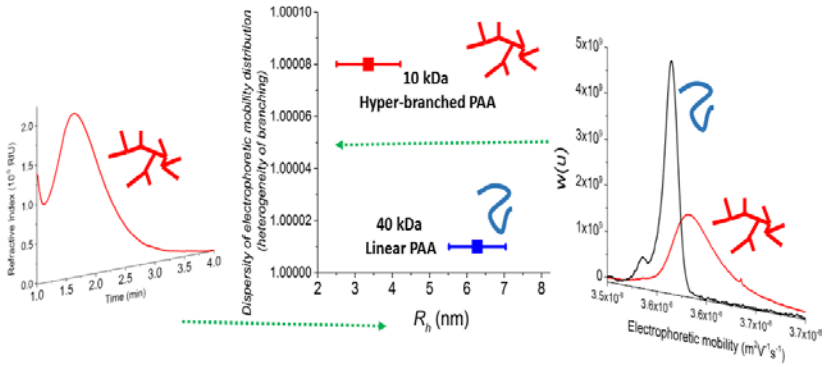
[2] Thevarajah, Gaborieau, Castignolles, Advances in Chemistry 2014, 2014, Article ID 798503.



Plot of Rh versus branching heterogeneity (centre). Measurement of Rh by AF4 (left) and branching heterogeneity by CE-CC (right). Error bars represent 1 standard deviation.

O5

CHARACTERIZATION OF THE MOLECULAR AND CHEMICAL STRUCTURE OF ACRYLIC EMULSION POLYMERS BY ASYMMETRIC FLOW FIELD FLOW FRACTIONATION COUPLED WITH A MULTI-ANGLE LIGHT SCATTERING DETECTOR

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Acrylic and styrene-acrylic copolymers prepared by emulsion polymerization represent an important group of synthetic polymers that find applications in the production of paints and adhesives. A series of methacrylate-acrylate and styrene-acrylate copolymers of various ratios of methacrylate or styrene to acrylate were synthetized using non-seeded semi-continuous emulsion polymerization and the molecular and in the case of styrene-containing copolymers also chemical structure was investigated by the combination of asymmetric flow field flow fractionation (AF4) and a multi-angle light scattering (MALS) detector. The lecture shows several applications of AF4-MALS for the analysis of these industrially important polymers that are often difficult or even impossible to be characterized by traditionally used size exclusion chromatography (SEC). The applications include (i) the determination of true molar mass distribution unaffected by non-SEC separation mechanisms or shearing degradation in SEC columns, (ii) quantification of nano-gel like fractions formed in the course of polymerization either purposefully by the addition of polyfunctional monomers or unintentionally due to the chain transfer to polymer, (iii) the determination of styrene fraction along the molar mass axis, (iv) identification and characterization of branching, and (v) characterization of the compactness of core-shell structures. Examples of these applications are presented in the lecture. In addition, the data obtained by AF4-MALS are contrasted with those acquired by SEC-MALS.

An example of AF4 separation of emulsion acrylic copolymer is shown in Figure 1 that demonstrates the ability of the method to efficiently separate these polymers over four orders of magnitude of molar mass. The root mean square (RMS) radius is determined simultaneously with the molar mass and the relation between the RMS radius and molar mass allows detecting and quantifying branching of polymer chains.

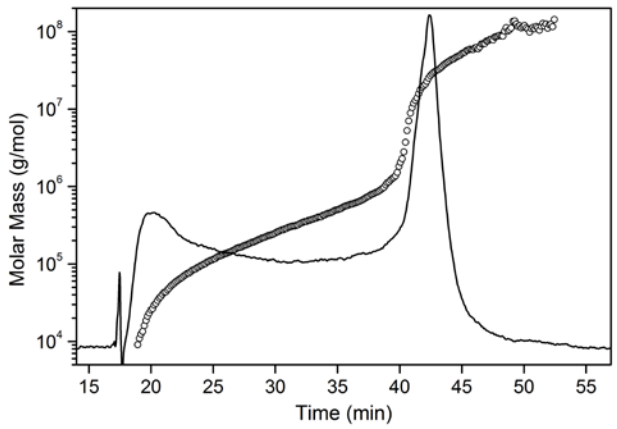

Figure 1:
Molar mass (o) versus retention time plot of acrylic copolymer prepared by emulsion polymerization. Fractogram recorded by a refractive index detector (line) is overlaid here. Data were obtained by an AF4 system Eclipse coupled with a MALS and a refractive index detector HELEOS and Optilab rEX, respectively (all Wyatt Technology Corporation).

O6

SIMULTANEOUS ON-LINE DETECTION OF SIO2, TIO2 AND AL2O3 PARTICLES IN TOOTHPASTE BY ASYMMETRIC FLOW FIELD-FLOW FRACTIONATION HYPHENATED TO TRIPLE-QUADRUPOLE INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

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Toothpaste is a complex mixture of chemicals including e.g. emulsifiers, surfactants, and particles such as SiO2, Al2O3 (abrasives) and TiO2 (pigment), which can be nano or micrometer sized. During its normal use, a fraction of toothpaste may be swallowed and thus the individuals may be exposed to these metal oxides. The size of the particles is a determining factor for their biological fate and the possible intestinal uptake of these particles. Therefore, in order to characterize these nano or microparticles, a method of analysis was developed aiming at simultaneous size separation of all three types of particles by asymmetric flow field-flow fractionation (AF4). Two strategies were tested and compared for preparing the toothpaste samples for analysis of its constituent particles by AF4: a simpler approach involving dilution of the toothpaste in aqueous solution and a more complex procedure consisting on microwave digestion of the toothpaste matrix with hydrogen peroxide. Multi angle light scattering was used for on-line size determination of the eluting particles, and triple-quadrupole inductively coupled plasma mass spectrometry (ICP-QQQ-MS) was invaluable for selective, simultaneous detection of all three elements under a fixed set of instrumental conditions. This talk will describe the methodological steps that were required for analyzing the three distinct particles present in the toothpaste, in particular the challenges that had to be addressed during sample preparation as well as the optimization work related to the AF4 separation and ICP-QQQ-MS detection.

O7

INVERSE SUPERCRITICAL FLUID EXTRACTION AS EFFICIENT TOOL FOR FFF SAMPLE PRETREATMENT AND ITS APPLICATION TO THE ANALYSIS OF SUNSCREENS

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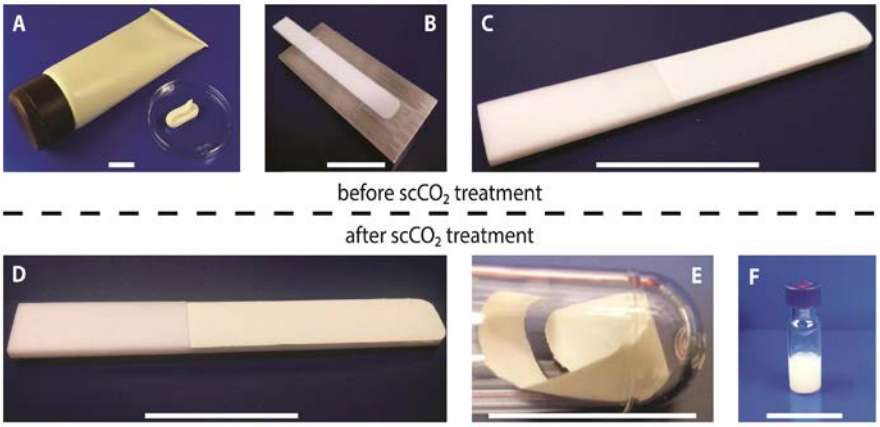
Engineered nanomaterials nowadays find their applications in a multitude of consumer products including e.g., food and cosmetics. A well-known example is the application of nanoparticulate titanium dioxide as UV-protection agent in sunscreen formulations. Here, titanium dioxide nanoparticles are incorporated in a complex matrix consisting of a variety of thickeners, emulsifiers and solubilizers in order to obtain a homogeneous formulation.

With existing and upcoming legislative regulations, e.g. the European Regulation EC No 1223/2009 on cosmetic products, the declaration of nanoparticulate ingredients in cosmetics has become mandatory. Therefore, strong efforts are currently undertaken in order to find methods for the reliable detection and characterization of nanoparticles in such complex matrices. A promising approach is the application of inverse supercritical fluid extraction (inverse SFE) and subsequent analysis using asymmetrical flow-field flow fractionation (AF4). Inverse SFE can be considered a "green" extraction method capable of gently removing matrix components without using potentially hazardous chemicals. AF4 allows the reliable fractionation of the nanoparticulate components, yielding detailed insight into the real particle size distribution of the sample.

In this presentation, we describe the application of inverse SFE for the efficient pretreatment of sunscreen formulations containing nanoparticulate titanium dioxide (AeroDisp® w740x, Evonik Industries). Subsequent analysis of the dried samples using AF4-UV-MALS not only revealed the excellent applicability of inverse SFE as an FFF sample pretreatment tool, but also allowed the unequivocal confirmation of the presence of nanoparticles in the investigated sunscreen formulations.



Samples based on semi-solid emulsions of oil and water, such as sunscreens, can be very challenging in terms of the necessary sample preparation. The figure shows a model sunscreen formulation at different stages of the inverse SFE using supercritical CO2. After processing, the residual material can be easily re-dispersed in an aqueous solution and directly analyzed by AF4. Scale bars are 25 mm.

O8

LIGAND DEPENDENT PARTICLE LOSSES OF GOLD NANOPARTICLES DURING AF4: ELECTROSTATIC VERSUS POLYMERIC STABILIZATION

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Asymmetrical Flow-Field Flow Fractionation (AF4) characterization of inorganic nanoparticles (NPs) is often associated with considerable losses of particles [1]. The development of measurement protocols with reduced losses requires knowledge on the particle loss mechanisms [2]. We investigate how particle-surface and particle-particle interactions lead to adsorption and agglomeration.

Here, we discuss results on ligand-dependent particle losses of gold nanoparticles (Au-NPs) during AF4 analysis. Citrate reduction was used to synthesize Au-NPs with a mean diameter of 12 ± 0.8 nm. We modified their surfaces with thiol-terminated polyethylene glycol ligands (PEG) to obtain Au-NPs with different hydrodynamic sizes, ligand densities and surface charges. The nanoparticle recoveries during AF4 characterization were quantified via UV-Vis transmission and elementary analysis of collected fractions in dependence of the ionic strength. The determined recoveries were correlated to their physico-chemical properties. Citrate stabilized particles showed the greatest losses; many of them adsorbed to internal surfaces other than the membrane. PEGylation increased recovery (from 50 % to 85 %) for short-chained ligands, whereas longer ligand chains caused the particles to adsorb at the membrane (visible in SEM) despite their enhanced colloidal stability.

We will present systematic results on the dependence of recovery on particle ligand shells and eluent composition. Membrane adsorption is not the only important loss mechanism - other internal surfaces are fouled by particles, too. We will discuss options for particle modification for the development of reliably reference particles.

[1] C. A. Soetebier, F. S. Bierkandt, S. Rades, N. Jakubowski, U. Panne, S. M. Weidner. Journal of Analytical Atomic Spectrometry 2015, 30, 2214-2222.
[2] F. v. d. Kammer, S. Legros, T. Hofmann, E. H. Larsen, K. Loeschner. TrAC Trends in Analytical Chemistry 2011, 30, 425-436.

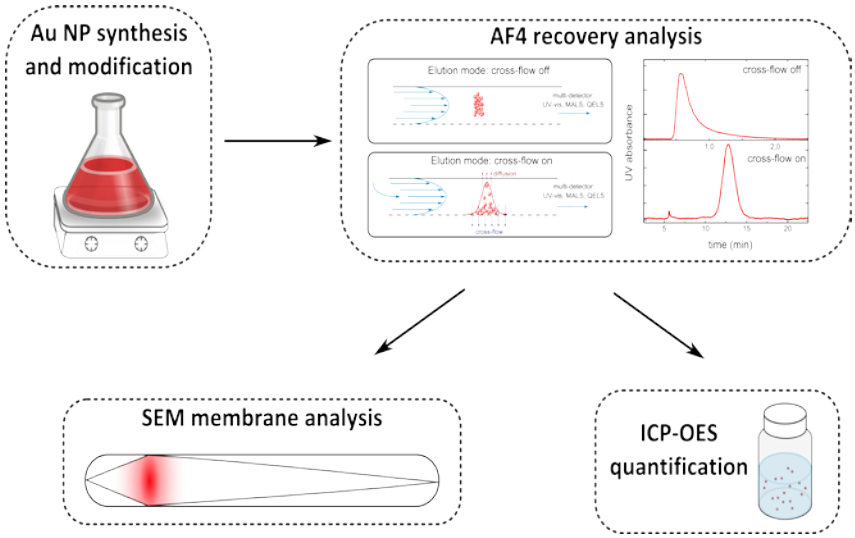

Figure 1: AF4 recovery investigation of modified Au-NPs using on-line recovery analysis, SEM characterization of used membranes and elementary analysis of collected fractions.

O9

COMPOSITION AND SIZE-BASED SEPARATION OF METAL HYBRID NANOPARTICLES BY THERMAL FIELD-FLOW FRACTIONATION

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In this work, thermal field-flow fractionation (ThFFF) is developed to separate and characterize iron oxide (Fe3O4), platinum (Pt), and platinum iron oxide (Pt-Fe3O4) metal hybrid nanoparticles (MHNp) by composition. MHNp incorporate multiple particle domains through solid-state interfaces to create individual nanostructures with multiple functionalities and synergistic properties[1]. Current understanding of the synthesis routes for these hybrids and their final structure-functional properties is hindered by insufficient characterization methods[2]. The ThFFF separation mechanism is based on the translational diffusion coefficient and the thermal diffusion (DT) of the particles. Hence, DT can be experimentally determined from ThFFF retention times. ThFFF separations of MHNp with sizes that varied by approximately 15% exhibited DT values that differed by as much as 30%. Existing (albeit controversial) theories show a correlation between particle DT and its surface and/or bulk composition and suggest that differences in measured DT may form the basis for composition calibration plots[3]. Our ThFFF data showed that the DT values for Pt-Fe3O4 MHNp fall between the values for the pure single component particles and are correlated to the mass percent of each constituent component. Using these calibration plots the average multi-lobed hybrid is determined to have a mass percent iron of approximately 67%. This is within 3% of elemental weight percent determined by energy-dispersive X-ray spectroscopy of the fractionated sample. Composition distributions constructed from online DT determination represent the first measurements of its kind for these complex hybrid nanoparticles. The observed composition sensitivity of ThFFF shows promise for the characterization of other complex nanoparticle systems such as bimetallic, alloy, core/shell and multi-domain nanoparticle systems.

[1] M. R. Buck, J. F. Bondi, R. E. Schaak. Nat. Chem. 2012, 4, 37–44.
[2] J. S. Beveridge, M. R. Buck, J. F. Bondi, R. Misra, P. Schiffer, R. E. Schaak, M. E. Williams. Angew. Chemie 2011, 123, 10049–10053.
[3] R. Piazza. Soft Matter 2008, 4, 1740.

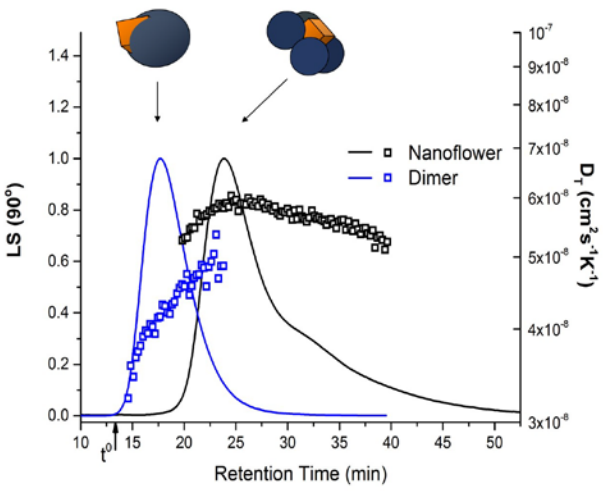

Figure 1:
Composition separation of similarly sized Pt-Fe3O4 MHNp by ThFFF

O10

DETECTION AND CHARACTERIZATION OF NANOPARTICLES IN FOOD AND BIOLOGICAL MATERIALS BY ASYMMETRIC FLOW FIELD FLOW FRACTIONATION HYPHENATED WITH INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

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The oral presentation was cancelled.

O11

CHARACTERISATION OF POLYDISPERSE TIO2: A STEP TOWARDS THE IMPLEMENTATION OF THE EC DEFINITION ON NANOMATERIAL

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The European Commission (EC) in 2011 published the recommendation on the definition of nanomaterial 'a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 – 100 nm'*. Titanium dioxide (TiO2) nanoparticles are one of the most heavily employed nanomaterials, they are used in paints, cosmetics, as catalysts, as food additives (also known as E171) in construction and building materials.

A strategy for the characterisation of polydisperse TiO2 materials by means of Asymmetric Flow Field Flow Fractionation (AF4) is presented. The main aim is to get a step closer to the implementation of the recommendation of the EC definition for a 'nanomaterial'. As a first step the importance of the sample preparation is presented, in order to disperse, i.e. to break up agglomerates and/or aggregates of the material and keep the material in suspension. In a next step different factors of the AF4 system were studied by experimental design in order to obtain a good characterisation of polydisperse TiO2 overcoming the limitations of the one factor at a time approach. Once the best working conditions of the system are obtained, the results from four different detectors (multiangle light scattering, dynamic light scattering, refractive index and UV-Vis) are presented in order to estimate the particle size distribution and the number-based particle size distribution of all the TiO2 as a further step towards in the implementation of the EC definition.

* European Commission, Commission Recommendation of 18 October 2011 on the definition of nanomaterial, Official Journal of the European Union. 2011/696/EU, (2011) 38-40.

O12

DOES A CENTRIFUGAL FIELD-FLOW FRACTIONATION SYSTEM EQUIPPED WITH AN UV-VIS ABSORPTION DETECTOR ALLOW CHARACTERIZING SILVER NANOPARTICLE SUSPENSIONS FOR SIZES AND CONCENTRATION?

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Silver nanoparticles (AgNPs) are of interest for a wide range of applications, which span from electronics, sensors, medicine, pharmaceutical, cosmetics and consumer products. The performances and applicability of AgNPs depends on their size and concentration, which must be accurately and precisely evaluated.

Centrifugal Field Flow Fractionation (CFFF) is able to sort AgNP suspensions, giving a very good characterization in terms of NP's size, however, UV-vis signal cannot be easily converted in a sample concentration profile in case of plasmonic nanostructures [1]. On the other hand, Optical Absorption Spectroscopy (OAS), with the support of the Mie theory, can easily give the concentration of AgNPs sample, whereas evaluation of NPs size is not straightforward [2].

In this work the CFFF, equipped with a multilambda UV-Vis detector, was used to obtain simultaneously the size distribution and the size-weighted concentration of five AgNPs suspensions (20, 30, 40, 60, 100 nm). The size results were supported by transmission electron microscopy (TEM) observations and DLS measurements, while the computed quantitative concentration profiles were compared with experimental Atomic Absorption Spectroscopy (AAS) data.

Alternatively to the use of more time consuming and expensive analytical techniques, this approach proves that a complete characterization of AgNPs samples is possible, with quite low errors in the computed sizes and concentration profiles.

[1] C. Cascio, D. Gilliland, F. Rossi, L. Calzolai, C. Contado. Anal. Chem. 86 (24), pp 12143–12151, 2014.
[2] V. Amendola, M. Meneghetti. J. Phys. Chem. C 113 (11), 4277-4285, 2009.

O13

CHARACTERIZATION OF SILVER NANOPARTICLES: LIMITATION AND ADVANTAGES OF FIELD-FLOW FRACTIONATION

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Silver nanoparticles (Ag NPs) are widely used in consumer products due to their excellent antibacterial properties. Their broad application has led to a variety of recent regulation on their use and labelling. Thus, a highly specific analytical method for their characterization and quantification is needed.

Due to their large separation range, field-flow fractionation (FFF) techniques are repeatedly applied for the analysis of NP. Limitations of FFF include quantification, sample loss and insufficient recovery rates. Another challenge can be non-ideal elution behavior of particles in complex and unknown matrices.

The possible sources for sample losses of Ag NP have been studied using an asymmetric flow FFF (AF4) in combination with inductively coupled plasma mass spectrometry (ICP-MS). The influence of different parameters, for example the sample concentration, on the recovery rates and sample loss has been investigated. Using laser ablation ICP-MS, the Ag deposition on the membrane was located and quantified. Our results identified ionic silver as the main sources of sample loss. These results can be useful for further method improvement.

However, when a Ag NP sample containing an unknown complex matrix is analyzed, FFF method optimization is challenging as the sample might show a shift in the retention times and lower recovery rates. In this case, ICP-MS experiment in the single particle mode (sp-ICP-MS) can be a useful addition to the FFF measurement. Here, upon assumption of spherical particles, the geometric diameters can be calculated. To overcome matrix effects in sp-ICP-MS measurements of complex samples, we propose sp-ICP-MS experiments using isotopic dilution analysis. This fast and easy approach can be helpful in order to interpret the FFF fractograms and advice the FFF method optimization process.

O14

CHARACTERIZATION OF SIZE AND SIZE DISTRIBUTION OF NANOMATERIALS: A COMPARISON OF SCANNING ELECTRON MICROSCOPY, DYNAMIC LIGHT SCATTERING, AND FLOW FIELD-FLOW FRACTIONATION METHODS

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Methods for the accurate determination of the size and size distribution of nano-sized materials are essential for nano- and biotechnology. Among the methods available, flow field-flow fractionation with multiangle light scattering and UV absorption detectors (FFFF-MALS-UV) is considered to be a one of the effective method than field emission scanning electron microscopy (FE-SEM) and dynamic light scattering (DLS) for determining the size and size distribution of nanomaterials [1]. However, the raw values of size and size distribution obtained using these three methods are number-, volume-, and z-averaged, respectively. In order to compare the size and size distribution determined using different measurement methods, it is necessary to transform the raw values into the same dimensionality of length.

In this study, we transformed the raw values obtained using the above mentioned three methods into the same dimensionality and found that the results for averaged size was qualitatively similar despite the differences between the measurement methods when the particles were dispersed well as narrow size distributed primary particles. Focused on DLS method, DLS is not reliable for measuring the apparent size distribution of materials over a wide size distribution because of the method's low resolution. Therefore, we also examined the coverage and accuracy of using experimental DLS method and theoretical approach to determine a submicrometer bimodal size distribution.

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[2] H. Kato, A. Nakamura, N. Noda. Material Express 4, 144-152 (2014).
[3] H. Kato, A. Nakamura, N. Ouchi, S. Kinugasa. Material Express 6, (2016) in press.

O15

BIOHYBRID STRUCTURES OF PROTEINS AND DENDRITIC GLYCOPOLYMERS CHARACTERIZED BY AF4

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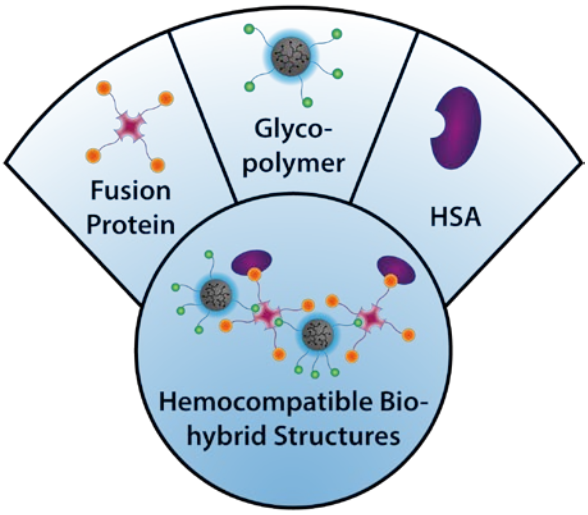
In the field of biomedical applications nanoscopic biohybrid materials with bioactive behaviour are very promising as delivery systems, therapeutics and diagnostics. These materials are combining properties of both – natural and synthetic – components. In this context, dendritic glycopolymers (DGP) exhibit an excellent biological behaviour for in-vitro and in-vivo applications[1] and their multivalent biotinylated derivatives are usable in the design and fabrication of complex biohybrid structures (BHS) triggered by polyassociation between avidin and biotin.[2,3] In these studies [2,3] several key characteristics (ligand-receptor stoichiometry; number, length and nature of biotin ligands; time of polyassociation etc.) and growth mechanism have been studied and it is postulated how defined BHS with narrow size distribution can be established.

Here, we present new aspects (e.g. steric hindrance combined with ligand properties) for the design and fabrication of BHS using the polyassociation between streptavidin and biotinylated DGP. Due to the controlled polyassociation for those BHS very narrowly distributed BHS obtained provide a model kit like size control by the ligand-receptor stoichiometry and properties of biotin ligands. To improve the hemocompatibility a streptococcal albumin binding domain (ABD) was used to interact with human serum albumin (HSA) and was fused with streptavidin (SA) to the fusion protein SA-ABD. The SA-ABD was used in the polyassociation process, but also for the conjugation of HSA with existing BHS using protein-protein interactions. Analytical method combinations with asymmetric flow field-flow fractionation (AF4), but also other characterization methods enabled us to smoothly evaluate the molecular structures and shapes of those BHS with and without HSA.

[1] D. Appelhans et al. Chem. Soc. Rev. 2015, 44, 3968.
[2] S. Boye et al. Macromolecules 2015, 48, 4607.
[3] F. Ennen et al. Biomacromolecules 2015, DOI: 10.1021/acs.biomac.5b00975.



Scheme of hemocompatible biohybrid structures based on biotinylated glycopolymers.

O16

CHARACTERIZATION OF GADOLINIUM NANOHYDROGELS FOR MRI BY FIELD FLOW FRACTIONATION (FFF) TECHNIQUES.

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4) *Institut de Chimie Moléculaire de Reims, UMR CNRS 7312, Groupe Chimie de Coordination, UFR des Sciences Exactes et Naturelles, Reims Cedex 2, France*
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The lymphatic system is an essential element of the immune system and participates in the spread of metastatic cells. Early detection of metastases in the lymph nodes is therefore essential for an early diagnosis. Molecular biomaterials (synthetic or natural biodegradable polymers) are the material basis of nanomedicine. Among them, hydrophilic and biocompatible polymers, such as chitosan and hyaluronic acid, can be crosslinked to form a hydrogel which have had wide applications in local drug delivery. Nanohydrogels especially combine the advantages of hydrogels and nanoparticles for drug formulation and delivery.

Gadolinium-based hypersensitive contrast agents for MRI of the lymph nodes have been synthetized leading to small size self-organized or self-assembled biopolymers nanohydrogels. The synthesis protocol could influence the size and size distribution of nanoparticles that thus should be scrutinized for their further use in medicine.

To this aim, DLS and AFM have been used to characterize these nanohydrogels, showing discrepancies in size determination. DLS exhibited systematically monodisperse population with elevated size values. Thus, a third approach by AF4-MALS was used, showing that the nanohydrogel suspensions may exhibit polydispersity depending upon the synthesis mode employed in contrast to DLS observations, and sizes are in the range of magnitude of those determined by AFM. FFF-based techniques are suitable for characterizing the size of nanohydrogels for imaging purpose, and may be later for therapeutic payload. A discussion will be given on the characterization of such nanohydrogels. The advantages, drawbacks and limitations of each characterization technique will be also considered. In a Pharmacopeia approach, these techniques must be coupled to complementary techniques for a full characterization of products but FFF could be envisaged to be used as a reference technique, with cGMP compliance as a final goal to meet.

O17

POLY(LACTIC-CO-GLYCOLIC ACID) NANOPARTICLES IN CELL MEDIUM USED AS BIOCOMPATIBLE SUBSTRATES IN PHARMACEUTICAL APPLICATIONS: COMPREHENSIVE CHARACTERIZATION WITH CENTRIFUGAL FIELD-FLOW FRACTIONATION COUPLED WITH ONLINE DYNAMIC LIGHT SCATTERING

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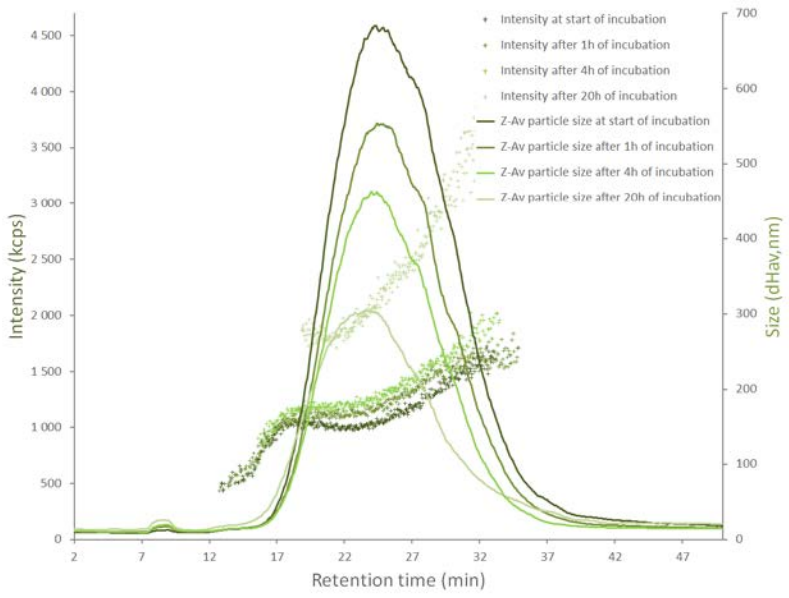
Centrifugal Field-Flow Fractionation (CF3) is a powerful subtechnique of Field-Flow Fractionation. While in Asymmetrical Flow Field-Flow Fractionation (AF4) separation is only based on the hydrodynamic size, CF3 additionally exploits density differences of the analytes resulting in an enhanced separation force and thus improved resolution. Moreover, Online Dynamic Light Scattering (DLS) enables recording of hydrodynamic sizes in real-time rendering this setup a powerful hyphenation (CF3-DLS) for the separation and characterization of nano- and microparticles over a wide size range.

In this presentation, CF3-DLS is used to characterize PLGA (Poly(lactic-co-glycolic acid)) sub-microparticles. In order to investigate their behavior under physiological conditions, the PLGA-particles were dissolved in cell medium and incubated for defined time durations at 37 °C. CF3-DLS analysis was subsequently performed using cell medium as carrier solution thereby closely mimicking the conditions during incubation. By these means, CF3-DLS provides valuable insights in the behavior of a biocompatible particle system, which shows promising properties towards the application in pharmaceutical applications, such as e.g. drug delivery.

This work was part of MINAC: Mechanisms of the Interaction of Nanoparticles with Cells

MINAC support code: 0315773D



The image displays CF3-DLS-fractograms of 200 nm PLGA NPs after different incubation times (0 h, 1 h, 4 h and 20 h) in RPMI 1640 (Hydrodynamic radii highlighted as dots in lighter colors to the corresponding intensity trace).

O18

POLY-ION COMPLEXES ANALYSIS WITH FRIT INLET FLOW FIELD-FLOW FRACTIONATION SYSTEMS

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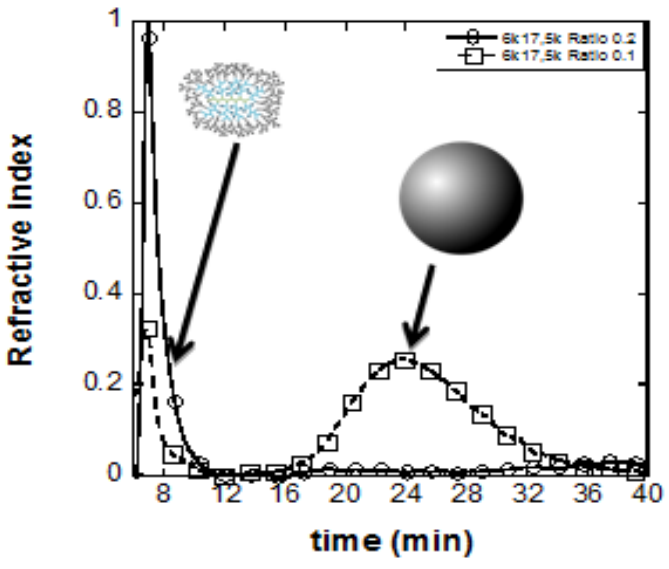
Poly-ion complexes (PICs) are self-assemblies based on the association of oppositely charged polyelectrolytes. They are increasingly assessed as drug vectors for nanomedicine. These charged complexes are highly challenging to study and characterize because of their small size and instability, particularly, when the monomers are <20 kDa. Hence, most studies to date focus on the PICs as formed and do not use a separation step to obtain insights into the formation mechanism, dynamics, multipopulation size distribution or even loading.

Initial experiments performed with AsFlFFF confirmed the high sensitivity of PICs to shear stress with the complexes being destroyed during the focusing step even when low flow rates are used. We describe here the use of Frit Inlet Flow Field-Flow Fractionation (symmetrical and asymmetrical) for the characterization of poly-ion complexes.

The use of Frit Inlet led to a very low loss of material, enabled the study of the PIC itself and showed numerous subpopulations that were not visible by the classical batch dynamic light scattering. We also examined the formation of the PICs by progressively increasing the ratio of the second polymer leading to the self-assembly, to examine the cooperativeness of the process. This showed different behaviours depending on the polymer used. The influence of salt concentration was also characterized and Frit Inlet FlFFF clearly showed quantitatively the rapid disappearance of PICs, in contrast with batch DLS. These studies demonstrate the importance of frit inlet flow FFF systems for analyzing fragile complexes and provide important new insights into the behavior of these drug carriers under different solution conditions.



Investigation of poly-ion complexes formation from monomer polylysine with PAA-PEO: fractogram of PEO-PAA 6k-17.5k DGL-3 PICs obtained with different concentrations of PEO-PAA by frit inlet Asymmetric Flow Field-Flow Fractionation

O19

SIZE CHARACTERIZATION AND QUANTIFICATION OF EXOSOMES BY AF4

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In the past few years exosomes have gained huge interest of scientific community since they show a great potential for human diagnostic and therapeutic applications. These vesicles have an important role in many physiological functions, ranging from intercellular communications, coagulation, angiogenesis to cell survival, and others. [1]

In the present work, asymmetric flow field-flow fractionation (AF4) technique coupled to a multi-angle light scattering (MALS) and UV detectors was used to detect and characterize exosomes. The emphasis was on the method development to size-separate, characterize and quantify the exosome population. Batch DLS (dynamic light-scattering), NTA (nanoparticle tracking analysis) and TEM analysis of unfractionated exosomes were also conducted to evaluate their shape and internal structure, as well as their number density. [2]

The results show that optimal fractionation is mainly governed by the chosen cross-flow conditions (optimal conditions: initial high gradient of 0.55 mL/min2 followed by lower gradient of 0.0036 mL/min2) and channel thickness (350 μm). AF4-MALS-UV and DLS results displayed the exosome sample to have broad size distribution and pointed to the presence of two particles subpopulations, the larger exosomes with solid sphere structure of nonuniform density and the smaller vesicle-like particles, which coeluted in AF4 together with impurities in early eluting peak. The Rh and number density of exosomes determined by NTA revealed consistency in terms of size and number density of larger exosome population determination by DLS and AF4- MALS. Compared to DLS and AF4-MALS results, NTA somewhat overestimates the size and the number density for larger exosome population, but it discriminates the smaller particle population.

[1] M. Simons, G. Raposo. Curr. Opin. Cell Biol. 2009, 21, 575-581.
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O20

CONTINUOUS MAGNETIC DEPLETION OF RED BLOOD CELLS FROM WHOLE BLOOD BY MAGNETIC SPLITT

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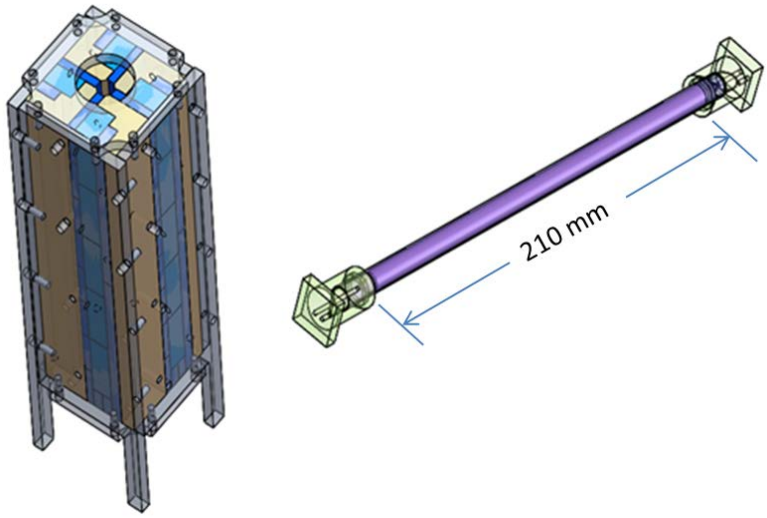
In biomedical research and in medicine, it is often desirable to remove red blood cells (RBCs) from whole blood, thereby enriching the white cell fraction, which might be investigated for stem cells or circulating tumor cells. The classical means of RBC removal includes centrifugation in a density gradient or exposure to hypotonic solution. Both techniques have the disadvantages of potential harm and significant losses to target cells, and integrate poorly with continuous processes, such as MEMS. Our approach takes advantage of the fact that RBCs become paramagnetic in oxygen depleted environments, converting oxy- to deoxy-hemoglobin and giving rise to unpaired electrons. Otherwise, oxy-hemoglobin is weakly diamagnetic as is the overall RBC. We have developed a magnetic SPLITT device comprising a quadrupole magnet and annular flow channel. The magnet assembly combines neodymium magnet blocks with carbon steel yokes for a maximum flux density, B, of 1.22 T. The aperture diameter is 9.65 mm and length is 203 mm. The flow channel employs high-resolution 3D-printed manifolds, each with dual flows that also support an axi-symmetric stainless steel rod and a cylinder. Dilute blood is placed in a stirred vessel while pure nitrogen is sparged to remove oxygen. Dilute whole blood was processed under variable flow rates to achieve optimal outcomes. More than 90% of RBCs were recovered in the enriched outlet at a total flow rate of 0.05 ml/min and inlet/outlet flow rate ratios of 0.5 and 0.55. Next, to mimic non-erythroid cells, 12-µm polystyrene microspheres (PS) were added to blood to yield a 90:10 RBC:PS number ratio. Again a flow rate sweep yielded an optimal total flow rate of 0.1 ml/min, with 97% RBC recovery in the enriched outlet at 98% purity. These findings are compared with modeling and presented through the method of Receiver Operating Characteristics to illustrate the tradeoffs in parameter selection.



MagSPLITT: Quadrupole magnet assembly and annular flow channel

O21

IMPACT OF ASYMMETRICAL FLOW FIELD-FLOW FRACTIONATION ON PROTEIN AGGREGATE STABILITY

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The impacts of asymmetrical flow field-flow fractionation (AF4) on protein aggregates are studied using online multiangle light scattering (MALS) and batch mode dynamic light scattering (DLS). Protein aggregates are a major concern in biotherapeutics because they may compromise product safety and efficacy.[1] Depending on the analytical method used, these complex species may be formed or destroyed.[2,3] It has been suggested that the dilution inherent to separation methods and stresses encountered during specific stages of AF4 may affect aggregation. To date, no systematic studies have been performed to investigate these potential impacts. This work examines the influences of carrier fluid composition, shear stress caused by sample injection, sample concentration during the AF4 focusing step, and dilution during the elution step. Heat stressed IgG1 samples were separated and fractions of low and high molar mass (M) aggregates were collected and then individually reinjected into the AF4 system. Aggregate fractions collected and reinjected in phosphate buffer dissociated almost entirely to monomer. Aggregates in citric acid buffer were partially stable upon reinjection resulting in dissociation to 25% and 5% monomer for the low and high M fractions, respectively. These results suggest that the buffer composition is important and low M aggregates may be more likely to dissociate to monomer than larger species. Increasing the duration of the focusing step during reinjection showed no significant changes in the percent monomer or the M in either fraction. Dilution of IgG1 aggregates did not result in a significant size change while shear stress of aggregates showed an increase in size. Furthermore, our calculations showed that sample dilution in AF4 is significantly lower than previously assumed. We also highlight the benefits of using AF4 retention time over detector-determined sizes for dilution-sensitive samples.

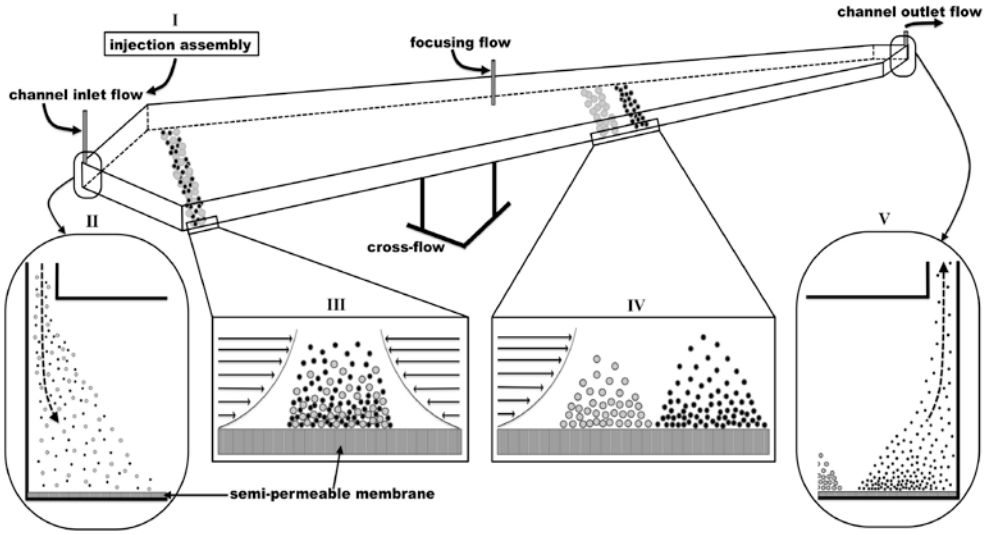

Figure 1: Schematic of steps during AF4 analysis: syringe injection (I), introduction to channel (II), focusing (III), fractionation (IV), and elution (V).

[1] A. S. Rosenberg. Aaps J. 2006, 8, E501–E507.
[2] V. Filipe et al. TrAC Trends Anal. Chem. 2013, 49, 118–125.
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O22

SEPARATION OF EXOSOMES WITH ELECTRICAL FIELD FLOW FRACTIONATION

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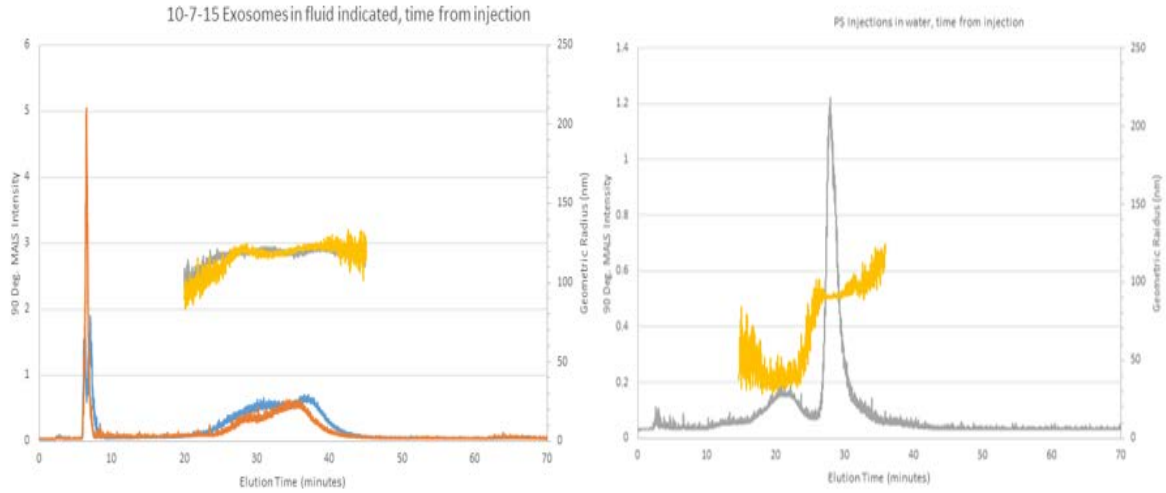
Exosomes are biologically important in cancer metastasis and their selective enrichment by a variety of traits (such as size, density, and antibody interactions) is commonly used. Exosomes may also be fractionated by their electrophoretic mobility using electrical field flow fractionation, but this technique is hampered to ineffectiveness at high (physiological) salt conditions. Exosome size and concentration is evaluated through the process of exchanging exosome buffers using buffer exchange spin columns and dynamic and static light scattering detection. Exosomes are shown to remain intact after long periods of time in substituted fluids and also withstand multiple buffer substitutions. E-FFF is coupled with MALS for the first time, and exosomes are fractionated in low-ionic solutions for the first time. Carboxylate modified polystyrene sphere standards and synthetically produced exosomes are fractionated under a variety of conditions and compared with the fractionation of biologically purified exosomes which separate based upon their charge. This work develops a method for an electrophoretic separation technique for biological particles (specifically exosomes) where buffer substitution followed by separation and characterization with E-FFF is possible.



90 degree MALS Intensity peaks for fractionation of exosomes, and polystyrene standards (51 and 200 nm diameter mixture) using E-FFF. Measured geometric radius is shown for the respective particles.

O23

SEDIMENTATION FIELD-FLOW FRACTIONATION OF NANO- AND MICROPARTICLES IN ROTATING COILED COLUMNS: THEORY AND APPLICATIONS

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Field-flow fractionation (FFF) is a very powerful and versatile set of liquid chromatography-like elution methods. However, conventional FFF separations occur in thin channels and the sample weight injected is usually less than 1 mg to avoid overloading. The fractionation in a rotating coiled column (RCC), which can be attributed to sedimentation FFF, enables the handling sample weight to be increased at least up to 1 g.

Regularities of the behaviour of nano- and microparticles of different size and origin in RCCs with different design parameters were systematically studied taking as example silica particles, latex beads, quartz sand, clay minerals, and other samples. The basic principles of the new FFF method were established.

The developed method was applied to the speciation analysis of polydisperse environmental samples, in particular, for the separation of soils into silt, clay and sand fractions. For the first time, nano- and submicron particles of street dust (e.g. road dust) have been separated, weighted, characterized by electronic microscopy, and quantitatively analyzed by ICP-AES and ICP-MS (after digestion). The elements that may be of anthropogenic origin (Zn, Cr, Ni, Cu, Cd, Sn, Pb) were found to concentrate mainly in nano- and submicron size fractions. It has been shown that the concentrations of Cr, Ni, Zn, Cu, Pb in the finest fraction (<0.3 □m) of street dust can be one order of magnitude higher than the concentrations of elements in bulk sample. Samples of contaminated dust affected by a metallurgic plant have shown particular patterns of element distribution between size fractions.

Study on the fractionation of synthetic samples has demonstrated the applicability of the method to the preparative separation and purification of metallic powders (e.g. aluminum-silicon alloy), chromatographic sorbents based on polystyrene-divinylbenzene, and other polydisperse materials.

O24

CONTINUOUS AF4 FOR THE FRACTIONATION AND PURIFICATION OF BIOMOLECULES AND NANOPARTICLES WITH THE USE OF MICROSTRUCTURED MEMBRANES

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We present a novel method and a device based on field-flow fractionation that is able to fractionate a feed solution of macromolecules into fractions according to their size. The key component that generates the continuous separation is a microstructured ultrafiltration membrane. Conventionally, only flat membranes are used in AF4 and any roughness of the membrane is considered unfavorable as it can cause extra peak broadening. However, we demonstrate that microstructured membranes with a periodic 'roughness' could be beneficial and could extend the applications of AF4.

Asymmetrical flow field-flow fractionation is a separation technique, alternative to size exclusion chromatography that operates in mild conditions because of the absence of a stationary phase. Last decade, it has gained significant popularity due to the rapid growth of nanotechnology and biotechnology. The continuous AF4 system that we have developed could be used as a semi-preparative technique but it could also be scaled up for industrial purposes or scaled down in lab-on-a-chip devices.

Up to date, continuous two dimensional field-flow fractionation systems can be found only sporadically in the literature, such as sedimentation-steric FFF or thermal FFF involving a rotating disk. However, the former separates microparticles and the later can be used mostly with organic solvents and in high temperatures. To our knowledge, our method is the first continuous FFF system that fractionates macromolecules in mild conditions and aqueous solvents.

O25

A NOVEL MAGNETIC FFF DETECTOR FOR THE QUANTIFICATION AND CHARACTERIZATION OF MAGNETIC NANOPARTICLES

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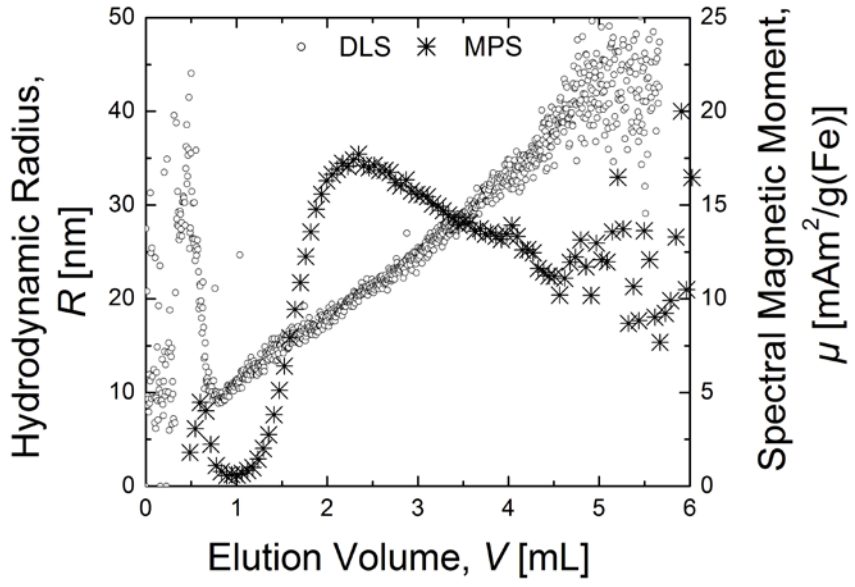
Magnetic nanoparticles (MNP) exhibit unique magnetic properties making them ideally suited for a variety therapeutic (e.g. hyperthermia and targeted drug delivery) and diagnostic (e.g. magnetic resonance imaging and magnetic particle imaging) biomedical applications. Depending on the desired magnetic effect MNP must meet special magnetic requirements which are mainly determined by their structural properties (e.g. size distribution). The hyphenation of chromatographic separation techniques with complementary detectors is capable to provide multidimensional information of submicron particles. Although various methods have already been combined for this approach, so far, no detector for the online magnetic analysis was used. Magnetic Particle Spectrsocopy has been proven a straightforward technique for specific quantification and characterization of MNP. It combines high sensitivity with high temporal resolution which are of prerequisite for a successful hyphenation with chromatographic separation. We demonstrate the capability of Magnetic Particle Spectroscopy (MPS) to specifically detect and characterize MNP under usually applied asymmetric-flow field flow fractionation (A4F) conditions (flow rates, MNP concentration, different MNP types). To this end MPS has been successfully integrated into an A4F multidetector platform including DLS, MALS and UV. Our system allows for rapid and comprehensive characterization of typical MNP samples for the systematic investigation of structure-dependent magnetic properties. This has been demonstrated by magnetic analysis of the commercial MRI contrast agents Resovist®, Endorem®, and Feraheme® during hydrodynamic A4F fractionation.



Magnetic particle spectroscopy (MPS) and dynamic light scattering fractogram of magnetic nanoparticles (Resovist(R)) during A4F analysis. The hydrodynamic size increased linearly with elution volume whereas the spectral magnetic moment reached a maximum at about 2.3 mL (corresponding to R=21 nm). The MPS online detection reveals a nonlinear magnetic signal dependence from hydrodynamic size.

O26

ONLINE COUPLING OF FFF AND FTIR SPECTROSCOPY

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The continuous development of new polymer materials, which are increasingly complex, evokes a need for more complex analytical techniques. The size distribution is one key characteristic of polymers. But its fractionation methods, namely size exclusion chromatography (SEC) and field-flow fractionation (FFF), lack in chemical sensitivity. This means they are not capable of providing information about the chemical composition correlated to molecular size. For such analysis one needs two- or multidimensional techniques. This is not only important for modern copolymers, but also to analyse polymer mixtures and blends in more detail and it is additionally useful for unknown samples.

We present a two dimensional method of FFF and online coupled FTIR spectroscopy. Compared to other multidimensional analytics correlating size and chemical composition this combination has the advantage of being very universally applicable. FFF can separate a broad range from dissolved molecules to undissolved particles and FTIR spectroscopy can also be equally used for both liquids and solids. FTIR spectroscopy can not only quantify but is furthermore very useful to identify different components present in the sample.

The main problems for online coupled FTIR measurements in liquid systems are the solvent signals and the low analyte concentration. The general idea and setup including the flow cell as well as the mathematical solvent suppression and first results of this new coupling technique will be presented.

O27

COMBINATION OF ELECTRICAL AND FLOW FIELD-FLOW FRACTIONATION TO MEASURE ELECTROPHORETIC MOBILITY OF NANOPARTICLES AND PROTEINS

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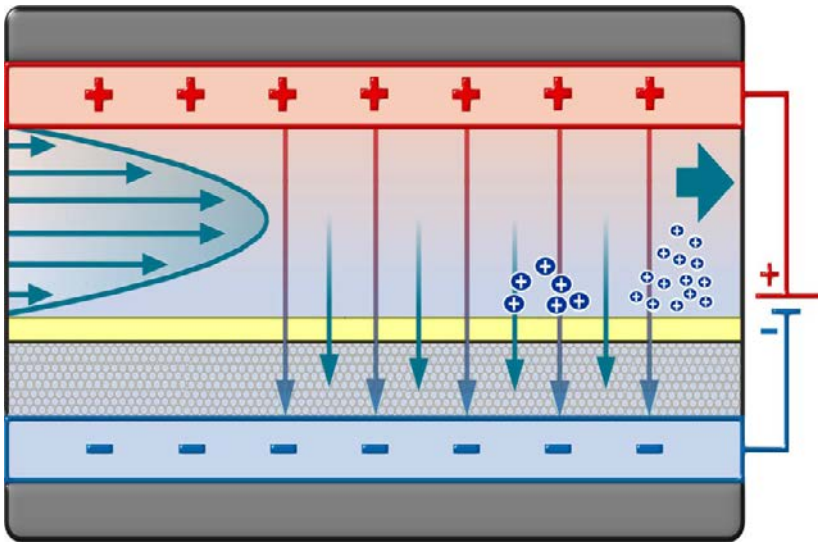
A new FFF method is presented which combines asymmetrical Flow-FFF (AF4) and Electrical FFF (ElFFF) in one channel (EAF4). The new method allows to measure electrophoretic mobility of eluting species. An AF4 channel is modified by adding two electrodes, one at the upper wall, the other beneath the frit of the accumulation wall. The measurement consists of a series of FFF separation experiments in which the electrical field strength is varied by applying different electrical currents, starting from 0 to typically 10 to 20 mA. A shift of retention time as a function of current or field strength is observed and from a plot of the electrical field strength versus drift velocity the electrophoretic mobility is obtained. The method is straightforward for homogeneous size populations where the shift in retention time of the peak maximum is taken for the mobility calculation. It can be used for populations with a broad size distribution if the size is measured by light scattering (either MALS or DLS). In this case the mobility as a function of size can be calculated across the distribution. The method has been validated by comparing mobility values measured by EAF4 and PALS for well known particle standards [1]. In this contribution we present results of the analysis of samples with a broad size distribution showing that the zeta potential is changing with size. Possible application of the method for the analysis of complex samples which contain species of different charge and polarity are discussed.

[1] C. Johann et al. (2015). Anal Chem 87(8): 4292-4298.



The schematic shows the principle of the electrical flow-FFF channel. Two electrodes are added to the AF4 channel which are used to create an electrical field which runs parallel to the cross-flow generated in the channel. In this way the drift velocity can be increased or reduced depending on the polarity of the field and the charge of the particles.

O28

URANIUM SPECIATION IN SOFT WATER USING ASYMMETRICAL FLOW FIELD-FLOW FRACTIONATION COUPLED WITH UV AND INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ASFLFFF-UV-ICP-MS)

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The uptake and toxicity of trace metals in aquatic organisms are strongly linked to speciation [1]. Uranium (U) can be present in a wide variety of chemical species, which can be divided into 3 predominant species: uranyl cation, uranyl hydroxides, and uranyl carbonates [2]. Important factors controlling the speciation are, for example, pH value, ionic strength, and availability of inorganic and organic ligands. It is important to evaluate the environmental impact of U under ecologically relevant conditions. It has been stated that U toxicity is predominantly caused by uranyl cation [3].

Speciation of uranium (U) in aqueous phase was studied in synthetic US EPA very soft water as a function of U concentration at different pH and concentration of fulvic acid (FA) and humic acid (HA). The results demonstrated the formation of small uranium polymeric colloids increased with U concentration and decreased with pH. In presence of humic (HA) or fulvic (FA) acids, significant fraction of U was associated with intermediate to higher than average molecular weight of FA/HA components. Concentration of U in complex form was increased with increased FA and HA concentration and pH suggesting low bioavailibity. However, higher molecular weight part was degraded due to UVB exposure transforming into lower molecular weight fraction. Around 50% of U was mobilized after 48 hrs UVB exposure. Thus, UVB could have impact on U speciation in natural lakes. This research suggests AsFlFFF-UV-ICP-MS as important separation and detection technique for understanding metals distribution in different DOM samples which may be key to understand metal transport and bioavailability.

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[2] G. Bernhard. Landbauforschung Völkenrode (2005) 55:139–148.
[3] H. Vandenhove, A. Cuypers, M. Van Hees, G. Koppen, J. Wannijn. Plant Physiol Biochem (2006) 44:795–805.

O29

DEVELOPMENT OF AN ANALYTICAL APPROACH USING CENTRIFUGAL FIELD-FLOW-FRACTIONATION HYPHENATED TO ICP-MS/MS FOR THE DETECTION AND CHARACTERIZATION OF TITANIUM DIOXIDE NANOPARTICLES IN THE ENVIRONMENT CHARACTERIZATION OF TITANIUM DIOXIDE NANOPARTICLES IN THE ENVIRONMENT

Florian Dutschke[1], Juan Soto-Alvaredo[2], Daniel Pröfrock[1], Andreas Prange[1], Jörg Bettmer[2]

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Since several years, the developing field of nanotechnology has gathered the attention of the scientific community. The increase of the application of manufactured nanoparticles NP in various sectors of human life raised concerns that the exposure to nanomaterials may result in significant adverse effects for both human health and the environment. One of the most frequently used manufactured nanomaterials are TiO2-NP, with a production volume of 15 kt per year. They are used for example in sunscreens and cosmetics with increasing production volumes. Initial Studies from the literature have shown that TiO2-NP can lead to oxidative stress in living organism also further studies showed adverse long term effects on marine organisms.

However, the fate and the quantities of TiO2 nanomaterials in the environment are still not sufficient studied. One of the main challenges related to the analysis of NP in environmental samples is caused by the low concentration and the wide size-distribution of the particles present in the environment as well as matrix related effects. Therefore new analytical approaches are necessary to address the different challenges related with the analysis of NPs in the environment.

The main part of this work will present the improvement of the detection and separation of TiO2 NP using centrifugal field-flow-fractionation hyphenated to ICP MS/MS, describing the main characteristics and improvements of this method. The following part will deal with the investigation of different industrial available TiO2-NPs with a strong focus on the characterization of the elemental composition as well as the different size-distributions using the techniques optimized in the first part. The last part will deal with the application of the developed methods for the analysis and the extraction of TiO2-NPs from marine sediment samples, while preserving the size-information and comparing the results with the characteristics found for the industrial used nanoparticles.

O30

TRACING AND QUANTIFICATION OF ISOTOPICALLY MODIFIED IRON NANOPARTICLES IN A SEDIMENT SLURRY MATRIX VIA AF4/ICP-SFMS

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Due to new promising properties of engineered nanoparticles (ENPs) several fields of application popped up during the last two decades. Hence, a release into the environment is most likely.

Particularly, with regard to the investigation of the fate of ENPs in environmental matrices (e.g., surface water) profound knowledge is still unclear to large extend. Especially the presence of “natural” nanomaterials (e.g., suspended sediment in rivers) exhibiting the same size range as well as elemental signature (e.g., Al, Fe, Ti, and non-metal Si) [1] counteracts a sensitive and unambiguous detection.

A promising, but so far barely applied analytical strategy for tracing ENPs is based on stable isotope labeling [2, 3]. For proof of concept isotopically labeled 57Fe-oxide ENPs were synthesized and spiked in re-suspended river sediment slurry matrix. Fractionation of the ENPs and natural iron fractions was achieved by means of asymmetric flow field flow fractionation (AF4) coupled on-line with inductively coupled plasma-sector field mass spectrometry (ICP-SFMS). By means of the isotopic fingerprint of the labeled 57Fe ENPs unambiguous tracing was enabled. Furthermore, on basis of our previous work [4] a reverse on-line isotope dilution approach for simultaneous quantification of the 57FeNPs was developed.

Our approach allowed for fractionation, quantification (LOD: 2.4 µg Fe L-1) as well as simultaneous unambiguous tracing of 57Fe ENPs in the presence of natural iron colloid fractions. Especially with regard to (environmental) fate studies our approach opens up new appealing opportunities and is adaptable to further ENPs exhibiting two stable, interference free isotopes.

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O31

UTILIZING ASYMMETRICAL FLOW FIELD FLOW FRACTIONATION AND HIGH RESOLUTION MASS SPECTROMETRY TO ASSESS THE ROLE OF DISSOLVED ORGANIC MATTER SIZE AND COMPOSITION ON MERCURY BIOAVAILABILITY

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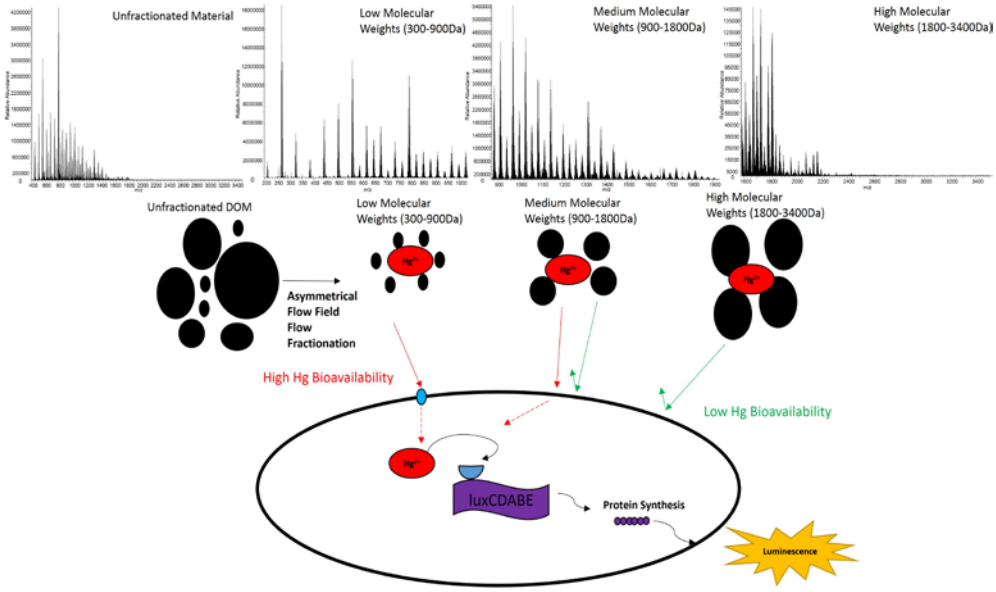
Dissolved organic matter (DOM) is classified as small (<0.45µm) hydrocarbons in aquatic systems from a variety of allochthonous and autochthonous sources such as terrestrial run off and microbial nutrient flux. While serving many important functions in aquatic systems, one major role of DOM is its ability to complex with many divalent trace metals, such as mercury (Hg). Information such as size and composition of organic ligands is of crucial importance as speciation and complexation impacts bioavailability and toxicity. In the present study, asymmetrical flow field flow fractionation (AF4) is utilized in order to separate DOM into size fractions of low molecular weight (LMW) (300-900Da), medium molecular weight (MWM) (900 -1800Da) and high molecular weight (HMW) (1800-3400Da) which were then exposed to 250pM of Hg to biological sensors. Biological sensors consisted of E.Coli cells containing a lux operon system that emits bioluminescence responses upon the uptake of Hg2+, allowing for a quantifiable response to Hg2+ bioavailability in the presence of various organic ligand MWs. Size fractions were also measured using high resolution mass spectrometry (HRMS) to determine molecular compositions of size fractions. Results suggest that small, protein-rich DOM promotes Hg2+ bioavailability whereas larger, aromatic species reduce Hg2+ uptake. The current research utilizes a variety of analytical and microbiological techniques to provide further insight as to how Hg2+ uptake is impacted by DOM.



AF4 was used to separate bulk samples of DOM into three categories: low molecular weight, medium molecular weights and high molecular weight. Separated DOM exposed to Hg, and cellular uptake was monitored using E Coli biological sensors. High resolution mass spectrometry was also used to determine composition of organic ligands present to examine how composition impacts Hg bioavailability.

O32

APPLICATION OF SELF-ADJUSTABLE SPLIT-FLOW LATERAL-TRANSPORT THIN CHANNEL FOR SEPARATION OF ENVIRONMENTAL MICROPARTICLES

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The new conception of SPLITT channel has been developed and tested with different analytical setups. It works in full feed depletion mode and, with its unique construction, it requires only one parameter that has to be adjusted, namely flowrate of the pump responsible for stable flow of carrier liquid at channel inlet. Thus, with this unique features, we name it self adjustable full feed depletion SPLITT (FFD-SPLITT-SA). This construction has been simplified to achieve easy-to-use fractionation tool of micrometer particles. This idea fits the requirements of both analytical and industrial purposes.

The conception has been tested with variety of materials: spherical silica, natural zeolites and bottom lake sediments. The size of collected fractions has been measured with laser diffraction particle size analyzer to prove the efficiency of achieved separation.

The results of first experiments with strictly defined size of the sphere silica particles revealed that short fractionation is very efficient with cut-of diameter nearly the same as expected and decent resolution comparable with commercial devices. However, the long term (24h) results of separation shows dramatic decrease of separation power through the sedimentation of particles in tubes and in the channel. Having in mind, that spherical particles are engineered rather than environmental presence, we have also tested natural materials. Both, kaolinite particles and lake sediments meet the criteria of separation in semi-analytical (short term) mode.

Additionally, we coupled FFD-SPLITT-SA channel directly to flame atomic absorption spectrometer (AAS) to test ability to fast online fractionation and detection of selected heavy metals for physical speciation. The results are optimistic and shows alternative, cost-efficient way to upgrade AAS to speciation methods of micrometer particles.

Acknowledgment: This paper was supported from the budget of NCN OPUS7 project no. 2014/13/B/ST4/04998.

O33

STUDY ON ENVIRONMENTAL EFFECTS OF NANOMATERIALS BASED ON HOLLOW FIBER FLOW FIELD-FLOW FRACTIONATION

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With the development of nanoscience and nanotechnology, the environmental effects and biological safety of nanomaterials, especially engineered nanomaterials, have sparked exponential growth of research. Silver nanoparticles (AgNPs), known as one of the most widely used nanomaterials, have drawn great interest due to their adverse effects on the environment and health. Given the highly dynamic properties of AgNPs, it is believed that AgNPs and ionic silver (Ag(I)) are coexisting and undergo inter-transformation in the environment. Herein, we firstly developed a novel method based on on-line coupling of hollow fiber flow field-flow fractionation (HF5) with inductively coupled plasma mass spectrometer (ICPMS) for characterization and quantification of different sized AgNPs and Ag(I). Based on the proposed method, identification, characterization, and quantification six Ag species (i.e., Ag(I) and five AgNPs with nominal diameter of 1.4 nm, 10 nm, 20 nm, 40 nm, and 60 nm) can be achieved. The applicability of the developed system was demonstrated by the good recoveries (71.1–108%) for both Ag(I) and 10 nm AgNPs determined in two spiked surface water samples. Furthermore, the proposed methods was successfully used to study the transformation of Ag(I) to AgNPs in the presence of natural organic matter under sunlight. Additionally, the unusual properties of carbon-based nanomaterials {e.g., graphene oxide (GO)} have led to a rapid increase in their production in a wide range of application. The GO inevitably released into the environment will also threaten human health. To figure out its fate and transport in aquatic environments, impacts of sunlight and humic acid on the aggregation of GO were investigated. Results showed that sunlight was ready to aggregate GO, while humic acid could greatly prevent GO aggregation mainly due to steric hindrance forces.

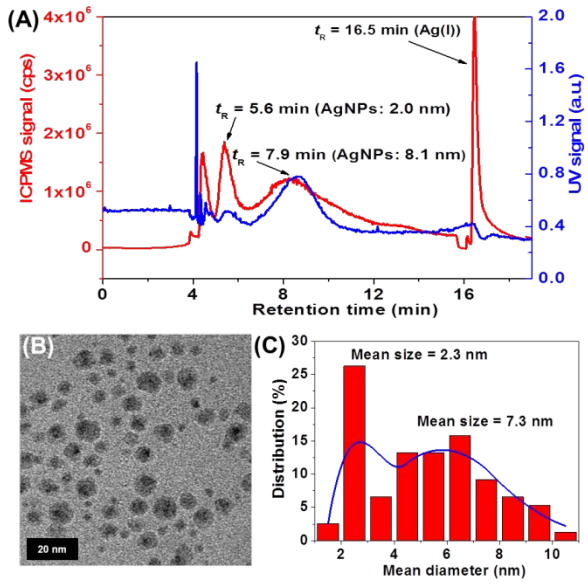

Figure 1: HF FlFFF/MCC-UV/DLS/ICPMS elution profile (A), TEM image (B), and histograms showing the size-distribution (C) of the photo-reduced AgNPs in NOM solution.

O34

MOLECULAR WEIGHT DISTRIBUTION OF MARINE DISSOLVED ORGANIC MATTER IN HIGHLY STRATIFIED ARCTIC OCEAN

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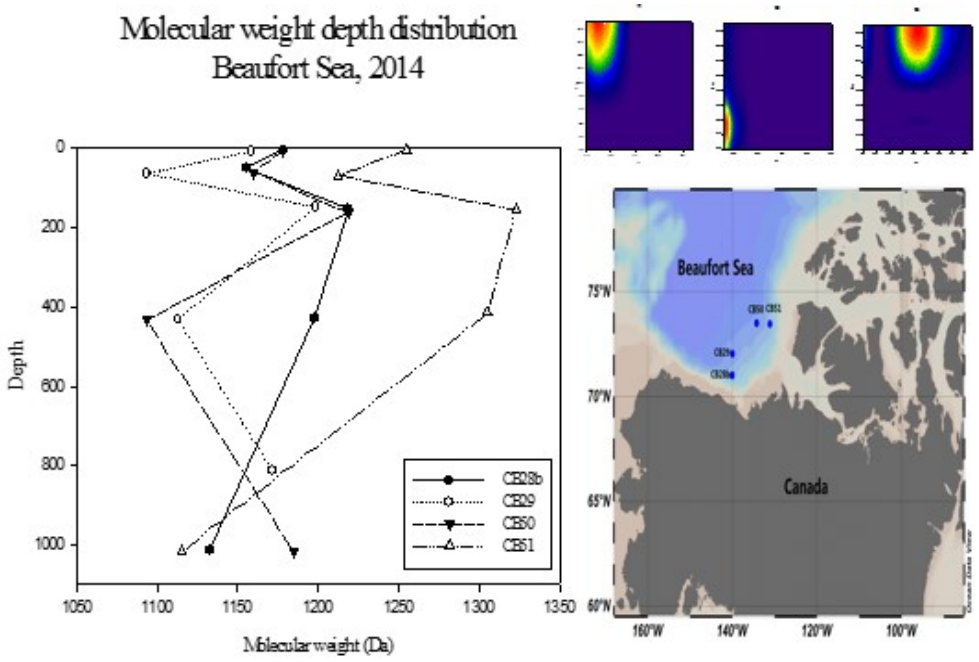
The molecular weight (MW) of dissolved organic matter (DOM) is considered as an important factor affecting the bioavailability of organic carbon and associated chemical species. DOM MW distribution was determined, for the first time, in the Beaufort Sea (Canada Basin) by asymmetrical flow field-flow fractionation (AF4) with UV-absorbance and fluorescence detection. The pH and conductivity of the carrier solution was similar to that of samples to preserve the native chemical environment of DOM. The molecular weight (MW) distribution of DOM was estimated using a series of organic macromolecules ranging from 479 to 14,300 Da. The apparent MW ranged from 1,093 Da to 1,320 Da, congruent with previous studies using high performance size exclusion chromatography and tangential flow filtration. Higher DOM MW was measured at the eastern-most station, likely due to the melting of sediment-laden ice commonly found in the eastern Beaufort Sea. The MW distribution was influenced by the origin of the water masses (Pacific- vs Atlantic- derived) in the Beaufort Sea. Indeed, a minimum in MW was observed in the summer Pacific-derived upper halocline (60 m depth), while higher MW DOM was associated with the winter Pacific-derived middle halocline (150 m depth). The higher MW DOM was also enriched in humic-like DOM as previously found. A decreasing trend was found from the middle halocline (150 m depth) to the Atlantic warm waters (400 m depth), congruent with intense mineralization in deeper waters. The Atlantic deep waters (1000 m depth) did not show any significant change in MW distribution (1115 Da to 1185 Da) across the study area, suggesting homogeneous DOM MW distribution in deep waters. The findings here indicate that water mass origin and mineralization processes can influence DOM MW in the open ocean.



The picture displays vertical distribution of DOM molecular weight determined by asymmetrical flow field-flow fractionation system on the left side, with a sampling map in the Arctic Ocean on the bottom right. Fluorescent components identified in the samples are shown on the top right.

O35

PREPARATIVE SEPARATION OF PARTICULATE FUNCTIONAL MATERIALS USING FIELD-FLOW FRACTIONATION IN ROTATING COILED COLUMNS

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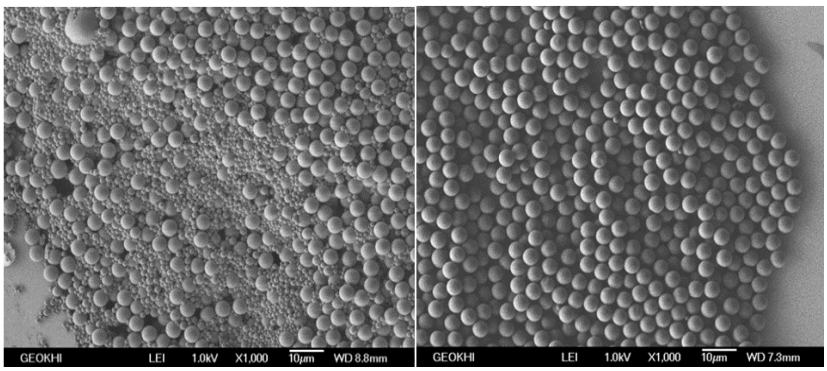
Properties of particulate functional materials are known to be strongly dependent on size distribution of particles. The synthesis of particles with required particle size distribution is a complex and sometimes impossible task. Therefore, separation (and hence purification) of synthesized particulate samples is an urgent necessity.

Field-flow fractionation (FFF) is powerful and versatile particle separation technique, which covers a size range from 1 nm to 100 μm. However, the limitation related to sample weight (about 1 mg) makes conventional FFF unable to be used for the preparative applications. Fractionation in a rotating coiled column (RCC), which can be attributed to non-conventional sedimentation FFF, enables this limitation to be avoided. The fractionation in RCC is achieved in a long column under a complex asymmetrical force field generated in planetary centrifuges. The weight of the sample to be fractionated is dependent on volume of the column. Therefore, the sample weight can be increased with increasing the column volume.

It has been shown that analytical RCC with total volume of 15 mL can be successfully used for the separation and purification of 100 mg of chromatographic sorbent (density 1.1 g/cm3). As a result, nearly monodisperse target fraction of 4.5 μm particles was separated as well as waste fraction consisted of 0.5–2 μm particles, particle fragments, and residues of sorbent synthesis. The scaling up experiments have been conducted using RCCs of greater total volume. It has been demonstrated that RCCs with volume of 73 and 453 mL enabled 0.5 and 3.0 g, correspondingly, of the initial polydisperse sorbent sample to be fractionated in a single run with the same efficiency as for analytical 15 mL column. FFF in RCC has been also applied to the separation of silumin (density 2.6 g/cm3) >20 μm particle fraction, which is used as a feedstock for additive manufacturing; the possibility of preparative purification of metallic powders being estimated.



Microphotographs of chromatographic sorbent sample before (left) and after (right) separation in the rotating coiled column

O36

APPLICABILITY OF ASFLFFF FOR INDUSTRIAL LIGNOSULFONATE ANALYSIS

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Sulfite pulping results in accumulation of large amounts of by-products containing lignosulfonates (LS) as the main component. Due to their structural characteristics LS are currently the most widely utilized lignins with respect to a non-energetic application. The search for more innovative utilization tactics still attracts a lot of attention in research and industry. The functional properties of LS are to a large extent dependent on the molar mass characteristics of the samples. However, there is a lack of fast and precise methods of LS molar mass analysis. Conventional size exclusion chromatography (SEC) is currently the method of choice but hampered by various shortcomings. Thus, the complex structure of the analyte and the presence of various impurities in sulphite spent liquors require tedious and time consuming sample purification and fractionation steps. In addition, LS like almost all lignins are known to emit fluorescence which disturbs light scattering detection and result in erroneous molar mass values. On the other hand the use of polymer standards for SEC column calibration also leads to deviations from the actual LS molar mass values due to differences in hydrodynamic volumes of the polymers.

Asymmetric flow field-flow fractionation (AsFlFFF) represents a good alternative to the conventional SEC technique with respect to LS molar mass analysis. It allows injection of non-purified industrial liquors resulting in a significantly simplified sample preparation procedure. A variety of critical AsFlFFF parameters including laminar and cross-flow rate, sample concentration, injection and focusing time were adjusted towards an optimal set of parameters for sample separation and recovery. The method´s applicability was tested using various industrial LS samples. The results were compared to those obtained by alternative techniques, such as APC, GPC and DOSY NMR. A critical overview on the advantages and obstacles of using AsFlFFF for LS analysis is presented.

O37

MICROFLUIDIC FREE FLOW ELECTROPHORESIS USING TUNABLE CONDUCTIVE PDMS POLYMER MEMBRANES

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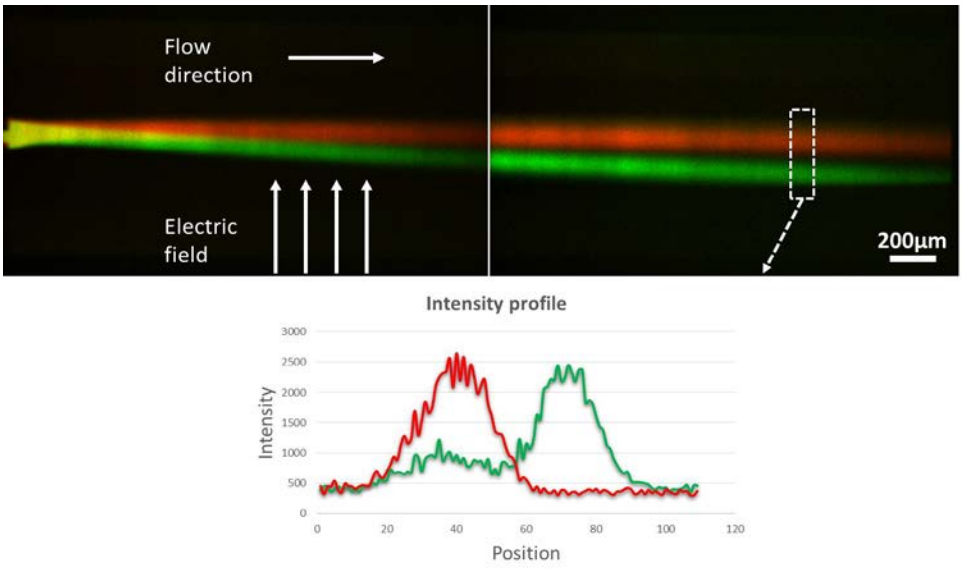
Free flow electrophoresis (FFE) utilizes a combination of fluid flow and electric field-induced electrophoresis to deflect analyte perpendicularly to the flow direction for sample separation or concentration. Several microfluidic FFE systems have been developed for biomedical, analytical and diagnostic applications. One major issue with miniaturized FFE systems is that the electrodes must be isolated from the main flow stream to prevent sample losses and electrode damage from electrolysis. Many strategies are used to isolate the electrodes from the main flow channel, including conductive gels, insulators and micro side channel arrays. However these techniques suffer from poor mechanical strength and screen a significant portion of the electric field from entering the flow channel. In this talk, we demonstrate a new type of tunable PDMS-based microfluidic FFE technique. Our approach utilizes easy-fabricated 3D metal electrodes, directly injected against the conductive PDMS membranes and integrated into the microchannel sidewalls. The conductive membranes are fabricated using nano-composites doped PDMS, and separate the electrophoresis channel from the gallium metal electrodes. These membranes are capable of preventing electrode contamination from electrolysis, while still providing sufficient electric field for FFE. The membranes also possess high mechanical strength and do not suffer from membrane breakdown caused by high electric field strengths. By adding different types of nano-composites like carbon blacks tubes or silver particles, the membranes also turn tunable for specific experiment designs. We first demonstrate the capabilities of these electrode-membrane systems for microfluidic separation and enrichment using fluorescent dye based FFE-isotachophoresis and FFE-zone electrophoresis. We then demonstrate its application for on-chip sample preparation by isolating different classes of biomolecules from mixtures and focusing them into concentrated bands.



Free flow zone electrophoresis separation of two fluorescent dye with designed tunable conductive PDMS membrane device. Fluorescein is shown in green and Rhodamine B is marked in red. The intensity profile demonstrates a complete separation of two molecules within a second after an electric field is applied

O38

BIOMAGNETIC SEPARATION WITH VARIABLE ELECTROMAGNETIC INDUCED FORCE FIELDS

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Biomagnetic Separation is a powerful tool to separate cells for further analysis using a magnetic field applied on a suspension. An important application are blood tests where the amount of high cost particles and the time for the separation are crucial.

To improve this method we developed a process using variable magnetic fields to accelerate the superparamagnetic particles in a sample container [1]. The aim is to achieve a high relative velocity between particles and cells in order to improve the complex formation between them. This results in a reduced separation time or the amount of particles needed to achieve the same separation results. Through simulations of the magnetic field as well as modeling of the acting forces, we optimized the geometry of the pole shoes of a customized electromagnet and investigated the stream and agglomeration behavior.

We used microscopic imaging to evaluate this method and the movement of the particles in a suspension during the application of the magnetic field. Due to the considerable influence of the streaming and separation rate, we also investigated the agglomeration behavior of the particles based on the used buffer and the influence of the magnetic field. In the used sample we proved and visualized a directed movement of the particles through trajectories.

Based on these results the experimental setup has been equipped with three customized electromagnets in a defined process of charging the magnets in order to induce a double circular particle stream and a high relative velocity to the cells. The proof-of-concept demonstrates an improved separation of E. coli from a suspension [2].

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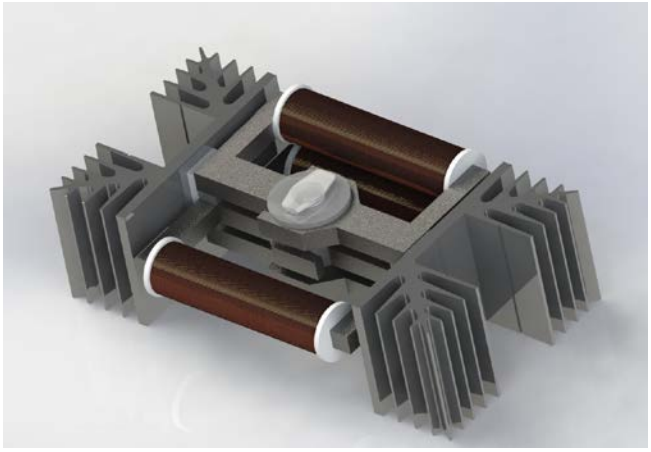
[2] R. Stange et al. Engineering in Life Sciences, (2015) 15, 727-732.



CAD-model of the experimental setup equipped with three customized electromagnets for the Biomagnetic Separation

O39

TRANSLOCATION OF MACROMOLECULES THROUGH POLYMER-BRUSH COVERED MICROCHANNELS

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The transport of macromolecules with different architectures through polymer-brush covered microchannels is investigated by means of molecular dynamics simulations. Star polymers with different functionalities and arm lengths are embedded in a polymer-brush bilayer of linear chains. The macromolecules are driven through the channel by a Poiseuille flow with various pressure gradients. A comparison between diffusion in equilibrium and under Poiseuille flows reveals changes of the transport properties depending on the architecture of the embedded molecules. While the self-diffusion for stars with intermediate softness is slower than for linear chains and stars with large functionality and short arms (the latter representing the limit of hard spheres), the current density of macromolecules in Poiseuille flows depends strongly on pressure gradient and decreases monotonically with increasing functionality. Our data for density and flow profiles as well as binary interactions among different species (brushes, solvent, macromolecular inclusions) reveal a complex translocation mechanism, which should be relevant in biological transport processes and micromechanical applications.

Abstracts of the posters

P1

CHARACTERIZATION OF STIMULI RESPONSIVE MICROGELS WITH FLOW FIELD FLOW FRACTIONATION

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Microgels are colloidally stable, solvent swollen particles that are lightly cross-linked. Owing to their versatility, they have utility in numerous biological applications like the delivery of therapeutics, microlenses and the development of non-fouling coatings. Additionally, their tunability with respect to functionality, mechanical properties and architectures further improves their scope and applicability. The presence of charged functional groups on microgels imparts unto them the property of being responsive to stimuli such as ionic strength and pH. Furthermore, microgels also reversibly respond to changes in temperature by undergoing a coil to globule transition through enhanced polymer-polymer interactions. The deformability of microgels can also be tuned by modifying their crosslinking density.

The characterization of microgels by FFF-F has the potential to provide a wide range of information about them, most importantly their size distribution and polydispersity index. This data in conjunction with that obtained via dynamic light scattering (DLS) can provide solution and individual characteristics for microgel dispersions. This knowledge can then be leveraged to optimize synthesis conditions for microgels suited to particular applications. Additionally, the purity of microgel dispersions can be determined by FFF-F, through the detection of soluble polymer fractions left over in solution post synthesis. Similarly, such analysis following core/shell microgel synthesis can provide useful information regarding the formation of secondary particles, which could otherwise perturb further particle analyses or utilization of these microgels in specific applications.

P2

NANOGELS BASED ON POLY(METHACRYLIC ACID)/ POLYACRYLAMIDE INTERPENETRATING POLYMER NETWORKS AS DRUG DELIVERY SYSTEM FOR VERAPAMIL HYDROCHLORIDE

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Interpenetrating polymer networks (IPNs) are a special class of polymeric materials which potential in the area of drug delivery still remains unexplored. The combination of two or more polymer networks results into a new material with structure and properties that differ significantly from those of its components. Many IPNs' properties are favorable for drug delivery application such as controllable porosity, swelling degree and "smart" behavior upon two or more external stimuli (temperature, pH, ionic strength, etc.).

The nanogels are swelled polymer networks based on hydrophilic or amphiphilic polymers within nanoscale size . They are successfully entering the field of controlled drug delivery as nanocarriers due to their hydrophilicity, good colloidal stability, inertness in the blood stream and their internal aqueous environment.

The aim of this investigation is to synthesize nanogels from the interpenetrating polymer networks of poly(methacrylic acid) and polyacrylamide and demonstrate their potential as drug delivery systems for verapamil hydrochloride. Nanogels with different compositions were obtained via inverse miniemulsion sequential polymerization by varying the ratio between the components. The prepared nanogels were characterized in terms of their zeta potential (ZP) and size under pH titration conditions. Assymterical flow field flow fractionation (AF4) method was successfully applied to evaluate the nanogels size as well as the verapamil hydrochloride loading efficiency in nanogels with different composition.

Acknowledgements: This research was financially supported from DAAD Project DNTS/Germany 01/12.

[1] Kabanov AV, Vinogradov SV, Angew Chem Int Ed Engl. 2009;48(30):5418-29

P3

GENERIC SAMPLE PREPARATION PROCEDURE FOR ISOLATING INORGANIC ENGINEERED NANOPARTICLES FROM COMPLEX MATRIXES

Milica Velimirovic[1], *Stephan Wagner*[1,2], *Fazel Abdolahpur Monikh*[1], *Frank von der Kammer*[1], *Thilo Hofmann*[1]

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The applicability of a previously developed multi-step generic sample preparation procedure for detection, characterization, and quantification of engineered nanoparticles (ENPs) in complex matrixes has been extended to commercial end products: 1) a powdered tomato soup which contains the anti-caking agent SiO2 (E551), and 2) a sunscreen which contains TiO2 as UV-filter.

The extended multi-step generic sample preparation procedure included: I) pre-characterization of the sample, II) homogenization of the sample, III) ENP isolation from the matrix, IV) ENP enrichment, and V) ENP stabilization. The mass recovery was calculated by elemental analysis of Si and Ti, respectively. The size distribution of the isolated ENPs was determined by asymmetric flow field-flow fractionation (AF4) coupled to multi-angle laser light scattering (MALLS). In order to quantify the mass concentration of the particles eluting from the AF4 an inductively-coupled plasma mass spectrometry (ICP-MS) was used.

Isolation of SiO2 (Si mass recovery > 90%) from the matrix was achieved by open vessel water bath acid digestion (HNO3/H2O2) method supported by microwave assisted digestion. Enrichment procedure and stabilization of SiO2 in suspension were performed prior to size characterization. The size distribution of commercially available pristine E551 was comparable with the size distribution of the SiO2 ENPs isolated from the powdered tomato soup.

TiO2 ENPs from the sunscreen were isolated (Ti mass recovery > 90%) by applying the open vessel digestion at water bath using HNO3:H2O2 as oxidation agent. AF4-MALLS-ICPMS analysis after stabilization of isolated TiO2 ENPs indicated that the size distribution was similar to the size distribution of pristine TiO2 ENPs.

Presented data indicate that the proposed multi-step generic sample preparation procedure might be a very important tool in standardizing the analytical methods for determining ENPs in complex matrixes, such as food and cosmetics.

P4

ASYMMETRIC FLOW FIELD-FLOW FRACTIONATION FOR CHARACTERIZATION OF HIGHLY CONCENTRATED CONJUGATES OF GOLD NANOPARTICLES AND ANTIBODIES

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Conjugates of gold nanoparticles (GNP) with antibodies are powerful tools for analytical purposes. Rapid immunoassay formats such as the lateral flow immunoassay are based on the use of these conjugates in highly concentrated form to reach their significant binding and high detected signals after minimal time of interaction. However, the conjugates state in highly concentrated preparations remains unclear. In the present study, we have applied asymmetrical flow field-flow fractionation (AF4) to answer this question. The influence of composition and concentration of the conjugate on its non-specific binding and aggregation was studied. The GNP with the average diameter of 15.3 ± 1.2 nm were conjugated with eight antibodies of different specificities. We found that while the GNP preparation had a zeta potential of –31.6 mV, the conjugates had zeta potentials from -5.8 mV to -11.2 mV (Zetasizer Nano ZSP, Malvern Instruments). To estimate the aggregation in highly concentrated solutions (18.6 nmol per load, OD520 was up to 80), the AF4 was accomplished by the Eclipse 3+ separation system (Wyatt Technology Corp.). To define the hydrodynamic and gyration radii of the conjugates, dynamic light scattering and multiangle laser light scattering techniques were used. Single conjugates and mix of eight conjugates (2.3 nmol per load) demonstrated equal average radii. The increased concentrations of the conjugates mix did not caused changes in form of chromatograms (see Figure, a), and the concentration dependence for their areas was strongly linear (see Figure, b). These results demonstrate the absence of non-specific binding and aggregation for concentrated conjugates preparations, as well as the efficiency of AF4 for characterization of the conjugates state.

This study was supported by the Russian Foundation for Basic Research (project no. 14-08-31563-mol-a).

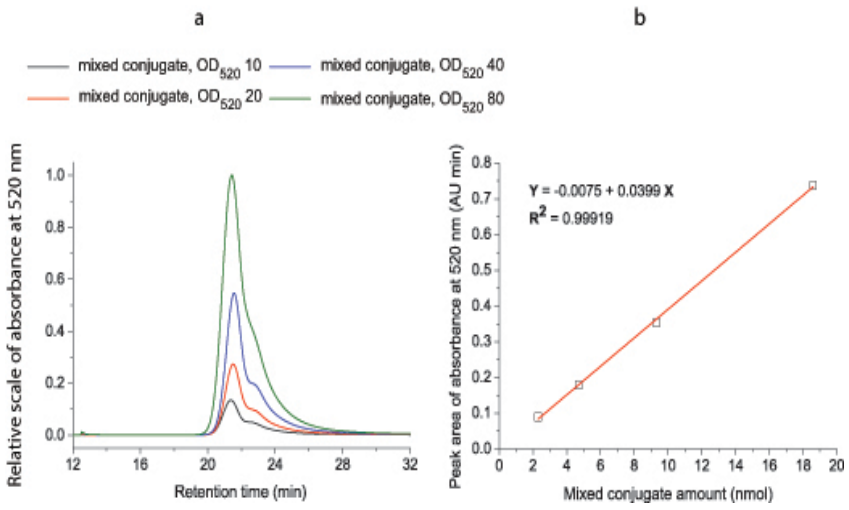

Figure: AF4 for highly concentrated mixed conjugate (a), calibrate plot of peak area versus conjugate amount (b). The separation was performed at the channel flow of 1 mL/min, focus flow of 1 mL/min and variable cross flow (linear rate gradient from 1.6 to 0.4 mL/min (from 18 to 22 min) and then at constant 0.4 mL/min (during 10 min).

P5

DETERMINATION OF MASS AND DENSITY OF NANOMATERIALS USING CENTRIFUGAL FIELD-FLOW FRACTIONATION, SINGLE PARTICLE ICP-MS AND TRANSMISSION ELECTRON MICROSCOPY

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In Centrifugal FFF (CF3), particle mass (or size) can be obtained from the retention time using the FFF theory, if the particle density is known. For particles with unknown density, particle mass and density can be measured by either carrying out the separation at different carrier densities or by combining CF3 with dynamic light scattering detection [1,2].

In this study, a similar methodology was applied to measure mass, size and density of monodispersed nanoparticles. Thereby, CF3 and single particle ICP-MS enable the determination of the mass and TEM the volume of the nanoparticle. The nanoparticle density can then be calculated from the obtained mass and volume data. The methodology was validated by measuring the density of differently sized polystyrene latex beads standards. The measured densities of the polystyrene standards were in the range of 1.04 g/mL to 1.05 g/mL. Data are presented for 60 nm sized silver-shelled gold nanoparticles indicating a porous rather than a solid structure of the investigated nanoparticles.

[1] Giddings JC, Karaiskakis G, Caldwell KD (1981) Separ Sci Technol 16: 607-618
[2] Caldwell KD, Jones HK, Giddings JC (1986) Colloid Surface 18: 123-131

P6

LARGE-SCALE SYNTHESIS AND SIZE CHARACTERIZATION OF SILICA NANOPARTICLES USING ASYMMETRICAL FLOW FIELD-FLOW FRACTIONATION (AF4)

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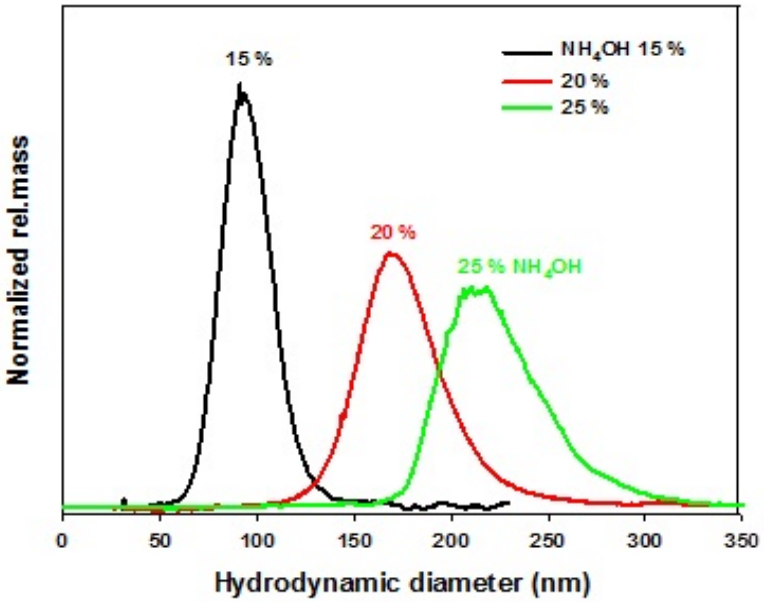
Industrial application of silica nanoparticles (NPs) is widespread such as food, medical, and biological industries. To measure particle size is important that determines characteristics of the silica nanoparticles. An accurate sizing technique is required for control in synthesis of silica NPs.

Several techniques have been used for size measurement of NPs including dynamic light scattering (DLS), electron microscopy (such as, scanning electron microscope (SEM) and transmission electron microscopy (TEM)), and field-flow fractionation (FFF).

In this study, silica nanoparticles were synthesized using sol-gel method by mixing ethanol, ammonium hydroxide, and tetraethyl orthosilicate (TEOS) at room temperature. A member of FFF family, asymmetrical flow FFF (AF4) was used for size characterization of the silica NPs. The AF4 results were compared with obtained results from SEM and DLS.

First, the synthesis was performed in a lab-scale (about 175 mL), where the effect on particle size and size distribution of various parameters were methodically investigated. Then Large-scale volume was extended to about 2 L. It was confirmed that, as the purity of TEOS or ethanol increases, the size of the silica NPs tended to decrease, while, as the concentration of ammonium hydroxide increases, the size of silica NPs tended to increase. Also, condition of large-scale synthesis was optimized that various parameter (the type, height, and the RPM of the agitator).



Size distribution of silica nanoparticles synthesized in a large scale at various concentration of ammonia.

P7

EL-FFF SEPARATION OF NANOPARTICLE MIXTURES

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Electrical field flow fractionation (EI-FFF) is a rapidly developing technique to separate and characterize nanoparticles on the basis of their size and charge (electrophoretic mobility). Much work is needed in El-FFF characterization as a technique since many particles for separation can be quite different from model particles such as polystyrene; likewise mixtures of particle types can demonstrate elusive nuances making them difficult to separate. The present work first evaluates standard El-FFF fractionations of 20-, 50-, 100-, 200-, and 400-nm diameter particles by retention time and measured size. The EI-FFF was connected to MALS detector for particle size analysis after separation. Various parameters affecting the separation were examined to get the best separation resolution. A fractionation of carboxylate and amine modified polystyrene had well resolved sizes and slightly different individual retention times, however, a separation of their mixture did not. Cyclical electrical field flow fractionation (Cy-EI-FFF) was also used to investigate the separation of other hard-to-separate mixtures: polysaccharides and their protein conjugates individually and also from polysaccharides-protein conjugates to get optimal separation.

P8

HYPHENATION OF FIELD-FLOW FRACTIONATION AND SINGLE PARTICLE ICP-MS FOR THE ASSESSMENT OF NUMBER-BASED PARTICLE SIZE DISTRIBUTIONS AT ULTRATRACE LEVELS

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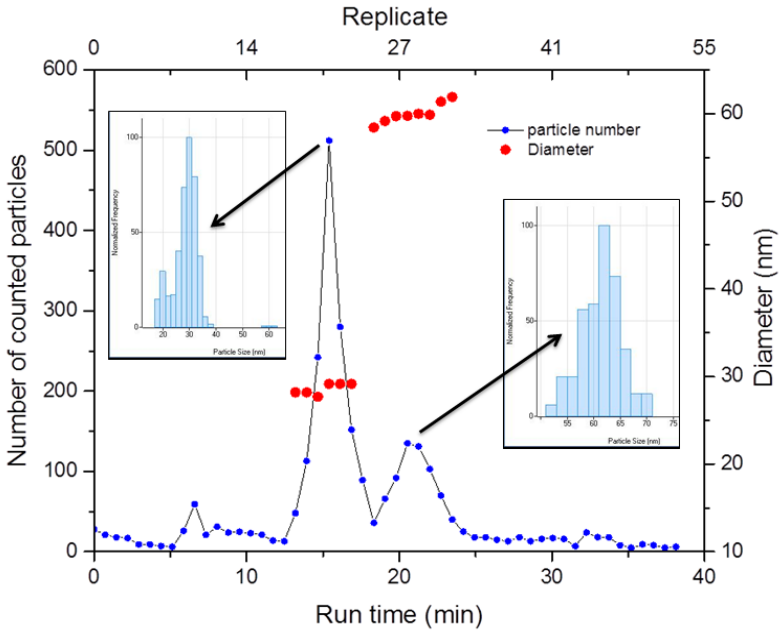
One of the challenges in characterization of complex nanomaterials in the environment is to obtain number based information such as concentration and size distribution at environmentally relevant concentrations. The combination of Field-Flow Fractionation and Inductively Coupled Plasma Mass Spectroscopy (FFF-ICP-MS) has been proven to be an essential analytical technique for characterization of environmental samples, but it lacks a direct measurement of particle numbers.

Single Particle ICP-MS (spICP-MS) is a new analytical technique to provide number based information for monodisperse metal and metal oxide nanoparticles at ppt concentration levels.

This presentation reports direct hyphenation of spICP-MS to both an Asymmetrical Flow and a Centrifugal FFF system. The spICP-MS was utilized as an online number detector for characterization of gold, silver, silica and titania nanoparticles as well as different mixtures thereof. The hyphenated technique was able to measure the number-based concentrations and size distributions of the nanoparticles by counting and sizing the respective nanoparticle mixtures in one single run.



The image displays the particle number-based fractogram of the mixture of 30 nm and 60 nm gold nanoparticles obtained by AF4-spICP-MS. The red circles represent the average replicate diameter measured by spICPMS.

P9

AF-4 TO CHARACTERIZE SULFATED GAG BUILDING BLOCKS FOR CELL INSTRUCTIVE BIOHYBRID HYDROGELS

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Biohybrid hydrogels composed of heparin and star-shaped poly(ethylene glycol) PEG has become a valuable platform for the sustained delivery of various cytokines to modulate cell fate decisions. The high negative charge of heparin is the base for the biomimetic, electrostatical conjugation of the cytokines. A variation of the GAG sulfation pattern and thus a modulation of the electrostatic interaction should allow to control the release kinetics from this materials.

This can be achieved by chemical hydrolysis to selectively remove sulfate groups followed by by maleimide functionalization to allow hydrogel formation with thiol terminated PEG by Michael type addition. Multiple analytical tools were explored to characterize the resulting GAG-building blocks. DLS batch measurements for heparin derivatives showed the presence of multiple populations' species for desulfated and maleimide conjugated heparin yet the concentration of the bigger sized species was not quantifiable. Accordingly, we systematically investigated the effect of desulfation and maleimide conjugation to the heparin's size and aggregation tendency by separation od different sized population and analyzed by both refractive index (RI) and DLS detectors. Desulfated heparin samples showed a narrow distribution of particle while maleimide conjugation to desulfated heparin caused a broader size distribution for the 6-O-N desulfated and 6-O-desulfated heparin derivates indicating the presence of multiple populations. The dual hydrophobic modification caused by the removal of the sulfate groups from the 6O and N positions and the introduction of hydrophobic maleimide groups resulted in a tendency of the heparin derivates to undergo self-association. As such, AF-4 coupled to RI and DLS detectors enabled the accurate quantification of the amount of self-aggregated species at different desulfated heparin derivates, which couldn't be achieved by DLS solely.

P10

ANALYSIS OF THERMOREVERSIBLY CROSSLINKING POLYMER NETWORKS BY TEMPERATURE DEPENDENT SIZE EXCLUSION CHROMATOGRAPHY

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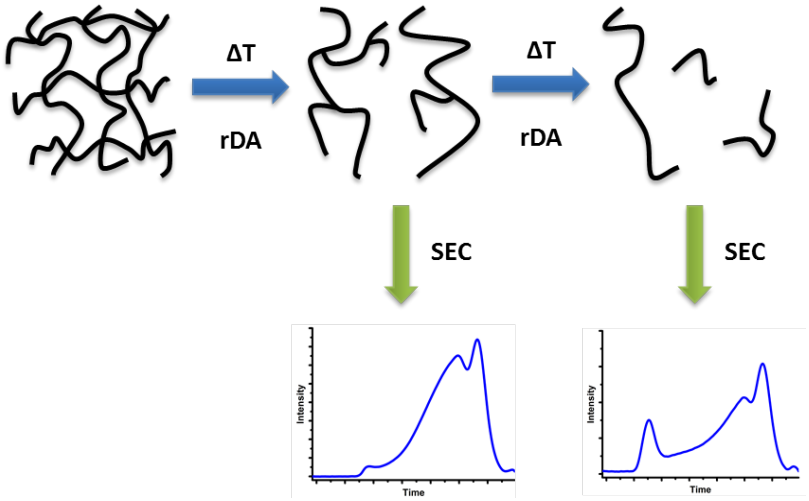
The analysis of polymers and especially cross-linked polymers such as networks was the aim of many studies. The difficulty of characterizing those compounds lies often in their limited solubility, which makes standard analysis techniques as NMR or SEC difficult. Hence, often an indirect approach for the analysis is chosen, where the network first undergoes a degradation process of the structure in order to obtain soluble compounds. These can be analyzed more easily and the gained results allow drawing conclusions of the network structure.

The objective of this work is the analysis of a thermoresponsive Diels-Alder (DA) polymer network that undergoes depolymerization via a retro Diels-Alder (rDA) reaction at increased temperatures. By temperature dependent size exclusion chromatography (TD SEC) the networks can be opened at high temperatures with subsequent in situ investigation of the obtained network fragments.[1] With the TD SEC it is possible to study the rDA network opening at well-defined temperatures. The combination of dRI, UV/Vis and viscosity detection gives detailed insights in molar mass, chemical composition and conformation of the dissolved network fragments.

[1] J. Brandt et al., Anal. Bioanal. Chem., Vol. 405 (2013), 8981–8993.



Schematically displayed decomposition of the DA networks with in situ investigation of the obtained network fragments via SEC.

P11

TEMPERATURE DEPENDENT SIZE EXCLUSION CHROMATOGRAPHY FOR THE IN SITU INVESTIGATION OF DYNAMIC BONDING/DEBONDING REACTIONS

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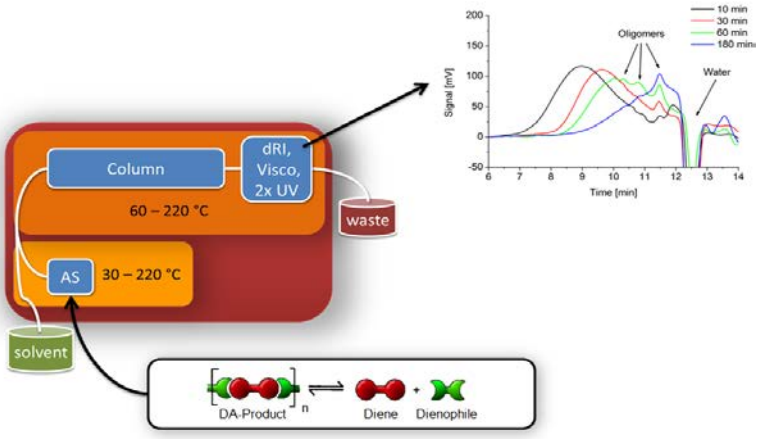
Polymers capable of thermally controlled reversible bonding reactions are promising candidates for stimuli responsive materials, as required for self-healing or drug delivery materials. In order to investigate how the dynamic reactions can be controlled, effective analytical tools are demanded that are capable of analyzing not only the polymers but can also monitor the respective bonding reactions [1]. Herein, we employ size exclusion chromatography in a newly developed temperature dependent mode (TD SEC) for the in situ characterization of polymers that undergo retro Diels-Alder (rDA) reaction at temperatures higher than 60 °C. Monitoring the evolution of the molar mass distribution of the polymers during the rDA reaction and evaluating the data quantitatively gives detailed information about the extent of the reaction and allows elucidating structural parameters that can be used for controlling the polymers debonding behavior [2–4].

In contrast to spectroscopic techniques, TD SEC analyzes only the size of the polymers, hence the polymers do not need to fulfill any particular requirements (e.g. presence of detectable functional groups) but only need to be soluble in the TD SEC, which makes the method universally applicable. Side effects that might bias the results are minimized by using a high temperature chromatograph that allows performing the analysis in a broad temperature range (60 – 200 °C) and in different solvents. Thus, the analysis can be performed under the exact conditions that are required for the bonding reactions and an in situ image is provided.

[1] J. Brandt et al., Adv. Mater., 2014, 26, 5758-5785.
[2] N. K. Guimard et al., Chem. Sci., 2013, 4, 2752-2759.
[3] J. Brandt et al., Anal. Bioanal. Chem., 2013, 405, 8981–8993.
[4] K. Pahnke et al., Chem. Sci., 2015, DOI: 10.1039/C4SC02908A



Schematic representation of the TD SEC analysis scheme.

P12

A BIOHYBRID TOPOLOGICAL DIVERSITY INVESTIGATED BY ASYMMETRICAL FLOW FIELD-FLOW FRACTIONATION

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Biohybrid structures formed by non-covalent interaction between avidin as a bridging unit and biotinylated glycodendrimers based on poly(propylene imine) (GD-B) have potential for biomedical application.[1] Therefore, an exact knowledge about molar mass, dispersity, size, shape and molecular structure is required. Asymmetrical Flow Field-Flow Fractionation (AF4) was applied to separate pure and assembled macromolecules according to their diffusion coefficients. The complex biohybrid structures consist of single components (avidin, differently valent GD-B) and nanostructures. These nanostructures were systematically studied depending on the degree of biotinylation and ligand-receptor stoichiometry by AF4 in combination with dynamic and static light scattering detection. This enables the quantification of composition and calculation of molar masses and radii, which were used to analyse scaling properties and apparent density of the formed structures. These data are compared to hydrodynamic radii obtained by applying the retention theory to the AF4 data. It is shown that depending on their architecture the molecular shape of biohybrid structures is changed from rod-like to spherical towards network-like behaviour (Fig. 1).[2]

[1] Ennen, F.; Boye, S.; Lederer, A.; Cernescu, M.; Komber, H.; Brutschy, B.; Voit, B.; Appelhans, D. Polym. Chem. 2014, 5, (4), 1323-1339.
[2] Boye, S.; Ennen, F.; Scharfenberg, L.; Appelhans, D.; Nilsson, L.; Lederer, A. Macromolecules 2015, 48, (13), 4607-4619.

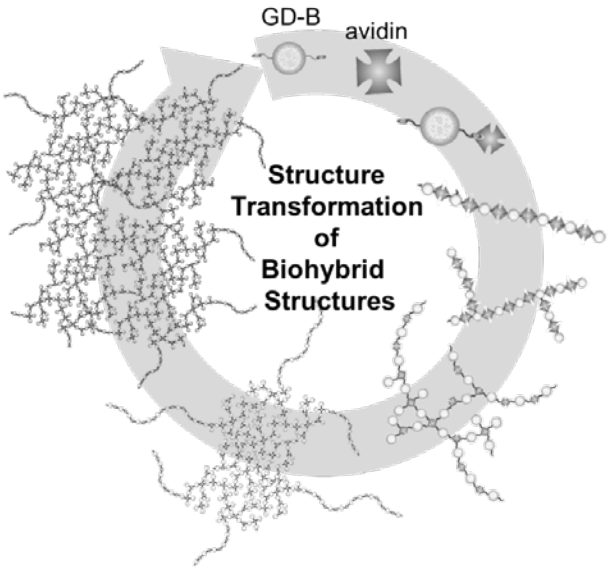

Figure 1: Schematic illustration of structure transformation (from 1D to 3D) of biohybrid structures formed by avidin and GD-B.

P13

HYDRODYNAMIC CHARACTERIZATION OF FUNCTIONAL POLY(ETHYLENE GLYCOL)S BY MEANS OF ANALYTICAL ULTRACENTRIFUGATION AND VISCOSIMETRY

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The solution behavior of pharmaceutically relevant polymers plays a pivotal role in many areas, in particular the life sciences. To this end, we present a combined hydrodynamic study of end-group functional, low dispersity, anionically synthesized poly(ethylene glycol)s (PEGs). Analysis is performed via viscosimetry, sedimentation velocity analysis of individual PEGs, as well as sedimentation velocity analysis in well-defined mixtures thereof.

Fundamental scaling relationships in the pharmaceutically relevant molar mass range of only a few thousand to tens of thousands of Daltons underlines the necessity of complementary hydrodynamic methods as the only means for a complete structural understanding of such and almost any polymer in solution. Our study indicates end group specific solvation of the macromolecules in dependence of absolute molar mass, information on which most other commonly utilized methods lack in significance and insight. We as well demonstrate that absolute methods as well account for small, though significant variations of scaling relationships and a physically sound estimation of hydrodynamic volumes, diameters, and diffusion coefficients. Our data clearly show a systematic deviation of other analytical methods (such as SEC) from that of the sedimentation velocity analysis. This is explained in particular by the estimation of molar masses based on first principle hydrodynamic analysis in the present study. This hydrodynamic methodology is shown being of pivotal significance not only for identification of physicochemical properties of polymers in solution, but as well paves the way for an understanding of controlled mixtures of different molar masses.

Finally, a differential observation of sedimentation velocity in small to intermediate molar mass mixtures indicates the opportunity to estimate key hydrodynamic characteristics in more complex polymer systems.

P14

SYNTHESIS AND CHARACTERISATION OF A POLY-THIOPHENE-OLIGODEOXYNUCLEOTIDE BLOCK COPOLYMER FOR THE SITE-SPECIFIC ATTACHMENT TO DNA ORIGAMI

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2) *Technische Universität Dresden, Mess- und Sensortechnik, Professur für Physikalische Chemie, Dresden, Germany*
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DNA origami structures [1] (Fig. 1) are formed via biomolecular self-assembly processes. They show great potential to act as molecular “breadboard” for opto-electronic devices such as nanoantennas because of their capability to arrange functional heteroelements site-specifically with a high spatial resolution. Such functional heteroelements can be conjugated polymers that are nanoscaled (semi-)conductors with appealing optical and electronic properties. The P3RT-type polythiophene-derivates are interesting species because of their controlled synthesis through the chain-growth mechanism [2].

To attach polythiophene to origami we synthesised a water-soluble, functionalised poylthiophene-derivate (P3(EO)3T) via Kumada catalyst-transfer polycondensation. An amine functionalised catalyst was chosen to provide the highest yield of functionalisation. Next, a modified, synthetic oligodeoxynucleotide (ODN) was covalently bound to the P3(EO)3T polymer, yielding the block copolymer P3(EO)3T-b-ODN. Further, we investigated the block copolymer and its single compounds (bare ODN and P3(EO)3T) with asymmetric flow field-flow fractionation in combination with UV detection. Here, we used the characteristic wavelengths of DNA and P3(EO)3T. The formation of the P3(EO)3T-b-ODN was visible in a shift in the chromatogram to higher molecular masses compared to the bare compounds.

[1] P. Rothemund, Nature 440, 297–302 (2006).
[2] A. Kiriy, et al., Macromol. Rapid Comm. 32, 1503–1517 (2011).

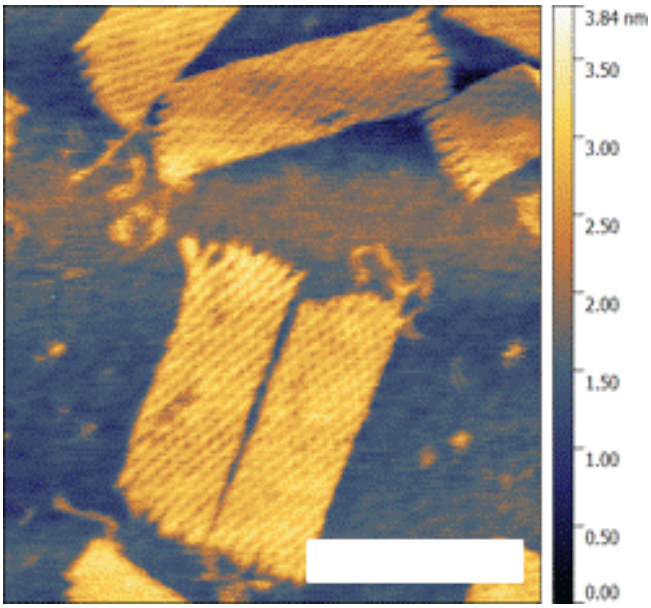

Figure 1: Liquid-AFM image of the target DNA origami structure, a 2D pad on which the block copolymer P3(EO)3T-b-ODN can be arranged. Scale bar 100 nm.

P15

POLYSACCHARIDE CHARACTERIZATION BY HF5 WITH ON-LINE MULTI-ANGLE STATIC LIGHT SCATTERING AND DIFFERENTIAL REFRACTOMETRY

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Polysaccharides are renewable macromolecules that have potential (either in native form or after structural modification) in various industrial applications as synthetic polymer substitutes. Accurate characterization of the molar mass and size of polysaccharides is an ongoing challenge, oftentimes due to structural complexity but also to the broad molar mass range and to the ultra-high molar mass of many polysaccharides. In this study, we coupled a hollow-fiber flow field-flow fractionation (HF5) system on-line to both multi-angle static light scattering (MALS) and differential refractometry (DRI) detectors and employed this system to characterize various structurally different polysaccharides (series of pullulans and dextrans, and an arabinogalactan) [1]. The detectors employed allowed the determination of molar mass across the peaks of the fractograms, even though the amount of polysaccharide injected onto the hollow fiber was relatively low. In addition to molar mass, hydrodynamic sizes (hydrodynamic radius, RH) of the polysaccharide samples were determined directly from the retention times of the HF5 experiments as well as off-line using quasi-elastic light scattering (QELS). In general, good agreement was obtained between the off-line QELS determined RH of the dextrans and pullulans and the values of this same radius as calculated from HF5 theory. Also, comparison of the size data obtained from both methods provided information about the separation process itself. In cases where retention times deviated from those predicted by theory, the reasons for these differences were discussed.

[1] Pitkänen L and Striegel AM. J Chromatogr A (2015) 1380:146-155.

P16

CHARACTERIZATION OF NATURAL RUBBER SAMPLES VIA THERMAL FIELD-FLOW-FRACTIONATION

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In this presentation the application of Thermal Field-Flow-Fractionation coupled with Multi Angle Light Scattering (MALS) detection, Refractive Index detection and Evaporative Light Scattering (ELSD) detection for the comprehensive characterization of natural rubber samples is discussed.

The determination of molecular weight, radii of gyration and fractal dimension for different natural rubber samples is presented. Using different detection principles not only molecular weight but also the molecular structure of the natural rubber sample can be determined. While Refractive Index detection and Evaporative Light Scattering detection are sensitive towards concentration of the sample Multi Angle Light Scattering (MALS) detection is sensitive towards molecular weight of the sample. It is shown that MALS detection is especially useful to measure high molecular weight parts of the sample with very low concentration.

It is shown that the technique of Thermal Field-Flow-Fractionation can be used to measure the molecular weight of natural rubber samples up to several million Dalton and molecular sizes up to more than 100 nm.

P17

MULTI-DETECTOR THERMAL FIELD-FLOW FRACTIONATION OF ELASTOMERS: RELATING ELASTOMER PROPERTIES TO THE THERMAL DIFFUSION COEFFICIENT

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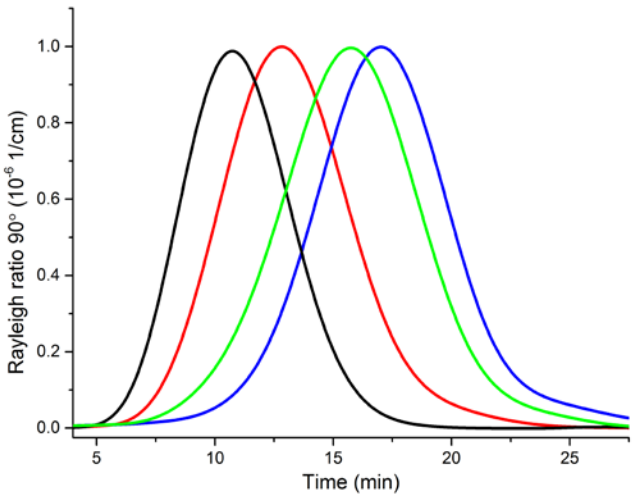
Industrial copolymers such as poly(butadiene) (PB) and poly(styrene-co-butadiene) (SBR) can contain distributions of composition and microstructure in addition to molar mass, all of which strongly influence the end properties of the elastomer. Currently there is no single method available that is able to provide information on all three of the distributions simultaneously.

Recently, it was shown that Thermal Field-Flow Fractionation (ThFFF) of PB separates based on microstructure in addition to size [1]; thereby providing an important advantage over size exclusion chromatography (SEC). The aim of our work was develop a method for the quantitative determination of PB and SBR microstructure and composition with multi-detector ThFFF. In addition, the ability to select between a size and microstructure based separation is desirable.

In ThFFF, the thermal diffusion of the polymer is related to its chemical composition as well as the solvent and has a major impact on retention time [2-4]. The choice solvent is important and dependent on the objectives of the analysis. Of the solvents investigated for the separation of PB, toluene was found to minimize the influence of microstructure while cyclohexane enhanced it. Additionally, THF was found to limit the molar mass separation of PB. SBR samples of varying monomer arrangements were able to be characterized by the molar mass, styrene content and microstructure. Future work will focus on correlating theoretical and empirical data to aid in the determination of microstructure directly from retention time.

[1] G. Greyling, H. Pasch, Macromol. Rapid Commun. 2014, 35, 1846.
[2] E.P.C. Mes, W.T. Kok, R. Tijssen, Int. J. Polym. Anal. Charact. 2003, 8, 133.
[3] J.R. Runyon, S.K.R. Williams, J. Chromatogr. A 2011, 1218, 6774.
[4] J.R. Runyon, S.K.R. Williams, J. Chromatogr. A 2011, 1218, 7016.



ThFFF of PB microstructures. 1,4 content = 94 % (black), 74% (red), 42% (green) and 12% (blue). ΔT = 65°C, Tcw = 27°C. Flowrate = 0.2 ml/min of THF. Peaks were scaled by peak maxima.

P18

SIMULTANEOUS IN-SITU MONITORING OF POLYMERIZATION REACTIONS VIA AF4 OR SEC COUPLED WITH MULTIDETECTORS

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Monitoring polymer properties during the reaction are important to understand the kinetics and mechanism of polymerization. In this work, we demonstrated that online monitoring with in-situ asymmetric flow field-flow fractionation (AF4) or size exclusion chromatography (SEC) coupled with an ultraviolet (UV) absorption、multi-angle light scattering (MALS)、differential refractive index (RI) and viscometer detectors are applied to monitor the polymerization reaction in NMP or DMF for obtaining absolute molecular weight、size and other polymer characteristics. Elution profiles can be used to monitor changes in the monomer and polymer concentrations that indicate whether the reaction occurs or not and provide a direct way to estimate the reaction rate of dynamic systems. Moreover, it is an efficient method for determining the end of the reaction and offering new strategies to optimize polymer reactions.

High Performance Asymmetric Flow Field-Flow Fractionation System Used for This Work.

P19

MULTIDETECTOR THERMAL FIELD-FLOW FRACTIONATION AS AN INNOVATIVE TOOL FOR MICROSTRUCTURE SEPARATION OF SYNTHETIC POLYMERS

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Polymer microstructure can greatly influence properties such as viscosity, solubility as well as mechanical and viscoelastic properties. However, separating polymers according to chemical composition and microstructure is challenging for traditional column-based techniques such as SEC.

Thermal field-flow fractionation (ThFFF) is a subtechnique of FFF that utilises a temperature gradient to drive analytes towards the accumulation wall of the channel. Of the various FFF subtechniques, ThFFF shows sensitivity towards chemical composition and as such has successfully been applied to address the analytical challenges associated with chemical composition. However, the question whether it is possible to separate polymers according to molecular microstructure is still an unexplored area.

It is shown that, in addition to chemical composition, ThFFF is capable of separating polymers according to microstructure. The capabilities of ThFFF to separate polymers according to microstructure is demonstrated by the separation of isotactic and syndiotactic poly(methyl methacrylate)s according to tacticity.

P20

STUDY ON MICROGEL-CONTAINING BUTADIENE RUBBERS (BR) USING THERMAL FIELD-FLOW FRACTIONATION COUPLED WITH MULTI-ANGLE LIGHT SCATTERING (THFFF-MALS)

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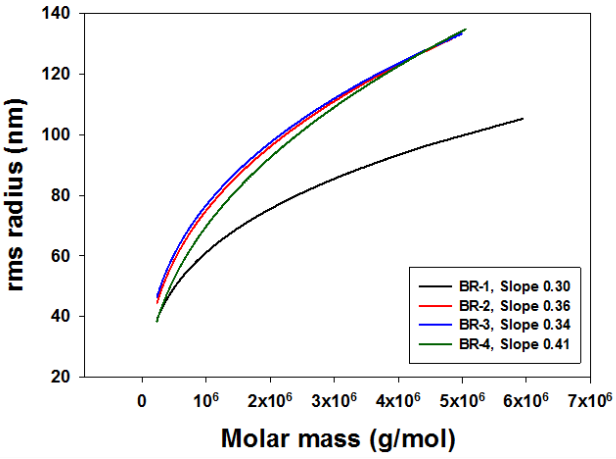
Molecular weight (MW) and the microgel content are important parameters of rubbers as they influence the physical, mechanical, and rheological properties of rubbers. Small difference in low or high MW fractions may result in significant differences in the end use properties. Thus, there is an increasing demand for accurate analysis of MW distribution and microgel content of rubbers.

Thermal field-flow fractionation coupled with multi-angle light scattering (ThFFF-MALS) was employed for characterization of microgel-containing of high cis-butadiene rubbers (cis-BR). A field-programming was used to optimize the separation of the sol and gel, where the temperature gradient was reduced according to a power function during analysis. ThFFF-MALS yielded the MW distribution of the sol, and the presence of microgel in the cis-BR.. Unlike ThFFF, size-exclusion chromatography (SEC) was unable to show the presence of microgel because the sample solutions were filtered prior to the injection. A correlation between the Mooney viscosity and RMS conformation of cis-BR was also investigated.



RMS conformation plot of cis-butadine rubbers (cis-BR)

P21

OPTIMISATION AND CHARACTERISATION OF THE SYNTHESIS OF MONO- METHOXY POLY (ETHYLENE GLYCOL)-BLOCK-POLY(4-VINYL PYRIDINE) (PEG-B-P4VP) BY ASYMMETRIC FLOW FIELD-FLOW FRACTIONATION (AF4) AND MULTIANGLE LIGHT SCATTERING (MALS)

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The synthesis of mono- methoxy poly(ethylene glycol)-block-poly(4-vinyl pyridine) (PEG-b-P4VP) have been achieved through two consecutive radical reactions with 1-vinyl-2-pyrrolidinone (VP) and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) consecutively. Those polymers have shown potential applications in the field of drug delivery due to the ability to form more complexes structures as micelles with low toxicity in in vitro cellular assays. The radical reactions have been controlled by reversible addition-fragmentation chain transfer (RAFT) allowing control of the number of monomers inserted in the polymeric chain. An optimisation of the whole synthesis was achieved by the systematised and controlled variations of the different components proportions in the reaction. The characterisation of the product of each radical reaction was performed by asymmetrical flow field-flow fractionation (AF4) connected to multi-angle light scattering (MALS) and differential refractive index detectors (dRI). The results show differences in the size and molar mass between polymers from the radical reactions of the synthesis, showing a path to control of the length of the polymer to be synthesised.

P22

CHARACTERIZATION OF VARIOUSLY BRANCHED DENDRITIC POLYETHYLENE BY SEC-LS AND AF4-LS

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Hyperbranched (hb) polymers combine unique physical properties with a convenient producibility and consequently exhibit a larger application potential as dendrimers. The invention of late transition metal catalysts created the opportunity of precisely synthesized polyolefin architectures for the first time.[1,2] This novel route gave way to molecules with controlled topologies and superior rheological properties. However, it is not completely understood how the molecular structure contributes to these features. Our work is focused on the complete elucidation of structure-property relations of hb polyethylene (PE). For this purpose size exclusion chromatography (SEC) was coupled to static and dynamic light scattering, viscosity and refractive index detectors enabling the determination of molar mass, viscosity, radius of gyration (RG) and the hydrodynamic radius (RH). The correlation of these parameters allows evaluating the properties of the molecule in solution. Variously branched hb-PE with molar mass between 30 – 400 kg/mol and RG ranging from 10 – 30 nm were investigated. The branching topology clearly affects essential molecular parameters like the ratio RG/RH, power law exponents (e.g. KMHS exponent) as well as the contraction factor and hence gives insight into the shape, topology and compactness of differently branched PE. However, a detailed characterization of branched polymers by SEC is often limited due to unusual elution effects.[3] Thereby, several segments of a branched molecule tend to get entangled inside the pores of the column packing material, elute later than the fraction corresponding to its hydrodynamic volume and falsify the required structural values.[4] In order to overcome anchoring phenomena of hb-PE in SEC, asymmetric flow field flow fractionation was used as an alternative separation method.

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P23

STRAIGHTFORWARD ANALYSIS OF PEG-PEPTIDE CONJUGATES

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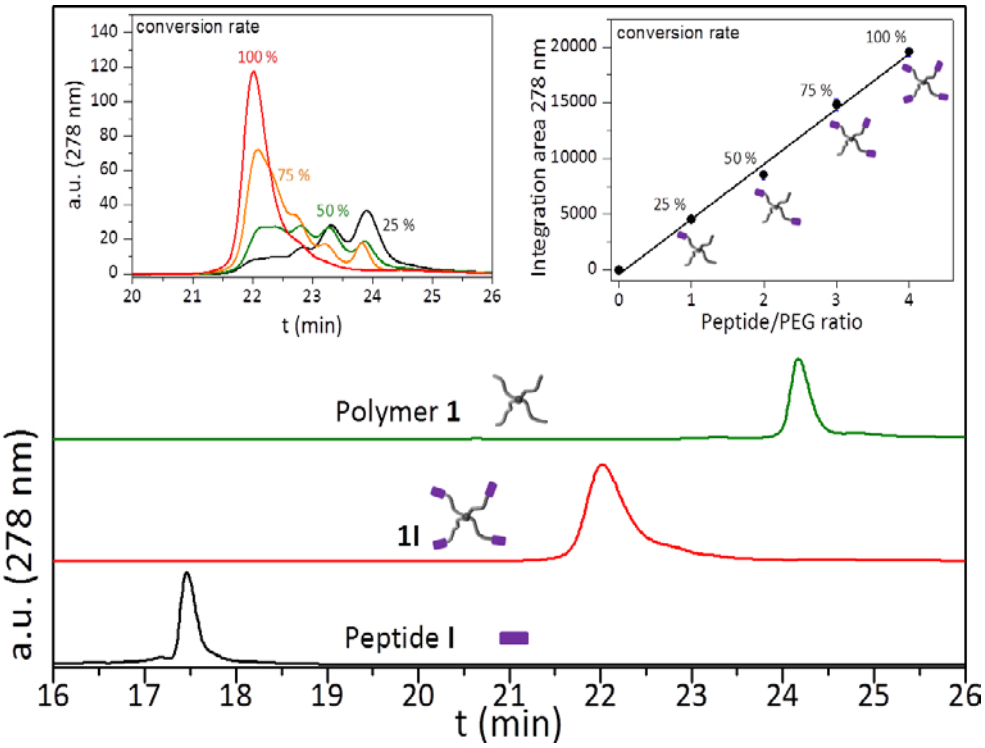
Synthetic poly(ethylene glycol) (PEG)-containing hydrogels with the aim to apply them for the treatment of human diseases or in regenerative therapies are one the most frequently used materials in ongoing research. While mechanical properties and the hydrogel shape can be controlled, it is often necessary to add biofunctional functionalities such as enzyme-cleavable or cell-adhesive peptides to the PEG chains via conjugation reactions in order to control cell behavior or differentiation. Often the materials are designed to be used in medical applications, therefore it is crucial to provide well-defined and well-characterized compounds. While reaction mechanisms and principles of combing synthetic polymers with nature-derived structures are well understood, analytical methods of analyzing such hybrid-materials need to be more established and optimized.

In this work, we coupled thiol-containing peptides to either linear, y-shaped, bifunctional, tetrafunctional or octafunctional maleimide-terminated PEG via “Click Chemistry” under neutral pH in aqueous solution. A combination and optimization of standard analytical methods such as Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), high & ultra performance liquid chromatography (HPLC/UPLC) was used to determine the properties of multifunctional PEG-peptide conjugates.



Reaction monitoring of four-arm PEG-Maleimide (Mw 10000 g/mol) and peptide I (H2N-GGPQGIWGQGGCG-CONH2). Functionalization degree of the PEG-peptide conjugate can be modified with peptide/PEG ratio.

P24

CHARACTERIZATION OF POLYMERIC VESICLES BY AF4

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In (bio-)medicine, polymersomes are a class of self-assembled macromolecules in artificial vesicles with bilayer morphology similar to liposomes. Polymersomes are made using amphiphilic synthetic block copolymers to form the vesicle membrane, and have radii ranging from 50 nm up to a few µm.(1, 2) Usually polymersomes contain an aqueous solution in their core and are useful for encapsulating and protecting sensitive molecules, such as drugs, enzymes, or other proteins.(3, 4) The polymersomes‘ membrane provides a physical barrier that isolates the encapsulated material from external media, such as those found in biological systems.

Asymmetric flow field flow fractionation (AF4) is a versatile separation method especially for the gentle separation and characterization of these molecular assemblies. Due to the lack of a stationary phase, interactions and shear forces are minimized. In this contribution, we will present pH sensitive polymersomes for the triggered loading and release of cargo. AF4 coupled to static and dynamic light scattering detection enables a comprehensive characterization of molar mass, molecular size and scaling behaviour of different loading stages of polymersomes.

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[2] Gaitzsch, J.; Appelhans, D.; Wang, L. G.; Battaglia, G.; Voit, B. Angewandte Chemie-International Edition 2012, 51, (18), 4448-4451.
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P25

CHARACTERIZATION OF PLASMA PROTEINS AND LIPOPROTEINS USING MICROCHANNEL ASYMMETRICAL FLOW FFF

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Field-Flow fractionation (FFF) is an elution-based separation technique which is capable of the rapid and high efficient separation of macromolecules, colloids and particles. Asymmetrical Flow FFF (AF4) is one of the FFF sub-techniques that has been broadly used in characterization of complex biological and pharmaceutical products. The commercial AF4 system commonly employs flat channels with different dimensions and aspect ratios (10-28 cm long and 2-10 cm wide). Annular channels (hollow fiber) have been also used in the AF4 system as an alternative geometry. The AF4 channel could be further downscaled to reduce channel volume (less dilution), sample consumption and operating cost.

A miniaturized flat AF4 channel (microchannel) with a size smaller than a credit card was constructed and examined for characterization of biological samples spanning a wide molecular weight and diameter range. The performance of the microchannel was tested using a mixture of plasma proteins. The resolution and reproducibility of the microchannel were found to be similar to those of standard channel. The microchannel was used to fractionate Human Serum Albumin (HAS) from different lipoprotein fractions. The baseline separation was also achieved between the high-density and low-density lipoproteins.

P26

USE OF AF4 TO STUDY THE CONFORMATION AND STABILITY OF INTERFERON-TAU

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Interferon-tau (IFN-tau) is a novel Type 1 interferon with potential as a therapeutic agent. The aggregation of IFN-tau in aqueous solution has been found to be affected by the choice of buffer, which can increase conformational stability by direct interaction with the protein. The rate of aggregation was investigated in the presence of tris, phosphate, and histidine buffer at pH 7. The aggregation was monitored using AF4. In addition, AF4 was employed to examine changes in size upon ligand binding and in the presence of denaturants. This study demonstrates that AF4 can be used to determine size changes for a protein pharmaceutical as a function of solution conditions. In addition, AF4 can also be valuable in measuring the aggregation rate of a protein at elevated temperature.

P27

ANALYSIS OF URINARY EXOSOMES BY ASYMMETRICAL FLOW FIELD-FLOW FRACTIONATION

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Exosomes, originating from multivesicular bodies (MVBs) in cells, are spherical-shaped cellular vesicles of which sizes range from 20 to 100 nm. Exosomes contain biological molecules in cells, such as RNA, proteins, and lipids, and transport the preceding molecules to other cells. Such cell-signaling process is known to be related to the immune system mechanism and it affects the transition of pathogens or growth of tumor cells. Therefore, exosomes have received great attention as indicator for development of various diseases and recent research focuses on finding possible disease related biomarkers in them.

The urinary exosomes are originated from renal or kidney cells and their size distribution is rather broad. Urinary exosomes are related with prostate cancer and urinary exosomes of prostate cancer patients have been reported to be smaller than those of healthy controls.

Conventionally, ultracentrifugation or flow cytometry has been utilized to isolate the exosomes from urine. However, preceding techniques are incapable of distinguishing exosomes from membrane vesicles that have relatively large size distributions. Compared to these techniques, flow field-flow fractionation has great advantages as its separation range is much broader.

In this study, asymmetrical flow field-flow fractionation (AF4) was utilized to separate and collect fractions of urinary exosomes. Size distribution from each fraction was confirmed using transmission electron microscopy (TEM) and specific types of proteins that eluted in particular fractions were identified through Western blot. Lastly, lipids were extracted from the collected fractions and using nLC-ESI-MS/MS, lipidomic analysis from each fraction was carried out.

P28

ASYMMETRIC FLOW FIELD FLOW FRACTIONATION METHODS FOR VIRUS PURIFICATION

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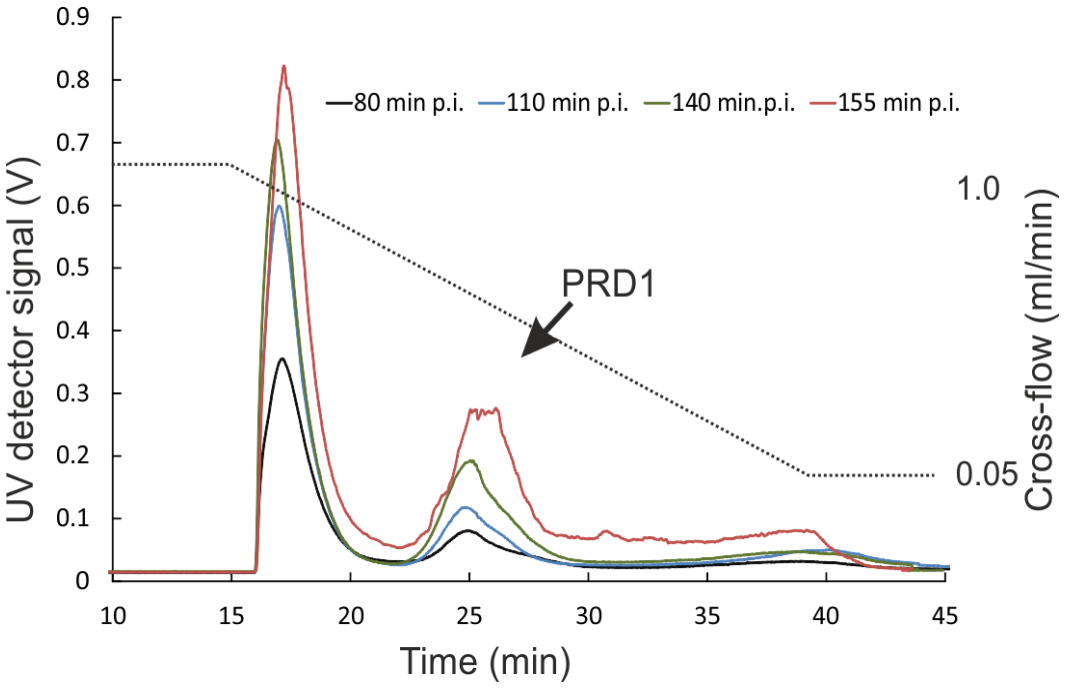
Detailed biochemical, biophysical and structural characterization of viruses require specimen of high quantity and quantity (purity). Consequently, the optimization of virus production and purification is an essential prerequisite for such approaches, but at the same time it is a laborious and time consuming process. The sizes of known viruses range from 20 nm upwards that limits the use of traditional chromatographic methods in virus purification. Asymmetric flow field flow fractionation (AsFlFFF) is an attractive alternative method for virus purification, as it is a rapid and gentle separation mechanism. Previous studies using AsFlFFF for viruses have focused on the qualitative and quantitative analyses of viruses and virus-like particles. Here, we optimized the AsFlFFF conditions to be used for purification of our model virus, bacteriophage PRD1, from various types of starting materials. PRD1 has an icosahedral proteinaceous capsid with a diameter of ~66 nm and molecular mass about 66 MDa. Virions are decorated with spikes of ~20 nm that are located at the five-fold symmetry axises. The protein shell covers an internal membrane that encloses the viral double stranded DNA genome. Our results show, that AsFlFFF is well suited for PRD1 purification. Furthermore, we show that AsFlFFF enables rapid real-time analysis of virus production in infected cells.



AsFlFFF analysis of culture supernatants from PRD1-infected Salmonella enterica cells. Representative fractograms at 80, 110, 140 and 155 min post infection are shown. Cross-flow gradient is shown in black (dashed), channel flow was 0.2 ml/min. Signal intensity (V) was measured with UV-detector at 260 nm. The peaks represent host-derived impurities and virus (arrow).

P29

PROFILING OF METALLOPROTEINS FROM PLASMA USING MINIATURIZED AF4 COUPLED WITH ICP-MS

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Selective analysis of metals in biological system is an emerging research topic and important for understanding the biological processes such as signal transduction, nerve transmission, and metabolism. Approximately one third of all proteins and enzymes inside human body contain metal cofactors or metalloid ions in their structures, but many of the critical roles of these metal ions remain unknown. Numerous kinds of metalloproteins are reported to be potential biomarkers for various diseases, and separation and detection with high sensitivity is indispensable in metallomics as their concentrations are extremely in biological samples.

In this study, on-line miniaturized asymmetrical flow field-flow fractionation (mAF4) channel coupled with inductively coupled plasma mass spectrometry (mAF4-ICP-MS) was adopted to profile metalloproteins from human blood samples. This integrated system not only separates proteins and other biological particles according to their sizes, but also on-line analysis of metals contained in proteins can be simultaneously accomplished. Five different types of standard metalloproteins, their size ranging from 29 to 669 kDa, were analyzed by mAF4-ICP-MS and mAF4-UV detector, and peak shapes and their retention times were compared to validate the applicability of mAF4-ICP-MS. In addition, the hyphenated system was applied for characterization of metal bound proteins from human plasma sample. Plasma samples from 10 control cases and 10 lung cancer patients were quantitatively analyzed and several metal bound proteins showed significant changes in their concentrations from lung cancer patients.

P30

PROGRAMMING CONSIDERATIONS IN MODIFIED FULL FEED DEPLETION MAGNETIC SPLITT DEVICE

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Typically SPLITT fractionation involves two inlet and two outlet flows. Full feed depletion (FFD) maintains the dual outlet flow while dispensing with the inlet carrier stream that causes dilution of the sample. We present a simplified FFD mag SPLITT, called Cell Genus (CG), that removes one of the outlet flows as a means to negatively deplete unwanted cells. The cells of interest, circulating tumor cells (CTCs), elute continuously through our quadrupole magnet and annular SPLITT channel and are recovered in the eluate fraction. Magnetic cells are captured and subsequently recovered by high shear carrier flow as the retained fraction, and these can be studied or discarded. Thus, CG sorting differs from others, such as MACS, that use ferromagnetic packing, with highly irregular magnetic force and flow geometries, causing non-specific capture. Moreover, the quadrupole produces regular field geometry in the transverse plane. The sorting is optimized by computer modeling so that the internal capture profile is uniform, through mobility inputs through our in-house device, the Single Cell Magnetometer.

The CG was employed as a negative cell separation platform to enrich non-EpCAM CMCs from blood. The approach involved depletion of red blood cells by hypotonic lysis, and depletion of white blood cells by immunomagnetic tagging with pan-leukocyte (CD45) antibody and CG sorting. With negative depletion, the CMC are enriched without being affected by immunomagnetic reagents. Initial studies used blood spiked with cultured SKMEL-28 cells, as well as malignant melanoma samples, indicating a CMC limit of detection of 10 cells/mL of blood. CMC Cytospin preparations were analyzed by immunofluorescence for known melanoma markers, Melan-A and S100. Samples obtained from healthy donor blood (n=5) had a baseline of 0-3 positive cells/mL blood. In contrast, samples from stage IV melanoma patients (n=8 exhibited significantly higher CMC counts ranging from 78 to 28,483 cells/mL.

P31

TUNABLE POLYMERSOMES BY PH-TRIGGERED ENCAPSULATION OF RHODAMINE

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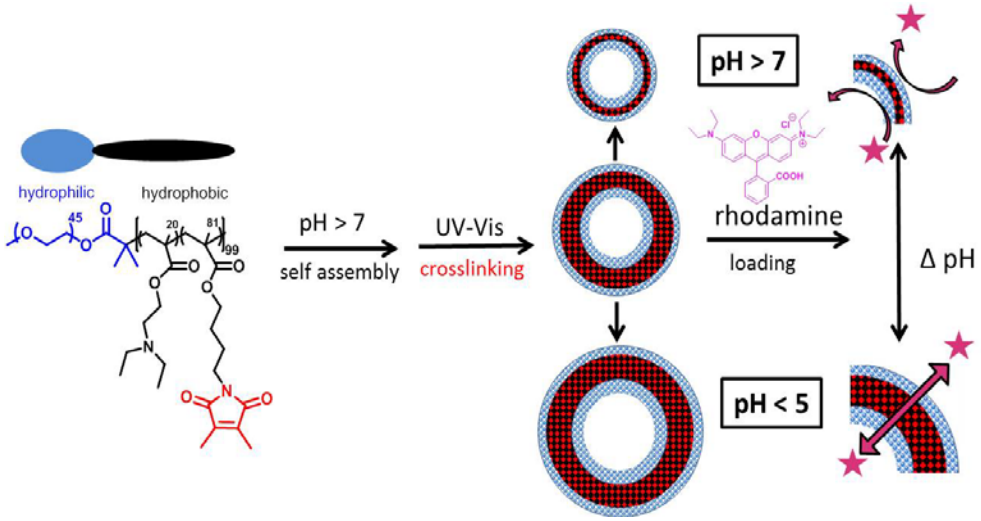
Artificial vesicles, which organize themself from amphiphilic copolymers in the aqueous medium, show great potential as carriers for drug delivery.

Polyfunctional triblock copolymers are applied as building blocks for the formation of vesicular polymersomes. ATRP is used for the synthesis of block copolymers with defined block lengths and great uniformity. The self-organization of these macromolecules should take place in a short time to bring up stable polymersomes of low dispersity in morphology and size [J. Gaitzsch, D. Appelhans, D. Gräfe, P. Schwille, B. Voit, 2011, Chemical Communications, 47, 12, 3466 - 3468.].

After their crosslinking by UV light, a higher stability as well as a pH triggered change of permeability reached via swelling and shrinking can be achieved. To study the pH regulated loading and release of drugs in vitro; the model substance rhodamine is used in our investigations. Here, we performed a variety of analytical methods such as DLS, SEC and AF4. The application of AF4 in combination with static and dynamic light scattering delivers information about the sizes and conformational properties of the polymersomes.

After post-encapsulation of rhodamine, the purification of non-encapsulated rhodamine by hollow fiber filtration (HFF) is examined and characterized by UV-Vis. The next step will be the quantification of the loading efficiency by AF4-UV, where free rhodamine and loaded polymersomes could be separated within one measurement.



Synthesis and organization of pH responsive Polymersomes

P32

ANALYZING ENZYME ENCAPSULATION IN SMART POLYMERSOME NANOREACTORS

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In recent years, large efforts were made to develop smart polymeric systems feasible for applications in drug delivery and synthetic biology.[1] Polymersomes, artificial analogues of liposomes, are promising candidates for such purposes. The resulting bilayer membranes of the polymersomes are thicker compared to liposomes resulting in mechanically and chemically much tougher vesicles.[2] Combining the incorporation of pH-responsive and photo crosslinkable units into the block copolymers leads to reversible swelling and shrinking behaviour of the polymersomes upon changes of the pH.[3]

Current work deals with the post-encapsulation of the enzyme esterase into the interior of swollen and photo-crosslinked polymersomes at acidic pH at which the enzymes are able to diffuse through the polymersome membrane. While the enzymes being accessible for the substrates and accordingly fully active at acidic conditions, no enzymatic activity could be detected at basic or neutral conditions after removal of non-encapsulated enzymes from the outer solution. In contrast to many studies aiming for the controlled release of encapsulated drugs from the polymersomes´ lumen,[4] no diffusion of enzymes to the outside could be detected regardless if enzymes are stored with switched “off” membrane at neutral pH or with switched “on” membrane at acidic pH up to 2 days, respectively. Thus, enzymatic nanoreactors are smoothly available without the need of their specific covalent attachment inside of polymersomes´ lumen while their activity can be switched on and off as response to pH. Among other analytical methods asymmetric flow field flow fractionation (AF4) was used to characterize the nanoreactors towards quantification of enzyme uptake as well as various structural parameters.

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[2] LoPresti et al., J. Mater. Chem. 2009, 19, 3576.
[3] Gaitzsch et al., Angew. Chem. Int. Ed. 2012, 51, 4448.
[4] Yassin et al., Small 2015, 11, 1580.

P33

BILE SALT MICELLES AND PHOSPHOLIPID VESICLES PRESENT IN ARTIFICIAL AND ASPIRATED HUMAN INTESTINAL FLUIDS: A FLOW FIELD-FLOW FRACTIONATION/ MULTI-ANGLE LASER LIGHT SCATTERING-STUDY

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Knowledge about colloidal assemblies present in human intestinal fluids (HIF) is of great importance for the in vivo dissolution and absorption of poorly water-soluble drugs, due to their drug-solubilizing ability.

The aim was to separate colloidal assemblies in simulated intestinal fluids (SIF) and HIF according to their size and to determine the size distribution of the fractions, using Asymmetrical Flow Field-Flow Fractionation (AF4) in combination with Multi-Angle Laser Light Scattering (MALLS) technique. AF4/MALLS-fractograms of HIF aspirates were compared with fractograms of a widely used bio-mimetic mimicking both fasted and fed state. Differences in size and assumingly morphology of the supramolecular assemblies present in the media required appropriate mathematical models applied in the size analysis of particle fractions. The Debye-plot and the hollow sphere model were used to analyze the smaller particles (micelles) and the larger particles (liposomes), respectively, both yielding fine fits with the respective particle fractions (fig. 1). UV-extinction was measured at 205 nm, being indicative of the presence of taurocholate and enabling differentiation between mixed-micelles and liposomes.

The fasted state SIF dispersion resulted in one peak, i.e. one group of homogenous particles with average diameter size DZ = 35.0 nm. The FaHIF dispersion resulted in a fractogram very different from its corresponding bio-mimetic, with the first two peaks representing particles too small for MALLS size analysis. Peak nr. 3 yielded a DZ = 43.8 nm and peak nr. 4 contained much larger particles, DZ = 149.8 nm, assumed to be phospholipid-vesicle structures (further supported by the absence of UV-extinction).

AF4/MALLS appears to be a feasible method to fractionate and size-determine supramolecular assemblies in both simulated and real intestinal media.

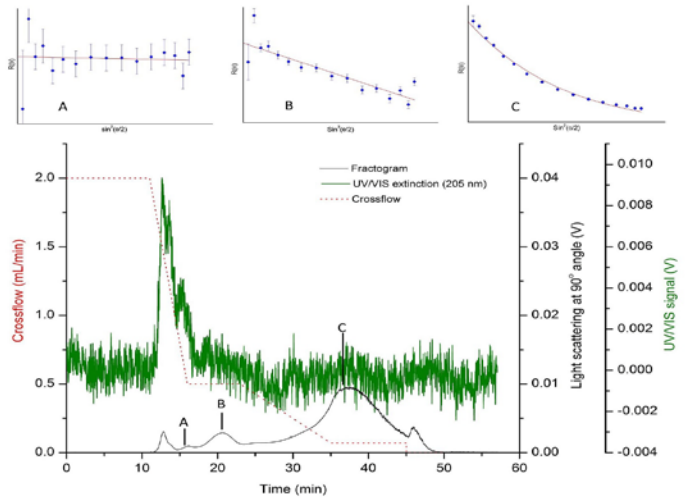

Figure 1: Obtained fractogram of FaHIF (black) together with crossflow (red) and UV extinction signal at λ=205 nm (green). The sample was filtered (0.45 um) prior to fractionation in the channel.

P34

AF4 CHARACTERIZATION OF NANOEMULSIONS OF LIPID DROPLETS COVERED BY A MONOLAYER OF SPHINGOMYELIN AND CHOLESTEROL

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AF4 coupled to a multi-angle light-scattering (AF4-MALS) together with dynamic light-scattering (DLS) and transmission electron microscopy (TEM) was used to study the size characteristics of nanoemulsions of lipid droplets (LD) (Vezočnik et al., 2015). Our study focused on the nanoemulsions of LD composed of a trioleoylglycerol core coated with a sphingomyelin (SM)/cholesterol (Chol) monolayer. In parallel, we studied large unilamellar vesicles (LUV) made of SM and Chol as a biophysically well-characterized vesicular species. Sonicated LD or extruded LUV were prepared at two different molar ratios (1/1, 4/1) of SM and Chol. In AF4-MALS various cross-flow conditions and mobile phase compositions were tested to optimize the separation of LD nanoemulsions and LUV particles. By coupling AF4 with MALS the average geometric radius, Rgeom, and the average root mean square radius, rms, of lipid particles were determined, whereas DLS in batch-mode gave the average hydrodynamic radius, Rh. From the shape factor defined as rms/Rh, the solid sphere structure of non-uniform density was determined for the nanoemulsions of LD (0.81-0.89), whereas the shape factor of the LUV was close to one due to shifting of the center of mass towards the particle periphery. The globular shape of nanoemulsions of LD and LUV was confirmed by particle visualization using TEM, however, their size appeared larger from the values determined by AF4-MALS and DLS, which could be ascribed to flattening of lipid particles on the surface.

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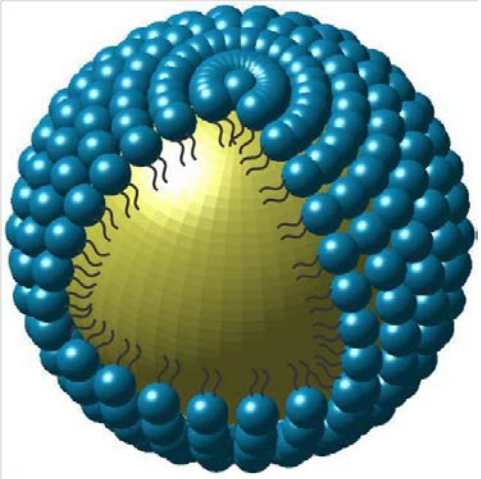

Figure 1: sketch of nanoemulsion of LD

P35

ANALYSIS OF THE PROTEIN CORONA USING ASYMMETRICAL FLOW-FIELD-FLOW-FRACTIONATION

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Nanomaterials are investigated as promising new drug carriers. They can potentially transport a drug directly to its target location without systemic effects and without losing a high amount of dose due to degradation.

As soon as a nanoparticle enters a biological fluid, its surface will be covered with proteins. This way, the nanomaterial receives a new surface, which then is responsible for the fate of the nanomaterial in the body. To understand and predict the behavior of the nanoparticle in the body, it is necessary to evaluate this so called ‘protein corona’. [1]

For the separation of free proteins in the medium from the protein-nanomaterial complex without disturbing the formed corona, only very few methods exist and for that reason it is very difficult to analyze.

A new approach for the above mentioned separation problem is the asymmetrical flow-field-flow-fractionation (AF-FFF). Using this technique, very small particles like proteins can be separated from the larger nanomaterial-protein complexes. Because AF-FFF does not contain a solid phase, less shear stress affects the sample, which increases the probability of isolating the particles together with the undisturbed protein layer, the so-called ‘soft’ corona.

For the experiment differently functionalized polystyrene nanoparticles were used, which were incubated with human blood plasma and with human serum albumin (HSA) as a single protein.

After separation, the obtained fractions were characterized with regards to size and protein composition with dynamic light scattering and SDS-polyacrylamide gelelectrophoresis. Like this, we were able to show that, indeed, free proteins can be separated from the nanoparticles with adsorbed proteins. Additionally, it was observed that the isolated corona differs in its composition from the ‘hard’ corona, which can be obtained after repetitive centrifugation of the protein-particle complexes.

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P36

COMBINING AF4-ICP-MS AND SP-ICP-MS WITH XAS TECHNIQUES FOR THE CHARACTERIZATION OF SOIL COLLOIDS INVOLVED IN THE MOBILIZATION OF ARSENIC

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Colloids formed by the weathering of mining and processing wastes may control the release of hazardous elements such as arsenic (As) into surface waters and contribute to long-distance contaminant transport and dispersion. The nature of the colloidal As determine its impact on As mobility and bioavailability and needs to be considered for the mitigation of colloidal As release from weathered mine wastes. In this study, we investigated the importance and mode of colloidal As mobilization from leached/weathered processing wastes and from sediments from the draining river bed and a more distant sedimentation pond.

Colloids with sizes 1000-10 nm were isolated by ultrafiltration and were later characterized for their composition, structure and mode of As uptake using a combination of flow field-flow fractionation coupled to plasma mass spectrometer (AF4-ICP-MS) and X-ray absorption spectroscopy (XAS) at the As and Fe K-edges. However, scorodite colloids from the processing wastes could not be measured by AF4 due to the high electrical conductivity of the colloidal suspension. To solve this problem, the single-particle inductively coupled plasma mass spectrometry (SP-ICPMS) was optimized for As single detection on the scorodite colloids. In single particle detection, the atoms of the analyte (i.e. a nanoparticle) produce a flash of gaseous ions when it is introduced into the ICP. The number of counts of this single pulse is related to the quantity of analyte atoms in the nanoparticle, and the frequency of the pulses is proportional to the number concentration of nanoparticles, which allows for the selective determination of dissolved arsenic and scorodite nanoparticles. The combination of X-ray absorption spectroscopy (XAS) and transmission electron microscopy (TEM) techniques provide molecular-scale information about the size and the As speciation in the colloidal scorodite, and therefore can be used as validation techniques for the SP-ICPMS analyses.

P37

TOWARDS TRACE LEVEL ANALYSIS OF SILVER NANO-PARTICLES IN ENVIRONMENTAL SAMPLES USING ASYMMETRICAL FLOW FIELD-FLOW FRACTIONATION COUPLED WITH UV, DLS AND ICP-MS

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Engineered nanomaterials (ENM) have become an essential part of our daily life. Being extensively used e.g., as UV-protection agent in sunscreen formulations (TiO2) or in sportswear due to their antibacterial properties (Ag), ENM finally end up in the environment, where their behavior and fate is still widely unexplored mainly due to the lack of appropriate analytical methods for their determination at trace levels in complex matrices [1,2].

In this presentation, as a part of the interdisciplinary research project "NanoUmwelt" funded by the German Federal Ministry of Education, we demonstrate the applicability of Asymmetrical Flow Field-Flow Fractionation (AF4) hyphenated with UV, DLS and ICP-MS [3,4] towards the determination of silver nanoparticles (AgNPs) in environmental samples such as river water, soil and fish. Besides the determination of alterations in the particle size distribution of the AgNPs via AF4-UV-DLS, we particularly focus on the reliable fractionation and quantification of AgNPs at concentration levels below ppb (µg/L) using AF4-ICP-MS. We hereby take advantage of the possibility to remove complex matrices directly in the AF4 separation channel prior to analysis.

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P38

ANALYSIS OF LOW AND INTERMEDIATE SIZED BETA-GLUCAN FROM BARLEY PRODUCTS AND THEIR RELATION TO PROTEINS AND THE CONSUMERS' HEALTH

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Cereal beta-glucan are well known to be beneficial for the consumers' health, causing a decrease in LDL cholesterol levels and preventing coronary heart diseases. Beta-glucan are also related to intestinal health; when fermented by the colonic microbiota they produce butyric acid, which can reduce and prevent inflammatory colonic diseases. Most of the health benefits have been attributed to the beta-glucan of high molar mass, therefore not much research has been done on the beta-glucan of small and intermediate molar mass. However, it is thought that the yield of butyric acid is higher from low molar mass beta-glucan, which is more readily fermented. In this study, different kinds of barley beta-glucan have been analysed with regard to their beta-glucan content, molar mass, molecular size and composition. Extracts were prepared from barley malts and Brewers' spent grain, since different varieties or processing conditions could influence beta-glucan characteristics.

Asymmetrical flow field-flow fractionation (AF4) was utilized in combination with multi angle-light scattering (MALS), differential refractive index (dRI) analysis, UV and fluorescence detection. We were able to detect beta-glucan in a large size range, with the lowest molecular weight below 2 kDa, and identified them by specific labeling with calcofluor. An interesting aspect was as well the influence of protein degradation on the size distribution of the beta-glucan. Total digestion with enzymes (pronase E, aminopeptidase M, prolidase) or trypsin treatment suggested a close relation between the presence of proteins and the high molar mass of beta-glucan, what lead us to the assumption of possible beta-glucan/ protein aggregates. Therefore, further experiments will focus on that relation.

P39

STUDY OF APTAMER – TETRACYCLINE COMPLEX BY ASYMMETRIC FLOW FIELD-FLOW FRACTIONATION

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Aptamers are oligonucleotides that bind target analytes with high affinity and specificity. We have studied this interaction by the use of asymmetric flow field flow fractionation (AF4). DNA aptamer 5-CGTACGGAATTCGCTAGCGGGCGGGGGTGCTGGGGGAATGGAGTGCTGCGTGCTGCGGGGATCCGAGCTCCACGTG-3 and its target analyte, antibiotics tetracycline (TC), were the studied pair.

AF4 was performed using autosampler and pump (Agilent Technologies Inc., USA) connected to a Wyatt Eclipse 3+ separation system (Wyatt Technology Corp., USA). Separations were performed using a 5-kDa MW cutoff regenerated cellulose membrane (Microdyn-Nadir GmbH, Germany) in the 275- mm channel with 350-μm thick spacer (Wyatt Technology Corp., USA). The channel was sequentially connected with a UV/VIS detector (Agilent Technologies Inc., USA), Dawn HELEOS II multi-angle light scattering detector, ViscoStar II viscometer and Optilab T-Rex refractometer (Wyatt Technology Corp., USA).

The Af4 technique allowed to register differences in structure and conformation of the aptamer caused by its heating (see Figure, a) and other actions. The aptamer-TC complex formation was studied with the use of TC – bovine serum albumin (BSA) conjugate. The TC concentration increase caused changes in form and appearance of new peaks of chromatograms (see Figure, b). The given effects correlate with the data about the TC – aptamer interaction obtained by circular dichroism and spectral methods.

The obtained results demonstrate the efficiency of the AF4 technique as tool to study the aptamer – low-molecular-weight compound interactions.

This study was supported by the Russian Science Foundation (Project No. 14-14-01131).

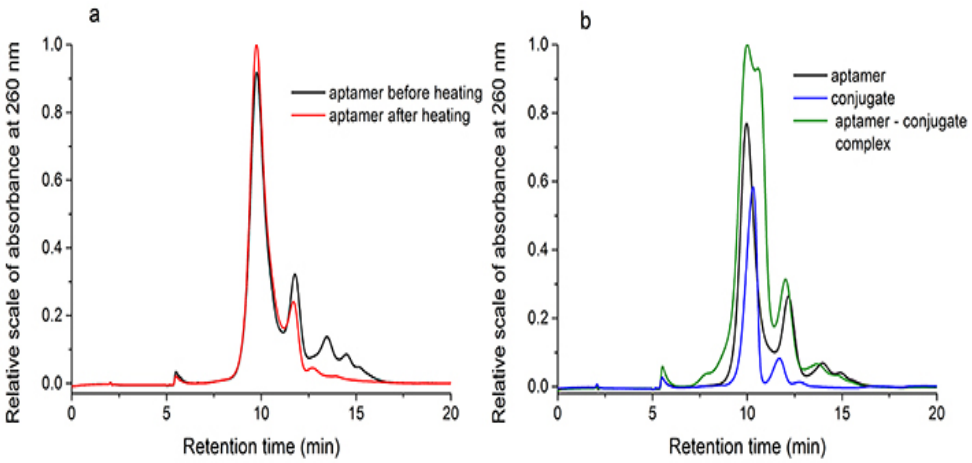

Figure. AF4 chromatograms. (a) The aptamer to TC before and after heating. (b) The aptamer to TC, TC-BSA conjugate and their complex.

P40

CHARATERIZATION OF MACROMOLECULES IN BEER USING ASYMMETRICAL FLOW FIELD-FLOW FRACTIONATION (AF4) COUPLED WITH MULTI-ANGLE LIGHT SCATTERING (MALS)

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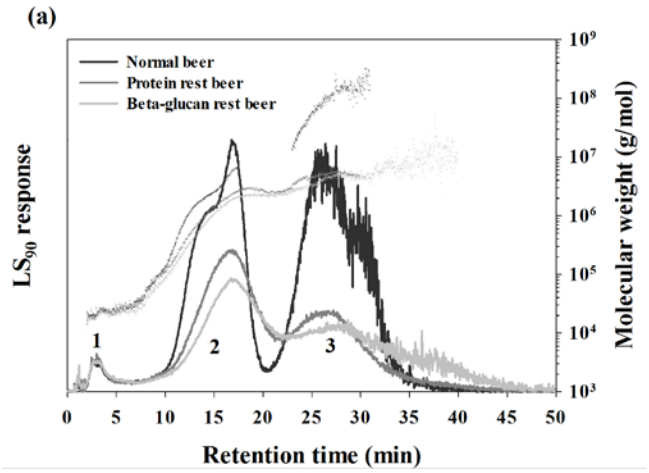
Beer is a complex mixture of various types of macromolecules such as proteins, and polysaccharides. The properites of macromolecules in beer are a result of brewing that involves various modifications of proteins and polysaccharides in the grist. It is known that properties of proteinaceous molecules are related to the foam stability and quality. The presence of some proteinaceous molecules may also result in a formation of haze. Furthermore, the presence of polysaccharides with high molecular weight tends to increase viscosity and turbidity of beer.

In this work, "American pale ale" beer was prepared using various mashing processes. Then the capability of AF4 coupled online with multi-angle light scattering (AF4-MALS) for determination of molecular weight distribution and the content of macromolecules in beers was investigated. The beer components were identified by enzymatic treatments. In addition, the correlation between beer's composition and the foam stability was investigated (an increases in concentrations of protein and beta-glucan are associated with increase in the foam stability. The results show that AF4-MALS may become a useful tool for monitoring the change in the macromolecular composition of beer.



AF4 fractograms for the three types of beer.

P41

COMPOSITIONAL EFFECTS ON THE ASSOCIATION OF CASEIN

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Because of its colloidal properties, casein as the main species of milk proteins is responsible for structure formation in fermented dairy products. Enzymatic cross-linking of casein by microbial transglutaminase (mTGase) has therefore been studied extensively as a possibility to improve the texture properties of yoghurt [1,2], with the latest work indicating that the properties of casein gels might be tailored by adjusting the number of cross-links to an optimum [3]. Based on these findings, we initiated a research project to investigate the gel forming ability of cross-linked casein in relation to its molecular properties [4].

In the first project stage we study the self-association of casein molecules to particles in aqueous solution depending on the casein composition. It is intended to vary the ratio between αS1-, αS2-, β-, and κ-casein, which is approx. 3:1:3:1 in bovine milk. Since the different casein types vary strongly in their hydrophobicity we expect a considerable effect on molecular interactions and thus on the formation of casein particles.

The β-casein content is reduced by membrane filtration [5], and rennet casein serves as a substrate with a reduced number of hydrophilic peptide chains. The preparations are acidified to pH 4.6 for disintegration of supramolecular structures by calcium phosphate removal, and redissolved in a 0.1 mol/L phosphate buffer at pH 6.8 to allow self-association as a consequence of hydrophobic interactions. Asymmetrical flow FFF coupled with static and dynamic light scattering is performed to evaluate the casein particles concerning molar mass, radius, and scaling behaviour as a function of casein composition. Analysis is also conducted for corresponding casein samples that were cross-linked by mTGase to different extents. For that, analytical size exclusion chromatography under denaturing conditions serves as a reference method to assess the enzymatic reaction based on the apparent casein polymerisation [3].

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P42

STRUCTURAL AND CONFORMATIONAL ANALYSIS OF β-GLUCAN FROM OAT AND BARLEY USING ASYMMETRIC FLOW FIELD-FLOW FRACTION (AF4)

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Health benefits, like lowering of LDL cholesterol as well as decreased glucose and insulin levels in blood, have been previously established for high molar mass mixed-linkage β-glucan from oat and barley. The effects are mainly attributed to the ability of β-glucan in forming viscous slurries in the gut although no comprehensive understanding exists. Characterization of β-glucan in the way they are present in food is a challenge as molar mass and molecular size can depend strongly on the extraction method used and the β-glucan is highly disperse in size, contains ultra-high molar mass species and aggregated structures.

In this work we developed a gentle extraction method to not modify the β-glucan and characterized them in terms of molecular size, molar mass, conformation in aqueous solution and the presence of proteinacious moieties linked to β-glucan utilizing asymmetric flow field-flow fractionation (AF4) coupled to different detectors (multi-angle light scattering MALS, fluorescence- and differential refractive index detection). Different fluorescent labelling methods (online and offline) are used to obtain chemical information over the size distribution. Conformational data for both polymers and aggregates are obtained from comparing hydrodynamic radii (from AF4) and root-mean-square radii (from MALS), Kratky plots (from MALS) and persistence length determination (from small angle x-ray scattering). The properties of the aggregated structures are also investigated with cryo transmission electron microscopy and compared with the above data.

The results show the presence of highly aggregated structures in addition to dissolved, highly disperse polymers. The structure and conformational properties of the aggregates are different between oat and barley β-glucan. The barley β-glucan showed surprisingly dense and well-defined aggregates.

P43

RECENT ADVANCES IN EL-SPLITT: A FLOW ADDITION WITH POROUS ELECTRODE

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An electrical and flow characterization was performed on a novel SPLITT device. This device incorporates aspects of electrical and flow field flow fractionation into a single device and can be used for continuous separations. One unique component is the development of an electrically conductive porous membrane. This membrane allows a transverse flow, performs size exclusion for flow-based separations, and also acts as an electrode for electrical separations. One method uses ultra-thin layers of sputtered platinum metal onto a polyethersulfone membrane, another uses the co-sputtering of both platinum and aluminum followed by subsequent etching of the aluminum layer resulting in nano-porosity. This method is capable of producing very large porous electrodes suitable for FFF and other applications. Supporting hardware to complete the SPLITT system was developed using simple syringe pumps, an isocratic pump, pressure transducers, appropriate valves, and restrictive tubing to control flows. The ability of the system to perform buffer substitutions is demonstrated for the removal of salts prior to performing electrical separations. An electrical characterization is performed on the SPLITT system and polystyrene particles are directed to continuously exit either a top or a bottom port depending upon the parameters set for separation.

P44

DEVELOPMENT OF CONTINUOUS TWO-DIMENSIONAL ASYMMETRICAL FLOW FIELD-FLOW FRACTIONATION FOR PARTICLES: PRINCIPLE, INSTRUMENT DEVELOPMENT AND APPLICATIONS

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Continuous two-dimensional field-flow fractionation (2D-FFF) is a field flow fractionation principle for continuous fractionation of macromolecules and particles [1]. In this technique the separation occur in a thin disc-shaped channel, where a carrier liquid flows radially from the center towards the perimeter of the channel, and a steady stream of the sample solution is introduced continuously at a second inlet close to the center. Under influence of the field, the sample components are separated in radial direction according to the analytical FFF principle. Simultaneously, the lower channel wall is rotating with respect to the stationary upper wall, while a shear-driven flow profile deflects the separated sample components into continuous trajectories that strike off at different angles over the 2D surface. Finally, the sample components are collected at the outer rim of the channel.

In this work a new 2D-FFF instrument design with asymmetrical cross flow as a force field was constructed according to the principles of AF4 and the continuous 2D-FFF. Instrument performance was tested using coloured samples. The effect of different flow parameters on fractionation was investigated.

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P45

SEMI-PREPARATIVE ASYMMETRICAL FLOW FIELD-FLOW FRACTIONATION FOR NANOPARTICLE CHARACTERIZATION

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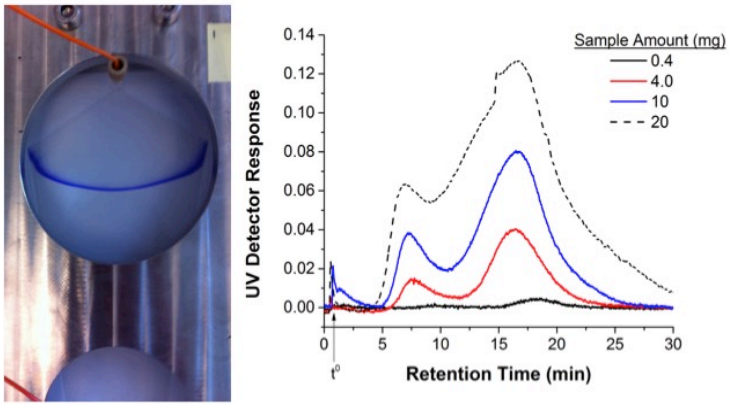
In this work, we investigate semi-preparative asymmetrical flow field-flow fractionation (sP-AF4) channel designs capable of handling large sample quantities (up to 20 mg) in a single analysis. Currently, separations of protein aggregates, biological particles, and other nanoparticles are limited to small analytical scale quantities. However, fractionation and purification of large analyte quantities is often critical for understanding their fundamental properties by further characterization and for subsequent applications.[1] Challenges associated with scaling-up the channel size and increasing sample amounts were considered, and a rational approach was used to design high performance semi-preparative channel spacers. Performance metrics were compared between an analytical AF4 channel and the sP-AF4 channel, and results showed that good resolution was maintained or improved in sP-AF4 separations of nano-scale materials. We have shown that a range of materials including hybrid metal nanoparticles, cancer-derived exosomes, and lipoproteins from human plasma can be fractionated in large quantities and narrowly dispersed size fractions can be collected for further analyses.



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P46

IONIC STRENGTH EFFECT ON RETENTION BEHAVIOR IN SEDIMENTATION FIELD-FLOW FRACTIONATION (SDFFF)

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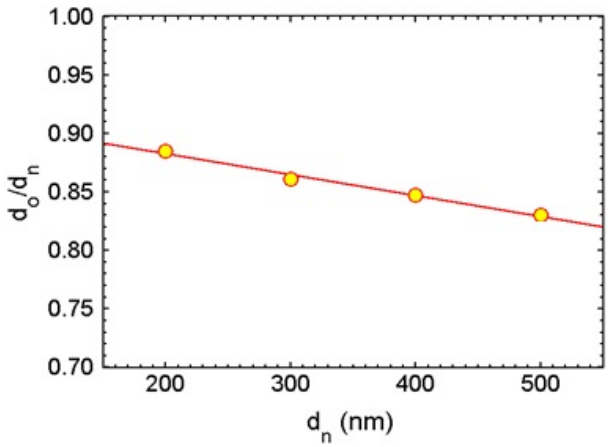
In the theory of field-flow fractionation (FFF), the parabolic velocity profile is often taken assuming no-slip boundary condition (BC) at the channel surface. This no-slip BC has some limitations, particularly when the hydrodynamic lifting forces or repulsive interactions are dominant over the attractive forces between the particles and wall. For instance, as shown in the figure, the hydrodynamic particle sizes estimated from the standard retention theory (SRT) for the polystyrene (PS) particles in pure water are smaller than the nominal values by about 12 – 18 % depending on the particle size. Some additives such as salts, surfactants, or buffering agents are usually employed to overcome this potential partial slip for the particles to have at the channel surface such that the retention behaviors of particles become as close as possible to the ideal no-slip BC.

In this study, the retention behaviors in SdFFF of PS latex beads of 200 – 500 nm in diameter is investigated as a function of the ionic strength. As expected, the estimated hydrodynamic sizes estimated from SRT tend to increase drastically with the ionic strength from pure water to ~1 mM, almost independent of the additive species such as FL-70, SDS, NaNO3, and NaN3 to control the ionic strength of the carrier liquid. In the near future, we will account for this strong dependence of the retention behaviors of the PS latex systems investigated above by means of the slip boundary model (reported in Langmuir 2012, 28, 10672-10681) instead of the standard retention theory that assumes the no-slip BC.



Ratio of do(calculated hydrodynamic diameter)/dn (nominal diameter).

P47

WHAT ARE THE ASSUMPTIONS BEHIND THE BASIC RETENTION EQUATION IN FFF?

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The characterization of the components of macromolecular or colloidal samples in field-flow fractionation (FFF) is usually performed by means of an equation that relates the retention time of a component to its interaction coefficient with the applied field. More precisely, the equation of the so-called basic retention theory of FFF is used. It expresses the retention ratio R to the basic FFF parameter, lambda, which is the reciprocal of the component Péclet number. Lambda is related to the parameter of interest for characterizing the component (for instance, particle size or molar mass).

Frequently, this basic equation is written in a simplified form, R = 6 lambda, which is a high retention limiting expression of the complete equation.

This basic retention equation, whether the complete or the simplified one, is based on several assumptions. It is important to be aware of them. Indeed, any failure to satisfy one or several of them leads to a more or less significant error in the determination of the component parameter of interest from its retention time. In this work, the various assumptions are listed under two groups, linked to the energetic and to the hydrodynamics of the FFF systems.

P48

COVALENT MODIFICATION OF ULTRAFILTRATION MEMBRANES FOR FLOW FIELD-FLOW FRACTIONATION

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We have developed a method to graft poly(N-isopropylacrylamide) (PNIPAM) brushes from regenerated cellulose (RC) ultrafiltration membranes while maintaining the flat surface and permeability required for their use in flow field-flow fractionation (FlFFF) channels. Undesirable analyte-membrane interactions is a potential problem encountered for FlFFF techniques, but can be minimized or eliminated by attaching antifouling chemistries to the membrane surface.[1] This attachment can be implemented by physisorption or covalent bonding. While the latter usually yields longer-term stability membranes, several challenges have hindered its application to the UF membranes used in FlFFF. These challenges include 1) large membrane areas must be modified (~30-40 cm2¬), 2) the membrane surface must remain smooth and flat, and 3) the membrane permeability cannot be significantly affected. The dilemma of modifying large membrane areas was resolved by using a modified FlFFF channel as the reaction vessel. Atom transfer radical polymerization (ATRP) was used to graft PNIPAM from the membrane surface [2] and the reaction mixture was re-circulated through the FlFFF channel reactor to facilitate even membrane coverage. Fourier transform infrared (FTIR) spectroscopy before and after ATRP confirmed the polymerization of PNIPAM on the surface of the RC membrane. Proton nuclear magnetic resonance (1H-NMR) also confirmed polymerization and allowed the monomer conversion kinetics to be monitored. Reaction conditions were selected so that the physical properties of the RC membrane were not significantly altered. However, water flux measurements decreased after polymerization suggesting that the presence of polymer brushes reduced the membrane pore size. Modified membranes were tested in a FlFFF channel and yielded successful fractionation of an IgG1 protein. The approach described is an important development forming necessary groundwork for future advances in FlFFF membrane modification.

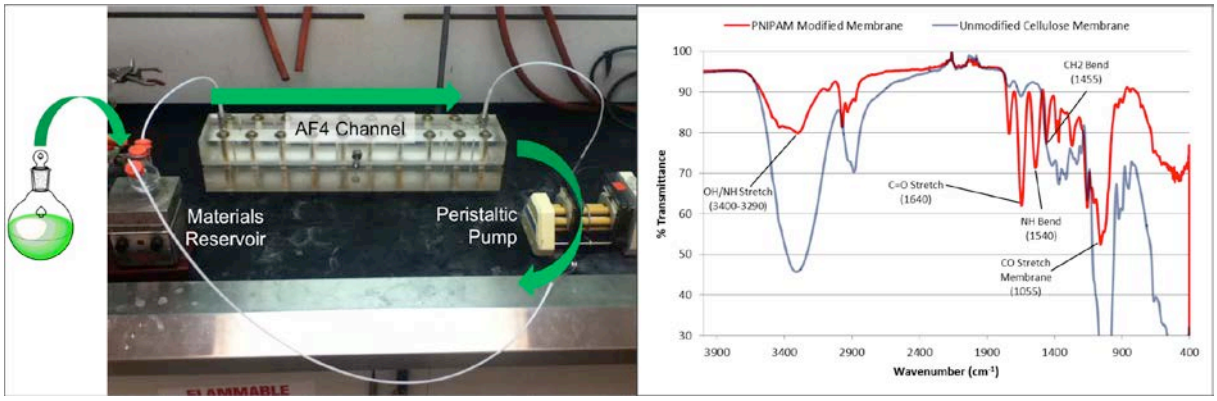

Figure 1. FlFFF channel reactor schematic (left) and FTIR spectra of the regenerated cellulose membrane before and after modification (right).

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P49

LENGTH SELECTION AND REPLICATION IN A THERMAL FLOW CHAMBER

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The replication of long nucleic acids is central to life. On the early Earth, suitable non-equilibrium boundary conditions would have been required to surmount the effects of thermodynamic equilibrium such as dilution and degradation of oligonucleotides. One particularly intractable experimental finding is that short genetic polymers replicate faster and outcompete longer ones, leading to ever shorter sequences and the loss of genetic information.

We show in theory and experiment that heat flux across an open chamber in submerged rock concentrates replicating oligonucleotides from a constant feeding flow and selects for longer strands (1). The thermal gradient triggers a complex interplay of molecular thermophoresis, external flow and laminar convection, where the latter drives strand separation and exponential replication. The experimental results are understood quantitatively based on the calculation of stochastic trajectories inside the chamber using a two-dimensional random walk model. It allowed to calculate lifetimes and thermal oscillation frequencies of the nucleic acids. In an intermediate range of external velocities, the superposition of flow fields retains strands of 75 nucleotides, while strands half as long die out, inverting above dilemma of the survival of the shortest (2). In a thermal cycler, the short strands outcompete the long ones. The combined feeding, thermal cycling and positive length selection opens the door for stable molecular evolution in the long-term micro-habitat of an asymmetrically heated porous rock.

P50

CHARACTERIZATION OF SYNTHETIC POLYMERS IN ORGANIC MEDIA USING ASYMMETRICAL FLOW FIELD-FLOW FRACTIONATION; DEVELOPMENT OF INDUSTRIAL APPLICATIONS

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Producing next generation of materials requires synthesis of new polymers with ever increasing complexity due to the need for diverse combinations of chemical and micro-structural features. Accordingly, molecular level characterization of these materials has become ever more relevant to establish structure-property relationships and to facilitate the design of materials with truly tailored properties. The molecular weight distribution and molecular architecture as a function of molecular weight are among the most important characteristics that need detailed characterization. Asymmetrical flow field-flow fractionation coupled with multi-angle light scattering (AF4-MALS) is used in Dow R&D to characterize complex polymeric materials in aqueous as well as organic solvent carriers, mostly to complement SEC analysis. On this poster we will show application developments of organic phase AF4-MALS in an industrial environment, where quality parameters such as accuracy, precision, robustness, and long-term stability are of utmost importance. Specific examples, such as, characterization of polyacrylic and polyol based samples will be presented. Some of the encountered challenges will also be outlined.